Breakthroughs in the Genetics and Breeding of Capsicum and Eggplant

Torino, 2-4 September 2013

XV Meeting on Genetics and Breeding of Capsicum and Eggplant

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EDITORS

Sergio Lanteri
Giuseppe Leonardo Rotino

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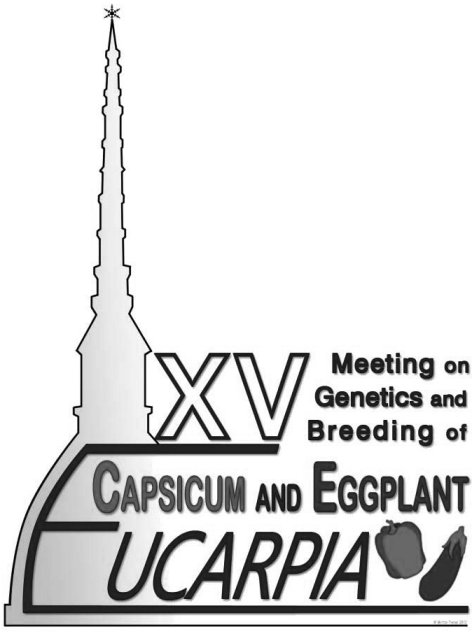

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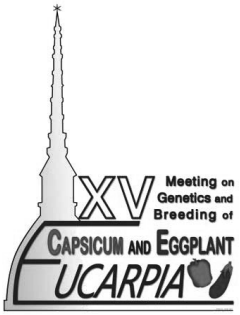

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ESASEM

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Evaluation and release of breeding material/cultivars, and seed production

Bio-chemical characters of eggplant (*Solanum melongena* L.) leaves and their correlation with the fluctuations of jassid (*Amrasca biguttula biguttula* (ishida)) populations.

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Abstract

The experiment was conducted to study the response of some selected cultivars of brinjal or eggplant (*Solanum melongena* L.) to shade light on the role of bio-chemical characters of eggplant leaves of the against the population fluctuation of jassid (*Amrasca biguttula biguttula)*. The brinjal is the host plant of jassid which is a very harmfull pest all over the world. The simple correlation and multiple linear regression equations were used to calculate the impact of chemical plant characters with population fluctuation of the jassid in the various selected cultivars of brinjal. The maximum variations were calculated between the selected eggplant cultivars and all chemical characters of the leaves showed significant variation between the selected varieties. Nitrogen, lipids, potassium, reducing sugar, phosphorus, calcium zinc, ADF, cellulose, NDF, and crude fat showed negative and significant correlation with the jassid population but potassium and phosphorus has non-significant correlation. On the other hand ash contents, crude protein, copper, magnesium, lignin had significant and positive correlation with the jassid population. The ash contents and ADF showed 76.1% and 12.1% respectively for the jassid fluctuation on the eggplant crop, while all other contents showed a minimal and negligible role for the fluctuation of jassid on the eggplant crop. The results showed that the bio-chemical characters of leaves of the brinjal plant play a very important role in the population fluctuation of jassid.

Keywords: Jassid, correlation, bio-chemical, characters leaves, eggplant, fluctuations.

Introduction

Brinjal or eggplant or aubergine (*Solaman melongena* L.) is an important crop which belongs to the family of Solanaceae. It is popular vegetable which is grown in the tropical and subtropical areas. It is called brinjal in Pakistan and India which is derived from the Arabic and Sanskrit words. In America and United Kingdom it is called eggplant which is named due to shape of fruits because some varieties have so much resemblance with the chicken egg. In French is called aubergine

The total area under brinjal farming has been estimated over 678,000 ha, which is about 37% of the world eggplant area, with a production of 10.50 million ton (FAO, 2007). Brinjal is very popular crop in the warmer areas of the Asia, being very popular in Pakistan, Indian, Bangladesh, China and the Philippine; it is also very common in Egypt, France, Italy and United States. In Pakistan different types of vegetables are cultivated on the 225.4 thousand hectare and its production is 2879.9 thousand tons (Anonymous 2004). Brinjal (*Solanum melongena* L.) is very important and very common vegetable through out the country except highest altitudes. Jassid (*Amrasca biguttula biguttula)* (Ishida) (Homoptera: Cicadellidae) is a major pest of numerous crops including cotton, eggplant, okra, potato, tomato, sunflower or cluster bean (Butani and Jotiwani, 1984: Hooda et al. 1997). Cotton Leaf hopper is serious pest of the brinjal crop (Ahmed 1986 and Mall et al. 1992) and

is a very important pest in the tropics and subtropics because the ecological conditions are often favorable for its growth on the host for the whole year. Certain genotypes, in spite of being less hairy are resistant to jassids (Singh et al. 1972), perhaps because of the presence of some biochemical compounds in the leaves of host plants (Singh et al. 1972). According to Barroga and Bernado (1993) the cause of death of jassids on the resistant varieties may largely be due to the nutrient deficiency, rather than the presence of toxic materials in the plants. Singh et al. (1972) concluded that the higher contents of minerals especially silicon, iron and magnesium in resistant strain increase the osmotic pressure of the cell sap thereby adversely affecting the feeding ability of jassids. Based on the facts mentioned above, the present project was initiated to relate chemical components of eggplant genotypes having different degrees of resistance and susceptibility, with the population of jassids.

Materials and Methods

Nine local genotypes of brinjal (Table 1*)* including 3 genotypes (Rubi, Vrib-01 and Culster king) showing susceptibility to jassids, three intermediate varieties (Vrib-0401, Vrib-9901 and Vrib-02-F1) and three resistant varieties (Nirala, Vrib-04 and Bemissal) against jassid were sown on19 February 2010 in the experimental area of the Post Graduate Agricultural Research Station (PARS), following a completely randomized block design with three replications. No plant protection measures were applied for the control of jassid. All the recommended agronomic practices were adopted during the experiment. The row to row distance was 36 cm and that of the plants with in the row 30cm. The plot size was maintained at 500 square meters during the study season. With the appearance of jassid-population, the data were recorded weekly.

For the counts on jassid population, 15 plants of each genotype in each replication were selected at random and tagged. The leaves were observed in such a way that one leaf at upper part of the first plant, one leaf of the middle part of the second plant and one leaf of the bottom part from the third plant of each variety of similar age was taken into account. So a total of 15 leaves were taken per treatment for recording the population data of jassids. The average population of nymphs and adults per leaf, for each genotype, was calculated by simple arithmetic means.

Various chemical components of plant were studied at the crop maturity when the plants were green. Green fresh leaves (500 g) of each selected variety of egg plant were taken from the top (leaves of 5 to 7 days age) and middle + bottom (full-grown leaves) parts of the plants from each plot, washed with distilled water and dried under-shade, followed by drying at 100 ± 5 º C in an oven for 12 hours. The dried material was cut into pieces, ground and passed through a sieve of 1.0 mm mesh. The samples were stored in dry polythene bags for chemical analyses. Crude protein and nitrogen percentage were determined by Kjeldahl method (Winkleman et al. 1986), while potassium was determined by a flame photometer, Model Jenway PFP-7 according to Page et al. (1982). Phosphorous concentration in plant samples was estimated using vanadate-molybdate UV-visible spectrophotometer (Chapmann and Pratt, 1961). Cupper, zinc, manganese, magnesium, iron and calcium in eggplant leaves were determined by study of the significant differences among treatments. Then means were compared by Duncan's multiple range test at P = 0.05. The data on various biochemical plant factors were correlated with the jassid population data. Multivariate regression models, by steps, were developed between pest-population and various biochemical plant factors. The data were transformed into square roots before proceeding with the analysis. Simple correlation was worked out, between the population and chemical factors individually and cumulatively, by using a Multiple Linear Regression Equation of the Type 1, viz., = A + b1x1 + X b2x2 X b3x3 X b4x4 …where population of jassids was taken as the response variables (Y) and the X represent the chemical factors in the equation. The data were analyzed using M. Stat Package (Steel et al. 1997).

Results and discussion:

The results presented in Table 2 showed correlation of individual traits with pest population /leaf. Nitrogen, lipids, magnesium, calcium, zinc, neutral detergent fiber, potassium and phosphorous in the leaves of brinjal plants showed negative and non significance correlation with values of jassid population. While protein, reducing sugar, acid detergent fiber, cellulose and crude fat also have negative but significant correlation with the jassid population.

On the contrary, ash contents and lignin have positive and most significant effect on the jassid population / leaf with correlation coefficient (Table 2). Hemicelluloses, protein, copper, manganese have positive correlation and showed the non-significant effect Table (2). Taking into account the above results, the factors which showed the important role with the population of jassid were analyzed for the multiple linear regressions with the objective to study the role of all factors in the population fluctuation. The data presented in the in the Table (3) showed that ash contents in the leaves of different genotypes of brinjal put maximum 76.1% variations in the population fluctuation of target pest jassid, while acid detergent fiber has explained the 12.1 % variations in population fluctuation. The values for reducing sugar, magnesium, calcium, cellulose, lignin, NDF and crude fat showed a minor role for the population fluctuation Table (2).

Table 1: Comparison of means for population of jassid on different selected genotypes of brinjal during 2010.

Name of genotypes	**Mean population per leaf**
Rubi	1.42 g
Vrib-01	1.67 f
Virb-02-F1	1.78 e
Vrib-0401	2.02 c
Vrib-04	3.03 b
Bemissal	3.36 a
Vrib-9901	1.92 d
Nirala	3.03 b
Culster king	1.67 f
LSD at P = 0.05	0.09
SE	$\pm$ 0.03

Means sharing similar letters are not significantly different by DMR Test.

Table 2: Correlation coefficient values between jassid population per leaf and various chemical plant characters.

Chemical plant characters	**r –Values**
Ash (%)	+0.873**
Nitrogen (%)	-0.206ns
Protein (%)	+0.064
Lipids (%)	-0.194ns
Reducing Sugars (%)	-0.739**
Potassium (%)	-0.353 ns
Phosphorous (%)	-0.153 ns
Magnesium (%)	-0.389ns
Calcium (%)	-0.430ns
Copper (%)	+0.207ns
Zinc (%)	-0.220ns
Manganese (%)	+0.013ns
Acid Detergent Fiber (%)	-0.921***
Cellulose (%)	-0.763**
Hemi-cellulose (%)	+0.240ns
Lignin	+0.837**
Neutral Detergent Fiber	-0.669*
Crude Fat	-0.848**

***=Significant at $P \leq 0.001$
** = Significant at $P \leq 0.01$
* = Significant at $P \leq 0.05$
ns = Non-significant

Table 3: Multiple linear regression equations along with coefficient of determination between jassid population per leaf on brinjal and various chemical plant characters.

	Regression Equation	**R2**	**Variation (%)**	**F. value/ SE**
**Y=	-4.735+1.779X1**	0.761	76.1	79.80/ ±0.347
**Y=	-10.265 = 2.367 X1** + 1.414 X2ns	0.779	1.80	42.27/ ±0.341
`**Y	9.245+2.278**X1+1.360X2-0.577X3	0.787	0.8	28.34/±0.342
**Y	9.165+2.280**X1+1.461X2-0.598X3-0.164X4	0.788.	0.1	20.42/±0.349
**Y	4.128+1.179**X1+2.75**X2-0.313X3-0.656X4-2.420X5	0.909	12.1	41.80/±0.234
**Y	2.938+1.494**X1+2528X2+0.0552X3-0.444**X4-3.780**X5+1.054X6	0.942	3.3	54.47/±0.191
**Y.	5.640+1.560**X1+2.211**X2+0.0269X3-0.514x4-4.114**X5+1.069**X6-0.416X7	94.6	0.40	47.49/±0.190
**Y	10.628+1.647X1+2.418X2-0.0638X3-0.592X4-4.395X5+1.070X6-0.985X7-0.710X8	0.962	1.6	56.30±0.164
**Y	12.862+1.464**X1+2.140**X2-0.0511X3-0.480X4-4.326**X6-0.960**X7-0.563*X8-1.088X9	0.967	0.5	55.64/±0.156

Where: *, Significant at P ≤ 0.05; **, Significant at P ≤ 0.01. ns: non significant

X1= Ash contents (%)
X2= Reducing sugars (%)
X3= Magnesium (%)
X4= Calcium (%)
X5= Acid detergent fiber (%)
X6= Cellulose (%)
X7= Lignin (%)
X8= Nuteral detergent fiber (%)
X9= Crude Fat (%)
R^2 = Coefficient of determination
SE = Standard error

The study was conducted to determine the role of chemical plant characters ash contents, lipids, protein, nitrogen, reducing sugar, ADF, cellulose, Hemicellulose, lignin, NDF, crude fat potassium, manganese phosphorus, calcium, magnesium, zinc, and copper on jassid population. These factors were correlated to higher or lower degree with the jassid population.

All the chemical plant characters of the leaves showed significant variations between selected varieties of brinjal; further more chemical plant characters as the effect of nitrogen, lipids, reducing sugar, magnesium, calcium, zinc, cellulose, NDF and fat in the leaves brinjal plant were significant and showed the negative correlation respectively with the jassid population and potassium and

phosphorous has negative correction and showed significant effects. Ash contents, protein, copper and magnesium had positive and non significant effects on the jassid population/leaf with correlation coefficient. The multiple linear regression analysis in chemical plant characters shows that ash contents contributed in pest fluctuation in 76.1%. In the present study, ash contents showed positive and high significant correlation with the jassid population in the brinjal. In other words it we suggest that ash contents showed higher contents in the leaves and caused the higher population of jassid on brinjal and vice versa. The present different results than those of Lit and Bernardo (1990) in which they study the chemical plant characters of brinjal and found that nitrogen, total sugars, fat and ash were negatively but significantly correlated with adult oviposition and larval feeding preference. The study on the contrary, shows similar results to those of Uthamasamy (1980) who reported that different minerals showed various responses towards population fluctuation of the jassid. In the present study the protein has positive and significant role on the population fluctuation. Singh (1988) found that the effect of protein was less significant on the prevalence of *A. biguttula biguttula.* Singh and Agarwal (1988) found that highly susceptible varieties contained considerably high amount protein as for compared to resistant varieties. The present study showed that proteins have positive and significant correlation with jassid population on brinjal. These results are confirmed by Singh and Taneja (1989) who found a positive correlation between protein contents and oviposition of jassid. Balasubramanian and Gopalan (1981) found that susceptible genotypes contain lower amount of reducing sugar as compared to resistant genotypes. Singh (1988) negatively correlated the total and non reducing sugar with *A. biguttula biguttula* population in leaves of resistance genotypes.

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'Abu' and 'Bhupendra'– two promising brinjal (*Solanum melongena* L.) varieties suitable for cultivation under changing climatic conditions of NE India

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Abstract

Two brinjal (*Solanum melongena* L.) selections viz., GB 09-12 and GB 09-05 along with 60 other accessions and 3 commercial controls were tested consecutively for a number of years in the Dept. of Horticulture, AAU, Jorhat (India) during the period 2009-2012. Multilocation testing of the lines was also done in different agro-climatic zones of Assam particularly in UBVZ, CBVZ, LBVZ and NBPZ. GB 09-12 and GB 09-05 exhibited highest average fruit yield of 27.3 and 27.7 t/ha, respectively which were 29.8 and 31.7% higher than the most popular commercial variety 'JC-1'(21.03 t/ha). Both of them exhibited an average yield increase of 50 – 80% and 30 – 60% respectively in different zones. Both varieties were ready for picking at 120 and 130 days respectively after sowing. They were found to be moderately resistant to bacterial wilt. The fruits of the former are long and of the latter are round, and are very attractive with high consumer preference. Both varieties are very good in taste and may be consumed cooked or roasted. They have been presented in the ZREAC and ATC meetings held at AAU in 2012 for release in the state of Assam. In the national level also, they have been being tested throughout the country through the AICRP programmes. 'GB 09-12' popularly known as 'ABU' and 'GB 09-05' as 'BHUPENDRA' are spreading very fast throughout the entire North Eastern States of India. Seeds are available from the Dept. of Horticulture, AAU, Jorhat.

Keywords*:* Abu, Bhupendra, brinjal, varieties, NE India

Introduction

Brinjal (*Solanum melongena* L.) is one of the most common, highly productive and popular vegetable crop widely cultivated in India and is believed to be originated in North Eastern part of India (Thompson and Kelly, 1957). This part of the country has accumulated a wide range of variability in this crop. There are many local and exotic varieties available and it is very important to evaluate and characterize the germplasm so that they materials of interest can be identified for commercial cultivation or used as genetic material in the breeding programmes (Bora and Saikia, 2012). Accordingly, from a large number of germplasm materials tested, two selections viz., GB 09-12 and GB 09-05 along with 60 other accessions and 3 commercial controls received under AICRP (VC) were evaluated in the North Eastern part of India. The performance of the varieties along with all desirable characteristics are presented in this paper.

Materials and Methods

The experiment was conducted at the experimental farm of the department of Horticulture, Assam Agricultural University, Jorhat during Rabi seasons of 2009-10 to 2011-12 with sixty-five materials of brinjal including PPL, JC-1 and Arka Nidhi as three national control varieties. The trial was laid out in randomized block design (RBD) with three replications. The seedlings were transplanted at a spacing of 60cm×45cm. All the recommended cultural practices were followed. The observations were recorded on five randomly selected plants per replication for each genotype for most of the desirable characters like plant height, number of fruits per plant, fruit length, fruit

yield, time to 50% flowering, single fruit weight (g) and days to 1st harvest. The plants were also observed for bacterial wilt incidence by counting the number of plants wilted out of the total number of plants expressed in percentage (Deka and Shadeque, 1986). The grading of the genotypes was done following the scale of Mew and Ho (1970) as resistant (R)- 0 to 20%, moderately resistant (MR)- 20 to 40%, moderately susceptible (MS)- 40 to 60% and susceptible(S)- above 60%. The promising selections were tested in different climatic zone of Assam also. The weather data for the crop seasons of all the years were also recorded for Jorhat condition.

Results and Discussion

The weather data presented in Table 1 show that there was great variation regarding temperature, relative humidity and rainfall from 2009-10 to 2011-12. During the experimental periods, there was rise in temperature by 0.5°C, a decline in RH by 7% and an increase in rainfall by 31.7 mm. This may be an indication that there was climatic change in the North East region even in a short period of years.

Great variation among the genotypes was observed for characters like fruit shape, colour, yield, fruit length and fruits per plant. Shape of the fruits varied from long to round or oblong. Different fruit colours like purple, white, pink etc. were observed (Anonymous, 2011).

Mean performance over years with respect to different quantitative characters for some promising genotypes is presented in Table 2. This table shows that GB 09-05 ranked first for 3 characters viz., fruit yield per plant, fruit diameter and single fruit weight. It was also found to be good for fruits per plant and days to 50% flowering also. GB 09-12 stood second position for fruit yield per plant, fruit length and days to 50 % flowering. It was found to be good for fruits per plant, and days to first harvest also.

The reaction of some genotypes to bacterial wilt is shown in Table 3. From this table, it is apparent that both the genotypes viz., GB 09-12 and GB 09-05 along with some others have shown resistant reaction to bacterial wilt. Bora et al. 2011 reported similar results with respect to a number of varieties including ‘Utsav’.

The yield performance in terms of q/ha at AAU, Jorhat and different Agroclimatic zones is presented in Table 4. The pooled results showed that both the varieties have shown yield increase of more than 25% over the best check variety in AAU, Jorhat and more than 50% increase over the local check variety in different agroclimatic zones of Assam. The fruits of GB 09-12 and GB 09-05 are very attractive and could be used for both cooking and roasting purposes. The consumer preference for both the varieties was also high. The benefit/cost ratio is also quite high for these varieties in comparison to the controls. The morphological characteristics of both the varieties are presented in Table 5. Fruits of GB 09-12 are long with light purple colour whereas those of GB 09-05 are round with dark purple colour. The incidence of shoot and fruit borer in both the varieties was low whereas phomopsis blight incidence was low in GB 09-05 but moderate in GB 09-12. They have been presented in the ZREAC and ATC meetings held at AAU in 2012 for release in the state of Assam. In the national level also, they are being tested throughout the country through the AICRP programmes. ‘GB 09-12’ popularly known as ‘ABU’ and ‘GB 09-05’ as ‘BHUPENDRA’ are spreading very fast throughout the entire North Eastern States of India the seeds of which are available at Dept. of Horticulture, AAU, Jorhat.

Conclusion

From the above results, it could be concluded that GB 09-12 and GB 09-05 are very good in respect of yield, disease and pest tolerance, consumer preference and economics of production and

are better than the existing varieties. Hence, they may be released for commercial cultivation in the North Eastern part of India.

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Table 1. Weather data during Rabi seasons of 2009-10 and 2011-12 and its variation.

Month/ year	Average Temperature (°c)			Average Relative Humidity (%)			Total Rainfall (mm)		
	2009-10	2011-12	Variation (+/-)	2009-10	2011-12	Variation (+/-)	2009-10	2011-12	Variation (+/-)
Sept.	29.4	29.4	(+/-) 0	82.5	81.5	-1.0	167.8	180.9	+13.1
Oct.	26.5	27.0	+0.5	82.0	82.5	+0.5	47.6	26.9	-20.7
Nov.	21.8	20.6	-1.2	83.0	83.0	(+/-) 0	22.4	18.4	-4.0
Dec.	17.9	18.0	+0.1	83.0	77.0	-6.0	2.1	19.4	+17.3
Jan.	16.7	15.9	+0.8	78.0	81.0	+3.0	9.8	15.7	+5.9
Feb.	18.4	19.2	+0.8	71.5	72.5	+1.0	10.3	10.3	(+/-) 0
Mar.	21.8	21.3	-0.5	76.0	71.5	-4.5	16.6	36.7	+20.1
Change in +/- direction			+0.5	-		-7.0			+31.7

Table 2. Mean performance of promising brinjal genotypes over years. Varieties ranking first and second are indicated in Roman numbers.

Genotypes	Fruit yield/ plant(kg)	Fruits/ plant	Fruit length (cm)	Fruit diameter (cm)	Single fruit weight (g)	Plant height (cm)	Days to 50% flower-ing	Days to 1st harvest
GB 09-12	2.1 II	11	28 II	7	180	56	120 II	140
GB 09-05	2.2 I	10	12	12 I	300 I	54	125	146
GB 09-02-01	1.2	8	21	4	200	64	130	129 I
GB 09-05-03	0.5	6	30 I	4	100	71 II	121	135 II
JB 09-03	2.1 II	5	14	8	250 II	60	134	150
GB 09-11-01	1.9	5	14	10 II	150	45	130	145
GB 09-10-14	1.2	4	12	5	150	52	136	146
JB 10-13	1.5	14 I	15	3	113	34	132	150
JB 10-14	1.2	11	11	6	163	36	128	155
JC-1©	0.6	8	16	5	195	85 I	130	150
JC-2©	0.8	5	14	9	250 II	66	118 I	140
SM 6-6©	1.2	13 II	15	7	58	43	135	150
PPL©	0.6	8	15	5	250 II	69	130	150
Arka Nidhi©	0.9	7	14	4	40	54	120 II	140
CD(5%)	0.8	5.0	6.0	4.0	51.0	10.0	7.0	6.5
CV(%)	8.5	6.3	5.3	3.6	8.3	6.3	2.8	3.5

Table 3: Reaction of some genotypes to Bacterial Wilt

Resistant (R)	BB-54, Utsav, Singnath, Bholanath, Arka Nidhi, GB 09-02-02, GB 09-16-02, GB 09-05, GB 09-12, JB 09-13, GB 09-05-03, GB 09-10-14
Moderately Resistant (MR)	GB 09-11-01, 10/BRBW RES-4, RCMBL-3, Khoruah-3, JB 10-13, JB 10-15, JB 10-18, SM 6-6
Moderately Susceptible (MR)	JB 10-17, JB 10-14
Susceptible (S)	09/BRBW RES-6, JB 10-19, PPL

Table 4. Yield (t/ha) performance of brinjal varieties in comparision to checks

Sl. No.	**Variety**	**Yield (t/ha) at AAU, Jorhat**				**(%) increase over best Ch.**	**Special character**
		2009-10	**2010-11**	**2011-12**	**Mean**		
1	GB 09-12	26.00	27.50	28.40	27.30	29.8	Very attractive fruit
2	GB 09-05	28.00	26.40	28.70	27.70	31.7	Very attractive fruit
3	JC-1©	18.82	21.03	23.24	21.03	-	-
4	JC-2 ©	17.53	20.54	18.22	18.76	-	-
Sl. No.	**Variety**	**Yield (t/ha) at different zones***		**(%) increase over Ch.**	**Duration (days)**	**Consumer preference**	**C/B ratio and Special character**
1	GB 09-12	22.00		65	120	High	1:2.80 /Good taste, suitable for cooking and roasting.
2	GB 09-05	20.70		55	130	High	1:2.72 /Good taste, suitable for cooking and roasting.
3	LC	13.3		-	-	Moderate	1:1.60 / -

*Different zones includes Upper Brahmaputra Valley Zone, Lower Brahmaputra Valley Zone, Central Brahmaputra Valley Zone and North Bank Plain Zone.

Table 5. Morphological and other characters of the brinjal varieties

Sl. No.	**Characteristics**	**GB 09-12**	**GB 09-05**	**Sl. No.**	**Characteristics**	**GB 09-12**	**GB 09-05**
1	Plant growth habit	Upright	Prostate	16	Fruit pedicel prickles	None	Few
2	Plant Spread (cm)	83	100	17	Fruit length-breadth ratio	4.0	1.0
3	No. of primary branches per plant	3	4	18	Fruit curvature	Curved	None
4	Petiole length(cm)	8	4	19	Fruit shape	Long	Round
5	Petiole colour	Green	Green	20	Fruit apex shape	Depressed	Depressed
6	Leaf blade length (cm)	20	15	21	Fruit colour	Light purple	Purple
7	Leaf blade width (cm)	12	11	22	Fruit color distribution	Irregularly striped	Uniform
8	Leaf blade lobing	Strong	Strong	23	Fruit flesh density	Moderately compact	Loose
9	Leaf blade tip angle	Acute	Interme-diate	24	No. of fruit harvest	3	3
10	Leaf blade colour	Green	Dark. green	25	Fruit position	Pendent	Pendent
11	No. of leaf prickle	None	None	26	Seediness	High	High
12	Corolla colour	Light purple	Dark purple	27	Seed colour	Light yellow	Dark yellow
13	Calyx colour	Light purple	Light purple	28	Seed size	Interme-diate	Large
14	Calyx spininess	Smooth	Smooth	29	1000 seed weight (g)	4.31	2.98
15	Fruit pedicel length (cm)	8.0	6.0	30	Biotic stress susc.	FB3PB5 **	FB3PB3 **

**FB =Fruit borer (3=low, 5=medium) ; **PB= Phomopsis blight(3=low, 5=medium).

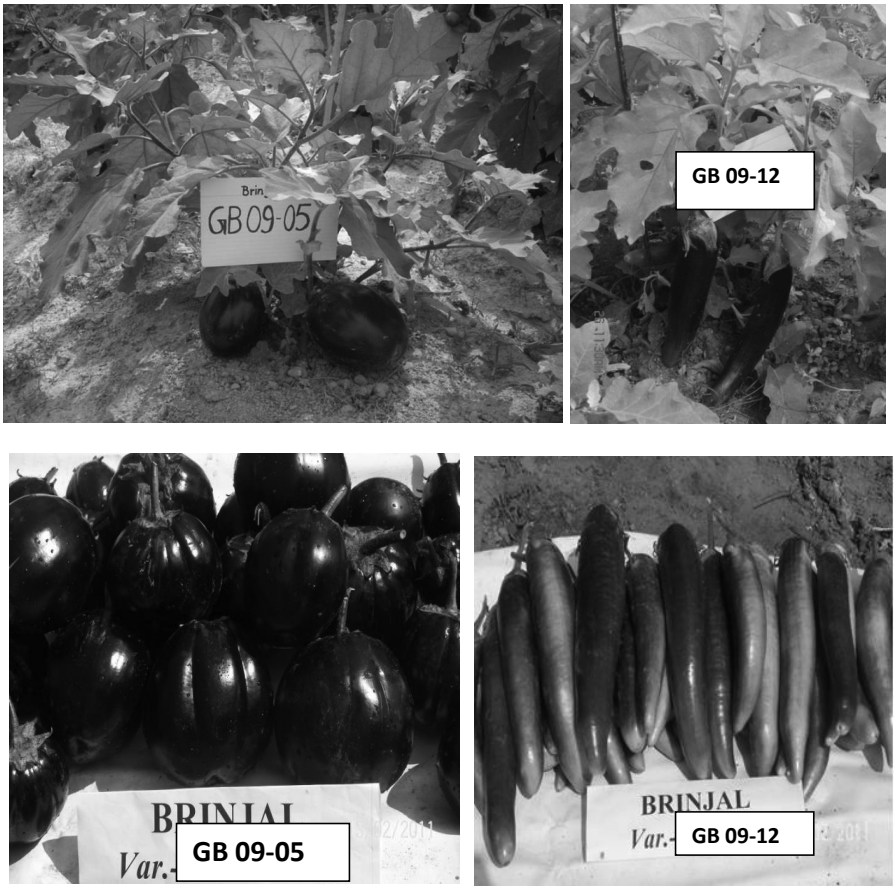

Fig 1. A comparative view of the brinjal varieties 'ABU'(GB 09-12) and 'BHUPENDRA'(GB 09-05)

Evaluation of new resistance-genes deployment strategies in the pepper *Capsicum annuum* for the durable management of root-knot nematodes.

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Abstract

The current restrictions on the use of chemical nematicides have contributed to increase root-knot nematode problems in horticultural crops. In this context, plant resistance appears as the most effective method of control, but the possible occurrence of virulent nematodes able to reproduce on *R*-plants may constitute a severe threat to this control strategy. In *Capsicum annuum*, resistance to RKN is controlled by several dominant genes — the *N* and *Me* genes. To implement a rational management of the *R*-lines increasing the durability of the *R*-genes, we tested several *R*-gene deployment strategies. Experiments were conducted in climate-controlled rooms, in greenhouses, and under 3-years-field agronomic conditions to compare i) the succession of the same *R*-genes every year, when introgressed in a partially resistant vs. a susceptible genetic background, ii) the alternance, ii) the mixed cultivation and iv) the pyramiding of two *R*-genes with different modes of action in a single genotype. At the plant level, we previously showed that the choice of the *R*-genes and the genetic backgrounds in which they are introgressed can lower the frequency of resistance breakdown, and that the pyramiding of two different *R*-genes in one genotype totally suppressed the emergence of virulent isolates. Here, at the field and rotation level, we confirmed these results and showed that i) alternating different *R*-genes in rotation is efficient to reduce the selection pressure of *R*-genes on the pathogens and allows to recycle broken *R*-genes, and ii) optimal cultivation practices of *R*-plants increase their "trap" effect and may decrease the amount of pathogens in the soil, below their damage threshold. These results are in good agreement with concepts recently developed from the analysis of other plant-pathogen interactions. The root-knot nematode model could thus contribute to generalize strategies for the breeding and management of *R*-cultivars strengthening and increasing the durability of qualitative resistances.

Keywords: Sustainable crop protection, breeding strategy, resistance gene deployment, virulence emergence, root knot nematodes, *Meloidogyne* spp., *Capsicum* spp.

Introduction

Plant-parasitic nematodes are among the most damaging and uncontrollable pests of cultivated crops causing severe economic losses in world agriculture, estimated to $US 121 billion per year and affecting 12.3 per cent of the word crop production (Chitwood, 2003). The specialized and intensive vegetable crops agriculture is becoming particularly vulnerable to a few species belonging to the group of root-knot nematodes (RKNs, *Meloidogyne* spp.), obligate plant endoparasites, found throughout the world, mainly in tropical, subtropical and warm-temperate areas in which several nematode generations can be completed per year. These polyphagous nematodes are one of the

main pathogens on many Solanaceous crops throughout the world (Khan and Haider 1991). The parasite pressure due to these soilborne pests in vegetable crops has increased steadily following the changes in pesticide legislation and the new regulations that have withdrawn the use of most chemical nematicides. These microscopic parasites are difficult to control, particularly because of their highly polyphagous nature and their ability to remain hidden in the soil or in plants. Host resistance is considered as an important component of integrated management of RKNs. Because few *R*-genes acting against these pests are currently available, it is urgently needed to protect them and promote their durability. In pepper, the *Me* genes, identified in local populations, control the main species of *Meloidogyne* (*M. arenaria, M. incognita, M. javanica, M. hapla*) (Hendy et al. 1985; Djian-Caporalino et al. 1999, 2001, 2007). Although they have only recently been used in plant breeding, a risk of some of these genes being overcome has already been demonstrated under laboratory experiments with high inoculum pressure of nematodes (Castagnone-Sereno et al. 2001; Djian-Caporalino et al. 2011). On this pathosystem, it was also shown that virulence is highly specific to a determined *R*-gene on which selection has occurred and that a reproductive fitness cost is associated to nematode virulence (Castagnone-Sereno et al. 2007; Djian-Caporalino et al. 2011). The adaptative significance of trade-offs between virulence traits and fitness-related traits suggests that, although the resistance can be broken, it may be preserved in some conditions. Beside the improvement of knowledge that is essential for a better sustainable management of plant resistance, the interest of the RKN model system is based on its originality compared to other plant pathogens. First, the parasitic pressure that is applied by RKNs to their host plants is theoretically low: small population size (a few hundreds of juveniles in one egg-mass as the total progeny of a female), long biological cycle (about eight weeks at 20°C). Second, the biological features of RKNs that govern their evolutionary potential should not favour the emergence of virulent populations: lack of sexual reproduction (obligatory mitotic parthenogenesis), active dispersal capacities reduced in soil. However, our previous studies in artificial conditions (Djian-Caporalino et al. 2011; Barbary et al. submitted) have shown that breaking resistance is dependent on the major resistance gene used, its allelic status (homozygous *versus* heterozygous) and the genetic background it has been introgressed in. These results are in good agreement with concepts recently developed from the analysis of very different plant-pathogen interactions: pepper-virus (Palloix et al. 2009) or rapeseed-*Leptosphaeria* (Brun et al. 2010). The RKN model studied here could thus contribute to the generalization of strategies for the breeding and management of resistant cultivars.

In this study, we evaluated several resistance-gene deployment strategies to implement a rational use of pepper *R*-cultivars, with the objective to improve the sustainable management of RKNs. Experiments were conducted in climate-controlled rooms, in greenhouses, and under 3-years-field conditions comparing i) the succession of the same *R*-gene every year, when introgressed in a resistant vs. a susceptible genetic background, ii) the alternance of single *R*-genes in rotation, iii) the mixture of genotypes bearing single *R*-genes sown in the same plot, and iv) the pyramiding of two *R*-genes in one genotype.

Materials and Methods

Plant material

The five pepper (*Capsicum annuum*) genotypes used in this work are inbred lines with differential resistances to RKN. Doux Long des Landes (DLL) is a susceptible cultivar. The two resistant haplo-diploid lines, DH149 and DH330 produced through *in vitro* androgenesis were previously described (Djian-Caporalino et al. 1999); they are homozygous for the *Me3* and *Me1* genes, respectively.The *Me3* gene induces early cellular necrosis in the root epidermis adjacent to the juveniles (Bleve-Zacheo et al. 1998). The selection of virulent variants against the *Me3* gene was achieved through strong selection pressure on avirulent *M. incognita* isolates. *Me1* induces a late hypersensitive reaction in the vascular cylinder of infected roots, thus inhibiting the

development of egg-laying females (Bleve-Zacheo et al. 1998). Under laboratory conditions, *Me1* prevents the emergence of *Me1*-virulent nematode genotypes, despite the implementation of drastic levels of inoculum (Djian-Caporalino et al. 2011). *Me3* and *Me1* are currently being introgressed by breeders into cultivars but they are not yet commercialized. Two F1 hybrid lines were also used, one carrying *Me1* in its heterozygous state in the DLL susceptible genetic background (F1 [DH330 x DLL]), and one combining the two mechanisms of resistance from *Me3* and *Me1* (F1 [DH149 x DH330]). All the lines were produced independently in insect-proof cages to eliminate outcrossing. Pepper seedlings were grown individually in 100 ml pots containing steam-sterilized sandy soil covered by a 1 cm layer of loam in climatic chambers maintained at 24°C (±2°C) with a 12-h light cycle and a relative humidity of 60–70%. Seven to eight-week-old plants (8-10 true leaves) were transplanted in the plots.

Design of the 3-yr field experiment

The experiment was carried out in a plastic tunnel belonging to the Chamber of Agriculture of Alpes-Maritimes (technical institute) in La Gaude (SE France). The tunnel was 224 m2 (28m x 8m). The soil had a pH of 8.2 with 46.68% of sand, 27.99% of loam, and 25,33% of clay. Total limestome was 171 g/kg. The soil temperature in the tunnel varied from 15°C in winter (December to April) to 25°C in summer (June to September) at 15 cm depth (Mediterranean climate). During the whole experiment, the tunnel received no phytosanitary treatment. It was subdivized in 52 microplots of one scare meter each, separated by one meter of bare soil between each plot. Before starting the experiment, nematode-susceptible tomatoes were cultivated for three consecutive years in non disinfected soil which was naturally infested with a mixture of *Meloidogyne incognita* and *M. arenaria*. The experiment was performed on 4 rows (1 meter apart) with two lines of fertirrigation drips (16 mm diameter tubes with 10 holes/ m² providing 2 L/h) by rank and the establishment of a non-degradable plastic mulch to prevent contamination between plots. The first year, the experiment received an organic amendment before the establishment of the plastic mulch. The third year, the experiment was only irrigated but not fertilized by the grower.

Six cultivation modalities were compared during 3 successive years: 1) the succession of the same *R*-gene (*Me1*), when introgressed in a resistant genetic background (DH330), 2) the succession of the same *R*-gene (*Me1*) when introgressed in a susceptible genetic background (F1 [DH330 x DLL]), 3) the alternance of single *R*-genes in rotation (*Me3* (DH149) the first year, *Me1* (DH330) the second year, then *Me3* (DH149) the third year), 4) the mixture of lines bearing single *R*-genes (*Me1* (DH330) or *Me3* (DH149), respectively) transplanted in the same plot, 5) the pyramiding of two *R*-genes (*Me3* and *Me1*) in one line (F1 [DH149 x DH330]) and 6) the susceptible cultivar (DLL) as control. Each scare-meter plot harboured five plants of a given modality from April-May to October, followed by five growing cycles of susceptible salads (*Lactuca sativa* cultivar Dedale-batavia), from November to February. Globally, repeats of 8 to 9 plots and 40 to 45 plants per genotype were tested, respectively.

Infestation parameters

Several infestation parameters were analysed along the 3 years. The gall index (GI) was determined for the roots of each pepper or salad plant using a 0 to 10 scale (Zeck, 1971). The number of infected plants per genotype tested was also recorded. To determine the RKN soil infection potential (SIP), 5 replicates of 1 kg-rhizospheric soil were sampled from each plot at 15 cm depth before and after pepper or salad cultivation. Two-month-old susceptible tomato plants (cv. Saint Pierre) were transplanted in pots filled with these soil samples and maintained in a climatic chamber (24°C±2°C, 14-h photoperiod). After 6 weeks, the number of egg masses (EMs) on the tomato plants was evaluated as previously described. To determine the reproduction rate (RR) of potentially virulent nematodes, EMs, if they were detected on a resistant pepper, were picked and inoculated on a 2-month-old resistant pepper carrying the same *R*-genes(s) and maintained in the climatic chamber. After 6 weeks, the roots were carefully washed with tap-water and examined

under a magnifying glass to detect EMs. If EMs were detected, they were reared by successive re-inoculations on 2-month-old resistant peppers carrying the same *R*-genes(s) according to the procedure of Jarquin-Barberena et al. (1991). After 2 generations, 10 EMs were picked up and the mean number of eggs per EM (i.e., the number of eggs produced by one female) was evaluated.

Statistical analysis

In order to compare the evolution of each data mean (SIP, RI, RR), a Kruskal-Wallis test was firstly carried out. Wilcoxon-Mann-Whithney unilateral tests were then used for comparisons in order to check if differences were significant. Bonferroni correction was consequently applied (significance level at α=0.05). Analyses were performed using the free software R (http://www.r-project.org/).

Results

Results on strength and durability of resistances

The evolution of the root infestation of peppers during the three successive years for the 6 modalities, respectively, is presented in Figure 1. As expected, the susceptible cultivar DLL, cultivated in naturally-infested plots, exhibited high infestation levels over the whole experiment (GI ranging from 9.2 to 9.4). Conversely, the 5 modalities that include R genotypes showed a significant reduction of the number of galls on their root systems, whatever the R gene(s) and the mode of use considered (i.e., alternance, mixture or pyramiding). However, differences were noticed among the 5 modalities. After one year of cultivation, the homozygous line DH330 did not show any gall (in monoculture or in alternation with DH149), while the level of infestation progressively increased during years 2 and 3 (GI raised up to 1.6 in year 3). The same trend was generally observed for the other modalities, the highest infestation level being observed in the case of the heterozygous F1 line [DH330xDLL] after the third year of cultivation (GI=3.7). The only notable exception is reported for the F1 [DH149xDH330] pyramiding *Me3* and *Me1*, which remained almost uninfested over the 3 years (GI ranging from 0 to 0.2). In order to evaluate the possible selection of *M. incognita* isolates virulent against *Me1* or *Me3* during the experiment, eggs recovered from R peppers were hatched and the resulting J2 used to reinoculate the same R genotype. Egg-masses sampled on HD149 contained more than 900 eggs on average, and a virulent line was successfully reared by successive re-inoculation on DH149 peppers. After 3 successive re-inoculations, the mean number of eggs per EM was 866.7±43.1 (18 replicates; data not shown). Numerous egg-masses were recovered from *Me1* peppers, either homozygous (DH330) or heterozygous ([DH330xDLL]), but they contained few eggs (<65 eggs per EM), and the nematodes obtained from these eggs did not survive to a successive inoculation, which impaired the selection of a *Me1*-virulent isolate. Very few EM were recovered from the F1 [DH149 x DH330] peppers combining *Me1* and *Me3*, and again no virulent population could be obtained after re-inoculation on R plants (data not shown).

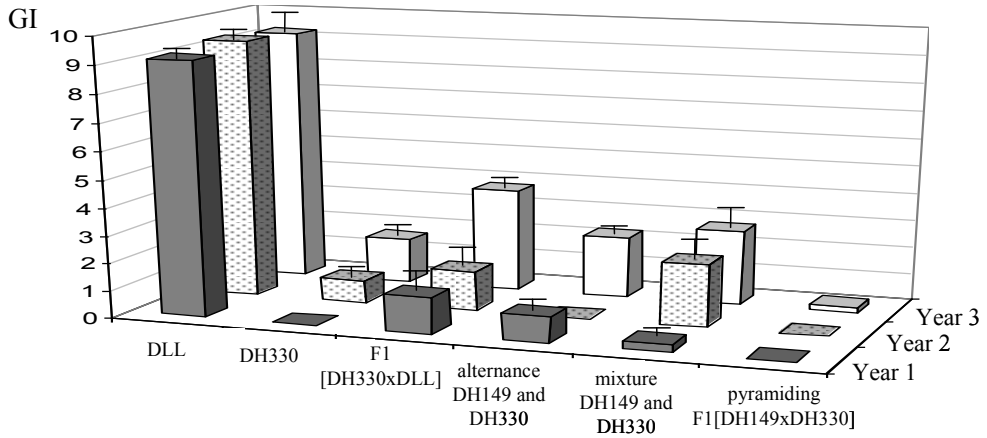

Figure 1: Gall index (GI) on peppers (mean of 40 to 45 replicates ± standard error).

Results on reduction of the soil infection potential ("trap" effect)

In the first year of the experiment, before planting the pepper genotypes, the SIP of the whole plot was moderate to heavy (the mean SIP for 52 microplots reached 546±71 EMs per plant) (Figure 2). A succession of susceptible plants each year (DLL in summer and salads in winter) greatly increased the SIP in corresponding microplots (from 456±140 to 1019±100 EMs in year 3). After 2 months of bare soil, no significant changes in SIP could be observed. Resistant peppers DH330, F1 [DH330xDLL], and mixture DH330 and DH149 did not significantly reduce SIP over three years of experimentation. In contrast, the results highlight the beneficial effects of two management strategies of resistance: the cultivation of hybrids combining two resistance factors and alternating rotation of varieties, each carrying a different resistance. In fact, DH149 doubled SIP (from 412±157 to 823±204) the first year with the developpement of a virulent population. Nevertheless, the rotation with DH330 significantly reduced SIP from 874±218 to 78±76 (91%). F1 [DH149 x DH330] combining *Me1* and *Me3* most strongly reduced SIP the first year (from 596±179 to 2.8±2.8, ie 99.5%), this reduction being almost complete in some microplots, when hairy root peppers were particularly developed through addition of an organic amendment and proper fertirrigation. This "trap plant" effect was maintained over the 3 years. The final level of reduction using this modality was 97.4% of the mean initial rate recorded in the 45 plots. These results are in agreement with those comparing the GI on susceptible salads each year after each pepper modalities (data not shown). After the first cultivation cycle of peppers, salads cultivated after the 5 modalities that include R genotypes showed a significant reduction of GI compared to salads cultivated after the susceptible cultivar DLL (mean GI=0 after pyramiding to 0.9 after DH149 compared to 1.5 after DLL). Alternance and especially pyramiding allowed protecting the salads during the 3 years : GI raised up to 4.3±0.3 in year 3 after DLL, 2±0.3 after alternance DH149, DH330, DH149, and only 0.6±0.1 after F1 [DH149 x DH330] peppers combining *Me1* and *Me3*.

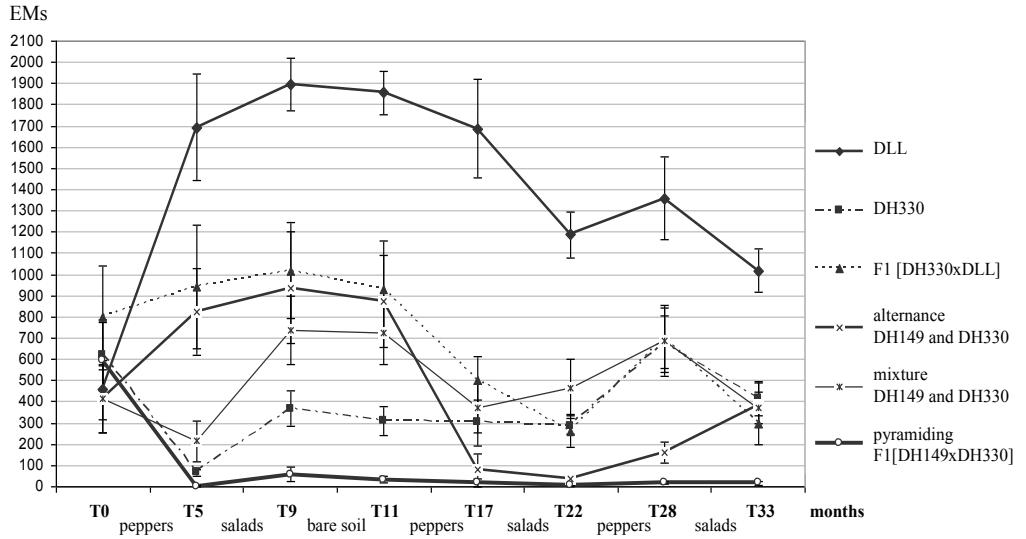

Figure 2: RKN soil infection potential (SIP) corresponding to the number of egg-masses (EMs) on susceptible tomato plants maintained 6 weeks in pot filled with 1 kg-rhizospheric soil sampled from each plot at 15 cm depth (mean of 8 to 9 replicates ± standard error).

Discussion

Our experimental data allow the identification of conditions strengthening the durability of qualitative resistances by lowering the emergence of virulent soil-borne pests and assess the time required for the sustainable improvement of soil health (reduction of parasite populations under their damage threshold) using the *R*-plants as "traps". Since resistance sustainability is influenced by the variation in (a)virulence and host range, we showed that two primary attributes of host resistance for resistance breeding and management are relevant: i) the value of resistance in crop self-protection, based on the level of resistance of the plant to injury caused by infection, and ii) the rotational value of different resistances in cropping systems for protecting subsequent crops, by reducing the selection pressure of each *R*-gene on the pathogens or by decreasing the amount of pathogens in the soil.

At the plant level, we showed, first, that the choice of the *R*-gene is of crucial importance. In fact, even in natural field conditions, one of the RKN *R*-gene (*Me1)* conferred a high level of resistance without being overcome (no virulent population obtained), while another one (*Me3*) was easily overcome and *Me3*-virulent natural isolates were generated by successive re-inoculation on *Me3*-peppers. Nevertheless, we showed that the genetic background in which the major *R*-gene is introgressed is important. In fact, the *Me1 R*-peppers with fifty percent of susceptible DLL background (F1 [DH330xDLL]) had more EMs compared to DH330. These results are confirmed by another study in which *Me3* and *Me1* were introgressed in either a susceptible or a partially resistant genetic background in either homozygous or heterozygous allelic status (Barbary et al. submitted). Confronting these genotypes to the high inoculation pressure of an avirulent *M. incognita* isolate demonstrated that the genetic background plays indeed an important role, whatever the allelic status (homo- or heterozygous) of the *R*-genes. These results are in agreement with laboratory experiments on other pathosystems such as tomato-*M. incognita* (Williamson and Roberts 2009), cotton-*M. incognita* (Wang et al. 2008), potato-*Globodera pallida* (Fournet et al. 2013), pepper-virus (Palloix et al. 2009), and in field experiments on rapeseed-*Leptosphaeria maculans* interaction (Brun et al. 2010). The authors suggested the presence of additional genes or

quantitative trait loci (QTL) that may have epistatic interactions with the primary resistance determinants, or may increase the number of virulence mutations required in the pathogen genome to breackdown the resistance. In pepper, experiments are now underway to detect and localize such QTL explaining the differences observed between susceptible and partially resistant genetic backgrounds, and to determine the effectiveness of their « protective » role on the major *R*-genes. Finally, we showed that the pyramiding of two different *R*-genes totally suppressed the emergence of virulent isolates, based on their complementary mode of action and was the more durable modality during the 3-years-field experiment. Moreover, this modality also controlled virulent laboratory selected and natural isolates overcoming one of both genes (data not shown). In theory, pyramiding into a single cultivar several *R*-genes that have the same spectrum of action but that differ in their mechanisms should provide a more durable resistance since mutational events at several avirulent loci would be required simultaneously to produce a new virulent pathotype (Mundt 1990). The probability of simultaneous mutations for virulence to two effective genes is much lower than for a single gene, as suggested by several simulation modeling studies (Porter et al. 2000; Zhao et al. 2003; Wang et al. 2007). However, a rather limited number of experimental studies have confirmed this hypothesis in plant pathosystems, especially for vegetable crops. The reason probably being that genotyping the pyramiding population needs reliable molecular markers that are not always at hand. The availability of molecular markers closely linked to each of the *Me R*-genes (Djian-Caporalino et al. 2007; Fazari et al. 2012) makes the identification of digenic genotypes possible and will help breeders to construct novel resistant pyramid genotypes.

At the field and rotation level, we further demonstrated that alternating different *R*-genes in rotation is efficient to reduce the selection pressure of the *R*-genes on the pathogens and to decrease virulent populations in fields (Figure 2). Previous studies (Castagnone-Sereno et al. 2001; Djian-Caporalino et al. 2011) showed indeed that neither natural nor selected *Me3*-virulent RKN isolates were able to reproduce on *Me1*-peppers. Such strict specificity of virulence could explain that, once virulent isolates are selected on a determined *R*-gene, alternance in the rotation with a different gene reduces the number of nematodes in the soil under their damage threshold, improving soil health. This finding offers the possibility of 'recycling' broken resistance genes in successive cycles of cultivation. We did not observed a significant protection of *Me3 R*-lines by *Me1 R*-lines when sown together in the same plot, except the first year, when the roots were highly developed due to organic matter (Fig. 2). Soil borne pathogens, including RKNs, have limited dispersion ability. So, mixture of *R*-lines could only be effective if the roots are intertwined. In this case, it can minimize the probability of resistance breakdown by decreasing the amount of pathogens in the soil. Implementing a root growth stimulation when using *R*-plants could increase the "trap plant" effect and thus decrease the amount of pathogens in the soil. Finally, we showed that the pyramiding of two different *R*-genes appears very promising as RKN "traps" plants, reducing up to 90% the infestation rate of the soil. When pyramiding remains difficult, the other *R*-genes deployment strategy - alternating – may benefit yields in the long-term by increasing the durability of the qualitative resistances.

To decrease the amount of pathogens and increase the durability of *R*-genes, the combination of *R*-plants and cropping techniques should also be tested. It is currently underway at INRA on RKN in protected vegetable cropping systems with financial support from the European Commission and from the French Ministry of Agriculture, Food and Fisheries (Gedunem project, launched in the framework of the INRA metaprogramme SMaCH – Sustainable Management of Crop Health). Results are expected to suggest rules for breeders and farmers for the sustainable management of disease resistance, re-engineering the agroecosystem to increase overall host diversity, at the species level as well as at the gene level, to reduce directional selection and present an evolutionary dilemma to the pathogen.

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Adaptability to Colombian Caribbean climate conditions of two new eggplant (*Solanum melongena* L.) cultivars.

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Abstract

In Colombia, the supply of improved eggplant varieties is scarce, as the crop is mainly grown by small farmers with low incomes which self-produce seeds. The aim of this study was to determine, leaf area index (LAI), leaf area ratio (LAR), relative growth rate (RGR) net assimilation rate (NAR) and phenology of two new varieties: CO-015 and CO-029 for assessing their adaptability to the Colombian Caribbean (8°45'N and 75°53'W) climate condition.

The variety CO-015 showed its maximum LAI (0.81) at 92 days after transplantation (DAT) while CO-029 showed an indeterminate trend. The LAR raised at the beginning of the season in both the varieties (61.3 cm^{-2} g) and then reduced to a daily rate of 0.37 cm^{-2} g ($R^2 = 0.95$) in CO-015 and 0.41 cm^{-2} g in CO-029 ($R^2 = 0.94$), while in both the RGR significantly increased up to 33 DAT and then decreased. Models that explain the RGR were: $Y = -0.0595 +0.0073 +4.1490 X-0.0001X^2 E-7X^3$ ($R^2 = 0.76$) in CO-015 and $Y = -0.0873 +0.0087 +4.9760 X-0.0001X^2 E-7X^3$ ($R^2 = 0.74$) in CO-029. The NAR increased up to 119 DAT (116.6 $g.cm^{-2}.day^{-1}$) in CO-015 while in CO-029 the highest value was detected at 92 DAT (99.58 $g.cm^{-2}.day^{-1}$). The setting of flower buds, flowers and fruits was analogous in both the varieties. The CO-015 produced an higher number of fruits of smaller size. On the basis of our results the variety CO-029 is more suitable for cultivation in the tested environmental conditions.

Keywords: Genotype environment interaction, growth analysis, crop physiology, crop phenology.

Introduction

In Colombia the area planted with eggplant is 209 hectares and the total production about 1.791 tons. The Córdoba region is the main growing area (274 acres, share of 42.95%), with a production of 769 tons (6.9 $t.ha^{-1)}$. The production decreases yearly of about 1.6%, due to both climatic changes and agronomic crop management (Agronet, 2013).

Eggplant is mainly grown by farmers self-producing seeds from heterogeneous populations, consisting of a mixture of genotypes which are the result of mutations, natural hybridization and segregation occurred over time (Hoyos et al. 1999). Furthermore, the agronomic crop management as well as the adverse environmental conditions limit the expression of the yield potential and the quality of the production; this hampers the possibility to meet the requirements of both national and international markets.

In view of the economic importance of this crop and its increased demand in countries like the U.S. and Canada, thanks to a support of the National Government and a collaboration between the University of Cordoba and Corpoica and small producers, two new cultivars adapted to the Colombian Caribbean environment have been released: CO-015 and CO-029. CO-015 is characterized by ovoid fruits of lilac color, and its potential yield is of 53.7 $t.ha^{-1}$, while CO-029 produces ovoid- long shaped fruits, dark purple, and its potential yield is of 55.3 $t.ha^{-1}$.

The aim of this investigation was to evaluate the growth related parameters and phenology of the two cultivars in the Colombian Caribbean climate conditions.

Materials and Methods

Location: The research was conducted during the first half of 2010 at the Agricultural Sciences Faculty of the University of Cordoba, located in Monteria - Colombia, 14 m.a.s.l, (8° 44' North latitude and 75° 53' West longitude), mean annual rainfall of 1346 mm, relative humidity of 84%, annual average temperature of 27.4 ºC, sunshine per year 2108.2 hours and living area called tropical dry forest (bs-T), as classified by Holdridge (Palencia et al. 2006).

Plant material. The two eggplant varieties CO-015 and CO-029 were assessed, which were obtained by breeding program performed at the University of Córdoba and Corpoica in cooperation with the small producers of the area.

Statistical analyses. Evaluation were carried out by adopting a randomized complete block with two treatments (varieties) and four replicates. In each plot 100 plants in 5 rows of 20 plants were planted, spaced at a distance of 1 m between rows and plants (area of 100 m^2).

Growth rates and development. Dry mass and leaf area were assessed every 15 days. Three randomly chosen plants per plot were subjected to dehydration in oven for 72 hours at 70 °C. The leaf area per plant was calculated using the regression model proposed by Cardona et al. (2009).

Response variables associated with growth rates and development are reported in Table 1.

Table 1: Equations applied for estimates of the growth rates.

Growth Rates	Equation	Units
Leaf area index (LAI)	LA/A_S	----
Net assimilation rate (NAR)	$(LnW_2 - LnW_1)/(t_2 - t_1)*(LnAF_2 - LnAF_1)/(AF_2 - AF_1)$	$g.cm^{-2}.day^{-1}$
Relative growth rate (RGR)	$(LnW_2 - LnW_1)/t_2 - t_1$	$g{\cdot}g^{-1}.day^{-1}$
Leaf area ratio (LAR)	$(LA_1/W_1 + LA_2/W_2)/2$	$cm^2.g^{-1}$

W_2: Final dry weight; W_1: Initial dry weight; t_2: Final time; t_1: Initial time; LA: Leaf area; As: Soil area; AF_2: Final leaf area; AF_1: Initial leaf area; Ln: Natural logarithm.

Phenology: Daily assessment was performed on 10 randomly chosen plant of each variety

Results

Leaf area index (LAI). Figure 1 highlights that the leaf area index progressively increased in both cultivars but was higher in CO-029 than in CO-015 as confirmed by mathematical models. CO-029 had higher development of the photosynthetic area (0.0095 cm^2 per day) and reached its maximum LAI (i.e. 1.18) at 144 days after transplanting (DAT). The rate of leaf development of the CO-015 was 0.0077 cm^2 and at 92 DAT reached its maximum LAI value (i.e. 0.81).

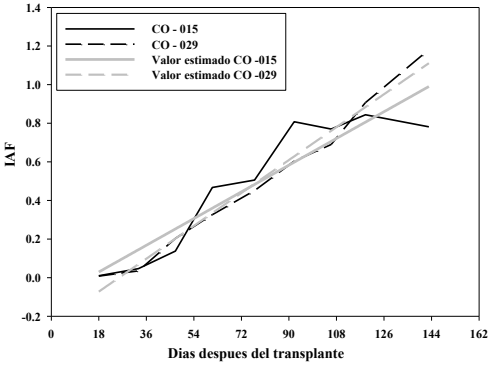

Figure 1. Leaf area index (LAI) of two improved varieties of eggplant (CO-015 and CO-029). Monteria, 2010.

Table 2. LAI model which explains the growth and development of two new varieties of eggplant.

Variety	Relationship	Equation	R^2
CO-015	Lineal	Y=-0.1090(0.1000)+0.0077(0.0012)X	0.8638
CO-029	Lineal	Y=-0.2428(0.0395)+0.0095(0.0005)X	0.9841

Net assimilation rate (NAR): As reported in Figure 2, the NAR values were high in both varieties during the early stages of development, afterwards progressively decreased showing a polynomial response (Table 3).The maximum rate was recorded at 119 and 92 DAT, with valuesof 116.66 and 99.57 $g.cm^{-2}.d^{-1}$ for CO-015 and CO-029, respectively.

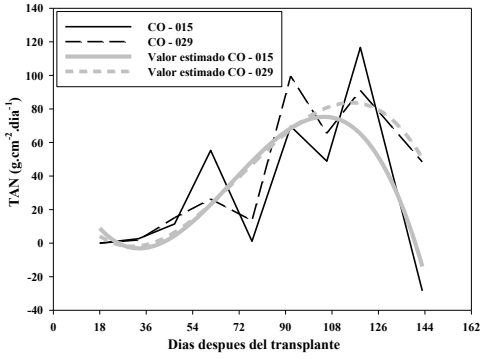

Figure 2. Net assimilation rate (NAR) of the two improved eggplant varieties: CO 015 and CO-029. Montería, 2010.

Table 3. Models that explain the trend of NAR at different growth stages of the two new varieties of eggplant: CO 015 and CO-029. Montería, 2010.

Variety	Relationship	Equation	R^2
CO 015	Polinomial	$Y=62.6554(90.1875)-4.4284(4.4630)X+0.0878(0-0618)X^2-0.0004(0.0003)X^3$	0.5685
CO 029	Polinomial	$Y=39.2362(54.3224)-2.9604(2.6882)X+0.0613(0.0372)X^2-0.0003(0.0002)X^3$	0.9841

Relative growth rate (RGR): The varieties showed a polynomial behavior in biomass production per unit of dry mass, with a rise in the early stages and subsequent gradual reduction. Figure 3 and Table 4, report the values equivalent to 0.11 and 0.12 g.day-1 at 33 and 47 DAT for CO-015 and CO-029, respectively. Likewise, the R^2 for each cultivar was similar and demonstrates the reliability of the selected regression model.

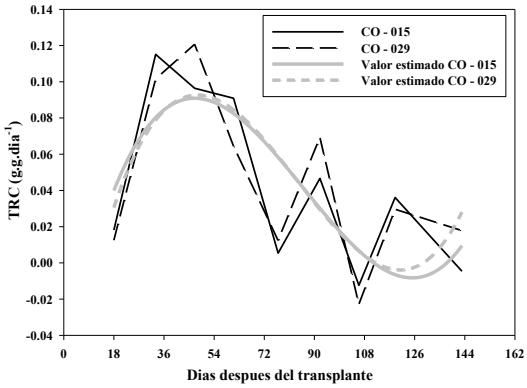

Figure 3. Relative growth rate (RGR) of the two improved varieties of eggplant (CO-015 and CO-029). Montería, 2010.

Table 4. Models that explain the behavior of RGR in different stages of growth and development of new varieties of eggplant. Montería, 2010.

Variety	Relationship	Equation	R^2
CO 015	Polinomial	$Y=-0.0595(0.0915)+0.0073(0.0045)X-0.0001(6.2722^{-5})X^2+4.149^{-7}(2.5579^{-7})X^3$	0.5926
CO 029	Polinomial	$Y=-0.0087(0.0953)+0.0087(0.0047)X-0.0001(6.5332^{-5})X^2+4.9760(2.6643^{-7})X^3$	0.5538

Leaf area ratio (LAR): The leaf area ratio at the beginning of the season was around 60 $cm^2.g^{-1}$ and showed a linear reduction of 0.37 and 0.41 cm^2-g^{-1} per day in CO-015 and CO-029 respectively. The R^2 values in both the varieties showed a to fit with a linear trend (Figure 4, Table 5).

Table 5. Models that explain the behavior of the LAR in the different stages of growth and development of new varieties of eggplant. Monteria, 2010.

Variety	Relationship	Equation	R^2
CO 015	Lineal	Y=64.5306(3.4206)-0.3722(0.0466)X	0.9208
CO 029	Lineal	Y=69.6786(4.6438)-0.4174(0.0605)X	0.8694

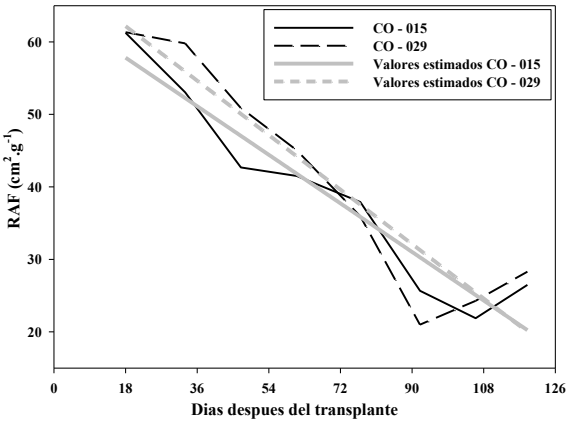

Figure 4. Leaf area ratio (LAR) two improved varieties of eggplant (CO-015 and CO-029). Monteria, 2010.

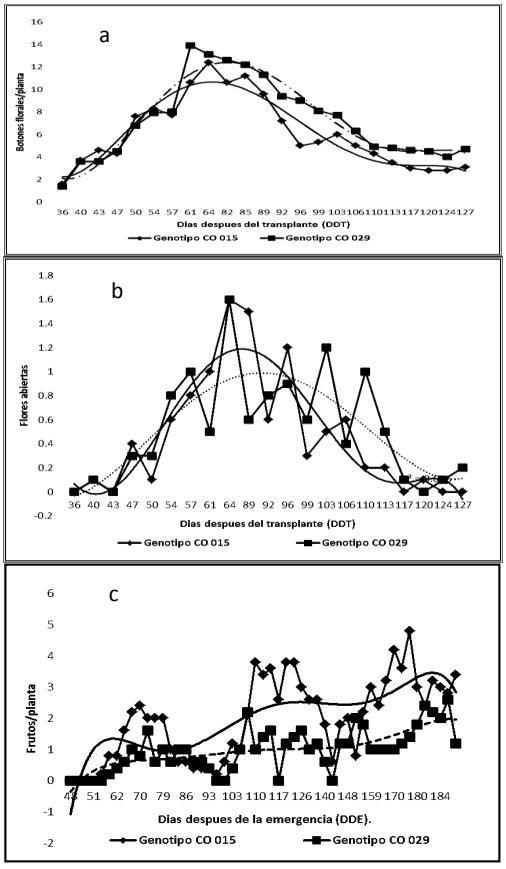

Figure 5. Flower buds (a), flowers (b) and fruit per plant (c) of the two improved varieties of eggplant (CO-015 and CO-029). Monteria, 2010.

Crop phenology

The production of flower buds, flowers and fruits followed an analogous trend in both varieties (Figure 5). The highest daily emergence of flower buds per plant was observed between 60 and 80 DAT (14 and 12 for CO-029 and CO-015 respectively). The daily number of flowers produced per plant showed its maximum value between 65 and 90 days, averaging 1.5 flowers/plant for both varieties. The daily number of fruit setting per plant showed an increasing trend after 70 days, recording highest values (4.8) in CO-015 at 75 days and in CO-029 (2.6) at 185 days. However, the higher number of fruits for CO-015 did not result in higher yields of fruit per hectare, probably due to higher rates of abscission and best growth efficiency of CO-029 at the end of the crop cycle as displayed in the NAR and RGR.

Discussion

The highest LAI values detected in CO-029 resulted in its greater efficiency in dry matter production since with the increase of IAF a side by side increase in crop growth was observed. Squire (1990) reports that, although the LAI critical value for different genotypes may be different, above a critical LAI the crop growth rate does not significantly increase. The high values of the NAR during the early stages of development was likely due to increases in photosynthetic efficiency, which in turn is stimulated by the high demands of photo-assimilates, water and mineral elements required for the formation of different reproductive organs fruits (Geraud et al. 1995). From the fruiting stage over, the plants reduced photosynthetic efficiency by increasing the un-assimilatory tissues, assimilates redistribution and senescence. Probably the fluctuations of the NAR, explain variation in foliar biomass production at every harvest cycle. The RGR progressively decreased in both varieties, presumably due to the highest energy request during the vegetative phase for plant growth and development, which gradually reduced with the increase in un-assimilatory tissues and decrease of incident radiation intercepted by the crop (Jarma et al. 2006). As expected, the LAR tends to decline over time because in the early growth stages plants invest most of the photoassimilates in establishing their photosynthetic apparatus while in a subsequent developmental stages the plants accumulate larger amounts of assimilates in the reproductive organs. The number of fruits was higher in CO-015, but was not related to final yields (data not shown), probably due to the higher rates of fruit abscission in juvenile state.

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Small farmers of eggplant of Córdoba department

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Development of hybrid chili: is it true F1 heterosis made from highly diverse parents?

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Abstract

Chile with small-erect fruit is highly appreciated in Asia, especially South-East Asia. To date, 'Super hot' is the only hybrid variety highly diffused in the market, however a wider range of chili varieties available in the market to serve various purposes is desirable. Superior F_1 hybrids are usually obtained from highly diverse parents. This study aimed at testing if highly diverse parents always produce heterosis in chili. Parental line selection was done by SSR analysis. Forty anchored SSRs distributed throughout the chili genome were applied to investigate the genetic diversity of 64 chili accessions with small erect-fruit, of which 62 were landraces and two were elite lines (Super hot parents). The SSR polymorphism information content (PIC) varied from 0.02 to 0.79, and the average number of alleles per locus was 3.37 (ranging from 2-8). The average chili genetic distance (GD) was 0.40 (ranging from 0.02-0.76). Cluster analysis using UPGMA (unweighted pair group with arithmetic means method) divided the 64 chili accessions into two main groups. Cluster I comprised 48 accessions and Cluster II comprised 16 accessions. The GD between the two elite lines was 0.35, and was used as criteria to select parental lines. Thirty five accessions were selected based on the GD criteria, and were then crossed with the two elite lines. A total 70 hybrids were obtained, however only 41 were successfully produced and planted in open field during rainy season for yield trial. Average yield per plant (YPP) of the hybrids were highly significant different (P= 4.3 e-15). Correlation analysis revealed that GD and YPP were not correlated with r = 0.056 besides the correlation between GD and heterosis was poor with r = 0.118. This study suggested that the highest diverse parents do not show the highest heterosis based on YPP analysis.

Keywords: genetic distance, simple sequence repeat, topcross, selection, chili pepper

Introduction

Importance of chili

Capsicum annuum L. is the most differentiated and widely cultivated *Capsicum* species worldwide and includes both pungent and non-pungent types. Chili is widely planted in South Asia, China, Korea and South-East Asia. A chili plant with small-erect fruit is highly prized in South-East Asia. This chili has high capsaicin, exploitable for pharmaceutical uses, and posses unique flavor, special aroma, shape, and color. The production area in Thailand in 2006-2007 was 75,954 hectares and the majority was planted with small erect fruit types (Khanobdee 2008). The market value for these chilies in 2012 was about $ 2.6 million (East West Seeds 2012 marketing data) and produced an high income for Thai farmers.

Advantage of F_1 hybrid and and their diffusion

F_1 hybrid cultivars are increasing in use due to their vigor, uniformity, disease resistance, stress tolerance and good horticultural traits including earliness, long shelf-life and giving consistent stable high yield. However the availability of F_1 hybrids of this chili types, characterized by high

yield and wide adaptation, is limited for Thai farmers. The leading F_1 hybrid at present is "Super hot". The knowledge of genetic diversity and relationships among sets of germplasm and the potential merit of the genetic diversity would be beneficial to select the appropriate parents to development superior hybrids. This research studied the genetic diversity, heterosis(yield per plant) and correlation of heterosis and SSR-based genetic diversity of chilies with small erect fruit, in order to select parental lines with good combining ability for the production of heterotic hybrids.

Materials and Methods

Plant material

Sixty-four accessions of small erect fruit types of chili (*Capsicum annuum* L.) comprised of 62 landraces and two elite lines ("Super hot" parents) were planted at Spa Agricultural farm in Thailand for observation and recording of fruit characters. The accessions comprised of 28 from Thailand, 14 from China, five from India , four from Vietnam, three from United States of America, three from Japan, two from Myanmar, and the rest were from Korea, Bangladesh, Laos and Indonesia.

Genetic diversity among accessions using SSR marker

Genomic DNA was extracted from five plants per accession using the modified CTAB method of Doyle and Doye (1987). The quantity and quality of genomic DNA pool were determined by Nano drop 8000 Spectrophotometer. A total of 100 SSRs were randomly selected from "Chili map of Genome Network" and Minamiyama et al. (2006) which were distributed across all 12 chili chromosomes. The DNA profile of each accession was done using M-13-tailed forward primers (Lorenz et al. 2001) synthesized by Integrated DNA Technologies, Inc. DNA profile of 64 accessions were performed in 96-well PCR plates, with each reaction containing 50 ng genomic DNA, 1μ mol/L of each primer, 2.5 m mol/L $MgCl_2$, 0.24 m mol/L of each dNTP, 1 × PCR buffer, and 0.45 U of *Taq* DNA polymerase. Reactions were carried out in a BIORAD Tetrad 2 Peltier Thermal Cycler (Hercules, CA). A Touch-Down PCR suitable for M-13- tailed primer was applied: denaturation at 94°C for 3 min, annealing temperature at 55°C, which was reduced to 0.5°C per cycle for the first ten cycles. PCR amplifications were then continued for 25 more cycles at 50 °C, annealing temperature and extension at 72 °C for 1 min, final extension at 72 °C for 10 min. The fragments were separated using 4.5% polyacrylamide gel run in DNA sequencer. The gel was stained with silver stained approach following the protocol described in SILVER SEQUENCE™ DNA Sequencing System (Promega, USA).

Genetic distance analysis

Polymorphic markers were manually scored as binary data with presence as "A" and absence as "B". A binary data matrix was constructed upon analyzing the DNA gel. All the bands were scored regardless of the band intensity. The polymorphism information content (PIC) was calculated by SJK (2002). Data analyses were conducted using the graphical genotype (GGT) program version 2.0 software (Van 1999). Dendrograms were constructed with the method of UPGMA (unweighted pair-group method with arithmetic averages) based on the genetic distance calculated with Jaccard coefficient (Jaccard 1908).

F_1 hybrid production

The genetic distance (GD) between the two elite lines (Super hot parents) was used as criteria to select parental lines. Three groups of parents were selected based on their GDs i.e., higher, lower, and equal to GD of the two elite lines. The selected parents were crossed with the two elite lines to produce hybrids.

F_1 hybrids evaluation

The hybrids were evaluated in open field for yield trial with three replications in a Randomize Complete Block Design (RCBD). The adjusted plot means were combined across replication and analyzed as a RCBD using R-program (Hornik, 2012). For fruit number and yield per plant, heterosis, heterobeltiosis, and standard heterosis were analyzed using the procedure given by Turner (1953). Heterosis was estimated by (F1 -MP) × 100/MP and heterobeltiosis by (F1 -BP) × 100/BP, where MP = Mid Parent, BP = Best Parent/Higher Parent. The same principle was used to estimate the commercial hybrid in comparison to the standard variety (Super hot).

Results

Genetic diversity among accessions using SSR marker

Of the total 100 SSR primers screened, 40 anchored SSR showed polymorphism within the 64 small erect fruit chili accessions. The SSR polymorphism information content (PIC) varied from 0.02 to 0.79, and the average number of alleles per locus was 3.37 (ranging from 2-8). The average chili genetic distance (GD) was 0.40 (ranging from 0.02-0.76). Cluster analysis using UPGMA divided the 64 chili accessions into two main groups (figure 1). Cluster I comprised 48 accessions within five subgroups, and Cluster II comprised 16 accessions within two subgroups. The GD between the two elite lines (3355, 3356) was 0.35, and was used as criteria to select parental lines. The accession number 3376 showed highest genetic distance with accession number 3355(GD=0.76) while accession number 3410 has highest genetic distance with 3356 (GD=0.72). The accessions showing the lowestmost GD (0.01) were 3405 and 3390. Thirty five accessions were selected based on the GD criteria from each subgroup, and were then crossed with the two elite lines to produced hybrids.

F_1 hybrids evaluation

A total of 70 crosses were made from 35 accessions, however only 41 hybrids were successfully produced. Yield trials of the 41 hybrids were performed in the open field during the rainy season. The average yield per plant (YPP) of the hybrids was highly significantly different (P=4.3e-15). The cross of 3356 x 3412 gave the highest average yield per plant(474.32 g). The heterosis was estimated by heterobeltiosis. The cross between 3355 x 3419 had the highest heterosis (2939%) and the cross between 3356 x 3408 had the lowest heterosis (-53.34%). The average yield/plant (g) and percentage of F_1 heterobeltiosis are shown in table 1.

Genetic distance and heterosis correlation

The average chili genetic distance (GD) was 0.40 The relationship among the 64 accessions of small erect fruit chilies showed that genetic distance was poorly correlated to hybrid heterosis with a correlation coefficient (r^2) of 0.118, while the correlation of genetic distance and average yield/plant among hybrids was not correlated with correlation coefficient (r^2) = 0.056.

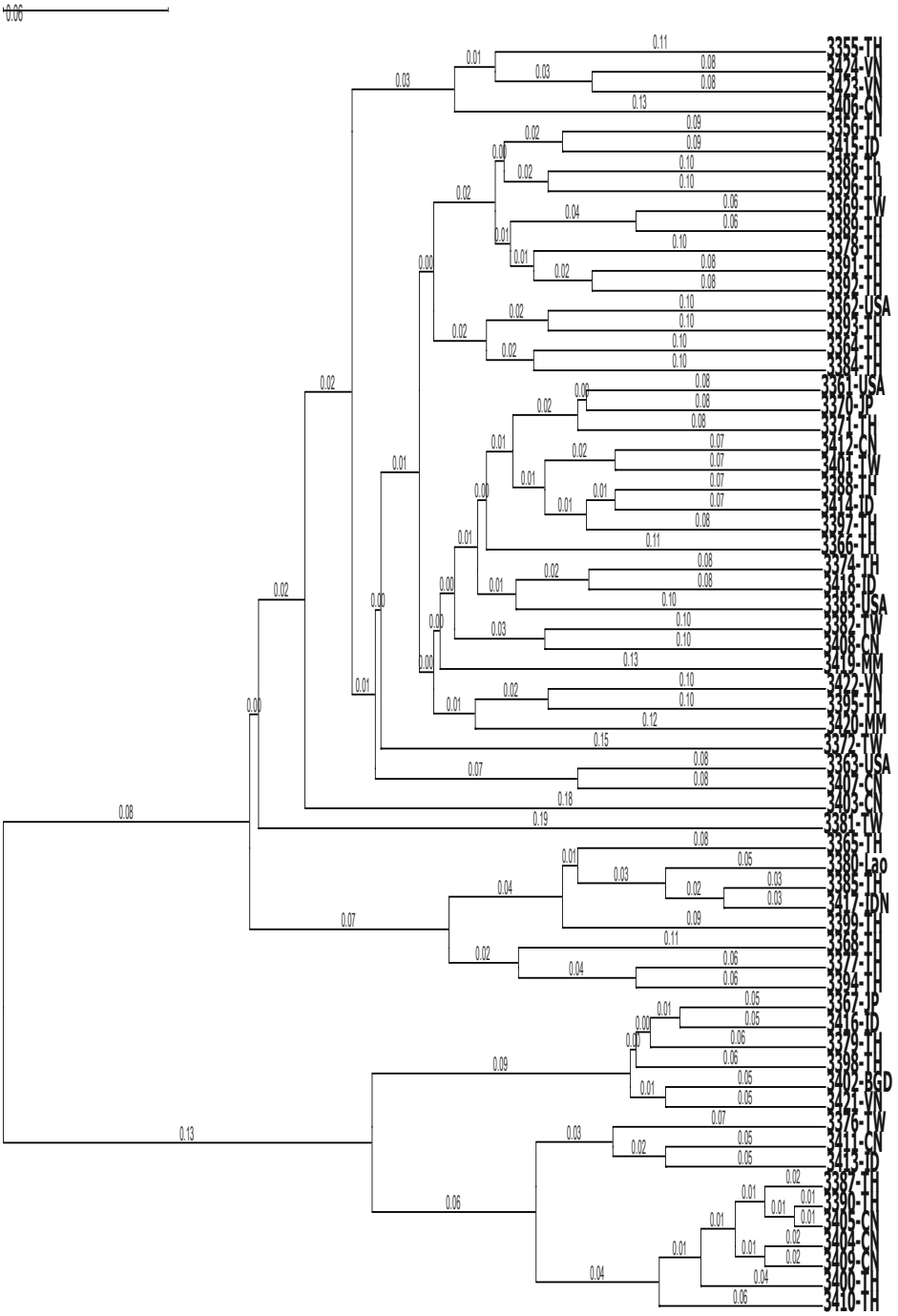

Fig. 1 Dendrogram of 64 small erect-fruited chilies based on SSR data

Table 1: The average yield/plant (g) and heterosis of hybrids

F1 hybrid	AVG yield/plant(g)		Genetic distance	Heterobeltiosis (%)
	BP1	F1***		
Super hot(3355 x 3356)	352.23	368.53	0.35	4.63
3355 x 3363	116.39	226.60	0.34	94.70
3355 x 3364	80.07	235.90	0.37	194.63
3355 x 3368	39.12	193.85	0.44	395.48
3355 x 3370	50.58	232.93	0.31	360.52
3355 x 3371	177.50	230.15	0.34	29.66
3355 x 3374	147.01	207.51	0.24	41.15
3355 x 3383	53.42	241.89	0.27	352.78
3355 x 3384	106.70	407.59	0.35	282.00
3355 x 3389	144.14	221.73	0.4	53.83
3355 x 3394	128.87	187.13	0.32	45.21
3355 x 3397	216.09	374.59	0.24	73.35
3355 x 3401	130.40	231.84	0.31	77.79
3355 x 3403	137.76	209.31	0.45	51.94
3355 x 3407	123.33	187.07	0.29	51.68
3355 x 3408	105.61	191.20	0.32	81.04
3355 x 3411	15.77	208.99	0.68	1225.54
3355 x 3413	226.17	322.53	0.67	42.61
3355 x 3415	225.08	303.68	0.38	34.92
3355 x 3416	271.28	306.88	0.49	13.13
3355 x 3419	8.72	265.16	0.34	2939.66
3356 x 3376	352.23	379.75	0.62	7.81
3356 x 3381	352.23	353.70	0.35	0.42
3356 x 3392	352.23	369.73	0.2	4.97
3356 x 3401	352.23	410.34	0.31	16.50
3356 x 3402	352.23	384.61	0.44	9.19
3356 x 3412	352.23	474.32	0.28	34.66
3356 x 3413	352.23	439.78	0.61	24.85
3356 x 3415	352.23	441.13	0.18	25.24
3356 x 3418	352.23	425.89	0.31	20.91

***Significant at $P \leq 0.001$

Discussion

Genetic diversity among accessions using SSR marker

Among the 64 accessions a close relationship was detected in cluster 2, that contain chilies from Thailand and China (3387, 3390, 3404, 3405, 3409, 3400, and 3410).The genetic distance in this cluster ranked from 0.01-0.09. The average genetic diversity of 0.4 is low for these germplasm, therefore they are belonging to same type (erect fruited) and same species (*C. annuum*). This results are in accordance with the ones of to Levebvre et al. (1993) and Heras et al. (1996) which highlighted, based on RFLP and RAPD data, that pepper cultivars in *C. annuum* L. have homogenous genetic background and lack genetic diversity. Also Lijun and Zou (2011) determined the genetic distance using ISSR and found low genetic polymorphism within *C. annuum* L. germplasm collections. The present results show that evaluation of GD in same type of chilies requires a high number of markers which should be link to trait of interest.

Genetic distance and heterosis correlation

Consistently from this study the relationship among the 64 accessions of small erect fruit chilies showed that 3355 x 3411 has the highest genetic distance (0.68) among the hybrids but the average yield/plant is less than 3355 x 3356, which has lower distance (0.35). This result indicates that the high genetic distance of parents does not give the highest heterosis based on average yield/plant analysis. Similar results were obtained by García et al. (2002) by studing the relationship between the genetic distance among parental lines using RAPD and by comparing the heterosis of yield in their F_1 hybrids; the correlation between the genetic distances matrix and the heterosis matrix was low ($r = 0.3281$) and non- significant; in other words, the genetic distances among parental lines was not related to heterosis of the hybrids. However, the limited genome coverage, or the possibility that the molecular markers used are not close enough to the genes controlling fruit yield in pepper, does not permit a final conclusion to be drawn about the relationship between genetic distance among parents and the heterosis of their F1s. The genetic basis of heterosis has been debated with respect to the relative importance of dominance, over -dominance and epistasis (Gulzar, 2011). The dynamic genome of an F1 hybrid is derived from its parents. The hybrid performance is quite different from the one of the parents, due to extensive differences in gene expression in hybrids as compared to parents. The patterns of gene expression in hybrids results from unique regulatory interactions in hybrids, which give rise in quantitative variants that may be responsible for the heterosis observed in the F_1 hybrid (Birchler et al. 2010; Hoccholdinger and Hoecker, 2007). For future studies on heterosis by its best, it is thus needed to understand the nature of dominance, epistatic properties of these genes and how they interact with the environment (Coors and Pandey, 1999).

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Investigation of combining ability and heterotic pattern of pepper (*C. annuum* L.) inbred lines developed for protected cultivation

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Abstract

Pepper (*Capsicum annuum* L.), is one of the most important vegetable species produced both in the world and in Turkey. Turkey is an important producer of pepper, in third place after China and Mexico. Greenhouse pepper production uses hybrid varieties. The use of hybrid varieties has also grown in open field production. Seed required for pepper production has been supplied from local private seed firms and has been imported through international seed companies. Development of new hybrid pepper varieties adapted to our region is an important issue. This study is a part of "İmprovement of F_1 Hybrid Vegetable Varieties and Qualified Lines in Turkey" project that is supported by TÜBİTAK to determine combining ability and heterotic pattern in pepper pure lines at the Vegetable Department of Bati Akdeniz Agricultural Research Institute (BATEM). Twenty five lines and two testers were crossed in the spring of 2011. Fifty hybrids and four commercial hybrids were tested in randomized complete block design with two replications in the single crop season in 2012 in unheated glasshouses. General combining ability and heterotic patterns of lines were evaluated with total fruit yields. Also, fruit characteristics were investigated for demands of market. General combining ability values of total fruit yield ranged from -3311 to 2869. As a result, 16 promising inbred lines were determined to cross for future new hybrid pepper varieties.

Keywords: Pepper (*Capsicum annuum* L.), inbred lines, combining ability, heterotic pattern, morphological characterization

Introduction

Capsicum genus of Solanaceae family includes about 30 pepper species including *Capsicum annuum* L. (Eshbaugh, 1970). *Capsicum* species can be grown in open field and in protected cultivation in all tropical and subtropical climates. Today, *C. annuum* is the most commercially grown and well known vegetable species (Onus, 2001). *C. annuum* has different types of peppers. Capia pepper type of the long pepper group in the classification by Bailey is named as *Capsicum annuum* var. *longum* (Vural et al. 2000).

Turkey is in third place in world pepper production and has an important potential after China and Mexico (Anonymous, 2012a). Antalya province has the most pepper production in Türkiye with open field and greenhouse production areas (Anonymous, 2012b).

Greenhouse pepper production uses hybrid varieties. Use of hybrid varieties has also increased in open field production. Seed quantity required for pepper production has been supplied by local private seed firms and has been imported through international seed companies.

Although Turkey's pepper seed needs are met by domestic production, import of pepper seed continues. While hybrid varieties are produced in protected cultivation, non-hybrid pepper varieties are mostly produced in open fields. The use of hybrid seeds has increased in open fields because of widespread use of ready seedlings in recent years.

Heterotic patterns are very critical for maximizing the expression of heterosis in hybrids. According to Menkir et al. (2003), a great number of inbred lines are available from a breeding program, breeders can not evaulate the combining ability of the lines in diallel crosses. For this reason, heterotic models are widely used in heterosis breeding. These models are mostly applied for

cross-pollinated plants, such as corn and sunflower. They has also become a need for self-pollinated plants. A positive correlation is widespread between genetic diversity of parents and heterosis (Riaz et al. 2001, Diers et al. 1996).

The determination of combining ability provides information of gene expression involved in quantitative traits (Falconer, 1979). Knowing combining ability and gene effects helps to select parents for development of hybrid varieties (Patel et al. 1998, Doshi and Shukla 2000, Kamble et al. 2009).

High heterosis can be obtained from crossing among inbred lines of different pepper heterotic groups. Therefore, specific crossings should be done by determining heterotic groups.

The aim of this study was to determine combining ability and heterotic groups of the inbred lines developed at of Batı Akdeniz Agricultural Research Institute (BATEM) for developing new pepper hybrid varieties.

Materials and Methods

Twenty-five capia type inbred lines and two tester lines developed by Batı Akdeniz Agricultural Research Institute (BATEM) were used for this study. Each inbred line was crossed to two testers, TK 1 and TK 2 to identify heterotic groups in the 2011 spring season.

The fruits were harvested on July 25, 2011. The seeds were extracted by hand. Four standard commercial hybrid varieties Belissa (Rijk Zwaan), Uygar, Urartu and Atris (Nunhems) were used to compare with fifty hybrids developed at BATEM for fruit yield.

Fruit Yield Trial for Combining Ability and Heterotic group

Seeds were sown on 26 August, 2011. Seedlings of hybrids were planted as 80-50x50 cm dimension (lines-beds x between plants), in randomized complete block design with two replicates, 7 plants in each replicate in greenhouse on 28 September, 2011.

Four fruit harvests were done from December 2011 to May 2012. Fruit yields were analysed on SAS program and differences among means were grouped by LSD ($p \leq 0.05$) multiple comparative method. General combining ability (GCA) of inbred lines was calculated (Kempthorne, 1957).

Determination of Heterotic Groups

Each tester group means were computed. Hybrid mean of each inbred line with each tester was compared with general line-tester hybrid mean. If inbredline-tester hybrid mean is greater than each of two tester means, then group is named as “both” and smaller than each of two tester means, then as “neither”. If inbredline-tester hybrid mean is greater than tester 1 or 2, then it is grouped with greater tester name, “T1” or “T2” (Table 2) (Menkir et al. 2003).

Morphological Characterizations

Flowering Days to 50%, Yield Per Plant (g/plant), Fruit Weight Average (g), Fruit Flavor, Fruit color, Fruit Length Average (cm), Fruit Diameter Average (mm) were recorded.

Inbred Line Selection for Spesific Combination Crossing Programme

Heterotic groups and the market demands in terms of fruit characteristics were taken into account for the determination of inbred lines for specific combination crossings.

Results and discussion

Combining Ability

A total of eleven inbred lines had positive GCA values ranging from 2869 (TK 30) to -3311 (TK 14) in fruit yield. Lines with positive GCA effects are generally considered to have good general combining ability. TK30,TK35, TK22, TK32, TK15, TK29, TK23, TK7, TK17, TK12 and TK 11 inbred lines showed positive GCA effects (Table 2). Ahmed et al. (1998) found that GCA values varied between -66,751 and 86,627 for fruit weight.

The means and estimates of GCA and SCA effects for fruit yield of the inbred lines are presented in Table 2. Mean yield of testcrosses varied from 6090 g to 13560 g. Eleven inbred lines showed positive SCA effects with Tester 1 (TK 1) but had negative SCA effects with tester 2 (TK 2).

Heterotic Grouping

Fruit yields were analysed on SAS program and differences among means were grouped by LSD ($P \leq 0.05$) multiple comparative method. Mean fruit yield of testcrosses were found to be significant ($P \leq 0.05$). The line x tester interaction (GCA) was not significant for fruit yield (Table 1).

Table 1. Analysis of variance for inbred lines evaluated in testcrosses with two testers in Antalya (Turkey) during the 2011-2012 single season.

Sources	DF	Sum of Squares	Mean Square	F Value	Pr>F
Tester	1	3378244.00	3378244.00	4.56	0.0378
Line	24	65926863.50	2746952.65	3.70	<.0001
Line x Tester	24	22992218.50	958009.10	1.29	0.2200
Replication	1	25644096.00	25644096.00	34.59	<.0001
Error	49	36332279.0	741475.1		
Corrected Total	99	154273701.0			

Mean yıeld were compared with yield testcrosses of each tester to determine heterotic group. Eight inbred lines were in both groups; four inbred lines were in T1 group, two inbred lines were in T2 group and eleven inbred lines were in neither group (Table 2) (Menkir et al. 2003).

Table 2. Mean Fruit Yield, General Combining Ability (GCA) effects, Heterotic Group and Selected Lines for Specific combining programme of 25 inbred lines evaluated in testcrosses with two testers in Antalya (Turkey) during the 2011-2012 single season.

Inbred Lines	Testcrosses Yield with Tester 1 (TK 1) (g)	Testcrosses Yield with Tester 2 (TK 2) (g)	GCA	SCA effects with TK 1*	HG	SL for SCAP
TK 30	**13310**	**11810**	2869	1118	**Both**	**S**
TK 35 (hot)	**13290**	**11750**	2829	1138	**Both**	**S**
TK 22	**9910**	**13560**	2044	-1457	**Both**	**S**
TK 32 (hot)	**9790**	**13320**	1864	-1397	**Both**	**S**
TK 15	**10100**	**12990**	1854	-1077	**Both**	**S**
TK 29	**13470**	9600	1844	2303	**T1**	**S**
TK 23	**11970**	**10260**	1424	1223	**Both**	**S**
TK 7	**9770**	**11970**	1179	-732.4	**Both**	**S**
TK 17	**10100**	**11365**	1041	-264.9	**Both**	**S**
TK 12	**10300**	9240	78.6	897.6	**T1**	**S**
TK 11	8360	**11150**	63.6	-1027	**T2**	**S**
TK 20	**9470**	9710	-101	247.6	**T1**	**S**
TK 18	9070	9840	-236	-17.4	**Neither**	**--**
TK 27	**9860**	8960	-281	817.6	**T1**	**S**
TK 25	8600	9710	-536	-187.4	**Neither**	**--**
TK 19	7820	9500	-1031	-472.4	**Neither**	**--**
TK 10	8600	8610	-1086	362.6	**Neither**	**--**
TK 13	7095	10010	-1139	-1090	**Neither**	**--**
TK 5	6970	**10070**	-1171	-1182	**T2**	**S**
TK 8	8960	8050	-1186	822.6	**Neither**	**S**
TK 28	9150	7800	-1216	1043	**Neither**	**--**
TK 9	7380	8940	-1531	-412.4	**Neither**	**--**
TK 16	7180	8380	-1911	-232.4	**Neither**	**S**
TK 6	6480	8210	-2346	-497.4	**Neither**	**--**
TK 14	6090	6670	-3311	77.6	**Neither**	**--**
Mean	**9323.8**	**10059**	**0**	**0**		
Std	**2050.7**	**1790**	**1657**	**978.7**		

GCA: General Combining Ability, **SCA**: Specific Combining Ability **SL for SCAP :** Selected Lines for Specific combining programme, **HG:** Heterotic group **Both**= Greater than two tester , **T1**= Only greater than tester 1 (TK 1),**T2**= Only greater than tester 2 (TK 2) **Neither**= Smaller than both tester, **S**= Selected Lines for Spesific combining ability programme * Same value in another cross with TK 2 but with completely opposite sign.

Results of Morphological Characterizations

Testcrosses and standard commercial hybrid varieties were characterized for time to 50% flowering and fruit features (Tables 3 & 4). Fruit shape, fruit size and fruit smoothness are important quality criteria of capia types for commercial market in greenhouse production. But, shorter and thinner fruits shape may be accepted in open field conditions.

Fruit features wanted market include fruit length between 18 and 20 cm, fruit diameter between 45 and 55 mm, fruit weight between 85 and 115 g, and light or dark green fruit color is permitted. Pepper lines reaching red fruit early are preferred, because fruits are usually harvested at full red color.

Table 3. Number of days to 50% Flowering, Yield Per Plant (g/plant), Average Fruit Weight (g), Fruit Flavor, Fruit colour, Average Fruit Length (cm), Average Fruit Diameter (mm) of 25 inbred lines evaluated in testcrosses with tester 1 (TK1) in Antalya (Turkey) during the 2011-2012 single term season.

HYBRİ	LINE	ND	YPP	AF	FF	FC	AFL	AFD
5	TK5	65	498	68.1	Sweet	DG	18	41.8
7	TK6	60	463	67.6	Sweet	G	18	43.5
9	TK7	57	698	79.1	Sweet	G	18.7	49.3
11	TK8	58	640	78.7	Sweet	G	21.3	48.1
13	TK9	59	527	81.9	Sweet	G	18.8	49.8
15	TK10	57	614	73.2	Sweet	G	17.9	40.7
17	TK11	57	597	76.8	Sweet	G	18.7	44.1
19	TK12	57	736	80	Sweet	DG	19.6	45.2
21	TK13	59	507	58.8	Sweet	G	16.7	42.9
23	TK14	57	435	74.5	Sweet	G	18.1	42.7
25	TK15	59	721	99.3	Sweet	DG	19.3	48.2
27	TK16	60	513	66.9	Sweet	G	20.2	48.3
29	TK17	59	721	80	Sweet	G	19.5	49.8
31	TK18	57	648	64	Sweet	G	19.3	43.3
33	TK19	59	559	79	Sweet	G	18.3	45
35	TK20	60	676	75.1	Sweet	DG	20	42.3
37	TK22	59	708	85.1	Sweet	LG	20.2	46.1
39	TK23	60	**855**	71.5	Sweet	G	18.6	44.9
43	TK25	59	614	75.8	Sweet	DG	18.7	43.5
47	TK27	59	704	58.2	Sweet	DG	16.9	37.3
49	TK28	57	654	54.4	Sweet	G	16	40.3
51	TK29	63	**962**	61.4	Sweet	G	17.9	40.9
53	TK30	59	**951**	75	Sweet	G	19.8	44.5
60	TK32	58	699	71.5	Hot	DG	19.3	44.3
62	TK35	60	**949**	81.7	Hot	G	18.3	46.8
MEAN TESTER 1		**59**	**666**	**74**			**19**	**45**
64 BELISSA		62	791	85.4	Sweet	G	19.4	51
65 UYGAR		60	1080	89	Sweet	G	19.8	49.3
66 URARTU		59	809	82	Sweet	DG	20	43.9
67 ATRIS		56	787	115	Sweet	DG	18.3	48.6
Mean std com		**59.2**	**867**	**93**			**19**	**48**

NDF: Number of days to 50% Flowering, **YPP:** Yield Per Plant (g/plant), **AFW:** Average Fruit Weight (g)
FF: Fruit Flavor, **FC:** Fruit color, **AFL:** Average Fruit Length (cm), **AFD:** Average Fruit Diameter (mm)
G: Green, **DG:** Dark Green, **LG:** Light Green

Mean 50% flowering day number for tester 1 hybrids, mean yield per plant, mean of fruit weight, mean of fruit length and mean of fruit diameter were calculated 59 days, 666 g/plant, 74 g, 19 cm and 45 mm, respectively. Mean number of days to 50 % flowering for tester 2 hybrids, mean yield per plant, mean of fruit weight, mean of fruit length and mean of fruit diameter were calculated 60,4 days, 719 g/plant, 76 g, 18 cm and 45 mm, respectively. As a result, 50 % flowering day number and average fruit length of tester 1 hybrids were found superior than tester 2 testcrosses. But, yield per plant and average fruit weight of tester two testcrosses were found higher than tester 1 hybrids. Average fruit diameter values of testcrosses were found equal each other for both testers. Hot hybrids were obtained from hot pepper parents.

Table 4. Days to 50 % Flowering, Yield Per Plant (g/plant), Average Fruit Weight (g), Fruit Flavor, Fruit colour, Average Fruit Length (cm), Average Fruit Diameter (mm) of 25 inbred lines evaluated in testcrosses with tester 2 (TK2) in Antalya (Turkey) during the 2011-2012 single term season.

HYBRİ	LINE	NDF	YPP	AFW	FF	FC	AFL	AFD
6	TK5	61	719	62.1	Sweet	G	17.8	43.9
8	TK6	61	586	60.8	Sweet	G	18.3	43.4
10	TK7	62	**855**	90.6	Sweet	DG	18.4	52.6
12	TK8	62	575	81.8	Sweet	G	20.2	48
14	TK9	58	639	75.7	Sweet	DG	16.1	47.8
16	TK10	61	615	73.3	Sweet	G	17.4	43.2
18	TK11	61	796	85.6	Sweet	G	17.9	43.8
20	TK12	59	660	74.4	Sweet	G	19.5	42.6
22	TK13	59	715	73.2	Sweet	G	16.1	45.3
24	TK14	58	476	81.5	Sweet	DG	18.5	47.2
26	TK15	63	**928**	95	Sweet	G	17.6	51.1
28	TK16	59	599	77	Sweet	G	19.5	46.4
30	TK17	60	**812**	74.6	Sweet	G	19.9	42.9
32	TK18	56	703	69.9	Sweet	G	18.2	43.7
34	TK19	61	679	77.3	Sweet	G	17.6	44.7
36	TK20	66	694	90	Sweet	DG	19	45.5
38	TK22	60	**969**	77.9	Sweet	G	18.7	46.9
40	TK23	61	733	90.6	Sweet	DG	19.2	49.4
44	TK25	61	694	81.3	Sweet	DG	17.7	44.8
48	TK27	60	640	65.2	Sweet	DG	17.5	42.3
50	TK28	58	557	71.5	Sweet	G	18.4	44.7
52	TK29	62	686	61.2	Sweet	G	17.5	40.3
54	TK30	60	**844**	75.9	Sweet	G	20.2	43.1
61	TK32	62	**951**	72.5	Hot	DG	17.4	41.6
63	TK35	58	839	60.8	Hot	DG	16.8	43.1
MEAN TESTER 2		**60.4**	**719**	**76**			**18**	**45**

NDF: Number of days to 50% Flowering, **YPP:** Yield Per Plant (g/plant), **AFW:** Average Fruit Weight (g) **FF:** Fruit Flavor, **FC:** Fruit colour, **AFL:** Average Fruit Length (cm), **AFD:** Averag Fruit Diameter e (mm) **G**: Green, **DG:** Dark Green, **LG:** Light Green

Inbred Line Selection for Specific Combination Crossing Programme

Yield of hybrids were considered to determine heterotic groups of inbred lines. But, inbred line selection for specific combination crossing programme is determined by heterotic group together with fruit morphological characterization of hybrids.

Eight inbred lines in group both, four inbred lines in group T1, two inbred lines in group T2 and two inbred lines in group neither were selected for specific combination crossing programme. Two inbred lines (TK8 and TK16) in group neither were selected due to fruit shape. Total sixteen inbred lines were identified for specific combination crossings with 8 male x 8 female.

Conclusions

Selection of superior inbred lines needs knowledge of combining ability of inbred lines and formation of heterotic pattern to improve new commercial hybrid varieties. These studies provide a way to develop the best combinations for breeders and prevent loss of time and labor.

Acknowledgements

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Development of new *Capsicum* cultivars at EMBRAPA (Brazil)

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Abstract

Brazil has a relatively long history of public and private *Capsicum* breeding programs. In the early 1980´s, EMBRAPA Vegetables, one of the research centers of the Brazilian Agricultural Research Corporation (EMBRAPA) launched its breeding program. Today this program is considered to be the largest public investment in hot and sweet pepper breeding. The strategy adopted by the program since its beginning considered as key elements: 1) the establishment of an ample germplasm collection to support the breeding effort; 2)a clear focus in the development of specific products demanded by the market, with about 80% of the total resources allocated to this segment; 3) allocation of about 20% of our breeding efforts to explore new opportunities; 4) capacity strengthening, through the involvement of a large number of trainees at the undergraduate and graduate level; 5) cooperative work with public and private universities, and 6) the establishment of agreements with the private sector. The germplasm bank established in the early 1980´s has been efficient in providing germplasm with variability, adaptability, yield and characteristics demanded by the breeding program. Some of the most recently-developed cultivars include: **BRS Brasilândia** (*C. annuum*, F1 hybrid for paprika, sweet), **BRS Sarakura** (*C. annuum*, OP for sauce, Jalapeño type, pungent), **BRS Garça** (*C. annuum*, OP for sauce, Jalapeño type, pungent), **BRS Seriema** (*C. chinense*, OP for pickled and fresh market, pungent), **BRS Moema** (*C. chinense*, OP for freh market and pickled, sweet), and **BRS Mari** (*C. baccatum*, OP for fresh and dehydrated markets, pungent). The breeding program has targeted different pepper types and species at different times. Most recent efforts address the development of new, uniform, high yielding, high nutrition, disease resistant *Malagueta* peppers (*C. frutescens*) as well as Habanero type cultivars (*C. chinense*), as recently demanded by the Brazilian market. Yellow Jalapeño, orange-fleshed Biquinho and multiple disease resistant F1 rootstocks for bell pepper are soon to be released as well.

Keywords: Chile pepper, breeding, disease resistance, genetics, variety

Introduction

Brazil has a relatively long history of public and private *Capsicum* breeding programs. An effort deserving to be highlighted was the public program led by Hiroshi Nagai in the 1960´s-1980´s, which released several peppers known as the Série Agronômico. Cultivar Agronômico 10, a conical sweet pepper, was a real landmark for the pepper producers due to its yields, adaptability and resistance to viruses.

In the early 1980´s, EMBRAPA Vegetables, one of the research centers of the Brazilian Agricultural Research Corporation (EMBRAPA) launched its breeding program. Today this program is considered to be the largest public investment in hot and sweet pepper breeding. Initial focus was given to the development of disease resistant lines and populations, and *Phytophthora capsici* was a major pathogen together with a potyvirus, *PVY*. A few years later, a tospovirus (TSWV - Tomato Spotted Wilt Virus) also became widespread in the *Capsicum*-producing areas in the country and the breeding program dedicated a substantial effort to finding sources of resistance to this virus.

The emphasis on disease resistance reflected not only the importance of diseases, the major production constraint in that period, but also the existence of a large group of plant pathologists at EMBRAPA Vegetables and at the Universidade de Brasilia, an important partner of EMBRAPA.

The strategy adopted by the program since its beginning considered as key elements:

the establishment of an ample germplasm collection to support the breeding effort;

a clear focus in the development of specific products demanded by the market, with about 80% of the total resources allocated to this segment;

an allocation of about 20% of our breeding efforts to explore new opportunities;

capacity strengthening, through the involvement of a large number of trainees at the undergraduate and graduate level;

cooperative work with public and private universities UnB, UENF, UFRRJ, USP/ESALQ, UFG, FTB,UCB), and

the establishment of agreements with the private sector.

Furthermore, the breeding program was structured taking into consideration the multiple functions that it actually performs in a developing country, i.e., the program serves as:

a *Capsicum* R&D platform, generating new cultivars, lines and population of interest to Brazil;

a *Capsicum* Knowledge Management & Knowledge Sharing platform (national meetings and an e-platform for discussion), moderated by the senior breeders, which have put together more than 500 professionals, mostly from the private sector, who have an interest in Capsicum-related issues in Brazil;

a *Capsicum* Services platform comprised of a genebank, seed production activities (with the private sector), and lab analysis of interest to the program and to the private sector (for example capsaicin/HPLC). The genebank, with over 4,000 accessions from different species, serves as the backbone to the research & development program.

Since the 1980´s, the *Capsicum* program has released several cultivars, inbred lines, populations and hybrids resistant to different pathogens which have had major impact in the country. As an example, one of the most recently-released cultivars, BRS Sarakura (Figure 1), an OP cultivar protected in the country, is responsible for a large share (>50%) of the hot pepper sauce made in Brazil, with over 2,000 ton harvested in 2012.

Figure 1: cultivar BRS Sarakura.

Materials and Methods

Germplasm bank

The genebank is a continuous undertaking initiated in 1980 and now over 4,000 accessions from Brazil and elsewhere are available to the breeding program. It has proven to be an invaluable source of new genotypes of interest to the Brazilian market as well as a source of important traits, such as disease resistance. Innovative approaches have been used to enrich the genebank, including the engagement of high school students. The passport information is organized in a databank (Figure 2) which includes photos of the accessions, and over 50 descriptors are used to characterize each genotype (http://www.cnph.EMBRAPA.br/paginas/servicos/banco_germoplasma_capsicum.htm). Morphological and molecular characterization is routinely performed in the collection, and a large percentage of the bank has been morphologically characterized following internationally-suggested descriptors (IPGRI). Control of seed stocks in the genebank is achieved through the use of a simple barcode system.

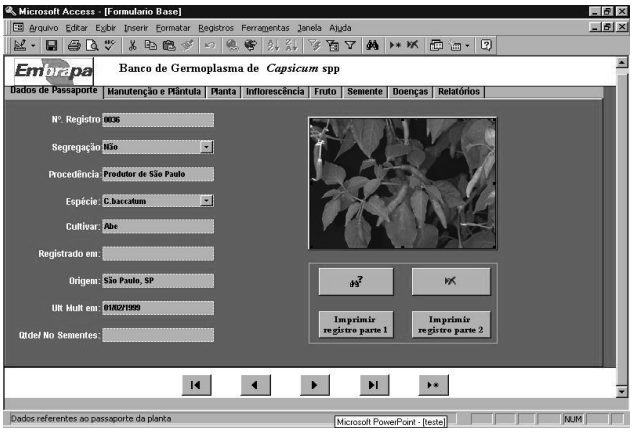

Figure 2: Screenshot of EMBRAPA´ *Capsicum* genebank.

Breeding new cultivars

Various breeding methods, including mixed methods, have been used to generate new hot and sweet peppers adapted to the different Brazilian regions. The first cultivar developed by the program was "Tico", a block-type sweet pepper developed for the Northeast of Brazil, resistant to *Cercospora*. Breeding projects have been mostly financed by public funds (EMBRAPA and CNPq), but projects funded by the private sector were also extremely important in bridging eventual demand gaps between these two sectors. In 2003, EMBRAPA gave stronger emphasis and support to breeding programs designed to assist small-scale farmers growing typical Brazilian peppers, in addition to the development of genotypes of interest to the medium and large farmers and agribusiness. At that time, most of those small-scale farmers pepper types were of limited interest to seed companies. All new cultivars have been registered in the National Cultivar Registry (**RNC**). Since this program is a public breeding effort, innovative arrangements had to be developed to ensure that the private sector, both Brazilian and international, timely produced and delivered seeds to growers. Non-exclusive licensing has been used and national and international companies have been marketing the new cultivars (Figure 3).

Figure 3: An example of a folder of the new BRS *Capsicum* marketed cultivars.

The flowchart below (Figure 4) provides a general graphic representation of the operation and flow of the breeding program.

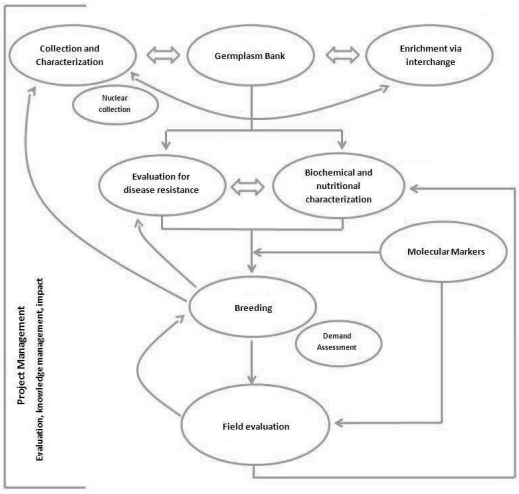

Figure 4: Flow chart of the various phases of the breeding program.

Molecular markers

Marker-assisted selection has more recently been used by the program, and molecular markers are being developed by the program (Embrapa Genetic Resources and Biotechnology). Perhaps the most significant contribution has been the development of a molecular marker to identify pungency, with 100% reliability, validated on hundreds of accessions and segregating populations (Figure 5).

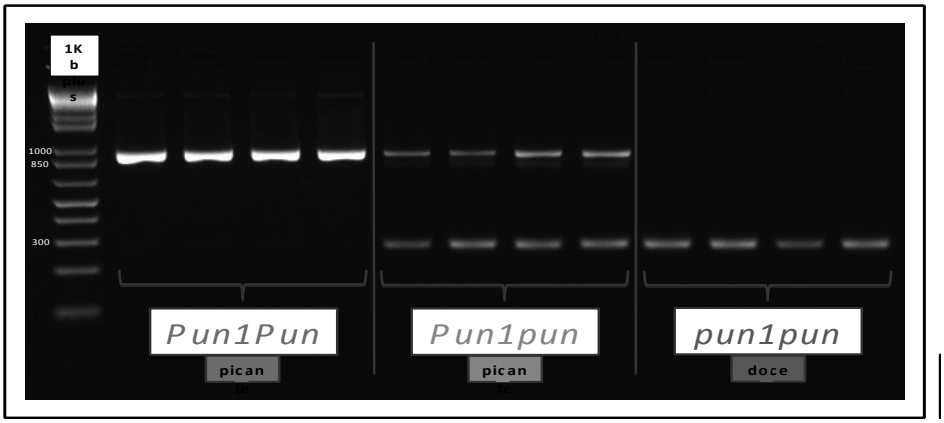

Figure 5: Molecular markers associated with pungency: homo- and heterozygous pungent (Pun1) and sweet (pun1) accession of *Capsicum*

Breeding beyond higher yields

In the past few years, increased attention has been given to pepper fruit quality, in addition to high, stable and sustainable yields. Vitamin C and aroma (volatiles) are considered important traits in the development of any new cultivar: An example for BRS Seriema is illustrated in Figures 6 and 7.

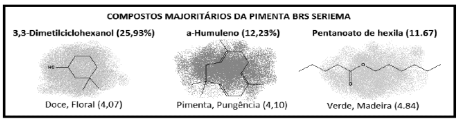

Figure 6: Major volatiles in the fruit of cultivar BRS Seriema.

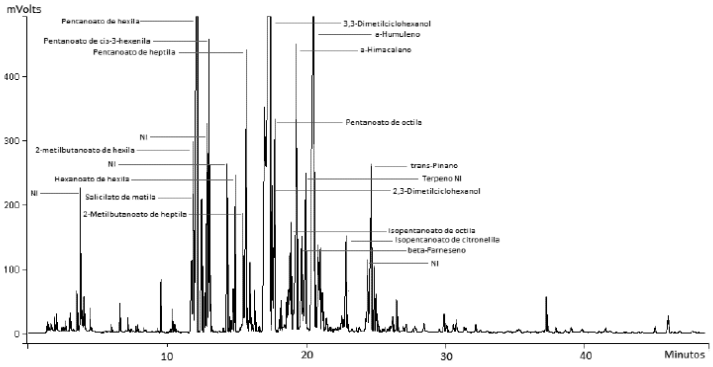

Figure 7: Complete chromatograph of volatiles for the newly released cultivar

Results and Discussion

The germplasm bank has efficiently supplied germplasm with variability, adaptability, yield and characteristics demanded by the breeding program. Some of the most recently-developed cultivars include:

BRS Brasilândia (*C. annuum*, F1 hybrid for paprika, sweet)

BRS Sarakura (*C. annuum*, OP for sauce, Jalapeño type, pungent)

BRS Garça (*C. annuum*, OP for sauce, Jalapeño type, pungent)

BRS Seriema (*C. chinense*, OP for pickled and fresh market, pungent)

BRS Moema (*C. chinense*, OP for freh market and pickled, sweet)

BRS Mari (*C. baccatum*, OP for fresh and dehydrated markets, pungent)

'BRS Mari' (*C. baccatum*), 'BRS Moema' (*C. chinense*), and 'BRS Seriema' (*C. chinense*) are the first three cultivars developed by EMBRAPA to support the small farmer as well as the larger entrepreneur. The breeding method used to develop these cultivars was mass selection and/or stratified mass selection for several cycles coupled with self-pollinations. 'BRS Mari' (Dedo-de-Moça type) is suitable for processing as dehydrated flakes or sauce, as well as for fresh market. This cultivar has shown multiple resistance to diseases, mainly Pepper Yellow Mosaic Virus (PepYMV), average resistance to *Oidium sicula*, *Xanthomonas* spp. and *Colletotrichum* spp. In Central Brazil, 'BRS Mari' showed high plant uniformity, excellent fruit quality and great potential yield, reaching 35 t/ha in six months (6,667 plants/ha). The most important traits of 'BRS Mari' are the high capsaicin content, approximately 90,000 Scoville Heat Units, and resistance to anthracnose. 'BRS Moema' (Biquinho type) is resistant to *Meloidogyne javanica* and PepYMV. This cultivar has presented high fruit and plant uniformity, with sweet, aromatic, flavored, and crunchy fruits, and it is consumed fresh or pickled. 'BRS Moema' has yielded 20 t/ha in six months of harvest (10,416 plants/ha). Finally, 'BRS Seriema' (Bode type) is a uniform, pungent and well-adapted cultivar to Central Brazil, consumed fresh or pickled.

The breeding program has been constantly challenged by – abiotic, biotic, economic and social factors, and today perhaps the biggest challenge in Brazil is the lack of labor for harvesting, which has demanded a new major effort to develop cultivars adapted to mechanical harvest, at both micro as well as macro scales of production.

New market demands include, in addition to the ever present demand for high yields, the development of cultivars adapted to organic production, high nutritional quality of fresh fruit and sauces, cultivars from previously unexplored groups, such as *Murupi*, and taste and aroma typical for the specific group of peppers, such as *Malagueta, Murupi, Bode, Cambuci, Cumari, Dedo-de-Moça*, among others.

Finally, it is noteworthy that the breeding program has targeted different pepper types and species at different times. Most recent efforts are concentrated on the development of new, uniform, high yielding, high nutrition, disease resistant *Malagueta* peppers (*C. frutescens*) as well as Habanero type cultivars (*C. chinense*), a more recent demand by the Brazilian market. Yellow Jalapeño, orange-fleshed Biquinho (Figure 8) and multiple disease resistant F1 rootstocks for bell pepper will soon be released as well.

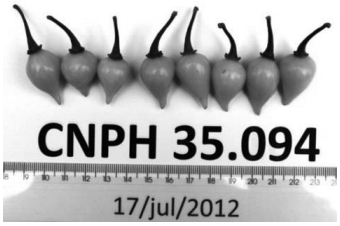

Figure 8: Fruit of orange-fleshed Biquinho.

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Studies on genetic divergence, heterosis and combining ability in paprika (*Capsicum annuum* L.)

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Abstract

Ninety four paprika accessions were evaluated for 17 traits, their heritability, genetic advance and genetic divergence. Higher phenotypic (PVC) and genotypic (GCV) coefficients of variation, and heritability coupled with high genetic advance was observed for number of fruits per plant including fresh fruit yield per plant, dry fruit yield per plant, 100 seed weight, number of seeds per fruit, capsanthin, capsaicin and oleoresin content. This indicates that high magnitude of variability for these traits and consequently more scope for their improvement through selection. Plant height, plant spread, and fruit diameter exhibited moderate PCV and GCV, suggesting the possible role of environment in expression of these characters. Mahalanobis D2 analysis classified the genotypes into 10 clusters indicating considerable genetic diversity. Forty crosses were attempted in line x tester design using the diverse parents belonging to different groups. Among 14 parents, 7 showed significant positive gca effect for dry fruit yield per plant with the maximum observed in LCA-428 (134.49) followed by LCA-436 (111.51) and LCA-432 (53.45). These parents had significant gca effects for number of fruits per plant, 100 seed weight, number of seeds per fruit, capsanthin content, days to 50 per cent flowering, days to maturity, fresh fruit yield per plant, weight of dry stalk less fruits per plant. The parent LCA-422 exhibited the highest negative gca effect for days to 50 per cent flowering, days to maturity, and significant gca effect in desired direction for number of seeds per fruit, 100 seed weight, and capsanthin content. LCC-422 is useful for incorporating earliness, low pungency and high color. Best five hybrids with high per se performance for fresh fruit yield per plant were LCA-436 x CA-960 (279.7), LCA-436 x Byadagi Dabbi (207.39), LCA-422 x CA-960 (217.19), LCA-431 x Byadagi Dabbi (220.3) and LCA-414 x Byadagi Dabbi (175.06). These hybrids also exhibited high sca effects for total yield per plant and yield components. Hybrids LCA-436 x CA-960 and LCA-436 x Byadagi Dabbi also exhibited significant sca effects for plant spread, number of fruits per plant, 100 seed weight, number of seeds per fruit and days to maturity. The crosses LCA-437 x KTPL-19 and LCA-437 x CA-960 had significant sca effects in desirable direction for capsaicin content. The cross LCA-414 x KTPL-19 showed desirable sca effects for quality (capsaicin and capsanthin). The crosses LCA-436x CA-960, LCA-436 x Byadagi Dabbi, LCA-422 x CA-960, LCA-414 x Byadagi Dabbi were the best for both yield and quality.

Keywords: Paprika, genetic divergence, combining ability, hybrid, capsanthin

Introduction

Paprika refers to certain types of chilli (*Capsicum annuum* L.*)* grown mainly for value-added powder and oleoresins for imparting color, flavor and aroma in various food preparations. The term paprika used by international spice traders for nonpungent (sweet) red capsicum powder has great commercial importance worldwide. This ground product is the basic material for producing

capsicum oleoresin. The demand for paprika oleoresin as a coloring agent has increased in international market especially in Europe and USA due to ban on artificial coloring substances. (Joshi et al. 1995). In 2011 chilli and paprika (dry) were globally grown on a total area of 1.94 million hectares with global production at 3.35 million tonnes. The global productivity of the chilli and paprika (dry) was 1.73 metric tonnes/ha. The value of the total chilli and paprika was 3.42 million USD. India is the world's leading producer occupying an area of 0.86 million hectares with a production of 1.5 million tons and productivity of 1.66 metric tons/ha (FAOSTAT, 2013). Importance of this crop is increasing to meet international demands. Developing good paprika hybrids will help the industry. Improvement in both quantitative as well as qualitative traits needs precise information on the nature and degree of genetic divergence, which helps in choosing the best parents for an efficient breeding programme.

Materials and Methods

The experiment was conducted during the June-May months of 2005 to 2007. The study was carried out at Regional Agricultural Research Station, Lam Farm, ANGRAU, Guntur, India (16°18' N and 80° 29'E). The experimental soil type was vertisols. The soil was medium in available N and available P_2O_5 and high in exchangeable K_2O. The present investigation was carried out with 94 paprika accessions from diverse geographical origin in India. The germplasm was studied for genetic variability, heritability, genetic advance as per cent of mean, genetic divergence, character association and path analysis, heterosis and combining ability for quality and economic characters. The data were recorded on 17 characters viz., plant height (cm), plant spread (cm),days to 50 per cent flowering, days to maturity, number of fruits per plant, fruit length (cm), fruit girth (cm),fruit shape index, fresh fruit yield per plant (g), dry fruit yield per plant (g),weight of dry stalk less chillies per plant (g),number of seeds per fruit, 100- seed weight (g), oleoresin content (%), capsanthin content (EOA colour value) and capsaicin content (%). Data were statistically analysed using methods described by Panse and Sukhatme (1967), Burton and De vane (1953), Lush (1940), Johnson et al. (1955), Mahalanobis (1928), Jackson (1991), Anderberg (1993) and Rao (1952).

Results and Discussion

The analysis of variance in the 94 paprika genotypes indicated highly significant differences among the genotypes for all 17 quantitative and qualitative characters studied, indicating the existence of adequate genetic variability among them. This was in accordance with the reports of Sarma and Roy (1995), Smitha (2005) and Biswadipchatterjee (2006). High heritability coupled with high genetic advance as per cent of mean were observed for all the traits except days to 50 percent flowering and days to maturity, indicating the influence of additive gene effects on the other 15 characters and hence simple selection may be effective for improving these traits. Higher PCV and GCV were observed for number of fruits per plant, fresh fruit yield per plant, dry fruit yield per plant, 100 seed weight, number of seeds per fruit, capsanthin content, capsaicin content and oleoresin content indicating the higher magnitude of variability for these traits and consequently more scope for their improvement through selection. Plant height, plant spread and fruit diameter exhibited moderate PCV and GCV estimate suggesting the possible role of environment in influencing these characters (Table 1).

The findings of multivariable (D square) analysis showed the random distribution of 94 paprika genotypes into ten clusters and there was no association of genetic diversity with geographical origin of parents. Principal component analysis identified three principal components (PCs), which contributed 78.47 per cent of cumulative variance. Dendrogram obtained by cluster analysis showed the sub-grouping of genotypes within the clusters, which was not possible through D2 analysis. Similar results were reported by Usharani (1996) and Biswadipchatterjee (2006) where 7 principal components were found to describe the maximum variance of the data set. Based on the inter- and

intracluster distance among the groups, fourteen parents were selected 3 each from both cluster IX and X and one each from clusters I, II, III, IV, V, VI, VII and VIII, keeping in mind the characters contributing for divergence so as to obtain better and desirable segregants (Table 2).

Among 14 parents, 7 had significant positive gca effect for dry fruit yield per plant with the maximum observed in LCA-428 (134.49) followed by LCA-436 (111.51) and LCA-432 (53.45). These parents had significant gca effects for number of fruits per plant, 100 seed weight, number of seed per fruit, capsanthin content days to 50 per cent flowering, days to maturity, fresh fruit yield per plant, weight of dry stalk less fruits per plant. The parent LCA-422 exhibited the highest negative gca effect for days to 50 per cent flowering, days to maturity and significant gca effect in desired direction for number of seeds per fruit, 100 seed weight, and capsanthin content. This parent will, therefore, be of use in breeding for earliness and less pungent and more coloured paprika.

For all the 17 characters studied except for fruit length, number of seeds per fruit, and 100 seed weight, about 50 per cent of the crosses recorded significant heterosis over the mid- parent indicating that both additive and non-additive type of gene action were of equal importance for these characters. For characters like plant height, fresh fruit yield per plant, dry fruit yield per plant, days to maturity and number of fruits per plant, the majority of the hybrids showed significant heterosis explaining the non-additive gene action, whereas for character like seeds per fruit, seed weight, capsaicin, only less than 50 per cent of crosses showed significant mid- parent heterosis, which meant that for these characters additive gene action was involved to a greater extent than non-additive type of gene action. However the combining ability analysis revealed more of SCA variance than GCA variance except for capsanthin content indicating that non-additive type of gene action was predominant for all the characters studied and additive gene effect for capsanthin content (Table 3).

The superiority of the hybrids in crosses was estimated over mid-parent, better parent and standard check for all the 17 characters studied. The cultivar, Byadigi Kaddi was selected as a standard check. For high productivity the crosses LCA-436 x CA-960, LCA-428 x KTPL-19, LCA-428 x LCA-424, LCA-436 x KTPL-19, LCA-432 x KTPL-19 were identified as the best heterotic combinations. They showed significant positive standard heterosis ranging from 299.3 to 349.8 per cent yield. These results indicated that the high productivity of these hybrids was associated with plant height, plant spread and number of fruits per plant. The hybrids LCA-436x KTPL-19, LCA-436x CA-960 also registered very low capsaicin content and good colour value compared to the check Byadigi kaddi. The hybrid LCA-437 x CA-960 is considered to be the best heterotic combination, which recorded high oleoresin content (16.7 per cent) maximum capsanthin content (7249 EOA units) and minimum capsaicin content (0.09%) (Table 4).

The cross LCA-414 x CA-960 and LCA-436 x B. Dabbi involved positive x negative general combination with common parent LCA-436, having highest and significant gca effect, demonstrating its value as good general combiner for the total yield per plant. Similarly the crosses LCA-437 x KTPL-19 and LCA-437 x CA-960 had significant sca effects in desirable direction for capsaicin content. The parents of the crosses were positive x negative general combiners. However, it can be observed that the cross LCA-414 x KTPL-19 can be considered the best as it has desirable sca effects for quality parameters both capsaicin and capsanthin. Similar results were reported by Gaddagimath (1992) Patel et al. (1997), Jagadeesh (2000) and Nandadevi et al (2003).

Conclusion

It can be concluded from the study that the lines LCA-414, LCA-422, LCA-431 and LCA-436 and the tester KTPL-19 and B. Dabbi gave the best F1 hybrids for potential yield. For quality parameters the lines LCA-433 and LCA-437 along with the tester KTPL-19 gave the best F1 hybrid. Multiple hybridization by combining most of the desired horticultural traits with high yield

and best quality parameters can be attempted as the lines exhibited considerable divergence and high magnitude of non-additive gene action for most of the economic characters.

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Table 1: Genetic parameters in respect of quantitative traits in paprika*

Characters	Mean	σ^2g	σ^2v	GCV (%)	PCV (%)	h^2 (%)	Genetic advance 5%
Plant height (cm)	95.2	199.6	210.2	14.8	15.2	95.0	28.4
Plant Spread (cm)	124.9	677.2	694.6	20.8	21.1	97.5	52.9
Days to 50% flowering	75.0	15.5	17.9	5.2	5.6	87.0	7.6
Days to maturity	110.0	81.6	85.1	8.2	8.4	95.9	18.2
Fruits/plant	126.0	1674.3	1712.2	32.5	32.8	97.8	83.4
Fruit Length (cm)	8.6	4.0	4.4	22.7	24.4	86.7	3.7
Fruit girth (cm)	2.3	1.8	2.0	28.6	30.6	87.5	2.6
Fruit shape index	3.7	0.6	0.7	37.0	40.1	85.1	1.4
Fresh fruit weight/plant (g)	517.7	33819.0	33892.0	35.5	35.6	99.8	378.4
Dry fruit weight/plant (g)	160.4	2412.0	2465.0	30.6	31.0	97.8	100.1
Recovery % (fresh to dry)	31.8	25.1	27.7	15.7	16.5	90.6	9.8
Weight of dry stalk less fruit (g)	124.6	1244.4	1271.4	28.3	28.6	97.9	71.9
Seeds/fruit	71.5	894.1	915.1	41.8	42.3	97.7	60.9
Test (100 seed) weight (g)	0.5	0.1	0.1	60.1	61.8	94.6	0.5
Oleoresin (%)	8.1	8.9	9.0	36.7	36.9	99.2	6.1
Capsanthin (EOA)	33625	388379000	392060600	58.6	58.9	99.1	40406
Capsaicin (%)	0.2	0.0	0.0	39.8	40.3	97.6	0.2

*Genotypic variation σ^2g, Phenotypic variation (σ^2v), Genotypic coefficient of variation GCV (%), Phenotypic Coefficient Variation PCV (%), Broad Sense Heritability % (h^2).

Table 2: Cluster groups and characters considered for selection for generating F1 hybrids

Cluster number	Selected genotypes in respective clusters	Characters considered for selection
I	Byadigi Dabbi	Low capsaicin content
II	LCA 428	Less number of seeds per fruit and low test weight.
III	KTPL19	High Oleoresin content and earliness
IV	LCA 422	Low capsaicin content
V	LCA 434	High seed content and test weight
VI	LCA 414	Maximum fruit length
VII	LCA 433	Maximum plant spread and desirable fruit shape
VIII	CA960	Maximum fruit diameter and minimum capsaicin Content
IX	LCA 427, LCA 436 and LCA 424	High colour values with maximum capsanthin content.
X	LCA 431, LCA 432 and LCA 437	Maximum plant height, maximum. number of fruits per plant, maximum fresh fruit weight, dry fruit weight and stalk less chilli weight per plant.

Table 3: General and specific combining ability variances and genetic components for 17 characters in paprika (*Capsicum annuum* L.)

Characters	Variance due to GCA	Variance due to SCA	COV (FS) L x T	F = 1		
				V_A	V_D	V_A / V_D
Plant height (cm)	40.09	87.37	264.94	80.17	87.37	1.08
Plant Spread (cm)	157.50**	930.03**	1585.86	315.61	930.03	2.95
Days to 50% flowering	2.1940**	17.845**	27.27	4.388	17.845	4.15
Days to maturity	4.249**	48.96**	66.32	8.498	48.96	5.76
No. of Fruits/Plant	1161.26**	2269.31	7190.30	2322.5	2269.3	0.98
Fruit Length (cm)	0.748**	2.084**	2.100	1.495	2.084	1.39
Fruit girth (cm)	0.012**	0.233**	0.233	0.025	0.23	9.2
Fruit shape	0.184**	0.6186**	1.401	0.368	0.6186	1.68
Fresh fruit wt/Pt (g)	15349.28**	25446.14**	90392.74	30699	25446	0.83
Dry fruit wt/pt (g)	2178.46**	2954.648**	12175.16	4356.91	2954.65	0.68
Recovery (%)	1.4927**	7.8219**	14.309	2.986	7.8219	2.62
Weight of Stalk less chillies (g)	1971.328**	2159.67	10518.88	3942.66	2159.67	0.55
No. of seeds /Fruit	-33.089	425.704**	279.63	+66.18	425.73	+6.43
100 seed wt (g)	0.006**	0.0132**	0.0385	0.0121	0.0113	0.94
Oleoresin content (%)	0.283**	3.2598**	4.414	0.567	3.2598	5.74
Capsanthin content (EOA)	66733047	327306	8261.90	133466094	32730	0.0024
Capsaicin content (%)	0.0013**	0.0088**	0.088	0.0026	0.0088	3.38

Table 4: Heterosis range for 17 quantitative traits in paprika

S.No.	Characters	Heterosis (range)		
		Mid parent	Better parent	Standard Check
1.	Plant height (cm)	-30.87 to 38.49	-35.96 to 22.63	-16.52 to 59.82
2.	Plant spread (cm)	-1.30 to 64.56	-2.72 to 61.40	-4.40 to 191.8
3.	Days to 50% flowering	-23.08 to 13.95	-28.57 to 12.09	-5.56 to 35.00
4.	Days to maturity	-22.83 to 9.57	-27.05 to 3.28	-11.59 to 25.17
5.	No. of fruits per plant	-69.54 to 56.03	-74.90 to 27.48	-1.31 to 330.5
6.	Fruit length (cm)	-46.53 to 14.53	-49.79 to 14.04	-48.71 to 9.28
7.	Fruit girth (cm)	-38.38 to 84.81	-41.84 to 67.82	1.76 to 186.27
8.	Fruit shape index (L/B)	-59.90 to 64.24	-69.14 to 36.70	-78.01 to –1.79
9.	Fresh fruit wt./plant (g)	-59.33 to 73.61	-62.67 to 77.82	0.5 to 349.84
10.	Dry fruit wt./plant (g)	-65.40 to 57.70	-68.16 to 82.10	14.68 to 51.61
11.	Fresh to dry recovery (%)	-14.94 to 38.23	-15.17 to 41.68	-38.87 to 31.39
12.	Weight of dry stalk less chilli (g)	-72.65 to 74.55	-74.46 to 77.40	-26.46 to 28.70
13.	No. of seeds per fruit	-34.46 to 87.27	-39.49 to 83.85	-53.91 to 17.39
14.	100 seed weight (g)	-51.2 to 96.9	-57.32 to 59.45	-60.85 to 62.77
15.	Oleoresin content (%)	-51.46 to 42.86	-56.97 to 38.16	-46.85 to 62.77
16.	Capsanthin content (EOA)	-72.28 to 52.76	-77.82 to249.31	-64.96 to 73.86
17.	Capsaicin (%)	-49.06 to222.73	-65.12 to 140.68	-57.38 to 32.79

MP: Mid Parent BP: Better Parent SC: Standard

Plate I. Paprika hybrids with high yield potential

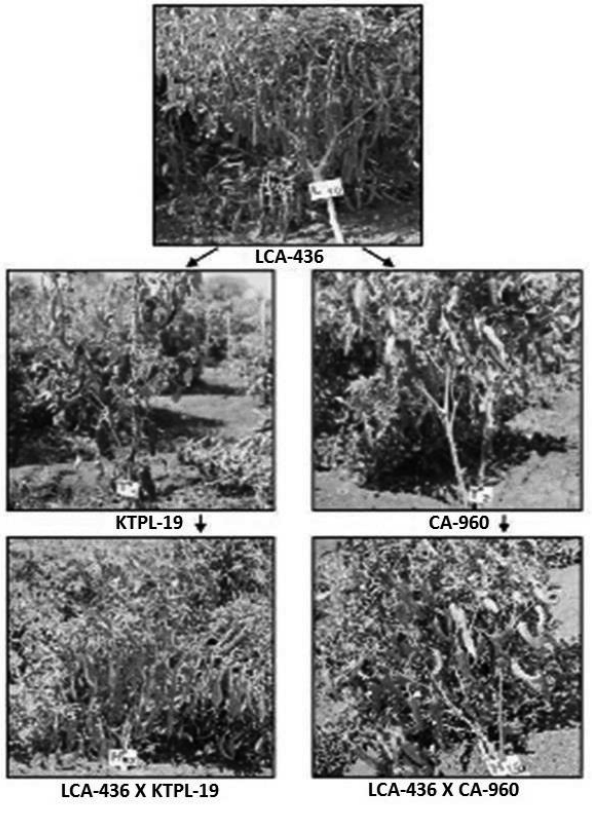

Plate II. Paprika hybrids with high Capsaicin content

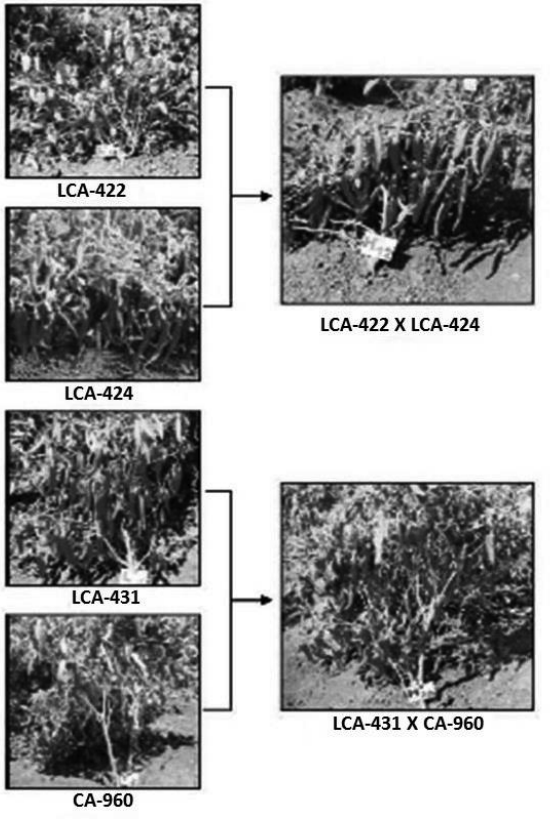

Plate III. Paprika hybrids with Capsanthin
LCA-433
LCA-424
LCA-433 X LCA-424
LCA-437
KTPL-19
LCA-437 X KTPL-19
Plate IV. Paprika hybrids with high Oleoresin content
LCA-437
CA-960
LCA-437 X CA-960
LCA-432
KTPL-19
LCA-432 X KTPL-19

Combining ability and heritability of fruit characteristics and capsacinoid content of pepper (*Capsicum annuum* L.) grown under high temperature

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Abstract

Pepper (*Capsicum annum* L.), the most important Solanaceous vegetable crop grown in open fields in Tunisia, is in great demand both in local (fresh and powder) and international markets ('harissa'). We investigated the combining ability and heritability of fruit parameters and capsaicinoid content in a diallel cross involvingsix divergent local hot pepper varieties. The magnitudes of variance due to general combining ability (GCA) as well as specific combining ability (SCA) were highly significant for all traits indicating the importance of both additive and non-additive gene action. The high GCA/SCA ratio for placenta length (10.27), fruit length (8.01), fruit diameter (6.46), capsaicin (1.57), dihydrocapsaicin (2.83) and nordihydrocapsaicin (4.62) indicate that these traits are predominantly controlled by additive gene action. Broad and narrow sense heritability was high for fruit length (77 and 91%) as well as for fruit diameter (73 and 81%) and capsaicinoid content (45 and 95%). Variety 'Chaabani' had the longest fruit, and the GCA effect was positive and highly significant, while 'BakloutiEssahel' and 'BakloutiKairouan' had the widest and heaviest fruit. Genetic effect analysis (S*ij*) of some crosses in which 'Chaabani' was one of the parents revealed the efficiencies to fruit length. High total capsaicinoids content was recorded for 'Rouge Long' (2080 µg.g^{-1}DW), 'PimentSisseb'(1747 µg.g^{-1}DW) and 'Chaabani' (840 µg.g^{-1}DW) having highly significant and positive GCA values (314**, 397** and 186**, respectively). The best crosses related to fruit characteristics and capsaicinoid content were 'Rouge long'x'Chaabani', 'PimentSesseb'x'Chaabani' and 'Chaabani'x'BakloutiEssahel'.

Keywords: *Combining ability, diallel crossing, fruit characteristics, capsaicinoids, pepper*

Introduction

In Tunisia, hot peppers (*Capsicum annum* L.) are the most important Solanaceous vegetable crop growing in open fieldsfrom late spring (May-June) to late summer (September-October). The unfavorable environmental conditions (high temperature and low relative humidity) that occur during such a cycle may have negative effects on plant growth, flower development, yield and fruit quality. High temperatures during fruit development result in reduced fruit yield in many crop plants, including tomato (Adam et al. 2001) and bell pepper (ALONI et al. 2001; Erickson and Markhart 2002). These latter authors reported that flower development after anthesis is the most sensitive stage to heat stress, leading to a reduction in fruit yield.

The active ingredients of *Capsicum* species are pungent capsaicinoids that are found in seed and fruit placentas, the main capsaicinoids in pepper fruits being capsaicin (C18H27O3N) (Garcès-Claver et al. 2007). Many studies have reported that the accumulation of pungency levels in pepper fruit are determined by two factors: the genetics of the plant and the interaction with the environment (Zewdie and Bosland 2001; Blum et al. 2002 and 2003). Estrada et al. (1999) suggested that temperature, light, fertilization and water supply affect the pungency level of fruits, although little research exists to support this claim.

In *C. annuum*, there is great variability in fruit traits, including colour, shape, length and especially pungency level, which have been poorly exploited (Lannes et al. 2007). The development

of a new variety with high quality fruit traits and high yield is one of the major goals of any breeding program. Geleta and Labuschagne (2004a) reported some factors that would be involved in crossing program: the characteristics to be improved, the type of inheritance of the characteristics and the source of available germplasm. These crosses are performed using analyses such as diallel crosses, which help in the selection of parents based on their genetic values and, in particular, their ability to combine to produce hybrids with promising segregating populations (Marame et al. 2009). Studying the effects of a diallel cross on yield and quality of pepper fruit, Patel et al. (1997) found that the effects of GCA were more important than the effects of specific combining ability (SCA) for some variables, namely fruit length, diameter and level of capsaicin.

In addition, the amount of capsaicinoids depends on the environment and on the genotype, and 34-36% of the total variation incapsaicinoids is controlled by the environment (Garcès-Claver et al. 2007). Zewdie and Bosland (2000b) and Blum et al. (2003) found that the genotype × environment interaction was highly significant, while Ben Chaim et al. (2006) confirmed that capsaicinoids are quantitatively inherited and that their biosynthesis is under genetic control.

Hot pepper local varieties characterized by high levels of capsaicinoids and capcenthin (responsible for the red colour of fruits) are usually recommended for open field culture. However, reports on diversity analysis and characterization of these varieties are almost lacking (Ben Mansour et al.2010). To the best of our knowledge, this paper represents the study which provides a comparison of some local varieties. Thus the aims of this work were:

To determine the heritability and the type of genetic effect on fruit characteristics and on the amounts of capsaicinoids,

To identify some parents/or crosses tolerant to high temperature with a high level of capsaicinoids.

Materials and Methods

Plant material and growth conditions

This experiment was carried out on the experimental station of the High Agronomic Institute of ChottMariem, Sousse, Tunisia. Half-diallel crossings were made of six divergent pepper parents (‘BakloutiEssahel’, ‘Rouge Long’, ‘Chaabani’, ‘Msarah’, ‘BakloutiKairouan’, ‘PimentSesseb’). The selection of parents was mainly based on their previously observed yield potential and on some qualitative traits including fruit quality, distinct morphological characteristics (Ben Mansour et al. 2010) and diversity of geographic origin.

During the 2008 field evaluation, these varieties had been self pollinated, and red fruit at physiological maturity were harvested; seeds were extracted, dried and conserved. In 2009, crossings were performed among all six parents without reciprocal crosses. Plants of parents were grown in a greenhouse in which day/night temperatures were optimal (25/14°C± 2°C). The half-diallel fashion to fit Griffing’s (1956) method 1 model 1 analysis was applied. The female flowers (five to seven flowers), chosen at bottom stage, were emasculated, and hand pollinated by using pollen collected from flowers of male parent at anthesis stage. General principles (Allard 1999) and techniques (Singh 2002) were followed in hybridization.

Fruits issued from these flowers were collected at maturity (red colour) and seeds were extracted and dried. In 2010, F1 generation crosses were grown together with their parents in an open field during summer season (from June to October). Each hybrid was placed between their two parents. The experiment was arranged as a randomized complete block design (RCBD) with 3 replications in plots of 5 x 4 m size with four rows to accommodate 60 plants per plot. The randomizing block system with 3 replications was applied. The experiment material was planted in well prepared soil using 3plants·m^{-2}. Fertilization and irrigation were performed according to the

needs (Chaux and Foury 1994). Day and night temperature and relative humidity were recorded, daily, using thermohygrograph (Fig. 1).

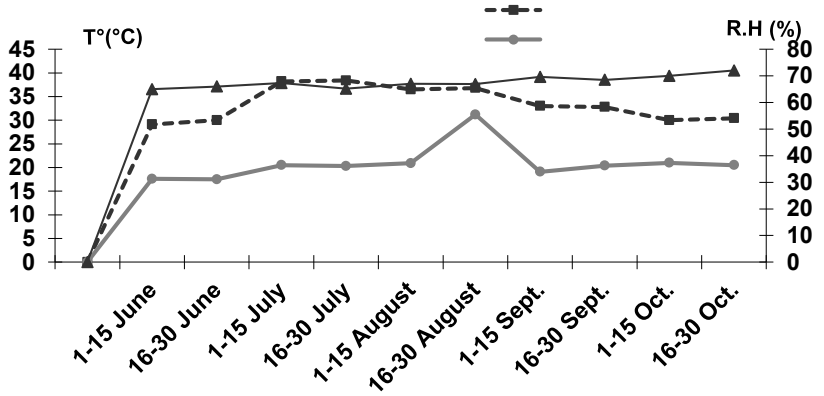

Figure 1: Evolution of day and night temperature (D T, N T) and relative humidity (R. H.) during the evaluation of crosses and parents in 2010.

Evaluated parameters

To estimate the effect of temperature on fruit characteristics, for all genotypes (parents and hybrids), 30 mature green fruits were harvested. The following parameters were recorded for each fruit: weight (g), length (mm), diameter (mm) measured at the middle of the fruit, placenta length (mm), pericarp thickness (mm) and seed number.

General and specific combining abilities

Analysis of the general and specific combining ability was carried out according to GRIFFING(1956) method 1, model 1. Narrow and broad sense inheritance of these traits was estimated by applying variance analysis and combining ability as following:

$\sigma^2_g = (CM_g - CM_e)/r$, with CM_g and CM_e respectively, square mean of parent and error (VERRIER et al. 2001), $\sigma^2_{gca}=(1/2)\ \sigma^2_a$ and $\sigma^2_{sca}= \sigma^2_d$ (GRIFFING 1956). Therefore, the broad and narrow sense heritability is:

$H^2bs = \sigma^2_g / (\sigma^2_g + \sigma^2_e)$.

$H^2ns = 2\sigma^2_a / (\sigma^2_g + \sigma^2_e)$.

Capsaicinoids evaluation

Whole or sliced green fruits of all genotypes and crosses were oven dried at 60°C for 2 to 3 hours (depending on sample size) and ground to a fine powder using a Kelner laboratory mill and stored in glass tubes at 20°C temperature prior to extraction. All samples were processed within 5 days. The capsaicinoids were extracted from 1.0 g of the ground pepper with acetonitril (1:10) by heating in an 80°C water bath for 4 h and they were swirled every hour (Collins et al. 1995). Samples were removed from the water bath and cooled to room temperature. The crude capsaicinoid extracts were purified and quantified by high performance liquid chromatography (HPLC) (Estrada et al. 1998) using a Spherisorb ODS2 C18 column with a photodiode array detector Waters 996 reading at 280 nm.Capsaiconoids standards (8-methyl-n-vanillyl-6-nonanamide for capsaicin and 8-methyl-n-vanillyl-nonanamide for dihydrocapsaicin) were obtained from Sigma Chemical Co. and were used for retention time verification and HPLC calibration.

Statistical procedures and data analyses

Analyses of variance for each trait were done using mean values of the 15 F1 progenies plus their 6 parents in order to study the variations among the 21 progenies. Data analysis was performed using SAS v. 6.0; One-way analysis of variance (ANOVA) was used to separate the means which were compared by Duncan's multiple range test (DMRT) at $P = 0.05$.

Results and Discussion

Fruit characteristics

The analysis of variance for combining ability (Table 1) revealed the existence of significant variation for all characters, indicating a wide range of variability among the parents. Highly significant variation ($P \leq 0.01$) of general (GCA) and specific (SCA) combining ability was recorded for all traits. Then, the magnitudes of variation indicated the importance of additive types of genes action in inheritance of these traits.These findings are in agreement with REDDY (2006) who studied the crosses of 14 genotypes of *C. annuum* and noted highly significant effects of general combining ability as well as of specific combining ability for fruit pepper characteristics (length, diameter and weight).

Table 1. Means squares of general combining ability (GCA) and specific combining ability (SCA), narrow-sense heritability (H^2ns) and broad-sense heritability (H^2bs) of fruit characteristics: length (L)(mm) diameter (D)(mm) weight (W)(g), pericarp thickness (Pth)(mm), plancenta length (PL)(mm) and number of seed (NS).

Source of variation	DF	L	D	W	Pth	PL	NS
Replication (R)	2	2.475^{ns}	30.000^{ns}	1.164^{ns}	0.120ns	54.330ns	95.728^{ns}
Genotype (G)	20	1121.34^{**}	100.993^{**}	110.698^{**}	1.495^{**}	1046.144^{**}	79.358^{**}
AGC	5	3263.368^{**}	275.957^{**}	143.422^{**}	1.707^{**}	3238.926^{**}	400.670^{**}
ASC	15	407.33^{**}	$42. 672^{**}$	99.790^{**}	1.424^{**}	15.216^{**}	1305.587^{**}
Error (E)	40	37.414^{**}	6.701^{**}	7.617^{**}	0.309^{**}	41.689^{**}	466.283^{**}
GCA/SCA	-	8.01	6.46	1.43	1.19	10.27	0.3
H^2ns	-	77	73	11	6	8	22
H^2bs	-	91	81	42	10	90	32
CV (%)	-	5.85	8.90	9.49	1 9.86	7.58	10.28

*;** significant differences, respectively, at 5 and 1% level; ns : no significant difference at 5% level

The higher GCA/SCA ratio indicates the predominance of additive gene action effect for all traits, especially for placenta length, fruit length and fruit diameter (10.27, 8.01 and 6.46 respectively). Although the latter ratio was low for seed number, pericarp thickness and fruit weight, showing that non additive (dominance or epistasie) gene action was important for controlling these traits. Ramalho do Rego et al. (2009) reported similar results.

Broad sense heritability showed high values, of 91%, 90% and 81%, respectively for the fruit length, placenta length and fruit width, moderate values for fruit weight and seed number characters (42% and 32%, respectively) and low values for pericarp thickness (10%). Narrow sense heritability showed high values for fruit length and fruit width (77% and 73%, respectively).

The observed high broad sense heritability estimates, except for pericarp thickness, indicated genetic variances with lesser effect of the environment and the potential effectiveness of selection of the hybrids for traits of interest. Low narrow sense versus high broad sense heritability for fruit weight and placenta length could be a sign of importance of dominance and inability of additive genetic component to achieve a high fruit yield and quality, because of the relationship of placenta length and the amount of fruit pungency (Zewdie and Bosland 2000).

Table 2 shows the means of fruit characteristics and the general combining ability effects of parents. Positive and significant highest general combining ability (GCA) effects for the fruit length

were recorded in 'Chaabani'(PCM) and 'Rouge Long' (RL) parents (11.72 and 9.30 for GCA and 116.2 mm and 105.1mm for mean of fruit length respectively). These latter parents have also the highest significant GCA effects for placenta length indicating that these traits would be improved by using these parents in cross breeding programmes for the accumulation of favourable genes.'Baklouti Kairouan'(PBK) showed the highest values of general combining ability (GCA) effects for fruit width and weight (4.53 and 3.37) while 'Baklouti Essahel'(PK) had the highest general combining ability (GCA) effects for fruit weight and pericarp thickness. The latter parents could be used in cross programmes to improve these traits. In the majority of the cases, good general combiners showed better mean performance for fruit characteristics (Table 2), indicating that the parent may be selected either on the basis of GCA, mean performance, or by combination of them. These results are in close agreement with those reported by Ahmed et al. (1999) and Geleta et al. (2004) with other peppers species.

Table 2. Means and general combining ability (gi) of fruit characteristics on six hot pepper parents used in a half- diallelcrossing:length (L)(mm) diameter (D)(mm) weight (W)(g), pericarp thickness (Pth)(mm), plancenta length (PL)(mm) and number of seed (NS).

	L		D		W		Pth		PL		NS	
Parents	**mean**	**gi**	**mean**	**gi**	**mean**	**gi**	**mean**	**Gi**	**mean**	**gi**	**mean**	**gi**
R L	105.1def	9.30**	25.0gh	-1.65**	21.0h	-1.10*	2.4c	-0.20*	86.7de	10.19**	195.0de	4.67^{ns}
PS	102.2def	5.63**	22.6h	-4.14**	20.6h	-3.33**	2.2c	-0.30**	82.9def	3.51**	183.9e	-6.14^{ns}
PM	95.2fgh	-1.12^{ns}	25.8fgh	-0.49^{ns}	23.8fgh	-1.42**	2.6bc	-0.13^{ns}	80.3ef	-0.57^{ns}	194.9de	-0.88^{ns}
PCH	116.2c	11.72**	28.9defg	-1.87**	30.1c	0.50^{ns}	3.0bc	0.11^{ns}	99.1bc	12.27**	201.0cde	4.35^{ns}
PB	56.2j	-19.72**	32.8 bcd	3.63**	22.2gh	1.97**	2.6bc	0.42**	37.5i	-19.2**	191.3de	-2.04^{ns}
PBK	90.0gh	-5.81**	35.5 bc	4.53**	31.4 c	3.37**	2.4c	0.10^{ns}	74.5fg	-6.2**	210.0bcde	0.04^{ns}

* means followed by the same letter are not significantly different (P<0.05) (global comparison, parents and their crosses); *;** significant differences, respectively at P<0.05 and P<0.01 ; ns: no significant difference at P≥0.05 (RL: Rouge Long, PS:PimentSesseb,PM: PimentMsareh, PCH: PimentChaabani, PB: BakloutiEssahel, PBK: BakloutiKairouan)

Evaluation of main seven crosses

Specific combining ability (SCA) and means for the traits of fruits characteristics on seven main crosses are indicated in Table 3. The highest means with a significant and positive SCA of fruit and placenta length were recorded on four crosses where PCH of RL varieties were used as parent (RL/PCH, PS/PCH, RL/PS and PCH/PB). These two genotypes (PL, PCH) were the best general combiners for fruit and placenta length and would be used as a source of these traits improvement; these traits highly appreciated for hot pepper fruits indicating high pungency (Zewdie and Bosland, 2000). In fact, these genotypes when they were crossed to 'BakloutiEssahel' (PB), highly appreciated in local market because of its highly pungency in spite of its low fruit length (Ben Mansour et al. 2010), improve fruit length. These results are in agreement of those of Marame et al. (2009) who have reported that fruit length is controlled by additive gene action rather than by environmental effect.

Capsaicnoids contents on green mature fruit

Table 4 shows the analysis of variance for general and specific combining ability and heritability of capsaicin, dihydrocapsaicin, nordihydrocapsaicin and total capsaicinoids on hot pepper green mature fruits. A highly significant effect of GCA as well as SCA was recorded for capsaicinoids compounds indicating an important variation among parents and the predominance effect of additive gene action on these characters. In addition the high value of GCA/SCA ratio for nordihydrocapsaicin and dihydrocapsaicn (4.62 and 2.83, respectively) indicated that these two compounds are controlled by additive gene action, while the moderate ratio for capsaicin and total capsaicinoids indicated the importance of non-additive gene action in inheritance of these traits and then the effect of environment.

Table 3. Means and specific combining ability (Sij) on the efficient seven crosses for fruit characteristics**:** length (L)(mm) diameter (D)(mm) weight (W)(g), pericarp thickness (Pth)(mm), plancenta length (PL)(mm) and number of seed (NS).

Crosses	L		D		W		Pth		PL		NS	
	mean*	Sij	mean	Sij	mean	Sij	mean	Sij	mean	Sij	mean	Sij
RL x PS	129.0^{ab}	9.47^{**}	28.2^{defg}	4.92^{**}	29.0^{cde}	4.38^{**}	2.6^{bc}	0.29^{ns}	107.7^{ab}	8.89^{**}	187.7^{c}	-20.90^{ns}
RL x PCH	138.3^{a}	25.48^{**}	26.0^{fgh}	-0.91^{ns}	31.0^{c}	4.40^{**}	2.6^{bc}	0.09^{ns}	114.6^{a}	19.80^{**}	207.3^{bcde}	-6.32^{ns}
RL x PB	102.8^{def}	8.71^{**}	35.7^{b}	4.65^{**}	40.9^{ab}	11.00^{**}	3.5^{b}	0.52^{ns}	86.3^{de}	10.24^{*}	251.8^{a}	39.10^{**}
PS x PCH	133.2^{ab}	11.24^{**}	17.0^{i}	5.99^{**}	24.6^{efgh}	-1.63^{ns}	2.9^{bc}	0.24^{ns}	110.7^{a}	9.85^{**}	242.9^{ab}	34.72^{**}
PCH x PB	107.2^{cde}	10.58^{**}	30.2^{def}	-0.69^{ns}	32.3^{c}	0.70^{ns}	3.1^{bc}	-0.21^{ns}	88.7^{cde}	10.53^{**}	198.7^{cde}	-13.67^{ns}
PCH x PBK	113.5^{cd}	-8.10^{*}	32.9^{bcd}	4.74^{**}	37.4^{b}	4.35^{**}	2.9^{bc}	-0.18^{ns}	93.8^{cd}	-7.91^{*}	212.9^{abcde}	-19.26^{ns}
PB x PBK	84.5^{hi}	-3.36^{ns}	43.3^{a}	2.59^{ns}	43.9^{a}	-1.35^{ns}	5.4^{a}	1.06^{**}	62.3^{h}	-6.56^{ns}	189.7^{de}	-32.90^{*}

* means followed by the same letter are not significantly different (P<0.05) according to Duncan's multiple range test (DMRT) (global comparison, parents and their crosses); *;** significant differences, respectively at P<0.05 and P<0.01 ; ns: no significant difference at P≥0.05 (PB : 'Baklouti essahel', RL: 'Rouge Long', PCH: 'Chaabani', PBK: 'Baklouti Kairouan', PS : 'Piment Sesseb').

Many studies reported that capsaicinoids content is affected by genetic and environment conditions (Contreras- Padilla and Yahia 1998; Estrada et al. 1999; Zewdie and Bosland 2000). Moreover, Harvell and Bosland (1997) reported that environment has stronger effect on capsaicinoids. In contrast, we found that genotype plays a major role in capsaicinoid contents, and GCA was significant for all capsaicinoids compounds.

Table 4. Mean squares of general (GCA) and specific combining ability (SCA), narrow-sense heritability (H^2ns) and broad-sense heritability (H^2bs) ofcapsaïcin(C), dihydrocapsaicin (DH), nordihydrocapsaïcin (NDH) and total capsaicinoïds (T.Cap)

Source of variation	**DF**	**C**	**DH**	**NDH**	**T. Cap**
Replication(R)	2	1510.87^{ns}	1322.45^{ns}	115.07^{ns}	6485.15^{ns}
Genotypes (G)	20	353168.37^{**}	416100.48^{**}	24546.79^{**}	1778134.75^{**}
AGC	5	485679.16^{**}	808085.29^{**}	59248.78^{**}	3124229.20^{**}
ASC	15	308998.11^{*}	285438.87^{*}	12979.46^{**}	1329436.59^{**}
Error (E)	40	2609.67^{**}	3618.41^{**}	436.81^{**}	11684.48^{**}
GCA /SCA		1.57	2.83	4. 62	2.35
H^2ns		24	55	77	45
H^2bs		84	95	93	95
C.V		7.46	9.25	16.17	7.40

*;** significant differences, respectively, at 5 and 1% level; ns : no significant difference at 5% level

Estimates for the combining ability effects on six parents involved in a half-diallel crossing showed that the parents Rouge Long (RL), PimentSesseb (PS) and pimentChaabani (PCH) have significant (P≤0.01) and positive general combining ability for most of the capsaicnoids compounds, including capsaicin, dihydrocapsaicin, nordihydrocapsaicin and total capsaicinoids (Table 5). RL and PS are good parents based on the higher content of total capsaicinoids (2082.4 and 1747.5 µg.g-1DW, respectively) and would be used in crossing programs to improve these traits. Despite the fact that the PCH parent does not have good combining ability for nordihydrocapsaicin it showed good combining ability for capsaicin, dihydrocapsaicin and total capsaicinoids. These results indicate the ability of these parents to produce fruits with high level of capsaicinoids under high temperature and could be used to perform these traits on others parents known by the low GCA, i.e. PM, PB and PBK parents.

In the majority of the cases, good general combiners showed better mean performance (Table 5), indicating that the parent may be selected either on the basis of GCA, mean performance or by a combination of them. These results are in close agreement with those reported by Ahmed et al.(1999) and Geleta et al. (2004) with other peppers species.

Therefore, in Tunisia there is a need for identifying local hot pepper varieties with stable and high amounts of capsaicinoids so the amount of pungency in the final product can be controlled. A genotype or cultivar that shows consistent performance across different environments and years for a given trait is considered stable. Our last findings (Ben Mansour et al. 2010) indicated that the most of these varieties showed a high level of capsaicinoids during two experimental years.

Table 5. Means (µg.g^{-1}DW) and general combining ability (gi) of capsaicin (C), dihydrocapsaicin (DH), nordihydrocapsaicin (NDH) and total capsaicinoïds (TCap) on green mature fruit of six parents used in a half- diallel crossing.

	C		DH		NDH		T Cap	
Parents	**mean***	**gi**	**mean**	**gi**	**mean**	**gi**	**mean**	**gi**
R L	670.56f	**59.72****	**1089.35c**	**183.46****	322.47a	**71.2****	**2082.39c**	**314.38****
P S	689.99f	**131.25****	**881.85de**	**223.65****	171.80d	**42.5****	**1747.48efg**	**397.41****
PM	682.43f	-0.06 ^{ns}	641.90g	-82.93**	133.45egf	-20.23**	1457.77h	-103.23**
P CH	**454.94g**	**124.47****	**324.43hi**	**56.11****	60.43ij	5.9 ns	**839.81jk**	**186.48****
PB	418.38g	**70.6****	288.58i	-156.03**	37.87ijk	-43.16**	744.84k	-269.79**
PBK	65.00i	-244.77**	38.79j	-224.27**	4.89k	-56.21**	108.70m	-525.25**

* means followed by the same letter are not significantly different (P<0.05DMRT) (global comparison, parents and their crosses); *;** significant differences, respectively at P<0.05 and P<0.01 ; ns: no significant difference at P≥0.05 (RL: Rouge Long, PS:PimentSesseb,PM: PimentMsareh, PCH: PimentChaabani, PB: BakloutiEssahel, PBK: BakloutiKairouan)

The top seven combinations selected showed a higher specific combining ability effects and values for the main capsaicinoids compounds, involving high x high (RL/PCH,PS/PCH), low x low general combing ability (PB/PBK) and high x low combinations parents (RL/PBK, PS/PB, PCH/PB) (Table 6). The higher values of capsaicin, dihydrocapsaicin as well as dihydrocapsaicin and total capsaiconoids were recorded on F1 hybrids, involving the best general combiner parents PS, RL and PCH: PS/PCH, RL/PCH, PS/PB, PCH/PB. The high x low combinations on RL/PBK, PS/PB, PCH/PB hybrids indicate the favourable additive effects of the high parent, manifested some complementary gene interaction effects (Sáanchez-Sánchez et al. 2010). These crosses could be recommended for the hot pepper fruit production in summer culture under high temperature in Tunisia. Commercial cultivars or hybrids with high capsaicinoids content would improve the quality of Tunisian 'Harissa',which constitutes a main type of pepper export.

Table 6. Top seven crosses selected on the basis ofmeans (µg.g-1DW), specific combining ability (Sij) for capsaicin (C), dihydrocapsaicin (DH),nordihydrocapsaicin (NDH) andtotal capsaicinoïds(TCap).

Crosses	C		DH		NDH		T Caps	
	mean	Sij	**mean**	Sij	**mean**	Sij	**mean**	**Sij**
RLxPCH	**1322.37b**	**460.87****	**1240.14b**	**360.73****	**319.95a**	**115.89****	**2882.46b**	**937.50****
RLxPBK	**931.29de**	**312.86****	**849.54def**	**333.11****	98.17hg	9.34^{ns}	**1879.00de**	**655.32****
PSxPCH	**1441.36a**	**508.32****	**1512.35a**	**592.75****	**245.24b**	**69.88****	**3198.95a**	**1170.97****
PSxPB	**878.00ed**	**140.03****	**797.45ef**	**89.98****	**149.13def**	**22.84***	**1824.57def**	**252.86****
PMxPCH	**866.04e**	**64.31***	628.30g	15.28^{ns}	103.16g	-9.45^{ns}	1597.50gh	70.15^{ns}
PCHxPB	**1041.99c**	**310.80****	**776.12f**	**236.21****	**123.96fg**	**34.27****	**1942.07cd**	**581.28****
PBxPBK	494.21g	14.53^{ns}	**408.29h**	**109.57****	**69.41hi**	**39.10****	**971.92j**	**163.20****

* means followed by the same letter are not significantly different (P<0.05 DMRT)(global comparison, parents and their crosses); *;** significant differences,respectively at P<0.05 and P<0.01 ; ns: no significant difference at P≥0.05 (PB : 'Bakloutiessahel', RL: 'Rouge Long', PCH: 'Chaabani', PBK:'BakloutiKairouan; PS: PimentSesseb)

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SESSION II

Molecular genetics and biotechnology tools

Lack of susceptibility factors: a novel breeding strategy for durable resistance

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Abstract

Plants are continuously attacked by a broad diversity of pathogens. World-wide farmers use large amounts of chemicals to secure crop yield. Breeding for disease resistance is a major objective of breeding activates in order to minimize the yield and quality loss associated with disease. Although, resistance can often be obtained by introgression of resistance genes (*R*-genes) from wild crop relatives, resistance conferred by *R*-genes is rarely durable. Recently, increasing research with the focus on suppression of plant immunity has led to the identification of a considerable amount of potential susceptibility genes (*S*-gene) in Arabidopsis. Taking the *Mlo* gene as the an example of *S*-genes, this review shows that *Mlo* orthologs are present in many plant species (including tomato and pepper) acting as a *S*-gene for powdery mildews (e.g. *Oidium neolycopersici* infecting tomato and *Leveillula taurica* infecting pepper*)*. With more examples of conserved *S*-gene function between Arabidopsis and crops, this review demonstrates how to impair plant *S*-genes, complementary to the introgression of *R*-genes, to achieve durable and broad-spectrum resistance.

Keywords: *Capsicum annuum*, MLO, powdery mildew, *Solanum lycopersicum,* susceptibility gene

Resistance breeding: resistance or susceptibility genes

High quality and productive crops are often susceptible to a multitude of different pathogens and pests. In practice, disease resistant crop varieties are commonly bred by the introgression of resistance (*R*) genes derived from wild crop relatives. However, it is rarely durable since race-specific resistance conferred by *R*-genes asserts selective pressure on pathogen populations promoting the appearance of new races rendering the introgressed *R*-gene ineffective. Pyramiding of *R*-genes has been proposed as a solution to this problem, but has so far not actively been deployed in varieties although from natural populations results are known which would suggest that this might be an effective form of resistance. Based on studies on effector-triggered susceptibility and by looking from a different point of view into host and non-host resistance, we have proposed a new breeding strategy by disabling plant susceptibility (*S)* genes to achieve durable and broad-spectrum resistance in crops (Pavan et al. 2010).

Several natural loss-of-function alleles of *S*-genes are known in agriculture that support the idea that impairment of specific host genes results in durable disease resistance, such as barley *mlo* mutants (Lyngkjaer et al. 2000) and the rice *pi21* mutant allele (loss-of-function of a proline-containing protein) for resistance to rice blast throughout a century of cultivation (Fukuoka et al. 2009). With increasing interest in the research topic on suppression of plant immunity, a considerable amount of potential *S*-genes has been identified in Arabidopsis (e.g. reviewed by Pavan et al. 2010). However, it is largely unknown whether orthologs of these Arabidopsis *S*-genes in crop species exist which are functional to corresponding crop pathogens.

So far, the best-characterized example on durable and broad-spectrum resistance by disabling *S*-genes is the *mlo*-based resistance to powdery mildew species in barley. The respective MLO protein isoforms modulate vesicle-associated defense responses at the cell periphery and the powdery mildew pathogen possibly exploits these proteins for successful host cell entry (Panstruga, 2005). In barley, mutations in a particular *Mlo* gene result in broad-spectrum powdery mildew resistance (Büschges et al. 1997). Barley *mlo* mutants have been known for more than 60 years and have been successfully employed in European barley agriculture for more than 35 years (Lyngkjaer et al.

2000), emphasizing the principal durability of *mlo*-mediated disease resistance under agricultural conditions. Taking *Mlo* as the target gene, this review (1) shows that *Mlo* orthologs are present in tomato and pepper acting as *S*-genes for two different powdery mildew species, namely *Oidium neolycopersici* and *Leveillula taurica*; and (2) demonstrates how to exploit *S*-genes, complementary to *R*-genes, to achieve durable and broad-spectrum resistance.

Presence and expression of the *Mlo* gene family in plant species

The first *Mlo* gene (*HvMlo1)* was identified in barley which is the only expressed gene copy of this (co-)ortholog cluster. A closely sequence-related paralog, *HvMlo2* (GenBank accession number Z95496) appears to represent a non-expressed pseudo gene. The transcription level of *HvMlo1* exhibited a noticeable increase at early time points (6 hours post inoculation) upon powdery mildew challenge in both the natural (*HvMlo1*) and mutant (*Hvmlo1*) genotypes. In other monocotyledonous plant species like *Oryza sativa*, *Triticum aestivum, Zea mays* and *Sorghum bicolor*, existence of *Mlo* homologues has also been shown (Devoto et al. 2003; Liu and Zhu, 2008; Sasanuma et al. 2010; Singh et al. 2012).

The *Mlo* gene family is well characterized in the model plant *Arabidopsis thaliana*. There are 15 *AtMlo* homologs(Devoto et al. 2003) in Arabidopsis, of which, four clades (with analysis of the AtMLO family only) have been revealed by phylogenetic analysis (Chen et al. 2006). All *AtMlo* genes exhibited altered expression patterns in different tissues as well as in response to various cues. However, none of the 15 *AtMlo* genes showed identical expression profiles between different clade members indicating their distinct functions in biological processes. Nevertheless, overlapping expression patterns between *AtMlos* in the same phylogenetic clade were observed. For example, the AtMLOs in Clade IV (AtMLO2, AtMLO6 and AtMLO12) showed abundant expression in leaf tissues, and appeared to be the most responsive AtMLO members under biotic stresses including biotrophic fungal pathogens *Erysiphe cichoracearum* and *Golovinomyces orontii,* the hemibiotrophic oomycete pathogen *Phytophtora infestans*, the necrotrophic fungal pathogen *Botrytis cinerea* and the bacterial pathogen *Pseudomonas syringae* (Chen et al. 2006). These mildew effective AtMLO (Clade IV in Chen et al. 2006) proteins are involved in modulating plant defense to powdery mildew responses (Consonni et al. 2006). AtMLO4 and AtMLO11 in Clade I (Chen et al. 2006) were shown to be predominantly expressed in the epidermal cells of the root meristematic zone and implicated in an Auxin dependent root thigmomorphogenesis (Chen et al. 2009). AtMLO7 in Clade III (Chen et al. 2006) was proven to be co-involved with the receptor-like kinase FERONIA in pollen tube reception (Kessler et al. 2010). The biological functions of the other AtMLOs are largely unknown.

In grape (*Vitis vinifera*) 17 *Mlo* homologs (*VvMlo*) have been identified. In total, six phylogenetic distinct clades were assembled by an analysis of the 17 VvMLO proteins with the whole MLO protein family from Arabidopsis, and selected MLO proteins from barley, rice, maize and tomato (Feechan et al. 2008). Interestingly, the VvMLO10 and VvMLO11 were grouped together with the AtMLO3 and formed the additional Clade VI. The AtMLO3 was considered as a single divergent lineage in the analysis of *Arabidopsis* MLO with those from the monocots (Devoto et al. 2003). Furthermore, comparative analysis retrieved four grapevine MLO homologs (VvMLO3, VvMLO4, VvMLO13 and VvMLO17) that are closely related to the Arabidopsis mildew effective MLOs (AtMLO2, AtMLO6 and AtMLO12). The *VvMlo3, VvMlo4* and *VvMlo17* showed up-regulation upon the challenge of grape powdery mildew fungus *Uncinula necator*, thus, have been considered as associated to powdery mildew susceptibility (Feechan et al. 2008).

The tomato genome sequence was recently published by the International Tomato Genome Sequencing Consortium (TGSC) (http://solgenomics.net/). In the tomato genome, 16 tomato *Mlo* homologs (*SlMlo*) could be identified (Zheng, 2012). Some of the *SlMlo* homologs (e.g. *SlMlo4, SlMlo7, SlMlo8, SlMlo14, SlMlo15* and *SlMlo16*) showed a tissue-specific expression, indicating

probably a specific function of those MLO proteins in different biological processes. These *SlMlos* were grouped in different clusters based on a phylogenetic analysis by aligning protein sequences of *SlMlos* with the MLO members of other plant species including the whole Arabidopsis MLO protein family. In addition to the presence of SlMLO members in the five clades corresponding to the Arabidopsis clades, Clade VI was identified containing SLMLOs which were grouped together with the AtMLO3 protein that was considered as a single divergent lineage in the analysis of *Arabidopsis* MLO with those from the monocots (Devoto et al. 2003; Zheng, 2012). Additionally, the expression profiles of the *SlMlo* gene family members were analysed in response to the tomato powdery mildew *O. neolycopersici*. *SlMlo1* transcripts showed considerable increase at 6 and 10 hours after *O. neolycopersici* challenge. In contrast, no other *SlMlo* homolog in Clade V (*SlMlo3*, *SlMlo5* and *SlMlo8*) showed up-regulation upon fungal infection. Surprisingly and contrast to expectation based on sequence homologies, the expression of *SlMlo4* and *SlMlo14* was also up-regulated at 10 hours after inoculation of *O. neolycopersici*.

With the available genome sequences of many plant species, such as cucumber, apple and etc., *Mlo* like sequences are being identified. Towards understanding the biological functions of the *Mlo* gene family, their expression patterns are being analysed in different plant species (Chen et al. 2006, Feechan et al. 2008; Winterhagen et al. 2008; Dry et al. 2010; Zheng, 2012). Molecular phylogeny and evolution of the plant specific *Mlo* family are being gradually uncovered.

Mlo homologs functioning as plant susceptibility genes in different plant species

In barley, the MLO protein was identified as a plant susceptibility factor manipulated by the powdery mildew fungus *Blumeria graminis* f. sp. *hordei* to cause disease (Büschges et al. 1997). The naturally occurring and mutation-induced recessive alleles of the *Mlo* locus confer a broad spectrum resistance against all known isolates of barley powdery mildew identified so far. The resistance conferred by the loss-of-function mutants in the *Mlo* gene prevents the mildew fungus from penetrating host cells, and thus prevents fungal haustorium formation (Peterhansel et al. 1997; Piffanelli et al. 2002). and thus prevents fungal haustorium formation (Peterhansel et al. 1997; Piffanelli et al. 2002). The later identification of natural and mutation-induced *mlo*-mutants in Arabidopsis, tomato and pea for resistance to different powdery mildews demonstrates a conserved requirement for MLO proteins in powdery mildew pathogenesis in plants (Bai et al. 2008, Consonni et al. 2006; Humphry et al. 2011; Pavan et al. 2011). In breeding practices, these findings imply the success in applying *mlo*-based resistance to combat powdery mildew disease in a wide range of crop species either by mining the natural variation in wild crossable species and/or by using conventional mutagenesis to generate mutations in the gene (Pavan et al. 2010).

In barley, pea and tomato, mutation in one *Mlo* gene is sufficient to confer full resistance to powdery mildews (Jørgensen, 1994; Büschges et al. 1997; Humphry et al. 2011; Pavan et al. 2011; Bai et al. 2008). On the other hand in Arabidopsis, deletion of three *Mlo* homologues (*AtMlo2, AtMlo6* and *AtMlo12*) is required for full powdery mildew resistance (Consonni et al. 2006). Phylogenetic analysis of *AtMlo* homologues has identified a special mildew effective clade, in which *AtMlo2, AtMlo6* and *AtMlo12* are grouped. This clade is labelled as having the *Mlo* gene members acting as susceptibility factors manipulated by the powdery mildews. In tomato, the mildew effective clade has four *SlMlo* homologues, (*SlMlo1*, *SlMlo3*, *SlMlo5* and *SlMlo8*), of which loss-of-function in *SlMlo1* resulted in resistance to *O. neolycopersici* (Zheng, 2012). Silencing *SlMlo3*, *SlMlo5* or *SlMlo8* did not result in a significant reduction of disease symptoms caused by *O. neolycopersici,* while, in Arabidopsis loss of function of individual *AtMlo* homologs (*AtMlo2, AtMlo6* and *AtMlo12*) in the mildew effective clade led to different levels of resistance against *G. orontii* and *G. cichoracearum* (Consonni et al. 2006) and *O. neolycopersici* (Zheng, 2012). These findings imply that the involvement of *Mlo* homologs in the susceptibility to powdery mildews is dependent on the plant species.

The expression of *Mlo* homologues in the mildew effective clade of different plant species has been shown to be induced upon powdery mildew infection. In barley, *Mlo* transcripts have been demonstrated to be increased at an early time point of the interaction (6 hours post inoculation) (Piffanelli et al. 2002). In tomato, *SlMlo1* transcripts showed considerable increase at 6 and 10 hours after *O. neolycopersici* challenge (Bai et al. 2008; Zheng, 2012). However, no other *SlMlo* homologs in Clade V (*SlMlo3*, *SlMlo5* and *SlMlo8*) showed pathogen-dependent up-regulation. This result suggested that *SlMlo3*, *SlMlo5* and *SlMlo8* may after all not be involved in the powdery mildew susceptibility of *O. neolycopersici* although they are closely related to the mildew effective *Mlo* homologs. Intriguingly, *SlMlo4* and *SlMlo14* showed up regulation upon *O. neolycopersici* infection. These two homologs are not in the clade containing MLO homologs involved in powdery mildew susceptibility. SlMLO4 is closely related to AtMLO4 and AtMLO11 which are in Clade III and are involved in the root thigmomorphogenesis. SlMLO14 is clustered with AtMLO7 in Clade I which is involved in the pollen tube reception. Furthermore, mutations in AtMLO4, AtMLO7 and AtMLO11 in Arabidopsis did not result in powdery mildew resistance (Consonni et al. 2006). Thus, it is logical to argue that silencing of these two *Mlo* homologs would likely not lead to resistance against tomato powdery mildew. However with the new data on the up regulation of these two homologs upon infection with the pathogen it will be very useful to create RNAi lines in which these two *SlMlo* homologues (and in view of these results also as many of the others) are silenced. If these two *SlMlo* homologues are powdery mildew effective, the claim on mildew effective clade *Mlo* homologues will be challenged. In view of these results it is however clear that the assumption that members of the same clade retain a similar biological function is no longer valid.

O. neolycopersici is epiparasitic and develops all structures except haustoria on the host surfaces. In contrast, *Leveillula taurica,* another powdery mildew species infecting both tomato and pepper, is endotrophic because fungal haustoria are produced in the mesophyll cells. So far *mlo*-based resistance is only demonstrated to confer resistance to epiparasitic mildews in barley, Arabidopsis, tomato and pea. Interestingly, the tomato *Slmlo1* mutant, which had full resistance to *O. neolycopersici* (Bai et al. 2008), showed incomplete resistance to *L. taurica* (Zheng, 2012). Currently, there are at least two *CaMlo* (*CaMlo1* and *CaMlo2*) present in the pepper genome. Interestingly, knocking down the expression of either *CaMlo1* or *CaMlo2* resulted in reduced susceptibility of pepper to *L. taurica* (Zheng, 2012), indicating that more than one *Mlo* homologue are involved in the susceptibility to endotrophic powdery mildew, *L. taurica*. Though silencing of *SlMlo3*, *SlMlo5* or *SlMlo8* in tomato did not give resistance to *O. neolycopersici*, the possibility cannot be ruled out that these homologues are involved in the susceptibility to *L. taurica*. Comparative analysis revealed a close homology between *CaMlo1* and *SlMlo3*, suggesting the possible involvement of *SlMlo3* in susceptibility to *L. taurica*. Thus, the *SlMl*o homologues (*SlMlo3, SlMlo5* and *SlMlo8*) that are phylogenically related to *SlMlo1* should be the first targets to be investigated in order to see whether silencing of these three homologs will reduce the susceptibility of tomato against *L. taurica*. Thus, for the same plant species, full resistance to different powdery mildew species likely requires mutations in different numbers of *Mlo* homologs. Therefore, the specificity or redundancy of plant MLO functions in powdery mildew interaction in different pathosystems requires attention. Hopefully, there is always one *Mlo* homolog having a major effort, such as the *AtMlo2* in Arabidopsis, which will simplify the use of *mlo*-based resistance in breeding practices.

A recently study has indicated the identification of the *CaMlo2* gene in pepper (Kim and Hwang, 2012) which represents a different allele of the *CaMlo2* gene isolated by Zheng (2012). Their results demonstrated that the newly isolated *CaMlo2* gene is involved in cell death response as well as formation of the reactive oxygen species (ROS). In addition, the *CaMlo2* was distinctly induced by the hemibiotrophic bacterial pathogen *Xanthomonas campestris* pv. *Vesicatoria*, the oomycete pathogen *Phytophthora capsici*, exogenous salicylic acid, methyl viologen, NaCl and drought stress treatment. Silencing of the *CaMlo2* could induce the resistance against *Xanthomonas*

campestris pv. *Vesicatoria* in pepper, while, overexpression of *CaMlo2* in Arabidopsis resulted in enhanced susceptibility to *Pseudomonas syringae* pv. *tomato* DC3000 and *Hyaloperonospora arabidopsidis*. The results of Kim and Hwang (2012) support the evidence obtained by Zheng (2012) that CaMLO2 is a negative regulator of defense response in pepper and also show that *mlo*-based resistance is not restricted to powdery mildews.

Perspective of plant S-gene in resistance breeding

Resistance and susceptibility are opposite sides of the same coin. However, most studies have for a long time focused on the resistance side, in search for plant *R*-genes. Recent studies on plant immunity have shown that a pathogen must suppress induced defence in order to infect a plant species, which otherwise would have been a non-host to the pathogen. Based on studies on effector-triggered susceptibility and by looking from a different angle into non-host resistance, we proposed that disabling plant *S*-genes might help to achieve durable and broad-spectrum resistance in crops (Pavan et al. 2010). Several natural loss-of-function alleles are known in agriculture that corroborate the idea that impairment of specific host genes results in durable disease resistance. Rice cultivars carrying the *pi21* allele have maintained durable rice blast resistance throughout a century of cultivation (Fukuoka et al. 2009). Barley *mlo* mutants show non-race specific resistance to the powdery mildew *Blumeria graminis* and have been successfully used in European agriculture for more than 35 years (Lyngkjaer et al. 2000).

Genetic dissection of disease susceptibility in Arabidopsis to powdery and downy mildew has identified multiple *S* genes whose impairment results in disease resistance (Vogel et al. 2000; Van Damme et al. 2005; Pavan et al. 2010). Although several of these *S*-genes have been cloned and characterized in more detail in Arabidopsis it is unknown to which degree their function in disease susceptibility is conserved among different plant species. As a proof-of-concept research, we have tested several tomato orthologs of Arabidopsis *S*-genes. Our recent results showed that ssilencing of the tomato orthologs *SlPMR4* and *SlDMR1* resulted in *O. neolycopersici* resistance in tomato, indicating that their *S*-gene functions are conserved across plant species (Huibers et al. 2013). Interestingly, no severe fitness costs in terms of reduced growth were found associated with *SlPMR4* silencing making this gene a promising target for mutagenesis to obtain suitable *Slpmr4* alleles for disease resistance breeding in tomato.

In spite of promoting pathogen proliferation and disease establishment, *S*-genes have not been eliminated during evolution. Evidence suggests that certain *S*-genes, besides being involved in plant-pathogen interactions, are required for other important physiological processes. For example, the rice *Xa13* gene is required for both the growth of *X. oryzae* and for pollen development (Chu et al. 2006). Loss-of-function mutations of such *S*-genes reduce fitness. Whereas, it has been also demonstrated that loss-of-function mutations of certain *S*-genes, such as *Mlo*, *Pmr4* and *Dmr4*, can result in broad-spectrum resistance without fitness cost (Vogel et al. 2000; Van Damme et al. 2005; Bai et al. 2008, Huibers et al. 2013). Valuable *S*-gene alleles mediating a high level of resistance with a minimum of associated fitness costs are expected to be rare. However, new techniques are available to enable the discovery and design of superior allele variants, which combine favourable disease resistance levels with no/less detrimental side effects. Such advanced technologies include TILLING (targeting induced local lesions in genomes) for allele mining (Kurowska et al. 2011); site-directed mutagenesis using Zinc-finger nucleases- and/or TALEN-based gene editing for allele design (Townsend et al. 2009). Thus, in addition to finding natural superior mutant alleles, it is in some cases feasible to alter exclusively the *S*-gene function without disturbing other biological functions.

For many crop species genome sequences are or will soon become available, which will facilitate the identification of orthologs of *S*-genes in other plant species. Assuming that their function in disease susceptibility is conserved among different plant species, as is the case for the

Mlo gene, desired mutations can easily be obtained by (targeted) mutagenesis approaches mentioned above and applied in breeding crops with durable resistance. Thus this review promotes the potential exploitation of orthologs of Arabidopsis *S*-genes in resistance breeding in crop species.

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Genetic control of quantitative variation of pigment content in pepper fruit

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Abstract

Fruit color is one of the most important quality traits of pepper. Studies on the genetic control of pigment content in pepper fruit focused mostly on monogenic mutations leading to change in fruit color. In addition to the qualitative variation in fruit color, quantitative variation in pigment content and color intensity exits in pepper giving rise to a range of color intensities from light to dark green and from light to dark red colors. However, the genetic basis of this variation has been very little studied. We analyzed quantitative variation of pigment content in a cross of a *Capsicum annuum* dark-green Poblano-type pepper with a *C. chinense* light-green pepper. We identified two major pigment content QTLs, *pc8.1* and *pc10.1* that control chlorophyll, carotenoids and alpha-tocopherol content mostly at the mature green stage and to a lesser extent in the red ripe stage. While *pc8.1* was predominant in the dark green x light green cross, *pc10.1* was predominant in a dark green x medium green cross, indicating the effect of the genetic background on the expression of the QTLs. Analysis of near-isogeneic lines (NILs) for the QTLs indicated that plastid compartment size is the major factor associated with variation in pigment content. The NILs were further compared for the content of fruit metabolites and physiological parameters.

Keywords: Pepper, fruit color, chloroplast biogenesis, QTL mapping

Introduction

Pepper fruit quality is attributed to a variety of traits, affecting visual appearance, flavor, chemical composition and nutritional value. Together, these traits determine consumer acceptance of the fruit. One route for improvement of crop quality is biofortification through enrichment of beneficial phytochemicals that exhibit health-promoting effects such as carotenoids, flavonoids and vitamins. Metabolic engineering of genes in biosynthetic pathways have been used in attempts to elevate beneficial phytochemicals in tomato fruit (Davuluri et al. 2005; Giuliano et al. 2008). However, this approach is currently not routinely amenable to pepper due to the lack of efficient stable transformation and since it relies on genetic modification (GM) that is not accepted yet in Israel and Europe. An alternative approach that relies on traditional plant improvement and utilizes sources of natural variation in the desired traits is therefore favored. A good example for utilizing natural variation to improve nutritional quality of the fruit is the use of *high-pigment* (*hp*) tomato mutants that are characterized by elevated contents of carotenoids, vitamins and flavonoids (reviewed by Levin et al. 2006). Although fruit ripening in tomato resembles that of pepper, similar mutants are not known to date in the latter crop.

Natural mutants that exhibit changes in immature and mature fruit color have been identified in pepper and several genes controlling this variation have been isolated (Paran and Fallik, 2010). In contrast, the genetic control of quantitative variation in pigment content and color intensity has not been studied extensively. We recently reported on QTL mapping of pigment content in a cross of dark-green and light-green parents (Brand et al. 2011). In this study two major pigment content QTLs, *pc8.1* and *pc10.1* that control chlorophyll content were identified. Initial characterization of the major QTL *pc8.1* indicated that the QTL exerts its effect via increasing chloroplast compartment size in the dark-green parent. Near-isogenic lines for the QTL (QTL-NIL) were constructed and partially characterized. In the present study we provide additional data on the effect of the QTL in the isogenic background.

Materials and Methods

Plant material

QTL mapping was performed in a cross of *Capsicum chinense* PI 152225 with light green immature fruit and *C. annuum* line 1154 with dark green immature fruit. To create QTL-NILs, 1154 was used as a recurrent parent in a backcrossing program using PI 152225 as the donor parent and selection of heterozygous plants at the *pc8.1* QTL by using the most significant marker (T1341). BC4F2 plants were fixed at the two alleles of the QTL and further selfing until BC4F4 created the NIL-light and NIL-dark lines used in the present study (Figure 1).

Fig. 1 Parental lines and *pc8.1* NILs used in this study. Bar = 5 cm

Chlorophyll measurements

Chlorophyll of mature green fruits, 4 weeks post-anthesis was measured from a total of 15 fruits from five plants for each parent and QTL-NIL by extraction with DMSO in the dark during 3 days of incubation. Chlorophyll content was measured by absorbance at 652 nm and was calculated by the following equation: Chlorophyll (μg g FW^{-1}) = 27.8 X A_{652}/g FW. Chlorophyll content was also measured in fully developed leaves of the parents and NILs.

Chloroplast measurements

Pericarp slices from 12 immature green fruits from four plants (14 days post-anthesis) were used to measure chloroplast number and size. Confocal microscopy (Olympus IX 81) was used to capture cell images and for excitation of chlorophyll autofluorescence. Image analysis (measuring number of plastids per cell, plastid length, and cell index-ratio of plastid area to cell area) was performed with ImageJ 1.37M software (National Institutes of Health, Bethesda, MD, USA, http://rsb.info.nih.gov/ij/).

Transmission electron microscopy (TEM)

Preparation of pericarp tissue from young immature fruit for TEM was done accordingly to Powell et al. (2012).

Yield and total soluble solids (TSS) measurements

For yield and TSS analyses, plants were grown in the open field in the Volcani Center Experimental Station in the summer of 2011. Yield was measured by weighting the total fruit yield from 10 individual plants three month after planting. TSS was measured in 10 plants for each parent (3 fruits per plant) in the mature green and ripe red stages using a refractometer on a drop of fresh juice extracted by a garlic crusher.

Results

Initial QTL mapping was conducted in an F2 population of a cross between the two parents that exhibit the maximum variation in chlorophyll content in *Capsicum* fruits. Lines 1154 (dark green fruit) and PI 152225 (light green fruit) differ approximately 20-fold in fruit chlorophyll content (83±8.1 and 4±0.2 μg g FW^{-1} for 1154 and PI 152225, respectively; Figure 2A). Two QTLs, *pc8.1* and *pc10.1* controlling chlorophyll content in immature fruit were identified in the F2 population and were verified in a BC2F2 generation, explaining 54% and 15% of phenotypic variation of the trait, respectively (Brand et al. 2011). In the present study we focus on characterization of the major QTL, *pc8.1,* using a pair of isogenic lines differing at the QTL alleles. NIL-dark had a fruit chlorophyll content that was not significantly different from the dark green parent 1154 (75±20 μg g FW^{-1}; Figure 2A), while the chlorophyll content of NIL-light (21±0.8 μg g FW^{-1}) was significantly lower than NIL-dark but was not as low as that of the light green parent PI 152225.

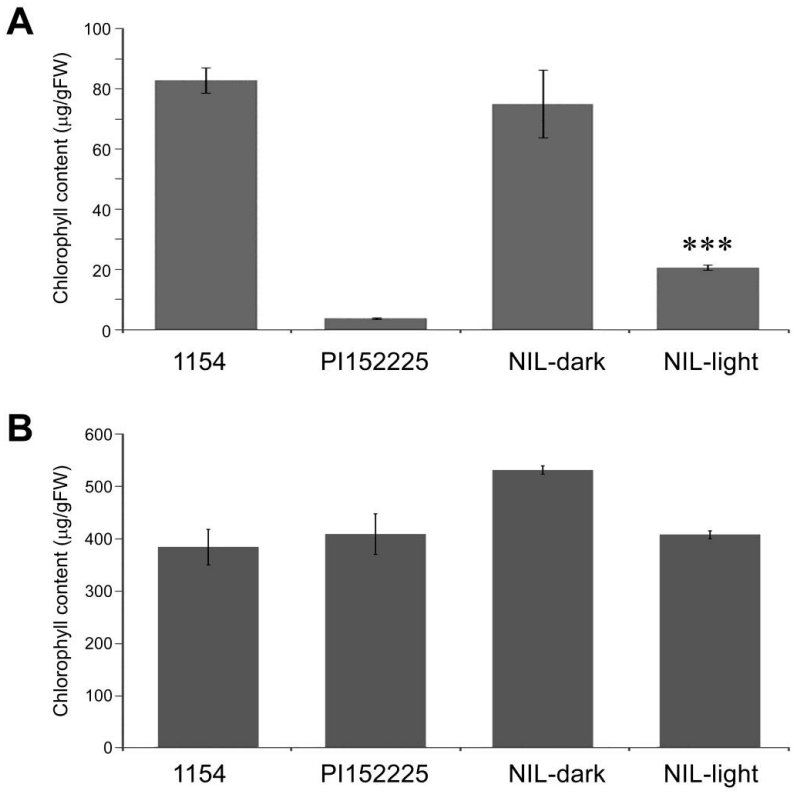

Fig. 2 Chlorophyll content in mature green fruit (A) and leaves (B) of parents and QTL-NILs for *pc8.1*. Asterisks indicate significant difference (***$P < 0.0001$, *$P < 0.01$) between NIL-dark and NIL-light by Student's t-test.

The two parents did not differ significantly in their chlorophyll content in the leaves (393±17.3 and 407±17.8 μg g FW^{-1} for 1154 and PI 152225, respectively; Figure 2B). However, NIL-dark had significant more chlorophyll in the leaves (534±7.9 μg g FW^{-1}; Figure 2B) than NIL-light (405±7.7 μg g FW^{-1}).

Chloroplast measurements of the parents and NILs using confocal microscopy, showed that the chloroplasts of the dark green parent 1154 were significantly larger and in higher number than those of the light parent PI 152225 (Figure 3). Similarly, the chloroplasts of the NIL-dark were significantly larger and in higher number than those of the NIL-light parent.

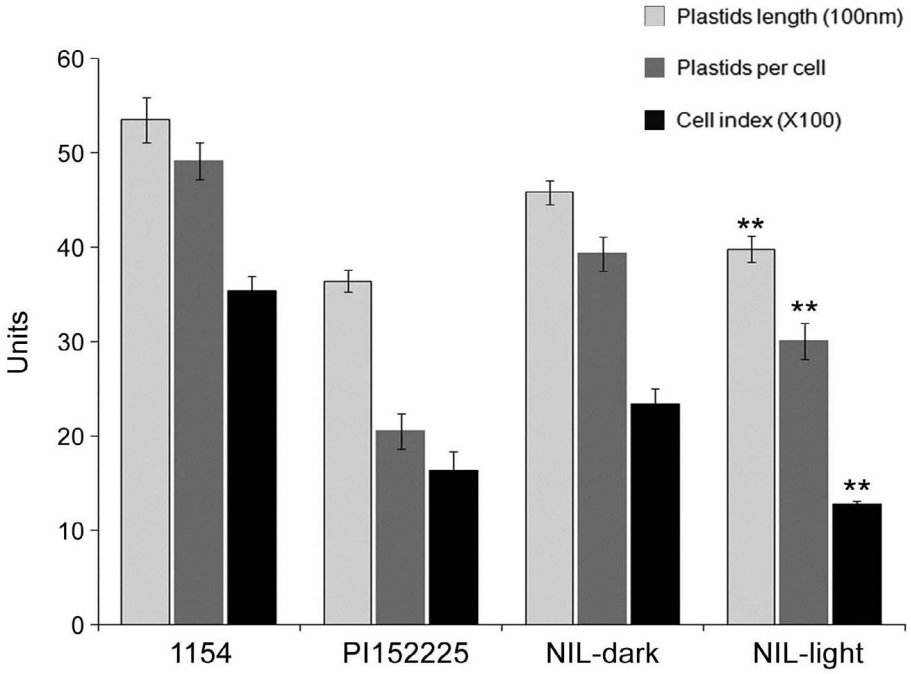

Fig. 3 Plastid compartment measurements of parents and QTL-NILs for *pc8.1*. Asterisks indicate significant difference (**$P < 0.001$) between NIL-dark and NIL-light by Student's t-test.

Accordingly, the grana in the chloroplast of the NIL-dark line were thicker than those of NIL-light (Figure 4). The chloroplast differences between the NILs indicate that *pc8.1* exerts its effect on controlling variation in fruit chlorophyll content via modulating chloroplast compartment size.

We further determined whether the change in chlorophyll content between the NILs is associated with a change in TSS content. Significant differences in TSS were observed in immature green fruits of the parents (6.9±0.23 for 1154 versus 5.1±0.2 for PI 152225) and NILs (6.3±0.25 for NIL-dark versus 5.2±0.11 for NIL-light, Figure 5). The parents also differed significantly in TSS in ripe red fruits (14±0.46 for 1154 versus 10.8±0.2 for PI 152225). However, the difference between the NILs in the mature green fruit stage was diminished at the ripe fruit stage, as the NILs did not differ in TSS in ripe fruits (14.9±0.29 for NIL-dark versus 14.7±0.15 for NIL-light). Total yield in NIL-dark was lower than in NIL-light, however, the difference was not significant (0.38±0.03 Kg and 0.44±0.06 Kg for NIL-dark and NIL-light, respectively (Figure 6).

Discussion

Chlorophyll content in pepper fruit is inherited as a quantitative trait and two major QTLs controlling variation in this trait have been identified (Brand et al. 2011). Characterization of the major QTL *pc8.1* using near-isogenic lines revealed that the QTL exerts its effect on chlorophyll content by regulating plastid biogenesis. The effect of plastid compartment size is evident

predominantly at the immature fruit, while in ripe fruit the effect is diminished as no significant difference in carotenoid content is observed between the NILs (Brand et al. 2011). Furthermore, while chlorophyll content in leaves is significantly affected by the QTL, its magnitude is smaller compared to variation in the immature fruit. Therefore, the effect of *pc8.1* on pigment content occurs predominantly at the immature green fruit.

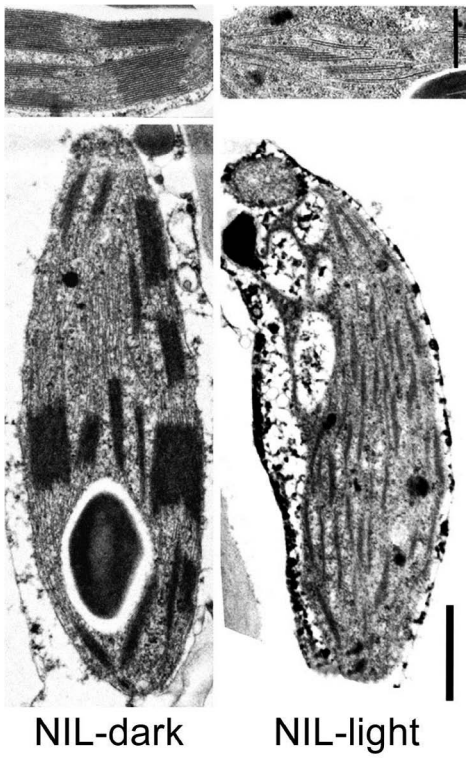

Fig. 4 TEM image of chloroplasts of fruits of NIL-dark and NIL-light.

Because accumulating increased chlorophyll content in the fruit has a potential to increase fruit photosynthesis (Hetherington et al. 1998) and as a consequence elevating carbohydrate content, this effect may improve fruit quality in dark green fruits because sugars has a major role in determining fruit flavor. We found that while *pc8.1* has a significant effect on TSS in green fruits, this effect is diminished in ripe fruit. Therefore, *pc8.1* may have a positive effect on fruit flavor by increasing total soluble content in immature green fruit or in ripe green fruits controlled by the *SGR* gene (Borovsky and Paran, 2008). Because the increase in TSS in dark green fruit is not associated with significant reduced yield, this further strengthens the potential use of the QTL in breeding for improved fruit quality in pepper.

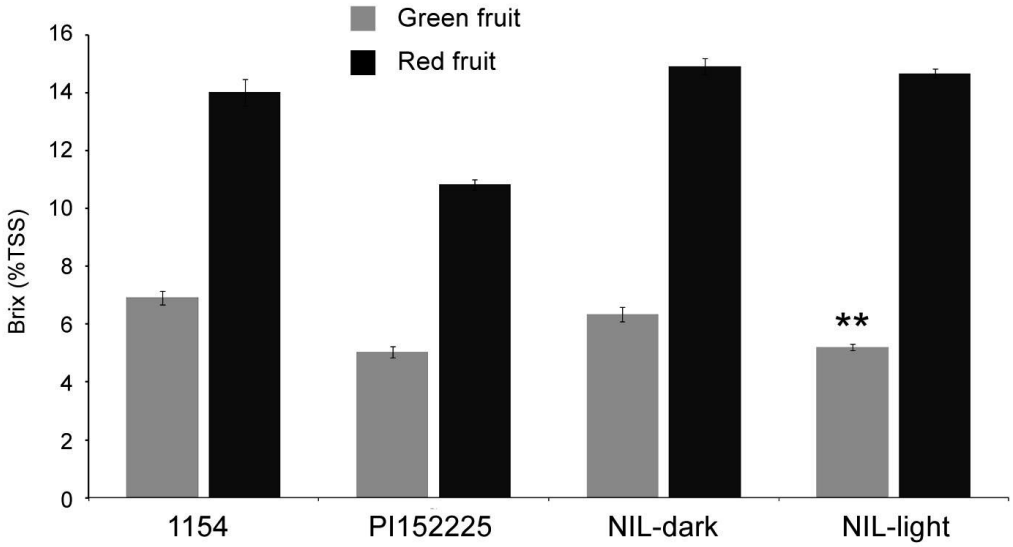

Fig. 5 Total soluble solids (TSS) of mature green and ripe red fruits of parents and QTL-NILs for *pc8.1*. Asterisks indicate significant difference (**$P < 0.001$) between NIL-dark and NIL-light by Student's t-test.

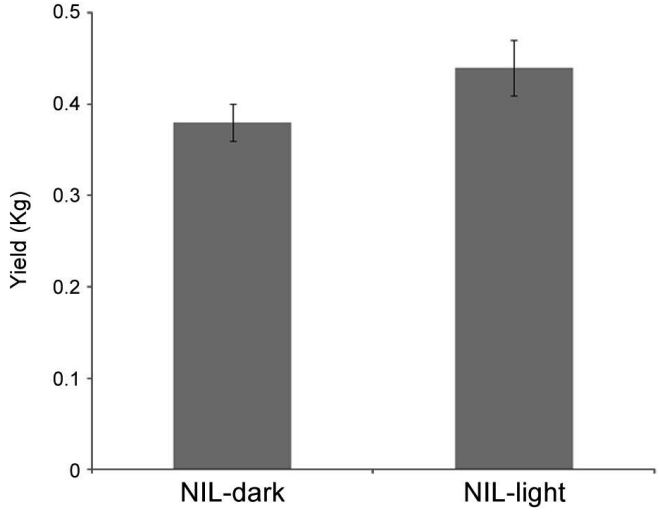

Fig. 6 Total yield of QTL-NILs for *pc8.1*.

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Genetic structure, linkage disequilibrium and first insights on association mapping in an eggplant (*Solanum melongena* L.) germplasm collection.

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Abstract

A panel of 191 eggplant accessions, which includes breeding lines, heritage varieties and selections within local landraces from Asia and the Mediterranean Basin was phenotyped in two locations for key breeding fruit and plant traits, and fingerprinted with 384 SNP (single nucleotide polymorphism) markers by Golden Gate assay (Illumina). A model based population structure analysis grouped the accessions into two main clusters, mostly related to the geographical provenance. The *linkage disequilibrium* (LD) of pair-wise loci analyses revealed a moderate large extension of the LD (4.8 cM for $r^2 > 0.15$), which is appropriate for genome wide association studies. The association analysis was performed through the mixed linear model (MLM), which takes into account both a kinship matrix and the sub-population membership of the accession, and which proved the most reliable among the ones tested. The detected associations were also compared with the QTL mapping results obtained using family-based linkage approaches.

Keywords: *Solanum melongena* – eggplant - association mapping - *linkage disequilibrium*

Introduction

Genome-wide association (GWA) studies in populations of unrelated individuals provide an efficient way to map complex traits (Rafalski, 2010). Compared to the widely adopted linkage analysis based on segregating populations derived from bi-parental crosses, the association mapping approach faces challenges that arise from the complex history of the populations under study (Hamblin et al. 2011). Its principle is to detect correlations between phenotypes and linked markers on the basis of *linkage disequilibrium* (LD) levels and patterns in the population in study. LD originates from the domestication, selection history and mating system of the crop under investigation (Hamblin et al. 2011), as it is expected to be higher in inbreeding than outcrossing species due to their higher homozygosity at a given locus, which leads to a lower rate of detectable recombination (Flint-Garcia et al. 2003). The extent of LD over the genome influences the association mapping strategy. If LD extension is high, the resolution will be low, fewer markers will be required and a whole genome scan approach may be performed. If LD is low, the resolution will be higher, an higher number of marker is required and a candidate gene analysis may be conducted (Rafalski, 2002).

Up to now no genome-wide association mapping, or genome scan, has been performed in eggplant. In this study we genotyped a panel of 191 eggplant accessions previously phenotyped for key plant and fruit traits with 384 SNP markers. Our objectives were to (i) assess the genetic structure of our collection, (ii) investigate the extent and pattern of LD in the eggplant genome as a function of genetic (map) distance, (iii) find out association between markers and key breeding traits and (iv) validate detected QTLs with the ones identified through a bi-parental population approaches.

Materials and Methods

Plant material and phenotyping.

A core population of 191 *S. melongena* accessions was identified within a wide collection of breeding lines, heritage varieties and selections within local landraces (Cericola et al. 2013). The entries originate from Asia (Oriental or Eastern – EA) and the Mediterranean basin (Occidental of Western - WE). The accessions were cultivated in two locations (Montanaso Lombardo - ML: 45 20'N, 9 26'E and Monsampolo del Tronto - MT: 42 53'N; 13 47'E, Italy) over the years 2010 and 2011 and each phenotyped for key breeding fruit and plant traits; six are here reported as an example (see Table 1). Phenotyping was based on the European Cooperative program for Plant Genetic Resource descriptors panel for Solanaceae (ECPGR, 2008) and the International Board for Plant Genetic Resource descriptors for eggplant (IBPGR, 1990). In each trial six plants per entry were planted in two completely randomized blocks. Local standard horticultural practices were applied.

The analysis of morphological data was based on the adjusted entry means (best linear unbiased predictors, BLUPs) of each trait. Variance components were determined using the restricted maximum likehood (REML) method by the following mixed linear model: $p_{ijsb} = l_j + y_s + g_i + r_{bjr} + e_{ijs}$; where p_{ijrb} is the phenotypic data of the i^{th} accession of the j^{th} location of the s^{th} year in the b^{th} agronomic replicate; l_j the effect of the j^{th} location; y_s the effect of the s^{th} year; g_i the effect of the i^{th} accession; r_{bjs} the effect of the b^{th} agronomic replicate within the j^{th} location by the s^{th} year and e_{ijs} the error term including the accession location and the accession year interaction.

Tab 1. Phenotypic variation of the six phenotypic traits considered in the whole collection

Trait*	Code	Average	ST. Deviation	Range	
				min	max
Fruit color	fcol	74.6	15.6	19.3	86.8
Calyx anthocianin	calan	2.4	2.1	0.0	5.0
Fruit weight	fw	257.0	122.3	22.0	253.0
Fruit diameter max	fdmax	7.4	2.6	2.5	12.5
Fruit lenght	fl	14.4	5.5	4.1	34.4
Leaf prickliness	lepri	0.4	0.6	0.0	5.0

*fcol was measured by means of a Konica Minolta Chroma Meter CR-400; calan and lepri were visually estimated in a range from 0 to 5; fw is in g; fdmax and fl are in cm.

SNPs assay.

Genomic DNA was extracted from young leaves of three plants per accessions. Genotyping was performed with a set of 384 SNPs markers developed by Barchi et al. (2011) from RAD-tag (restriction associated DNA tag) derived sequences, of which 339 were previously mapped (Barchi et al. 2012). Genotyping was achieved using the Golden Gate assay (Illumina, San Diego, CA), UC Davis Genome Center with automatic allele calling implemented with GenCall software (Illumina). Two genotypes of the collection were represented twice in each genotyping assay to provide an internal control. Markers showing a Minimum Allele Frequency (MAF) value lower than 0.05 were discarded from further analyses.

Population structure.

To depict genetic relationships between the 191 accessions the Un-weighted Pair Group Arithmetic Mean (UPGMA) dendrogram and Principal Coordinates Analysis (PCoA) were employed. The *STRUCTURE* v2.3.4 software (Pritchard et al. 2000) was applied to estimate the number of sub-populations in the complete set of accessions using the admixture model for the ancestry of individuals and correlated allele frequencies. Population structure was modeled with a burning of 100,000 cycles followed by 50,000 Markov Chain Monte Carlo (MCMC) repeats. The Evanno transformation method was implemented to infer the most likely number of populations (K) (Evanno et al. 2005). The accessions were sorted into sub-populations based on their maximum membership probabilities (threshold level of 70%), the ones not showing a clear membership were classified as admixtured. The pair-wise kinship coefficients between the accessions were estimated using *SPAGeDi* software (Hardy and Vekemans, 2002).

Linkage disequilibrium analysis.

The decay of LD over genetic distance was investigated by plotting pair-wise r^2 values against the distance (cM) between markers of the same chromosome. In order to evaluate the effect of the population differentiation on the r^2 estimation, we considered three different models as proposed by Mangin et al. (2011): r^2 (the simple estimation of the disequilibrium between markers without any corrections), r^2s (which account for the population structure information provided by the software *STRUCTURE*), and r^2sk (which is corrected both considering the *STRUCTURE* and the kinship matrix information). A non-parametric regression line was fit to the data using second-degree locally weighted scatterplot smoothing method (LOESS, Breseghello and Sorrells, 2006).

Association mapping.

The association analyses were accomplished on the whole collection of 191entries by means of *TASSEL* v4.0.25 software (Bradbury et al. 2007). The following statistical models were tested: (i) Simple General Linear Model (GLM, Naive-model), (ii) the Structured Association Model (GLM, Q-model), taking into account only subpopulation membership of the accessions provided by *STRUCTURE* software, and (iii) the Mixed Linear Model (MLM, K+Q-model), taking into account both pair wise relationship provided by the kinship matrix and the structure information, as described by Yu et al. (2006). To deal with the multiple testing we converted the p-value into q-values (Storey, 2002) by using the *QVALUE* package for R. The estimation of the overall proportion of true null hypothesis $\pi 0$ was based on λ range set from 0 to 0.95 by 0.05 and the smoother method was applied (Storey and Tibshirani, 2003).The q-values lower than 0.05 were considered significant.

Results and Discussion

Phenotypic/genotypic characterization and population structure.

A wide phenotypic variation was displayed for the six traits in study (Table 1). Histograms representing the normal distribution of the fruit-related traits (fruit weight, length and diameter) are reported in Figure 1. Variance component calculated by REML confirmed that genotypic variance was significant for all the traits ($P < 0.001$).

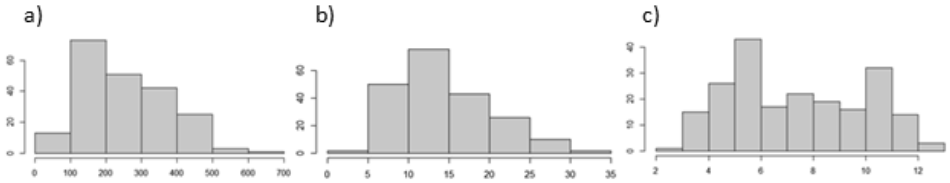

Fig 1. Histograms showing the distribution of a) the fruit weight, b) the fruit length, c) the fruit diameter.

Among the 384 SNPs applied for genotyping, 46 (12%) were discarded as could not be scored in an unambiguous manner or showed MAF values lower than 5%. The remaining 338 loci, of which 331 had known map position (Barchi et al. 2012), were maintained for further analyses. Figure 2 reports the UPGMA dendrogram along with the output of *STRUCTURE* analysis and Principal Coordinate Analysis. The overall shape of the dendrogram, the PCoA spatial subdivision as well as the ΔK provided by the Evanno transformation clearly designated a two sub-population subdivision as the better description of the population structure. The genotypes were thus sorted, according with the level of membership provided by *STRUCTURE*, into two clusters: A, including 67 and B including 89 genotypes, while 34 accessions, not showing a clear membership, were classifies as admixured (Fig. 2).

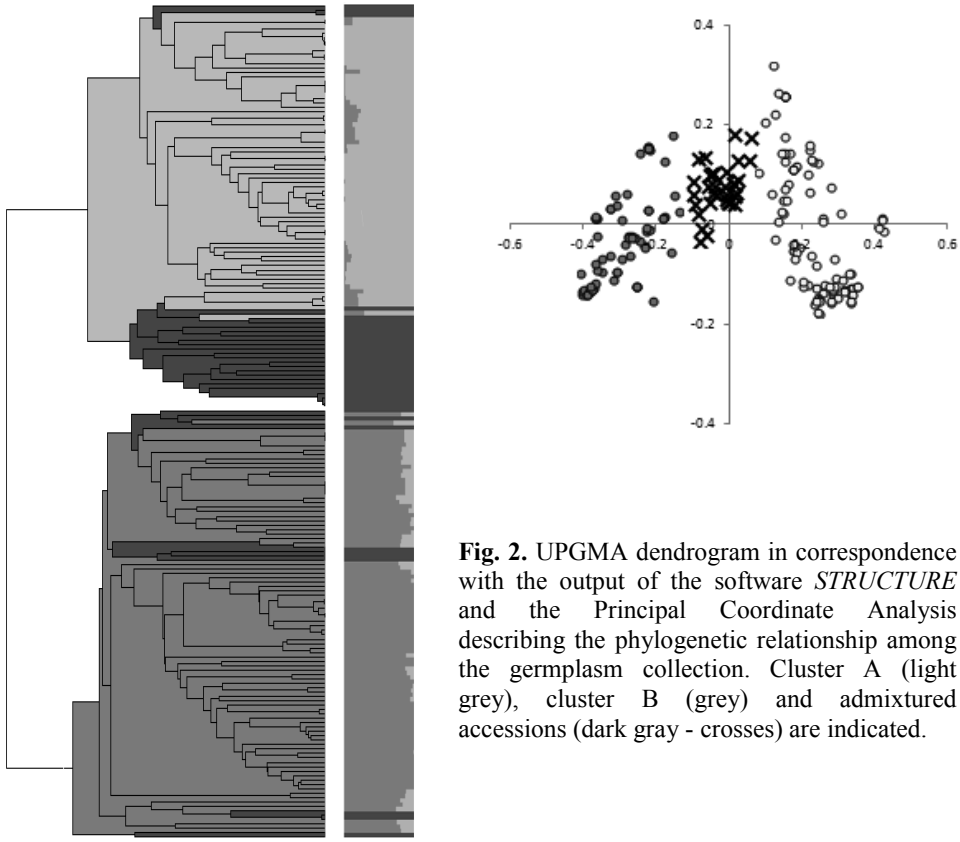

Fig. 2. UPGMA dendrogram in correspondence with the output of the software *STRUCTURE* and the Principal Coordinate Analysis describing the phylogenetic relationship among the germplasm collection. Cluster A (light grey), cluster B (grey) and admixtured accessions (dark gray - crosses) are indicated.

Linkage disequilibrium analysis

To obtain an estimate of the mapping resolution in our population, we analysed the extent of the LD. A threshold of $r^2 = 0.15$ was considered to define loci in LD (Fig. 3). On the basis of the r^2 model (with no correction for the population structure) the association between intra-chromosomal markers alleles extended (on average) over a genetic distance of 4.8 cM, with an average r^2 on the whole genome of 0.15. This rather high level of LD extension is attributable to the prevailing selfing of eggplant and it is comparable to the ones detected in other Solanaceous species (Robbins et al. 2011, Xu et al. 2013, Fricano et al. 2012). Such extent of LD is well suited to genome-wide association studies, because even regions that are only sparsely covered by markers may still show sufficient LD for marker-traits association studies.

In order to evaluate the possible bias of the population structure in determining the association between markers both the r^2s and the r^2sk model were considered (Fig. 3). On the basis of the r^2s model an extension of the LD over 3.9 cM, and with a whole genome average of r^2 equal to 0.07,was observed; vice versa on the basis of the r^2sk model the association extended over 3.4 cM, with a whole genome average of r^2 equal to 0.04. The reduction of the estimate mean of the LD over the genome indicates that the population structure may affect association estimates and determine spurious associations between markers

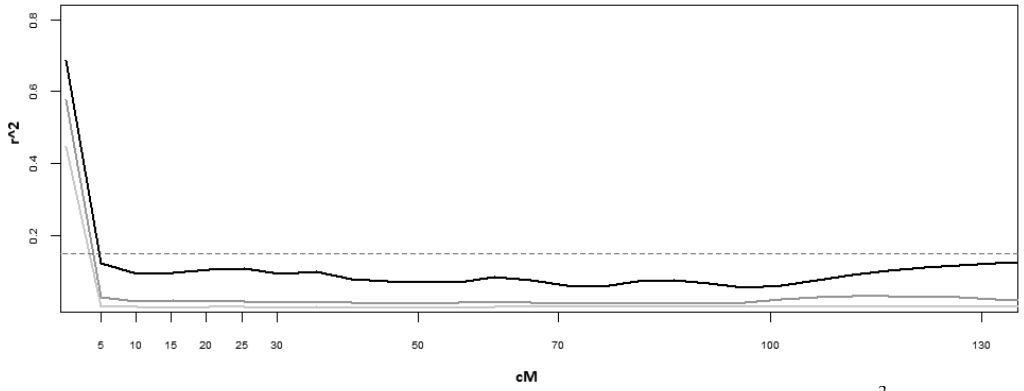

Fig. 3. Locally weighted scatterplot smooth regression of the LD decay plots. The r^2 model (black trend line), the r^2s model (dark gray trend line) and the r^2sk model (light grey trend line) decay plots are reported. The dashed line indicates the threshold level for the LD ($r^2 = 0.15$).

Association mapping and comparison with previously mapped QTL

Several statistical methods have been developed to overcome the influence of undetected population structure on association mapping results (Pritchard et al. 2000; Price et al. 2006; Yu et al. 2006). Here we applied three different models for estimating associations between markers alleles and morphological traits. The comparison of the probabilities obtained with the theoretical uniform distribution of *p-values* showed that the GLM Naive-model, with no correction for the population structure, leaded to several spurious associations. The GLM Q-model, which accounted for the subpopulation level of membership provided by the software STRUCTURE, improved the reliability of the results. The MLM-model, accounting both for STRUCTURE output and the kinship matrix of the observed *p-values* distribution provided results comparable to the theoretical one and was thus the one adopted. This is in agreement with previous studies (Yu et al. 2006; Atwell et al. 2010) reporting that the MLM-model was the more efficient for the population structure corrections.

After the *q-value* correction, 31 associations between markers alleles and phenotypic traits were found significant, and regions carrying QTLs scattered over 10 eggplant chromosomes were identified (Tab. 2). Manhattan plots showing SNPs associated with the traits were used to visualize the identified association between QTL and eggplant genome regions (Fig. 4). Table 2 reports a comparison between results described in literature and obtained through bi-parental QTL mapping studies (Doganlar et al. 2002; Barchi et al. 2012; Toppino et al. 2013) and our own. About 46% of the QTLs coincided with the previously identified chromosome location, while the remaining associations represent novel QTL.

Table 2. List of the marker-trait associations detected for each phenotypic trait considered, together with their chromosomal location based on the SNP-based eggplant map (Barchi et al. 2012) and the comparison with associations reported in literature.

Association mapping			**Family based QTL mapping**		
Trait	N°of associated markers	Chromosomes	N° of loci	Chromosomes	Reference
Fruit color	10	1, 5, 7, 10, 11	2	8, 10	Doganlar et al. 2002
Calyx anthocyanin	6	5, 10	4	5, 6, 8, 10	Barchi et. al 2012
Fruit weight	2	1, un-mapped	3	2, 9, 11	Doganlar et al. 2002
			5	2, 3, 4, 12	Toppino et al. 2013
Fruit diameter max	5	1, 3, 8, un-mapped	2	1, 11	Doganlar et al. 2002
			5	2, 3, 7, 11, 12	Toppino et al. 2013
Fruit lenght	3	1, 5, 11	2	2, 9	Doganlar et al. 2002
			6	1, 2, 3, 7, 8, 11	Toppino et al. 2013
Leaf prickliness	5	2, 6, 7, 8	1	6	Doganlar et al. 2002
			1	8	Toppino et al. 2013

Conclusions

Because of the practical limitations of QTL mapping in bi-parental populations, association mapping has gained wide acceptance as an efficient method for mapping QTLs in plant populations. In this study, several associations between SNP markers and fruit and plant trait were identified by analyzing a large number of unrelated genotypes. Some previously reported major QTLs as well as novel QTLs were identified, highlighting the reliability of our results. Although some of the newly discovered QTLs need to be confirmed, several of these loci are expected to be the result of the wider variability assayed in the present association study. Some QTL regions previously identified were no longer spotted. Some of these "lost QTLs" can be strictly related with the specific crosses performed in family based studies, since they are carried by one of the parental lines but not sufficiently represented in our germplasm collection. Anyhow, our results highlight the need to increase the molecular markers coverage of the *S. melongena* genome.

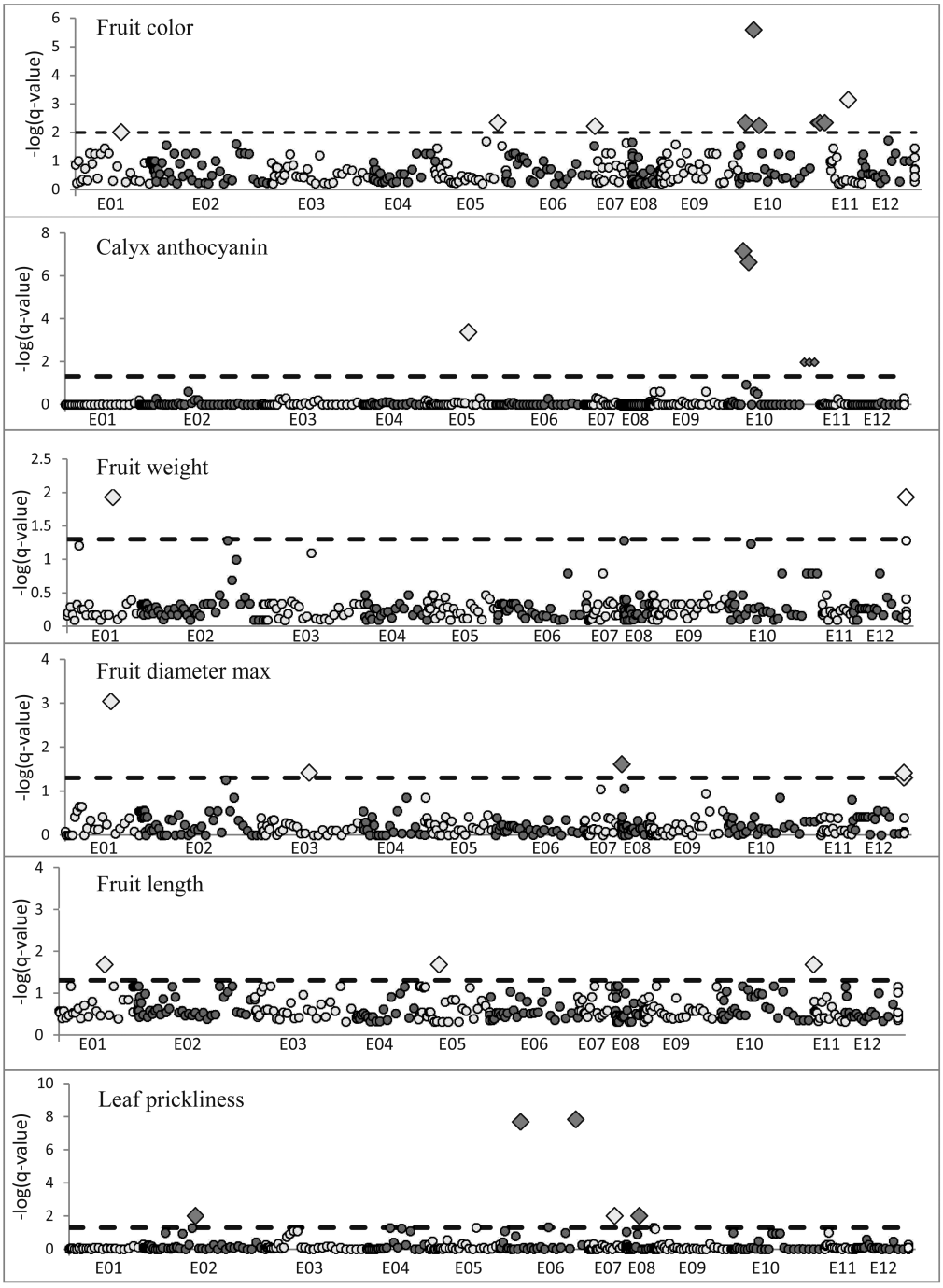

Fig. 4. Manhattan plots showing SNPs associated with the six traits in analysis. Vertical axis represents -log10(P) values of the P-value of the marker trait association. The peaks above minimum threshold of 1.3 (P-value = 0.05) can be considered as significantly associated. The diamond shaped points indicate significant associations.

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Molecular characterization of resistance-breaking isolate of *Tomato Spotted Wilt virus* on pepper

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Abstract

In Hungary resurgence of Tomato spotted wilt virus (TSWV) frequently causes heavy crop losses in pepper production since the mid nineties. Management of TSWV control was first directed against the thrips (using different insecticides or plastic traps), and against weeds as host plants of the virus and the thrips. Later on *Tsw* resistant gene was introduced into different types of pepper. In 2010 and 2011 sporadically, but in 2012 more frequently a resistance breaking strain of TSWV on resistant pepper cultivars was observed in the Szentes region (Hungary). It is supposed that outbreaks of TSWV infection was due to the fact that protection against the thrips, *Frankliniella occidentalis,* was neglected and some effective pesticides (like Unifos 50 EC) were withdrawn.The presence of a new resistance breaking strain was demonstrated by virological (test- plant, serological and RT-PCR) methods. Earlier experiments indicated the location of the determinant responsible for breakdown *Tsw* resistance in S RNA, one of the three genomic ssRNA segments of TSWV. For this reason our aim was to characterize the S RNA of Hungarian resistance breaking isolates of TSWV and to compare them to other TSWV isolates.

Keywords: pepper, *Tomato spotted wilt virus*, resistance breaking isolates, molecular characterization, S RNA

Introduction

Tomato spotted wilt virus (TSWV) is the type member of the genus *Tospovirus* (family *Bunyaviridae*), causes an important disease of horticultural and agronomic crops. The virus distributed worldwide is having extremely broad host range and is now considered as one of the ten most economically destructive plant viruses (Goldbach and Peters 1996, Tomlinson 1987, Adkins 2000). TSWV is transmitted by thrips in a persistent manner (Whitfield et al. 2005). The virion varies in size from 80 to 120 nm and has spherical enveloped character (Prins and Goldbach 1998). The genome of TSWV consists of three ssRNA segments: small (S) and medium (M) RNAs have ambisense coding strategies, whereas the large (L) RNA is of negative polarity.

In Hungary TSWV was described in 1972, but the virus was not considered as an important pathogen. In 1995 very severe damage of TSWV infection was observed in tomato and pepper production in the Szentes vegetable growing region (Hungary). The introduction and spread of western flower thrips (*Frankliniella occidentalis*), an efficient TSWV vector, in that time certainly played an important role in TSWV emergence (Gáborjányi et al. 1995).

Management of TSWV control was first directed against the thrips using different insecticides or plastic traps, and against weeds as host plants of the virus and the thrips. Later on *Tsw* resistant gene (Black et al. 1996) was introduced into different types of pepper (conical white, long pale green hot and sweet, tomato shape, spice pepper and blocky types) (Csilléry unpublished). Pepper cultivars carrying *Tsw* resistance gene upon TSWV inoculation show necrotic local lesions on the leaves or other parts of the plant without systemic infection.

In 2010 and 2011 sporadically, but in 2012 more frequently systemic virus symptoms were observed on resistant pepper cultivars in Szentes region (Bese et al. 2012; Csilléry et al. 2012; Salamon et al. 2010). The presence of new resistance breaking strain of TSWV was proved by virological (test-plant, serological and RT-PCR) methods. It was demonstrated that TSWV can adapt very rapidly to plant resistance, and the *Tsw* resistance gene was broken down only a few years after its deployment in pepper crops (Roggero et al. 2002; Thomas-Caroll and Jones 2003; Margaria et al. 2004; Sharman and Persey 2006).

Despite of the economical importance, Hungarian TSWV isolates occurring in resistant pepper plants have not been characterized and there was no available information about the main component of TSWV epidemics. Earlier experiments predicted the determinant for breakdown *Tsw* resistance locating to S RNA (Jahn et al. 2000; Margaria et al. 2007). For this reason our aim was to characterize the S RNA of Hungarian resistance breaking isolates of TSWV and to compare them to other TSWV isolates.

Materials and Methods

Virus isolates.

TSWV isolates originated from infected pepper plants collected in Hungary, Italy and Portugal (Table 1). Fruit samples were collected from plants exhibiting typical symptoms of virus infection, stunting, mosaic, chlorotic and/or necrotic spots, rings and distortion on the leaves and fruits (Figure 1). The isolates were used for ELISA serological tests, RT-PCR and maintained by mechanical inoculation into *Nicotiana tabacum* cv. *Xanthi*-nc test plants.

Table 1. Origin of TSWV isolates sequenced in this study.

Isolates	Type[1]	Host plant	Origin	Date of collection
TSWV-Szeg-12/Bre-1	RB	pepper	Szegvár, Hungary	06.2012
TSWV-Szeg-12/Bre-2	RB	pepper	Szegvár, Hungary	06.2012
TSWV-Szen-09/CIB	N	pepper	Szentes, Hungary	06.2009
TSWV-Szen-05/CIB	N	pepper	Szentes, Hungary	06.2005
TSWV-Port-12/pepper-1	RB	pepper	Amadora, Portugal	9.2012
TSWV-Port-12/pepper-2	RB	pepper	Amadora, Portugal	9.2012
TSWV-Italy-12/pepper	RB	pepper	Verona, Italy	9.2012

[1] – RB: resistance breaking TSWV strain, N: normal strains

RNA extraction, RT-PCR and sequencing.

Total RNA was extracted from leaves of *N. tabacum* cv. Xanthi-nc plants systemically infected by TSWV or from infected pepper fruits using the Spectrum Plant Total RNA Kit (Sigma) following the manufacturer's instructions. RT-PCR reactions for synthesis of first-strand cDNA were performed with Revert Aid H Minus First Strand cDNA Synthesis Kit (Thermo Science). Specific primers TSWV-184NSsforward (5'- GG CTG TAG CAG AGA GCA ATT GTG TCA TAA TTTT-3') and TSWV-186NSsreverse (5'-GGA CAT AGC AAG ATT ATT TTG ATC CTG-3') amplified the 1404 bp fragment of NSs region, while TSWV-185CPforward (5'-AAT TTC TCC GCA ATC TAT TTC AGT TG-3') and TSWV-183CP reverse (5'-GGG GAT CCA GAG CAA TTG TGT CAA TTTT-3') amplified 1720 bp fragment of N and the non-coding regions. PCR reaction was performed in 25µl - 50 µl final volume. Amplification consisted of one cycle at 94°C for 5 min, followed by 35 cycles of 30 sec of denaturation at 94°C, 1 min of annealing at 50°C; and 2 min of extension at 72°C; and then one cycle of final extension for 10 min at 72°C. PCR products were electrophoresed in 1% agarose and stained with ethidium bromide. PCR Products were

purified using Silica Bead DNA Gel Extraction Kit (Fermentas) and subsequently cloned into CloneJet (Fermantas) or pGEM-T Easy Vector (Promega, Madison USA). All clones were sequenced by BAYGEN (Szeged). The obtained sequences were analyzed with the CLC Sequence Viewer 6.8.1.

Results

Sequences of two genome regions covering the S RNA segment were determined for 5 resistance breaking (RB), and 2 normal isolates of TSWV. The NSs fragment was 1404 bp and N fragment was 1720 bp long. The sequence identity among the TSWV isolates was between 95 – 100 % (Table 2.). There was 100 % identity in the NSs gene sequences between isolates TSWV-Szeg-12/Bre-1 and TSWV-Szeg-12/Bre-2, and TSWV-Port-12/pepper-1 and TSWV-Port-12/pepper-2, respectively. All isolates were resistance breaking. The identity between normal and resistance breaking (RB) Hungarian TSWV isolates were higher than that observed between Hungarian RB isolates and Italian or Portuguese RB isolates. Sequence identity of N region between TSWV-Szen-05/CIB and TSWV-Port-12/pepper-1 isolates was only 96 %.

Phylogenetic tree of the NSs region was constructed from the TSWV isolates sequenced in this study and compared to some sequences available in the GenBank (See Table 3, Figure 2).

The BLAST analysis revealed that the sequences of isolates TSWV-Szeg-12/Bre-1, TSWV-Szeg-12/Bre-2 and TSWV-Szen-09/CIB were over 99% identical with Bulgarian TSWV isolates (AJ411877, AJ4118779). The TSWV-Szen-05/CIB isolate (collected in 2005) was very similar to the French and Italian TSWV isolates (FR692836, HQ830186). The TSWV isolates from Portugal and Italy showed 99 % identity with Spanish pepper TSWV isolates (FR692930, FR693011, FR693023).

Phylogenetic analysis showed/indicated that the European cluster could be further divided into several geographical subpopulations. Hungarian TSWV isolates from 2009 and 2012 belongs to the Bulgarian subgroup, TSWV isolate from 2005 belongs to the French subgroup and TSWV isolates from Portugal and Italy form a separate subgroup in the European cluster.

Discussion

Some outbreaks of TSWV infection have been reported in Hungary since the virus was first described. First outbreak in the mid nineties was connected to the introduction of its efficient vector *Frankliniella occidentalis* to Hungary. The second outbreak in 2012 was coupled with the appearance of new resistance breaking TSWV isolates. The NSs sequence differences of normal and RB isolates of TSWV were less than 1 %. TSWV RB isolates from Hungary differed from Portuguese and Italian RB isolates. Further investigations are still needed to elucidate the differences between normal and resistance breaking TSWV strains.

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Table 2. Sequence identities (%) of the NSs gene of TSWV isolates sequenced in this study.

	NSs	1	2	3	4	5	6
1	TSWV-Szeg-12/Bre-1						
2	TSWV-Szeg-12/Bre-2	100					
3	TSWV-Szen-09/CIB	99	99				
4	TSWV-Szen-05/CIB	99	98	98			
5	TSWV-Port-12/pepper-1	96	96	96	97		
6	TSWV-Port-12/pepper-2	96	96	96	97	100	
7	TSWV-Italy-12/pepper	95	95	95	96	98	98

Table 3. NSs gene sequences of TSWV isolates used for phylogenetic analysis and sequence identity (%) comparing with TSWV isolates of this study. (The pepper isolates are in bold.)

Origin	**Host**	**Acc. Num.**	TSWV-Szeg-12/Bre-1	TSWV-Szeg-12/Bre-2	TSWV-Szen-09/CIB	TSWV-Szen-05/CIB	TSWV-Port-12/pepper-1	TSWV-Port-12/pepper-2	TSWV-Italy-12/pepper
Bulgaria		D13926	99	99	99	98	96	96	96
Bulgaria	tobacco	AJ418777	99	99	99	98	96	96	95
Bulgaria	tomato	AJ418779	99	99	99	98	95	95	95
France	dahlia	FR692827	98	98	99	99	96	96	95
France	tomato	FR692835	98	98	99	99	96	96	95
France	lactuca	FR692831	98	98	99	99	96	96	95
USA	dahlia	AY744478	98	98	99	99	96	96	95
France	sow-thistle	FR693032	98	98	99	99	96	96	95
France	tomato	FR692839	98	98	99	99	96	96	95
France	tomato	FR692836	98	98	99	99	96	96	95
USA	dahlia	AY744476	98	98	99	99	96	96	96
Italy	**pepper**	**HQ830186**	**98**	**98**	**99**	**99**	**96**	**96**	**96**
USA		AF020660	98	98	98	98	96	96	95
China	tomato	HQ402595	96	96	96	96	96	96	95
Spain	**pepper**	**FR692930**	**96**	**96**	**96**	**95**	**99**	**99**	**98**
Spain	**pepper**	**FR693011**	**96**	**96**	**96**	**96**	**99**	**99**	**98**
Spain	**pepper**	**FR693023**	**96**	**96**	**96**	**95**	**99**	**99**	**98**
Colorado		AY744475	96	96	96	96	99	99	98
Sicilia	**pepper RB**	**DQ431237**	**98**	**98**	**99**	**99**	**96**	**96**	**96**
Italy	chrysanthemum	FR692824	98	98	99	99	96	96	96
France	tomato	FR692837	98	98	99	99	96	96	95
France	tomato	FR692833	98	98	99	99	96	96	95
S-Korea	**pepper**	**HM581942**	**97**	**97**	**97**	**96**	**96**	**96**	**95**
S-Korea	**pepper**	**HM581939**	**97**	**97**	**97**	**96**	**96**	**96**	**95**
Serbia	**pepper**	**GU369737**	**98**	**99**	**99**	**99**	**96**	**96**	**95**
Bulgaria	tobacco	AJ418778	98	98	98	97	95	95	95
Brazil	tomato	NC_002051	94	94	94	94	96	96	95
China	salad	JN664252	96	96	96	95	95	95	95
S-Korea	**pepper**	**AB643672**	**96**	**96**	**97**	**96**	**96**	**96**	**95**
S-Korea	**pepper**	**AB643671**	**96**	**96**	**96**	**96**	**96**	**96**	**95**
Spain	tomato	AY744480	95	96	95	95	99	99	98
Spain	tomato	AY744479	95	96	95	95	99	99	98
Spain	**pepper RB**	**HE600702**	**95**	**95**	**95**	**95**	**99**	**99**	**98**
Italy	**pepper RB**	**DQ915946**	**95**	**95**	**95**	**95**	**98**	**98**	**99**
Italy	**pepper RB**	**DQ376179**	**95**	**95**	**95**	**95**	**98**	**98**	**99**

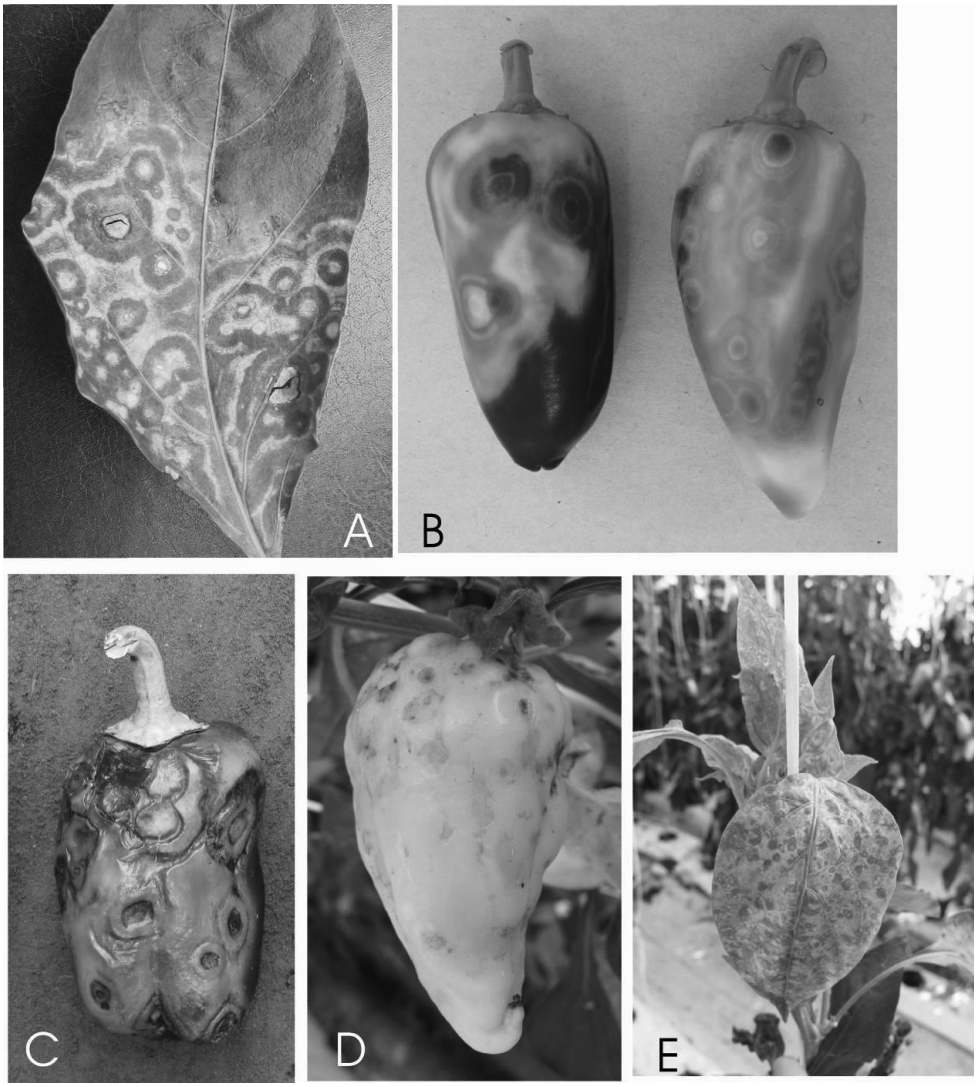
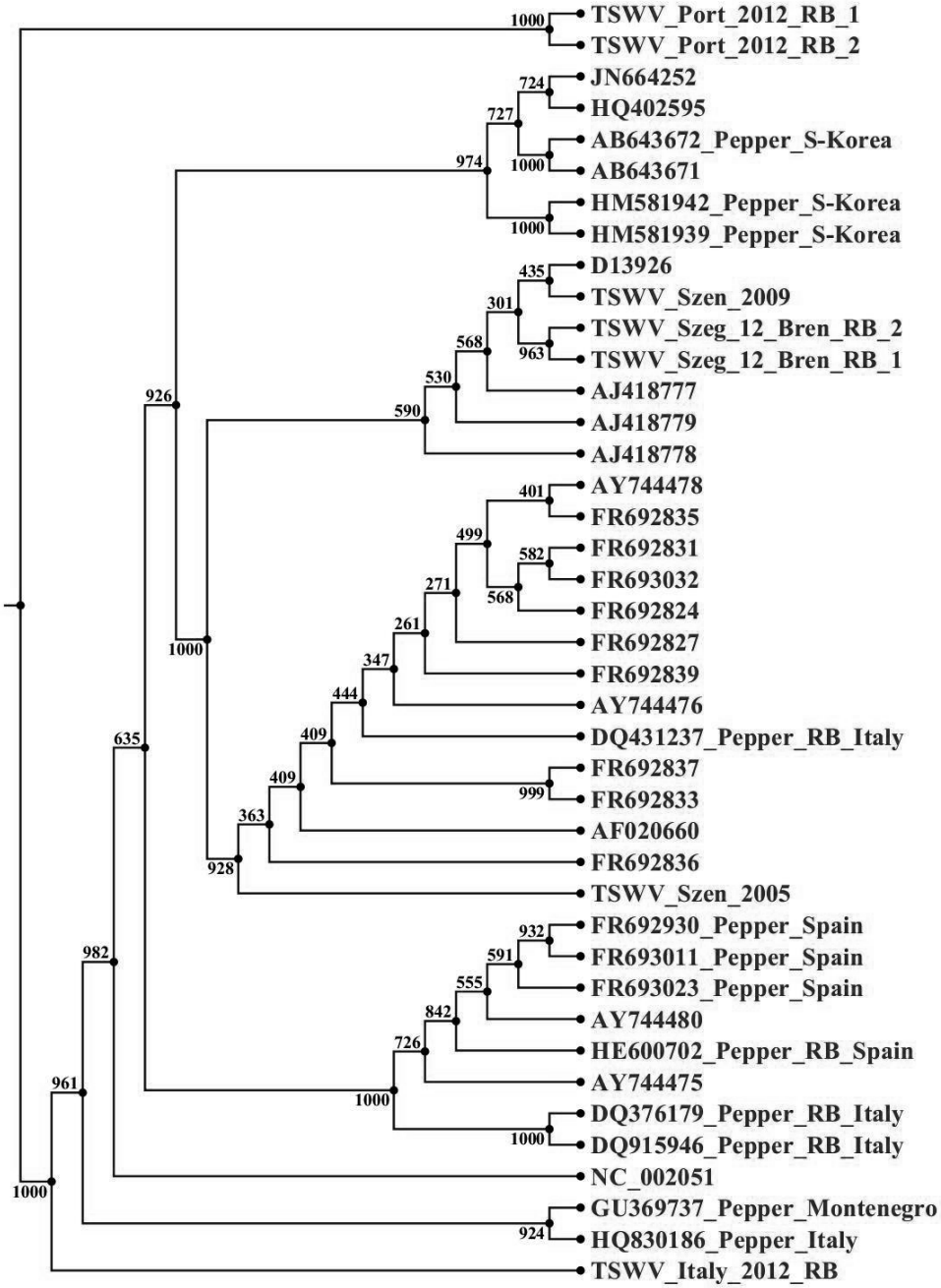


Figure 1. Symptoms of TSWV infection on pepper. Chlorotic spots and rings on leaves (A) and fruits (B), necrotic spots and rings on fruits of resistant variety (C). Symptoms of RB isolate of TSWV on resistant pepper fruit (D) and leaves (E).

Figure 2. Phylogenetic tree of the NSs gene of TSWV isolates sequenced in this study and of some additional isolates available in GenBank.



Mapping of candidate genes involved in the improvement of the nutraceutical quality of eggplant

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Abstract

The high content in phenolic compounds, mostly chlorogenic acid (CGA), in the fruit flesh of eggplant (*Solanum melongena*) confers nutraceutical properties to this crop. *Solanum incanum*, a wild species closely related to eggplant, presents higher phenolics content than the cultivated species. Given that hybrids between both species are completely fertile, *S. incanum* is an interesting source of variation to increase the phenolics content of eggplant. Given that a genetic map based in an interspecific cross between *S. melongena* and *S. incanum* is available, and that the genes involved in the pathway of the synthesis of CGA are known, we undertook the mapping of these genes. We amplified correctly six candidate genes encoding enzymes involved in the synthesis of chlorogenic acid: PAL (phenylalanine ammonia-lyase), C4H (cinnamic acid 4-hydroxylase), 4CL (4-coumaroyl: CoA-ligase), C3H (p-coumarate 3-hydroxylase), HQT (hydroxycinnamoyl CoA quinate hydroxycinnamoyl transferase), and HCT (hydroxycinnamoyl-coA shilimate/quinate hydroxycinnamoil transferase). Furthermore, given that polyphenol oxidases (PPOs) are involved in the browning of the fruit flesh of eggplant caused by oxidation of phenolics, mapping of PPOs would also be of interest for the molecular breeding of eggplant. Therefore, we also amplified six genes encoding enzymes polyphenol oxidases (PPO1 to 6). The next step is to investigate the co-localization of the genes involved in the CGA synthesis pathway with QTLs for CGA content and also of the PPO cluster with flesh browning QTLs. This may allow the development of functional markers for marker-assisted selection for high CGA content and low browning in eggplant.

Keywords: Chlorogenic acid, polyphenol oxidases, *S. incanum, S. melongena*

Introduction

The term nutraceutical was coined by Dr. Stephen DeFelice in 1989 as a portmanteau of the words “nutrition” and “pharmaceutical”, and can be defined as “a food (or part of a food) that provides medical or health benefits, including the prevention and/or treatment of a disease" (Kalra, 2003).

The most remarkable aspect in eggplant composition is its high content in phenolic compounds (Hanson et al. 2006). Among the different horticultural species, eggplant (*Solanum melongena* L.) is ranked amongst the group of vegetables with highest antioxidant capacity (Cao et al. 1996; Stommel and Whitaker, 2003; Prohens et al. 2007). Its main phenolic compounds are hydroxycinnamic acid conjugates, which are located mainly in the fruit flesh. Chlorogenic acid (5-*O*-caffeoylquinic acid) is the major hydroxycinnamic acid conjugate and represents between 70-95% of the total polyphenols in eggplant flesh (Stommel and Whitaker, 2003). These compounds have an antioxidant capacity similar to that of ascorbic acid (Kinsella et al. 1993) and are highly stable to cooking processes.

Chlorogenic acid (CGA) is the ester of caffeic acid and (-)-quinic acid. It has been demonstrated that it has multiple beneficial properties for human health, such as antioxidant, anti-inflammatory, cardio-protective, anti-carcinogenic, anti-diabetic and anti-obesity (Plazas et al. 2013). There is a great diversity in the content of CGA in eggplants, influenced mainly by developmental stage, storing conditions and environmental factors. The wild ancestor of common eggplant, *S. incanum*

L., has a high CGA content (Ma et al. 2011; Prohens et al. 2013; Stommel and Whitaker, 2003). This trait can allow for the selection of individuals with high CGA content after an interspecific cross between *S. incanum* and *S. melongena* and the following backcross generations, since they form fully fertile individuals (Plazas et al. 2013; Prohens et al. 2013).

As in other fruits and vegetables, such as apples or artichokes, the oxidation of phenolics in eggplant flesh due to its exposure to the air results in browning and a consequent loss of apparent quality (Rubatzky and Yamaguchi, 1996; Macheix et al. 2005). When an eggplant is cut open, the destruction of the cellular compartments allows for the orthodiphenolic substrates (hydroxycinnamic acid derivatives) to be accessible to polyphenol oxidases (PPO), which catalyze their oxidation to quinones, which in turn react non-enzymatically with oxygen, amines, amino acids, proteins and sulfhydryl compounds to give brown-colored compounds which are responsible of the browning (Ramírez et al. 2002).

Taking into account the preference of consumers and the industry for varieties with white flesh and low degree of browning, future varieties with high polyphenol content must also have a moderate flesh browning (Prohens et al. 2005). For this reason, the search for genetic resources with a high phenolic content and low browning is of great interest for the improvement of the nutraceutical quality of eggplant (Prohens et al. 2007). Various authors have found differences in PPO activity, which is required for the enzymatic browning, in different eggplant varieties (Doganet al. 2002). Therefore, the simultaneous selection of low PPO activity and high poliphenol content could result in a higher antioxidant capacity and low browning.

The present study focused in the mapping of candidate genes that encode enzymes involved in CGA synthesis. It is also aimed at the amplification and positioning of genes that encode PPO enzymes.

Materials and Methods

Plant material and DNA extraction

Material used consisted of a variety of *S. incanum* (MM577; parental P1), a variety of *S. melongena* (AN-S-26; parental P2), the F1 hybrid resulting from the cross of *S. incanum* x *S. melongena*, and 91 individuals of the first backcross generation (BC1), resulting from the crossing of the hybrid F1 x parental P2 (*S. melongena*). Genomic DNA was extracted from flesh leaves on the parents, F1 and BC1 population according to CTAB method procedure (Doyle and Doyle, 1987). The quality of DNA was checked on 1% agarose gels and the DNA concentrations were measured with a Nanodrop ND-1000 spectrophotometer.

Search for sequences based in syntenic relationships

The sequences of candidate genes implicated in the CGA synthesis pathway were obtained through a search on Genomic Network webpage (Bonbarely et al. 2011). The sequences of PPO genes implicated in the browning process were obtained from Shetty et al. (2011). Given that, up to now, the eggplant genome has not been sequenced; eggplant-related sequences from tomato, potato and pepper were used with the BLAST program and a local database with 16245 eggplant genes available in VegMarks webpage (Fukuoka et al. 2010).

Primer design and PCR amplification

The design of primers was carried out with the program Primer3 (v.0.4.0., Rozen and Skaletsky, 2000) and the amplification of genes was carried out in PCR thermocyclers (Mastercycler ep gradient S, Eppendorf) according to the following protocol: denaturalization at 94º C for 5 min, 35 cycles at 94º C for 30 s, annealing at 55º C for 1 min, extension at 72º C for 2 min, and a final

extension at 72º C for 10 min. In case of nonspecific amplifications, additional tests with higher annealing temperatures and/or lower $MgCl_2$ concentrations were carried out. If no amplification product was obtained, annealing temperature was lowered and/or higher $MgCl_2$ concentration was used.

Sequencing and search for SNPs

The amplification product was purified and sequenced with an automatic sequencer of capillary electrophoresis and fluorescent detection *ABI PRISM3100-Avant* (Applied Biosystems). Results were analyzed with Sequence Scanner v1.0 (Applied Biosystems). The program Blast2Seq (NCBI) was used to compare the parental sequences in order to detect SNPs.

Search for CAPS and HRM analysis

SNPs found were transformed in cleaved amplified polymorphic sequences (CAPs) markers with CAPs Designer software (Mueller et al. 2005). In those cases in which there was a nucleotide change (SNP) but no CAP marker development was possible, primers to detect the change with High Resolution Melting (HRM) were developed.

Mapping of the candidate genes

An interspecific map was used for the mapping of candidate genes. Linkage groups were established at a LOD ≥3 and map order was determined using maximum recombination fraction θ=0.4. Kosambi (1994) mapping function was used to convert recombination units into genetic distances (cM).

Results and discussion

Chlorogenic acid biosynthetic pathway

The 6 genes involved in the chlorogenic acid synthesis pathway (Comino et al. 2007, 2009; Joët et al. 2010; Mahesh et al. 2007; Menin et al. 2010; Niggeweg et al. 2004) (Figure 1.) were amplified and mapped according to the syntenic position with the tomato genome (Wu et al. 2009). The results from each gene are shown below.

Phenylalanine ammonia-lyase (PAL) gene

The sequence of the ortholog gene in tomato has a high identity with the eggplant unigene OVS02A18A obtained by Fukuoka et al. (2010). In order to have a higher probability to find a polymorphism between both parents, primers to amplify the only intron present in this gene were designed. The amplification product was sequenced and compared, and we found one SNP (T/C) that was converted into a CAPs marker using the restriction enzyme RsaI. The sizes of the bands were of 960 bp and 260 +700 bp for *S. melongena* and *S. incanum* respectively. The candidate gene was mapped into the linkage group 9 at 15.0 cM from the linkage group end.

4-Coumaroyl:CoA-ligase (4CL)

Using a tomato ortholog sequence it was possible to find the eggplant unigene SmFL38N19A in our local database. Primers that amplify a region that comprises both gene introns were developed. Sequences were analyzed finding a polymorphism (A/G) that allowed us to develop a CAPs marker using the restriction enzyme ScaI. The sizes of the bands were 1000 pb and 554 + 446 pb for *S. melongena* and *S. incanum* respectively. The candidate gene could be mapped into the linkage group 3 at 90.3 cM from the linkage group end.

Hydroxycinnamoyl-coA shikimate/quinate hydroxycinnamoyl transferase (HCT)

Using a tomato ortholog sequence the eggplant unigene ROT01O23W was identified. The gene in tomato shows a quite large size (5369 bp) and consists of three exons and two introns. The first

intron has 148 bp and is located in the 3'-UTR region and the second intron has approximately 3500 bp. We tried to amplify the second intron without success and subsequently the two exonic areas were amplified and sequenced. No polymorphism between the two parental sequences was found. We tried to find intron information looking for homology in the eggplant sequences obtained by RAD tags sequencing (Barchi et al, 2011). Using BLAST search, a positive contig (22573:15433) was detected and primers that partially amplify the second intron were developed. An amplicon was obtained and after the analysis a SNP (T/A) was found. The polymorphism was detected in the population using HRM. The gene was mapped into the linkage group 3 at 89.0 cM from the linkage group end.

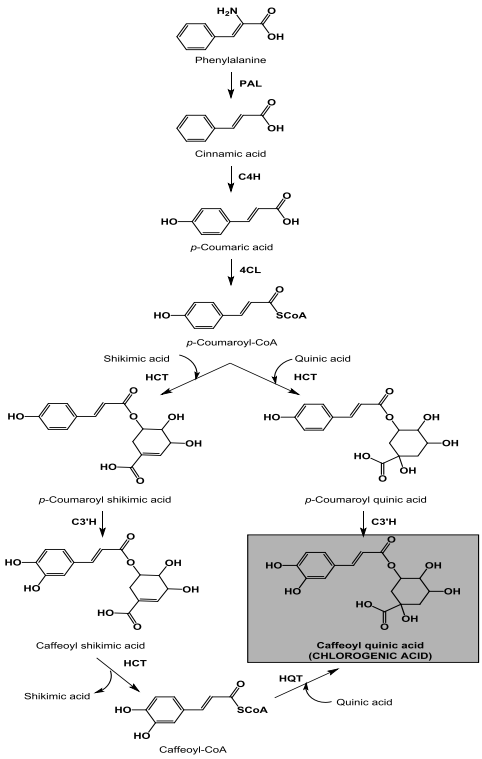

Figure 1. Biochemical pathway for the synthesis of chlorogenic acid in eggplant (Plazas et al. 2013).

Cinnamic acid 4-hydroxylase (C4H)

The sequence of the ortholog gene in tomato was used to find the corresponding unigene in a local database of eggplant unigenes. As a result, the SmFL27M04A unigene was found and primers were developed to amplify the first intron. The amplicon was sequenced and a SNP (A/G) was located but we were not able to develop a CAP marker. New primers were designed in order to detect this polymorphism with high resolution melting (HRM) (Figure 2). Following this strategy, the gene was mapped at the end of the linkage group 6 (107.1 cM) from the linkage group end.

p-Coumarate 3-hydroxylase (C3'H)

Using tomato ortholog sequence the eggplant unigene YFR01I20A was identified. Primers were developed to amplify the second intron of the gene. Analysis of the sequences showed an Indel (TT) between the two parents (Figure 3). Using this polymorphism a CAP marker, using ApoI restriction

enzyme, was developed. The sizes of the bands obtained were 450 bp and 300+150 bp in *S. melongena* and *S. incanum* respectively. The candidate gene was mapped into the linkage group 1 at 86.1 cM from the linkage group end.

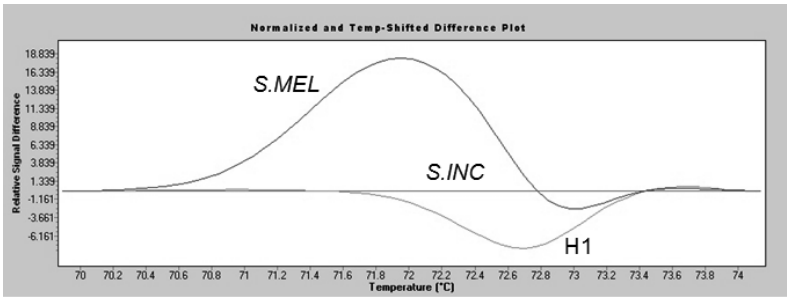

Figure 2. Amplification of SNP in C4H gene in both parents (*S. melongena*, *S.MEL*; *S. incanum*; *S.INC*) and in the F1 hybrid (*H1*) by high resolution melting (HRM).

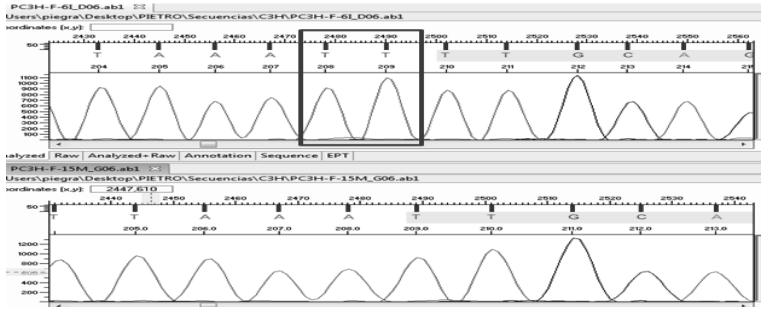

Figure 3. Detection of an Indel between the parental *S. incanum* and *S. melongena* using the program Sequence Scanner.

Hydroxycinnamoyl CoA quinate hydroxycinnamoyl transferase (HQT)

Using tomato ortholog sequence the eggplant unigene YFR01H03A was identified. We tried to amplify the single intron of the gene (2121 bp), but we were not able to obtain a clear band probably due to the large size or secondary structures. Primers designed to amplify also gave a non-specific product. We used the parental cDNA to amplify different exon areas avoiding the intron. A SNP (A/G) polymorphism was found, and the BC1 population was genotyped by HRM. The candidate gene was mapped into the linkage group 7 at a distance of 3.0 cM from the linkage group end.

Polyphenol oxidases

PPO genes in tomato have no introns, present high homology and are located in the middle of chromosome 8 in a cluster (Newman et al. 1993; Thipyapong et al. 2007; Tran et al. 2012), This features complicate the search of polymorphisms. In eggplant, Shetty et al. (2011) isolated and sequenced six PPO genes. To search polymorphism in our parents, sequences of these six genes were obtained from NCBI database (http://www.ncbi.nlm.nih.gov/). The sequences were aligned with the program ClustalW2 (http://www.ebi.ac.uk/Tools/msa/clustalw2/) and specific primers were designed for each of them. We could amplify and sequence all of them except PPO6, which was amplified from *S. melongena* but not from *S. incanum*. This result led us to speculate that this

polyphenol oxidase gene presents substantial changes or is not present in the wild relative *S. incanum.*

SNPs polymorphisms were found in the other five genes. CAPs could be developed for PPO1 (C/A), PPO2 (C/G), PPO4 (G/A) and PPO5 (G/A and T/G). For PPO3 SNP (G/A) polymorphism was found but we were not able to develop a CAP marker. Therefore HRM was used to genotype the BC1 population.

Among the 6 genes that encode PPO enzymes in eggplant (Shetty et al. 2011), we could correctly map 5 of them (PPO1, PPO2, PPO3, PPO4, PPO5) in the linkage group 8 in agreement with the synteny observed with the tomato genome (Wu et al. 2009). PPO1, PPO2, PPO3 and PPO4 genes were located at a distance of 40.0 cM from the linkage group end, while PPO5 is found a little bit separated (35.5 cM from the linkage group end) (Figure 4). These findings suggest that PPO genes also form a cluster in eggplant genome (Newman et al. 1993; Thipyapong et al. 2007; Tran et al. 2012).

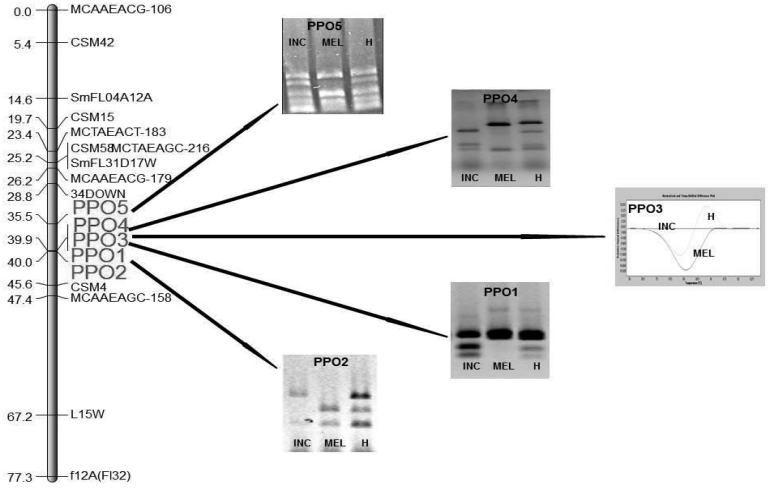

Figure 4. Mapping of PPO genes in linkage group 8 of eggplant.

These results are promising since they will aid in the marker-assisted selection (MAS) process to select eggplant varieties with favorable alleles that simultaneously have low PPO activity and high polyphenol content, therefore having products with higher antioxidant capacity and low browning. The next step is to investigate the co-localization of the genes involved in the CGA synthesis pathway with QTLs for CGA content and also of the PPO cluster with flesh browning QTLs.

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Analysis of a complex QTL region controlling the broad-spectrum resistance to *Phytophthora capsici* root rot by comparative mapping and association study in pepper germplasm

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Abstract

While no R gene has been reported for resistance to *Phytophthora capsici* in pepper, all studied resistant accessions display a major effect QTL on chromosome P5. By clustering 14 QTLs detected on P5 from independent studies, we identified 3 linked metaQTLs. The QTL *Pc5.1* confers a broad-spectrum resistance to the 12 tested isolates, collected worldwide. A fine mapping of QTL *Pc5.1* delimited the locus to less than 0.4 cM. The physical map based on BAC libraries delivered a reference sequence covering ~1.1 Mb. Structural and functional gene annotation identified a huge proportion of transposable elements and a few ORFs. One ORF shows homology with a transcript differentially expressed in *P. capsici*-infected peppers, and exhibits SNPs between resistant and susceptible lines. By constructing a trait-specific core-collection and by sequencing strategic positions on the locus of interest, we are achieving an association study in order to identify the causal SNP(s). Identifying the responsible gene should facilitate marker-assisted selection, and the study of the molecular crosstalk between plant and Oomycete pathogen.

Keywords: *Capsicum* spp., *Phytophthora capsici*, quantitative disease resistance, QTL, candidate gene, germplasm collection, association analysis

Introduction

Plant diseases are major threats to crop production since pathogens and insects reduce yield potential by at least a quarter. Because governments strongly discourage pesticide use considering risks for human health and the environment, both identification of resistant genitors within genetic resources and breeding for broad spectrum resistance (BSR) to biotic stresses are continually major concerns for end-users. Many major-effect race-specific resistance genes (R genes), involved in gene-for-gene interactions and associated with the hypersensitive response (HR), have been introduced into various cultivars. When widely deployed in environments favourable to disease, however, improved cultivars may succumb. Conversely, it is often assumed that resistances under polygenic control are typically not race-specific, not involved in HR, nor overcome (Lindhout 2002; Hu et al. 2008; Ayliffe et al. 2008; Kou & Wang 2010). Quantitative trait loci (QTLs) conferring partial BSR are therefore considered valuable potential sources for improving durable resistance.

The fungal-like eukaryotic pathogens belonging to the Oomycete genus *Phytophthora* are resurgent and affect many economically important crops worldwide. *P. capsici*, first described by Leonian in 1922 on chili pepper (*Capsicum* spp.) in New Mexico (USA) and causing root rot, crown rot, fruit rot and foliar blight, is today responsible for one of the most destructive and widespread disease affecting pepper production worldwide. The control of *P. capsici* diseases in most developed countries relies extensively on fungicide applications despite their progressive banning and their limited efficacy. Additionally, sources of resistance to *P. capsici* are rare in *Capsicum* diversity, since very few resistant accessions were identified during previous screenings of genetic resources (Kimble & Grogan 1960, Candole et al. 2010, Cantet et al. submitted). The few well-known sources of resistance belong to *C. annuum*, although a small number of sources have also been reported in *C. baccatum* and *C. frutescens*. As no race-specific R genes have been reported, the pepper genitors exhibiting partial quantitative resistance are of particular agronomic interest. Breeding programs and mapping studies have therefore far concentrated on the few *C. annuum* genitors of resistance (Palloix et al. 1990; Thabuis et al. 2003). Mapping data indicate that resistances to *P. capsici* are under polygenic control and that a QTL region on pepper chromosome P5 has consistently been shown to play a major role on resistance (Mallard et al. 2013).

To investigate the major effect QTL region consistently detected on chromosome P5, our study had three strategic objectives: i) to determine whether this QTL region would correspond or not to the same locus in the different resistant genitors; ii) to assess its resistance spectrum; iii) to explore its genetic variability in a pepper core-collection in association with the quantitative evaluation of *P. capsici* resistance in the whole INRA germplasm collection of *Capsicum* spp.

Materials and Methods

Plant material

Three INRA pepper intraspecific *C. annuum* mapping progenies were considered: HV (H3 x Vania), PY (Perennial x Yolo Wonder (YW)), and F5YC (YW x Criollo de Morelos 334 (CM334)) described by Lefebvre et al. (2002) and Barchi et al. (2007). H3 and YW are susceptible to *P. capsici.* Vania, Perennial and CM334 are partially resistant. Vania was derived from the *P. capsici* resistant accession *C. annuum* PI201234 (PM217). Progenies and parental lines had previously been assessed for stem and root resistance to *P. capsici* isolates Pc101 or Pc197 for QTL detection according to the experimental design described in Lefebvre and Palloix (1996), Bonnet et al. (2007), and Thabuis et al. (2003).

By marker-based haplotyping analysis, we chose 11 lines from the 3 mapping progenies that carry the allele associated with resistance at markers located within the major effect QTL *Pc5.1*, and 8 lines with the allele associated with susceptibility at the same markers. These 19 lines varied for alleles at the other *P. capsici* resistance QTLs detected by Thabuis et al. (2003) and Bonnet et al. (2007).

A core-collection of 60 accessions of *Capsicum* spp. was constituted from the INRA pepper collection to describe the polymorphism at *Pc5.1* candidate genes (see Sage-Palloix et al. 2013, in this issue). The core-collection is structured into two subsets: the “ingroup” subset dedicated to intra-species analyses and the “outgroup” subset that will be used to perform inter-species analyses. Attention was paid to balance phenotypes of high resistance, moderate resistance and susceptibly to *P. capsici* (Cantet et al, in prep).

Pathogen material, resistance assessment and experimental design

The artificial "stem inoculation test" was performed with 4 *P. capsici* isolates (Pc101, Pc107, Pc197 and Pc204) as described by Lefebvre & Palloix (1996). Plants were grown in a nursery greenhouse until the six-leaf stage, and a 4-mm mycelium plug of *P. capsici* was deposited on a

decapitated stem. Inoculated plants were kept in growth chambers with a 12-h-photoperiod at 22°C night/24°C day. From the day of inoculation onwards, the pathogen progressively grew to the bottom of the stems, causing stem necrosis. The length of necrosis was measured at 3, 7, 10, 14, 17, and 21 days post-inoculation (dpi). Then we calculated the speed of the necrosis spread for each scoring date (S3, S7, S10, S14, S17 and S21).

The 5 parental lines and 19 selected lines were tested against the 4 *P. capsici* isolates. For each pathogen isolate, six plants of each line were inoculated.

Sequencing of Pc5.1 candidate genes

Candidate genes were amplified either by standard PCR or long-range PCR (Cantet et al. in prep), and libraries for the 60 accessions were constructed to sequence on Illumina HiSeq 2000. The nucleotide diversity was calculated from the consensus sequence of the 60 accessions, using DnaSP v5.10, and haplotype networks were constructed by TCS software v1.21.

Data analysis

Statistical analysis was performed using the statistical software R version 2.14.1 (R Development Core Team, 2011). Analyses of variance (ANOVA) were performed for the resistance measurements on the 19 selected lines to determine the effects of the variables 'Plant genotype', 'Locus *Pc5.1*' and 'Isolate' along with the interaction 'Locus *Pc5.1* x Isolate'. Comparisons of all pairs of adjusted means were performed using Tukey's test with a probability level of $P < 0.05$.

Segregation data for each marker were analysed using MAPMAKER/EXP software, version 3.0. Markers were mapped onto the previously constructed P5 linkage groups (Bonnet et al. 2007; Thabuis et al. 2003). The three chromosome P5 maps were constructed independently and aligned using anchor markers.

QTLs contributing to *P. capsici* resistance were independently detected de novo on the improved maps of the three INRA pepper progenies. Phenotypic datasets previously analysed by Thabuis et al. (2003) and Bonnet et al. (2007) were used along with QTL Cartographer software for this analysis. To detect significant QTLs, a permutation test was performed to estimate the appropriate critical LOD threshold for each trait using the composite interval mapping (CIM) model. LOD thresholds were determined after 1000 permutations, corresponding to a genome-wise significance level of alpha = 0.05.

To determine the most likely number of "real" QTLs on chromosome P5 from the QTLs detected in independent studies (published and from this paper), individual genetic maps were compiled, and a meta-analysis was performed using BioMercator, version 2.0 (http://www.genoplante.com/) (Arcade et al. 2004). The meta-analysis clustered projected individual QTLs. The best clustering model having the lowest Akaike criteria value indicated the optimal number of meta-QTLs that explains the observed QTL distribution on chromosome P5.

Results and discussion

Anchor markers and de novo *QTL detection in INRA pepper chromosome P5 maps*

Three markers permitted the alignment of the 3 INRA P5 maps within the *Pc5.1* confidence interval (AFLP E38M61-139, RFLP GC015_1, CAPS Mfvt_M22). In addition, we mapped 7 new CAPS markers derived from tomato sequences of chromosome T4 available on the SGN website and 11 SSR, COSII and CAPS markers from published pepper chromosome P5 maps (Table 1).

Three resistance QTLs, *Pc5.1*, *Pc5.2* and *Pc5.3*, were detected on the P5 chromosome of HV and PY maps (Fig.1). A single QTL, corresponding to *Pc5.1*, was detected on the P5 chromosome of F5YC map. Individually, QTLs explained between 6.71 and 52.72% of the phenotypic variation (R^2). The QTL *Pc5.1* affected several resistance components in the three progenies, whereas *Pc5.2*

and *Pc5.3* only affected a few resistance components. *Pc5.1* had a larger R^2 value and additive effect than the other two QTLs.

Table 1 New CAPS markers mapped onto the INRA pepper chromosome P5 maps

Unigene / EST / Marker added on INRA P5 pepper maps	Corresponding tomato BAC containing a sequence homologous to the unigene / EST		Corresponding tomato marker homologous to the unigene	Primers for *Capsicum annuum* amplification (5'-3')		Enzyme used for CAPS	INRA map in which the marker has been mapped
	BAC name	Tomato markers contained in the BAC sequence		Forward primer (5' - 3')	Reverse primer (5' - 3')		
Tomato chromosome T4-derived CAPS markers							
SGN-U196349	C04HBa0070F01	T1068	-	CCTGGGAGAGGAGTCCTACA	GCAAGAAACAGCGCCTTTAG	*Nla*IV	HV
SGN-U204895	C04HBa0070F01	T1068	-	TGTCGATGTTACAAGGCCATA	CATGCGGTGACAATACCAAG	*Hpy*CH4IV	F5YC
SGN-U202638	C04HBa0049A17	TG370, TG437	-	GCTTTGAAGATGAGGCAAG	GGTGTACACATCGCCAGAT	*Hph*I	F5YC
SGN-U198114	C04SLm0040B16	TG123	-	TTGGGCTCAATTAAACCATACA	GCACCCCTTGATTGAGAGAA	*Alw*NI	HV
CK901616	C04HBa0008H22	T1792, C2At1g8620, C2At1g8630, C2At1g8640	-	CTCCAAATCGTGTCTGGTCA	ATCACGCTTCCTTCACATCC	*Hph*I	F5YC
SGN-U197890	-	-	TG437	CCATATGGTCCTCCTCCAGA	CTTTCAACCACTTCGGCAAT	*Hph*I	PY
SGN-U196183	-	-	T1261	CACACGTTTCTGGGAGATGA	TCAGCAGCCTTGATGATGTC	*Hha*I	HV
Pepper chromosome P5-derived CAPS markers							
Sn-2 (a)	-	-	-	TTCGATCCACACCATCATCT	TCCTTCAATGGCTTTCCATC	*Eco*RI	HV
P5-SNAP-CM (b)	-	-	-	TCATGAGGTTGCTATTAAG ATTGGTCCTGTTATATA	CATAGAAAGGGATATCATCT GGTACATGCAGAAA	*Hpy*188I	F5YC

(a) Primers for the Sn-2 CAPS marker were designed using the Genbank sequence X79231.1 (Pozueta-Romero et al. 1995).
(b) Primer sequences for the CAPS P5-SNAP-CM marker were supplied by Kim et al. (2008).

Comparison of QTL locations between pepper chromosome P5 maps

The addition of markers on the INRA chromosome P5 maps reinforced their connection with published maps in which *P. capsici* resistance QTLs have been reported (Kim et al. 2008; Minamiyama et al. 2007; Ogundiwin et al. 2005; Sugita et al. 2006; Truong et al. 2012). The integration of fourteen individual maps from independent studies (published and from this paper) produced a chromosome P5 consensus map containing 199 markers over 175 cM. Clustering of 14 individual resistance QTLs against *P. capsici* resulted in 3 meta-QTLs, namely MetaPc5.1, MetaPc5.2 and MetaPc5.3 (Fig. 1). Their confidence intervals ranged between 2.21 and 4.61 cM. The projection of the QTL detected on the top of P5 by Kim et al. (2008) positioned it above MetaPc5.3. The QTL Phyto-P detected by Ogundiwin et al. (2005) was not included in the meta-analysis due to the lack of anchor markers with other published maps. The meta-analysis reduced the number of resistance QTLs by 4.6-fold and the QTL confidence interval mean by 3.7-fold (mean CI for individual QTLs = 12.29 cM, mean CI for meta-QTLs = 3.30 cM).

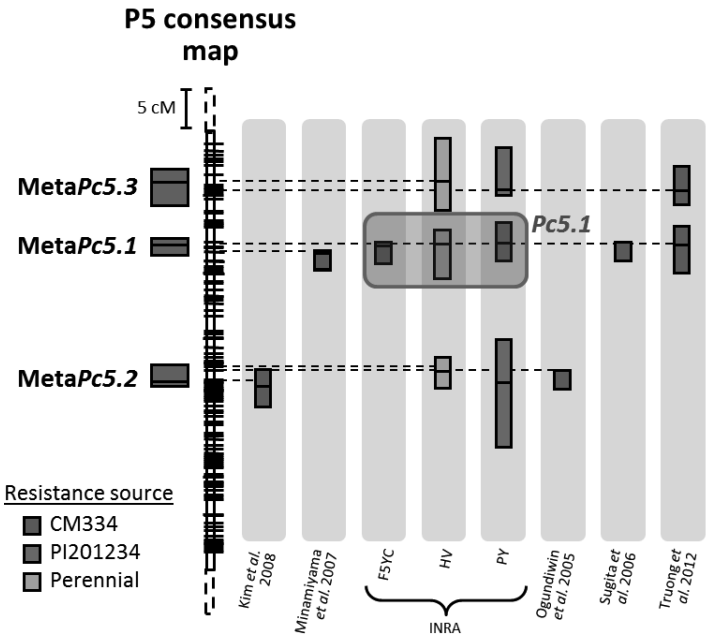

Figure 1: Consensus map of pepper chromosome P5 with projected individual resistance QTLs to P. capsici and computed meta-QTLs. Confidence interval and maximum LOD thresholds of each QTL are represented. For each map, corresponding references are indicated. The consensus map is only partially represented due to the high density of markers.

Effect of QTL Pc5.1 *on genetically distinct P. capsici isolates*

Tukey's test clearly separated susceptible and resistant genotypes. YW and H3 were susceptible to the 4 isolates we tested and displayed a comparable level of susceptibility. On the contrary, Vania, Perennial and CM334 were resistant. For isolates Pc107 and Pc197, CM334 were significantly more resistant than Vania and Perennial (Fig. 2).

Progeny lines with the resistant allele at *Pc5.1* were more resistant than those carrying the susceptible allele, regardless of the progeny considered. The QTL *Pc5.1* exhibited a significant effect on all resistance components assessed with the 4 isolates ($P < 0.0001$) and explained between 55 and 70% of the variation according to the component.

A significant 'isolate' effect was detected for all resistance components ($P < 0.0001$), and resistant parental lines actually behaved differently with tested isolates. Tukey's test classified the 4 *P. capsici* isolates into 3 levels of aggressiveness, with Pc107 and Pc197 being the most aggressive, Pc204 exhibiting an intermediate aggressiveness and Pc101 being the least aggressive.

Lastly, the interaction '*Pc5.1* x isolate' was not significant for REC ($P = 0.609$). While it was significant for the two other resistance components ($P < 0.0001$ for IND, $P = 0.003$ for STA), it explained less than 4% of the observed variation. We therefore concluded that no major host differential reaction occurred with the tested isolates.

Those data with the meta-analysis result demonstrate that *Pc5.1* is active in various genetic backgrounds and against the 12 tested isolates collected worldwide: France, Turkey, North America, Japan, Korea, and Taiwan. As a major BSR QTL against *P. capsici* with a robust effect in different backgrounds, *Pc5.1* should be the major target for breeders.

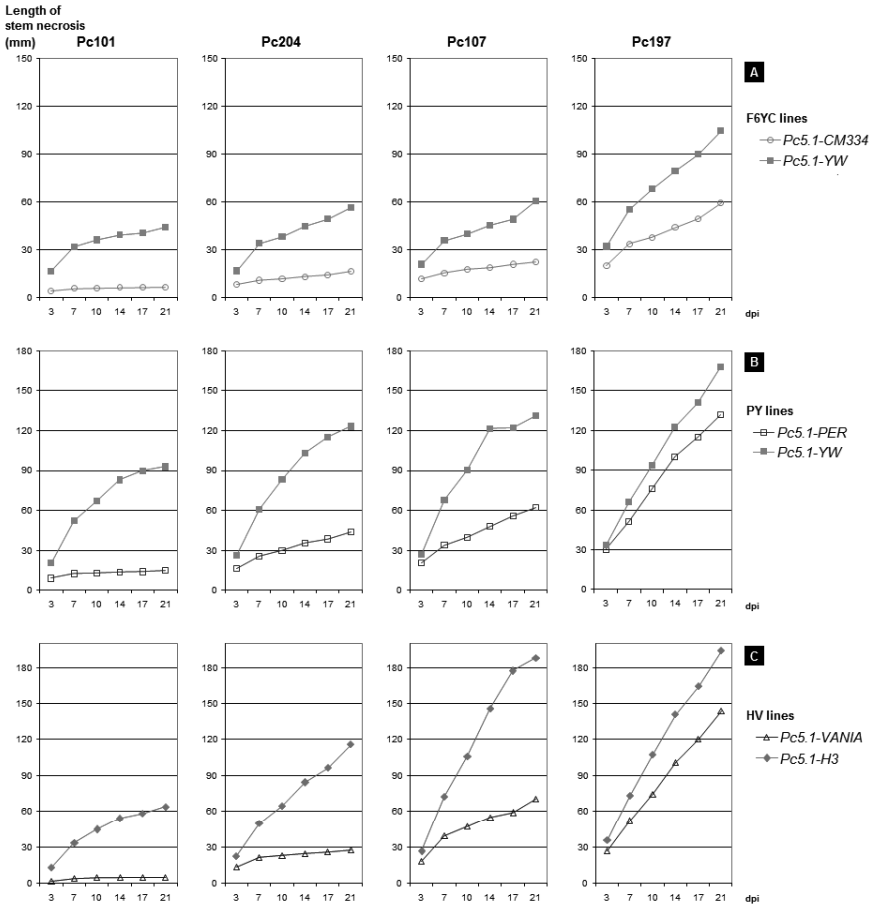

Figure 2: Mean length progression of stem necrosis over 21 days post inoculation (DPI). Stem necrosis was assessed in lines possessing the resistant or the susceptible allele at QTL Pc5.1 for F6YC, PY and HV progenies (A, B, and C, respectively) after inoculation with *P. capsici* isolates Pc101, Pc107, Pc197 and Pc204.

Fine mapping and physical map of QTL Pc5.1

The position of the locus *Pc5.1* was first downsized to less than 5 cM in the F5YC RIL progeny (Bonnet et al, 2007). Thanks to a positional cloning approach, it was yet downsized to less than 0.4 cM by screening a large selfing progeny derived from an introgression line heterozygous at *Pc5.1* in a fixed YW genetic background. Then, we constructed a physical map by anchoring BAC clones to the fine genetic map, and sequenced BAC clones constituting the minimum tilling path. Assembling of reads delivered a reference sequence of ~1.1 Mb. This sequence contained more than 90% of repeated elements corroborating the finding of Park et al (2011). Structural and functional annotation delivered less than 10 ORFs, with predicted functions for a few of them. One ORF shows homology with a transcript differentially expressed in *P. capsici*-infected peppers, and exhibits SNPs between resistant and susceptible lines.

Pattern of polymorphism at candidate genes for QTL Pc5.1

Six of the *Pc5.1* candidate genes and 14 loci distributed all over the pepper genome were successfully amplified for more than 55 accessions of the 60-accessions-core-collection. Their sequencing yielded between 4×10^5 to 202×10^5 of reads per accession. The entire trimming process removed 4% to 33% of sequenced reads depending on accessions. After applying filtering parameters, a total of 124 SNPs and no InDels were detected at candidate genes which corresponded to 9.8 SNPs per 1-kb.

Haplotype analysis of Pc5.1 *candidate genes for QTL*

Haplotype networks of candidate genes identified two major haplotypes, H1 and H2, for all analysed genes. H1 was mostly exhibited by accessions assigned to the susceptible and intermediate resistance clusters. H2 was exhibited for all candidate genes by exactly the same resistant accessions. This result indicates that candidate genes are under purifying selection.

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Capsaicin activity on cutaneous microcirculation in diabetes. A capillaroscopic study

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Abstract

The cutaneous physiological neurovascular (C and Aδ-fiber/endothelium) mechanism is altered in diabetes. Because Capsaicin stimulates Transient Receptor Potential Vanilloid 1 (TRPV1) present on fiber C causing vasoactive response, we wanted to determine in vivo whether Capsaicin could modify microcirculatory alterations present in diabetes capillaroscopic pattern. We applied on nailfold of 20 diabetic subjects the Capsaicin oleoresin and using capillaroscopic models, by employing combined pharmacological approaches, we studied the cutaneous capillaries. Using reflected capillaroscopy at 200x magnification, we analysed length, caliber, presence of volutes and tortuosities of nailfold capillaries and by means of frame to frame method we determined the flow rate. To verify the vaso-activity of Capsaicin, we adopted Lunedei's Test and CO_2 Cryotest. A significant increase of cutaneous capillaroscopic flowmetry was found. Application of Capsaicin oleoresin provides early evidence that Capsaicin markedly modify the cutaneous microcirculation in diabetes. In particular, we found that modifications occurred in both venous and arterial branch of capillaries and that the greatest improvement occurred in the venous branch of the capillary with total disappearance of venous stasis by reducing the caliber and improving venous return. Pre-treatment with chronically applied Capsaicin resulted in the almost complete disappearance of the vasal response during application of Lunedei's Test and in similar to normal skin recovery time in CO_2 Cryotest. These findings suggest an original capillary axon reflex response to Capsaicin-sensitive nerve terminals in the human diabetes skin which results in an improvement of the cutaneous micro vascular functional damage.

Keywords: capsaicin, diabetes, TRPV1, cutaneous microcirculation, videocapillaroscopy, microangiopathy

Introduction

Cutaneous microangiopathy is a frequent complication of diabetes; however its pathogenesis is still unclear. Several mechanisms by which diabetes may cause microangiopathy have been hypothesised such as oxidative damage, protein kinase C overactivity, increased glycation end-products (AGEs), and excess sorbitol formation. It has been widely demonstrated that microangiopathy affects the skin in diabetic patients, with loss of capillaries and an ensuing decrease in perfusion reserve (Ngo et al. 2005). Dysfunctions of C and Aδ nerve sensory fibres and endothelium play a major role in the pathogenesis of diabetic microangiopathy (Kilo et al. 2000). C Fibres in particular express the TRPV1 receptor, which is a member of the TRP (Transient Receptor Potential) family of ion channels. TRPV1 receptors are activated by high temperature (>42 °C), protons, arachidonic acid metabolites, and endocannabinoids, and is critically involved in mechanisms of peripheral nociceptive sensitization underlying the development of chronic pain (reviewed by Spicarovà and Palecek, 2008; Romanovsky et al. 2009). TRPV1 activation promotes vasomotor responses by stimulating the release of vasoactive molecules, such as Calcitonin Gene-Related Peptide (CGRP) from nerve endings (Harada et al. 2007).

Capsaicin (8-methyl-N-vanyllil-6-nonenamide), the active component of the chili peppers, behaves as a superagonist of TRPV1 receptors, causing an initial activation followed by receptor desensitization and defunctionalisation of peripheral nociceptors. These properties set the ground for the use of capasaicin patch in the treatment of chronic pain (Anand et al. 2011). TRPV1 activation causes neurovascular responses, characterized by vasodilation and increased vascular permeability (Baluk et al. 1997), and can also cause cell cycle arrest and apoptotic cell death (Bode and Dong, 2011). Hence, we hypothesized that skin application of capsaicin could have beneficial effects on cutaneous microcirculatory dysfunction associated with diabetes. We examined this possibility with the aid of a videocapillaroscopy technique.

Materials & Methods

A group of 20 patients (14 females, 6 males) who were hospitalized in the Dermatology department, aged 50 to 70 years, with a 2-5 year history of diabetes mellitus type II with no clinically evident complications were selected for the study. All patients were undergoing treatment with variable doses of insulin. The patients underwent an accurate clinical examination before testing. The videocapillaroscopic machine was a Zeiss OMP1.

Each patient was examined while in sitting posture after an acclimatisation of 30 minutes at a room temperature of 20-26°C. Videocapillaroscopy at 50x and 200x magnification was performed on the nailfold of the fourth finger of the left hand. The following parameters were evaluated: number of vessels (increased, decreased, normal), distribution, morphology, length, presence of volutes and tortuosities and flow rate. To increase initial transparency during morphological videocapillaroscopic examination, a drop of immersion oil was placed on the nailfold.

After the initial videocapillaroscopic evaluation, 15% Capsaicin oleoresin (2ml) was applied topically on the nailfold and the same parameters were examined at 5 and 15 minutes from application. 15% Capsaicin oleoresin was applied to all patients once a day during 7 days with a dropper, directly on the nailfold of the fourth finger of the left hand. In order to verify the vasoactive effect of a one-week topical application of Capsaicin, all patients were examined by Lunedei's Test and CO_2 Cryotest after 7 days of capsaicin oleoresin application. Lunedei's Test is performed by applying a tourniquet above the elbow and waiting 2 minutes at 5mmHg above the patients systolic pressure, then 5 minutes at 5 mmHg below patient's systolic pressure. The tourniquet is then removed and the presence of ecchymoses using videocapillaroscopy is examined, chronometering and recording the time taken for normal microcirculation to appear. CO_2 Cryotest consisted in application of a balloon filled with cold water (12 or 15°C) to the left hand, until the skin temperature of 26'C was reached. The cold water balloon was subsequently removed and the reactive vasodilatation was observed. The time taken for the vasodilatation to disappear was then chronometered and recorded.

Results

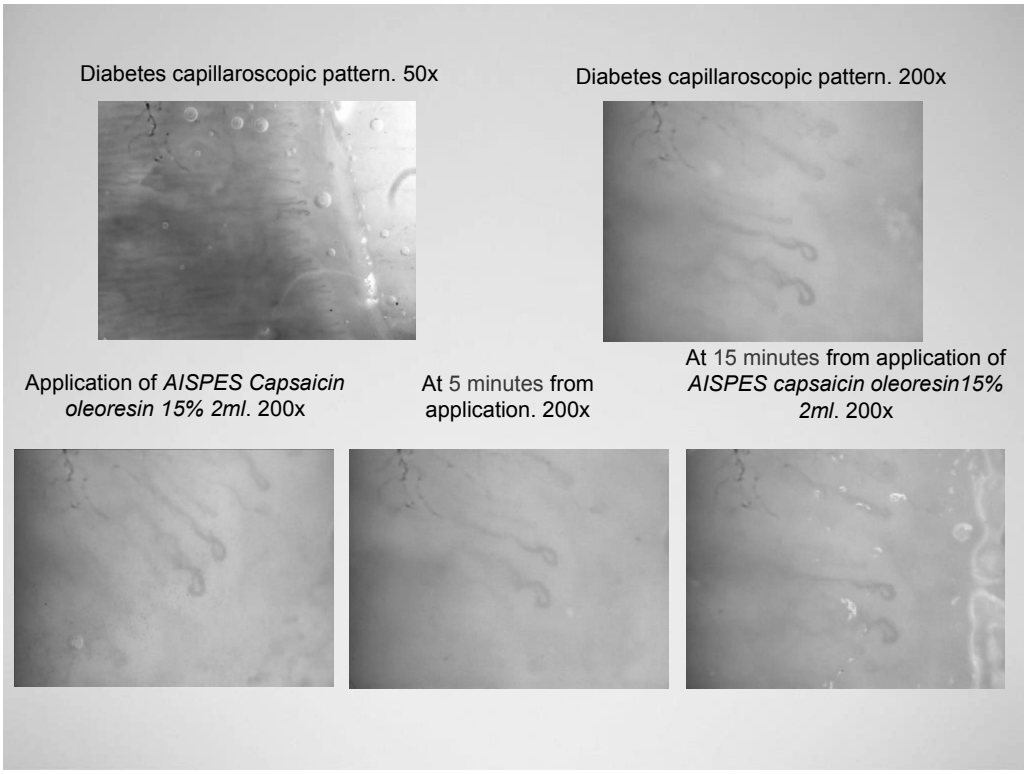
In the 20 diabetic patients evaluated at time 0, the following morpho-functional aspects were found by capillaroscopic evaluation: (i) decrease in capillary calibre, (ii) dilatation and tortuosity of capillaries in all nailfolds, (iii) decreased flow rate, and (iv) several volutes in capillary morphology. At 5 min from the application of 2 ml of 15% capsaicin oleoresin, we observed a reduction of capillary calibre and an increase in flow rate in both venous and arterial branches of capillaries, while we did not find changes in the number or distribution of vessels. At 15 min from capsaicin application, we found a marked reduction of capillary calibre and disappearance of venous stasis because of reduced vessel diameter. Finally, a 7-day treatment with capsaicin caused an almost complete disappearance of the abnormal vascular response to the Lunedei's Test and normalized the recovery time to CO_2 Cryotest.

Table 1: Patient characteristics and capillaroscopic features

PATIENT CHARACTERISTICS AND CAPILLAROSCOPIC FEATURES																	
PATIENT	SEX	AGE	YEARS OF DIABETES	CALIBRE			VOLUTES			TORTUOSITIES			FLOW RATE		
				T0	T5	T15	T0	T5	T15	T0	T5	T15	T0	T5	T15
1	F	52	2	++	+	+	++	++	+	++	++	++	+	+	++
2	F	64	4	++	++	+	++	++	+	+++	++	++	+	++	+++
3	F	69	5	+++	+++	+	+++	++	++	+++	++	++	-	++	++
4	F	65	4	++	++	+	+	+	+	+++	++	++	++	++	+++
5	F	66	4	++	+	+	++	++	+	++	++	++	++	+++	+++
6	F	51	2	++	++	++	+	+	+	+++	++	++	-	+	++
7	F	70	5	+++	+	+	+++	++	++	+++	++	++	+	++	+++
8	M	55	3	++	+	+	++	+	+	++	+	+	+	++	++
9	M	58	5	++	++	+	++	++	++	+++	+	+	+	+++	+++
10	M	64	4	+++	++	+	+++	++	++	++	+	+	-	+++	+++
11	M	54	3	+++	++	+	++	++	++	++	+	+	+	++	++
12	M	63	5	++	+	+	+	+	+	+	+	+	+	++	++
13	M	68	3	++	+	+	++	++	+	++	++	++	-	++	++
14	F	60	5	++	++	+	++	++	+	+	+	+	-	++	+++
15	F	60	4	++	+	+	++	++	+	+	+	+	+	++	+++
16	F	55	3	+++	++	+	++	++	+	++	++	++	+	+++	+++
17	F	57	3	+++	++	+	++	++	+	++	++	++	++	+++	+++
18	F	68	5	++	++	+	++	++	++	++	++	+	++	+++	+++
19	F	65	2	++	++	+	++	++	++	++	++	++	-	+++	+++
20	F	67	2	+++	++	+	++	++	+	++	++	+	+	++	+++

Table legend: - decrease + no modification ++ increase +++ marked increase

Figure 1: Capillaroscopic images of a patient of the study



Discussion

Capsaicin is widely studied for its role in pain transmission and in mechanisms of nociceptive sensitization (see introduction and references therein). However, a large body of evidence suggests that TRPV1 receptors, the molecular targets of capsaicin, are expressed not only in neurons but also in non-neuronal cells, such as smooth muscle cells and endothelial cells of blood vessels (Yang et al. 2010). Capsaicin causes vascular responses, which result from neurogenic mechanism (vasodilation, increase in vascular permeability) (Harada et al. 2007), as well as from a direct action of capsaicin on TRPV1 receptors expressed by the endothelium (vasodilation) or smooth muscle cells (vasoconstriction) (Kark et al. 2008).

We have shown here that skin application of 15% capsaicin oleoresin caused a local improvement in microvascular function in diabetic patients. The precise mechanisms underlying this beneficial effect of capsaicin in diabetic patients remain to be determined. Diabetic neuropathy is known to be associated with a reduced production of CGRP, a vasoactive molecule that is under the control of TRPV1 receptors in nerve terminals, and causes neurogenic vasodilation (Davidson et al. 2006). Activation of TRPV1 by capsaicin may cause an increased CGRP release, thereby restoring the blood vessel response to stimulation of sensory nerve endings (Rosenbaum et al.2004). However, this mechanism cannot explain the beneficial effect seen after 7-day treatment with capsaicin, which should result into TRPV1 desensitization and a lower secretion of CGRP and other vasoactive compounds from nerve terminals. It is possible that TRPV1 receptors expressed on endothelial cells are refractory to capsaicin-induced desensitization (for example, because of a high receptor reserve), and their activation stimulates nitric oxide formation (with resultant vasodilation and vasoprotection) during the 7-day treatment with capsaicin. This hypothesis warrants further investigation on isolated capillary vessels or cultured endothelial cells obtained from control or diabetic patients.

In conclusion, we have shown that topical capsaicin application may be beneficial for the management of microvascular complications in diabetic patients. Further studies are needed to establish whether, and to what extent, this particular approach can be applied to the treatment of diabetes complications.

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Arranging BAC clones covering *cop8.1*, a major QTL controlling parthenocarpy in eggplant (*Solanum melongena*)

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Abstract

Parthenocarpy is an effective trait for stable fruit setting under unfavorable conditions like extremely high/low temperatures. Genetic analysis of parthenocarpy has been extensively studied in tomato. However, each parthenocarpy-causing genes studied in tomato has problems associated with its use such as pollinated flowers producing a very low seed set. Aside from this, in eggplant, which belongs to the same genus, *Solanum*, as tomato, we developed the parthenocarpic Japanese eggplant cultivar, 'Anominori' using the parthenocarpic resources, 'Talina' in 2006.'Anominori' can show normal seed production and parthenocarpic fruit setting without any harmful trait. Genetic analysis of parthenocarpy in eggplant was started in 1998, and using DNA markers, we performed QTL analyses with two F_2 populations (ALF2/NAF2). ALF2 was derived from the cross of 'LS1934' (non-parthenocarpic line collected in Malaysia) x 'AE-P03' (parthenocarpic line developed in NIVTS) and NAF2 was derived from the cross of 'Nakate-shinkuro' (non-parthenocarpic Japanese domestic species) x 'AE-P03'. 'AE-P03' was derived from a cross of 'Nakate-shinkuro' x 'Talina' and additional multiple self-pollinations. As a result, we mapped two QTLs on chromosome 3 and 8, respectively. And the percentage of phenotypic variance explained (PVE) of *Cop3.1* was 6.3% in ALF2 and 10.6% in NAF2. The PVE of *Cop8.1* was 45.7% in ALF2 and 29.7% in NAF2, which explained 45.7% (ALF2) and 29.7% (NAF2) of variation. In this study, we focus on the main QTL, *Cop8.1* and aim to isolate the causative gene by map-based cloning. For achieving the goal, we developed near isogenic lines (NILs) derived from the cross of ALF1 ('LS1934' x 'AE-P03', a donor parent) and 'LS1934' (a recurrent parent). NILs and NIL-derived lines can be effectively used to deal with a target QTL by blocking genetic background noise. As a result of parthenocarpic test of NILs and NIL-derived lines, *Cop8.1* was mapped on a region between a couple of markers. In addition, we screened the BAC clones covering above region. Consequently, the causative gene was narrowed down to genomic region wholly covered by two BAC clones. Now, we start to determine the sequence of the above two BAC clones, and clarify the responsible sequence of *Cop8.1*. In the near future, we could confirm the effect of *Cop8.1* by means of developing transgenic plants, and the findings, likely obtained by this study can also create a wide range of ripple effect on parhtennocarpic study of other solanaceous species.

Keywords: parthenocarpy, eggplant, QTL, map-based cloning

Introduction

The initiation of fruit set normally depends on successful pollination and fertilization (Gillaspy et al. 1993). After pollination, the level of the endogenous auxin indole-3-acetic acid is increased in eggplant (Lee et al. 1997), and it is well known that synthetic auxins such as 4-chlorophenoxyacetic acid stimulate the growth of unpollinated ovaries. Focusing on this finding, Rotino et al. (1997) developed transgenic eggplants with the *iaaM* gene from *Pseudomonas syringae* that exhibited increased auxin biosynthesis in their cells and organs (Gaudin et al. 1994). In the transgenic eggplants, fruit set without fertilization, and the fruits developed parthenocarpically from emasculated flowers (Acciarri et al. 2002; Rotino et al. 1997).

Genetic studies of parthenocarpy, particularly in tomato, have identified eight parthenocarpic genes—*pat* (Beraldi et al. 2004; Mazzucato et al. 1998), *pat-2* (Fos et al. 2000; Fos et al. 2003), *pat-*

3/*pat-4* (Fos et al. 2001; Gorguet et al. 2008), *pat4.1*/*pat5.1*, and *pat4.2*/*pat9.1* (Gorguet et al. 2008)—with potential applications in the production of parthenocarpic fruits. Five of these sources—*pat*, *pat4.1*, *pat4.2*, *pat5.1*, and *pat9.1*—have been mapped on genetic linkage maps (Beraldi et al. 2004; Gorguet et al. 2008). Using these maps, attempts at map-based cloning have been made, and parthenocarpy-causing genes may soon be isolated. However, each parthenocarpy-causing gene identified in tomato has problems associated with its use, such as pollinated flowers producing a very low seed set (Mazzucato et al. 1998), genetic background controlling the level of parthenocarpy (Fos et al. 2000), different sizes of seeded and seedless fruits being set (Fos et al. 2001; Gorguet et al. 2008), and decreased parthenocarpy at low temperature (Gorguet et al. 2008).

Genetic analysis of parthenocarpy in eggplant, which belongs to the same genus, *Solanum*, as tomato, began in 1994 at the NARO Institute of Vegetable and Tea Science (NIVTS; Mie, Japan) with the crossing of a European parthenocarpic cultivar, Talina, and a Japanese non-parthenocarpic cultivar, EPL1 (Yoshida et al. 1998). Segregation tests in F_2 and BC_1F_1 populations suggested that parthenocarpy was controlled by a single major gene (Yoshida et al. 1998). In a subsequent experiment with a different cross combination—an European parthenocarpic cultivar, Mileda, by a Japanese non-parthenocarpic line, ASL-1—progeny testing confirmed the existence of a dominant single major gene (Kuno and Yabe 2005). Doganlar et al. (2002) reported a detailed interspecific linkage map of eggplant compared with tomato, and using this linkage map, Frary et al. (2003) performed advanced quantitative trait locus (QTL) analyses of morphological traits in eggplant. Recently, Barchi et al. (2010) reported an intraspecific linkage map. Most of the above mentioned markers, developed by Doganlar et al. (2002) and Barchi et al. (2010), were RFLP (restriction-fragment-length polymorphism) and AFLP (amplified-fragment-length polymorphism) markers, respectively. Therefore, it is difficult to apply those markers to detailed genetic studies on other populations of different intraspecific crosses, because of the low numbers of polymorphisms. To solve this problem, Nunome et al. (2009; 2003a; 2003b) developed simple sequence repeat (SSR) markers for *S. melongena* species, and Fukuoka et al. (2012) developed single nucleotide polymorphism (SNP) markers derived from expressed sequence tag (EST) sequences to fill in the gaps and complete the detailed integrated linkage map. Consequently, we could conducted the QTL analyses of parthenocarpy (Miyatake et al. 2012).

We report here the development of the segregating population for starting map-based cloning of the main QTL for parthenocarpy, and narrowing down the candidate region of the main QTL by the novel SNP markers designed by means of comparing the eggplant genome with that of tomato. The purpose of this study was to close the cloning of the responsible gene for parthenocarpy and discuss the prospect for studies of the mechanisms of parthenocarpy in eggplant.

Materials and Methods

Plant materials

For genetic analyses, we developed two populations of F_2 plants derived from the crosses between two non-parthenocarpic eggplant lines, LS1934 and Nakate-Shinkuro, with a parthenocarpic line, AE-P03, which we denoted ALF2 ('LS1934' × 'AE-P03') and NAF2 (Nakate-Shinkuro × AE-P03). In addition, for detailed mapping of the responsible genes, we developed NILs (BC_4F_2 populations) derived from the cross of ALF1 and 'LS1934' (a recurrent parent). And we crossed them and self-pollinated for developing NIL-derived lines underlying the minor factor, *Cop3.1* to evaluate the effect of *Cop8.1* accurately. For verifying the effect of the detected QTLs, we also developed the population of 128 BILs (ALBIL: F_9) derived by single-seed descent from reciprocal crosses between an F_1 plant (LS1934 x AE-P03) and LS1934, and three additional populations of F_2 progeny, crossing the Japanese non-parthenocarpic pure bred varieties, Aodaimaru, Kamonasu, and Satsuma-Shironaganasu (all *S. melongena*) with AE-P03, which we denoted as AAF2, KAF2, and SAF2, respectively.

Plant growth conditions and evaluation of the level of parthenocarpy

We grew the plants in the winter–spring of 2004–05 (ALF2: $n = 135$), 2007–08 (NAF2: $n = 93$), 2009-10 (AAF2, KAF2, SAF2) and 2011-2012 (NILs, NIL-derived lines) in a warming greenhouse at a minimum air temperature of 15 °C, and natural lighting, having sown the seeds in September 2004, 2007 and 2011, respectively. One to two months after sowing, ALF2/NILs seedlings were transplanted into 21/30-cm-diameter plastic pots, and NAF2 seedlings were transplanted into the ground. A few days before anthesis, 5 to 10 flowers were emasculated, and 2 months later we measured the length of all 5 to 10 fruits and checked for the absence of seeds.

Fruits at least as long as the maximum length of the emasculated non-parthenocarpic parents (LS1934, 60 mm; Nakate-Shinkuro, 70 mm) were scored as normal, and those shorter were scored as malformed. If a cavity was observed inside the fruit, the fruit was evaluated as malformed, even if it was longer than the standard.

We calculated the level of parthenocarpy (L_p) as the ratio of the number of normal fruit set (N_{normal}) to that of the total fruit set (N_{total} = number of emasculated flowers except dropped flowers):

$$L_p = N_{normal}/N_{total} \times 100 \qquad (1)$$

SSR analysis

Total of 1,054 SSR markers developed by Nunome et al. (2009) were screened for detecting polymorphisms among four eggplant lines, AE-P03, LS1934, Nakate-Shinkuro and WCGR112-8. Following these data, we chose the markers which were polymorphic among as many combinations as possible, and labeled with fluorescent pigment (FAM/VIC/NED/PET). Among the labeled markers (total of 453 markers), we screened for usable markers for mapping in the ALF2/NAF2 populations. PCR amplification and fragment analysis were carried out as described by Miyatake et al. (2012).

SNP analysis

We screened for polymorphic SNP markers designed from the sequences of hypothetical intron regions estimated by the comparison of the tomato genome sequence and the orthologous EST sequence sets of eggplant, tomato, and potato (Fukuoka et al. 2012). All SNPs markers developed by Fukuoka et al. (2012), total of 630 markers, were screened for detecting polymorphisms among parents of ALF2/NAF2 populations . To genotype these SNPs, we used the Tm-shift genotyping method originally reported by Fukuoka et al. (2008).

Map construction

We genotyped 135 ALF2 and 93 NAF2 F_2 plants with the markers. Fukuoka et al. (2012) created an integrated linkage map of eggplant (LWA2010) derived from LS1934 × AE-P03 ($n = 93$) and LS1934 × WCGR112-8 ($n = 90$). Detailed data of the integrated linkage maps and the molecular markers are available from the Vegmarks database at http://vegmarks.nivot.affrc.go.jp. Among the 954 markers mapped on it, we selected suitable markers for the QTL analyses on ALF2 and NAF2 populations, on the basis of map position and the polymorphic data among parents. We used MAPMAKER/EXP 3.0b software (Lander et al. 1987) to determine the linkage groups (LGs) and the order of the markers by Kosambi's mapping function (Kosambi 1943) with a LOD threshold of 3.0 and a map distance of <50 cM.

QTL analysis

QTL analyses were performed using the levels of parthenocarpy of the F2 populations and the linkage map from each experiment. Composite interval mapping (CIM) was performed using Windows QTL Cartographer software v. 2.5 (Wang et al. 2005) with the parameter settings of model 6 and forward and backward stepwise regression with a threshold of $P < 0.05$. The genome

was scanned at 2-cM intervals. One thousand permutation tests were performed to establish empirical LOD thresholds (ALF2, 3.7; NAF2, 3.6) at the 5% level for experiment-wise Type I errors (Churchill and Doerge 1994).

SNP discovery around the Cop8.1 locus

For fine mapping and map-based cloning of *Cop8.1*, we compared the genome of eggplant and tomato following the orthologous markers reported by Fukuoka et al. (2012). Furthermore, we picked up the genes mapped on the tomato genome region corresponding to that of the *Cop8.1* in eggplant. Then, we selected eggplant genes which were orthologous to these tomato genes and we designed primers on them to discover the SNPs. After that, we genotyped these markers using the segregating population.

Results and Discussion

Evaluation of the level of parthenocarpy

All of the emasculated flowers of LS1934 and Nakate-Shinkuro developed into malformed, unseeded fruits. AE-P03 usually sets normal-sized fruits, even without pollination. F_1 plants derived the cross of LS1934 × AE-P03 and Nakate-Shinkuro × AE-P03 exhibited partial parthenocarpy, suggesting dominant inheritance of this trait. The two F_2 populations, ALF2 and NAF2, showed a continuous distribution of the level of parthenocarpy. Aside from this, results of Shapiro-Wilks test with p-values of 5.544e-07 (=ALF2) and 1.264×10^{-4} (= NAF2) suggested that these data didn't follow the normal distribution. The both results were summed up that parthenocarpic trait was likely controlled by a few gene, but not a single gene.

Linkage map and map comparison

We used two sets of F_2 populations and constructed linkage maps. After screening the polymorphic markers available for the ALF2/NAF2 populations, we selected 118/125 SSR markers and 132/51 SNP markers. The map constructed using these markers consisted of 12/15 LGs covering a total map distance of 1414.6/1153.8 cM. By comparing the two maps and the integrated linkage map, we assigned all LGs for ALF2 and NAF2 to those of LWA2010. However, three gaps on the NAF2 linkage map resulted in 15 non-converged LGs. To confirm their genome coverage, we compared them with the integrated linkage map of eggplant (LWA2010, Fukuoka et al. submitted, http://vegmarks.nivot.affrc.go.jp) using common markers. The integrated linkage map fully corresponded to the original tomato map, which was estimated to cover the whole genome, using the orthologous SNP markers (data not shown).

QTL analysis

We detected four QTLs with LOD > 2.5 in the ALF2 population and three in NAF2. Among these QTLs, we detected two that we denoted *Cop3.1* and *Cop8.1* (Fig. 1). Comparison of the eggplant linkage map with that of tomato using the common markers showed that the LGs on which the QTLs were mapped corresponded to chromosomes 8 (*Cop8.1*) and 3 (*Cop3.1*) (Fukuoka et al. 2012). The major QTL, *Cop8.1*, was detected with LOD values of 23.8 in ALF2 (PVE = 45.7) and 7.9 in NAF2 (PVE = 29.7), which are higher than the empirical threshold values (ALF2, 3.7; NAF2, 3.6) in the same region of chromosome 8 (Fig. 1). The ranges of the LOD peaks above the empirical threshold were approximately 22 cM (ALF2: emf21A23–emh11J10) and 24 cM (NAF2: emf21A23–ecm023) (Fig. 1). The LOD values of *Cop3.1* were 4.2 in ALF2 (PVE = 6.3) and 3.0 in NAF2 (PVE = 10.6) (Fig. 1). The LOD value of *Cop3.1* in ALF2 was higher than the empirical threshold (3.7), but that of *Cop3.1* in NAF2 was lower than the empirical threshold (3.6) (Fig. 1). The QTL positions detected in this study did not correspond with those of tomato already reported (Fos et al. 2000; Fos et al. 2001; Gorguet et al. 2008; Mazzucato et al. 1998), and there have been

no reports of genetic studies of parthenocarpy in other solanaceous fruit vegetables. Therefore, *Cop8.1* and *Cop3.1* are novel parthenocarpic loci in solanaceous species.

We genotyped 128 BIL plants with two markers (est_cpa03j24 and est_ls502j22) nearest to the parthenocarpy QTLs detected in the ALF2 population, and grouped them with the genotypes of those markers. The BIL plants with non-parthenocarpic parental homozygous genotypes at the est_cpa03j24 marker locus (nearest to *Cop8.1*) expressed a lower level of parthenocarpy (5.6%), and those with parthenocarpic parental homozygous genotypes at the same locus showed a higher level of parthenocarpy (56.1%). This demonstrates the effect of *Cop8.1* in raising the level of parthenocarpy. However, in case of the other QTL, *Cop3.1*, we could not clearly detect positive effect. Results indicate that *Cop8.1* has a major effect, that is, it contributes to the stable expression of the parthenocarpic trait in any situation, while *Cop3.1* has a minor and limited effect, depending on the effect of Cop8.1. In the additional populations of F_2 progeny of crosses between various Japanese non-parthenocarpic cultivars and AE-P03 (AAF2, KAF2, and SAF2), *Cop8.1* contributed to parthenocarpic fruit development regardless of the genotype of *Cop3.1* (data not shown). This indicates that by using these two markers, emf21H22 and emh11J10, both linked to *Cop8.1*, we may be able to select fully parthenocarpic individuals at the seedling stage from a wide range of genetic backgrounds.

Narrowing down the genetic region of Cop8.1, and arranging BAC clones for map-based cloning

Following the parthenocarpic effect of the detected QTLs, we focused on the main QTL, *Cop8.1*, and we developed the NILs for map-based cloning of this gene, that enabled us to judge the existence of the responsible gene clearly (Fig.2). We clarified that *Cop3.1* supported the stable gene expression of *Cop8.1*. As a result of the screening of SNPs around *Cop8.1,* targeted 163 genes, we detected 20 SNPs. Genotyping the segregating population using these SNPs markers, we could narrow down the genetic region of *Cop8.1* to a couple of markers. Comparing the tomato genome, the candidate region corresponded to 50kb in tomato. And that region was covered by two BAC clones of 'AE-P03'.

Conclusion

In greenhouse cultivation of eggplant, unfavorable conditions such as high or low temperature decrease the amount of fertile pollen in the air, thereby restricting successful fertilization. As a practical measure, synthetic auxins are used to improve the fruit setting rate. However, exogenous application of 4-chlorophenoxyacetic acid takes time and places a heavy burden on workers. Saito et al. (2009) developed a parthenocarpic cultivar, Anominori, for which Talina, a paternal line of AE-P03, was used as the parthenocarpic resource. Anominori does not have an agronomically unfavorable trait like those seen in parthenocarpic tomato resources (Fos et al. 2000; Fos et al. 2001; Gorguet et al. 2008; Mazzucato et al. 1998; Saito et al. 2009). Thus, the flanking DNA markers suitable for selecting parthenocarpic genes may be useful as a new tool for developing a wide variety of parthenocarpic eggplant cultivars aimed at practical use. As breeding parthenocarpic cultivars in eggplant takes a lot of time and labor (Saito et al. 2009), this finding enables for the first time a systematic breeding program using DNA markers for parthenocarpic cultivars in solanaceous species. Moreover, we clarified that synteny was conserved around *Cop8.1* between eggplant and tomato, and we used it to make novel SNP markers for fine mapping. Now we start detecting the polymorphism between 'AE-P03' and other non-parthenocarpic resources to narrow down the candidate genes and analyzing gene expression of them. We hope to reach the advanced goal of isolating *Cop8.1*. Considering the novelty of these QTLs, finding the detailed genetic and physiological mechanism of parthenocarpy would affect not just the study of eggplant, but also the study of all solanaceous fruit vegetables.

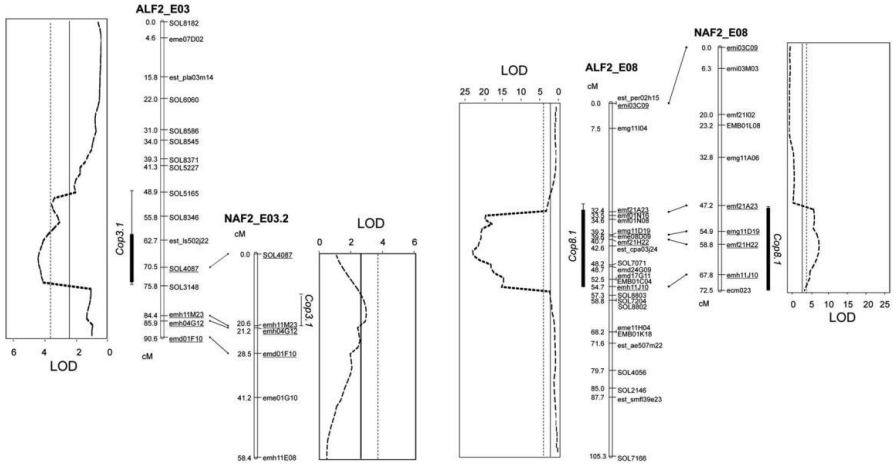

Fig.1 Comparision of the two linkage maps (left ALF2, right NAF2) near the *Cop3.1* and *Cop8.1* gene. Underlined names indicate the common markers and their positions are connected with lines. The lines drawn on the side of the QTL names (the *Cop3.1* gene and the *Cop8.1* gene) mean the range of detected QTL regions with a LOD score higher than 2.5, and similarly, the black bars mean the region with a LOD score higher than threshold calculated using one thousand permutation tests (ALF2: 4.7, NAF2: 3.9).

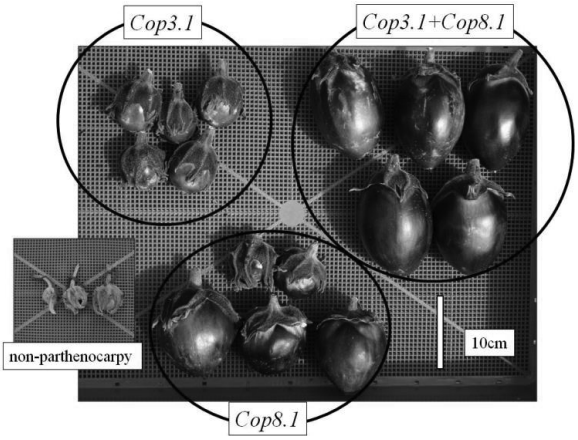

Fig.2 The shape of emasculated fruits of non-parthenocarpic line (LS1934), partially parthenocarpic lines with the Cop3.1 or the Cop8.1 gene and parthenocarpic line with both of them in NILs.

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Improvement of an isolated microspore culture protocol for Spanish sweet pepper (*Capsicum annuum* L.)

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Abstract

Anther or microspore cultures can be used as biotechnological approaches to accelerate the production of pure lines, needed to obtain commercial hybrids. Isolated microspore culture is technically more complex but more efficient than anther culture. Microspore culture in pepper (*Capsicum annuum* L.) was previously proven efficient for Asian hot pepper types. This technique has not been refined yet in Spanish sweet peppers. Thus, the aim of this work was to find an optimal protocol for isolated microspore culture in 'Herminio', a Spanish sweet pepper type. First of all, each of the individual stages of microsporogenesis and microgametogenesis were correlated with different stages of bud and anther development, in order to find easily measurable and visible morphological markers to relate with the microspore stages optimal to induce androgenesis (vacuolate microspore and young bicellular pollen). In order to prevent the low percentage of microspores effectively induced towards embryogenesis and to improve the quality of the embryos obtained, we tested the effect of some key parameters, including the evaluation of different microspore plating densities, and of different concentrations of sucrose and antibiotic.

This work proved that microspore culture in 'Herminio' might be possible after some modifications of the initial protocol, but there is still much work to do in order to consider pepper as an efficient androgenic system.

Keywords: Androgenesis, biotechnology, embryogenesis, microsporogenesis, microgametogenesis.

Introduction

Androgenesis can be defined as a set of biological processes that produce an individual from a male nucleus. Techniques based on androgenesis, such as anther or microspore culture, can be used to produce double haploid (DH) lines, needed to obtain commercial hybrids. With this approach, the typical 7 – 8 generations of classical inbreeding are reduced to just one *in vitro* generation, reducing the time and resources needed(Seguí-Simarro, 2010). Anther culture is a simple method applicable to a wide range of crops. Isolated microspore culture is technically more complex but more efficient than anther culture (Forster et al. 2007). There are protocols to produce DHs in more than 250 species, but the efficiency of these protocols is still very low. Just a few species such as rapeseed (*Brassica napus),* tobacco (*Nicotiana tabacum*), barley (*Hordeum vulgare*) or maize (*Zea mays*) are considered as model systems (Seguí-Simarro et al. 2011).

Pepper (*Capsicum annuum* L.) is considered one of the three solanaceous crops that could be defined as recalcitrant (together with eggplant and tomato). Microspore culture in pepper has been proven efficient for Asian hot pepper types (Kim et al. 2008; Supena et al. 2006a; Supena et al. 2006b). Although some positive results have been published with sweet pepper types (Lantos et al. 2009, Lantos et al. 2012), there is still room for the optimization of the methodologies.

The identification of buds and anthers with microspores/pollen at the optimal stage to be induced towards androgenesis is an essential preliminary step to have an optimized protocol. For the vast majority of inducible species, it is widely agreed that induction can only be achieved in a narrow time frame revolving around the first pollen mitosis (Seguí-Simarro, 2010). This means that buds and anthers containing mostly late, vacuolate microspores (VM) and just divided, young

bicellular pollen (YBP) must be precisely identified. This is not a trivial issue, since out of this time frame, induction efficiency drops dramatically or is nearly impossible (Seguí-Simarro, 2010). Pepper is not an exception to this rule. Indeed, the stages most sensitive to inductive treatments revolve around the VM and YBP (Irikova et al. 2011), exactly the same described for most of the known inducible species. Thus, it is essential to identify suitable morphological markers, predictors of the precise developmental stage of the microspore or pollen grain within the anther.

Other improvable aspects are induction efficiency and embryo anatomy. Microspore plating density is known to affect the efficiency of microspore embryogenesis. Kim et al. (2008) implemented the isolated microspore culture protocol by doubling the microspore plating density during the inductive treatment. However, there are efficient protocols for microspore culture in several species such as rapeseed, cereals like wheat and rye, and also a recalcitrant solanaceous crop such as eggplant (*Solanum melongena*), where the same microspore plating density is kept during the whole culture (Custers et al. 2001; Gustafson et al. 1995; Ma et al. 2004; Corral-Martínez and Seguí-Simarro, 2012). Therefore, it is important to establish the optimal microspore plating density in each case to improve the efficiency of microspore embryogenesis. Compared with other osmotica like polyethylene glycol, the presence of high levels of sucrose was shown to affect the morphology of microspore-derived embryos in *Brassica napus*. Underdeveloped and aberrant cotyledons and also reduced suspensors were observed in embryos cultured with high levels of sucrose (Ilic-Grubor et al. 1998). A negative effect of sucrose was also described in eggplant, where experiments with 2% of sucrose produced more calli per anther than higher sucrose concentrations (Miyoshi, 1996). In pepper, the efficiency of microspore induction can be seriously compromised by endogenous microbial contamination, which is known to be a potential problem in plant tissue culture (Kneifel and Leonhardt, 1992). Antibiotics have been used in isolated and shed-microspore culture systems of different pepper genotypes to keep the cultures free of endogenous contamination (Lantos et al. 2009; Lantos et al. 2012; Supena et al. 2006b).

The protocol of isolated microspore culture demonstrated as efficient in hot pepper (Kim et al. 2008), still needs major modifications to be considered efficient in sweet pepper cultivars. The efficiency of microspores induced towards embryogenesis and the quality of the microspore-derived embryos obtained with this protocol is still very low. Thus, the aim of this work was to find an optimal protocol for isolated microspore culture in a Spanish sweet pepper variety, using as a control the protocol described by Kim et al. 2008. We determined the easiest and more accurate morphological markers to correlate the visible changes of anthers and buds during flower development with the corresponding stages of the microspores and pollen grains contained in them (Parra-Vega et al. 2013). Some modifications of the control protocol have been tested, including microspore plating density and lower concentrations of sucrose. Furthermore, the minimum inhibitory concentration of antibiotic needed to avoid contamination while allowing microspore induction towards embryogenesis has been analyzed.

Materials and Methods

Plant material and growing conditions

Seeds of the commercial F1 hybrid 'Herminio' (Lamuyo Type, from Syngenta Seeds) were used to obtain donor plants. Plants were grown in the COMAV glasshouses at the Universitat Politècnica de València under 25ºC and natural light.

Microspore stage determination

Flower buds ranging from 2 to 8 mm long were selected and manually excised. Bud, anther and calyx lengths were measured using an electronic digital caliper. Anther purple pigmentation was categorized into four pigmentation levels: (1) no purple pigmentation, (2) pigments in the apical end, (3) pigments covering most of the distal (petal-facing) surface, and (4) full pigmentation of the

distal surface of the anthers. A minimum of ten buds (two anthers per bud) were considered for each stage analyzed. A minimum of 300 randomly chosen microspore/pollen grains per anther were counted. The different microspore/pollen developmental stages present in a single anther were observed and counted with a Zeiss (Axiovert 40 CFL) inverted microscope. Numerical data were processed and graphically represented using a Microsoft Excel spreadsheet.

Isolated microspore culture

Flower buds at the appropriate stage (containing mostly vacuolate microspores and young bicellular pollen) were manually excised. Anthers were extracted from the buds, selecting the ones containing anthers with the appropriate purple pigmentation. According to Kim et al. (2008), microspores were isolated in sucrose-starvation medium, plated on a density of 2×10^6 microspores/ml and pretreated at 31ºC for three days. After inductive treatment, microspores were transferred to liquid NLN culture medium supplemented with 10% of sucrose, diluted to a density of 10^6 microspores/ml. Dishes were incubated at 25ºC during one month.

Characterization of the microspore culture system

Fourteen days after inductive treatment, observations of the isolated microspores were carried out with a Zeiss (Axiovert 40 CFL) inverted microscope. At least one third of the dishes were observed in each experiment. A minimum of 300 microspores were counted per dish. The percentage of induced microspores was calculated dividing the number of microspores showing one or more cell divisions by the total number of microspores. One month after inductive treatment, the total number of calli and embryos obtained were counted.

Study of the effect of sucrose concentration and microspore plating density

Different microspore densities during the inductive treatment (10^6/ml and 2×10^6/ml), and different sucrose concentrations in the culture medium (5% and 2.5%) were tested.

Determination of the minimum inhibitory concentration of antibiotic

The high level of contamination in pepper microspore culture forced us to add 200 mg/l of sodium cefotaxime from the onset of microspore pretreatment. Different concentrations of antibiotic were tested as explained in Results in order to determine the minimum inhibitory concentration of antibiotic that allow microspores to induce them towards androgenesis.

Results and discussion

Determination of morphological markers to correlate bud and anther development with the optimal stage for androgenesis

First, we studied the parallel development of microspore/pollen grains (Fig. 1A-G), flower buds (Fig. 1A'-G') and anthers (Fig. 1A''-G''), covering from tetrad to the mature pollen stages. We focused on the stages of vacuolate microspores (VM) and young bicellular pollen (YBP) because the transition between these two stages has been described as the most sensitive stage to inductive treatments (Irikova et al. 2011). VMs were characterized by a thick exine wall where apertures were clearly visible (Fig. 1D). At this stage petals were slightly longer than sepals (Fig. 1D') and anthers turned to dark yellow and presented slightly purple pigmentation at the apical end (Fig. 1D''). The transition of VM to YBP (Fig. 1E) was characterized by changes in bud size, a widening of the bud receptacle, and the clear emergence of petals out of the calyx (Fig. 1E'). Purple pigmentation of the apical end of the anthers was rather evident at this stage (Fig. 1E'').

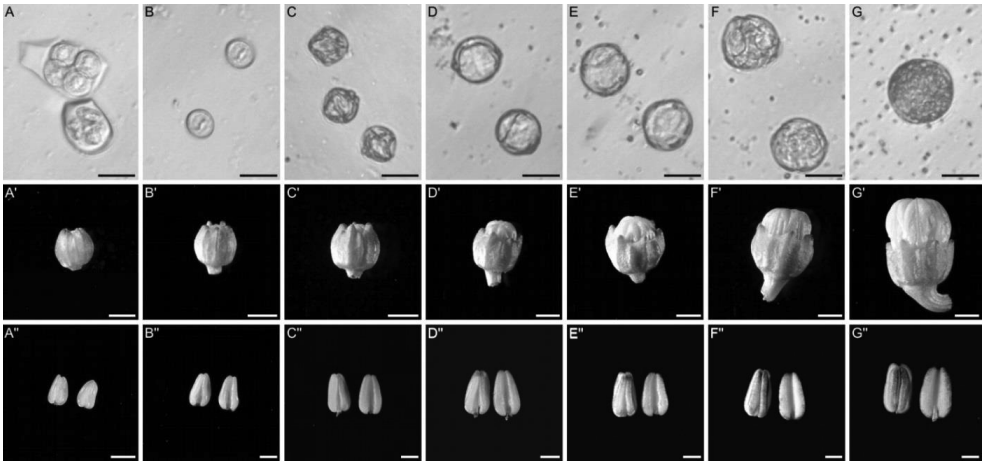

Fig. 1. Changes during microspore/pollen (A-G), bud (A'-G') and anther development (A''-G''). A: Meiocytes and tetrads. B: Young microspores. C: Mid microspores. D: Vacuolate microspores. E: Young bicellular pollen. F: Mid pollen. G: Mature pollen. Anther are presented in A''-G'' at their distal (left) and proximal sided (right). Bars: A-G: 10 µm; A'-G'': 1 mm.

Next, we compared the evolution of microspore/pollen development with anther and bud increases in length. Figs. 2A, B shows the percentage of microspores/pollen at each developmental stage, represented by the size of corresponding bubble. Anther and buds grew in parallel to microspore/pollen development. With respect to the identification of the optimal stage for induction, we looked for the ranges containing mostly VM and YBP (light bubbles in Fig.2). In case of doubt, we selected the range with a majority of VM with respect to YBP, since they were at a stage immediately prior to mitosis, and would enter it soon. 'Anthers contained these stages at 2.50 to 2.99 mm of length (Fig. 2A), whereas buds contained these stages at 4.00 to 5.00 mm of length (Fig. 2B). The anther purple pigmentation patterns were also correlated with microspore/pollen development (Fig. 2C). According to the pigmentation levels established in 'Material and methods', the higher percentage of VM and YBP was observed exclusively in anthers with purple pigmentation at the apical end (pigmentation level 2). As the last morphological criterion, we assessed the proportion of calyx length with respect to the whole bud. This criterion allows for a quick visual identification of suitable buds. In order to verify if this criterion could be reliable, we analyzed the linear correlation between bud length and calyx/bud ratio values (Fig. 2D). A clearly positive linear correlation was observed, with a linear regression coefficient (R) of 0.93. This proved that we could use this criterion as an accurate morphological marker. Therefore, buds with a calyx covering about 80-90 % of the total bud length should be selected to maximize the presence of VM and YBP.

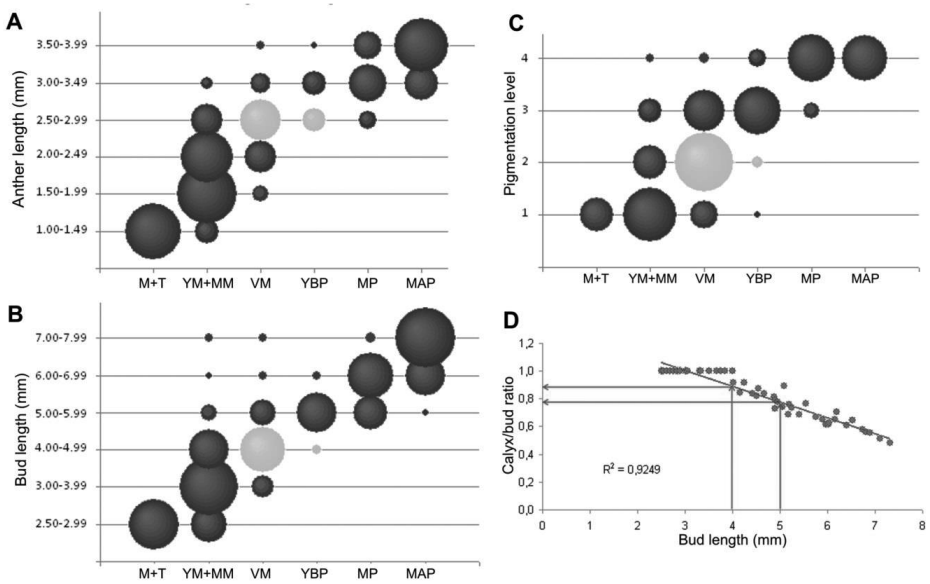

Fig. 2. Correlation of anther length (A), bud length (B), anther pigmentation (C) and calyx/bud ratio (D) with microspore/pollen development in cv 'Herminio'. Percentages of microspores/pollen at each stage are proportionally represented by bubbles. Light bubbles correspond to the stages where most vacuolate microspores and young bicellular pollen are found. For d, dots are the data points used to calculate the regression line. M+T meiocytes and tetrads, YM+MM young and mid microspores, VM vacuolate microspores, YBP young bicellular pollen, MP mid pollen, MAP mature pollen.

The criteria to identify microspore/pollen stages must be precise, but also easy to use and quickly measurable. Bud and anther length could be considered as the most precise markers, but they may not be the most useful in practice due to the time needed to excise and measure every single bud and anther. These time-consuming procedures would significantly delay the experiments and could compromise microspore/pollen viability. Anther pigmentation has been suggested as the most reliable criterion for anthocyanin-producing cultivars, since it is less genotype dependent (Regner, 1996). However, it is based on the visual estimation of pigmentation, which may reduce its accuracy. In addition, it is a destructive procedure. Calyx/bud ratio is not a destructive marker that could be applied to the buds still *in planta*, avoiding excision. However, its limitation could be the reduced accuracy, since in practice it may be difficult to visually distinguish between ratios of calix covering about 70, 80 or 90% of the total bud length.

All these considered, we propose a combination of calyx/bud ratio and anther pigmentation as a precise, easy and quick marker. In practice, buds having a calyx/bud ratio of about 80-90%would be selected *in planta,* and then, in the laminar flow hood, only those anthers showing purple pigmentation at the apical end would be isolated and used. Thereby, the combination of both morphological markers will provide more accuracy and the selection of anthers having microspore/pollen at the optimal stage will be faster and easier.

Study of the effect of sucrose concentration and microspore plating density

Sugar concentration (Figs. 3E, F), it clearly affected the number of induced microspores (Fig. 3E) and the number of calli and embryos obtained (Fig. 3F). Lower sucrose concentrations gave rise to reduced numbers of induced microspores and of embryos obtained. A half of the control sucrose

concentration (10%), gave rise to a reduction of about one third the number of induced microspores, calli and embryos with respect to control experiments. Since high levels of sucrose were shown to affect the morphology of microspore-derived embryos in rapeseed (Ilic-Grubor et al. 1998), and our results demonstrated that lower levels of sucrose decreased the efficiency of microspore-derived embryos in 'Herminio', a possibility to implement the protocol could be to test other osmotica, such as polyethylene glycol, in order to avoid the negative effect of sucrose on microspore-derived embryos.

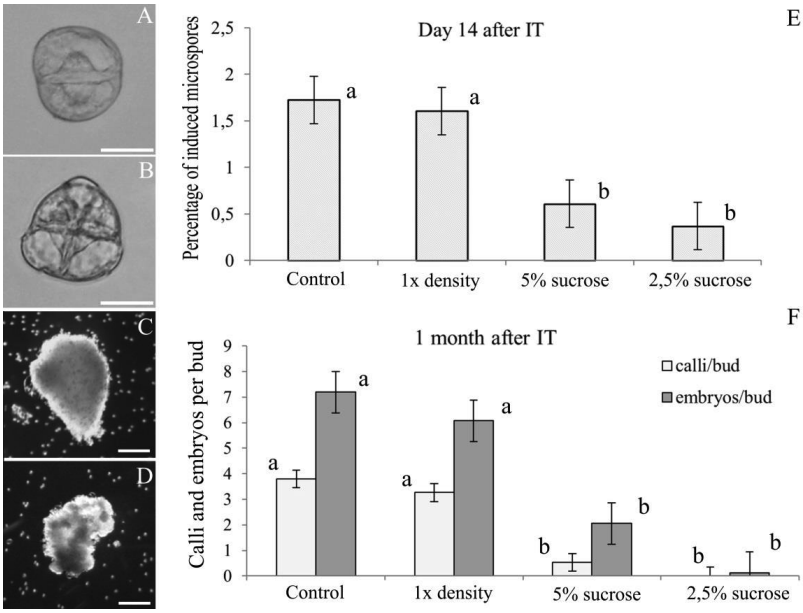

Fig. 3. A: Induced microspores with 2 divisions. B: Induced microspores with 4 divisions. C: Microspore-derived embryo obtained in control after 1 month of the isolation. D: Microspore-derived callus obtained in control after 1 month of the isolation. E: Percentage of induced cells obtained 14 days after inductive treatment (IT) F: Number of calli and embryos obtained per bud 1 month after inductive treatment (IT). Bars: a, b: 20 µm; c, d: 250 µm. 1x density: microspore plating density of 106 microspores/ml during inductive treatment instead 2×106 microspores/ml (IT in control). 5% and 2.5% sucrose: NLN liquid media supplemented with those concentrations of sucrose instead 10% of sucrose (control).

The results of using a microspore plating density of 2×10^6 microspores/ml during IT (Control in Figs. 3E, F:) were not statistically different from those obtained by keeping the same microspore plating density (10^6 microspores/ml) all over the culture (1x density in Figs. 3E, F:). These results showed that duplication of the microspore density during the inductive treatment has not a significant impact of microspore induction. Thus, the inductive treatment could be simplified by keeping the same microspore density during the entire process.

Determination of the minimum inhibitory concentration of antibiotic

Fourteen days after inductive treatment, the percentage of induced microspores with different concentrations of cefotaxime was measured. As seen in Fig. 4, there were no statistically significant differences between 200, 150 and 100 mg/l of cefotaxime. However the number of induced cells dramatically decreased with antibiotic concentrations under 100 mg/l. When microspore isolations were carried out with antibiotic-free medium, they were completely contaminated and microspores

were not induced towards androgenesis. Therefore, the minimum concentration of cefotaxime in 'Herminio' microspore cultures should be 100 mg/l.

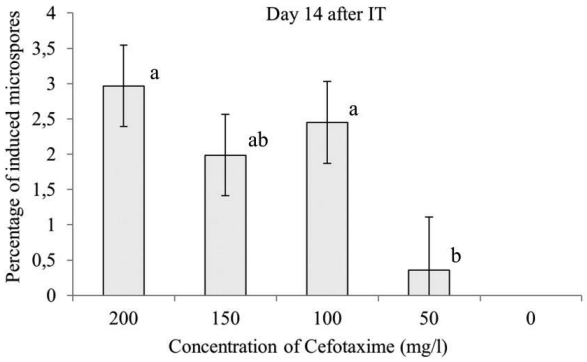

Fig. 4. Percentage of induced microspores at different concentrations of cefotaxime (mg/L). Data measure at day 14 after inductive treatment (IT).

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QTLs analysis of anthracnose (*Colletotrichum acutatum*) resistance in pepper (*Capsicum* spp.)

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Abstract

Pepper (*Capsicum* spp.) anthracnose, caused by various fungal species of *Colletotrichum* spp., is a serious disease worldwide leading to heavy yield and income losses in pepper production. The inheritance of PBC932, a *Capsicum chinense* variety with strong resistance to *Colletotrichum acutatum*, was studied in a BC_1 mapping population derived from an interspecific cross between *Capsicum annuum* inbred lines 77013(susceptible recurrent parent) and PBC932 using QTL (quantitative trait locus) analysis method. Resistance test was performed on detached mature green (35～40 d after flowered) and red (45～50 d after flowered) fruits using the microinjection method with a inoculation of 1 μL 5×10^5 conidia·mL^{-1} conidia suspension under laboratory conditions. The disease resistance level was evaluated in three methods, disease incidence, true lesion diameter and overall lesion diameter. The interspecific linkage map was constructed composed of 14 linkage groups including 385 markers (SSR, InDel or CAPS), covering a length of 1310.2 cM. Inclusive Composite interval mapping (ICIM) undertaken on genotyping and phenotyping data revealed 9 QTLs and 3 QTLs for resistance to *C. acutatum* at mature green and red fruit stage, respectively. Interestingly, the main effect QTLs $AnR_{GO}5$, $AnR_{GT}5$ and $AnR_{GD}5$ for resistance of mature green fruit stage, and $AnR_{RO}5$ and $AnR_{RT}5$ for ripe red fruit stage, and one minor QTL $AnR_{RD}5$ explained the most anthracnose variance in red fruit disease incidence, were mapped in the same marker interval, InDel and HpmsE116 (interval of 9.6 cM), which might indicated that this locus was region for major gene associated with resistance to *C. acutatum* and that resistance at both stages was controlled by the same genes in PBC932. When compared with reference pepper maps indicated that this locus is located on the pepper chromosome P5.

Keywords: Pepper; Anthracnose; *Colletotrichum acutatum*; Resistance; QTL mapping

Introduction

Pepper (*Capsicum* spp.) is an important vegetable crop all over the world as spice and food, sometimes can extract components of color dyes and medications. Pepper anthracnose (*Colletotrichum* spp.), mainly caused pre- and post-harvest fruit rot, leading to severe economic losses in tropical and subtropical areas including China, Korea, India, Indonesia and Thailand, has become one of the main barriers to pepper production (Hartman and Wang 1992a; Voorrips et al. 2004; Poonpolgul and Kumphai 2007; Kim et al. 2008a; Than et al. 2008a, 2008b； Xia et al. 2011). Pepper anthracnose is caused by *Colletotrichum* spp., including *C. acutatum* (teleomorph *Glomerella acutata*), *Colletotrichum gloeosporioides* (teleomorph *Glomerella cingulata*), *C. capsici* (a synonym of *C. dematium*), and *C. coccodes* (Park and Kim 1992), of which *C. acutatum* and *C. gloeosporioides* are the most destructive and widely distributed (Voorrips et al. 2004; Sarath Babu et al. 2011). These pathogens primarily attack pepper fruits at both green and red fruit stages, and sometimes can also cause lesions on pepper leaves and stems. Typical anthracnose symptoms on pepper fruits are sunken necrotic tissues, with concentric rings of acervuli. These blemishes have led to marketability decreasment (Than et al. 2008). Although applications of fungicides and integrated management are used for disease control, it brings in negative effects on farmer income and health. The most economic and environmentally friendly method is to developed resistant varieties; however, we are desperately short of anthracnose-resistance commercial cultivar.

Several sources of resistance to anthracnose have been identified mostly in *C. baccatum* and *C. chinense* (AVRDC 1999), and researchers have used these sources to study the inheritance of anthracnose resistance (Lin et al. 2002; Pakdeevaraporn et al. 2005; Voorrips et al. 2004; Lee et al. 2010; Kim et al. 2010). Genetic studies show that the resistance inheritance pattern varies depending on the resistance source and the *Colletotrichum* isolate. Resistance to *C. acutatum* 'Coll-153' in the resistance line '0038-9155', derived from *C. chinense* 'PBC932', was controlled by two complementary dominant genes (Lin et al. 2007), and resistance to *C. acutatum* 'KSCa-1' in *C. baccatum* PI594137 is controlled by a single dominant gene (Kim et al.，2008b) at green fruit stage. Wheras resistance to *C. acutatum* 'KSCa-1' in *C.annuum* 'AR', derived from 'PBC932' and to 'MJ5' in *C. baccatum* PBC80, is inherited by a single recessive gene (Kim et al. 2008a; Mahasuk et al. 2009b) at green fruit stage. At red fruit stage, resistance to *C. acutatum* 'Coll-153' in '0038-9155' is controlled by duplicate recessive genes (Lin et al. 2007), whereas by a single dominant gene in PBC80 to 'MJ5' (Mahasuk et al. 2009b). In addition, Yoon and Park (2005) reported that resistance to *C. acutatum* 'KSCa-1' in PBC81 was inherited dominantly and quantitatively at untold fruit stage.

Some reports have found that resistance to *C. capsici* was inherited recessively. Cheema et al. (1984) found that resistance to *C. capsici* was inherited recessively, with significant epistatic effects. And resistance to *C. capsici* '158ci' in *C. chinense* PBC932 at green and red fruit stage, and resistance to *C. capsici* 'ThSCc-1' in *C. annuum* 'AR'and 'Daepoong-cho' at green fruit stage were inherited through a single recessive gene (Pakdeevaraporn et al. 2005; Kim et al. 2008a; Mahasuk et al. 2009a). In contrast, some researches found that resistance to *C. capsici* was inherited dominantly, to *C. capsici* '158ci' in the *C. annuum* breeding line '83-168' (Lin et al. 2002), and to *C. dematium*, a synonym for *C. capsici*, in *C. annuum* 'Chungryong' (Park et al. 1990b).

Some studies found that resistance to anthracnose was inherited by polygenic genes (Voorrips et al. 2004; Lee et al. 2010; Kim et al. 2010). One major and three minor QTLs to *C. gloeosporioides* resistance, and one major and one minor QTLs to *C. capsici* resistance were identified in an interspecific *C. annuum* Jatilaba × *C. chinense* PRI95030 population (Voorrips et al. 2004). Lee et al. (2010) identified two major QTL (*CaR12.1* and *CaR12.2*) for *C.acutatum* resistance and two major QTLs (*CcRC* and *CcR9*) for *C. capsici* resistance in an interspecific *C. baccatum* PBC81 × *C. annuum* SP26 population. And Kim et al. (2010) identified two major QTLs and 16 minor QTLs that influenced *C. acutatum* resistance in an intraspecific *C.baccatum* pendulum Golden-aji.

This study was carried out to evaluate the inheritance of anthracnose resistance to *C. acutatum*, the dominant pathogen of anthracnose in China, in an interspecific population from a cross between *C. annuum* and *C. chinense*, and to identify some QTLs with effect on resistance, which would be of value in future breeding programs.

Materials and Methods

Plant materials

The female parent is inbred line 77013(*C. annuum*, sweet bell pepper) susceptible to *C. acutatum***,** developed in Institute of Vegetables and Flowers (IVF), Chinese Academy of Agricultural Sciences (CAAS), China. The resistant variety PBC932 (*C. chinense* - hot pepper) is the male parent, presented by DR. Wang Tiancheng, Asian Vegetable Research and Development Center (AVRDC). By backcrossing the interspecific F_1 with the susceptible parents, a BC_1 population was generated.

Together with the BC_1 population, 3 plants of each parental material and 9 F1 plants were grown with normal field management in 2012 in a plastic greenhouse of IVF, CAAS, China. 186 plants of BC_1 were used to as the mapping population, and gained fruits with artificial pollination.

Pathogen

An isolate of ***C.*** *acutatum*, collected using the single-spore isolation method modificated (Ho and Ko, 1997) from naturally infected pepper fruit in Hunan and Shanxi province field by Wei Ran, Institute of Vegetables and Flowers. The isolate was maintained on potato dextrose agar (PDA). The isolate was subcultured in liquid culture of PDA and incubated on a shaking table for 3 days in dark at 28℃ with a rotation speed 100r·min $^{-1}$. The liquid culture was filtrated through four layers of gauze, and then the suspension was adjusted to a concentration of 5×10^5 spores·mL^{-1} with sterile distilled water, using hemocytometer.

Bioassay of anthracnose resistance

Artificial inoculation was performed on detached mature green fruits(35～40 d after flowering) and mature red fruits (45～50 d after flowering) respectively, using the microinjection method developed at the AVRDC(1999) with slight modification. The healthy fruits were harvested from greenhouse-grown individual plant. The fruits were washed in order by tap water, distilled water, 75% ethanol to remove various germs on the fruit surface, and then air dried. Inoculation was conducted at a 1 mm depth using a microinjector comprised of a Micro Syringe^{TM} model 1705 TLL and a dispenser (Hamilton PB600-1, Repeating Dispenser, Reno, NV, USA). Each fruit was injected with 1μl of prepared conidial suspension per site; and one to four sites were inoculated depending on the fruit size. Inoculation was conducted with three replications, each with at least three fruits. The inoculated fruits were incubated with the inoculated sites facing upwards in plastic boxes (50×25×20 cm), on four layers of filter paper which was moistened with 150 ml distilled water. The boxes were sealed tightly with plastic wrap to maintain the relative humidity at greater than 95% and incubated under 28℃ in the dark for 48h. Finally, the plastic wrap was removed and the fruits were incubated for five more days under the same conditions. Fruits harvested from 77013, PBC932 and F_1 were also inoculated and incubated in the same conditions.

Disease incidence (D, as a percentage of infected sites per total inoculated sites), overall lesion diameter (O, as an mm value averaged over all inoculated sites, including those that did not develop lesions), and true lesion diameter (T, as an mm value averaged over all lesions that actually developed) were measured as index of resistance assessment (Yoon et al. 2004). Lesions showing bacterial rot were not measured. In total, there were 6 resistance evaluating methods: GD, GO, GT, RD, RO, RT, where G and R means green (G) and red (R) fruit stage, respectively.

Data analysis

Distributions of disease incidence, overall lesion diameter and true lesion diameter at mature green and red stage were analyzed using the statistical analysis system (SAS 9.1.3) software and Excel 2007 (Microsoft office 2007).

Construction of linkage map

Total genomic DNA was extracted from young leaves of parents, F_1 and BC_1 mapping population seedlings by CTAB method with relatively minor modification (Fulton et al. 1995). DNA quality was checked and quantified by BioSpec-nano (UV-VIS Spectrophotometers), and then adjusted the concentration of DNA to 25 ng·μL^{-1}. Initially the parents, 77013 and PBC932, were screened for polymorphism with 815 SSR (Huang et al. 2001; Lee et al. 2004; Minamiyama et al. 2006; Yi et al. 2006; Huang et al. 2011), 1 InDel (Wang 2011) and 228 out of 295 CAPS markers (Wu et al. 2009). Polymorphic markers identified between 77013 and PBC932, were subsequently used for genotyping the BC_1 mapping population.

The PCR amplifications of SSRs and InDel were performed as described by Huang et al. (2011) and Wang (2011). PCR products were then separated on 8% non-denaturing polyacrylamide gel and visualized by silver staining method. The PCR amplifications and restriction enzyme digestions of CAPS markers were performed as described by Wu et al. (2009). The digested PCR products were

then separated on a 2% agarose gel electrophoresis, and the DNA bands were visualized and photographed with the Gel Doc 2000 (Bio-Rad). All the markers were scored codominantly.

Mapping was performed using JoinMap 4.0 software (Van Ooijen, 2006) with a population type code, BC_1. Markers were grouped at an LOD score of 3.0, where recombination fraction was converted into map distances in centiMorgans (cM) using Kosambi function (Kosambi 1944).

QTL mapping

The QTLs associated with anthraconose resistance was identified by the QTL ICIMapping software version3.2 (http://www.isbreeding.net/) using ICIM-ADD and ICIM-EPI analysis methods. Briefly, for ICIM-ADD analysis, the *P* values for entering variables (PIN) and removing variables (POUT) were set at 0.0001 and 0.0002, and the scanning step was 0.1 cM; for ICIM-EPI analysis, the PIN and POUT were set at 0.0001 and 0.0002, respectively, and the scanning step was 5.0 cM. A LOD threshold of 2.0 was chosen to declare a putative additive QTL as significant, while 5.0 to a pair of putative epistatic QTL. The proportion of observed phenotypic variance explained (PVE) by each detected QTL and the corresponding additive effects were also estimated. The QTL nomenclature followed this: ***An***thracnose ***R***esistance at ***G***reen (or ***R***ed) fruit stage under ***O***verall lesion diameter (or ***T***rue lesion diameter, or ***D***isease incidence) on chromosome P***x*** (*x*: number of chromosome), for example $AnR_{GD}5$.

Results

Anthracnose resistance phenotyping

A set of 186 BC_1 plants derived from a susceptible female and recurrent inbred lines 77013 and highly resistant male parent PBC932 for *C. acutatum*, along with the parental and F_1 genotypes, was phenotyped for resistance level at both fruit stages, mature green and mature red, both with three resistance indexes, disease incidence, true lesion diameter and overall lesion diameter, totally 6 phenotyping methods.

The mean disease score of parent PBC932 showed lower values than 77013 in all the six phenotyping methods. While the mean disease score of F_1 fell in between PBC932 and 77013, and skewed toward the resistant parent PBC932 in all the six phenotyping methods. The distributions of resistance in different phenotyping method are shown in Fig. 1. In the BC_1 mapping population, the ranges of resistance were 6.25～100%, 0～100%, 1.22～22.43 mm, 0～22.10 mm, 2.13～22.43 mm and 0～22.17 mm for GD, RD, GO, RO, GT and RT respectively.

Construction of linkage map

Of the 1111 markers screened on parental genotypes, 402 SSRs, 1 InDel and 38 CAPSs showed high quality and polymorphic amplification. Finally a total of 387 polymorphic markers including 351 SSRs, 1 InDel and 35 CAPSs, which can produce clear and polymophic applicants when screened among the

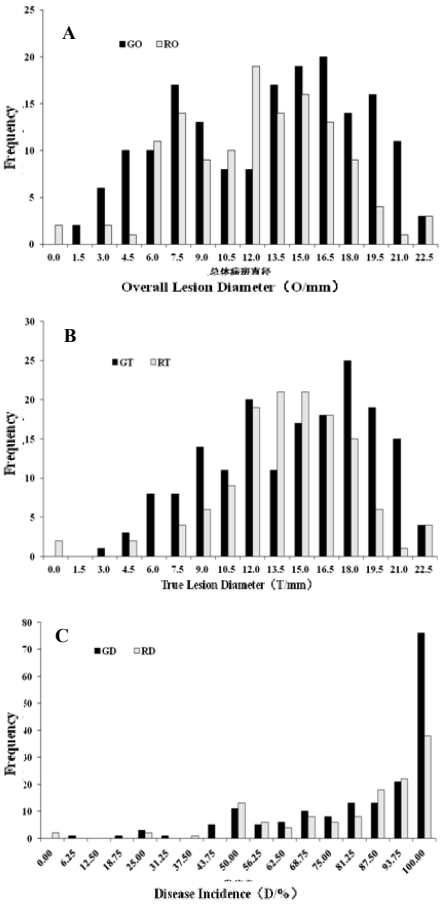

Fig. 1. Distribution of anthracnose resistance in BC_1 population depending on three scoring methods (A: overall lesion diameter; B: true lesion diameter; C: disease incidence) and fruit stage (mature green or red fruits)

mapping BC_1 population, were selected to construct the linkage map. By importing all genotyping data into JoinMap 4.0 for linkage map analysis and construction, 385 markers (Gpms198 and C2_At4g16580 remained unlinked to any of the linkage groups) were assigned to 14 linkage groups spanning 1310.2 cM, with an average maker interval of 3.40 cM. The number of markers mapped on per linkage group varied from 4 (P 1b and P 11b) to 68 (LG 1), while the length of linkage group varied from 21.8 cM (P 11b) to 152.1 cM (P4).

The linkage map constructed in this study was compared to maps previously published (Yi et al. 2006, Lee et al. 2008, Wu et al. 2009, Zhang et al. 2010, Mimura et al. 2012).Comparison of these maps showed a lot of common markers with which have been mapped on corresponding chromosome, and with these common mapped markers as a bridge, all the linkage groups were matched to one unique pepper chromosome except LG1 linkage group, because markers belongs to chromosome 1 and 8 couldn't be separated.

QTL mapping

A total of 9 and 3 additive QTLs on P3、P5、P7、P10 and P12 chromosomes were detected by ICIM-ADD analysis for all the indexes associated with *C. acutatum* resistance at mature green and red fruit stages, respectively. And no epistemic QTLs were detected by ICIM-EPI analysis.

There were 5 QTLs $AnR_{GO}3$, $AnR_{GO}5$, $AnR_{GO}7$, $AnR_{GO}10$ and $AnR_{GO}12$, 1 QTL $AnR_{GT}5$ and 3 QTLs $AnR_{GD}5$, $AnR_{GD}10$ and $AnR_{GD}12$ controlling resistance at mature green stage under indexes of overall lesion diameter, true lesion diameter and disease incidence, respectively. Phenotypic variance explained by these QTLs ranged from 2.52 to 63.38%, and the total phenotypic variance explained by these QTLs was 73.88%, 60.50% and 43.29% in GO, GT and GD, respectively. The major QTLs $AnR_{GO}5$, $AnR_{GT}5$ and $AnR_{GD}5$ were located at the same maker interval - InDel and HpmsE116 - on chromosome P5 (Table 1), explaining 62.38%, 60.50% and 33.17% phenotypic variance in GO, GT and GD.

Fewer than at the mature green stage, there were one QTL $AnR_{RO}5$, one QTL $AnR_{RT}5$ and one QTL $AnR_{RD}5$ controlling resistance at ripe red stage under overall lesion diameter, true lesion diameter and disease incidence, respectively. Phenotypic variance explained by these QTLs was 15.24%, 15.90% and 9.31% in RO, RT and RD, respectively (Table 1). The two major QTLs $AnR_{RO}5$, $AnR_{RT}5$ and one minor QTL $AnR_{RD}5$ were located at the same maker interval- InDel-HpmsE116-on chromosome P5.

The resistance alleles of all these five major QTLs were come from resistance parent PBC932 and contributed to the increase of resistance to *C. acutatum*. And QTLs, which explained the most phenotyping variance, were all located at the same marker interval, InDel and HpmsE116, in a 1.6 cM region, and the nearest marker was InDel. This might indicate that this region covers the major resistant genes.

Table 1 Main-effect QTLs detected by ICIM analysis for anthracnose resistance in a BC_1 interspecific population

QTLs	Chr	Position	Flanking Marker	Interval	LOD	PVE (%)	Add
$AnR_{GO}5$	P5	1.2	InDel-HpmsE116	9.6	32.26	62.38	8.61
$AnR_{GT}5$	P5	0.8	InDel-HpmsE116	9.6	31.91	60.50	7.28
$AnR_{GD}5$	P5	1.6	InDel-HpmsE116	9.6	12.26	33.17	0.25
$AnR_{RO}5$	P5	0.0	InDel-HpmsE116	9.6	4.49	15.24	3.60
$AnR_{RT}5$	P5	0.0	InDel-HpmsE116	9.6	4.70	15.90	3.12
$AnR_{RD}5$	P5	0.0	InDel-HpmsE116	9.6	2.65	9.31	0.13

Discussions

The total length of our linkage group was 1310.2 cM, compared with other maps (Kang et al. 2001, Lee et al. 2004, Yi et al. 2006, Lee et al. 2011, Prince et al. 1993,Livingstone et al. 1999), the map distance calculated by JoinMap is probably shorter than that by Mapmaker (Bradeen et al. 2001). All our linkage groups were successfully assigned to the corresponding pepper chromosome except LG1 which was a fusion of chromosome P1 and P8. This result suggests that our map has a relatively high coverage rate of the pepper genome. As a result, LG1 represented two pepper chromosomes, P1 and P8. Such pseudo linkage of chromosome 1 and chromosome 8 has been reported in interspecific (*C. annuum* and *C. chinense*) and intraspecific (*C. annuum*) populations (Yi et al. 2006; Barchi et al. 2009; Lee et al. 2009). Wu et al. (2009) proposed that such pseudo linkage may occur resulting from reciprocal translocation of the two chromosomes between the parents of the mapping population. Interestingly, our linkage map can serve as an anchor for the alignment of major pepper maps, as it contain so many new generation markers (SSR and CAPS) which offer tremendous advances in cost, efficiency, throughput and sensitivity for plant genomics, and a lot of common markers to published maps.

Using ICIM analysis, we identified a total of 9 QTLs and 3 QTLs for resistance to *C. acutatum* at mature green and red fruit stage, respectively (Table 1). The major QTLs $AnR_{GO}5$, $AnR_{GT}5$ and $AnR_{GD}5$ resistant to *C. acutatum* at mature green and the major QTLs $AnR_{RO}5$, $AnR_{RT}5$ at mature red stage were located at the same marker interval, InDel and HpmsE116, nearest to InDel (Table1), indicating that PBC932 has a same resistance gene at both fruit stages, or two different but closely linked genes. However, *C. acutaum* resistance in '0038-9155(derived from PBC932)' and *C. baccatum* PBC80 were inherited by different and unlinked genes at both fruit stages, which is different from our results. Compared with the heritabilities of *C. acutatum* resistance at mature green and red fruit stage, total phenotypic variances explained by the QTLs identified at mature red fruit stage in our study and the QTL number were much lower than at mature green stage, suggesting that resistance to C. acutatum might affected by environmental conditions, or that there were some QTLs with higher PVE failed detection in our linkage group. The InDel marker was developed by Wang (2011) from an AFLP marker, which was highly correlated with anthracnose resistance at the green mature stage in resistant material CCA0038-9155(derived from PBC932). Interestingly this marker was the closest linked maker to our major QTLs, and we further assigned this marker to pepper chromosome P5. This will not only provide us an effective marker for marker-assisted selection in anthracnose resistance breeding program, but also narrow the region of resistance gene in PBC932, which is important for further study about anthracnose resistance gene.

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Mapping of QTLs for key breeding traits in eggplant (*Solanum melongena* L.)

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Abstract

A previously developed intraspecific F_2 population of 156 individuals, obtained by crossing the eggplant (*Solanum melongena* L.) breeding lines '305E40' x '67/3' and employed for developing an SNP-based genetic map, was used in the present study for the phenotypical characterization in two locations in Italy and QTL (quantitative trait locus) analyses. Of the more 40 morphological, physiological and biochemical features analyzed, here we report about the analyses of 23 traits including the yield-related ones. Up to 7 QTLs per trait were identified and the percentage of phenotypic variance explained (PVE) by each QTLs was up to 93.7%**.** Most of the QTLs were confirmed in both locations and for almost all the traits one major QTL (PVE ≥ 10%) was spotted at least in one location. The genetic control of traits was stable across locations. For the most interesting traits, evidence for putative orthologous QTLs with tomato is discussed. The present results supply valuable information to eggplant breeders on the inheritance of important traits, and provide potential tools to assist breeding.

Keywords: *Solanum melongena*, RAD markers, QTLs, agronomic traits, synteny

Introduction

Eggplant genome is relatively unexplored, especially if compared to those of the other major Solanaceae crops tomato, pepper and potato. Genetic maps based on both inter-specific (Wu et al. 2009) and intra-specific crosses (Barchi et al. 2010; Fukuoka et al. 2012) have been developed in the last few years. Recently, we combined the developed Restriction-site Associated DNA (RAD, Miller et al. 2007) approach with Illumina DNA sequencing to effect the rapid and mass discovery of both SNP and SSR markers in eggplant for mapping purposes as well as QTL analysis (Barchi et al. 2011). A subset of 339 newly developed SNPs, together with 2 HRMs, 33 SSRs, 27 COSs, 11 RFLPs and 3 CAPS (415 total markers) were used for the construction of a genetic map which includes 12 LGs spanning 1.390 cM, with an average map distance of 3.8 cM. Thanks to RFLP and COSII markers, and to the syntenic relationships between the eggplant and tomato genomes, the chromosome assignment of the LGs identified was made available.

In comparison to tomato, potato and pepper (Bradshaw et al. 2008; Tanskley et al. 2004; Rao et al. 2003) a limited information is available on QTLs controlling key breeding and quality traits in eggplant. Nunome et al. (2001) discovered QTLs for the fruit colour and shape in a RAPD and AFLP based intraspecific eggplant genetic map. Doganlar et al. (2002) and Frary et al. (2003) identified some QTLs for breeding traits; however these studies were based on an interspecific F_2 population, thus partially limiting the extension of their results to breeding programs. QTLs associated to resistance to *Ralstonia solanacearum* and parthenocarpy were recently identified using intraspecific F_2 map populations (Lebeau et al. 2012; Miyatake et al. 2012).

The purpose of the present work was to identify the genomic regions underlying fruit quality and yield-related traits using the RAD-tag based intraspecific linkage map we recently developed (Barchi et al. 2012). QTLs for the considered traits were identified, located in the genetic map and syntenic relationships with other Solanaceae species highlighted.

Materials and Methods

Plant materials

A population of 156 F_2 plants was previously obtained by crossing the eggplant lines '305E40' and '67/3', strongly differing for the horticultural and plant traits here studied (Barchi et al. 2012). The female parent, '305E40', is a double-haploid introgression line derived from a somatic hybrid between eggplant and *S. aethiopicum* gr. *gilo* (Rizza et al. 2002), displaying the resistance to the soil-borne *Fusarium oxysporum* f. sp. *melongenae* coded by the locus *Rfo-sa1* (Toppino et al. 2008). The highly homozygous '67/3' line lacks of the resistance locus *Rfo-sa1*.

Plant phenotyping

The F_2 mapping population, together with both the parental lines and their F_1 hybrid, were cultivated in two locations, Montanaso Lombardo (ML) and Monsampolo del Tronto (MT). In each trial, 4 cuttings per each F_2 plant were planted in two completely randomized blocks and phenotyped for key fruit and plant traits. Phenotyping was performed according to the European Cooperative program for Plant Genetic Resource descriptors panel for Solanaceae (ECPGR, 2008) and the International Board for Plant Genetic Resource descriptors for eggplant (IBPGR, 1990). Biometric fruit characteristics (see Table 1) were obtained from 5 representative fruits from each block. To calculate the total yield (ty), total number of fruit (tyfn) and the mean fruit weight (tyfw) per segregant, data of number and weight of the fruits at the commercial ripeness were collected weekly along the entire harvesting season, for a total of 12 harvestings. Data collected during the first 5 harvests were used to calculate early production (ey), early number of fruit (eyfn) and the early yield mean fruit weight (eyfw). The colour of the various organs was collected using arbitrary scales as reported in Table 1. *F. oxysporum* artificial inoculation was performed on 27 plantlets of F_3 families to infer the phenotype of the F_2 individuals.

Statistical analysis and QTL detection

Analysis of variance was applied to estimate genotypic/environmental effects. The broad-sense heritability (h^2_{BS}) values were calculated as $\sigma^2_G/(\sigma^2_G + \sigma^2_E/n)$, where σ^2_G represent the variance in *g* and σ^2_E the residual variance and n the number of blocks; normality, kurtosis and skewness were assessed for each trait with the Shapiro-Wilks test (α=0.05). Correlations between traits were estimated using the Spearman coefficient. Principal component analysis (PCA) for the data collected at M L. was carried out using Past software (Hammer et al. 2001) to determinate which traits were most effective in describing the population. QTLs detection was determined by both interval mapping (Lander and Botstein, 1989) and Multiple QTL Mapping (MQM) (Van Ooijen, 2004). QTLs were considered as 'major' when they explained more than 10% of the total variability for the considered trait. LOD thresholds for declaring a QTL to be significant (α=0.05) were established by applying 1,000 permutations. QTL effects were estimated on the basis of Markov Chain Monte Carlo (MCMC) method. The syntenic regions of the available genome tomato sequence (http://solgenomics.net/organism/Solanum_lycopersicum/genome) were investigated for identifying candidate genes and transcription factors co-localizing with the eggplant identified QTLs.

Results and Discussion

Phenotypic variation and inter-trait correlations.

The parental lines displayed significant contrasted phenotypes for most of the traits analyzed in both environments (Tab. 1). The '305E40' line produces longer, narrower and lighter fruits with less locules, a longer peduncle than '67/3' and the flesh show a green ring next to the peel. The plants are resistant to *F. oxysporum*, have an upright growth habit, low anthocyanin pigmentation on leaves and stem, prickles present on calyx and leaves, and produce a higher number of light pink flowers per inflorescence. On the contrary, the parental line '67/3' have a prostrate growth habit, highly pigmented leaves and stems, with reduced prickliness and bears violet flowers giving origin to shorter, larger, heavier purple fruits with more locules; thus, in spite of the lower number of fruits produced, its total and early yield was higher than the one of '305E40'.

Table 1 List of the traits (unit of measurement) analyzed and their code, means, standard deviations (SD), coefficients of variation (cv) and broad sense heritability for the traits. Significant mean difference among parental values (Wilcoxon test) is reported (*p<0.05).

Trait	Code	Env.	Parents means ± SD			F1	F_2 population mean ± SD	cv	h^2_{BS}
			305E40	67/3					
Total. yield (gr)	ty	ML	3088±494.0	5325.2±1113.4	*	8166.7±777.8	7912.6 ± 2783.3	0.35	0.84
		MT	2624.2±600.7	3783±783.7	*	4342.2±460.5	4389.5 ± 1561.3	0.36	0.42
T. yield fruit number	tyfn	ML	22.75±4.65	16.25±2.62	*	29.25 ± 2.75	41.61 ± 11.89	0.29	0.84
		MT	21.25±3.5	15.75±3.09	*	23.5±2.64	31.63 ± 9.78	0.31	0.49
T. yield av. fruit weight	tyfw	ML	137.1±14.76	326.02±20.16	*	279.55 ± 15.16	185.90 ± 32.07	0.17	0.91
		MT	122.5±10.33	240±11.77	*	185.02±7.10	134.23 ± 24.51	0.18	0.54
Early yield (gr)	ey	ML	1769.5±444.0	2743.2±557.6	*	4403.2±940.0	2797.1 ± 988.1	0.35	0.85
		MT	1527±404.07	1852.5±612.6	*	2577.7±265.5	2324.6 ± 760.7	0.33	0.18
E. yield fruit number	eyfn	ML	12±2.45	8±1.63	*	14.5±2.38	14.06 ± 4.27	0.30	0.83
		MT	10.25±1.71	7±2.16		11.75±1.26	13.13 ± 3.79	0.29	0.68
E yield av. fruit weight	eyfw	ML	147.12±16.2	343.78±23.15	*	301.98±18.43	195.3 ± 36.17	0.19	0.86
		MT	148.60±28.99	264.69±20.39	*	219.69±10.72	176.73 ± 40.66	0.23	0.86
Fruit weight (gr)	fw	ML	153.9 ± 32.0	392.75 ± 70.51	*	383.5 ± 65.76	252.33 ± 56.48	0.23	0.84
		MT	180.4 ± 20.5	294.75 ± 44.27	*	304 ± 38.46	214.65 ± 40.8	0.19	0.88
Fruit length (cm)	fl	ML	21.83 ± 2.98	9.88 ± 0.43	*	17 ± 0	14.15 ± 1.87	0.13	0.91
		MT	20.33 ± 5.57	8.17 ± 1.46	*	11.08 ± 1.97	11.46 ± 1.65	0.15	0.74
Fruit diameter max (cm)	fdmax	ML	4.40 ± 0.21	10.52 ± 0.75	*	8.05 ± 0.35	7.05 ± 0.85	0.12	0.91
		MT	4.45 ± 0.40	8.33 ± 1.28	*	6.33 ± 0.89	6.03 ± 0.65	0.11	0.60
Fruit shape	fs	ML	4.96 ± 0.57	0.94 ± 0.07	*	2.11 ± 0.23	2.05 ± 0.4	0.20	0.96
		MT	4.54 ± 0.99	0.98 ± 0.11	*	1.74 ± 0.08	1.93 ± 0.37	0.19	0.92
Pedulcle length (cm)	pedl	ML	5.82±1.05	2.87±0.67	*	4.43 ± 1.11	5.77 ± 0.99	0.17	0.90
		MT	4.90 ± 1.27	3.63 ± 1.04		4.35 ± 1.56	4.58 ± 0.69	0.15	0.69
Fruit calix prickliness (0-3)	fcpri	ML	1.42 ±0.49	0.5 ± 0	*	1.5 ± 0.71	1.05 ± 0.45	0.43	0.86
		MT	1.65±0.44	0.63 ± 0.22	*	1.62±0.49	1.27 ± 0.39	0.31	0.64
Fruit firmness (kg/cm^2)	outfir	ML	2.42 ± 0.57	2.13 ± 0.22		2.63 ± 0.13	2.32±0.42	0.18	0.79
		MT	3.05 ± 0.61	2.13 ± 0.57	*	3.48 ± 0.18	2.81±0.65	0.23	0.63
Number of locules	slon	ML	3.67 ± 0.58	8 ± 1.87	*	4 ± 1.41	4.29 ± 0.95	0.22	0.63
		MT	4.17 ± 0.75	5.50 ± 0.57	*	4.75 ± 0.50	4.23 ± 0.77	0.18	0.63
Flesh green ring (0-1)	gring	ML	1 ± 0	0 ± 0	*	1 ± 0	0.66 ± 0.47	0.72	1.00
		MT	1 ± 0	0 ± 0	*	0.88 ± 0.25	0.61 ± 0.44	0.72	0.98
Plant growth habit (1-3)	hab	ML	3 ± 0	1 ± 0	*	2 ± 0	2.25 ± 0.72	0.43	0.80
		MT	3 ± 0	1 ± 0	*	2 ± 0	2.11 ± 0.82	0.50	0.42
N° flower per inflorescence	flwin	ML	4 ± 0	1 ± 0	*	2 ± 0	2.93 ± 1.2	0.46	0.33
		MT	5.5 ± 0.71	1 ± 0	*	2 ± 0	3.06 ± 1.51	0.31	0.47
Corolla colour	corcol	ML	1 ± 0	5 ± 0	*	3.9 ± 0.15	3.95 ± 1.61	0.41	0.94
		MT	1 ± 0	5 ± 0	*	4 ± 0	3.57 ± 1.6	0.45	0.84
Adaxial leaf lamina anthocyanin	adlan	ML	0 ± 0	3 ± 0	*	1.5 ± 0	1.57 ± 0.92	0.58	0.93
		MT	0.5 ± 0	3 ± 0	*	1.5 ± 0	1.5 ± 0.83	0.55	0.92
Stem anthocyanin	stean	ML	1.25 ± 0.29	3 ± 0	*	2.2 ± 0.3	2.4 ± 0.52	0.22	0.89
		MT	1 ± 0	3 ± 0	*	2.25 ± 0.35	2.32 ± 0.52	0.22	0.82
Calyx anthocyanin	calan	ML	1.12± 0.25	3 ± 0	*	1.8 ± 0.3	2.2 ± 0.55	0.25	0.90
		MT	0.75 ± 0.35	3 ± 0	*	1.75 ± 0.35	2.02 ± 0.68	0.34	0.88
Fruit color (1-30)	fcol	-	13,8 ± 0,42	24,55 ± 2,29	*	18,5 ± 0,71	22,01 ± 4,71	0,2	0.85
Fusarium resist.	fores	-	100 ± 0	0 ± 0	*	1 ± 0	0.66 ± 0.30	0.46	-

The yield-related traits (yields, weight and dimensions of fruit; Figure 1) as well as most of the other bio-morphological traits (data not shown) displayed a normal distribution. Comparing data of total and early yield, during the first 5 weeks of harvesting the parental line '67/3' produced a lesser number of fruits (averaging 1/3 of the total), with respect to '305E40', but their average weight was higher if compared to that of the fruits produced along the entire season. On the contrary, the line '305E40' produced a higher number of fruit during the early season, averaging 1/2 of its total production, but of similar weight of the ones produced along the entire season. In both the environments tested, the F_1 hybrid displayed values between the parental lines for almost all the traits in study with the exception of the total and early yield, for which the F_1 hybrid displayed higher values. In the F_2 progeny, the heritability value was overall high, (with the exception of number of flowers per inflorescence) and ranged from 0.63 to 1. Transgressive segregation (detected when F_2 individual recorded a trait value higher or lower by at least two standard deviations than the higher or lower scoring parental line) was observed in both ML and MT locations for several traits, and above all the yield traits, both towards '67/3' and '305E40' parent.

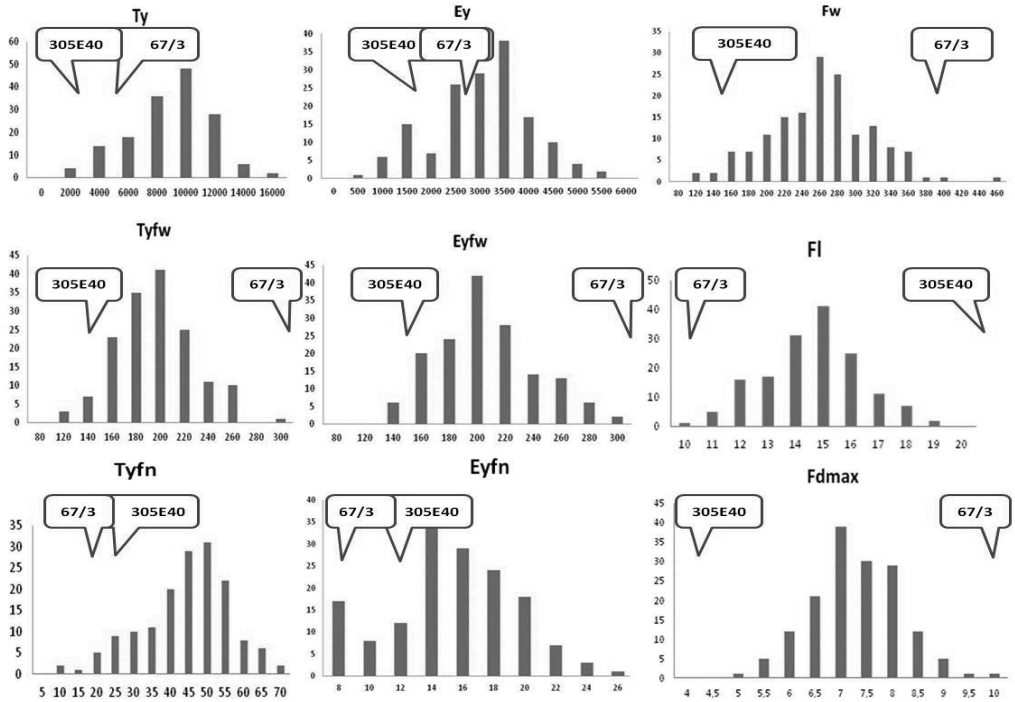

Figure 1. Histograms showing the distribution of the traits Ty (total yield), Ey (early yield), Fw (fruit weight), Tyfw (average fruit weight of total yield), Eyfw (average fruit weight of early yield), Fl (Fruit length), Tyfn (number of fruit of total yield), Eyfn (number of fruit of early yield), Fdmax (fruit diameter)

For sake of simplicity, data about Spearman correlation are not shown. Significant inter-trait correlations were detected both within and across locations. Across-locations the correlation values ranged from +0.285 for nlo to + 0.897 for fw. In both ML and MT the traits related to production (e.g. fw, fl, fdmax, tyfn, ty, tyfw, ety, eyfw) were always strongly positively correlated to each other, while the fruit shape displayed an highly significant negative correlation with fruit weight and diameter. The highest values of positive correlation were between data of early production and their co-respective of total yield production. Positive correlations were also found between leaf

anthocyanin intensity and steam and calyx anthocyanin intensity (+0.78, and +0.83, respectively) and between fruit and flower color (+0.73).

Principal Component Analysis.

The multivariate analysis (data not shown) revealed that the first three components were the most relevant to represent the whole phenotypic variability in the F_2 population as they overall accounted for 56.1% of the total variance. Considering the correlation coefficients of each trait with respect of each of the three principal components, it can be observed that the first component, which explained the 28.8% of the total variance, was negatively correlated with all the traits regarding plant production (early and total yield), habitus and fruit dimensions (fruit length, maximum diameter and weight, peduncle length) and positively correlated with *F. oxysporum* resistance. The second component, which explained 14.0% of total variance, was positively correlated with all the traits regarding the presence of anthocyanins (in leaves, stem and calyx) and also and with flowers and fruit color, and negatively correlated with fruit shape. The third component, which explained the 13.2% of total variance, was positively correlated with the number of locules. As expected, all the individuals of the F_2 population fitted in a single group and could be discriminated principally considering the yield-related traits and presence of anthocyanin (Figure 2).

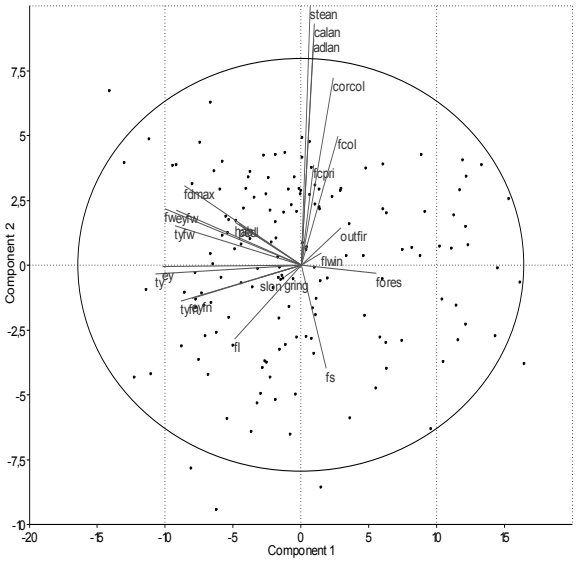

Figure 2. Plot of the distribution of traits and F_2 progeny according to the first two PCA components.

***QTL identification*.**

A total of 112 QTLs (of which 68 can be considered as major QTLs) were identified and mapped on 11 chromosomes (only chromosome 9 had no QTL) by phenotyping 23 traits in the two locations; 61 QTLs (of which 36 major) were identified in ML and 51 (32 major) in MT. Of all the major QTLs, 26 were in common between the two environments, 8 QTLs were found only in ML, 2 were found only in MT, 2 were found as major in ML and present as minor in MT and, finally, 4

were found as major in MT and present as minor in ML (Table 2). For each trait, from 1 to 4 major QTLs were identified. The major QTLs identified had LOD values ranging from 4.8 (fcpri) to 93.6 (gring) with a PVE of 12.8% and 93.7% respectively; for all the key yield-related traits (total and early yield, number and weight of fruits) high values of PVE were also detected. Clusters of QTLs were found in almost each chromosome. The high values of inter-trait Spearman correlations (data not shown) between some traits suggest that their strictly correlation; as a result, the major QTLs controlling them are either closely located in the same chromosomic regions or coincident. Chromosome E02 contains a big cluster of QTLs related to early and total yield, and to many fruit features (weight, length, diameters, peduncle length and firmness), encompassing a region spanning 12 cM and containing also the QTL for the resistance to *F. oxysporum,* as well as the resistance locus *Rfo-sa1* introgressed from *S. aethiopicum* (Toppino et al. 2008). Chromosome E12 contains clusters of QTLs for early and total yield, average weight, fruit weight, diameters, fruit shape, number of locules and number of flowers/ inflorescence.

On chromosome E08, a cluster of QTLs for leaf prickles, fruit shape, fruit length and peduncle length is positioned just at the beginning of the chromosome. Finally, cluster of coincident major QTLs for all traits for anthocyanin pigmentation (except for corcol in ML) mapped to a site on chromosome E10. Moreover, chromosome E05 harbored coincident major QTLs responsible for the presence of anthocyanins in both locations. All the positive alleles for increased anthocyanin content derived from '67/3'.

Trait	QTLs				LOD/PVE	Chromosomes
	ML		MT			
	Total	Major	Total	Major		
ty	1	1	1	1	25.6/ 53.0	**2**
tyfn	1	1	1	1	24.8/51.9	**2**
tyfw	5	2	3	2	20.4/31.1	**2**, 3, 8, 11, **12**
ey	1	1	2	1	24.2/51.3	**2**
eynf	1	1	2	1	20.6/45.6	**2**
eyfw	5	2	4	1	23.2/35.9	**2**, 3, 8, 11, **12**
fw	3	1	4	2	22.0/40.0	**2, 3**, 4, **12**
fl	6	4	3	3	12.4/17.8	**1, 2, 3**, **7**, 8, **11**
fdmax	5	2	2	2	19.4/30.2	**2, 3**, 7, 11, **12**
fs	7	2	6	3	20.0/28.2	1, **3,** 4, **7**, 8, **11**, 12
pedl	5	3	3	2	9.9/17.1	1, **2a, 2b, 4**, 8
fcpri	1	1	1	1	4.8/12.8	7
slon	1	1	1	1	9.2/23.9	**12**
gring	1	1	1	1	93.6/93.7	8
hab	3	3	1	1	5.4/14.2	**1, 8, 10**
flwin	1	1	1	1	6.8/18.2	**12**
corcol	1	1	2	1	34.1/63.7	**5**, 10
adlan	2	1	4	1	45.0/60.9	5, 6, 8, **10**
stean	2	2	3	2	36.6/53.6	2, **5, 10**
calan	4	2	3	1	52.2/74.1	**5**, 6, 8, **10**
outfirm	4	2	2	2	7.3/14.6	**1, 2**, 3, **5**
fcol	3	2	-	-	42.2/64.1	**5a, 5b**, 8
fores	1	1	1	1	51.7/69.2	**2**
Total	61	36	51	32		11

Table 3. Number of total and major QTLs identified in the two locations (ML and MT), the maximum LOD and PVE (Percentage of Variance Explained) values scored for the QTL of each trait, and chromosomes involved (in bold the chromosomes carrying major QTLs).

Candidate gene identification based on evaluation of synthenic regions with tomato.

Search for genes and transcription factors in the tomato syntenic regions allowed to identify the gene encoding a chalcone synthase (*CHS*) located on T5, and the genes encoding UDP glucose anthocyanidin 3-0 glucosyltansferase (*3GT*), *an2* and *ant1* located on T10 in orthologous regions on chromosome E10 harbouring QTLs for stean, calan and adlan QTLs. Moreover, regions harbouring QTLs for fruit shape and dimensions and weight in tomato are syntenic with regions controlling the same traits in eggplant. the tomato QTL *fw2.4* (Grandillo *et al.* 1999) on chromosome T02, the QTLs *fw3.2* (Zhang et al. 2012) and *fs3*.a on T3, the *sun* gene on T07 (Xiao et al. 2008), the QTL *fs8.1* (Grandillo et al. 1996) on T08 and the genes *FASCIATED* (*FAS*) and *fw11.3* (Huang and Knaap 2011) on T11 appear to be respectively orthologous to eggplant regions positioned on chromosomes E02, E03, E07, E08 and E12 where several QTLs for fruit dimensions and weight clustered. In the same region, also Doganlar et al. (2002) identified the QTL *fs7.1* controlling fruit shape while Frary et al. (2003) the QTL involved in fruit set. Finally, the QTL for the green ring revealed homology with the tomato sequence corresponding to a ferredoxin family protein whose hortologous in *Arabidopsis* is known to play a role in Photosystem I (Voss et al. 2011) and thus, in chlorophyll production.

Conclusions

Our intraspecific segregating population derived from two highly contrasting parents for agronomically key traits coupled with their SNP-base linkage map allowed to locate, for the first time in eggplant, several major QTLs related to yield (early and total) and its components (number and weight of fruit), to the fruit dimension and shape as well as to other important characteristics such as fruit firmness, number of seed locules, fruit peduncle length, prickles and anthocyanin content in different plant organs and plant growth habitus. Major QTLs clustering for different traits were evidenced. The high value of LOD scores, the elevated percentage of variability explained and the stable chromosomal localization of the discovered QTLs in the two locations make them both suitable for dissecting these genetically complex traits in *S. melongena* and promising to apply molecular breeding approaches to develop better-adapted eggplant varieties. The survey of the tomato genome allowed the identification of putative orthologous candidate genes and transcription factors, which represent potential additional genomic resources for marker assisted selection programs and for further synteny studies with tomato and other solanaceae species.

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QTL mapping of thrips resistance and metabolites in pepper (*Capsicum* spp.)

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Introduction

Thrips are small insects but may cause big losses in pepper cultivation. Direct damage results from feeding on leaves, flowers and fruits, while indirect damage is caused by the transmission of viruses, especially TSWV. Earlier we developed bioassays for resistance to two thrips species (*Frankliniella occidentalis* and *Thrips parvispinus*) and showed that non-choice, detached leaf and leaf punch assays correlated well with whole-plant assays. Based on both whole-plant assays and detached leaf and leaf punch assays we identified several accessions with strong and medium resistance to these species (Maharijaya et al, 2011). The strong resistance in two *Capsicum annuum* and one *C. baccatum* accessions was shown to reduce oviposition by adult *F. occidentalis* females and to almost completely block the development of L1stage larvae into L2 and further stages (Maharijaya et al, 2012; also a poster in this meeting).

Materials and Methods

We obtained an F2 population derived from the cross between one of these resistant accessions (*C. annuum* Ac1979) as female parent and the susceptible *C. chinense* 4661 as male parent. Both accessions were obtained from the Centre of Genetic Resources, the Netherlands. The F2 population consisted of 196 individuals. We constructed a linkage map with 170 AFLP and SSR markers, spanning 1630 cM. Linkage groups were assigned to physical chromosomes based on SSR markers corresponding to previously published maps (Yi et al. 2006; Lee et al. 2009; Wu et al, 2009; A. Palloix, pers. comm.). In this F2 population we assessed resistance to *F. occidentalis* using detached leaf assays, in which we scored leaf damage and the survival of L1 larvae to the L2 and pre-pupal stages.

Results and Discussion

Also we used GC-MS and LC-MS for untargeted metabolite profiling on young, fully opened leaves of unchallenged plants. Altogether we detected 729 distinct metabolites, after grouping the mass peaks into “centrotypes” based on retention time and intensity pattern over the samples (Tikunov et al. 2011).

We found one single highly significant resistance QTL which affected all three resistance parameters. The maximum LOD score for this QTL varied between 18.7 and 21.3 for the three parameters.

Of the 729 detected metabolites 275 (38 %) segregated in the F2 population. For the detection of metabolite QTL a LOD threshold of 3.6 was applies. This resulted in the detection of at least one QTL for 242 (88 %) of these 275 segregating metabolites. Some of these metabolites overlapped with the resistance QTL indicating a possible role in the resistance.

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Proteomic analysis of cytoplasmic male sterility in pepper

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Abstract

With the purpose to explore the molecular mechanism of cytoplasmic male sterility (CMS) in pepper (*Capsicum annuum* L.) at the proteome level, we examined the anther proteomes of a cytoplasmic male sterile line FS1030A and its maintainer line FS1030B, at the abortive stage occurring before tetrads came into being, which were determined by Transmission Electronic Microscopy (TEM) technique. Two-dimensional difference gel electrophoresis (2D-DIGE) and LC-MS/MS analysis were employed to determine the differentially expressed proteins. About 1070 anther protein spots were detected for each gel, and 13 were expressed differently in FS1030A and FS1030B. Six proteins showed up-regulated expression and seven proteins showed down-regulated expression in FS1030A compared to FS1030B. Eight out of these thirteen proteins were identified which matched with Cu/Zn superoxide dismutase, glutathione S-transferase, ketol-acid reductoisomerase of capsicum. And most of them were associated with metabolism and stress resistance. These findings can provide a good basis for further studies of CMS in pepper.

Keywords: Pepper, cytoplasmic male sterility(CMS), 2D-DIGE

Introduction

Pepper (*Capsicum annuum* L.) is an important vegetable worldwide, often cross-pollinated, having significant redominant heterosis. Cytoplasmic male sterility (CMS) can improve seeds production efficiently, and have been used in many crops and vegetables abroad, including pepper. While the molecular mechanism of CMS in pepper is still not clear, several CMS-specific DNA markers have been developed [1-4], but their application is limited. The cooperation of genes in nuclear and cytoplasm results in the CMS trait. However, we still cannot determine how many genes are working. With this purpose, we applied 2D-DIGE coupled to LC-MS/MS spectrometry to identify the key proteins while CMS occurring to illuminate the molecular mechanism of pepper CMS at the proteome level.

Materials and Methods

Plant materials and anther preparation

FS1030A, a cytoplasmic male sterile line and its maintainer line FS1030B were grown in plastic tunnel in the farm of Beijing Vegetable Research Center, Beijing Academy of Agriculture and Forestry Sciences in spring.

Transmission Electronic Microscopy (TEM) technique was used to observe the pollen abortive stage. According to continuous observed results from spring 2010 to spring 2011, the abortion was found to occur at the early stage of tetrad. Buds with the youngest leaves on sides half unfolded and calyx wrapping corolla tightly, corresponding to the early stage of tetrad, were picked out for anther isolated for 2D-DIGE analysis. The chosen anthers were immediately frozen in liquid nitrogen and then stored at -80℃ refrigerator for further analysis.

Protein samples preparation

Anther proteins were extracted using a trichloroacetic acid (TCA)-acetone protocol, according to Sheoran et al. [5] with some modifications. Frozen anthers were powdered in liquid N_2 for 15 min, and homogenized with 15 μl chilled TCA/acetone at -20 ℃ and 300 μl β-ME, and the mixture was centrifuged at 13000 rpm for 20 min at 4 ℃. After two washes with acetone, the pellets were freeze-dried and proteins were extracted with buffer containing 50 mM Tris base, 2% SDS, HCl (pH 8.0), deionized water, and 2% β-ME, by vortexing at room temperature. Then 30% sugar and saturated phenol (PH 8.0) were added and vortexed well. The phenol phase was recovered by centrifugation at 13000 rpm for 20 min at 25 ℃ and re-extracted once. The proteins were precipitated more than 60 min at -20 ℃ with 5 times volume of cooled ammonium acetate/methanol at -20° and recovered by centrifugation at 13000 rpm at 4°. Then after 3 washes with acetone, the proteins were solubilized in rehydration buffer which containing 7 M urea, 2 M thiourea, 4% w/v CHAPS, 65 mM DTT, 0.2% w/v Bio-Lyte, 0.001% bromophenol blue. The supernatants were collected and were centrifuged for 70 min at 13000 rpm to remove particulate materials. The protein concentrations were determined by the Bradford method (Bio-Rad), and confirmed by SDS-PAGE. Protein samples were stored at -80°.

Cyanine dye labeling

Both sample of FS1030A and FS1030B had three replicates to reduce the biological variation. The pH of protein samples were adjusted to 8.5. FS1030A and FS1030B proteins were randomly labeled with Cy3 and Cy5, while the internal standard was prepared by pooling a mixture of equal amounts of the six protein extracts and labeling with Cy2, using 400 pmol of fluorochrome/50 μg of protein. Labeling was performed for 30 min on ice in the dark. Then reactions were quenched by incubating with 1μl of 10 mM lysine on ice in the dark for 10 min.

2-DE

Fifty microgram of each Cy3- and Cy5-labeled protein samples of FS1030A and FS1030B were combined with 50 μg Cy2-labeled internal standard. Then an equal volume of 2× sample buffer (20 mg DTT, 20 μl ampholyte/1ml) was added to the sample. After homogenizing and centrifuging at 12000 rpm for 30 s, labeling was performed on ice and in the dark for 10 min. The volume was made up to 450 μl by adding rehydration buffer (45 mg DTT, 50 μl ampholyte/5ml). Then for the isoelectrofocusing step (IEF), IPG strips (24cm, pH 3-10, GE Healthcare) were rehydrated with labeled samples in the dark overnight in the rehydrated buffer. The first-dimension isoelectrofocusing (IEF) was performed using an Ettan IPGphor System (GE Healthcare) for a total of 80 kV- h at 20℃. Prior to the second dimension analysis (SDS-PAGE), the strips were first treated with equilibration buffer (6M urea, 2% SDS, 30% glycerol, 50 mM Tris-cl, pH8.8, and 1% w/v DTT) for 15 min for reduction, and then treated with the same solution containing 4% iodoacetamide instead of DTT for another 15 min for alkylation. The IPG strips were over layered onto 12% homogeneous polyacrylamide gels precasted with low-fluorescent glass plate and

electrophoresed for 20 mA/gel using an Ettan DALT Twelve caster. The samples images of Cy2, Cy3 and Cy5-labeled were scanned on the Typhoon 9410 scanner (GE Healthcare) with excitation/emission wavelengths specific for Cy2, Cy3 and Cy5 of 488/520, 532/580, 633/670 respectively.

DIGE analysis

DeCyder 6.5 software (GE Healthcare) was used to analyze the DIGE images for relative protein detection and quantification between FS1030A and FS1030B. Protein spot detection and matching between gels were performed automatically and manually confirmed. Only the spots presented in both gels of at least two out of three replicate experiments and presented in all gels of Cy2-labeled internal standard were considered. Student's t-test ($p<0.05$) was used to detect the significant differences of spot relative abundances in anthers of CMS FS1030A compared with FS1030B. Significant protein spots with a 1.5-fold change were selected and retained for further analyses.

In-gel Digestion

Protein spots were excised from preparative gels and destained in 50% acetonitrile (ACN) and 25 mM ammonium bicarbonate. After vibrating for 20 min, the supernatant was discarded. Then the gel fragments were dehydrated with 100% ACN and digested with 0.01 μg/μl trypsin at 4 ℃ for 30 min and digested in 25 mM ammonium bicarbonate at 37 ℃ for at least 15 h. The peptides were extracted by digesting the gels with 5% TFA at 40 ℃ for 1 h, ultrasonic processing for 3 min and subsequently incubating in 50% ACN, 2.5% TFA at 30℃ for 1 h, ultrasonic processing for 3 min. All supernatants were pooled and lyophilized completely and stored at -20 ℃.

LC-MS/MS

Tryptic digests were solubilized in solvent A (5% v/v acetonitrile, 01% v/v formic acid), and 19 μl were injected into a nanoLC-MS/MS Ultimate system (Dionex) interfaced on-line to a linear ion trap LTQ (Thermo Finnigan^{TM}). Peptides were eluted using a 20-40% linear gradient of solvent B (80% v/v acetonitrile, 0.1% v/v formic acid) in first 90 min, and then the mobile gradient was changed into 40-100% of solvent B in 15 min, following 100% of solvent B for 10 min. Then the next sample was eluted. Mass spectra were acquired using the data dependent acquisition, under the relative collision energy of 35%, with a spray voltage of 1.8 kV. Mass spectral analysis consisted of a full MS and following 5 MS/MS, and the most intense 5 ions were selected for analysis for each MS spectrum, and a dynamic exclusion window was applied during the analysis.

Protein identification

The spectra were processed using Bioworks 3.1 software (Thermo Finnigan^{TM}) and analyzed by MASCOT version 2.3 software (Matrix Science Ltd). Tandem mass spectra were searched against the NCBI nonredundant database (*Capsicum*-August, 2012). The search parameters were as following: the enzyme semitrypsin; a fixed modification, carbamidomethyl (cysteine); variable modifications oxidation (methionine); monoisotopic mass values; unrestricted protein mass; 0.8 Da and 0.5 Da for peptide and fragment mass tolerance, respectively; one missed cleavage; instrument type, ESI-TRAP. A minimum of two peptide match and the protein with a Mascot score above 70 was considered identified.

Results

The TEM technique showed that the abortion occurred before tetrads coming into being, and two kinds of main structural changes in the tapetum were observed in CMS FS1030A. First, during the meiosis of microspore mother cells (MMCs), some of the tapetal cells were enlarged, elongated and developed into multilayer cells. They squeezed the MMCs excessively, which narrow the powder chamber much. The MMCs degenerated, vanished and failed to develop into tetrads, eventually leading to microspore abortion. Second, some of the premature tapetal cell layer degenerated, and then the remnants of the collapsed tapetum and crushed MMCs formed a visible dense belt in the anther locule. The MMCS presented irregular shapes, and eventually degenerated for lack of nutrition.

The main purpose of this study was to estimate the diversity of anther proteome between a CMS line FS1030A and its maintainer line FS1030B, while the abortion occurring. We used 2D-DIGE to generate the protein expression profiles of two samples (Fig 1). Equal amounts of two protein samples labeled wih Cy3 and Cy5, respecively, were analyzed on the same gel, together with a pooled sample as an internal standard labeled with Cy2. Each sample had three replicates that were loaded on three different gels. The protein expression patterns of two samples were similar, and up to 1070 matched spots were detected in our gels using the Decyder 6.5 (GE) software.

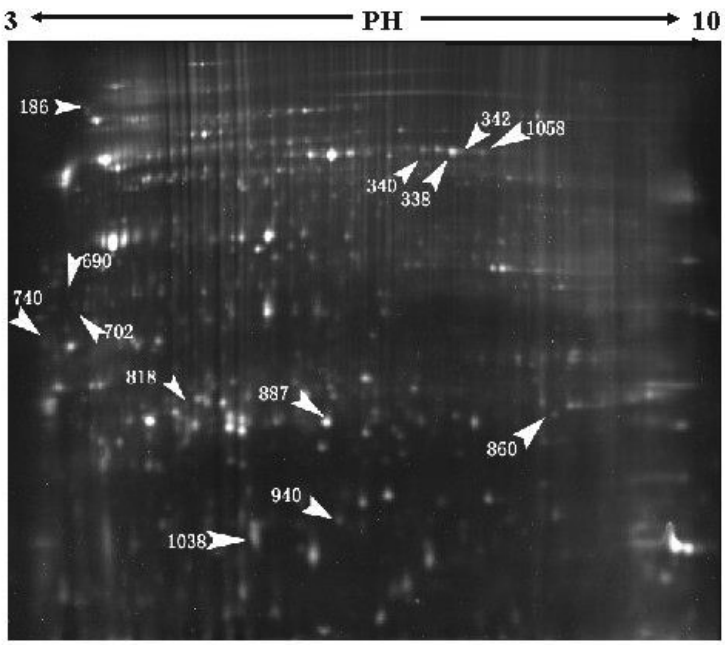

Fig1. DIGE image of interior label (labeled with Cy2) FS1030A (labeled with Cy3, green) and its maintainer line FS1030B (labeled with Cy5, red) anther separated with IPG 3-10 strips. Green spots reveal highly expressed proteins in FS1030A and red spots in FS1030B anthers, the bright white spots represent equal protein levels. The results correspond to the analysis of Decyder 6.5(GE).

Quantitative analysis resulted on 13 spots significantly variable and above the 1.5-fold change threshold, including 6 up-regulated spots and 7 down-regulated spots in FS1030A anthers compared with their levels in FS1030B anthers.

All the 13 spots were excised from the preparative 2D gels and sequenced by LC-MS/MS. Information including spot number, PI/MW, sequence coverage, protein name and other data of all 13 spots are listed in Table 1. Eight protein spots out of thirteen were successfully identified. Several proteins were identified from more than 1 spot such as putative branched-chain alpha-keto acid dehydrogenase E3 subunit (spots 342 and 1058), glutathione S-transferase (GST) (spots 860 and 887), suggesting that these spots may have relation with post-translational modifications of proteins. The proteins identified were mainly corresponding to branched-chain alpha-keto acid dehydrogenase E3 subunit, Cu/Zn superoxide dismutase, glutathione S-transferase, lipoamide dehyelrogenase (LPD), ketol-acid reductoisomerase (KARI), and actin. Their functions were chiefly about participation in cell signal transduction, amino acid biosynthesis and stress resistance.

Discussion

Although for *Capsicum* the genome information is still not available and the protein database capacity is limited, in our research eight out of thirteen (61.5 %) distinctly differential spots were identified using NCBI protein database of capsicum, instead of other plant species or *Capsicum* EST data. It is different from former proteomic research of plants [6-9]. More accurate protein information can be obtain by using the same species protein database; indeed 8 out 13 proteins were identified using *Capsicum* protein database, and in our opinion, results were better than those obtained by using NCBI protein database of Green Plants. The protein information what we got is exact.

Three enzymes were down-regulated in FS1030A anthers, relative to FS1030B, including LPD, KARI and branched-chain α-keto acid dehydrogenase E3 subunit. LPD is a subunit of several multienzyme complexes which includ the brantched-chain α-ketoacid dehydrogense consisting of three protein components, E1, E2 and E3[10]. Thus, LPD and branched-chain α-keto acid dehydrogenase E3 all play crucial roles in different metabolic pathways and control carbon flow in the citric acid cycle. The branched-chain α-ketoacid dehydrogense complex in *Arabidopsis* has been suggested as an alternative carbon energy source, important for the degradation of branched-chain amino acids during stress-induced sugar starvation[11,12]. KARI is the key enzyme which catalyzes amino acid synthesis[13]. The decreased activity of three important enzymes in CMS line FS1030A anther may cause male sterility.

Other three proteins with higher spot volume in FS1030A, compared to FS1030B, were actin, GST (spots 860 and 887), and Cu/Zn superoxide dismutase. Recently many studies suggest actin has important functions in the cytoplasm and nucleus and controls various cellular functions and organisme development [14,15]. GST has been proved to play important roles in plant stress resistance[16,17]. Cu/Zn superoxide dismutase is important for early metabolic cellular defense against stresses [18]. Increased stress resistance indicates some abnormal events causing male sterility were occurring in FS1030A anther.

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Table 1 Identification of differently expressed proteins by MS/MS

Spot number	Accession	PI/MW(kDa)[c]	Sequence Coverage(%)	MASCOT Score	Description
186↑	gi|83423376	4.96/ 32.3	25	100	actin [Capsicum chinense]
338↓	gi|44662784	6.71/53.3	31	188	lipoamide dehydrogenase [Capsicum annuum]
340↓	gi|193290660	6.50/63.3	63	384	putative ketol-acid reductoisomerase [Capsicum annuum]
342↓	gi|193290670	6.38/53.9	44	200	putative branched-chain alpha-keto acid dehydrogenase E3 subunit [Capsicum annuum]
690↓	gi|171854659	5.77/55.0	25	69	putative aspartic protease [Capsicum chinense]
702↓	gi|83423376	4.96/32.3	25	52	actin [Capsicum chinense]
740↑	gi|171854657	4.98/80.2	7	56	putative Hsp90-2 [Capsicum chinense]
818↑	gi|395434121	5.97/98.7	19	34	glycoside hydrolase family 3 protein [Flavobacterium sp. F52]
860↑	gi|224708780	5.41/24.9	64	258	glutathione S-transferase [Capsicum annuum]
887↑	gi|224708780	5.41/24.9	40	77	glutathione S-transferase [Capsicum annuum]
940↓	gi|395435209	4.97/12.1	8	39	regulator of chromosome condensation, RCC1 [Flavobacterium sp. F52]
1038↑	gi|171854653	5.13/15.3	67	760	putative Cu/Zn superoxide dismutase [Capsicum chinense]
1058↓	gi|193290670	6.38/53.9	33	174	putative branched-chain alpha-keto acid dehydrogenase E3 subunit [Capsicum annuum]

Accession: NCBI accession number.
PI: Theoretical isoelectric point.
Sequence coverage (%): the percent of the matched peptide in relation to the full-length sequence.

SESSION III

Genetic resources

Capsicum germplasm bank maintained by EMBRAPA Vegetables, Brazil

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Abstract

Brazil is the most important center of genetic diversity for the genus *Capsicum.* Some species are considered to occur exclusively in Brazil and this emphasizes the need to adequately maintain the germplasm *in situ* and/or *ex situ.* Embrapa Vegetables' *Capsicum* germplasm bank was established in 1980 and today it has over 4,000 accessions, being the largest and most diverse *Capsicum* collection in Brazil, with domesticated species, semi-domesticated, and wild species. This bank provides the genetic base for a large breeding program implemented by Embrapa, which has released several open-pollinated (OP) and hybrid cultivars for both small-scale farmers as well as for the large entrepreneurs. The *Capsicum* breeding program has a multidisciplinary and multi-institutional team; activities include germplasm collection; conservation and documentation; morphological, molecular, cytogenetic, disease resistance, and nutritional characterization and development of inbred lines, OP, and hybrid cultivars with a focus on disease resistance. Collection expeditions have taken place in the Amazon and the Atlantic Forest (Eastern Brazil) where wild populations with high extinction risk were thoroughly sampled. During the last twelve years, 1112, 627, and around 300 accessions were characterized for morphological, molecular, and disease resistance characteristics, respectively. Knowledge and information generated by characterization have been organized in an Access software-based databank. The *Capsicum* germplasm collection has presented broad genetic variability for fruit and plant traits such as color and shape, aroma, pungency, multiple disease resistance, and plant architecture. Novel work includes the evaluation of volatiles as well as processing characteristics, the later in collaboration with the private sector. This variability has contributed to the development of new cultivars and hybrids adapted to Brazilian conditions and market niches, with superior performance.

Keywords: Variability, characterization, conservation.

Introduction

The conservation and characterization of genetic resources of *Capsicum* spp. has been strategic for obtaining superior individuals that combine desirable traits for different segments of the production chain (Ribeiro and Reifschneider, 2008). By the 1940´s, the centers of origin of domesticated species were considered unlimited sources of genetic variability. The expansion of the agricultural frontier and the indiscriminate use of land, without concern for preserving the environment and genetic resources for future generations, can lead to the extinction of many wild relatives of cultivated species, including the genus *Capsicum* (Ribeiro, 2000). The possibility of a decrease in the genetic diversity within a species and related wild species has led the scientific community to advocate for the maintenance of genetic resources of different plant species. Currently, most of the public and private national and international research institutions maintain a germplasm bank or collection (Ribeiro, 2000).

Genetic resources of a domesticated species include wild species, landraces and special types, populations, inbred lines or OP varieties, obsolete varieties, and hybrids (Nass et al. 2012). Plant breeders usually appeal to the germplasm bank to generate new cultivars, which are more productive, disease resistant, with higher nutritional quality or with other characteristics of interest.

In Brazil, the most important *Capsicum* germplasm collection is maintained by EMBRAPA, Embrapa Vegetable Crops and Embrapa Genetic Resources and Biotechnology (Embrapa Cenargen), in Brasilia, D.F. The germplasm collection has a bit over 4,000 entries represented by OP varieties, inbred lines, hybrids, populations, and landraces of the five cultivated species, and dozens of semi-domesticated and wild species. The regeneration of this germplasm is periodically done, and this is critical for the safe conservation of Capsicum species that risk extinction. Morphological, molecular, and disease resistance characterizations are important because they identify duplicates and generate a database useful to breeders. Collection of wild species and germplasm exchanged among national and international institutions are also strategic procedures to maintain the variability of the genus.

Materials and Methods

Enrichment of germplasm bank

Enrichment of the *Capsicum* genebank was made through exchange of genotypes with national and international research institutions, by collection, and by expeditions in the Amazon and in Atlantic Forest (Eastern Brazil), where wild populations are at high risk.

Multiplication of accessions

The multiplication of *Capsicum* spp. accessions was performed simultaneously under greenhouse conditions at Embrapa Vegetables, Brasilia, Brazil. Five plants of each accession were grown using standard growing practices. Around 100 accessions were multiplied and characterized each year. Self-pollinated and O.P. seeds were harvested and kept separately. Seeds were manually extracted, washed in water, pre-dried at 32^{o}C for 48 hours, dried in an incubator at 40^{o}C for 48 hours, packaged in aluminum foil bag and stored in a cold storage chamber at 4^{o}C. Around 400 accessions are maintained at -20^{o}C in a basic collection (Colbase) by Embrapa Cenargen. A bar code system was developed for documentation, coding and control of seed stock of the germplasm bank. From 2000 to 2012, 1158 accessions including different *Capsicum* species (domesticated, semi-domesticated and wild species) were multiplied.

Morphological characterization

Morphological characterization was performed according to the *Capsicum* descriptors (IPGRI, 1995) using a total of 56 descriptors, as follows: 18 for vegetative part, 13 for inflorescence traits, 22 for fruit and 3 for seeds. Descriptors pungency, aroma and fruit position were also added because they are important for the breeding program. Different species were identified using a classification key for domesticated species, domesticated and semi-domesticated varieties of *Capsicum* genus occurring in Brazil (Ribeiro et al. 2008).

Disease resistance characterization

Around 300 *Capsicum* spp. accessions were evaluated for resistance to most important pathogens that affect pepper production in Brazil: bacterial wilt (*Ralstonia solanacearum* biovars 1 and 3), bacterial spot (*Xanthomonas campestris* pv. *vesicatoria*), phytophthora root rot (*Phytophthora capsici*), Tospovirus (Groundnut Ring Spot Virus - GRSV, Tomato Spotted Wilt Virus - TSWV, Tomato Chlorotic Spot Virus - TCSV), Potyvirus (Pepper Yellow Mosaic Virus - PepYMV, Potato Virus Y - PVY) and powdery mildew (*Oidiopsis taurica*).

Molecular characterization

Molecular characterization was performed for 627 accessions using 55 RAPD (Random Amplified Polymorphic DNA) markers developed by Embrapa Cenargen (Buso et al. 2003). RAPD markers were used for the analysis of genetic similarity among accessions, and the similarity matrix was made based on Jaccard and agglomerative hierarchical analysis by UPGMA.

Documentation

Data of characterization, passport information and a digital photo of each accession have been organized in an Access software-based databank.

Results and Discussion

Brazil is an important center of diversity of the genus *Capsicum* and has the largest number of wild *Capsicum* species, which emphasizes the need to properly maintain this genetic diversity in a germplasm bank. More than 150 accessions of domesticated and semi-domesticated species were collected in the Amazonian region, and 50 accessions of wild species were collected in the Atlantic Forest. Accessions of *C. chinense* collected in the Amazonian region showed huge diversity of fruit shape, positions, color, size and degree of pungency. Many genotypes exhibited characteristics of interest and, therefore, have high potential for immediate use in the breeding program. The Amazon basin is the diversity center of *C. chinense* species (IBPGR, 1983). Three new *Capsicum* species were recently described by Barboza and Bianchetti (2005): *C. pereirae*, *C. friburgense* and *C. hunzikerianum* (Fig.1). These species are from the East coast Atlantic forest.

In the past twelve years, 1158 accessions of the *Capsicum* germplasm bank of Embrapa Vegetable Crops were multiplied by self-pollination and from these, 1112 were characterized morphologically. Out of these, 432 were classified as *C. annuum* L. var. *annuum*, 118 as *C. baccatum* var. *pendulum* (Wild.) Eshbaugh, 428 as *C. chinense* Jacquin, 101 as *C. frutescens* L., 1 accession as *C. pubescens* Ruiz & Pavon, 28 accessions as semi-domesticated and 4 accessions as wild species (Table 1). Morphological characterization has allowed the identification of similar accessions and genetic variability within each species. The use of morphological descriptors has also been crucial to the registration and protection of cultivars, for the establishment of a core collection, development of base populations, besides adding value to conserved germplasm that could be used in the breeding program.

Molecular markers have been useful tools in assessing the genetic diversity of germplasm banks. The genetic similarity analysis performed on 627 accessions organized these accessions into four major groups, subdivided into species by genetic similarity (Buso et al. 2003). Accessions of the cultivated species *C. annuum*, *C. baccatum*, *C. chinense* and *C. frutescens* were grouped according to their respective classification. Groups of *C. chinense* and *C. frutescens* were closer to the group of *C. annuum* than accessions of *C. baccatum*. Wild species have formed a separate group with about 30% similarity with cultivated species. The classification of most wild accessions corroborates the morphological classification, i.e., *C. flexuosum* was closest to cultivated species.

For disease resistance, 328 accessions were evaluated for resistance to *Ralstonia solanacearum,* 329 for *Xanthomonas campestris* pv. *vesicatoria*, 307 for *Phytophthora capsici*, 273 for *Leveillula taurica* and 399 for different virus (Tospovirus and Potyvirus) (Table 1). Sources of resistance or tolerance to *Ralstonia solanacearum* (Lopes and Quezado-Duval, 2001; Lopes and Boiteux, 2004) and to *Xanthomonas campestris* pv. *vesicatoria* (Lopes and Quezado-Duval, 2001) and Phytophthora root rot (Ribeiro et al. 2003) were identified in accessions of domesticated species including *C. annuum*. Accessions of *C. frutescens* had good levels of resistance to viruses (Lima et al. 2011). Accessions with resistance to one or more pathogens and from different species of *Capsicum* were successfully incorporated to the breeding program. In the past twenty years, a significant number of OP and hybrid cultivars were released: a bell pepper with resistance to *Cercospora* (cultivar Tico), three OP cultivars of typically Brazilian hot peppers (BRS Seriema, BRS Mari and BRS Moema) and three Jalapeño cultivars developed in partnership with processing companies (BRS Ema, BRS Garça, BRS Sarakura) (Reifschneider and Ribeiro, 2012). Moreover, several lines resistant to different pathogens were made available to the national and international research institutions. Examples include: CNPH 148 (resistant to *Phytophthora* root rot), CNPH 703

(resistant to several species of *Xanthomonas* spp; Poulos et al. 1991) and CNPH 679 (resistant to Tospovirus), which have been used by public and private breeding programs in Brazil and abroad.

The information generated by the characterization has been organized in an Access software-based databank (Fig. 2). The germplasm presents broad genetic variability for plant and fruit traits such as plant architecture, multiple disease resistance, color, shape, aroma, and pungency.The availability of characterization data (morphological, molecular and disease resistance) in a database has facilitated the access to the genetic diversity maintained in the *Capsicum* genebank by breeders and to select desirable genotypes for the breeding program. This documentation is available on the website http://www.cnph.embrapa.br/paginas/servicos/banco_germoplasma_capsicum.htm.

Novel research lines have been incorporated to the *Capsicum* program like characterization for volatile compounds related to aroma, vitamin C, oleoresins, anthocyanin and antioxidant activity in the fruits. A new project has been initiated and focuses on germplasm characterization for resistance to arthropods and nematodes and the establishment of a core collection of *C. frutescens*.

Table 1. Number of accessions of Embrapa Vegetables' *Capsicum* germplasm bank conserved and with morphological, molecular and disease resistance characterization (2000 to 2012).

Species	Conservation	Characterization						
		Morphol.	Mol.	Disease resistance				
				RS	XCV	PC	LT	Viruses
C. annuum L. var. *annuum*	432	432	354	207	210	209	206	208
C. baccatum var. *pendulum* (Wild.) Eshbaugh	118	118	62	39	37	38	9	37
C. chinense Jacquin	428	428	96	57	57	35	36	76
C. frutescens L.	101	101	101	24	24	24	21	59
C. pubescens Ruiz & Pavon	1	1	-	-	-	-	-	-
C. annuum var. *glabriusculum* (Dunal) Heiser & Pickersgill	10	10	1	1	1	1	1	1
C. baccatum L. var. *baccatum*	11	11	-	-	-	-	-	11
C. baccatum L. var. *praetermissum* (Heiser & Smith) Hunziker	7	7	3	-	-	-	-	7
C. flexuosum Sendtner	1		1	-	-	-	-	-
C. villosum Sendtner var. *villosum*	4		4	-	-	-	-	-
C. dusenii Bitter	2		2	-	-	-	-	-
C. buforum Hunziker	2		2	-	-	-	-	-
C. parviflorum Sendtner	1	1	-	-	-	-	-	-
C. campylopodium Sendtner	1	-	1	-	-	-	-	-
C. chacoense Hunziker	15	-	-	-	-	-	-	-
C. hunzikerianum Barboza & Bianchetti	1	1	-	-	-	-	-	-
C. friburgense Bianchetti & Barboza	1	1	-	-	-	-		-
C. pereirae Barboza & Bianchetti	1	1	-	-	-	-	-	

RS= *Ralstonia solanacearum* biovares 1 e 3 , XCV= *Xanthomonas campestris* pv. *vesicatoria*, PC= *Phytophthora capsici*, LT= *Leveillula taurica*, Viruses= Potyvirus (Pepper Yellow Mosaic Virus - PepYMV, Potato Virus Y - PVY), Tospovirus (Groundnut Ring Spot Virus - GRSV, Tomato Spotted Wilt Virus - TSWV, Tomato Chlorotic Spot Virus - TCSV).

Figure 1. Three new species identified.

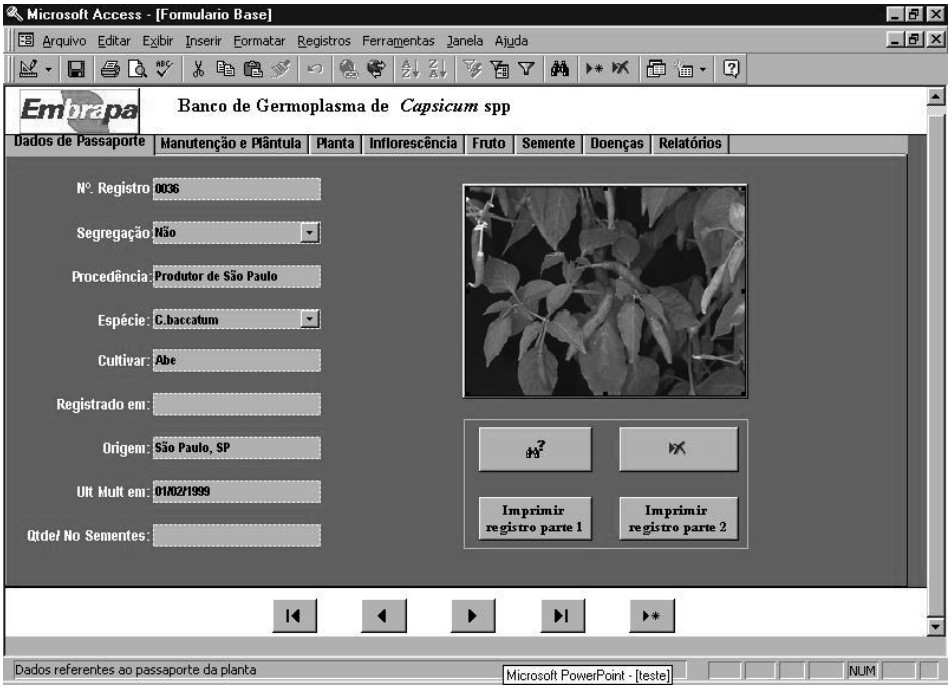

Figure 2. Characterized *Capsicum* germplasm is organized in an Access software-based databank.

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Wild Capsicums: identification and *in situ* analysis of Brazilian species

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Abstract

Capsicum is well-known for its cultivated species, spices and vegetables consumed worldwide, but it also includes ca. 30 wild species native from Central and South America. In order to gain knowledge about wild *Capsicum* species, to compare fruiting and flowering features with cultivars, and to characterize their natural habitat, three field trips took place to the Brazilian Mata Atlantica.

The campaigns took place in February and June 2011 and April-May 2012, for which at least nine endemic wild Capsicums were found; populations of uncertain species identity were also observed. The species differ considerably from the cultivars and even from wild taxa from W-NW South America. Some features that are worth to highlight are (i) the dimensions that the plants can reach (woody shrubs up to 3-4 m tall, e.g. *C. hunzikerianum* and *C. schottianum*), (ii) the variability of the corolla pigmentation in some species (e.g. *C. schottianum*), and (iii) the different levels of fruit pungency.

Most findings were located inside protected areas while outside those areas the findings were sporadic. The progressive anthropization of the territory and the intensive use of the land have reduced the natural habitat of the wild species, narrowing their areas of distribution. As a consequence, the conservation status of the Brazilian wild Capsicums may be considered vulnerable, especially for those species confined to particular environments (e.g. *C. friburgense*). It would be advisable to promote conservation strategies for these species, both *in situ* and *ex situ*.

Keywords: Biodiversity, *Capsicum*, endemism, morphology, genetic resources

Introduction

Capsicum L. is well-known for its cultivated species, spices and vegetables consumed worldwide, but it also includes ca. 30 wild species native from Central and South America (Table 1). Wild crop relatives are important genetic resources since they can be used to improve yields and the quality of crops, and also be the source of pests and diseases resistance genes. Therefore, the knowledge of the wild diversity (reliable species identification, phylogenetic relationships, reproductive constraints, habitat, etc.) is a key factor.

Important progresses have been made in recent years regarding the systematics of the wild Capsicums (Barboza & Bianchetti 2005; Nee et al. 2006; Barboza et al. 2011), but the delimitation of some species is still a matter of debate due to the morphological variability observed among different specimens (cf. Bianchetti et al. 1999; Buso et al. 2002). This situation is recurrent in the entire range of distribution of the genus.

Since the major recent advances in the understanding of wild Capsicums have been focused in Brazil (Barboza & Bianchetti 2005; Barboza et al. 2011), our efforts were concentrated in this territory. Actually, at least 15 wild *Capsicum* species, 12 of them endemic, can be found in this country. Therefore, in order to gain knowledge about wild *Capsicum* species, to compare fruiting and flowering features with cultivated varieties, and to characterize their natural habitat, field trips took place to the Brazilian Mata Atlantica.

Materials and Methods

Three campaigns to the Mata Atlantica in SE Brazil were organized in February and June 2011, and April-May 2012. The routes to be followed were defined using published information of specimens studied (Bianchetti et al. 1999; Buso et al. 2002; Barboza & Bianchetti 2005; Kaplan Barbosa 2009) and labels from herbaria vouchers available on-line. Over 6000 km were covered across four states: Minas Gerais, Paranà, São Paulo and Rio de Janeiro (Fig. 1). The explorations took place in the open field and also inside the following protected areas: Parque Estadual do Ibitipoca (Minas Gerais), Parque Natural do Caraça (Minas Gerais), Parque Nacional Serra do Órgãos (Rio de Janeiro), Estação Biológica de Boracéia (São Paulo), and Reserva Biológica do Alto da Serra de Paranapiacaba (São Paulo).

The species were identified in the field following the original descriptions, images available on on-line databases of several herbaria (e.g. MO, NY, F), and using the key of Barboza & Bianchetti (2005). Macroscopical observations were recorded and compared to previous data. Trichomes were studied *in situ* under a digital Moritex light microscope, using macro photography. The habitat of each species was characterized as regards to vegetation cover, solar irradiance, altitude, and their range of distribution was defined using GPS information.

Results

A total of 10 wild *Capsicum* species were found, nine of them endemic from Brazil, namely: *C. friburgense*, *C. hunzikerianum*, *C. mirabile*, *C. pereirae*, *C. praetermissum*, *C. recurvatum*, *C. schottianum*, and *C. villosum* found in their natural environments, and *C. caatingae* from the "Caatinga", which was cultivated in the Universidade Federal de Viçosa (Minas Gerais). Besides, the non-endemic *C. baccatum* var. *baccatum* was also found. In addition, several populations of uncertain specific identification were also found, as previously reported (Bianchetti et al. 1999; Bianchetti 1996; Buso et al. 2002; Barboza pers. comm.). In these cases the plants have been named according to their similarities with known species, that is 'aff. *flexuosum*', 'aff. *mirabile*-1', 'aff. *mirabile*-2', 'aff. *recurvatum*', and 'aff. *schottianum*'; a particular population had combined features of two species and was named after the locality where it was found, that is to say *Capsicum* 'cunha'.

Morphology

To summarize the observations on the vegetative organs and flowers, the more remarkable features registered are the following:

General characteristics: (woody) shrubs (Fig. 2); mostly stellate to rotate corollas (Fig. 3 A, B, D, F, G, J-M); green, yellow, brown, and/or purple spots in the corolla (Fig. 3 A, B, D, G, J, K), eventually dominant lilac, pink or purple pigmentation (Fig. 3 I, M); geniculate pedicels (Fig. 3 E, F, L), upright at flowering in most species, pendant, not geniculate in a few cases.

Capsicum schottianum was the most variable species along its range of distribution. The more variable features are: the dimension of the plants (shrubs up to 4 m tall; Fig. 2 A), the shape of the leaves (from ovate to almost lanceolate), and the corolla pigmentation (purple spots may be large to absent; Fig. 3 A).

Capsicum villosum is noticeable for the dense pubescence (Fig. 3 B, C) and long pluricellular trichomes.

Capsicum mirabile is characterized for the purple pigmentation in the corollas (Fig. 3 D), particularly strong in the buds (Fig. 3 E), and for linear-lanceolate leaves.

Capsicum recurvatum has up to 10 horizontal or curved calyx teeth (Fig. 3 F) that may have different length among individuals.

Capsicum pereirae shows pendant flowers with brownish burgundy and green spots (Fig. 3 G) and particular coriaceous leaves (Fig. 3 H). Stamens of different lengths may be observed, which is a novel feature registered for the species. Since in 'old' flowers all the stamens have the same length, the differences observed may be due to asynchronous post-anthesis filament elongation.

Capsicum hunzikerianum outstands by being shrubs up to 3 m tall with the largest flowers among Brazilian species.

Capsicum friburgense appears as the most different species because of the urceolate-campanulate corolla, purely lilac (Fig. 3 I); as a novel feature, the variable length of the calyx teeth among flowers can be mentioned.

Capsicum caatingae belongs to the Caatinga biome from NE Brazil, very different from the Mata Atlantica where it was found in cultivation. Nevertheless, the plants grew up to 5 m tall and showed a main stem of ca. 10 cm of diameter. This species is peculiar due to the inflorescences formed for 5-18 flowers, which have small, white/violet corollas with green spots inside (Fig. 3 J); a basal constriction observed in the fruiting calyx has not been yet described.

The plants of uncertain identification show a few differences in contrast to known species, like a different pattern of corolla pigmentation in 'aff. *flexuosum*' (Fig. 3 K), the absence of calyx teeth in 'aff. *recurvatum*' (Fig. 3 L), dominant pink or purple pigmentation in the corolla in 'aff. *mirabile*-1' (Fig. 3 M), and the presence of calyx teeth of variable length in 'aff. *schottianum*'. Other features that deserve to be mentioned are the dimensions of the 'aff. *recurvatum*' plants (up to 3-4 m tall; Fig. 2 D), and the profuse flower production in 'aff. *mirabile*-1'. As regards *Capsicum* 'aff. *mirabile*-2', it seems to be the most distinct from its reference species since the plants are more pubescent, and they have much narrow, elongated leaves and white buds.

Capsicum 'cunha' shared calyx features with *C. recurvatum* while its pubescence pattern showed some similarities with *C. cornutum*, but the corolla was completely white.

Young shoots arising from stolons were frequently observed around the main stem of an individual.

Special attention was paid to the pubescence because the Brazilian Capsicums show repeatedly a definite type of trichomes. Microscopic observations revealed non-glandular simple, uni- and pluricellular, antrorse trichomes (Fig. 3 N), present on vegetative organs as well as on pedicels and calyces; the density of the trichomes may be different between the adaxial and abaxial surfaces of the leaves. The longest hairs and in higher densities were observed in *C. villosum*.

The fruits are small globose or globose-depressed, pendant berries (Fig. 3 O), up to 1 cm wide. The fruits change slightly from green when immature to mostly golden-yellow or golden-green at maturity. The exceptions are *C. baccatum* var. *baccatum*, *C. praetermissum* and the plants referred as 'aff. *flexuosum*', which have red berries. Although the pungency is perceptible to the taste, when present, the burning sensation is brief and not strong.

Habitat and distribution

The typical environment where the plants were usually found is a transition zone between light and shadow, like the edges of clearings or along roadsides. Inside the protected areas visited, the plants are also intermingled within the closed vegetation (Fig. 2). All the species were found at relatively high altitudes, from 500 m to ca. 2000 m, except for *C. recurvatum* that may grow almost at sea level. Most of the findings were located inside protected areas while outside those areas they were more sporadic.

Most species grow in nearby areas which sometimes partially or totally overlap (e.g. *C. villosum* and *C. schottianum*). At least two zones of higher diversity could be delimited (no. 1 and 2 in Fig. 1 B). Nevertheless, some species were found in a single location within each zone (e.g. *C. friburgense*

in no. 2 or *Capsicum* 'aff. *flexuosum*' in no. 1 -Fig. 1 B-). The more extensive areas were recorded for *C. recurvatum* and *Capsicum* 'aff. *recurvatum*' (Fig. 1 B). The most isolated population belongs to *Capsicum* 'aff. *mirabile*-2', within the Parque Natural do Caraça, in the northernmost point visited.

Some species or populations are confined to small areas. Such is the case of *C. friburgense* that lives in an area of ca. 1000 m^2, where just a few dozen plants could be counted. Another case is *C. pereirae*, which was found only within the Parque Estadual do Ibitipoca, in a few thousands m^2 area, where it grows in very particular conditions, inside humid caves with high environmental humidity and scarce sunlight (Fig. 2 C). Despite our efforts for finding this species in its northern position (Castelo, Rio de Janeiro, an area transformed mostly in coffee fields), no signs of the species were found. The populations of uncertain identification were also small and unique to a given location.

Excluding a few exceptions, all the populations found were reduced, sometimes formed by only 1-2 individuals. Since in many cases the plants were found along roadsides, it was frequently seen that they had been pruned with the surrounding vegetation to clear the roads. This was even observed along the trails inside protected areas. For instance, the only plant of *C. cornutum* found in 2011 in the Reserva Biológica do Alto da Serra de Paranapiacaba (G. Barboza, pers. comm.) had been cut off. Often not even offspring could be found in the surrounding areas, except in the case of Estação Biológica de Boracéia (*C. hunzikerianum*) and the Parque Natural do Caraça (*Capsicum* 'aff. *mirabile*-2').

Discussion

In contrast to the cultivated *Capsicum* species, throughout the entire assemblage of the wild species of the genus, the plants are shrubs, woody in many cases, the fruits are usually small and there is a wide diversity of patterns in the corolla pigmentation (e.g. Hunziker 1961; Eshbaugh et al. 1983; Barboza & Bianchetti 2005; Nee et al. 2006; Barboza et al. 2011). Among the species of the Brazilian Mata Atlantica there is a generalized set of features that characterize the group, with a certain degree of variation for some of them. That is the case of the shape of leaves and corollas, the presence/length of teeth in the calices, the patterns of corolla pigmentation, and the fruit color. Nevertheless, it is worth mentioning the extreme exception regarding the corolla shape and color of *C. friburgense*, and the red fruits found in *Capsicum* 'aff. *flexuosum*', *C. praetermissum* and *C. baccatum* var. *baccatum*. It is noteworthy that the two latter species with red fruits are closely related to the cultivated species of the *C. annuum* complex (Walsh & Hoot 2001; Guzmán et al. 2009), while *Capsicum* 'aff. *flexuosum*' shares this red fruit feature with *C. flexuosum*, although their affinities are still controversial. Indeed, Buso et al. (2002) found *C. flexuosum* nested among the *C. annuum* and *C. baccatum* complexes, while Moscone et al. (2007) suggested a rather isolated position for this species. Exceptions aside, a sort of continuum is observed for several morphological features in the Capsicums from SE Brazil, with variations combined in different ways among species. The relative homogeneity across species leads us to presume a recent differentiation between them, which might be still in progress. To this regard, the occurrence of populations of uncertain specific identification, according to the current knowledge, would be another evidence of this phenomenon.

As many other species growing in wild forests, like the Brazilian Mata Atlantica, the wild Capsicums are threatened with extinction due to the reduction and modification of their habitat. A clear example of the situation in the genus involves *C. lanceolatum*, whose habitat was destroyed by extensive agricultural and population pressure, and only after 50 years it was rediscovered in Guatemala (Bosland & Gonzalez 2000). In the case of the *Capsicum* species growing in the Mata Atlantica, although some are frequently found (e.g. *C. schottianum* and *C. villosum*), most of them have narrow distributions, where only small populations can be retrieved. The signals of recent

pruning and the lack of offspring in several cases evidence the risky situation of these species, which seems to be even more risky for species that grow in very particular and restricted environments. That is true for *C. friburgense* that lives in a reduced area periodically subjected to intensive pruning and weeding since it is on the way to communication antennas of oil platforms settled in the Atlantic Ocean. Another strongly endangered species seems to be *C. pereirae*, because of the particular conditions of its preferred environment.

The populations of unidentifiable plants were also reduced in number of specimens and distribution. However, their situation is difficult to assess because it cannot be affirmed whether they are deviations of known species, hybrids or independent taxa. This underlines the importance of the taxonomic work, which is still needed, since "we can only discover if biodiversity is in decline if we can reliably identify its components over and over again, allowing monitoring to determine trends", as stated by Knapp et al. (2004).

We mentioned the frequent presence of lateral shoots born from stolons. To that regard, it has been registered that the wild Capsicums have a reduced radical system and thin and elongated stems and branches, prone to form lateral shoots (Bianchetti et al. 1999), which may eventually become independent. As a result, cloned plants could be found within a population and therefore the genetic variability in that population would be low. In the end, this feature would also be detrimental for the conservation of these species if the diminution of their environments continues.

The richness of the wild Brazilian Capsicums diversity is undeniable, and it still needs exhaustive studies and field explorations to inventory more accurately the species and to understand their relationships. The wild species need to be known and protected because of their inherent value and also because they are valuable genetic resources for cultivated Capsicums breeding. However, the progressive anthropization of the territory and the intensive use of the land had reduced their natural habitats by narrowing their areas of distribution. As a consequence, the conservation status of the wild Brazilian Capsicums may be considered vulnerable (following IUCN -2012- criteria), especially for those species confined to particular environments (e.g. *C. friburgense*). It would be advisable to promote conservation strategies for these species, both *in situ* and *ex situ*, to prevent the loss of these valuable genetic resources.

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Table 1. Informal grouping of currently recognized *Capsicum* species (based on Barboza & Bianchetti -2005-, Barboza et al. -2011, Eshbaugh et al. -1983-, Heiser & Smith -1958-, Hunziker -1961, 1998, 2001-, Nee et al. -2006-, and Nee -pers. comm.-).

***Capsicum annuum* complex**
C. annuum L.
C. chacoense Hunz.
C. chinense Jacq.
C. frutescens L.
C. galapagoense Hunz.
SE Brazil
C. campylopodium Sendtn.
C. cornutum (Hiern) Hunz.
C. friburgense Bianchetti & Barboza
C. hunzikerianum Barboza & Bianchetti
C. mirabile Mart.
C. pereirae Barboza & Bianchetti
C. recurvatum Witas.
C. schottianum Sendtn.
C. villosum Sendtn.
Possible transition species
C. caatingae Barboza & Agra
C. flexuosum Sendtn.
C. longidentatum Agra & Barboza
C. parvifolium Sendtn.

***Capsicum baccatum* complex**
C. baccatum L.
C. praetermissum Heiser & Smith
***Capsicum pubescens* complex**
C. cardenasii Heiser & Smith
C. eshbaughii Barboza
C. eximium Hunz.
C. pubescens Ruiz & Pav.
C. tovarii Eshbaugh, Smith & Nickrent
W South America
C. caballeroi M. Nee
C. ceratocalyx M. Nee
C. coccineum (Rusby) Hunz.
C. minutiflorum (Rusby) Hunz.
C. dimorphum (Miers) Kuntze
C. geminifolium (Dammer) Hunz.
C. hookerianum (Miers) Kuntze
C. lanceolatum (Greenm.) C.V. Morton& Standl.
C. rhomboideum (Dunal) Kuntze
C. scolnikianum Hunz.

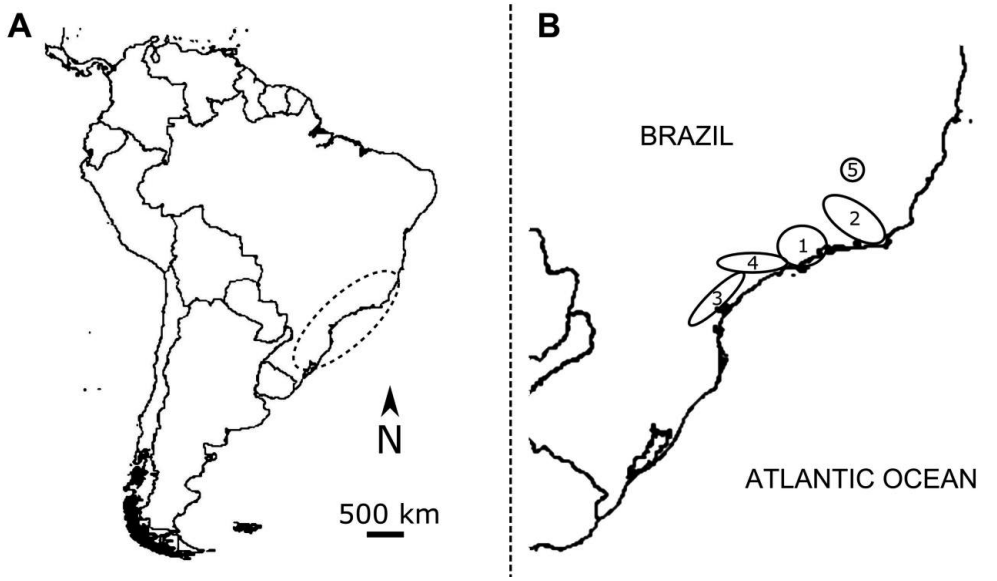

Figure 1. Area of study. **A**. Area explored in SE Brazil (dotted ellipse). **B**. Areas where wild Capsicums were found: **1**. *C. baccatum* var. *baccatum*, *C. hunzikerianum*, *C. mirabile*, *C. praetermissum*, *C. schottianum*, *C. villosum*, *Capsicum* 'aff. *flexuosum*', *Capsicum* 'aff. *mirabile*-1', *Capsicum* 'cunha'; **2**. *C. friburgense*, *C. pereirae*, *Capsicum* 'aff. *schottianum*'; **3**. *C. recurvatum*; **4**. *Capsicum* 'aff. *recurvatum*'; **5**. *Capsicum* 'aff. *mirabile*-2'.

Figure 2. Growth habit and habitat of wild Brazilian Capsicums. **A**: *C. schottianum* near Caraguatatuba (São Paulo). **B**: *C. villosum* in Casagrande (São Paulo). **C**: *C. pereirae* in the mist of Parque Estadual do Ibitipoca caves (Minas Gerais). **D**: *Capsicum*'aff. *recurvatum*' inside the Reserva Biológica do Alto da Serra de Paranapiacaba (São Paulo).

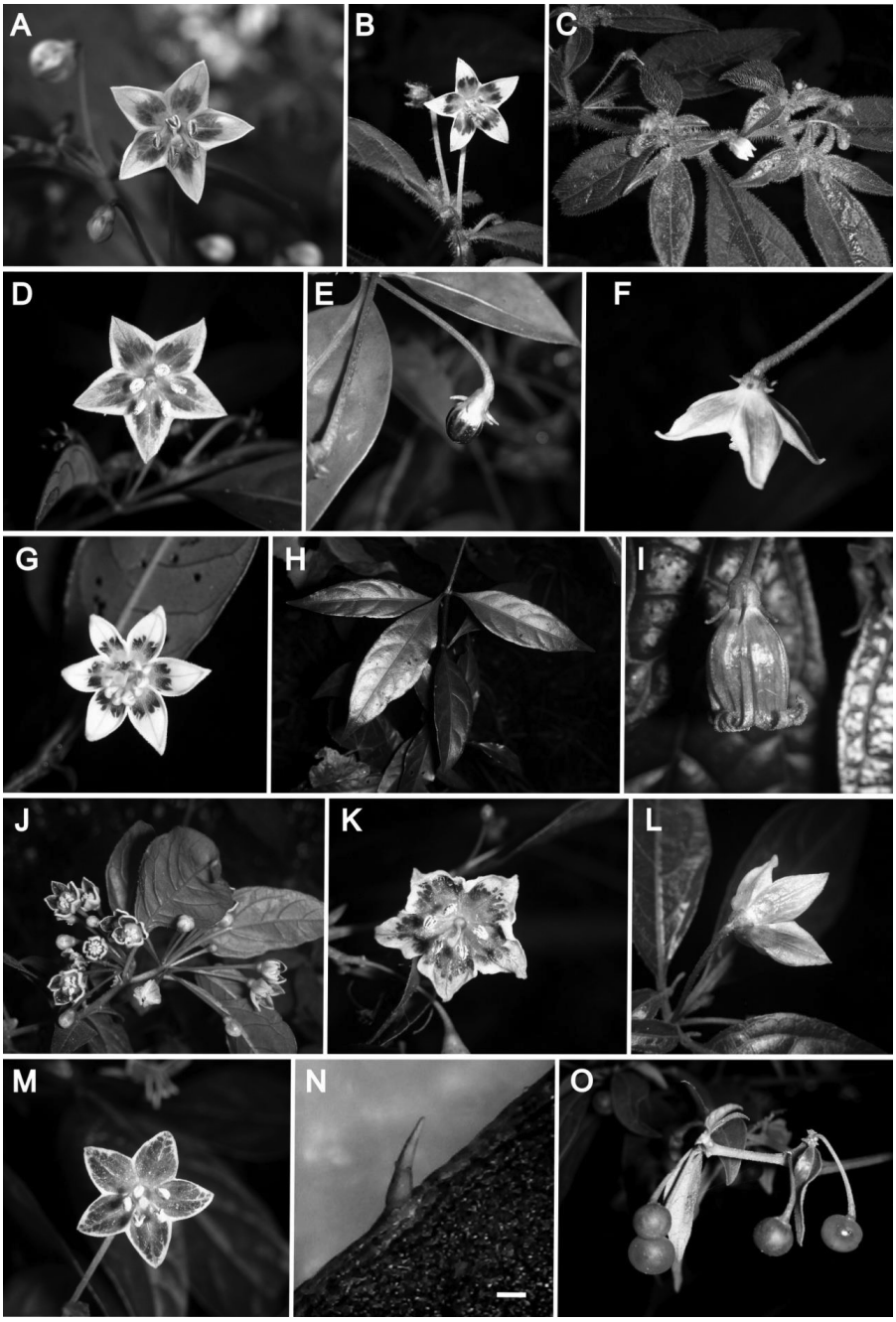

Figure 3. Features of wild Brazilian Capsicums. **A**, **B**, **D**, **F**, **G**, **I**, **K**, **L**, **M**: flowers of *C. schottianum* (**A**), *C. villosum* (**B**), *C. mirabile* (**D**), *C. recurvatum* (**F**), *C. pereirae* (**G**), *C. friburgense* (**I**), *Capsicum*‘aff. *flexuosum’* (**K**), *Capsicum*‘aff. *recurvatum’* (**L**), and *Capsicum*‘aff. *mirabile*-1’ (**M**). **C**: detail of pubescence of *C. villosum*. **E**: bud of *C. mirabile*. **H**: leaves of *C. pereirae*. **J**: inflorescence of *C. caatingae*. **N**: foliar trichome of *C. mirabile* (bar: 100 µm). **O**: fruits of *C. schottianum*.

Introduction to a collection of generative organs pepper mutants

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Abstract

We distinguished between two types of mutations: mutations that affect the *vegetative organs* and the *generative organs*. At the International Pepper Conference (2012) we presented the *vegetative organ pepper mutant* collection and here we present the *generative organ pepper mutants*. The collection is based on our breeding work of the last 40 years during which we analyzed more than half a million items of self-pollinated plants and the samples received from different foreign laboratories We studied the flower (peduncle, calyx, corolla, anther and stigma) the fruit (colour, form, size) and the seed (colour and size). Note that the following list is provisional because we still have to confirm some of these mutations with allelic tests.

Keywords: *Capsicum*, spontaneous mutants

Introduction

During the 60-80 years of intense genetic research on spontaneous mutations of pepper (*Capsicum annuum* L.) a huge number of results were published (Deshpand 1933, 1939, Odland & Porter 1938, Smith 1950, Kormos & Kormos 1955, Peterson 1959, Cook 1961, Bergh & Lippert 1964, Pochard 1970a, Shifriss 1973, Daskalov 1974, 1977, Csilléry 1980a,b, 1983, 1985, 2012, 2013, Subramanya & Ozaki 1983, Hurtado-Hernandez & Smith (1985), Lightbourn et al. 2007, 2008). The first gene list summarising the previously published mutations compiling altogether 50 genes was issued by Lippert et al. (1965). In their later list, Lippert et al. (1966) documented 75 genes. In 1980, Csilléry listed 46 new mutants described from and maintained in his own collection (Csilléry 1980). Greenleaf (1980) published a list of 63 genes, whilst in 1983 Csilléry described further 67 of his own mutants. Based on later publications, the list of Daskalov & Poulos (1994) included 212 genes. The most complete gene list was published by Wang & Bosland (2006) compiling 292 genes.

Of the practical benefits of mutations, evidence is provided by several F1 hybrids, the mother line of which possesses either a recessive marker gene (mostly anthocyanin less – al) or a recessive male sterility (ms) gene. The presence of a marker gene enables the fast and unambiguous determination of the purity of a hybrid seed lot, and when the mother is male sterile, 100% of the F1 seeds are of hybrid origin. In the latter case, seed production might become much more economical and cheaper.

Materials and Methods

In the past 40 years, we analysed the self-pollinated progenies, of more than half a million genetic lineages. From the beginning, we have monitored the development of all the lots, right from the germination till fruiting. We have never treated any line with mutagenic agents, thus all the found and described mutants are of spontaneous origin. However, our collection includes also several mutants derived from mutagenic treatments, which were obtained from foreign colleagues.

Our main breeding goal was to produce cultivars resistant to pathogens and pests. Since the eligible sources of genetic resistance are almost exclusively wild *Capsicum* species, our breeding material is nearly entirely derived from interspecific hybrids (*C. annuum x C. chinense, C. chinense x C. annuum, C. annuum x C. frutescens, C. frutescens x C. annuum, C. annuum x C. chacoense*). The extraordinarily high number of spontaneous mutations in the interspecific progenies may be caused by a possible mutagenic effect of interspecific hybridisation, but environmental conditions (intense background radiation or sunspot activity, nuclear disaster of Chernobyl) might have played a role in the background. We are aware that our interest on plant genetic diversity is far beyond the average, i.e. we discovered ourself almost all the alterations of the plant material. Naturally, we

found spontaneous mutants not only among the progenies of interspecific hybrids, but also among the progenies of our old, open pollinated cultivars.

During our surveys, we have documented three such lineages amongst which the frequency of observed mutations was above the average.

One of the lineages derives from the line **Rez363** obtained from a cross between *C. annuum* and *C. frutescens*. This line is used in the breeding process since it presents an outstanding tolerance to CMV. Although *C. annuum* relatively easily hybridises with *C. frutescens*, the hybrid plants of *C. frutescens x C. annuum* often suffer strong reduction of fertility, so we observed significant incompatibility between both species. Among the offspring of **Rez363** self-pollinated and back-crossed with *C. annuum* we found *xantha, lutescesns, mosaic, wilty, filiform, datura leaf* and *dwarf* mutants.

The second lineage derives from a spice pepper line (**S78**) containing the gene *Bs2*. *Bs2* gene originates from the species *C. chacoense*. A notable fact about this species is that fertile interspecific hybrids with *C. annuum* are produced only when this latter is used as female. Completely sterile plants with abnormal (*stamenless*) flowers result from the reciprocal cross (*C. chacoense* used as female), so we also observed quite remarkable incompatibility between the two species. Despite the multiple back-crosses, there are many fertility problems of pepper lineages containing the gene *Bs2*. Among the descendants of **S78** self-pollinated and back-crossed with *C. annuum,* the following mutants were found: *lutescens, mosaic, viburnum leaf, wilty, small leaf, bullose leaf, rogose leaf, dwar, prostrata*.

The third lineage is more interesting than the previous ones, since it derives from the dihaploid (DH) line we obtained from the self-pollinated progeny of a Hungarian Cecei-type white pepper (**C571**) from USA. **C571** also contains genes of *C. chinense* and *C. chacoense*, because some specimens are resistant to the viruses TMV and TSWV. **C571** is remarkable for a further reason. Indeed, an important method of the current pepper breeding programs is producing DH plants via *in vitro* anther or microspores culture. Plant breeders emphasise that homozygous lines can be produced quickly, in one step by using this method. This is basically true, yet they forget that living creatures are not genetically constant entities, but they are changing, i.e. spontaneous mutations occur also in DH lines that are however considered to be stable. Nevertheless, this means that the homozygosity of DH plants is only temporary, and mutations may appear any time. If these mutations are phenotypically discernible, many breeders would deduce that the mother plant was accidentally cross pollinated. Although such events might happen, the rate of cross-pollination in pepper is quite low, app. 5% (Csilléry et al. 1987). Consequently, it is worth to pay attention to these plants displaying altered genotype/phenotype, because they may harbour beneficial spontaneous mutations. Between 1992 and 2008, within the frame of a collaboration between the Agricultural Biotechnology Center of Gödöllő and the Budakert Ltd., several thousands of DH plants have been produced by Dr. Judit Mitykó. Although DH plants are theoretically homozygous, fruits and seeds of their progenies were collected plant by plant. We found several spontaneous mutants among these progenies, but the lineage **C571 DH66** was outstandingly remarkable. Indeed, among its offspring we observed *virus like leaf, rugose leaf, small leaf, horizontal stem, procumbent hypocotyls, dwarf, rust brown seed* mutations. Presumably, the lines **Rez363**, **K78** and **C571 DH66** contain transposons or so-called mutator gene(s) (Cammack et al. 2008) that caused the outstanding number of mutations.

Our collection includes several such mutants that were earlier obtained or maintained by geneticists and breeders who sent them to us. We are especially grateful to the following colleagues who provided a great number of mutants: Bob Bergh (110 mutants), Edmond Pochard (16), Chen Shifriss (5), Stefan Daskalov (5), Bob Heisey (5).

We maintain more than 250 mutations in our collection. In several cases, the maintenance is rather difficult due to the complete or partial lethality (*xantha, lutescens, mosaic, wilty*) or to male or female sterility (*male sterile, male-female sterile, proliferous plena, compound inflorescence*) of

the mutants. These special mutations can only survive in heterozygous material, which considerably increases both the number of the required plants and the cost of the maintenance.

Discussion

When describing the mutants we use the terminology proposed previously by Lippert et al. (1966) and Rick (1970). As a 'wild-type' control genotype we suggested using the French cultivar Doux des Landes (small pointed fruits), being a small, productive and easily cultivated cultivar, instead of the modern and previously recommended large, blocky-type, California Wonder, of American origin. Unfortunately, we are unaware of any scientific group using this cultivar for producing isogenic lineages of the mutations. Our work having begun in the 1980's ceased due to financial reasons and for our current studies we changed our initial strategy and we presently maintain the mutations in early fruiting, sweet (i.e. containing no capsaicin), spice pepper cultivars bearing drooping dark green fruits, which are economically important in Hungary.

We are frequently asked why it is so important for us to maintain mutant plants. The first part of the answer is that the complete traditional genetic map of the pepper regrettably has not been constructed yet, and for this process it is indispensable to dispose of a number of mutant traits. If the previously genetic mapping using trisomic lineages (Pochard 1970b) was hardly successful, genetic mapping with molecular methods has yielded admirable results. But constructing the traditional genetic map has still to be completed and the described mutants should be involved in molecular mapping projects, as well.

As we have already mentioned in the Introduction, some mutations can be used as markers in hybrid seed production, such as the ones easily recognised at the seedling stage. This is the case for stem (*anthocyanin less*) or cotyledon (*lutescens, mosaic*) colour, for cotyledon shape or arrangement (*rugulose leaf, rugulose leaf extra*). A further category consists of auxotrophic and semi-lethal mutants (*xantaha, albino, dwarf, small leaf, wilty*), the physiological aspects of which have not been studied so far. An exception is the *light sensitive mosaic – lsm* mutant (Csilléry 1980), which can be applied as a self-eliminating trait. Finding *brown seed* colour – as a unique trait – would be also of remarkable importance, since if a cultivar would contain such a gene, its seeds would be easily distinguished from those of the concurrent companies merely based on the plant phenotype. Besides, this would make the seed staining procedure applied by several companies unnecessary. Phenotypic marker genes are increasingly applied in hybrid seed production. Testing F1 plants possessing a phenotypic marker is far less expensive than using molecular markers (in a plot of some square meters, several thousands of plants can be examined). The use of male sterility mutations is of uneven frequency. Due to the world-wide expansion of the (cheap and creditable) Asian seed production, male sterility was less used, at some stage, by breeders for the production of F1 hybrids. However, we are presently finding male sterile (mother) plants among the F1 hybrid plants we examine in a continuously increasing frequency. We hardly think that the use of male sterility can be avoided in the future. The cost of seeds transportation and the rate of unemployment in the European countries will require the re-evaluation of the Asian seed production, but first of all the huge expenses spent on seed production should be reduced.

The following compilation contains the generative mutant phenotypes of the pepper that are maintained in our collection, most of which were found by us. Several mutations affecting generative organs are associated to alterations on the vegetative parts; that are also discussed here. In case of some mutants, the biliteral abbreviations used in previous publications were changed, and triliteral ones were applied instead, in order to ease the distinction between them. (For instance, *long peduncle – lop* instead of *lp*.) In other cases, instead of the previously applied 'fancy names' we proposed new genetic names describing the mutation more precisely. For example, we recommend changing the genetic name *hungarian tricolor – ht* to *white shoulder – whs*. Moreover, the name *hungarian tricolor* (written in lower case due to being a recessive mutation) is also grammatically incorrect.

Peduncle:

- *upright (up)* – the peduncle of both the flower and the fruit points upwards. Several wild *Capsicum* species (*C. frutescens, C. baccatum, C. praetermissum*) bear such peduncles. The length and thickness of the peduncle impedes the evaluation of the fruit position. It is a recessive trait, although in some F1 plants the peduncle is not completely pendent.

- *long peduncle (lop)* – the length and thickness of the peduncle varies remarkably. Usually the blocky types bearing larger fruits have thick and long peduncles. The Turkish Dolma types have conspicuously thin and long peduncles. The pointed, ribbed fruit of the *lop* mutant is of mean size, yet its peduncle has remarkable length (10-12 cm) and low diameter (3-4 mm). It is a recessive feature; however in some parental combinations F1 generation has peduncles somewhat longer than that of the normal parents.

- *short peduncle (shp)* – the peduncle is 2-3 cm in length.

- *Fruit base (Fb)* – According to the capital in the genetic name, it is a dominant trait. The peduncle of the fruit widens in a club-like manner right under the shoulder of the fruit. The fruit is rather easily separated from the peduncle: this is an important feature of spice pepper cultivars, since during their processing, these parts should be detached from each other.

Calyx:

- *Long calyx (loc)* – sepals are discernibly long (3-4 cm), somewhat resembling the long and thin sepals of the tomato. Long sepals hide the petals both in the buds and at the early stages of flowering. The trait is obvious also on the mature fruit. It can be applied as a fruit marker.

- *petalous calyx (pet)* – Discernible as early as in the green buds; the sepals are completely light green, the tips being even white in colour, like the petals. Subsequent to flowering, the sepals wither together with the petals, thus no sepals are present on the fruit. This feature is as a perfect fruit marker.

Corolla:

- *closed flower (cf)* – This flower mutation was described by Subramanya and Ozaki in 1983. The corolla splits quite hardly or only at the very tip of the petals. The cross pollination is almost impossible, because self-pollination occurs even within the closed, bubble-like flower.

- *plena (ple)* – The number of the petals multiplies (just like in the rose flower), but the anthers do not change significantly. Fertility is somewhat decreased; fruit just rarely develops and it is distorted, since anthers get jammed together with the petals.

- *Yellow spot (Ys)* – The characteristic feature of certain wild species (*C. baccatum, C praetermissum, C. eximium*) is the greenish-yellow marking around in the entrance of the corolla tube. By hybridisation of the species, the trait was successfully transferred into *C. annuum*. *Ys* is a monogenic and dominant gene, in back-cross tests we observed that the intensity of the greenish-yellow colour is determined by several genes.

Anther:

- *anthocyanin less (al1)* – The presence or absence of this very trait can be easily determined right from the seedling stage to that of the economically ripened fruit maturity. We observed the *anthocyanin less* phenotype (*al1*, previously called *alh*, *alf*) two times, in lineages of different genetic origins (*alh* – cv. *H*atvani, *alf* – cv *F*ehérözön). A French breeder, colleague of us (Jean Louis Nicolet), however also found it in his own collection (preliminary name: *alj* – *J*ean). The identity of *al1*, *alh*, *alf* and *alj* was proven by allelic tests. Gene *al1* is closely linked to the *L* gene controlling resistance to the tobacco mosaic virus (Csilléry and Ruskó 1980). The gene *al1* does not cause the total absence of anthocyanins, since among extreme conditions slight anthocyanin production may occur. Similarly, the *al* gene (previously: *ala* - *A*lbena) of the Bulgarian Albena cultivar does not induce totally *anthocyanin less* phenotype, either. We gave the preliminary name *sal* (*als* – *S*oroksári) to another mutant gene, which in homozygous form shuts down anthocyanin synthesis in all plant cells, even amongst extreme conditions. At the end of our studies, it got the genetic name *anthocyanin less totally – alt*. Another *anthocyanin less* mutant was preliminarily denominated *aln*, because in plants with this gene the nodes (*aln* – *N*ode) completely lack

anthocyanins or bear only a pale purple patch, while the anthers are completely purple. Moreover, both the filament and the style are extremely purple in colour. Interestingly, the F_2 progeny of the cross *al1* (syn. *alh, alf, alj*) x *aln* bear filaments, styles and anthers of *al1* phenotype, i.e. slightly purple colour. Till the results of further studies we keep the genetic name *aln*.

- *male sterile (ms)* – These mutants have the most important economic interest. The best-known male sterile mutants are those found by Pochard (1970a), Shifriss (1973) and Daskalov (1974), but only the *ms1* mutant of Shifriss and the *ms3* and *ms5* mutants of Daskalov are commonly used in practice. The male sterile mutants of French origin were found to have disadvantageous effects in the F1 hybrid generation (Lamuyo), thus they are not applied in breeding. Csilléry (1989) recommended the use of maternal lineages containing two or more male sterility genes for the efficient hybrid seed production.

- *male female sterile (mfs)* – The anther wall modifies into a petal-like structure, it is completely *anthocyanin less* (!) and no viable pollen is produced. When finding this mutant, we regarded it as a perfect male sterile one, but in cross tests with plants of normal genotype we observed seedless fruits; the placenta bore only some filiform appendages. It can be maintained only in heterozygous form. Previously, we found it in a plant of conic, light green fruits, yet later it was lost. Nevertheless, we recently found it again in a spice pepper line as well as in a conic, white type. The phenotype is rather similar in case of the plants from the three different origins.

Stigma:

- *long stigma (lst)* – The filament is extremely long, and the style elevates the stigma above the anthers, thus the rate of cross-pollination is high.

- *fastigiated stigma (fst)* – This characteristic also implies another phenotypic trait, the fasciation of the stem. The shoot axis curls back like a crosier and it is highly fasciate. The plant has only fertile pollen grains, the female organs are sterile. The distorted flowers slightly resemble the composite head of the sunflower.

Fruit:

- *white shoulder (whs)*- syn. *hungarian tricolor (ht),* – No chlorophyll is synthesised in the shoulder region of the pericarp, thus it remains ivory even at the physiological fruit ripeness as in the Hungarian white cultivars. This mutation has also been observed in three lines of independent origin (in an American Blocky, an Italian Blocky and a Hungarian spice pepper). Instead of the previously applied, grammatically incorrect *hungarian tricolor – ht*, we propose the more accurate genetic name *white shoulder – whs.*

- *gluey fruit (gf)* – The outer surface of the pericarp is matt and gluey. The histological studies of this mutant and post-harvest water loss investigations are discussed in the paper of Erős-Honti et al. (2013) in the present issue. Due to the rapid water loss after harvest, this mutant is important for spice peppers breeding.

- *Soft fruit (S)* – The fruit of most wild *Capsicum* species softens when physiologically ripen, like that of tomato. This ancient, monogenic, dominant trait is present also in the Mexican cv. Serrano of *C. annuum*.

- *Red, light red, salmon pink, peach, orange, orange yellow, lemon yellow and white fruit* colour at physiological maturity. Hurtado-Hernandez & Smith (1985) and Thorup et. al. (2000) investigated thoroughly the colour of the economically ripen fruits. Three independent genes interact and result in eight possible phenotypes, when plants with completely white fruit are crossed with red fruited genotypes. The inheritance of the brown colour was studied by Smith (1950) for the first time.

Other generative organs:

- *proliferous plena (prp)* - stamina are transformed into petals, and interior to their whorl are equally transformed carpels. The latter forms a closed structure, resembling a pistil but inside of it, petaloid and carpelloid, laminar structures alternate in successively overlapping whorls. Occasionally, ovules also develop on the carpelloids. The plant is completely sterile. This mutation can be maintained only in heterozygous form.

- *compound inflorescence (cin)* – at the end of the sympodia, dichasial structures are formed with repeated branching as pseudo-umbels do. However, flowers are, reduced and rudimental. The size of the repeatedly branched structure is variable. Recently, we found a similar alteration in three independent lines. In one case, the structure of the dichasial fork is almost inconspicuous, of 1-2 cm in length, whilst in another lineage it is the longest, up to 8-12 cm. The plants are completely sterile; the mutation is maintained only in heterozygotes.

- *racemosa (rac)* – this mutant bears a completely distorted, racemose structure instead of flower and fruit. In the first stages, the flower buds are indistinguishable from the normal ones, yet later another stalked bud-like structure develops from the bud. Sometimes, this raceme-like structure displays up to sixth to eighth order branches.

- *first fruit deformed (ffd)* – The flower and the fruit of the first fork are distorted and fruit is seedless. Nevertheless, the next sympodium is normal and the next flower is fertile.

- *chappy (chy)* – at full maturity the whole exocarp is covered with transversally oriented small suberised (corky) cracks interrupting the smooth cuticule. Relation to analogous phenotype in Mexican cultivars (Jalapeno) is still unexplored.

Seed:

- *rust brown (rb)* - the mature seeds are rust brown, as well as the secondary xylem of the stem. This phenotype is caused by mutations of the maternal tissues of the seed coat. The preliminary genetic names of the up-to-now found 8 brown seed mutants are *gold seed – gos, rust brown seed – rb1-7*. According to our genetic analyses summed up so far, *gos*, *rb1* and *rb2* mutants are identical, whilst *rb3* is another gene (Csilléry 2013). Accordingly, histological differences were found between the seeds of *rb3* and those of *gos*, *rb1* and *rb2* mutants (Erős-Honti and Csilléry 2013). *Rust brown seed* mutants cannot be applied as marker genes in hybrid seed production (due to the maternal origin of the seed coat), but they are promising candidates as marker genes for cultivar protection.

- *green seed (grs)* – The seed coat of the mature seed is slightly greenish, dirt yellow. So that to prevent the spread of seed-borne viruses, seeds are treated with NaOH solution. Both fresh and the previously dried, seeds – being yellow originally –turn into greenish-grey in colour as a response to the NaOH bating if they contain the *grs* gene. The treatment slightly decreases the chance of germination.

Acknowledgements

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Software-aided seed image analysis of gene bank collections of Nigerian pepper (*Capsicum annuum* L.)

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Abstract

Characterization of germplasm collections is a routine function in gene banks, a task for which seed morpho-metric measurements by digital imaging analysis had been identified as a fast and reliable method. In this study, ten seed morpho-metric variables namely seed length, seed width, seed thickness, seed diameter, seed area, embryo angle, shape factor, roundness factor, flatness index and circularity index were measured and/or calculated with the aid of digital imaging software (Veho™) from captured images of 20 accessions of pepper landraces in the germplasm collections at the NACGRAB gene bank, Nigeria. The digital seed metric data were subjected to multivariate analyses. Seed area, length, diameter, roundness and shape factors showed highest Eigen vectors in the first principal component axes which contributed 72.91% of total variance. These variables thus constitute potential descriptors for cultivar discrimination of pepper seeds by digital imaging analysis. Single linkage clustering analysis (SLCA) showed two major clusters and two minor clusters of the Nigerian pepper landrace collections based on the seed size and shape metrics. The clusters is a classification tool for genotype discrimination in future evaluations of cultivar identity by seed metrics, and thus improve efficiency in management of the genetic resources for pepper breeding.

Keywords: Capsicum, genetic resources, genotype identification, seed geometry, digital imaging, multivariate analysis

Introduction

In Nigeria, the National gene bank at the National Center for Genetic Resources and Biotechnology (NACGRAB) holds 40 accessions of popular local landraces of pepper as seeds. Nonetheless, the distinction of pepper genotypes in the gene bank and indeed in tropical Africa remains largely unresolved (Grubben and El Tahir, 2004). Traditionally, assessment of genotype distinction often depend on morphological observations on the field in Grow Out Tests (GOT) complemented with laboratory evaluation involving ocular observation of seeds with the purity board. The approaches are time and resource consuming, have low reproducibility and possess a degree of subjectivity which is hard to quantify. Hence, there is need for fast, reliable and economical methods for evaluating distinctness and genetic purity of seed lots.

Seed shape and size had been considered as important parameters for seed grading. The various metric measurements on seeds are very important quantitative variables for determining size and shape of seeds and for genetically classifying seeds. In this regard, use of digital imaging of seed metrics to quantitatively discriminate cultivars offers more accurate evaluation of genetic similarities than manual ocular visualization (Eevera et al. 2009; Vijaya Geetha et al. 2011; Daniel et al. 2012). Image analysis techniques with computer vision also offer versatility because it can derive advanced information from seed images like colour analysis (Dell'Aquila, 2006; Grillo et al. 2011). Furthermore, there are advantages of speed, precision and extended usefulness of captured images in databases. Thus the objectives of the study were: (1) to identify digital seed metric parameters that most discriminates the Nigerian pepper landraces and (2) to establish clusters of the pepper germplasm collections in Nigeria based on the seed morpho-metric data.

Materials and Methods

Seeds of twenty pepper accessions were obtained from the NACGRAB gene bank, Ibadan, Nigeria. All the accessions were landrace collections from different parts of Nigeria (Table 1).

Digital seed imaging analysis was conducted on the seeds at the Federal University of Agriculture, Abeokuta, Nigeria. Thirty seeds of each accession were randomly sampled for digital imaging analysis in three replications with the aid of a digital imaging software (Veho™, UK) installed on a USB digital microscope. The seeds were placed under the USB microscope lens to capture and store the images in a folder. Measurements were taken by loading the captured images to the measurement window of the software where measurement icon menus were used to analyse the seed. Before actual measurements, the system was calibrated to millimeters under x40 magnification. The parameters measured on captured seeds were: (1) Seed length (SL), measured as the distance between two points stretching from the base of the embryo axis to the tip of the seed; 2) Seed width (SW), the length of a line drawn across the widest section of the seed taken at a right angle to the length axis of the seed; 3) Seed thickness (ST), the distance between the two opposite flat sides of a seed, measured using a caliper; 4) Seed diameter (SD), determined as the diameter of a circle drawn around the seed, touching all edges; 5) Seed area (SA) determined as the area of a circle drawn around the seed and touching all edges; 6) Embryo angle (EA), the value of the angle created by 2 lines joined at the base of the seed and subtending to the tip of the seed on the 2 sides of the embryo axis. Seed shape parameters were derived from the above measured parameters and included shape factor, roundness factor, flatness index and circularity index. Shape factor (SF) was estimated according to Eevera et al. (2009) as ratio of SW to circumference of a circle drawn around the seed:

$$SF = SW / 2 (\pi * SA)^{0.5}$$

Roundness factor (RF) was estimated according to Grillo et al. (2011) as:

$$RF = 4 \times SA / \pi \times SD^2$$

Flatness index (FI) was the calculated ratio of the sum of SL and SW to ST as:

$$FI = SL + SW/ 2 ST$$

Circularity index (CI) was calculated as the square root of the ratio of actual area of seed to the area of a circle with the same circumscribed shell (SA). The estimation was done as:

$$CI = \sqrt{(A/SA)}$$

Where A is the actual area of the object, SA is the area of a circle with a diameter equal to the circumscribed diameter or length of the object (Vijaya Geetha et al. 2011).

Analysis of variance was done on each of the digital seed metric parameters with accessions as treatments. The data were also subjected to principal components analysis (PCA) and single linkage cluster analysis (SLCA). The PCA was used to generate a correlation matrix of the seed morphometric traits, estimate eigenvectors to quantify contributions of each trait to variability in the landrace population, and chart the accessions on principal component plots. The SLCA was used to generate a dendrogram of phylogenetic relationships among accessions in the population based on the seed morphometric traits. All analyses were done with procedures of SAS (SAS Institute, Inc., Cary, NC).

Results and Discussion

Analysis of variance showed significant effects of genotype ($P< 0.05$) on seed size and shape metric parameters (Table 2). Descriptive statistics of the digital measurements showed consistent seed size differences among the accessions, with accession 3 having maximum values and accessions 5 and 11 having minimum values of most of the digital traits (Table 2). Significant effects of genotype on seed metrics of Nigerian pepper signify the possibility of grouping the landraces based on seed metric descriptors as shown in earlier studies involving grain seeds (Keefe and Draper, 1986; Eevera et al. 2009; Daniel et al. 2012), lentils (Dell'Aquilla 2006), and indeed pepper seeds (Dell'Aquilla, 2004). Recent advances in computer image analysis of seeds had made it possible to categorize and group cultivars according to slight quantitative differences in seed traits that would be indiscernible in traditional ocular seed examination.

The principal component analysis summarizes the multivariate data into several principal components and identifies parameters that best separates the genotype entries (Johnson 1998). The first 3 principal components accounted for 94% of the total variation among the genotypes for seed metric parameters (Table 3). Eigen value of the first principal component was 7.3, which accounts for up to 72.91% of the total variance compared to principal component 2 which had Eigen value of 1.21 accounting for 12.10% of total variation. Values of Eigen vectors of seed metric parameters in the first principal component axis were highest in seed area and shape factor with the vector loading of 0.367, while seed length, roundness factor and seed diameter also had Eigen vectors ~ 0.35. Eigen vector score was below 0.35 for other parameters and was negative in embryo angle in the first principal component. Eigen vectors loadings in the first principal component axes accounting for 72% of total variance, was highest in seed area, length, diameter, roundness and shape factors. Identification of efficient descriptors was essential for application of digital seed imaging to genotypes discrimination (Granito et al. 2003). In this study, seed area, length, diameter, roundness and shape factors were the traits indicated with highest descriptive abilities and potentially the most effective digital seed metric descriptors for pepper. Daniel et al. (2012) reported similar contributions of seed length and seed area to discriminating tropical maize inbred lines by digital seed imaging.

Classification analyses of genotypes help gene banks to effectively manage germplasm collections serving as decision support tools for eliminating duplicates, building core genetic collections and creating mapping populations for efficient delivery of genetic resources for breeding (Boerner 2006). Figure 1 shows the plot of PC1/PC2 in which accession 3 was shown as an outlier, suggesting that the pepper accessions clustered along the mean values of seed size metrics. Many of the large-seeded accessions including 1, 4 and 20, clustered in the +PC1 quadrants while the small-seeded accessions like accession 5, 11 and 19 clustered in the –PC1 quadrants. The SLCA support this observation because at <0.5 minimum linkage (ld) distance, two major clusters were formed, and three minor clusters at >0.5 ld. Cluster A, comprised of accessions 1, 2, 4, 12,16, 17, 18 and 20, includes mostly large seeded genotypes, while cluster B had genotypes 6, 8, 9, 10, 11, 13, 14 15, 19, 20 and 17. Accessions 3 and 5 with extreme values of small and large seed metrics respectively formed minor clusters C and D (Figure 2). The classification based on PCA thus grouped the Nigerian pepper genotypes to large sized seeds, medium sized seeds and small sized seeds. Classification based on the SLCA was similar to the PCA classification of the pepper landraces offering a statistical basis for grouping the Nigerian pepper seeds based on digital seed morpho-metric measurements. Thus result of this study constitutes background classification data for identifying unknown samples and evaluation of genetic purity of test samples (Grillo et al. 2011).

Conclusion

The study demonstrated the capability of computerized imaging techniques for discriminating genotypes of pepper. The seed morpho-metric parameters evaluated in this study are useful

discriminators of pepper genotypes, seed length, area, shape and roundness factor being the most important seed metric parameters for discrimination. The classifications obtained based on these parameters constitute data for discriminating the Nigerian pepper landraces and genetic purity evaluation of test seed samples. Further studies on digital imaging of exotic and improved cultivars along with the local landraces will broaden the image database improve application of digital analysis for discriminating pepper cultivars. Correlations of seed digital image characterization with plant agronomic and molecular characteristics are future studies to validate discrimination by digital imaging of seeds.

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Table 1. List and passport data of pepper landraces obtained from the NACGRAB gene bank.

Entry accession number	Gene bank accession number	State and (region*) of collection
1	NG/AT/APR/09/008	Ogun (SW)
2	NG/SA/01/09/053	Niger (NC)
3	NG/AA/MAY/09/051	Ekiti (SW)
4	NG/AA/MAY/09/013	Osun (SW)
5	NG/SA/07/204	Kaduna (NW)
6	NG/SA/01/09/050	Kaduna (NW)
7	NG/AA/SEP/09/010	Ekiti (SW)
8	PEPPER 106	Oyo (SW)
9	NG/SA/09/007	Kaduna (NC)
10	NG/AA/SEP/09/034	Ekiti (SW)
11	NG/SA/JAN/09/042	Kaduna (NW)
12	NG/SA/JAN/09/036	Niger (NC)
13	NG/AT/APR/09/009	Ogun (SW)
14	NG/AA/SEP/09/027	Ekiti (SW)
15	NG/AT/APR/09/011	Ogun (SW)
16	NG/TB/OCT/09/006	Oyo (SW)
17	HOT PEPPER	Niger (NC)
18	NG/SA/07/007	Kano (NW)
19	NG/AO/MAY/09/007	Oyo (SW)
20	NG/AT/APR/09/006	Ekiti (SW)

*Regions in which the states are located *i.e.* SW = South West, NW = North West and NC = North Central. All accessions were collected in 2009.

Table 2. ANOVA F values of accession effect and descriptive statistics of seed morpho-metric parameters in Nigerian pepper accessions

	Seed length	Seed width	Seed thickness	Seed diameter	Seed Area	Embryo angle	Shape factor	Roundness factor	Flatness index	Circularity index
F value	9.20**	9.89**	2.0*	10.43**	13.43**	4.46**	13.43**	13.99**	4.71**	9.77**
Mean	1.23	0.99	0.26	1.28	1.23	57.94	1.094	2.76	0.29	0.97
Minimum (Accession)	1.01 (11)	0.80 (11)	0.23 (5, 11)	1.00 (5)	0.87 (5)	49.74 (13)	0.74 (5)	1.34 (11)	0.23 (5, 10, 11)	0.89 (5, 11)
Maximum (Accession)	1.67 (3)	1.13 (3)	0.29 (9)	1.67 (3)	2.21 (3)	67.78 (8)	1.39 (3)	8.01 (3)	0.37 (3)	1.15 (3)
Standard error (n=30)	0.006	0.004	0.0006	0.056	0.015	6.460	0.013	0.05	0.002	0.002

* Significant F at P = 0.05; **Significant F at P = 0.01.

Table 3. Eigen vectors of seed morpho-metric parameters, Eigen values and percentages of variations accounted for by the first 3 principal components axes of the *Capsicum* genotypes.

Seed metric parameters	PC 1*	PC 2	PC 3
Seed length	**0.357**	0.002	-0.058
Seed width	0.331	-0.232	0.154
Seed thickness	0.119	0.844	0.018
Embryo angle	-0.116	0.060	0.970
Seed diameter	**0.348**	0.071	-0.063
Seed Area	**0.367**	-0.100	0.051
Shape factor	**0.367**	-0.100	0.051
Roundness factor	**0.353**	0.004	0.022
Flatness index	0.339	0.354	0.039
Circularity index	0.323	-0.278	0.136
Eigen value	7.29	1.21	0.93
% of total variance	72.91	12.10	9.35
% cumulative variance	72.91	85.02	94.37

*Bolded values in PC 1 indicate highest contributions to total variation among the pepper landraces.

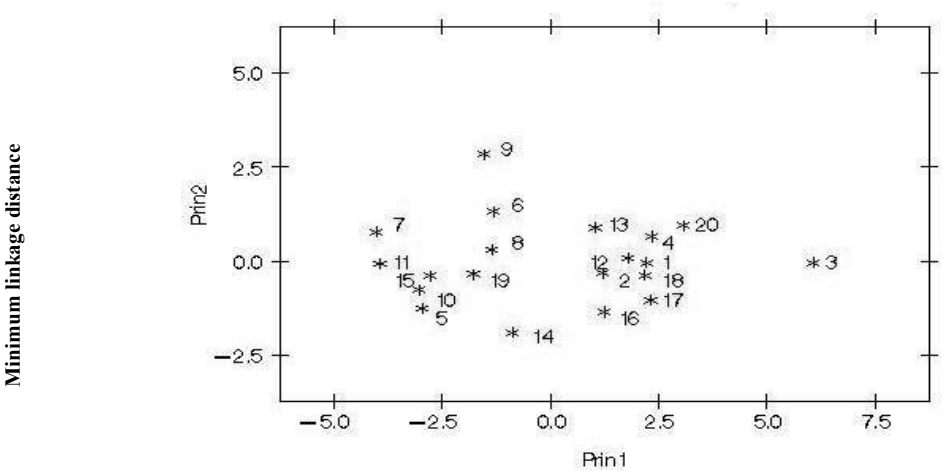

Figure 1. Plots of first two principal components showing clusters of Nigerian pepper landraces based on digital seed morpho metric data. Numerals next to symbols are accession numbers.

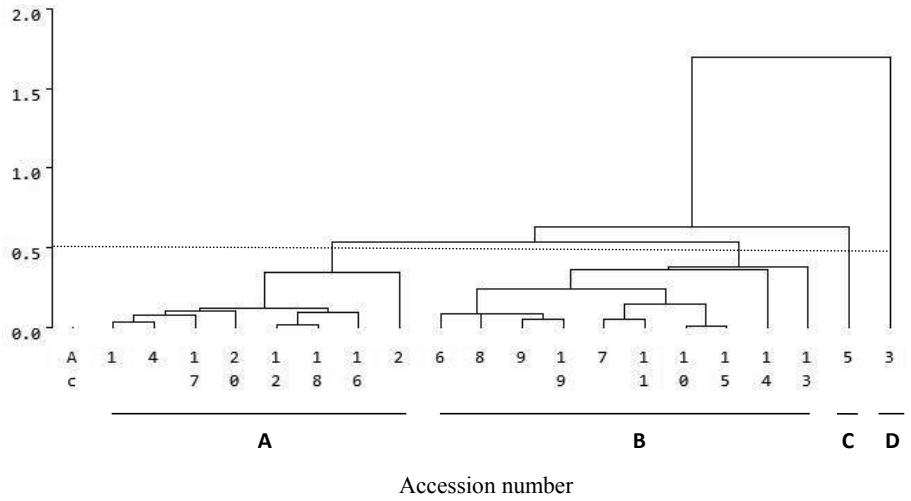

Figure 2. Dendrogram of relationships among Nigerian landrace pepper collections based on digital seed morphometric traits showing 2 major clusters (A and B) formed at 0.5 minimum linkage distance and two minor clusters (C and D).

Potentialities of wild relatives for eggplants and eggplants rootstocks breeding.

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Abstract

Cultivated eggplants (*S. melongena*, *S. aethiopicum*, *S. macrocarpon*) and their wild relatives belong to genus *Solanum* subgenus *Leptostemonum*. Although most species of this subgenus are native from the American continent, the eggplants and their closest relatives originate mostly from Africa and Asia. The largest living collections of wild *Solanum* species are maintained at Radboud University (Nijmegen, The Netherlands) and at INRA (Montfavet, France). Part of these collections has been characterized for traits of agronomic interest during the so called EGGNET project (1999-2004) within the frame of the European Union GENRES programme, project. *Verticillium* wilt and root knot nematode (*Meloidogyne incognita*) resistances have been screened for by artificial inoculation on young plantlets. Good levels of resistance have been identified in other *Solanum* species than the well known and partially resistant *S. torvum* and *S. sisymbriifolium*. Graft affinity experiences using the wild material as rootstock and *S. melongena* as scion have been carried out on young as well as on adult plants and various levels of grafting affinity have been identified. The characterization results obtained are discussed in the light of the present understanding of interspecific cross compatibility between wild and cultivated eggplants. The results obtained clearly indicate the high potential of the wild diversity for eggplants and eggplants rootstocks breeding, as well as the need to intensify the characterization of these collections for traits of agronomic interest.

Keywords: Eggplant, *S. melongena*, *S. aethiopicum*, *S. macrocarpon*, wild *Solanum*, *Verticillium*, *Meloidogyne incognita*, resistance, grafting, genetic resources, breeding, rootstock

Hot peppers for happiness and wellness: a rich source of healthy and biologically active compounds

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Abstract

The genus *Capsicum* is nowadays widely spread. Fruits are not only used for food (spice), but also for therapeutic and wellness purposes (pain relief, rheumatic illnesses, breast and prostate cancer, obesity, heart diseases, hair loss treatments, and so on). These bioactivities are due to the abundant presence of alkaloids, capsaicinoids, which are found only in the fruits of *Capsicum* species. Fruits are also rich in many other antioxidant molecules, vitamins and carotenoids as compared to fruits and vegetables we regularly eat. In this view, chili pepper is one of the most important detoxifying edible products in the world! Five species of chilies have been domesticated, *C. annuum* L. being the most widely diffused in Italy and used in both domestic and industrial processing. Consequently, it is important to identify cultivars with higher levels of capsaicinoids and antioxidants, for their use as dietary supplement in food and/or as nutraceutical. This work refers to the quantitative analysis of pungency and vitamin C contents in fruits of ten *Capsicum* cultivars, together with their characterization through molecular markers.

Keywords: *Capsicum*, Pungency, Capsaicinoids, Vitamin C, Nutraceuticals, RAPD Molecular Markers

Introduction

The peppers (*Capsicum* spp.) belong to Solanaceae family. This genus originated in new world and rapidly spread to all continents after the discovery of the Americas – maybe the varieties encountered by Colombus were Habanero and Scotch Bonnet like, belonging to C. *chinense*. Currently, five domesticated species of peppers are known: *C. annuum* L., *C. baccatum* L., *C. chinense* Jacq., *C. frutescens* L., and *C. pubescens* Ruiz et Pav. The most spread and widely cultivated species is *C. annuum*. Within this species, two major classes of varieties were selected by the domesticators and bred by the breeders, first with fruits rich in pungent compounds and used as spice, called chili or hot pepper, and second with non-pungent fruits used as vegetables, called bell/sweet pepper or capsicum (Siviero 2002).

The importance of hot pepper fruits in improving human health and wellbeing has shed a new light on the use of this genus, which until recently was commonly known only for its spicy flavor and taste, which possibly contributed to the end of the Crusades (Wright 2007).

The pungent compounds belong to a class of derivatives of phenylpropanoids, the most abundant being capsaicin and its analogues, called capsaicinoids, which account for the hotness. Capsaicinoids are pungent and fat soluble molecules, mostly present in the epidermal cells of the placenta, and, to a lesser extent, in seeds. Capsaicinoids are synthesized in the placenta tissue by the condensation of vanillylamine (via the phenylpropanoid pathway) with a chain of fatty acid. In seeds, capsaicinoids are not synthesized, but their presence is only due to a contamination from the nearby tissues (Aza-González et al. 2011).These alkaloid molecules have proven to have biological effects due to the activation of the transient receptor potential vanilloid type 1 (TRPV1) (Caterina et al. 1997). Capsaicin binds to the receptor TRPV and thus produces analgesia by depleting substance P in nociceptor neurons. Consequently, these insensitive nerves lose their capacity to deliver mediators involved in neurotransmission and inflammation mechanisms. Hence capsaicin and its

analogues are used to cure pain associated with chronic and inflammatory diseases. These properties, pain relief, weight reduction, cardiovascular benefits, gastroprotection, pruritus decrease have been explored to develop new therapeutic agents in the future (Hayman et al. 2008).

Other classes of phenylpropanoid derivatives, which are non pungent analogues of the former one possessing similar biological effects, have been recently described: capsinoids, and capsiconinoids (Tanaka et al. 2009). For these motives *Capsicum* extracts have been used in medicine as previously reported and, recently, even in cosmetics (Hayman et al. 2008., Luo et al. 2011).

Studies have described the capsaicinoid biosynthetic pathway, but little is known about the genes controlling the expression of the individual molecules and the control of their expression. Stewart et al. (2005) showed that a putative capsaicinoid synthetase gene co-segregated with the pungency trait, and was mapped in close proximity to *Pun1*, which might modulate the level of pungency (Stewart et al. 2005, Blum et al. 2002). Blum et al. (2002) identified a QTL, termed *cap*, located on chromosome 7, which is associated with the quantitative variation of capsaicinoids in a F_2 generation from a cross of a non-pungent with a pungent pepper genotypes (Blum et al. 2003).

Pepper fruits also possess other molecules, which are beneficial to humans, such as vitamins C and A, and carotenoid pigments with antioxidant activities (Zhuang et al. 2012). Antioxidants are defense mechanism of living cells against oxidative damage and thus become important tools in the prevention of a variety of degenerative diseases, including ageing, cancer, diabetes, and cardiovascular diseases. Among the carotenoid pigments, capsanthin and capsorubin are known to be exclusive of the genus *Capsicum* and are responsible for the final red fruits, while the green colour of the fruit is principally due to the presence of chlorophyll, typical of the chloroplast, and in minor extent to carotenoids and xanthophylls (De Masi et al. 2007).

In a previous paper, we reported on capsaicinoids and carotenoids evaluation in various Italian *Capsicum* cultivars (De Masi et al. 2007). Adequate characterization of chili accessions is needed to assist germplasm utilization by breeders. In the present contribution, we report on the capsaicin and vitamin C contents of ten *Capsicum* accessions from different origins. In addition, a description of genetic diversity of these accessions has been investigated using molecular approach.

Materials and Methods

Plant materials

The ten *Capsicum* accessions used in the present study were selected from a larger collection of hot pepper held at the Institute of Plant Genetic (IGV) of the National Research Council, on the basis of the pungency trait (Sarli et al. 2004). They were obtained from the "Accademia Italiana del Peperoncino", Diamante (CS), Italy, and commercial sources (Table 1). Out of these, five were *C. annuum*, three *C. chinense* and two *C. baccatum*. Plants were grown under controlled conditions in a greenhouse at the IGV. Fruits were collected at commercial maturity for chemical analysis, and young leaves were harvested two weeks after emergence for genetic analysis.

All chemical reagents used in this study were of Analytical Grade (Sigma Aldrich, Italy), or otherwise stated.

Capsaicinoid analysis

Capsaicin was examined according to the American Spice Trade Association (ASTA) method 21.3 (1998) with some modifications (De Masi et al. 2007). Fresh fruit tissues (2 to 10 g samples) were homogenized and mixed with 50 mL of dichloromethane:methanol (2:1). The organic layer was filtered, through Whatman filter paper, and then evaporated at room temperature. The dried samples were suspended in 25 mL ethanol and each extract was further filtered through a 0.45 μm

nylon filter, before the HPLC analysis. Standards of capsaicin and dihydrocapsaicin were obtained from Sigma-Aldrich (Italy). Standard curves were prepared using suitable linear working range of capsaicin and dihydrocapsaicin serial dilutions from 25 to 1000 mg/kg. An HPLC instrument (Thermo, Italy), equipped with UV detector and connected with a computer, was used for chromatographic analyses. The separation was carried out using a RP C18 column (250 x 4.6 mm) (Phenomenex, USA). The injection volume was 20 µL and the elution was performed in 60 min with a mobile phase made of ACN:water (3:2), containing 1% of acetic acid, at a flow rate of 1.0 mL/min. For each sample in study, the result was the average value of three determinations. Conversion of ASTA Pungency Units to capsaicin content of a Chilli of 1 part per million (ppm) joints to 16 Scoville units (Table 2).

Vitamin C analysis

Vitamin C in fresh fruits at commercial maturity was estimated by the 2,6-dichlorophenol indophenol method using a spectrophotometer at a wavelength of 520 nm (Durust et al. 1997). Vitamin C content was calculated on the basis of an adjusted calibration curve of L-ascorbic acid standard. For all samples results were mean value of three determinations.

DNA isolation and RAPD analysis

Genomic DNA was isolated from fresh young leaves through the protocol of the PureLink-Plant Total DNA Purification Kit (Life Technologies, Italy). Leaves from 10 individual plants from each population were bulked to constitute a unique homogeneous sample of 1.5 g. Each sample was ground into powder in a mortar with the help of liquid nitrogen and samples were processed following the manufacturer instructions. DNA quality was checked by gel electrophoresis, and also by spectrophotometer (260/280 absorbance ratio).

The ten DNA samples were analyzed by 16 arbitrary oligodeoxynucleotide primers (10-mer). These primers were selected based on their reproducible amplification pattern in a RAPD PCR test (Table 3). The PCR assay was done in a 50 µL reaction mixture containing 3 mM $MgCl_2$, 200 µM of each dNTP, 20 pmol of single primer, 10 ng of pepper genomic DNA as template and 2.5 U of Stoffel Fragment Taq DNA polymerase in a reaction buffer provided by manufacturer. A Veriti 96 well thermal cycler (Applied Biosystems, USA) was used with a program previously described (De Masi et al. 2007). Positive and negative controls were included and each experiment was performed in triplicate to verify the reproducibility of the procedure. The RAPD amplicons were separated by 1X TBE buffered electrophoresis on 2% (w/v) agarose gel. The resulting bands in the gel were visualized under UV light and digitalized by a Gel Doc System (BioRad, USA).

Results and Discussion

Average values of capsaicin and vitamin C are shown in figures 1 and 2. Ascorbic acid concentration did not show great variation from sample to sample and between pepper species, with values ranging from 123,4 to 199,80 mg/100 g (FW) of Portafortuna and Peter pepper (*C. annuum*) respectively.

The average value of capsaicin ranged from 3,8 to 12 mg/g of FW, in Campana (*C. baccatum*) and Habanero (*C. chinense*), respectively. The capsaicin and vitamin C amounts of the pepper varieties obtained in this study are similar to those reported in other *Capsicum* varieties from the literature, except for little difference due to environmental and growth factors, which generally affect secondary metabolites in plants (De Masi et al. 2007).

The ratio of ascorbic acid over capsaicin contents was also calculated for all cultivars under investigation. The results showed a very intriguing behavior: the cultivar with the lowest value of capsaicin content possesses the highest value of vitamin C. It can be observed from the current study that the cultivar Campana (*C. baccatum*), with the lowest capsaicin/vitamin C ratio of 2.80, is

superior to the other cultivars in terms of capsaicinoids/ascorbic acid contents ratio. It is interesting to notice that the regression of this ratio over vitamin C content shows a negative regression value of R=-0,44, although with a not very significant value (p ~1), while the regression of the actual values of capsaicin and vitamin C only show a very low regression value (R=0,10). This indication might help *Capsicum* breeders in selecting cultivars with superior nutritional and functional composition in vitamin C, which is a well-known antioxidant and a biologically active compound.

The genetic molecular analysis differentiated all the cultivars (Figure 3) but no correlation between genetic diversity and fruit pungency could be observed. The conclusion is that the loci considered by the RAPD analysis are not associated with the genetic determinants of the spicy taste. Possibly increasing the number of molecular markers, it would be possible to identify specific markers able to assist selection for pungent/non pungent character. Similarly, Lee et al. (2005) developed SCAR markers of the *C* locus to detect presence/absence of pungency, useful in breeding programs.

The capsaicinoids are a class of phenylpropanoid derivatives present only in the genus *Capsicum*. Their biosynthesis and accumulation are genetically determined traits, and different genotypes show a great variation in the reciprocal contents of all the phytochemicals of interest to human health, not only capsaicinoids. Moreover, it has been shown that variation within the same genotypes is the result of interaction with environmental growth conditions.

Several experiments have demonstrated that the factors affecting capsaicin accumulation are different. The actual determinants of capsaicinoids and their analogs synthesis and accumulation remain unknown (Aza-González et al. 2011). To our best knowledge, the only gene determining whether pungency is present or not is *Pun1* (Stewart et al. 2005).

Our previous reports showed a high level of inter-varietal polymorphism among hot pepper populations, but varietal selection based on agro-morphological and chemical characters is difficult and selection is only effective when plants are grown under the same growing conditions (De Masi et al. 2007). DNA markers are suitable for species and varietal identification, genetic purity assessment, helpful in breeding and genetic conservation, as they are not affected by environment or growth phase. The utility of RAPD markers has been extensively demonstrated in detecting genetic variation in local populations and in screening biodiversity in plants species including hot pepper (De Masi et al. 2007, De Masi et al. 2005, De Masi et al. 2006).

Our work is still in progress to further characterize the extent of genetic diversity using molecular marker with aim to assist future breeding for health related compounds in pepper fruits.

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Table 1. List of accessions employed in this study

N.	Accession	Species
1	Aji Panca	*C. chinense*
2	Campana	*C. baccatum*
3	Cedrino	*C. annuum*
4	Cedruccio	*C. annuum*
5	Habanero	*C. chinense*
6	Peter pepper	*C. annuum*
7	Portafortuna	*C. annuum*
8	Ramiro	*C. baccatum*
9	Scotch Bonnet Jamaican	*C. chinense*
10	Zimbabwe Bird	*C. annuum*

Table 2. Hotness rating of *Capsicum* accessions in study

Scoville Heat Units (SHU)	Referring pepper varieties	Pepper accessions
855,000-1,000,000	Bhut Jolokia	
350,000-577,000	Red Savina Habanero	
100,000-350,000	Habanero, Scotch Bonnet	Aji Panca, Cedrino, Cedruccio, Habanero, Peter, Portafortuna, Scotch Bonnet Jamaican, Zimbabwe Bird
50,000-100,000	Thai	Campana, Ramiro
30,000-50,000	Cayenna, Tabasco	
20,000-30,000	Manzano	
2,500-20,000	Jalapeno, Serrano, Yellow Wax	
1-2,500	Anaheim, Cascabel, Mexican, Pimento	
0	Sweet Bell	

Table 3. Arbitrary primers (10-mer) used for genetic analysis of hot pepper population

primer name	base sequence (5' - 3')	GC content (%)
AE19	GACAGTCCCT	60
AG14	CTCTCGGCGA	70
AK10	CAAGCGTCAC	60
AN10	CTGTGTGCTC	60
AN19	ACCACGCCTT	60
AX01	GTGTGCCGTT	60
AX08	AGTATGGCGG	60
E10	CACCAGGTGA	60
E11	GAGTCTCAGG	60
G07	GAACCTGCGG	70
G12	CAGCTCACGA	60
G19	GTCAGGGCAA	60
U1	AGGGGTCTTG	60
U3	GGGTTTAGGG	60
U4	GACAGACAGG	60
U19	TGGGAACGGT	60

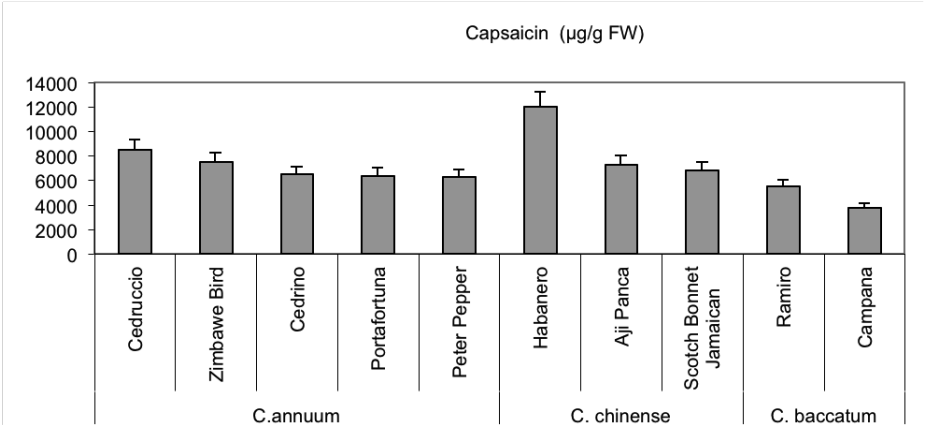

Figure 1 Capsaicin values of the samples analysed

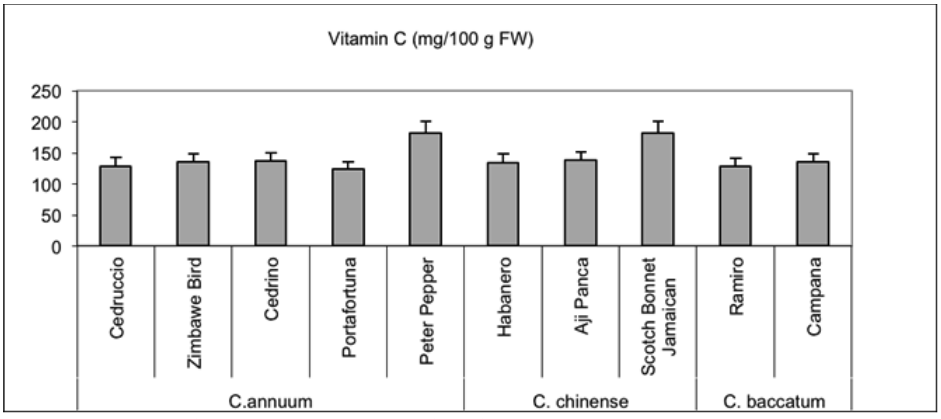

Figure 2 Vitamin C values of the samples analysed

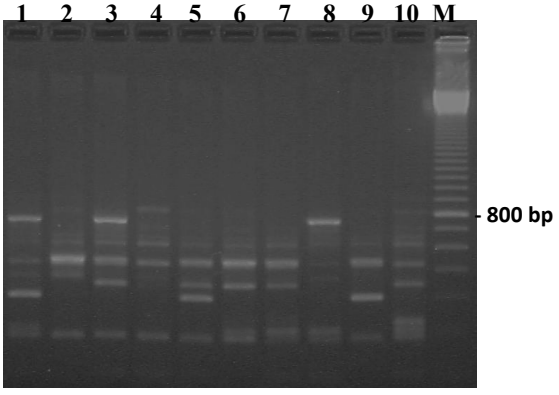

Figure 3 DNA fingerprints obtained by using primer U1 for RAPD-PCR amplification of chili pepper genomic DNA. Lane **1**: Aji Panca; lane **2**: Campana; lane **3**: Cedrino; lane **4**: Cedruccio; lane **5**: Habanero; lane **6**: Peter pepper; lane **7**: Portafortuna; lane **8**: Ramiro; lane **9**: Scotch Bonnet Jamaican; lane **10**: Zimbabwe Bird. Lane **M**: 100 bp DNA Molecular weight marker.

Management of pepper resistance to *Meloidogyne incognita* populations in South East Spain

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Abstract

The major phytopathological problem of the pepper crop in southeastern Spain is *Meloidogyne incognita*. The difficulty of the introduction of resistance to this nematode in commercial varieties, plus the restrictions on the use of soil fumigants have led to the use of rootstocks resistant to *M. incognita.* The emergence of nematode populations virulent to *Me7* gene when rootstocks carrying this resistant gene are repeatedly used on the same soil, demands the establishment of strategies for resistance management. Three rootstocks, with different resistance genes, were evaluated in Murcia (southeast Spain) on non-disinfected soil in four greenhouses naturally infested with *M. incognita*. Two greenhouses had nematode populations virulent to *Me7* gene and the other two had populations avirulent to this same gene. Out of the two greenhouses infested with virulent populations to *Me7* gene, one was in process of reducing the nematode populations by managing the use of rootstocks with *Me1* and *Me7* genes, while the other is in process of selection for virulence to *Me7* gene. The rootstocks evaluated were: Terrano (Syngenta Seeds) *Me1* gene carrier; Atlante (Ramiro Arnedo S.A.) *Me7* gene carrier, and IMIDA2 (obtained at the Instituto Murciano de Investigación y Desarrollo Agrario y Alimentario) *Me7* gene carrier. As controls there were two susceptible varieties, either Gacela (Syngenta Seeds) in three of the greenhouses or Traviata (Rijk Zwaan) in the other one. The rootstocks carriying *Me7* gene resulted infested at the same level as the susceptible variety in the greenhouse where virulence was being selected. By contrast, the other greenhouse where resistance had been managed for the last 4 years, these rootstocks showed a low level of infestation. In these two greenhouses (infested with nematode populations virulent to gene *Me7*) the rootstock Terrano, *Me1* gene carrier, was not infested. In the two greenhouses infested with populations avirulent to *Me7* gene, the rootstocks carrying this gene showed no galls in one of them, while in the other they showed significantly less infestation than the reference susceptible variety. In these greenhouses the performance of Terrano rootstock was the same of that shown in greenhouses with populations virulent to gene *Me7*. Our results show that strategies of management of resistance genes can be a useful strategy to control populations of *M. incognita* in greenhouse cultivation.

Keywords: *Capsicum annuum*, rootstocks, resistant gene, nematode, greenhouses.

Genetic structure of the INRA *Capsicum* spp. collection using SSR loci: refining the wild origin of cultivated *C. annuum* and impact of human selection on the structuration of genetic diversity in cultivar types.

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Abstract

Germplasm collections of cultivated plants constitute the source for further genetic progress and gained interest with approaches for tracking allelic variants associated to phenotypic variations within core collections. In order to explore the structure of genetic variation in pepper (*Capsicum* spp.) and to select core-collections maximizing the genetic and the phenotypic diversity, a pepper collection including 1352 non redundant accessions from 11 *Capsicum* species from 89 different countries was genotyped using 28 microsatellite (SSR) markers spanning the whole genome. Model-based analysis structured the collection into 6 clusters, with 3 clusters separating the main species complexes, including cultivated species and wild relatives, according to botanic classification (*C. frutescens/C. chinense, C. baccatum, C. pubescens*), and 3 additional clusters for *C. annuum*. The relationships between the cultivated *C. annuum* species and the wild relative (*C. annnuum* var *glabriusculum*) was refined. The 3 *C. annuum* clusters were significantly distinct for plant and fruit descriptors corresponding to cultivar types, showing that the genetic structuration of this cultivated species was strongly impacted by the long term human selection of landraces in primary as well as secondary diversification centres. We settled nested core-collections of 8, 16, 32, 64 and 128 *C. annuum* accessions capturing from 37% to 90% of the genetic diversity for further sequencing efforts and establishment of high through put genotyping assays. By compiling phenotypic and genotypic data, a larger core-collection of 332 accessions was established, capturing 97% of the *C. annuum* genetic and phenotypic diversity for further genetic association studies.

Keywords: *Capsicum*, germplasm collection, human selection, cultivar types, microsatellite, core collection

Introduction

In plant breeding, genetic diversity is the essential source of genetic progress which motivated the collection, maintenance and characterization of genetic resources for most cultivated plant species since the early 20th century. For most cultivated plants, the domestication process caused a loss of genetic variability compared to wild plant populations (Hammer et al. 2003;, Tang et al. 2010). Contrarily, thousands of years of human selection in multiple environments and cultural contexts, provided new mutants and allele combinations of agricultural interest which had poor probabilities to be retained under natural selection pressure. The thousands of local cultivars issued from field selection is a wide source of diversity for alleles of agricultural interest and local adaptations. Their contribution to further genetic progress and to the restoration of biodiversity in agrosystems is at least as promising as wild accessions, related species or exogenous gene sources.

Beyond the collection of these resources, their exploitation depends on our ability to characterize it. Association mapping or linkage disequilibrium (LD) mapping recently developed to track the allelic variants associated to phenotypic variations directly within core collections of plant genotypes, i.e. subsamples of genotypes which represent the genetic diversity of the crop with a minimal redundancy (Marita et al. 2000; Gupta et al. 2005; Zhu et al. 2008). The approach provides

access to multiple alleles and thus increases the efficiency of genetic resources exploitation. However, testing for statistical associations between genotypes and phenotypes in a population is directly affected by the presence of groups of related accessions with different allele frequencies (population structure) which may lead to false associations (Freedman et al. 2004). The core-collection also has to maximize the genetic diversity found in the whole collection and to span the full range of phenotypic variation (Ranc et al. 2010). Thus, analyses of the structure of the genetic diversity within the whole collection of accessions together with an evaluation of the range of phenotypic variation are prerequisite to the selection of core-collections for SNP mining and for association or LD mapping.

Since its domestication in pre-columbian times, peppers were subjected to successive migrations events through Atlantic and Pacific oceans to Europe, Africa and Asia. Trade routes between Europe, Middle-East and Asia also promoted reciprocal exchanges, so that complex introduction processes spread peppers throughout most tropical, mediterranean and temperate regions of the world (Somos 1984; Andrews 1984). In these secondary diversification centers, thousands of landraces have been selected for 4 to 5 centuries by growers to fit new environments and local consumption habits and trade, resulting in the phenotypic diversity of pepper cultivars (Nuez et al. 1996; Bosland and Votava 2000). The taxonomic structuration of the *Capsicum* genus was established from a multidisciplinary approach (Pickersgill et al. 1979; Pickersgil 1991) giving evidence for 5 distinct cultivated species originating from distinct domestication events, and grouped into 3 genetic pools. *C. annuum, C. frutescens* and *C. chinense* form the first genetic pool (the white flowered species) which was related to the wild progenitor *C. annuum* var *glabriusculum*. *C. baccatum* and the wild species *C. microcarpum* form the second genetic pool and *C. pubescens* together with the wild species *C. eximium* and *C. cardenasii* form the 3rd genetic pool. Since the nineties, the species nomenclature was consolidated (Barral and Bosland 2002) and analyses using isozymes, nuclear and chloroplastic DNA markers confirmed this structure and increased our knowledge of the relationships between wild and domesticated species (Walsh and Hoot 2001; Toquica et al. 2003; Ibiza et al. 2012). Many evaluations of genetic diversity were also published, showing that DNA polymorphism rate is rather constant within cultivated species whatever the markers used and generally higher than the polymorphism observed in other autogamous *Solanaceae* like tomato. Analyses of the structuration of genetic diversity reported the relationships between phylogenetic clusters and geographic distribution when species are considered in their primary diversification centers (Hernandez-Verdugo et al. 2001; Votava et al. 2002; Aguilar-Melèndez et al. 2009; Albrecht et al. 2012; Gonzalez-Jaral et al. 2012; Moses and Umaharan 2012; Pacheco-Olvera et al. 2012) and the narrow genetic basis of sweet and large fruited *C. annuum* cultivars compare to exotic landraces (Lefebvre et al. 2001; Tam et al. 2009).

These studies were always performed among restricted sets of accessions ($10<n<200$). Considering a larger panel of *Capsicum* genotypes, should provide a more complete view of the differentiation between pepper cultivars and landraces worldwide and enable us to establish core-collections for further studies of the impact of selection on genetic diversity (Nicolaï et al. submitted). With this aim, we genotyped the INRA *Capsicum* collection which includes 1352 non redundant accessions from 89 different countries, with a large majority of *C. annuum* landraces, but also representatives of 10 additional cultivated or wild species (Sage-Palloix et al. 2007), using 28 SSR loci. Model-based analysis structured this collection into 6 clusters, including 3 distinct clusters for *C. annuum*, which were related to large cultivar types differing in plant and fruit traits as a result of selection. These data were used to establish core collections with different sizes for further SNP mining or genetic association studies.

Material and Methods

Pepper germplasm collection and phenotypic trait measurements.

The pepper (*Capsicum* spp.) germplasm collection maintained at INRA *Unité de Génétique et Amélioration des Fruits et Légumes* includes 1352 non redundant accessions from 11 *Capsicum* species which were collected since 1959 from 89 distinct countries (Sage-Palloix et al. 2007). *Capsicum* accessions are mostly landraces from the cultivated species: *C. annuum* (1063 accessions, including 27 wild *C. annuum glabriusculum*), 92 *C. chinense*, 51 *C. frutescens*, 107 *C. baccatum*, 18 *C. pubescens* and representatives of 6 wild species: 13 *C. chacoense*, 3 *C. cardenasii*, 2 *C. eximium*, 1 *C. galapagoense*, 1 *C. microcarpum* and 1 *C. praetermissum*. These accessions are maintained and multiplied by controlled selfing. Accessions and passport data are registered in the European Solanaceae Network: http://www.ecpgr.cgiar.org/germplasm_databases/list_of_germplasm databases/crop_databases/crop_database_windows/pepper.html). The collection was phenotyped for 21 plant and fruit descriptors and resistance to several pathogens as in Sage-Palloix et al. (2007).

DNA extraction and microsatellite genotyping.

DNA was extracted from pools of leaves of 6 young plantlets per accession as described by Fulton et al. (1995). A set of 28 published microsatellite markers was chosen on the basis of their distribution on the genetic map, spanning 11 of the 12 pepper chromosomes (available on demand). PCR amplifications were performed in a 10 µL reaction volume containing 25 ng of genomic DNA as template. Forward primers were 5'-end labelled with FAM, VIC, or NED for analysis on an Applied Biosystems 3730xI DNA Analyzer. GeneMapper 3.7 software (Applied Biosystems) was used to evaluate the size of the alleles.

Genetic diversity and structure analysis.

The number of allele, the number of genotypes, the Nei's unbiased gene diversity index (He), the observed heterozygosity (Ho), were calculated using the PowerMarker version 3.25 software (Liu and Muse 2005). To infer the population structure of the pepper collection, we used the model-based clustering algorithm implemented in the computer program Structure version 2.3.3 (Pritchard et al. 2000). From multilocus genotypes this algorithm identifies determined number (K) of clusters that have distinct allele frequencies and assigns portions of individual genomes to these clusters. We used the admixture model assuming correlation among allele frequencies. Ten runs were taken into account for each tested value of K, ranging from 1 to 10. In each run, we used a burn-period of 500,000 Markov Chain Monte Carlo iterations and then 250,000 iterations for estimating the parameters. The optimal K value (Kopt) was inferred according to Evanno et al. (2005). Individuals were assigned into a cluster when their proportion of membership into this cluster was higher than 50%. Genetic distance matrices between pairs of accessions were estimated from an index of dissimilarity based on the simple matching method for SSR alleles, and the standardized Euclidean distances for quantitative phenotypic traits. The graphical representation of the neighbour joining trees and principal coordinate analyses were performed with the DARwin 5.0.158 software (Perrier and Jacquemoud-Collet 2006).

Core collection sampling.

For sampling core collections, we used the Maximization (M) algorithm implemented in MSTRAT software version 4.1 (Gouesnard et al. 2001) which permits to maximize the number of alleles captured in the sample (allelic richness). Core collection's minimal size and accessions sampling were performed in 20 replicates with 30 iterations per replicate. The core collections were built using all SSR data alone (nested core collection) or together with phenotypic alleles for 3 plant phenotypic traits (flowering earliness, primary axis length and number of leaves), and 3 fruit traits (fruit length, fruit diameter and pericarp thickness). Phenotypic alleles were inferred from quantitative phenotypic data splitted into 10 classes of equal amplitudes.

Results and Discussion

Microsatellite diversity across the Capsicum species.

The diversity pattern of the 28 SSR loci across the 1352 *Capsicum* spp. accessions revealed 3 to 47 alleles per locus with an average of 18.2 alleles. The observed heterozygosity was very low (<0.085), as expected from accessions maintained and multiplied through selfing. Null alleles (no amplicons) were found for 2 to 3 SSRs in *C. pubescens, C. cardenasii, C. eximium*, *C. baccatum* and *C. chacoense*. Despite the 1352 *Capsicum* accessions were previously screened to remove redundant accessions according to passport and phenotypic descriptors, a few accessions displayed the same multilocus genotypes and were removed from further analyses, leading to a final panel of 1210 accessions. The allelic diversity (He index) was maximum in the *C. annuum* var *glabriusculum* sub-species (0.78) and lower but rather similar in the other species (0.47 to 0.59 for He).

Genetic structure of the collection.

The optimal number of genetic clusters in the complete collection (1210 accessions) was determined at 6 (Kopt = 6, Figure 1). The clusters 1, 2 and 3 included all the cultivated *C. annuum* accessions and displayed some admixture. The clusters 4, 5 and 6 displayed a clear cut structure with no or very few admixture. The 4th cluster included all the *C. frutescens* and *C. chinense* accessions, together with *C. galapagoense*. The 5th cluster included all the *C. baccatum* and *C. microcarpum* accessions. The 6th cluster included all the *C. pubescens, C. eximium*, *C. cardenasii*, *C. praetermissum* accessions and also the 10 *C. chacoense* accessions. This model based clustering closely corresponded to the known taxonomic groups of the *Capsicum* genus, except for the *C. annuum* var *glabriusculum* accessions which were distributed in several clusters.

A phylogenetic tree for the whole collection, based on Nei's genetic pairwise distances was constructed using UPGMA procedure. This tree generally confirms the previous model based clustering, but brings more precisions in agreement with the taxonomic classification of *Capsicum* species (Figure 2). Indeed, the *C. chacoense* accessions are clearly separated from the *C. pubescens* accessions, similarly, the *C. chinense* accessions are clearly in a distinct branching than the *C. frutescens* accessions. The wild *C. annuum* var *glabriusculum* were distributed in different branches: at the root of and within the *C. annuum* branches (14 accessions originating from Mexico and North-America) or at the root of the *C. frutescens and C. chinense* branches (11 accessions originating from Central America), or close to the *C. chacoense* group (2 accessions from Columbia). Most of these accessions were given by B. Pickersgill in 1976 (former var. *aviculare*) who suggested this subspecies to relate to the 3 cultivated white flowered species (Pickergill et al. 1979). Further analyses (Pickersgill 1991; Moscone et al. 2007) revealed distinct karyotypes in those accessions, with one or two pairs of (sub)telomeric chromosomes which are specific of *C. frutescens* and *C. chinense* or of the domesticated *C. annuum* respectively. Interestingly, their position in the tree validates and refines their distribution in the white flowered group and suggests distinguishing the wild relatives of *C. annuum* which originated from Mexico and North America from the wild relatives of *C. frutescens* and/or *C. chinense* originating from Central and South Americas.

Finally, the cultivated *C. annuum* accessions are distributed in several branches in the lowest half of the tree with a large group corresponding to the previous cluster 1, but displayed a slightly more complex pattern for the previous clusters 2 and 3 which are subdivided into several subgroups.

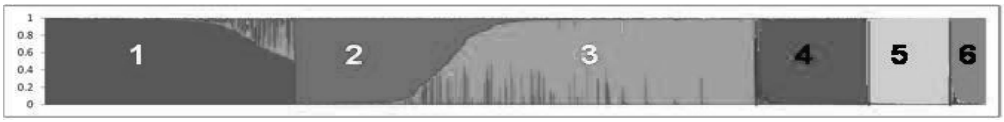

Figure 1: Model-based structuration in the whole *Capsicum* collection (1210 accessions) based on allelic variants at the 28 SSR loci. Six clusters were defined following the method of Evanno et al. (2005). Clusters 1, 2 and 3 included 908 cultivated *C. annuum* and 9 var *glabriusculum*, cluster 4 included 136 *C. frutescens C. chinense* , *C. galapagoense* plus 4 *var glabriusculum* accessions, cluster 5 included 105 *C. baccatum* and *C. microcarpum* accessions, cluster 6 included 34 *C. chacoense, C. pubescens, eximium, cardenasii, praetermissum* and 14 var *glabriusculum* accessions.

Genetic and phenotypic diversity of the 3 clusters of C. annuum.

The model based analysis with the Structure software delivered 3 distinct clusters within the *C. annuum* accessions (Figure 1) with admixted accessions. These 3 *C. annuum* clusters differ in their genetic diversity, with a higher He value for the cluster 1 (He 0.64) than for clusters 2 and 3 (He 0.44 and 0.40 respectively). In a first attempt to explore the diversity in phenotypes and origin of these 3 clusters, their means for plant and fruit parameters were compared and revealed significantly different average values for most plant and fruit descriptors (Figure 3).

The cluster 1 was characterized by late flowering plants (+ 3 days), with a long primary axis (28 cm) developing at least 14 leaves before flowering. Fruits from these accessions were small in length and particularly in diameter (1.9 cm in average) resulting in an elongated shape (4.7 times longer than large), with a pointed blossom end, and a thin pericarp (1.5 mm). That is characteristic for most small and elongated fruited peppers which represent 82% of the accessions from this cluster. This cluster was highly diversified in the geographic origins of the accessions, including traditional Mexican cultivars (‘Pasilla’, ‘Anaheim’, ‘Serrano’ types), from Asia (‘Perennial’ from India, ‘Nanjing early’ from China) and from Africa (‘H3’ from Ethiopia, ‘Chatah’ from Sudan). These late flowering and small-fruited cultivars mostly originate from subtropical areas but the cluster also includes many cultivars that became traditional in temperate and mediterranean countries like ‘Espelette’ pepper from France which appeared close to the Mexican ‘Pasilla Apaseo’.

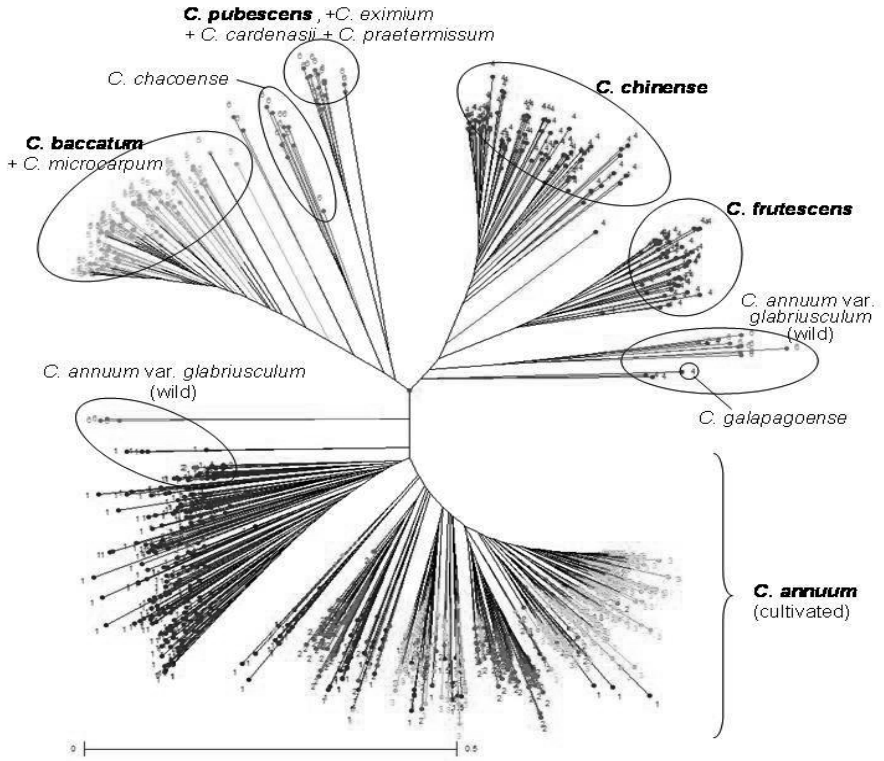

Figure 2: Phylogenetic tree showing the genetic diversity of the pepper germplasm collection (1210 accessions). The tree was produced using the neighbor-joining UPGMA method based on the 28 SSR markers.

The cluster 2 is characterized by early flowering plants (-3.5 days in average) with shorter primary axis (22 cm) bearing a lower number of leaves (9.5). The fruits are longer and larger than the previous cluster, resulting in a 2.8 length/width ratio with an obtuse apical end and a much thicker pericarp (4.2 mm). This corresponds to the triangular and horn shaped peppers but also to some elongated peppers, which represent 40% and 35% of the accessions respectively. Considering the geographic origins, this cluster displayed a clear predominance of central European origin. Indeed, 147 of the 201 accessions from cluster 2 (73%) are local cultivars originating from central Europe (Hungary, former Yugoslavia and Czechoslovakia, Romania, Poland, South Russia, Bulgaria) whereas these countries represent only 18% of the origins of the whole *C. annuum* collection. This cluster can be characterized by the traditional cultivars with elongated fruits like 'Hatvani', conical fruits with light green or ivory immature color like 'Podarok Moldavia', 'Feherozon', 'Cecei', but also a few blocky fruits with ivory color like 'Bela Krupna' or 'Paradicsom'. Another characteristic of this cluster is the presence of the traditional Turkish cultivars with horn or conical shaped fruits (10 landraces from the 'Sivri' and 'Carliston' types).

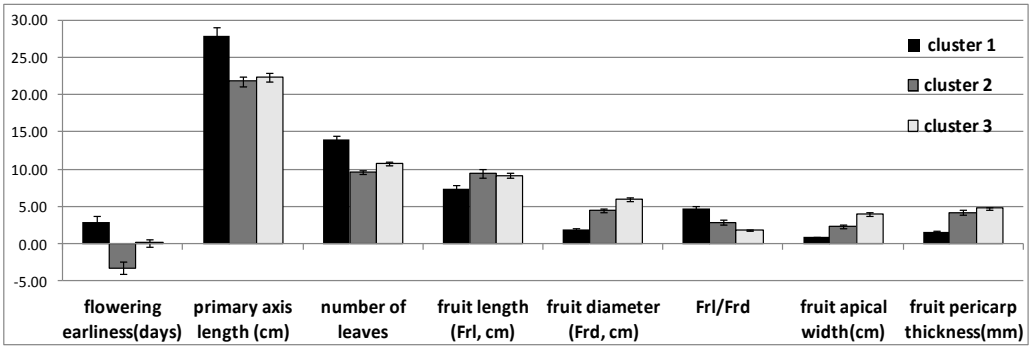

Figure 3 : Average values of the 3 cultivated *C. annuum* clusters defined by the model-based analysis for 8 plant and fruit traits. Vertical bars represent the 95% confidence interval.

The cluster 3 included plants with intermediate earliness but axis growth and development close to the cluster 2. Fruits are close to the cluster 2 in length but significantly larger (6 cm) resulting in an average length/width ratio of 1.8. The mean apical end is much more large and lobate and the pericarp is thick (4.8 mm). This clearly corresponds to the large fruited peppers with blocky or rectangular shape which contribute to 61% of the accessions of this cluster. Geographic origins in this cluster are diversified, including the traditional cultivars with very large (up to 600g) and rectangular fruits from Mediterranean Europe ('Largo de Reus' and 'Largo Valenciano' from Spain, 'Lagnes' from France), the large blocky fruits from Italy ('Quadrato Asti'), smaller blocky fruits from USA ('Yolo Wonder', 'California Wonder'), from Netherlands ('Mavras'), Poland ('Oda'), China ('Zao Feng', 'Ben Xi'). In this cluster were also located several accessions with thick pericarp but triangular, tomato, cherry or heart shaped fruits like 'Fresno' or 'Cherry bomb' from USA, 'Morron Conserva' or 'Ñora' from Spain. These cultivars present an admixted genome between the cluster 3 and 2 or 1.

Except for landraces from cluster 2 which originated from Central Europe and Turkey, the structuration by geographic origins of the clusters 1 and 3 was weakly visible. A more detailed analysis within each cultivar type reveals clusters of accessions with common geographic origins. However, these clusters also include cultivars collected from exotic countries. Genetic differentiation between cultivar types interferes or tends to dominate differentiation between geographic origins. It also attests the numerous and complex migration events of pepper genotypes, their adoption in a new country resulting from human migration and local selection.

Construction of core collections.

Sub-samples of 8, 16, 32, 64, and 128 accessions of *C. annuum* were selected, based on their genotypes at the 28 SSR loci. In this strategy, the accessions from the smaller samples were included in the successive larger samples (nested core-collections) These successive core collections captured 37 %, 55 %, 71 %, 85 %, and 89 % of the alleles from the whole *C. annuum* collection. The M strategy algorithm, using alleles at SSR loci, permits to select the smallest samples while maximizing the genetic diversity which maybe favourable for sequence diversity analyses and SNP mining. However the deficit in accessions from the cluster 3 (large fruited cultivars) and cluster 2 (early flowering plants and conical or long fruited cultivars) in these small core collections affects their representativeness for horticultural traits, which may not be favourable to association analyses with these traits. Thus, a larger core collection of *Capsicum annuum* was built with the objective to optimize the contribution of the *C. annuum* clusters, and to maximize both the genetic and phenotypic diversity. This was achieved, using the same M strategy, but based on their alleles at the 28 SSR markers and on their 'phenotypic alleles' for 6 primary traits

(flowering date, axis length, number of leaves, fruit length, fruit width, pericarp thickness). The resulting core-collection included 332 accessions distributed in the 3 *C. annuum* clusters proportionally to the gene diversity observed in each cluster (142 accessions from cluster 1, 97 from cluster 2, and 93 from cluster 3). This final core collection captured 97% of the SSR as well as phenotypic alleles from the whole *C. annuum* collection.

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An updated look at the taxonomy of the brinjal eggplant complex.

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Abstract

The brinjal eggplant, *Solanum melongena* L. is an economically significant crop, cultivated across much of the world. The taxonomic history of the brinjal eggplant complex has nevertheless presented us with a profusion of misidentifications and nomenclatural difficulties, as well as a complex puzzle of synonymous names. As a result, a consistent and workable system of classification for this important group has so far been lacking. This has had significant implications for the assessment of the genetic resources of this crop. Furthermore, the interpretation of genomic data generated by molecular marker analysis has often been compromised by the lack of a reliable taxonomic framework, and our understanding of the phylogeny and domestication of the brinjal eggplant may consequently have been limited. A survey of previous studies based on a wide range of methodologies, including the most recent genomic analyses, is given in this paper. Based on the morphological and ecogeographical assessment of around 2500 herbarium specimens, an updated and simplified taxonomic scheme for the brinjal eggplant complex is proposed. It consists of four species which include wild relatives from Africa and the Middle East, wild and semi-cultivated relatives from South and Southeast Asia, landraces from South and Southeast Asia, and modern cultivars grown worldwide.

Keywords: brinjal eggplant complex; domestication; genetic resources; taxonomy; wild relatives

Introduction

The brinjal eggplant complex.

The cultivated vegetable crop, *Solanum melongena* L., known as the brinjal eggplant (Lester & Hasan, 1991), has several wild and weedy relatives found in parts of Asia, the Middle East and Africa. Collectively, they are referred to as the brinjal eggplant complex (Samuels, 2010a). This group was first described as series *Incaniformia* Bitter (Bitter, 1923), and comprised 28 species found across Africa and Asia. Series *Incaniformia* is generally included in section *Melongena* (Miller) Dunal, which is placed in subgenus *Leptostemonum* (Dunal) Bitter, the "spiny solanums". Since Bitter's treatment of series *Incaniformia* 90 years ago there have been no other detailed studies of this group. Lester & Hasan (1991) adopted an informal taxonomic scheme for brinjal and its allies, and Lester et al. (2011) briefly covered this group in the wider context of their survey of the genus *Solanum* in Africa.

Variability.

The highly variable nature of most *S. melongena* allies has caused difficulties with identification. Morphology in spiny solanums shows great diversity between groups of closely-related species, or even within species themselves; the brinjal eggplant complex is no exception. Heteromorphism is strongly evident in many taxa, whereby shoot and leaf morphology may vary considerably between plants in the juvenile phase and those in the mature phase. Vigorous, young shoots often have a thicker indumentum, heavier covering of prickles, and larger leaves, compared with shoots on older plants. In the past, dried specimens from young and old shoots from the same plants have even been identified as being from different species.

Ferals, adventives and hybridisation.

S. melongena is believed to form feral populations which have diverged away from cultivation, and "wild" populations of brinjal are known to occur in India (Chaudhary, 1995; Sekara et al.

2007). Several wild spiny *Solanum* species which produce fruits with food or medicinal value are often semi-cultivated and encouraged to grow around village settlements in parts of Asia. Examples of these are *S. violaceum* Ortega, *S. virginianum* L. and *S. trilobatum* L. used in traditional medicine and as vegetables. All are able to hybridise with *S. melongena* (Daunay & Hazra, 2012). Many such relatives of brinjal are typically adventive, and can rapidly colonise disturbed ground along roadsides, amongst crops in small-scale cultivations and on the edges of villages. If growing within close proximity of brinjal cultivations, the potential for hybridisation will be considerable, and identification of the resultant populations will be problematic.

Figure 1. Detail of shoot of *S. campylacanthum* showing leaf variation.

Photo: courtesy of Ł. Haliński, University of Gdańsk.

Materials and Methods

This study was based largely on the comparative morphology of approximately 2500 herbarium specimens. These comprised loans from BR, G, HBG, JE, M, W, WU, and Z, which were examined at BIRM, whilst specimens housed at BM, K, RO and SING were examined in situ. In addition, access was made to online herbarium specimen collections at B, E, G-DC, L, LISC, P, Radboud University (Netherlands) Solanaceae Database, and the Solanaceae Source database. Many of Bitter's type specimens, and some of those of U. Dammer, were amongst these. Wherever possible, specimens were determined formally (initially by R. Lester and, subsequently, by the present author). Child's (1988) English translation of Bitter's (1923) *Solana Africana* proved indispensable during the herbarium study, as it alleviated the need to refer to the original, lengthy German and Latin texts.

In addition, an ecogeographic survey (Samuels et al. in prep.) based on the examination of around 800 herbarium specimens from K and BR was undertaken. Specimen label data relating to collector, date, provenance, locality, habitat and ethnology was analysed using BRAHMS and AGIS software. This provided a detailed insight into the floristics and ecology of *S. incanum s.l.* from Africa and the Middle East.

Results and Discussion

Based on the studies outlined above the following scheme is presented as an updated conspectus of series *Incaniformia*-the brinjal eggplant and its nearest wild relatives. For reasons of space, detailed lists of synonyms and their types are not given here; for further details see: Bitter (1923), Lester et al. (2011) and Samuels (1996).

Subgenus *Leptostemonum* (Dunal) Bitter

Section *Melongena* (Miller) Dunal

Series *Incaniformia* Bitter

S. campylacanthum Hochst. ex A. Rich.

subsp. *campylacanthum*

subsp. *beniense* (de Wild.) *comb. nov.*

subsp. *panduriforme* (Drège ex Dunal) J. Samuels

S. lichtensteinii Willd.

S. incanum L.

S. melongena L.

subsp. *melongena*

subsp. *cumingii* (Dunal) *comb. nov.*

Solanum campylacanthum **Hochst. ex A. Rich., Tent. Fl. Abyss. 2: 102. 1850. Type: Abyssinia, nr. Adua, 25 Nov. 1838, *Schimper 1082.* Lectotype: P; isolectotypes: P, BM, G-DC, K, W.** http://sonneratphoto.mnhn.fr/2012/03/08/11/P00344532.jpg.

Subsp. *campylacanthum* (Samuels, 2012a).

Common synonyms: S. bojeri Dunal, D.C. Prodr. 13 (1): 344. (1852); *S. cerasiferum* Dunal, DC., Prodr. 13 (1): 365 (1852); *S. aureitomentosum* Bitter, Feddes Repert., Beih. 11: 18 (1912); *S. delpierrei* De Wild.,Bull. Jard. Bot. État Bruxelles 4: 398. 1914; *S. stellativillosum* Bitter, Feddes Repert., Beih. 16: 226 (1923); *S. stellativillosum* Bitter var. *makinduense* Bitter, Feddes Repert. Beih 16: 228.

Characteristics: highly polymorphic group of more or less pubescent, more or less armed shrubs; prickles almost always recurved; up to 2m or more high; with ovate-lanceolate to lanceolate, more or less lobed leaves; up to 50 violet or purple flowers in each inflorescence, often with several to many hermaphrodite flowers; infructescence of several to many fruits, up to 3.5cm diameter, bitter. *Distribution:* centred in tropical eastern Africa and encompassing southern Sudan, Ethiopia, Eritrea, Somalia, Kenya, Uganda, Tanzania, Zanzibar, Comoro Islands, Burundi, Rwanda, eastern Democratic Republic of Congo, N. Zambia, Malawi. Found between sea level and 2,850m altitude and 17deg. N. to 13 deg. S. The extended presence of *S. campylacanthum* is also a useful indicator of overgrazing in the grasslands of East Africa.

*Commentary: w*ide variation in certain aspects of vegetative morphology, such as density and colour of indumentum, stellate hair form, and prickle density is common, particularly in several localised ecotypes, first recognised by Bitter (1923). Pending further investigations, two such examples: *S. aureitomentosum* Bitter and *S. stellativillosum* Bitter, both limited to upland eastern Africa, are synonymised here with subsp. *campylacanthum*.

Morphologically intermediate forms which lie between subspp. *campylacanthum* and *panduriforme* have been noted (e.g. Bitter, 1923; Lester et al. 2011; Samuels et al. in prep.). Knapp et al. (2013) viewed them as aspects of a north-south clinal variation in morphology (covering most of the length of Africa) of *S. campylacanthum*. This latter view is not supported here. Even with an extremely broad *S. campylacanthum* species concept, the *campylacanthum-panduriforme* extremes should be accounted for taxonomically, and the obvious geographical disparity shown by these taxa (Samuels, 2010b) can be usefully recognised by subspecific differentiation. In the present treatment, the majority of variation is subsumed into subsp. *campylacanthum* (generally distributed in north-east and eastern Africa), whilst plants of subsp. *panduriforme* (generally distributed in southern Africa) display more uniformity.

Although recognised by several authors (Bitter, 1923; Knapp et al. 2013; Lester et al. 2011) *S. cerasiferum* Dunal is rejected in this treatment. The name is based on a strongly-lobed variant of subsp. *campylacanthum* from Sudan (holotype at G-DC), with which it is synonymised here.

The root of subsp. *campylacanthum* extends 20-30 cm. below soil level and will produce suckers readily, often a considerable distance away. This feature, along with the capacity to regenerate from isolated root portions, enables the formation of expansive clonal stands (Samuels, 1996). This may give a false impression of uniformity amongst what may appear to be large numbers of individuals.

Subsp. *beniense* (de Wild.) *comb. nov*. *S. beniense*, Pl. Bequaert. i. 419 (1922). Type: Democratic Republic of Congo, Beni, grassy savannah, 4 April 1894, *J. Bequaert 3402*. Holotype: BR; isotypes: BR, K. www.br.fgov.be/RESEARCH/COLLECTIONS/HERBARIUM/zoomifyimaging.php?filename=0000008993038.

Common synonyms: S. homblei De Wild., Repert. Spec. Nov. Regni Veg. 13: 141 (1914); *S cerasiferum* Dunal var. g*randiflorum* Bitter, Repert. Nov. Spec. Regni Veg. 16: 286 (1923).

Characteristics: uniform, herbaceous or shrubby plants, branches with moderate covering of stellate hairs, always armed with straight or slightly curved prickles; ovate, sinuately lobed leaves, up to around 20 cm. long; inflorescences with around 6-8 flowers, mostly only 1 hermaphrodite per cluster; fruits up to about 3.5 cm. diameter, bitter.

*Distribution: s*avannah grassland, central Africa.

Commentary: collections of this subspecies are only known from central Africa and, although it is morphologically close to subsp. *campylacanthum*, it is regionally separate from it. Subsp. *beniense* differs by its uniformly sinuate leaf margins, acute, extended leaf tips, and laterally flattened, deltoid prickles. All specimens housed at BR show a characteristic, uniform morphology and, on this basis, it should be recognised at subspecific level. Collections of closely-related plants from the Democratic Republic of Congo exhibit strong armature of prickles up to 7.5 mm long on shoots and leaves, elliptic leaf laminae with long petioles; these forms seem to constitute a new species and further study is needed to confirm this.

Subsp. *panduriforme* (Drège ex Dunal) J. Samuels, K. Bull. 67: 401-411 (2012). As *S. panduraeforme* in DC, Prodr. 13 (1): 370 (1852). Type (as *S. panduriforme*): South Africa. Transkei, banks of River Mbasche, below 1000 ft., in scrub, January 1832, *Drège s.n.* Holotype: G-DC ; isotypes: P, K. http://sonneratphoto.mnhn.fr/2012/02/22/1/P00343022.jpg

Common synonyms: S. delagoense Dunal, DC Prodr. 13 (1): 349 (1852); *S. trepidans* CH Wright, in K. Bull. 1894: 128 (1894); *S. tuntula* De Wild., in de Wildeman, Pl., Beq. 1: 438 (1922).

Characteristics: uniform group of finely tomentose, sparsely armed or unarmed shrubs, sub-shrubs or herbaceous perennials; up to 2m or more high; with elliptic, entire to sub-entire leaves; up to 12 violet flowers in each inflorescence, usually only one flower (more rarely up to 3) hermaphrodite; infructescence of up to 3 fruits, up to 2.5cm diameter, bitter.

Distribution: centred in E. and S.E. Africa extending across Kenya, Uganda, Rwanda, Burundi, Tanzania, E. Zaïre, Mozambique, Malawi, Zambia, Angola, Namibia, Botswana, Zimbabwe, South Africa, Lesotho and Swaziland. Found between sea level and 2,500 m. altitude and 4 deg. N. to 30 deg. S.

Commentary: as for subsp. *campylacanthum*, there is a capacity for the production of clonal stands as a result of sucker formation some distance away from the parent plant (Samuels, 1996). The root stock also plays a major role in regeneration of plants after fire, drought or frost in savannah and scrubland habitats.

***Solanum lichtensteinii* Willd., Enum. Pl. 1: 238 (1809). Type: S. Africa, Cape of Good Hope, interior region, 1808 from seed cultivated in Berlin, *Willdenow s.n.* Holotype: B.** http://www.ville-ge.ch/musinfo/bd/cjb/chg/adetail.php?id=152303&base=img&lang=en

Common synonyms: S. incanum L. var. *lichtensteinii* (Willd.) Bitter, *Repert. Spec. Regni Veg.* 16: 278 (1923); *S. subexarmatum* Dunal, DC Prodr. 13 (1): 367 (1852).

Characteristics: uniform group of xeromorphic, densely tomentose, armed shrubs or sub-shrubs, 0.5-2m high; with narrowly ovate, repand leaves; up to 5 white (or more rarely violet) flowers in each simple inflorescence, usually only one flower (more rarely up to 3) hermaphrodite; infructescence of up to 3 fruits, up to 4.5cm diameter, bitter. *Distribution:* centred in southern Africa, extending across Zambia, Angola, W. Malawi, Zimbabwe, Botswana, Namibia and South Africa. Found growing between 600m and 1830m above sea level and restricted to the southern hemisphere, between 8 deg. S. and 33 deg. S. This species is often found thriving in dry savannah habitats.

Commentary: this species is superficially similar to *S. incanum*, but clearly differs from it by the triangular leaf lobes and the tendency for dried specimens to present with conspicuous ridging of the stems and main branches. The large, persistent tap-root may penetrate down to 0.5m. below soil level, and provides the main means of regeneration after drought, frost or savannah fires.

***Solanum incanum* L., Sp. Pl. 1: 188 (1753). Type, presumed from N.E. Africa/Middle East, *Herb. J. Burser Vol. IX No. 20.* Neotype: LINN.** http://sonneratphoto.mnhn.fr/2012/02/17/3/P00049842.jpg

Common synonyms: S. coagulans auct. non Forsskal; S. sanctum L., Sp. Pl. (ed. 2) 1: 269 (1762), nom.ilegit. superfl.; *S. unguiculatum A*. Rich., Tent. Fl. Abyss. II: 102 (1851).

Characteristics: uniform group of xeromorphic, densely tomentose, armed perennial shrubs; up to 2m high; with broadly ovate, sub-entire to repand leaves; up to 15 purple or violet flowers in each simple inflorescence, usually only one flower (more rarely up to 3) hermaphrodite; infructescence of up to 3 fruits, up to 3.5cm diameter, bitter, but sometimes edible.

Distribution: centred in N.E. Africa, including Egypt, Sudan, Eritrea, Djibouti, Ethiopia, and extending southwards into Somalia and Kenya, westwards into southern Niger, northern Nigeria, Benin, southern Ghana, Burkina Faso, eastern Mali, and also into the Middle East through Israel, Jordan, Saudi Arabia, Yemen (including Socotra), Oman, Iraq, southern Iran, and as far east as northern Pakistan and northern India. Found between 240 m. below sea level to 2,400 m. above sea level, and between 35 deg. N. and 3 deg. S. Similarly to *S. lichtensteinii*, the xeromorphic adaptations of this species allow it to survive arid environments, and it is commonly found in semi-desert conditions. It is often found growing near dried up watercourses or streams in otherwise arid environments.

Commentary: the distinction of *S. incanum* L. from its close relatives remained unclear for many years after it was first described by Linnaeus (1753). Many herbarium sheets in the UK and Europe comprising pre-1900 collections of *S. incanum* bear the name *S. coagulans* Forssk. This is a common misidentification as *S. coagulans* is a distantly-related species with distinctive flowers and fruits, found in N.E. Africa, Egypt and Yemen.

There have been varying views on the distribution of *S. incanum*-in particular its occurrence in India. Daunay et al. (2001), Lester & Hasan (1990, 1991) and Lester et al. (2011) described the distribution of *S. incanum* as restricted to parts of Africa and the Middle East. However, the easterly range of this species extends into Pakistan (Goodman & Gafoor, 1992; Yousaf et al. 2010) and north-western India (Clarke, 1885 [as *C. coagulans* Forssk.]; Singh, 2009). The Saharo-Sindian phytogeographical region extends eastwards from Africa and the Middle East into south Baluchistan, Sind and Punjab in Pakistan, and on into Punjab and Rajasthan in India (Thorhaug, 2008), which is part of the north-western arid zone of that country. Several other, closely-related spiny *Solanum* species such as *S.*

cordatum Forssk. and *S. forskalii* Dunal also have Saharo-Sindian distributions and, like *S. incanum*, they are well-adapted to arid and semi-desert conditions.

***Solanum melongena* L., Species Plantarum (1): 186 (1753). Type: "Old World tropics," no collector details or date. Lectotype: LINN.** http://www.linnean-online.org/2605/

Subsp. *melongena* (L.)

Common synonyms: S. ovigerum Dunal, Hist. Nat. Solanum: 210 (1813). There are over fifty synonyms for *S. melongena* L. (Samuels, 2012b) and detailed coverage can be found in Rao (2011).

Characteristics: annual or perennial shrubs or sub-shrubs; up to 0.30-1.5 m. high; armed or unarmed; young shoots often violet-suffused, indumentum densely pubescent; leaves ovate, up to around 25 cm. long, always more or less lobed, more or less repand; inflorescences often with 1 hermaphrodite flower only, sometimes andromonoecious with several flowers; fruits more or less pendulous, ripe fruit sometimes remaining greenish, otherwise ripening to yellow or orange; fruit in landraces globose, ovoid, or oblong, skin green, white, or purple, sometimes with stripes, up to 3 per infructescence, up to 5 cm. long, bitter or non-bitter and edible; fruit in modern cultivars globose, ovoid, oblong, or snake-like, skin white, yellow, purple, or pinkish, sometimes with stripes, up to ca. 30 cm. long, mostly only 1 per infructescence, sometimes several, slightly bitter/ non-bitter and edible. *Distribution:* landraces ("*S. melongena* group G/*S. ovigerum* Dunal" [Daunay et al. 2001]) are restricted to the South Asia to Southeast Asia region, and grown in gardens and small plots. Modern cultivars ("*S. melongena* group H" [Daunay et al. 2001]/*S. melongena* L. *s. str*.) are found across the world and grown in a variety of situations, ranging from small-scale farming in Asia, to intensive, protected cropping installations in Europe.

Commentary: common names are: brinjal (South Asia), terong/talong (Southeast Asia), brinjal eggplant (English), *aubergine* (French), *berenjena* (Spanish), and *baadanjaan* (Arabic). *S. ovigerum* has often been employed as the name for landraces of *S. melongena*. The name *S. ovigerum* Dunal *s. str*. (Dunal, 1813) should only be applied to plants with sparsely-prickled stems and peduncles (as for some landraces) it also only applies to forms which have ovate-oblong fruits, whereas many landraces tend to have globose fruits. Under subsp. *melongena*, landraces are grouped along with modern cultivars.

Many authors subscribe to the view that *S. melongena* was domesticated in India (e.g. Isshiki et al, 1994; Samuels 2013), probably around 2000 BC (Samuels, 2010a). Meyer et al. (2012) showed the close genomic connection between *S. incanum* and *S. melongena* landraces; this may be an indication of the process involving domestication of *S. melongena* from *S. incanum*, solely in India (Samuels, 2013). Towards the end of the first century B.C. in India there was probably an initial spread of brinjal eastwards, following the movements of Buddhist monk missionaries (who, as strict vegetarians favoured the meat-like flesh of brinjal) using the Silk Route. Thus, *S. melongena* probably reached China near the turn of the millennium, corresponding to the findings of Wang et al. (2008).

The use of cultivar groups (as recommended by the International Code for Nomenclature of Cultivated Plants) is needed for a more detailed classification. For landraces these should be regionally based, reflecting the three main centres of diversity in the Indo-Burma, Southeast Asia and Mediterranean regions. Unfortunately, this is beyond the scope of the present work.

Subsp. *cumingii* (Dunal) *comb. nov. S. cumingii* Dunal, DC., Prodr. 13 (1): 363 (1852). Type (as *S. cumingii*): Philippines, *Cuming 443*. Holotype: G-DC; isotypes: G-DC, K, L. http://sonneratphoto.mnhn.fr/2012/02/15/1/P00379572.jpg

Common synonyms: S. undatum auct. non Lam.

Characteristics: fairly uniform group of erect, annual or perennial shrubs, up to 2m. tall; young shoots and branches becoming more sparsely pubescent; always armed with straight prickles,

slightly laterally flattened, rarely slightly curved; leaves, up to around 15 cm. long, more or less repand, ovate-lanceolate, ovate-elliptic, or obovate-lanceolate, with prickles on the petiole, mid-rib and secondary veins; inflorescences andromonoecious, usually with solitary hermaphrodite flowers; fruit a globose berry, up to 3 cm. diameter, bitter, but edible. *Distribution:* South and Southeast Asia, from India to Philippines and southern China.

*Commentary: s*ubspecies *cumingii*, also known as wild brinjal, corresponds to the erect, moderately prickly, semi-cultivated shrubs of gardens and disturbed vegetation, or *S. melongena* group F (Lester & Hasan (1991). Following the suggestion of Mace et al. (1999), plants corresponding to group F are classified here as a subspecies of *S. melongena*. Prickles in all other members of series *Incaniformia* (excluding *S. insanum sensu* Lester & Hasan, 1991) have leaf armature which is almost solely limited to the mid-ribs and petioles. Subsp. *cumingii* is the only example which also bears prickles on the secondary veins. Plants tend to be found growing as weeds in and around villages and waste places wherever brinjal is grown, and are sometimes collected for consumption. This parallels the close relationship between nearest relatives and several other domesticated members of subgenus *Leptostemonum*, whereby the wild or semi-domesticated relatives tend to be prickly, adventive forms. An example, also from section *Melongena*, is the domesticated Gboma eggplant, *S. macrocarpon* subsp. *macrocarpon*, and its near relative *S. macrocarpon* subsp. *dasyphyllum* (Schumach) Lester, Jaeger & Child. Subsp. *dasyphyllum* is found growing in or around human settlements and has edible fruits (Lester et al. 2011).

Subspecies *cumingii* seems more likely than *S. insanum* (which is a taxonomically unreliable taxon) to be the "feral form" of *S. melongena* referred to by Lester & Hasan (1991). *S. insanum sensu lato* (including *S. insanum* L./*S. melongena* group E of Lester & Hasan, 1991) is a complex assemblage of "progenitor and derived forms," according to Karihaloo and colleagues (Karihaloo & Rai, 1995; Karihaloo & Gottlieb, 1995). The correct application of *S. insanum* L. has remained doubtful and confusing ever since its first use, and it continues to be used in various, inconsistent ways. It is a current example of a "*nomen ambiguum*," or ambiguous name. Recurrent problems with application, as well as an unstable species concept and questionable taxonomic legitimacy (Meyer et al. 2012) have meant that the name may no longer be usefully employed and should be considered for rejection.

Figure 2. *S. melongena* subsp. *cumingii* young plant, Malaysia.
Photo: courtesy of Ł. Haliński, University of Gdańsk.

Brinjal eggplant relatives in the "eggplant clade"

The "eggplant clade" (Weese & Bohs, 2010) is based on phylogenetic analysis and may contain several other species apart from those listed above, but which nevertheless are distinct from those in series *Incaniformia*. One such species suggested by Weese & Bohs is *S. linnaeanum* Hepper & P-M.L. Jaeger, found wild in the Cape Province of South Africa. However, it seems that limited sample size and provenance, as well as the possibility of introgression with *S. lichtensteinii*, may have influenced the phylogenetic position that Weese and Bohs (2010) assign to it. Their assumptions of interfertility between *S. linnaeanum* and *S. melongena* are unfounded as, at the time

of writing, there is no published evidence of hybrids between *S. linnaeanum* and *S. melongena* produced via the natural germination of fertile seed resulting from cross-pollination. Lester et al. (2011**)** affirm that *S. linnaeanum* belongs to section *Melongena* Dunal (as does brinjal and its allies), but differs widely from brinjal in several morphological characters. Lester et al. classify *S. linnaeanum* as part of series *Sodomela* Lowe. In the light of this information, judgement on the relationship between *S. linnaeanum* and brinjal should be postponed pending more conclusive studies.

S. umtuma Vorontsova & S. Knapp was described from south-eastern Africa recently (Vorontsova & Knapp, 2012). Morphologically it resembles the allopatric *S. cerasiferum*, and the sympatric *S. macrocarpon* subsp. *dasyphyllum*. One of the main distinguishing features of *S. umtuma* is the presence of secondary leaf lobes, also found in subsp. *dasyphyllum*. Citing unpublished genomic analyses, Knapp et al. (2013) describe *S. umtuma* as closely related to *S. linnaeanum*. Superficially, it appears to belong to section *Melongena*, but its precise taxonomic status is unclear. It is suggested here that it may be of hybrid origin, probably involving subsp. *dasyphyllum*.

S. rigidum Lam**.** has also been described as a close relative of *S. melongena* and its allies (Knapp et al. 2013) and may be a member of the eggplant clade (Stern & Vorontsova, unpubl.) It is a low-growing, prickly shrub with ovate leaves bearing sinuate-angulate lobes, and white flowers (Lamarck, 1797), and bears a superficial resemblance to *S. cerasiferum*. However, as an endemic of the Republic of the Cape Verde Islands, 350 miles off the coast of Senegal, its relationship to species of series *Incaniformia* is not clear.

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SESSION IV

Breeding strategies

Prediction model for fresh fruit yield in aromatic peppers (*Capsicum annuum* L.) grown over years

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Abstract

It is frequently of interest to identify those components which contribute to the complex trait 'yield'. This study aimed to develop models for predicting fresh fruit yield in aromatic *Capsicum annuum* through multiple linear regression analyses. Ten genotypes of aromatic pepper were evaluated for 3 years in the Faculty of Agriculture farm, University of Nigeria Nsukka. All the genotypes were raised in nursery baskets at the Botanic Garden, using a growth medium of 3:2:1 of top soil, poultry manure and river sand. The experimental design was a randomized complete block design of 3 replication and ten plots per block. Data were collected on morphological and yield component traits, and fruit yield. The analyses of data were done for each of the years and then the combined analyses. Yield components with strong and significant correlation coefficients were regressed to establish relational functions with fresh fruit yield. Fruit yield could be predicted using the combined effects of number of nodes per plant, number of leaves per plant and number of fruits per plant with 87.6% accuracy in the 3 year combined analysis. Linear regression for the single effects of each of the yield components were also used to predict fruit yield. The models developed could predict fruit yield in *C. annuum* with 62.5%, 61.7%, and 57.2% accuracy using any one of these yield components, number of nodes, number of leaves and number of fruits, respectively. The combined effects gave higher predictive value than the single effects of the traits. The models developed were validated by extrapolating the values and comparing with actual yield data. Produced model could be used in predicting the fresh fruit yield in *C. annuum*. Inferences drawn from the functions developed were discussed as they affect breeding for high yield in aromatic peppers.

Keywords: aromatic peppers, yield components, prediction model, fruit yield, regression

Introduction

Peppers are cherished in many diets of Nigerians. Pepper is the only source of capsaicin, an alkaloid that is a digestive stimulant and important ingredient of daily diet (Bosland and Votava, 2000). The fruit colour is due to the presence of total carotenoid pigments. Consumption of pepper in Nigeria accounts for about 40% of average daily intake either in diets as stimulants due to the presence of the alkaloid capsaicin or as condiments for flavouring and colouring food. Nigeria is the largest producer of pepper in Africa covering about 50% of total Africa production (Adetule and Olakojo 2006). Nigeria was named as number 12 among top producing countries of dry chillies and peppers – 148508 – 50000 metric tons in 2008 FAO report (Hays 2009).

Crop production efforts are directed towards yield optimisation. Optimising yield can either be via agronomic practices and/or breeding. Yield components play important role in many crop research programmes. It is frequently of interest to identify those components which contribute most to the complex trait 'yield' (Piepho 1995; Baiyeri and Mba 1997). Information on functional relationship among components of growth and yield will enhance research efforts to breed for high yield in aromatic peppers. It will aid breeders to make both direct and indirect selection for high

yield via components that have the strongest bearing on yield. Baiyeri and Mba (1997) reported that knowledge of the strength of the relationship and/or interdependence of yield components will help plant breeders to focus their selection efforts on components that favour faster yield improvements.

Yield improvement programmes of pepper have indicated that some genotypes perform better than others under certain environmental conditions (Mattei et al. 1971). Hays (2009) equally reported that production of pepper is characterised by large fluctuations in the number of fruits and the final fruit yield per genotype over environments. Effects of random environments on *C. annuum* genotypes in relation to yield and its components have also been reported (Abu et al. 2011). There is therefore the need to develop functional relationships with data on yield and yield components in the different years of genotypic assessment and in the combined analysis. This will guide both the breeder and the farmer on components to select in order to improve yield, it would equally help in constructive yield prediction even at the vegetative stage of the plants. Estimating the model across years would give insight into the possible effects of the random environment on the accuracy of the prediction. The aim of this work was to develop prediction models for yield in *C. annuum* in each of the years and the combined analysis.

Materials and Methods

Ten aromatic pepper genotypes were used in this study, five were obtained from the pepper germplasm of the Department of Crop Science, University of Nigeria Nsukka while the the other five genotypes were bought from the open market. All the genotypes were grown for 3 cycles in the Botanical garden before the onset of the experiment. These genotypes were evaluated in the field under rain fed conditions for 3 years in the Faculty of Agriculture Research Farm, University of Nigeria, Nsukka. Nsukka lies within latitude 06^0 51' N, longitude 07^0 29' E and an altitude of 400m above sea level. Randomized Complete Block Design (RCBD) of three replications was used. Each block was divided into ten plots measuring 2.9m x 2m ($5.8m^2$). The seeds of the different genotypes were raised in nursery baskets before transplanting to the field. The nursery medium was a 3:2:1 mixture of top soil, poultry manure and river sand, respectively (Uguru 1996). Transplanting was done at four weeks and with twelve plants per plot and ten plots per block. The plant spacing is already stated above 45cm x 60cm intra - and inter – row spacing, respectively (Bosland and Votatava, 2000). The seeds collected after each year's sowing were used for the following year's planting. Data were collected on morphological and agronomic characters for each of the three years. Number of nodes per plant and number of leaves per plant were obtained by numerical count starting at 100 days after planting (DAP) from block one to block three. Number of fruits per plant was also obtained by counting the number of fruits at each harvest. At the end of harvesting the total number of fruits for each plant in each plot was added together. The mean number of fruits per plant per plot in each block was recorded as number of fruits per plant prior to analysis. Fresh fruit weight was obtained from the weight of fruits at each harvest per plant, this was added together as above at the end harvest and the mean estimated. The fruit yield per hectare was estimated based on yield per plant and the plant population per hectare.

The data collected on yield components were regressed with fruit yield in each of the years and then in the combined analysis. Prediction models were developed for each of the years and the 3 year combined analysis.

Results and Discussion

Regression models were developed to predict the effects of yield components on fresh fruit yield. By using these models, fruit yield could be successfully predicted at onset of flowering via the number of nodes and number of leaves per plant. Significant ($P = 0.001$) simple and multiple regression existed among yield components – number of nodes, number of leaves and the number of

fruits, with fresh fruit yield. The functional equations developed are shown on table 1. The level of significance of the regression and the standard error of estimation (SEE) support their usefulness for prediction. Pepper plants inherently keep growing and flowering at the nodal regions with a consequent increase in nodal and leave formation even at fruiting. Yield prediction using the number of nodes and number leaves could serve as pre-flowering index in predicting fruit yield; this could guide the breeder/farmer on whether to increase inputs on agronomic practises in order to improve the yield.

Number of nodes per plant (NP) versus fruit yield (FY)

Predictions for the fresh fruit yield using linear regression across the 3 years of genotypic evaluation and in the combined analysis for the number of nodes per plant showed the following functions (Table 1).

FY = -1.53 + 0.035NP

FY = 0.66 + 0.016NP

FY = 0.271 + 0.025NP

FY = -2.244 + 0.032NP

The fitted equation explained 90.4%, 10.8%, 81.5% and 62.5% of the fruit yield using the number of nodes for each of the three years and in the 3 year combined analysis (Table 1). Note that year 2 was not significant, however the high r^2 values obtained in other years could be used to predict fruit yield at the vegetative stage of the crop when agronomic inputs can still be applied to increase expected yield. The significant influence of the number of nodes on fruit yield could be positively exploited. Nodes are the flowering points in *C. annum* peppers. This implies that selection for high nodal formation in a breeding programme targeted to increase fruit yield would be advantageous. Equally, the breeder/farmer could adopt agronomic practices that would increase high nodal formation before and during flowering. Number of nodes among other traits has been reported to be of great economic importance in pepper production (Nandadevi and Hosamani, 2003).

Number of Leaves per plant (LP) versus fruit yield (FY)

FY = -1.031 + 0.068LP (r^2 = 83.9%)

FY = 4.96 + 0.005LP (r^2 = 0.4%)

FY = 0.216 + 0.043LP (r^2 = 82.5%)

FY = -0.36 + 0.04LP (r^2 = 61.7%)

The above functions showed that fruit yield was 83.9%, 82.5% and 61.7% dependent on the number leaves per plant in year 1, 3 and combined analysis, respectively. The model was not significant in year 2 (r2 = 0.4%), hence the high values of SEE (Table 1 year 2). Vegetative parameters as number of nodes and leaves per plant could serve as pre-fruiting prediction index in C. annum. The number of leaves being the photosynthetic source is vital to fruit setting, development and maturity. These vegetative parameters could serve as indices for pre-fruiting prediction. These early predictions could enable the breeder/farmer to increase agronomic practices that would cause increased luxuriant growth via these two parameters and indirectly improve the yield.

Number of fruits per plant (FP) versus fruit yield (FY)

The coefficient of determination across the years for number fruits seems weak, though significant. The fitted equations could explain fruit yield via the number fruits per plant by 62%, 75.5%, 53% and 57.2%, across the 3 years and in the combined analysis, respectively. The weak expression could possibly be that the number of fruits per plant affects fruit yield via itself and

individual fruit size – single fruit weight. The developed equations, coefficients of determination and SEE are shown on table 1. Based on observed correlations, Lahbib et al. (2012) reported the number of fruits as one of the characters that were effective on yield.

Combined effects of the three traits on fruit yield

FY = -1.452 + 0.018NP + 0.022LP + 0.044FP (r^2 = 92.3%)
FY = -0.4 + 0.01NP – 0.005LP + 0.09FP (r^2 = 81.5%)
FY = -0.153 – 0.005NP + 0.042LP + 0.031FP (r^2 = 88.6%)
FY = -1.237 + 0.001NP + 0.029LP + 0.055FP (r^2 = 87.6%)

The multiple regression equations of the joint effect of the three yield components showed that fruit yield is 81.5% - 92.3% dependent on the collective effects of these three traits across the years and in the combined analysis. In all the years of genotype evaluation and the combined analysis of 3 years data, the combined effects of the three traits viz: number of nodes per plant, number of leaves per plant and number of fruits per plant gave higher predictive values than individual traits. This implies that breeding efforts based on the joint effects of these 3 traits would produce a shift in mean value under selection faster than that focused on individual traits. The estimates from the 3 year data analysis were significant, having a range of probability level from 0.004 – 0.01. The single effects could explain fruit yield by 62.5%, 61.7% and 57.2% while the combined effects of the 3 traits explained 87.6% of the fruits yield in the combined analysis of the 3 years (Table 1). The model developed from the joint effects of the traits in the 3 year combined analysis is as follows: FY = -1.237 + 0.001NP + 0.029LP + 0.055FP.

The actual yield values plotted against the predicted yield based on the models developed are shown in figures 1 – 4. The yield values predicted from the joint effects of the 3 traits across the years had closer relationship with the actual yield due to their higher coefficients of determination. The extrapolated chart of the actual and predicted yield (Figure 3) gave a sharper figure as the components explained high predictive values except the number of fruits per plant that was relatively low (53%). The actual and predicted yield in the combined analysis (figure 4) showed close relationship as explained by the coefficients.

Year effects on prediction models

The combined effects of the three yield components in year 2 explained the fitted equation as high as 81.5% even when two out the three traits were not significant when considered alone. The curve of actual yield in relation to predicted yield showed a flatter tendency than the other years especially with the number of nodes and number of leaves that had a non significant coefficient of determination (Figure 2). Kumar and Dubey (2001) reported that correlation coefficients are specific to the material and environmental conditions, emphasizing that associations between quantitative traits are subject to environemntal fluctuatations.

The estimation of the model for each year and in the combined analysis offered the opportunity of checking the accuracy of the prediction across the random environment of the three years. The linear regressions of year1 and 3 were significant with high predictive accuracy, while the year 2 was not significant for number of leaves and number of nodes. The observed random environment of the second year seemed to be unfavourable and this may have affected genotypic expression and association link between traits.Drastic variation in the random environment could bring variation in the expected validity of the model. This seems to suggest that models are different or inaccurate when environmental conditions are not met. Where there are erratic whether conditions which differed widely from that of the preceding years, prediction may not be sufficiently accurate due to the major influence of the environment on the gene expression. The wide variations exhibited by *C. annum* genotypes in character manifestation across the random environment of derived savannah ecology have been sufficiently discussed (Abu et al. 2011). Marcelis and Gijzen (1998) reported that the accuracy of prediction of cucumber yields depends on accuracy of whether prediction. Crop

yield is the result of complex interaction among factors of soil, atmosphere, plant genotypes and management practices adopted (Sehgal 2013). Costa and Coelho (2009) reported that agriculture is an economic activity that strongly depends on climate and weather information. The complex interaction of crop with various factors and of the factors among themselves make crop yield modelling a difficult task (Sehgal 2013). These are all suggestive of the fact that developed functions are subject to influence by other factors or information outside the conditions for the experiment where they were developed. The year 2 distribution curve showed flatter tendencies than the other years and the combined analysis; this is also a pointer to the inability of the genotypes to fully express inherent ability due to weather conditions.

Conclusion

Fruit yield could be effectively predicted – above 80% accuracy, via the combined effects of number of nodes per plant, number of leaves per plant and number of fruits per plant. Yield improvement programme based on the selection of these 3 traits would increase fruit yield. Adopting agronomic practices that would encourage luxuriant growth – more number of nodes, leaves and invariably number of fruits, could significantly increase fruit yield in aromatic peppers. Fluctuations in random environment could affect the validity of the predictions via its effect on yield components; however, accuracy of predictions could be maintained by a combination of these three traits in estimating the coefficient of determination rather than the use of individual traits in linear regression analysis.

Table 1: Predictive functions, correlation, coefficients of determination and standard error of estimates (SEE) of yield components of *C.annuum* across the years of genotypic evaluation and the combined analysis.

Traits	Functions	R	R^2(%)	SEE
	YEAR 1			
No of nodes/plt	FY = -1.53 + 0.035NP	0.95	90.4	0.6
No of leaves/plt	FY = -1.031 + 0.068LP	0.92	83.9	0.8
No of fruits/plt	FY = 0.058 + 0.145FP	0.79	62.0	1.3
Combined effects of the traits	FY = -1.452 + 0.018NP + 0.022LP + 0.044FP	0.96	92.3	0.6
	YEAR 2			
No of nodes/plt	FY = 0.66 + 0.016NP	0.33	10.8	2.9
No of leaves/plt	FY = 4.96 + 0.005LP	0.06	0.4	3.1
No of fruits/plt	FY = 1.81 + 0.096FP	0.89	75.5	1.4
Combined effects of the traits	FY = -0.4 + 0.01NP – 0.005LP + 0.09FP	0.9	81.5	1.5
	YEAR 3			
No of nodes/plt	FY = 0.271 + 0.025NP	0.903	81.5	1.0
No of leaves/plt	FY = 0.216 + 0.043LP	0.908	82.5	0.9
No of fruits/plt	FY = 2.313 + 0.067FP	0.728	53	1.5
Combined effects of the traits	FY = -0.153 – 0.005NP + 0.042LP + 0.031FP	0.941	88.6	0.9
Combined analysis of 3 years data (Mean of means)				
No of nodes/plt	FY = -2.244 + 0.032NP	0.79	62.5	1.0
No of leaves/plt	FY = -0.36 + 0.04LP	0.79	61.7	1.1
No of fruits/plt	FY = 1.92 + 0.077FP	0.76	57.2	1.1
Combined effects of the traits	FY = -1.237 + 0.001NP + 0.029LP + 0.055FP	0.94	87.6	0.7

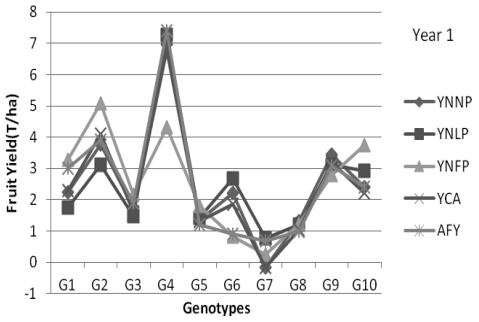

Figure 1: Actual and predicted yield across genotypes in year 1

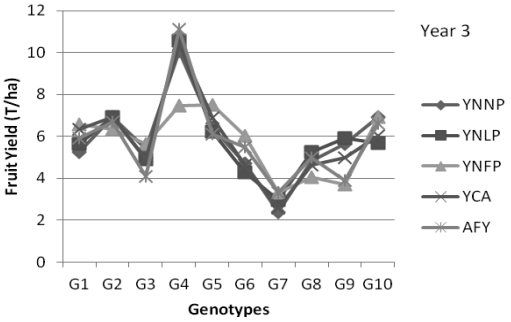

Figure 3: Actual and predicted yield across genotypes in year 3

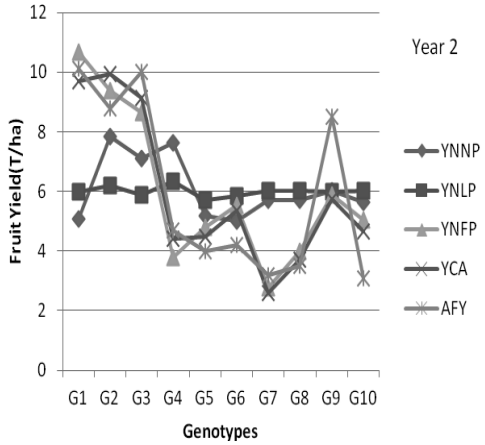

Figure 2: Actual and predicted yield across genotypes in year 2

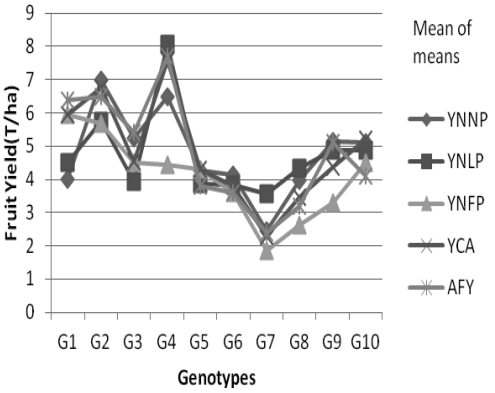

Figure 4: Actual and predicted yield across genotypes in the 3 year combined analysis.

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Multivariate QTL analyses and predictions of yield related traits in pepper

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Abstract

Yield is a key trait in pepper and is often measured simultaneously with other traits over several environments. The understanding and genetic improvement of yield may benefit from the joint analysis of yield with its related traits simultaneously. Linear mixed models have emerged as a flexible approach that correctly model underlying variance-covariance structures among the traits and between environments simultaneously. In this study, we applied four different QTL approaches based on linear mixed model on five yield related pepper traits measured across four environments. We evaluated the performance of the approaches in terms of the number of QTLs detected for each trait and their explained variance. The QTL models are a single-trait single-environment approach (STSE), a multi-trait approach (MT), a multi-environment approach (ME), and a multi-trait multi-environment approach (MTME). We further compared prediction accuracies between STSE and MT models. The predictions were subjected to a five-fold cross validation. Our results showed that multi-trait and/or multi-environment QTL analyses are more powerful and effective to map pleiotropic QTL and QTL by environment interactions than performing STSE analysis. The multivariate models further showed improvement over STSE in terms of both number of QTLs and the explained variance. MTME clearly outperformed all the other methods. With MTME, nine QTLs explaining 51% of genetic variation were identified for yield in the autumn trial in Spain as against three, three and six QTLs explaining 37%, 29% and 43% from STSE, ME and MT analyses, respectively. The MT model for yield in SP2 had prediction accuracy of 0.53, against 0.42 from the STSE model. These results confirmed that multivariate analyses of traits have better capabilities to unravel complex traits than single trait approach. Our result showed that trait's prediction accuracy depends not only on prediction model of choice and traits genetic architecture but also on the environment.

Keywords: Pepper, Quantitative Trait Loci, Multi-Trait-Multi-Environment, Genetic Correlation, Pleiotropy, Prediction Accuracy.

Introduction

When measurements are obtained for several traits on a plant simultaneously, it is only proper to consider analysing such traits multivariately instead of univariate analyses. This is even more so as biological processes are usually interdependent. Also, good varieties are known to show combined optimal values for several traits simultaneously. Many of such traits are often genetically correlated and proper QTL mapping could show if the correlation is due to a QTL simultaneously affecting many traits i.e. pleiotropy. Also, for several uncorrelated traits, the overall experiment type-I error (α) can easily be computed. However, the formula for such computation is not correct if some of the traits are correlated (Weller et al. 1996). In univariate analysis of correlated traits, the sampling variances of estimated parameters tend to be higher and the hypothesis tests show lower statistical power. The joint analysis of multiple traits has been shown to improve the power and precision of QTL mapping. It has also helped in improving the selection of some primary traits with low heritabilities or difficult to measure by exploiting their genetic correlations with other traits (Jiang and Zeng, 1995).

Also, measurement for yield and other important traits of agronomic importance may be done across a number of environments and may thus exhibit genotype by environment interactions (GEI). When dealing with unravelling the genetic architecture of such traits, their QTLs have to be analyzed by considering the combination of the QTL under different environment using the so-called QTL by Environment Interaction (QEI) analysis (Boer et al. 2007). The use of QEI would allow QTL categorization according to the stability of their effects across different environments. A 'constitutive' QTL is consistently detected across most environments, while an 'adaptive' QTL is detected only in specific environmental conditions or increases in expression with the level of an environmental factor (Vargas et al. 2006).

Earlier studies in pepper breeding focused mostly on univariate analyses of traits in single environments (Alimi et al. 2013a; Barchi et al. 2009; Ben Chaim et al. 2006; Mimura et al. 2010; Rao et al. 2003). In this study, aside from the univariate analysis, we implemented three different multivariate QTL modelling strategies to analyse data on a recombinant inbred line (RIL) pepper population (Alimi et al. 2013b; Voorrips et al. 2010; www.spicyweb.eu). These QTL modelling strategies are multi environment (ME), multi trait (MT) and multi-trait multi-environment (MTME) analyses. We modelled genetic correlations within (between traits in a given environment) and between environments, and explicitly test the presence of QEI and pleiotropic QTLs. Furthermore, we investigated and compared the accuracies of predictions from STSE and MT models. The QTL analyses and prediction accuracies were evaluated with five yield related traits measured across four environments. The traits and environments were selected from the EU-SPICY data (Alimi et al. 2013a).

Materials and Methods

Genotypic and Phenotypic Data

The traits selected here are taken from the four SPICY project phenotypic experiments (Alimi et al. 2013a). The mapping population is from sixth generation (F_6) of the segregating recombinant inbred lines (RILs) of an intraspecific cross between the large – fruited inbred cultivar 'Yolo Wonder' (YW) and the pungent small-fruited cultivar 'Criollo de Morelos 334' (CM 334) of pepper plant. DNA was extracted from 149 RILs to produce information for 455 markers assembled into 12 pepper chromosomes, covering 1705cM. The majority of markers used are SNP and SSR markers.

Phenotypic evaluations of the RILs were carried out via designed experiments across two locations; Spain (SP) and Netherlands (NL). The trials were done under both spring (1) and autumn (2) weather conditions in 2009. This gave a total of four trials (i.e. environments); Netherland trial in spring (NL1), Netherland trial in autumn (NL2), Spain trial in spring (SP1) and Spain trial in autumn (SP2). The five studied traits are total dry weight fruit (DWF) which represents fruit yield for these experiments, mean increase in leaf area index per unit time (LAI) and light use efficiency (LUE) which is a measure of dry matter production per megajoule of intercepted radiation. Other traits include total number of harvested fruits (NF) and the proportion of total biomass due to fruit (PF). Phenotypic characteristics for these traits including trait distributions and heritabilities are given in Alimi et al. (2013a).

Univariate QTL Model

The single-trait single-environment (STSE) model was of the form:

$$y_i = \mu + \sum_{j=1}^{m} x_{ij}\beta_j + e_i, \qquad (1)$$

where y_i was the phenotypic response of genotype i, μ the population mean, β_j was the additive effect of marker j. Genetic predictors were calculated at all marker positions and intermediate positions for those marker intervals that were larger than 5cM, giving a total of 639 evaluation

points. The genetic predictor for genotype *i* at genomic evaluation point *j* is denoted by x_{ij}, and e_i was the residual term.

Multivariate QTL Model

Three different types of multivariate QTL models were implemented. These include

Multi-Environment (ME) QTL model where each trait was evaluated over the four trials with the aim of investigating genotype-by-environment interaction (GEI) and QTL-by-environment interaction (QEI). The ME model was of the form:

$$y_{ik} = \mu + E_k + \sum_{j=1}^{m} x_{ij}\beta_{kj} + g_{ik} + \varepsilon_{ik}, \quad (2)$$

where E_k was the environmental mean deviation from the population mean, β_{kj} was the environment-specific effect of the additive genetic predictor at evaluation point *j*, g_{ik} represented the genetic effect of genotype *i* for environment *k*, and ε_{ik} represented the non-genetic component. We assumed that the vectors $g_i = (g_{i1}, \dots, g_{ij})$ follow a multivariate normal distribution with zero mean and an unstructured variance-covariance (VCOV) matrix G i.e. $\mathrm{g_i} \sim \mathrm{N}(0, \mathrm{G})$. This model accounts for genetic correlations between traits.

Multi-Trait (MT) QTL model: The MT model is a joint analysis of the five traits within each environment. The model specification is similar to that of ME except that E_k in equation 2 was replaced by T_p which represented the trait mean deviation from the population mean i.e.

$$y_{ip} = \mu + T_p + \sum_{j=1}^{m} x_{ij}\beta_{pj} + g_{ip} + \varepsilon_{ip}, \quad (3)$$

This model allows us to explicitly model genetic correlations between traits in each environment via pleiotropy by specifying unstructured VCOV matrix among each pair of traits.

Multi-Trait Multi-Environment (MTME) QTL model: This is a joint analysis combining all the traits across the four environments in a single mixed model analysis. ME and MT models were extended by allowing the response (y) to be a vector of trait-environment (TE) combinations.

$$y_{ir} = \mu + TE_r + \sum_{j=1}^{m} x_{ij}\beta_{rj} + g_{ir} + \varepsilon_{ir}, \quad (4)$$

The trait-environment mean deviation from the population mean was represented as TE_r while other model parameters are as explained above. With the MTME model, GEI and genetic correlations between traits were simultaneously modelled.

The number of significant QTLs and their explained variance were compared among the four models. QEI and pleiotropic effects were also investigated. The QTL analyses were performed using the QTL facilities in GenStat 15 (VSNi, 2012).

Finally, prediction accuracies were estimated for STSE and MT models. Prediction accuracy was defined as the correlation between phenotypic values and predicted breeding values. The predicted breeding values were obtained through a five-fold cross validation process with ten replications each. For each prediction runs, four of the five subsets were used as the training set for model fitting while the last subset were predicted based on the fitted individuals. This was repeated until all the subsets have been predicted.

Results and Discussion

Genetic Correlations and Comparison of results from QTL models

The pepper traits considered showed positive and mostly uniform correlations between environments (Table 1). These between-environment correlations were generally moderate to high, ranging from 0.34 for LUE between NL2 and SP1 (i.e. NL2.SP1) to 0.79 for LAI between SP1 and SP2 (i.e. SP1.SP2), with the majority of the correlations above 0.6. The results from the ME

analysis showed very significant non-crossover (quantitative) QEI (i.e. QTL effects between environments differ only in magnitude but not in direction) which supported the uniform correlations observed among environments. As an example, the ME analysis revealed three QTLs for DWF in most of the environments (except NL1 with two QTLs). Two of these QTLs were constitutive i.e. they showed consistent significant effects across the four environments.

Table 1 Trait genetic correlations between environments.

	NL1.NL2	NL1.SP1	NL1.SP2	NL2.SP1	NL2.SP2	SP1.SP2	Mean
DWF	0.72	0.60	0.61	0.53	0.62	0.58	0.61
LAI	0.73	0.76	0.70	0.67	0.75	0.79	0.73
LUE	0.64	0.45	0.60	0.34	0.64	0.36	0.51
NF	0.70	0.55	0.54	0.49	0.65	0.41	0.56
PF	0.69	0.65	0.67	0.54	0.72	0.57	0.64
Mean	0.70	0.60	0.62	0.51	0.68	0.54	0.61

Table 2 Genetic correlation of traits within each environment

Trait	DWF	LAI	LUE	NF		Trait	DWF	LAI	LUE	NF
NL1						SP1				
LAI	0.07					LAI	0.09			
LUE	0.13	0.11				LUE	0.40	-0.34		
NF	0.85	0.01	0.23			NF	0.80	0.12	0.37	
PF	0.90	-0.18	-0.11	0.76		PF	0.93	-0.18	0.34	0.74
NL2						SP2				
LAI	0.19					LAI	-0.04			
LUE	0.26	0.32				LUE	0.22	0.01		
NF	0.86	0.09	0.36			NF	0.60	-0.01	0.36	
PF	0.91	-0.07	-0.03	0.76		PF	0.89	-0.37	0.03	0.51

The within-environment correlations among traits were mostly consistent in sign and magnitude among the environments (Table 2). DWF, NF and PF were highly correlated. This is expected since there is usually a direct relationship between number of harvested fruits and fruit weight. Also, PF was computed from total fruit weight and total plant biomass. The MT analysis revealed pleiotropic QTLs which are consistent with genetic correlations among the traits. As an example, five QTLs were detected for yield in SP1 environment. All the five QTLs were also detected for PF while three of the QTLs also influenced NF, LAI and LUE. However, two of the QTLs showed crossover pleiotropy between yield and LAI and between yield and LUE.

Factorial combinations of traits and environments and their joint analysis through the MTME revealed a total number of 17 regions as harbouring putative QTLs (Figure 1). As the MTME model fully utilizes covariance structures between environments and among traits within environments, it thus increased the power of QTL detection with increased precision. Table 3 displays number of QTLs together with their explained variance for each of the five traits in the four environments using the four QTL methods. There is a clear gain in the number of QTLs and their explained variances using multivariate QTL methods over univariate method. Also, jointly accounting for correlations among environments and among traits within an environment result in detection of far more QTLs than simply accounting for correlation among environments or among traits within an environment alone. As an example, 3, 3, 5 and 8 QTLs were identified for DWF in SP1 using SE, ME, MT and MTME QTL methods respectively explaining about 25%, 40%, 41% and 42% of genetic variations respectively. Most of the QTLs picked up in simpler methods were also detected in more complex methods. The three QTLs picked up for DWF in SP1 by STSE method were also detected by ME, MT and MTME methods. Many of the yield increasing alleles originated from the large fruited YW parental line. Many of the additional QTLs detected in MTME were of small effects. Also the QTLs already detected from simpler methods had reduced explained variances in

MTME. This might be related to the so called "Beavis effect" as simpler models resulted in overestimation of some effect sizes (Beavis, 1997).

Figure 1 Significant QTL positions from MTME model for five yield related traits (DWF, LAI, LUE, NF & PF) across the four environments. The top section shows the *P*-values of tests for QTL main effects. The bottom section shows heat maps along the genome for each trait, where blue indicates QTLs with significant effect from YW allele while red indicates QTLs with significant effect from CM334 allele. Most of the QTLs showed pleiotropic and QEI effects.

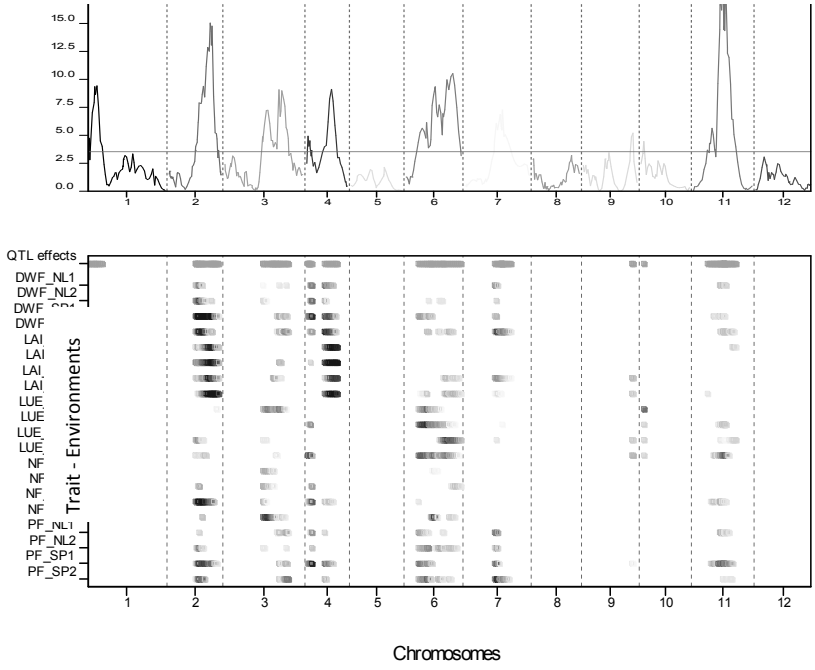

Table 3 Comparison of Number of QTLs (#QTL) and Explained Variance ($H^2_{(qtl)}$) from STSE, ME, MT and MTME models for five yield related traits measured across four environments.

		Number of QTLs (#QTL)					QTL Variance Explained ($H^2_{(qtl)}$)						
Trait	Method	NL 1	NL2	SP1	SP2		NL1	NL2	SP1	SP2		Avg. #QTL	Avg. $H^2_{(qtl)}$
DWF	STSE	1	2	3	3		18	18	25	37		2.3	24.5
	ME	2	3	3	3		21.8	24.9	39.6	28.7		2.8	28.8
	MT	2	2	5	6		18.9	13.1	40.8	43.2		3.8	29.0
	MTME	5	7	8	9		26.1	34.7	42.3	50.6		7.3	38.4
LAI	STSE	2	3	2	2		33	48	22	42		2.3	36.3
	ME	4	4	6	5		37.4	49.7	57.8	42.7		4.8	46.9
	MT	2	4	5	4		30	39.9	31.6	50.9		3.8	38.1
	MTME	4	6	7	8		34.2	42.1	47.8	54.7		6.3	44.7
LUE	STSE	2	1	1	4		26	14	17	31		2.0	22.0
	ME	3	2	2	3		25.8	19.5	11.6	22.5		2.5	19.9
	MT	4	2	4	4		26.3	13.4	21.4	27.8		3.5	22.2
	MTME	5	5	7	8		26	22.4	41.6	38.9		6.3	32.2
NF	STSE	0	1	4	3		0	10	31	34		2.0	18.8
	ME	4	2	2	3		20.4	14	15.2	33.5		2.8	20.8
	MT	2	3	4	4		7.4	20	16.3	38.6		3.3	20.6
	MTME	2	4	6	4		10.6	24.3	33.4	27.9		4.0	24.1
PF	STSE	0	0	4	3		0	0	32	26		1.8	14.5
	ME	4	3	6	3		42.1	34.1	66.6	36.4		4.0	44.8
	MT	3	4	5	5		15.7	28.9	32.5	24.1		4.3	25.3
	MTME	6	7	7	7		32.5	41.3	37.3	39.3		6.8	37.6

Table 4: 5-fold with 10 replications cross validated predictive accuracies for five yield related traits in four environments using single-trait single-environment (STSE) and multi-trait (MT) QTL models.

	NL1		NL2		SP1		SP2		
Trait	STSE	MT	STSE	MT	STSE	MT	STSE	MT	Mean
DWF	0.21	0.16	0.28	0.11	0.47	0.52	0.42	0.53	0.34
LAI	0.48	0.40	0.67	0.61	0.37	0.46	0.59	0.64	0.53
LUE	0.44	0.46	0.32	0.27	0.27	0.39	0.28	0.45	0.36
NF	0.05	0.07	0.01	0.04	0.37	0.42	0.41	0.43	0.23
PF	0.10	0.15	0.33	0.16	0.38	0.51	0.29	0.47	0.30
Mean	0.26	0.25	0.32	0.24	0.37	0.46	0.40	0.50	0.35

Prediction Accuracies of STSE and MT models

Prediction accuracies of each of the traits (Table 4) showed direct relationship to QTL genetic architecture of the traits. Traits with higher explained variance in the QTL study were also better predicted and vice versa. Using STSE method in NL1, no significant QTL was picked up for NF, hence the trait was very poorly predicted (0.05), while two QTLs with 33% explained variability were picked up for LAI, hence the trait was highly predicted (0.48). Also, for each of the five traits, prediction accuracies differed among environments, irrespective of the method employed. Most of the traits were better predicted in Spanish trials than in Netherlands trials. For example, yield was better predicted in Spanish trials (0.42 - 0.53) than in NL trials (0.11 - 0.28). This could be caused by poor fruit set in NL trials. Furthermore, for each of the traits, prediction accuracies differ between STSE and MT models. It is expected that multi-trait model should have better predictive power than single-trait model. This is mostly true in our case for SP trials but not for NL trials especially NL2. This showed that in situations where phenotypic data were simultaneously collected on a large number of traits, using multivariate QTL method that properly model underlying variance-covariance structures among the traits would lead to improved predictive

power than performing single trait analyses. In multi-trait model, information sharing among correlated traits helped to increase prediction accuracies for traits with hitherto low accuracies e.g. prediction accuracy for LUE in SP2 increased from 0.28 to 0.45 when handled multivariately.

Concluding remarks

We showed that in situations such as the EU-SPICY project, where phenotypic data on a number of traits have been collected in multiple environments, using QTL methods that properly model underlying VCOV structures among the traits and between environments led to improved power to detect more QTLs than performing individual trait/environment analyses. The joint analysis was especially suitable for complex traits (such as yield) whose genetic variations are usually due to a large number of QTLs of smaller effects which might go undetected with single trait/environment analysis. The five traits considered showed positive and mostly uniform correlations between environments. Many of the detected QTLs showed quantitative QTL-by-environment interactions which corroborated uniform correlations between environments. Also pleiotropic effects were observed for many of the QTLs, which resulted from relationships between the traits they govern. Pleiotropy may also suggest redundancy between the measured traits, which could be avoided to decrease the cost of experiments. Such pleiotropic effects were more accurately studied by explicit modelling of the correlation/covariance structure among the traits through a joint multi-trait analysis. We also showed that predictive accuracy of traits depends not only on prediction model of choice and traits genetic architecture but also on the environment. This is an indication that though these traits are genetically determined in any given environment, their degree of expression differs from one environment to the other indicating presence of genotype-by-environment interaction and QTL-by-environment interaction. In furtherance of this study, we intend to explore prediction accuracies from univariate and multivariate genomic prediction models and compare with prediction accuracies from QTL models. We would also investigate an indirect prediction approach where yield is predicted from underlying component traits coupled with environmental information through crop growth modelling strategies (Chapman, 2008; van Eeuwijk et al. 2010). This approach has the ability to help in dissecting QTLs responsible for complex traits on which direct selection may be difficult Yield predictions from the crop growth model would be implemented within and across environments.

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Towards the deciphering of the genetic factors involved in durability of plant major resistance genes to root knot nematodes in pepper

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Abstract

Root-knot nematodes (RKNs), *Meloidogyne* spp., are extremely polyphagous plant parasites worldwide. Since the use of most chemical nematicides is being prohibited, genetic resistance is an efficient alternative way to protect crops against these pests. However, few resistance genes (R-genes) are available and some nematode populations may become able to overcome them with time. Sustainable management of these valuable resources is thus a key point of R-gene durability. In pepper (*Capsicum annuum*), *Me3* is a dominant major R-gene, currently used in breeding programs, that controls *M. arenaria*, *M. incognita* and *M. javanica*, the three main RKNs species. It was introgressed in either a susceptible or a partially resistant (i.e., that shows reduced symptoms) genetic background in either homozygous or heterozygous allelic status. Doux Long des Landes (DLL) was used as susceptible recipient pepper line and Yolo Wonder (YW) as a partially resistant one. Challenging all these genotypes with a high inoculation pressure of an avirulent *M. incognita* isolate demonstrated that i) the efficiency of the R-gene in reducing the reproductive potential of RKNs is strongly affected by the plant genetic background, ii) the allelic status of the R-genes has no effect on nematode reproduction. These results highlight the primary importance of the choice of both the R-gene and the genetic background into which it is introgressed during the selection of new elite cultivars by plant breeders.

Keywords: *Meloidogyne* spp., *Capsicum annuum* (pepper), *Me* resistance genes, dosage allele effect, resistance durability, quantitative resistance

Introduction

Root-knot nematodes (RKNs), *Meloidogyne* spp., are considered as one of the most damaging pathogen in the world (Trudgill and Blok 2001). A reliable way of controlling these polyphagous endoparasitic worms is the use of chemical nematicides. However, the use of such compounds was drastically restricted in the past years because of environmental and public health issues. Now, one of the best alternatives to cope with nematode infestations relies on the deployment of resistance genes (R-genes), which represent an efficient, environmentally safe and economically sustainable method of control (Djian-Caporalino et al. 2009). As a consequence, many breeding programs are being developed in order to introgress the desired R-genes into elite cultivars and/or rootstocks. However, not only the R-gene itself may completely account for the observed resistance phenotype. Indeed, effects linked to the plant genetic background have been recognized to modify levels of nematode resistance in several crops (Jacquet et al. 2005; López-Pérez et al. 2006; Wang et al. 2008). Additionally, in several pathosystems, including plant-nematode interactions, it was shown a dosage effect of the R-gene alleles on the pathogen multiplication (Collmer et al. 2000; Jacquet et al. 2005; Chintamanani et al. 2008).

Since resistance sources against RKNs are limited, management of the available R-genes is of crucial importance. As RKNs exhibit noteworthy capacities of adaptation to their environment,

including R-genes, the emergence and spread of virulent nematode populations constitutes a severe threat to R-gene durability (Castagnone-Sereno 2002, 2006; McDonald and Linde 2002).

In pepper (*Capsicum annuum* L.), several dominant R-genes have been identified and well characterized for their spectrum of resistance against RKNs, i.e., the *Me* genes and the *N* gene (Hare 1956; Hendy et al. 1985a, 1985b; Djian-Caporalino et al. 1999; Thies et Fery 2000). Some of them have been mapped and co-localised in a cluster on the pepper P9 chromosome (Djian-Caporalino et al. 2001, 2007; Fazari et al. 2012). One of these dominant R-genes, *Me3*, displays a broad spectrum of resistance to the three main RKN species, i.e., *M. incognita*, *M. arenaria* and *M. javanica*. Currently, it is being actively exploited in breeding programs.

The present work aims at evaluating the influence of the genetic background of pepper genotypes on the expression of the resistance to RKNs conferred by the *Me3* R-gene, using either a susceptible or a partially resistant genetic background. Allele dosage effect of this gene was also tested to evaluate the relevance of hybrid varieties *versus* inbreed lines on R-genes efficiency.

Materials and Methods

Plant material

Pepper (*Capsicum annuum* L.) genotypes used in this work were Yolo Wonder (YW), Doux Long des Landes (DLL) and DH149. These inbred lines were selected for their differential resistance to *Meloidogyne incognita*. DLL is a highly susceptible pepper cultivar; YW is a partially resistant (i.e., shows reduced symptoms) cultivar. DH149 is a resistant doubled haploid (DH) lines produced through *in vitro* androgenesis (Dumas de Vaulx et al. 1981) from the intraspecific F1 hybrids (PM687 x YW). DH149 carries the single dominant resistance allele *Me3* (Hendy et al. 1984).

In order to introgress the *Me3* allele into the DLL (very susceptible) or YW (partially resistant) genetic backgrounds, the resistant parental line DH149 was crossed with the susceptible parental lines DLL and YW (recurrent parents) and the two F1 hybrids were backcrossed (BC) with their respective recurrent parental lines. At each backcross cycle, the heterozygous resistant plants were sorted using molecular markers linked to the *Me3* allele and self-pollinated to generate a segregating population for the R-gene (BC-S1). The progenies issued from the first backcross (BC1 and BC1-S1) were used.

Plant allelic status determination

Total genomic DNA was isolated from 100 mg of fresh leaf material as described by Fulton et al. (1995). After RNAse treatment, DNA concentration and purity were measured with a NanoDrop 2000 spectrophotometer (Thermoscientific) and adjusted to a final concentration of 20 ng/µL for PCR.

BC1-S1 plants carrying *Me3* were genotyped with SCAR_N, a codominant marker linked to this gene, in order to discriminate *Me3* homozygous susceptible ($Me3^+/Me3^+$), homozygous resistant (*Me3*/*Me3*) and heterozygous ($Me3/Me3^+$) plants in both DLL and YW genetic backgrounds, according to a standard procedure (Fazari et al. 2012).

Nematode material

The RKN isolate used in this study was *M. incognita* Morelos from the collection maintained at INRA research centre in Sophia Antipolis. It is avirulent towards *Me3* gene. Because of the mitotic parthenogenetic mode of reproduction of *M. incognita* (Triantaphyllou 1985), all the second-stage juveniles (J2s) that hatched from a single egg mass were considered as a clonal line. Prior to multiplication, this isolate was specifically identified according to its isoesterase electrophoretic pattern (Dalmasso and Bergé 1978) and/or by SCAR PCR (Zijlstra et al. 2000).

Experimental procedures and evaluation

Pepper seedlings were sown individually in 9-cm plastic pots containing steam-sterilized sandy soil covered by a 1-cm layer of loam. At least twenty replicates (individual plants) were performed for each control genotype (i.e., DLL, YW, DH149 and each F1) and one hundred and twenty BC1-S1 plants were grown. This was done to ensure to obtain at least twenty replicates of each genotype (homozygous susceptible, homozygous resistant and heterozygous). The whole experiment was conducted in a climatic chamber maintained at 24°C (±2°C) with a 12-h light cycle and a relative humidity of 60–70%. Six to seven-week old plants (4-6 true leaves) were inoculated with a water suspension of 5,000 hatched second-stage juveniles (J2s) obtained in a mist chamber, from previously inoculated susceptible tomato roots (cultivar Saint Pierre). Six to seven weeks after inoculation (i.e., a duration that allowed completion of the nematode life cycle), plants were harvested, carefully washed individually with tap-water, and stained for 10 min in a cold aqueous solution of eosin yellow (0.1 g/l water), to specifically stain egg masses (EMs) (Roberts et al. 1990). The roots were rinsed and examined under a magnifying glass. The number of EMs was counted for each plant and the average number of EMs was calculated for the different genotypes, providing their disease severity (DS). In addition, for each genotype, the frequency of plants exhibiting more than five EMs in relation to the number of inoculated plants was computed, giving their disease incidence (DI) ranging from 0 to 1.

Statistical analysis

All the statistical analyses were performed using the free software R (http://www.r-project.org/). First, to check the good fit of the expected segregation of the BC-S1 populations, a χ^2 test was performed. In order to investigate a possible effect of the genetic background and/or a dosage allele effect, non-parametric tests were further applied to compare the DS of the different genotypes. When the Kruskal-Wallis test was significant, Wilcoxon-Mann-Whitney bilateral tests with a significance level at α=0.05 were carried out using Bonferroni correction.

Results

Homozygous susceptible (*Me3*$^+$/*Me3*$^+$), homozygous resistant (*Me3*/*Me3*) and heterozygous (*Me3*/*Me3*$^+$) BC1-S1 plants were sorted using SCAR_N, a codominant marker linked to *Me3*. With both recurrent parents DLL and YW, the observed segregation of *Me3* fitted the expected segregating ratio as revealed by a χ^2 test at α=0.05 (Table 1).

Genetic background	Allelic status at the *Me3* locus	Number of plants	χ^2 (1:2:1)
BC1-S1 [(DH149 x DLL) x DLL]	Homozygous susceptible *Me3*$^+$/*Me3*$^+$	19	X-quared = 4.1538
	Homozygous resistant *Me3*/*Me3*	23	df = 2
	Heterozygous *Me3*/*Me3*$^+$	62	p-value = 0.1253
BC1-S1 [(DH149 x YW) x YW]	Homozygous susceptible *Me3*$^+$/*Me3*$^+$	35	X-quared = 3.2182
	Homozygous resistant *Me3*/*Me3*	22	df = 2
	Heterozygous *Me3*/*Me3*$^+$	53	p-value = 0.2001

Table 1 Observed segregation ratio at the *Me3* allele in progenies with DLL (upper part) or YW (lower part) genetic backgrounds from a self-pollinated heterozygous resistant backcross 1 plant
BC1-S1=Backcross 1 Self-pollinated; DH149=Double Haploid 149 line (resistant genotype); DLL=Doux Long des Landes (susceptible genotype); YW=Yolo Wonder (partially resistant genotype)

All the genotypes were infested with a high pressure inoculum (5,000 J2s) of *M. incognita* Morelos avirulent isolate. The Kruskal-Wallis test revealed that there was a significant effect of the plant genotype on nematode reproduction (χ^2=363.71, df=10, p-value<10^{-3}). Consequently, the

mean values of the different genotypes were compared each other to determine which one(s) provided the best efficiency against RKNs.

As expected, DLL exhibited a high number of EMs (DS=1579.7) whereas YW showed a moderate one (DS=462.4). For both genotypes, EMs were detected on the root system of all inoculated plants (DI=1.00). The plants of the susceptible BC1-S1 ($Me3^+/Me3^+$) genotypes were all infested (DI=1.00) and they exhibited numerous EMs, but less than their respective susceptible parents DLL and YW. The EM number of susceptible BC1-S1 ($Me3^+/Me3^+$) plants in the DLL genetic background was much higher than in the YW one (DS=490.9 and DS=116.1, respectively) (Fig. 1).

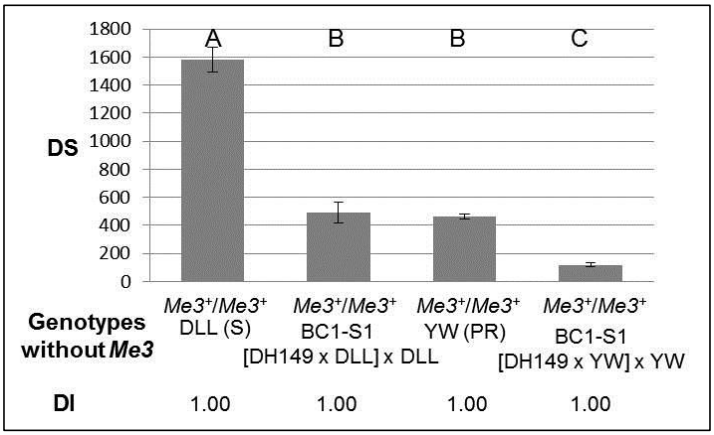

Figure 1 Disease severity (DS) and disease incidence (DI) of different pepper genotypes inoculated with a high pressure inoculum (5,000 J2s) of an avirulent isolate of *M. incognita*. DS=average number of egg masses/plant; DI=number of plants exhibiting more than five egg masses in relation to the number of inoculated plants; $Me3^+/Me3^+$=homozygous susceptible at the *Me3* locus; BC1-S1= Backcross 1 self-pollinated; DLL=Doux Long des Landes (susceptible genotype or genetic background); YW=Yolo Wonder (partially resistant genotype or genetic background); DH149=Double Haploid 149 line (resistant genotype). Bar=standard error. Different letters mean significant differences (Wilcoxon-Mann-Whithney bilateral tests at α=0.05 after Bonferroni correction).

DH149 confirmed its resistant status with a mean number of EMs close to zero (DS=4.4) and a few number of plants affected (DI=0.17). There was no significant differences between the average EM number of the two F1 hybrids (DH149 x YW) and (DH149 x DLL) which appeared resistant (DS=0.2 and DS=0.9, respectively). Different results were obtained with the BC1-S1 plants heterozygous or homozygous at the *Me3* allele. In the YW genetic background, the number of EMs of the BC1-S1 plants, heterozygous or homozygous at the *Me3* allele, did not significantly differ from DH149 and both genotypes had a similar rate of infested plants. In the DLL genetic background, the number of EMs was significantly much higher than in the DH149 one (DS=30.8 and DS=49.1, respectively) and the rate of infested plants was important (DI=0.68 and DI=0.61, respectively). Comparing *Me3* heterozygous to homozygous resistant BC1-S1 plants within the same genetic background (DLL or YW) did not reveal significant differences (Fig. 2).

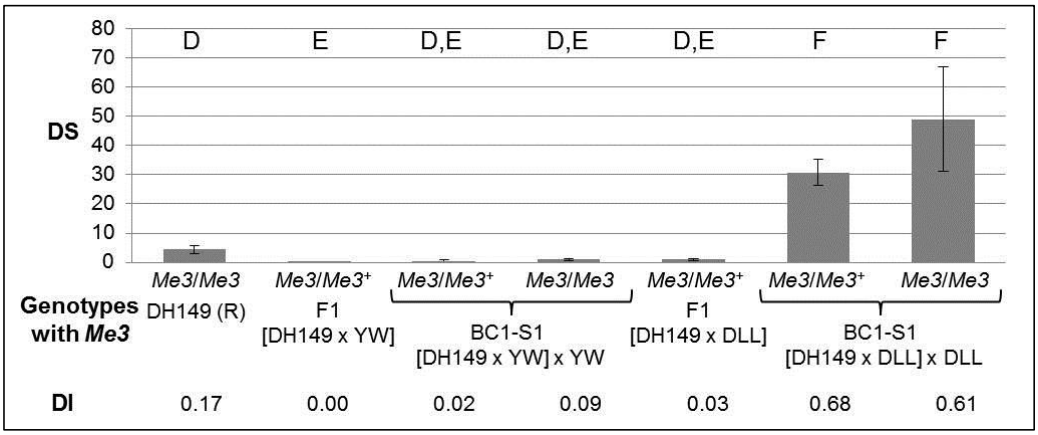

Figure 2 Disease severity (DS) and disease incidence (DI) of different pepper genotypes inoculated with a high pressure inoculum (5,000 J2s) of an avirulent isolate of *M. incognita*. DS=average number of egg masses/plant; DI=number of plants exhibiting more than five egg masses in relation to the number of inoculated plants; *Me3*/*Me3*=homozygous resistant at the *Me3* locus; *Me3*/*Me3*$^{+}$=heterozygous at the *Me3* locus; BC1-S1= Backcross 1 self-pollinated; F1=Hybrid F1; DLL=Doux Long des Landes (susceptible genetic background); YW=Yolo Wonder (partially resistant genetic background); DH149=Double Haploid 149 line (resistant genotype). Bar=standard error. Different letters mean significant differences (Wilcoxon-Mann-Whithney bilateral tests at α=0.05 after Bonferroni correction).

Discussion

In order to explore the effect of the plant genetic background on R-gene efficiency, *Me3* was introgressed into a susceptible (i.e., DLL) or a partially resistant (i.e., YW) pepper genetic background. Compared with the donor resistant parental lines, the DLL or YW genetic background surrounding the R-gene was increased of 50% in F1 hybrids and of 75% in BC1-S1 plants. The different genotypes were challenged with a high inoculation pressure of *M. incognita* and their ability to resist to this pathogen was evaluated. The main observation was that plants with *Me3* in a susceptible genetic background were more easily attacked than in a partially resistant one. Nematode reproduction was higher when the part of susceptible genetic background surrounding the R-gene was increased. This result is in agreement with studies on other pathosystems. Influence of the plant genetic background on R-gene efficiency to nematodes was shown in tomato (López-Pérez et al. 2006) and cotton (Wang et al. 2008). It was also demonstrated that the genetic background was able to modulate the expression of a R-gene in rice, conferring more or less resistance efficiency against a bacteria (Zhou et al. 2009). Further research needs to be conducted to determine the genetic factors, within the plant genetic background, that may explain the discrepancies from a pepper genotype to another. The homozygous susceptible BC-S1 plants at the *Me3* locus but carrying a residual genome part of the resistant donor parental line always showed a lower number of EMs than their respective parental recurrent lines DLL and YW. This difference may indicate the presence of resistance quantitative trait loci (QTLs) controlling the quantitative differences in the level of resistance, as observed between YW and DLL genetic backgrounds. To date, no QTLs were found against RKN in pepper. In that respect, a QTL analysis is currently on-going on this biological material, as we strongly suppose that the protective effect of the plant genetic background on R-genes is provided by such quantitative resistance factors.

The second objective of this study was to evaluate an eventual dosage effect of the *Me3* allele on the reproductive potential of RKN. Heterozygous and homozygous genotypes at the R-gene locus, in the same genetic background and at the same level of introgression, exhibited the same

level of resistance. Thus, the number of alleles of the R-gene did not significantly influence the nematode reproduction. This result indicates that there is no dosage effect of the *Me3* allele on nematode proliferation. This finding is in agreement with other studies on the dosage allele effect of several other R-genes against RKN (Bost and Triantaphyllou 1982; Cap et al. 1993; Thies and Fery 2002; Cortada et al. 2009). Seemingly, other studies raised opposite conclusions (Tzortzakakis et al. 1998; Jacquet et al. 2005). However, it is noteworthy that in the studies quoted above, authors tested the homozygous *versus* heterozygous status of the R-gene in non-homogenous genetic backgrounds. Conversely, we took care of this issue in our own study, and results confirmed that it is important to consider these parameters when investigating dosage allele effect of a R-gene. Indeed, in most cases, comparing a F1 genotype (i.e., heterozygous) with the corresponding homozygous BC-S1 genotype would have led to conclude that there was a dosage allele effect, but this assertion is invalidated when comparing homozygous to heterozygous BC-S1 genotypes, which differ only for the allelic status of the R-gene. The difference observed between the F1 and the homozygous BC-S1 genotype was due to the proportion of genetic background surrounding the R-gene, not the number of alleles. The homogeneity of the genetic background is very often disregarded in dosage allele studies, whereas it is of major importance.

The same kind of experiment was performed on *Me1*, another pepper dominant major R-gene used in breeding programs since it controls *M. arenaria*, *M. incognita* and *M. javanica*. As for *Me3*, *Me1* efficiency was influenced by the plant genetic background, with higher level of infestation on genotypes with a DLL genetic background than with a YW one. Similarly to *Me3*, no dosage effect of the *Me1* allele was shown on the reproductive potential of RKNs (data not shown).

In the present study, the crucial role of the plant genetic background in resistance to RKNs was clearly demonstrated using the *M. incognita*/pepper pathosystem as a model. This point can have direct practical implications on breeding strategies. It is of major importance for breeders to take into account the genetic background into which they introgress major R-genes, in order to increase their efficiency and likely improve the lifetime of new elite varieties released on the market. Thus, one of the best alternatives to avoid nematode damages without impairing *Me3* (and *Me1*) efficiency would be to combine them with partial resistance factors (i.e. QTLs). This strategy would take simultaneous advantage of these R-genes, which provide total resistance to the three main RKN species, and of QTLs, which theoretically reduce the level of infestation. In addition to increased resistance efficiency, we suspect that a partially resistant genetic background may have a protective role on *Me3* and may prevent it from being quickly overcome. One might expect that the reduction of the nematode reproduction due to the partially resistant genetic background surrounding *Me3* (or *Me1*) may decrease the risk of resistance breakdown by RKNs and may increase its durability. This hypothesis is supported by several studies on different pathosystems which proved that the durability of R-genes was dependent on the plant genetic background into which they were introgressed (Palloix et al. 2009; Brun et al. 2010; Fournet et al. 2012).

Another point that can have direct practical implications on breeding strategies relies on the absence of dosage effect of *Me3* (and *Me1*) allele on the reproductive potential of RKNs. Consequently, since the proportion of hybrids in commercial cultivars has been increasing, taking advantage of the possibility to cumulate R-genes against different pathogens in an heterozygous status, using *Me3* (or *Me1*) in hybrid varieties is not an issue, as long as it is into a suitable genetic background.

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Improvement of resistant eggplant lines against Fusarium (*Fusarium oxysporum* Schlecht. f. sp. *melongenae)*

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Abstract

Most of the eggplant cultivars grown are susceptible to Fusarium wilt caused by *Fusarium oxysporum* Schlecht. f. sp. *melongenae*. Although grafting appears to be an effective way to control Fusarium, there are some problems encountered (incompatibility, need for qualified labor, higher cost of seedlings, etc.) in this method. Use of improved resistant eggplant varieties against the disease is more advantageous for producers. In this study, resistant genotype LS1934 and susceptible inbred line BT35 were crossed to improve resistance in breeding lines. To eliminate the undesirable characteristics of the resistant genotypes, they were backcrossed two times with susceptible genotypes. Classical and molecular methods were used to determine the reaction of backcross progenies to the disease. Root-dip method was performed for inoculation of the pathogen. Seedlings at the 2-4 true leaf stage were inoculated with conidial suspensions for five minutes. Disease symptoms were not observed in the resistant plants while susceptible plants died. The SCAR marker Me8/Em5 was also used to determine resistant and susceptible plants and results from the classical method were verified with the SCAR marker.

Keywords: *Solanum melongena*, backcross, inoculation, root-dip method, marker-assisted selection

Introduction

Eggplant is a cultivated solanaceous species widely grown of many countries (Daunay, 2008). It is attacked by many insect pests and diseases (Sidhu et al. 2005). A main factor affecting eggplant production is its susceptibility to soil-borne diseases. Fusarium wilt is one of the most serious diseases in eggplant cultivation (Rotino et al. 2004). The wilt disease caused by *Fusarium oxysporum* Schlecht. f. sp. *melongenae* (FOM) was identified in both Europe and Asia in eggplant growing areas (Joffe & Palti, 1974; Steekelenburg, 1976; Cappelli et al. 1995; Altinok, 2005). Different control methods are used for the management of this pathogen. Chemical control is known to be the most efficient method. However, pesticides may represent a hazard. Physical and cultural practices are more environmental friendly for control of this disease. These include crop rotation, fallow, deep ploughing, and soil solarization (Katan, 2000; Gamliel et al. 2000). The use of resistant cultivars is a desirable approach to control the disease. Some varieties tolerant/resistant to different biotic and abiotic stresses have been developed. However, it was shown in a screening experiment that almost all commercial eggplant cultivars were susceptible to Fusarium wilt (Stravato & Cappelli 2000). Further work on resistance breeding is of much importance in this regard (Sidhu et al. 2005). Studies on sources of Fusarium resistance in eggplant revealed that resistance was found in some non-commercial *S. melongena* forms and its wild types, i.e., *Solanum indicum*, *S. aethiopicum*, aculeatum Group (also found in literature as *S. integrifolium* Poir.), *S. aethiopicum* Gilo Group*, S. torvum*, *S. incanum*, *S. violaceum* and *S. sisymbriifolium* (Rizza et al. 2002; Gousset et al. 2005; Boyaci et al. 2012). Inheritance of resistance to FOM in some wild *Solanum* species of eggplant (Rotino et al. 2001; Toppino et al. 2008) and cultivars (*Solanum melongena*) (Boyaci et al. 2011) was determined to be controlled by single dominant genes. The primary aim of the present study was improvement of resistant eggplant lines against *Fusarium oxysporum* Schlecht. f. sp. *melongenae*.

Materials and Methods

This study was carried out at Bati Akdeniz Agricultural Research Institute, Antalya, Turkey (BATEM) between 2010 and 2012. Two plant materials were used, resistant line LS1934 (Boyaci et al. 2011) and susceptible pure line BT35, developed according to the pedigree method at BATEM. Pathogenic strain AF of *Fusarium oxysporum* Schlecht. f. sp. *melongenae* isolated from naturally infected eggplant vascular tissues (Boyaci et al. 2012) was used in artificial inoculations. The SCAR marker (Me8/Em5) (Mutlu et al. 2008), 426 bp in size, was utilized as a tool for marker-assisted selection.

Breeding strategy

The backcross breeding method (Harlan and Pope, 1922) was used. LS1934 which has a single dominant gene (Boyaci et al. 2011) was used as the source of resistance while BT35 was the recipient line. The F1 plants were self-pollinated and F2 progenies were obtained. F2 progenies were backcrossed with the recurrent parent BT35 to produce BC1F1 progenies. F2 and BC1F1 progenies were evaluated before flowering and plants carrying rr alleles for the gene were discarded. Backcrossing was performed two times for recovering the recurrent parent (Fig 1).

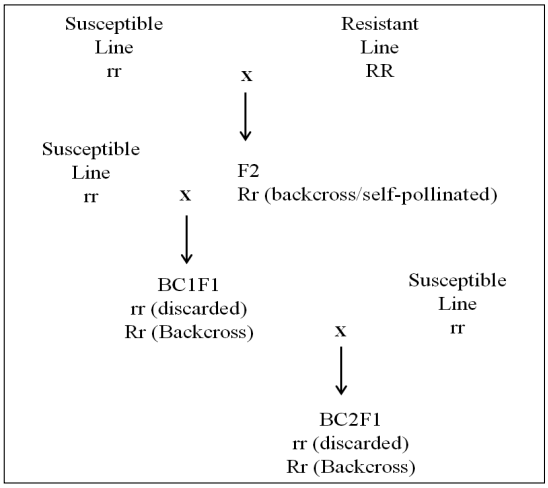

Figure 1. Backcross breeding method used to incorporate FOM resistance from LS1934 into susceptible BT35 breeding line.

Bioassay

The susceptible plants with rr genotype in the F2 and BC progenies were identified by a disease resistance test using artificial inoculation. For these assays, seeds of F2 and BC progenies were germinated in sterilized peat moss. The seedlings at the cotyledon stage were transplanted to wide pots. The plantlets were grown until they had 2-4 true-leaves in a greenhouse. The plants were inoculated by a root-dip method according to Cappelli et al (1995). For producing inocula, AF isolate (FOM) was incubated to produce colonies on PDA at 24°C in the dark for 10 days. Preparation of inocula was performed from this culture. Eight mm disks containing sporulating fungus were cut using a sterile cork borer from the PDA plates and transferred into 250 ml Erlenmeyer flasks containing liquid culture prepared according to Pitrat et al. (1991). All flasks were incubated at 24 to 25°C on a rotary shaker set at 50 rpm for 8 days. The suspensions were filtered through cheesecloth. The inoculum was adjusted to $1x10^6$ conidia/ml spore suspension as determined with a thoma slide. For inoculation, seedlings were removed from the trays, roots were

first washed with tap water and then wounded by trimming the tips. Plant roots were immersed into spore suspension for five minutes (Gordon et al. 1989; Zink and Gubber, 1986). The roots of control plants were immersed in distilled water. After inoculation, five-plant was immediately transplanted into a sterile pot (180x165 mm) containing pasteurized sand, peat and perlite mix and kept under controlled climatic greenhouse conditions: 60% humidity, 25 ± 2^0C day and 20 ± 2^0C night temperatures. Five plants were planted in each pot. The resistant plants that show no symptoms were chosen for backcrossing 28 days after inoculation.

DNA extraction and PCR

DNA was extracted from 0.2 g fresh fresh eggplant leaves from the growing tips of parent and segregating population seedlings. A CTAB (cetyltrimethylammonium bromide)-based modified DNA extraction procedure (Aldrich and Cullis 1993) was used for DNA extraction. Fresh leaves were crushed using a drill in a tube including extraction solution (1.4 M NaCl, 20 mM EDTA, 100 mM Tris-HCL (pH 8), 2% CTAB and 0.2% beta-mercaptoethanol). The tubes were mixed gently by inversion and incubated at 65°C for 30 min. The tubes were gently agitated during incubation to keep the extract mixed. After appropriate incubation, the incubation mixture was allowed to cool to room temperature. The tubes were mixed gently by inverting several times followed by adding chloroform-isoamyl alcohol (24:1) and centrifugation at 13 000 rpm for 10 min at 25–30 °C. The clear aqueous phase was transferred to a new tube and 2/3 volume of isopropanol in cold ice was added and mixed gently by inversion until the DNA appeared. The pellets were washed twice with 0.75 mL washing buffer (76% ethanol and 10 mM of ammonium acetate) to eliminate interphasic debris. The pellets were drained by inverting tubes onto a paper towel and then resuspended in 50 µl double distilled water and stored at -20°C until the end of the study. DNA was run on a 1% agarose gel stained with ethidium bromide. The DNA samples were photographed under ultraviolet light. DNA concentration was adjusted approximately 10 ng/µl and used as a template in PCR reactions.

Each PCR reaction was carried out in a total reaction volume of 15 µl containing 10 to 15 ng genomic DNA, 1.5 µl 2.5 mM dNTP (deoxyribonucleotide triphosphate mix-VIVANTIS), 1.5 µl 10X PCR Buffer (VIVANTIS), 0.3 µl Go *Taq* polymerase (VIVANTIS), 1.5 µl $MgCl_2$ (25 mM) and 1 µl each forward and reverse primers. The PCR reactions were performed in an Eppendorf Mastercycler Gradient. The amplification profile consisted of an initial denaturation for 5 min at 94°C followed by 35 cycles of PCR amplification under the following parameters: 1 min at 94°C, 45 seconds at the annealing temperature of 57°C and 50 seconds of primer elongation at 72°C. A final incubation at 72°C for 5 min was programmed to allow completion of primer extension. The amplified PCR products were separated electrophoretically in 1.5% agarose gels using 0.5X TBE buffer for 2 h at 120 V and photographed under UV light. A 100 bp ladder was used as a molecular weight marker.

Results

Genetic variation was observed in the F2 population derived by selfing of F1 hybrids obtained by crossing the susceptible and resistant parents. Whereas the resistant F2 plants showed no symptoms in, the susceptible plants died three to four weeks after artificial inoculation. The SCAR marker was specific to the *S. melongena* chromosome segment containing the resistance allele and amplified a single 426-bp band in resistant plants. The 426-bp band was present in 41 plants of tested 52 plants in the F2 segregating population (Fig 2). Thus, classic test results were verified with SCAR marker. To eliminate undesirable characteristics etc. fruit size and fruit color, selection was performed to choose the best individuals in the F2 segregating population. Thus, 25 selected resistant plants were backcrossed with the recurrent parent, BT35. Then 100 BC1F1 progenies derived from the backcross were tested with the molecular method and 42 plants were determined as resistant. A second backcross was performed after selection of 20 BC1 for desirable traits.

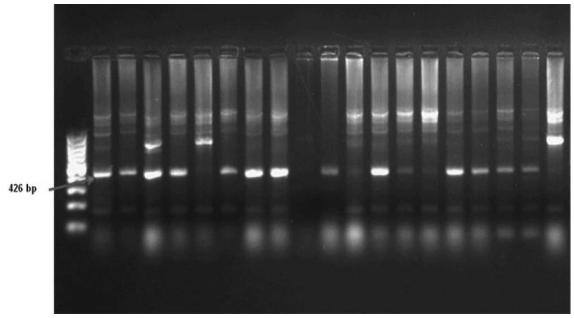

Figure 2. PCR screening of F2 population using the SCAR marker (Me8/Em5) which gave a diagnostic 426 base pair product.

Discussion

Fusarium is one of the most destructive pathogens of eggplant. Until now, resistant commercial varieties have not been developed. Traditionally, breeders have used wild relatives as donor parents for transferring resistance genes from wild type to commercial cultivars. Unfortunately, interspecific crosses between eggplant and its related species generally fail. In order to overcome this barrier, breeders use biotechnological methods like organogenesis, somatic embryogenesis, anther culture, protoplast culture, etc. (Kashyap et al, 2003). Interspecific hybrids derived from crossing eggplant with the closely related species *S. integrifolium* (Monma et al. 1997; Okada et al. 2002; Rotino et al. 2002) and *S. aethiopicum* (Rizza et al. 2002) were developed for use as rootstock. Although grafting appears to be an effective way to control Fusarium, there are some problems such as incompatibility, need for qualified labor, higher cost of seedlings, etc. In addition, more desirable plant traits were maintained in the hybrids as compared to interspecific crosses. The durability of disease resistance in breeding lines may be affected by the evolutionary potential of the pathogen (McDonald and Linde, 2002). However, variability has not been identified by genetic and molecular studies of *Fusarium oxysporum* f. sp. *melongenae* populations (Altinok & Can, 2010). Molecular markers tightly linked to the resistance gene are useful tools for disease resistance selection (Mutlu et al. 2008). It was shown that the SRAP marker (Me8/Em5) (426 bp in size) was a useful tool for selection of a resistance gene in eggplant breeding.

The improved Fusarium-resistant lines will allow development of resistant commercial eggplant varieties in short-term breeding programs. As they are members of the cultivated species of eggplant (*S. melongena*), these genotypes have various advantages for breeding programs.

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A taste of sweet pepper: insights in the biochemical and sensory aspects of pepper flavor

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Abstract

To better understand and predict the complex multifactorial trait flavor, volatile and non-volatile components were measured in fresh sweet pepper (*Capsicum annuum*) fruits throughout the growing season in a diverse panel of twenty-four breeding lines, hybrids, several cultivated genotypes and one gene bank accession. Biochemical profiles were linked to individual taste attributes, that were objectively quantified by a trained descriptive expert panel. Metabolic contrasts between genotypes were caused by clusters of volatile and non-volatile compounds, which could be related to metabolic pathways and common biochemical precursors. Clusters of phenolic derivatives, higher alkanes, sesquiterpenes and lipid derived volatiles formed the major determinants of the genotypic differences. Flavor was described with use of 14 taste attributes of which the texture related attributes and the sweet-sour contrast were the most discriminatory factors. We used a Random Forest regression approach to relate the taste attributes to the (non-)volatile data and to determine importance of individual compounds. Fructose and (E)-2-hexen-1-ol were highly correlated with aroma, fruity/apple taste and sweetness. New relations were found for fruity/apple taste and sweetness with compounds like p-menth-1-en-9-al, (E)-β-ocimene, (Z)-2-penten-1-ol and 1-methyl-1,4-cyclohexadiene. Based on overall biochemical and sensory results, the perspectives for flavor improvement by breeding are discussed.

Keywords: Sensory evaluation; Biochemical profiling; Metabolomics; Multivariate analysis; Random Forest

Introduction

Pepper fruits (*Capsicum annuum*) are widely consumed either as a fresh vegetable or dried as a spice. Especially, sweet peppers are eaten fresh or processed, as unripe (green or white) or ripe (*e.g.* red, yellow and orange) fruits. Fruits are commonly used in diets because of their typical color, pungency, taste and/or distinct aroma (Govindarajan 1985). In the breeding process of pepper, flavor has been identified as a chance for added value creation and consumer satisfaction (Verkerke 2000). However, flavor is a complex multifactorial trait (combination of taste, aroma, mouth feel, sight and sound) and in case of a biological product like pepper, is influenced by environmental factors during growth and post-harvest processing. As a consequence, until now it has been difficult to measure pepper flavor in a high-throughput and quantitative way. So far, only some studies have been performed to understand the role of various volatile and non-volatile components in fresh pepper flavor (e.g. Luning et al. 1994; Rodriguez-Burruezo et al. 2010), but a clear understanding of

the interaction between them and their effect on flavor is lacking. In the present study we characterized flavor over different harvests in a broad germplasm panel from a commercial breeding program completed with a few cultivated genotypes and a gene bank accession. Flavor was objectively quantified by thorough biochemical profiling in combination with sensory evaluations by a trained expert panel. The overall results make it possible to link individual flavor attributes to volatile and non-volatile components and to develop models for prediction of sensory descriptors over harvests. Our results form a starting point for a better understanding of pepper flavor and targeted improvement by breeding.

Materials and Methods

Experimental setup

In this study the 24 non-pungent *Capsicum annuum* accessions described in Eggink et al. (2012a) were used (Table 1). In short, the genotype panel consisted of elite pepper breeding lines and hybrids (provided by the breeding company Rijk Zwaan), several cultivated genotypes (landraces and old hybrids) and one gene bank accession. In 2008, the genotypes were grown in soil in greenhouse at Rijk Zwaan (De Lier, The Netherlands), according to Dutch pepper management conditions with 2.5 plants/m^2. All genotypes were grown in three randomized plots of five plants. From the beginning of May till the end of September 2008, all completely (95-100%) colored fruits were harvested, counted and weighed on a (bi)weekly base. In that period 3 harvests (29 May, 31 July and 4 September) were used for biochemical measurements and sensory evaluation.

Sensory analysis and metabolic profiling were performed as described in Eggink et al. (2012a). In short, the 24 *C. annuum* accessions were evaluated by a trained descriptive sensory panel. This expert panel scored 14 attributes on a scale from 0 to 100 to describe the taste sensation in the mouth/throat, which were the attributes: crunchiness, stickiness of the skin, toughness, juiciness, sweetness, sourness, aroma intensity, grassiness, green bean, carrot, fruity/apple, perfume, petrochemical and musty taste. The smell of the fruits was not evaluated separately. For biochemical profiling, the fruit samples from the three harvests were analyzed in a single headspace SPME-GC-MS experiment and derived GC-MS profiles were simultaneously processed. In total 254 putative volatile compounds were detected of which reliable identities (mass spectra match factor $\geq$ 700 and identity probability rank $\geq$ 2, Mihaleva et al. 2009) could be assigned to 129 of these. In addition to this, the concentration of sugars (fructose and glucose) was measured by enzymatic determination (Velterop and Vos 2001). Anion exchange chromatography was used for citric, malic and ascorbic acid determination based on standard protocols (Dionex Corporation, Sunnyvale, CA; http://www.dionex.com/ Application Note 143 "Determination of Organic Acids in Fruit Juices"). Sugar and acid measurements were completed by dry matter content and total soluble solids (brix) determination.

Data analysis

The sensory data was analysed per harvest in Genstat version 12 using a linear mixed model REML (residual maximum likelihood) analysis with genotype, replicate and their interaction as fixed terms. Sessions (tasting sessions) within replicate/genotype combinations and panelists within sessions were taken as random terms. Mean values were calculated per genotype per replicate after a correction for session and panelist effects and removal of strong outliers (if the absolute value of a standardized residuals was larger than three residual standard deviations).

Principal components analysis (PCA) as implemented in GeneMaths XT version 2.0 was used for visualizing relationships between and among metabolites and attributes. For these analyses the metabolite data sets were log transformed and mean centered. Pearson's correlation coefficient was used as a measure for metabolite-metabolite correlations.

A Random Forest (Breiman, 2001) regression approach was used to relate each sensory attribute (response) to the volatile and non-volatile data (predictors) and to determine importance of the individual volatiles and non-volatiles. A double ten-fold cross-validation approach was used to optimize the number of variables for each decision rule in the random forest (the 'mtry' parameter in the R function to perform Random Forest) and to estimate the mean square error (on independent test samples). The performance of the models is expressed by the prediction R^2, which is calculated from the out-of-bag samples (Breiman, 2001). This R^2 value therefore is not a goodness-of-fit of the data at hand but an estimate of predictive accuracy on independent (left-out) samples. Variable importance was estimated by the increase in mean square error (MSE) after permutation (Breiman, 2001).

Results

Genotypes

In correspondence to the genetic diversity in our collection of 24 non-pungent *Capsicum annuum* accessions, a high degree of variation was found for fruit size and yield. Fruits ranged from 5-22 cm in length and 2-8.5 cm in width, within the fruit types blocky, dulce italiano, dolma, kapya, lamuyo, conical and elongated (Table 1). The majority of the genotypes were red, as this is the predominant color in cultivated material; yellow and orange genotypes were less represented. Total yield was measured throughout the complete growth period (May through September) and ranged from 6.6-15.3 kg/m^2.

Table 1. Description of *Capsicum annuum* genotypes evaluated for fruit quality attributes

Genotype	Origin	Source country	Fruit type	Size[a] (cm)	Color	Yield[b] (kg/m^2)
Mazurka[c]	Elite	Netherlands	Blocky	8 x 8	Red	12.1 ± 0.1
Hybrid 1	Elite	Netherlands	Blocky	8 x 8	Red	12.8 ± 0.8
Line A	Elite	Netherlands	Blocky	8 x 8	Red	11.7 ± 0.3
Line B	Elite	Netherlands	Blocky	8.5 x 8	Red	9.6 ± 0.9
Line C	Elite	Netherlands	Blocky	8.5 x 8	Red	12.9 ± 0.3
Line D	Elite	Netherlands	Blocky	8 x 8	Red	9.1 ± 0.7
Line F	Elite	Netherlands	Blocky	9 x 8	Yellow	11.8 ± 1.3
Line G	Elite	Netherlands	Blocky	8 x 8	Yellow	15.3 ± 0.6
Line H	Elite	Netherlands	Blocky	8 x 8	Yellow	12.9 ± 0.5
Line I	Elite	Netherlands	Blocky	8 x 8.5	Yellow	14.6 ± 1.4
Line J	Elite	Netherlands	Blocky	8 x 8.5	Orange	12.4 ± 2.4
Line K	Elite	Netherlands	Mini block	5 x 5	Orange	7.2 ± 1.1
Hybrid 2	Elite	Italy	Dulce italiano	20 x 4	Red	10.9 ± 2.0
Hybrid 3	Elite	Italy	Dulce italiano	22 x 4.5	Red	13.0 ± 0.8
Line L	Elite	Italy	Dulce italiano	22 x 4	Red	11.5 ± 0.5
Line M	Elite	Italy	Dulce italiano	18 x 4.5	Red	9.8 ± 1.4
Line O	Elite	Italy	Dulce italiano	22 x 4	Red	11.6 ± 3.0
Line E	Elite	Turkey	Dolma	7 x 6.5	Red	8.7 ± 1.1
Line N	Elite	Turkey	Kapya	12 x 4	Red	8.8 ± 1.1
Piquillo	Cultivated	Spain	Conical	9 x 4	Red	6.6 ± 0.7
Buran	Cultivated	Poland	Lamuyo	10 x 7	Red	11.0 ± 0.3
PBC1405	Gene bank	AVRDC, Taiwan	Elongated	18 x 2	Red	8.8 ± 1.0
Vania	Cultivated	France	Lamuyo	14 x 8	Red	10.1 ± 0.9
Maor	Cultivated	Spain	Blocky	8 x 8	Red	12.0 ± 0.8

[a] Size is indicated by length x width, [b] Average yield and standard deviation in the harvesting period May through September, [c] Standard variety (reference to *e.g.* Luning et al. 1994).

Volatiles are correlated according to metabolic pathway

Hierarchical cluster analysis (HCA) was performed on measurements from the first harvest, using intensity patterns of all 254 volatiles, concentrations of sugars (fructose and glucose) and acids (malic, citric and ascorbic acid) plus total soluble solids (brix) and dry matter measurements. Few clusters of highly correlated compounds were found, which are shown as dark colored blocks in the correlation matrix (Fig. 1). NIST library matching results indicated that nine of these blocks contained compounds that have a common biochemical precursor or belong to the same metabolic pathway: phenolic derivatives (a,i), higher alkanes (c), sesquiterpenes (d), lipid derivatives (e), terpenoids (f,h) and saturated acid derivatives (g). In our 24 genotypes also the majority of primary metabolites as well as brix and dry matter content, clustered together. Specifically, the sugars glucose and fructose grouped with citric and ascorbic acid, brix, 3-methyl-butanoic acid, 3-methyl-3-butenylester and several compounds of unknown identity (cluster b, Fig. 1). Malic acid did not cluster with the other primary metabolites.

Biochemical and sensory contrasts

Both the metabolic and sensory data from the combined three harvests were subjected to principal components analysis (PCA, Fig. 2). The biochemical data revealed two major types of metabolic contrasts within the 24 pepper genotypes (Fig. 2A). Firstly, a clear separation between the gene bank accession PBC1405 and all other cultivated and elite genotypes. Secondly, PCA revealed the variation in metabolite content within the cultivated and elite genotypes, separating the yellow blocky genotypes F, G and I from the red conical genotypes N and Piquillo. The third, fourth and fifth principal component (PC) accounted for approximately 5% explained variance each. Adding PCs however, did not obviously contribute to further separation (with biological relevance) of the genotypes. The separate grouping of PBC1405 in the PCA plot was mainly caused by a higher relative abundance of 20 volatiles in this accession compared to the other genotypes. Eight volatiles of those were solely detected in PBC1405: hexanoic acid hexyl ester, β-ionone, propanoic acid, 2-methyl-, 1-methylbutyl ester, 3,7-dimethyl-6-octen-1-ol formate, (Z)-1,1,3,5-tetramethyl-cyclohexane, 3,3-dimethyl-cyclohexanol, 2,4-dimethyl-3-pentanone and 1-tridecene. Of the other twelve volatiles, trace amounts could be detected in fruits of also a few non-PBC1405 genotypes. Nine volatiles, from the twenty making the difference between PBC1405 and the other genotypes, belonged to the higher alkanes and sesquiterpenes (cluster c/d, Fig. 2B). The metabolites which were most discriminative between yellow genotypes F, G and I versus all other genotypes, were mainly of phenolic origin (cluster i, Fig. 2B): copaene, 1,4-dimethoxy-benzene, benzoic acid, ethyl ester, 2-ethyl-1-hexanol, 2-octanone, camphor, thymol and benzophenone. The fourth yellow genotype H, remarkably, did not cluster with the yellow genotypes F, G and I, but positioned among red and orange genotypes. Lipid derived volatiles (cluster e, Fig. 2B), including (E)-2-heptenal, (E,E)-2,4-heptadienal, hexanal, (E)-2-hexen-1-ol, neopentane and 2-hexenal, were principally responsible for the separation of the red conical genotypes N and Piquillo.

For the sensory data, the first two principal components explained 66.5% of the variation in the data, with the first PC alone accounting for 50.4% and PC2 accounting for 16.1% of the variation (Fig. 2). PCA revealed two sensory contrasts within the pepper genotypes, *i.e.* a juiciness versus stickiness/toughness contrast (vector 1) and secondly a sweet/fruity versus sour/grassy contrast (vector 2, Fig. 2D). The six attributes carrot, bean, grassiness, petrochemical, musty and perfume taste, which are situated close to the origin hardly contributed to visualize the genotype variation. The contrast along vector 1 is texture related, whereas vector 2 mainly describes the basic sweet-sour taste contrast. In our material vector 1 distinguished the yellow blocky genotype F from the red conical cultivar Piquillo, mainly based on their texture differences, with F having juicy fruits and Piquillo having very tough fruits with a sticky skin (data not shown). The dulce italiano genotypes

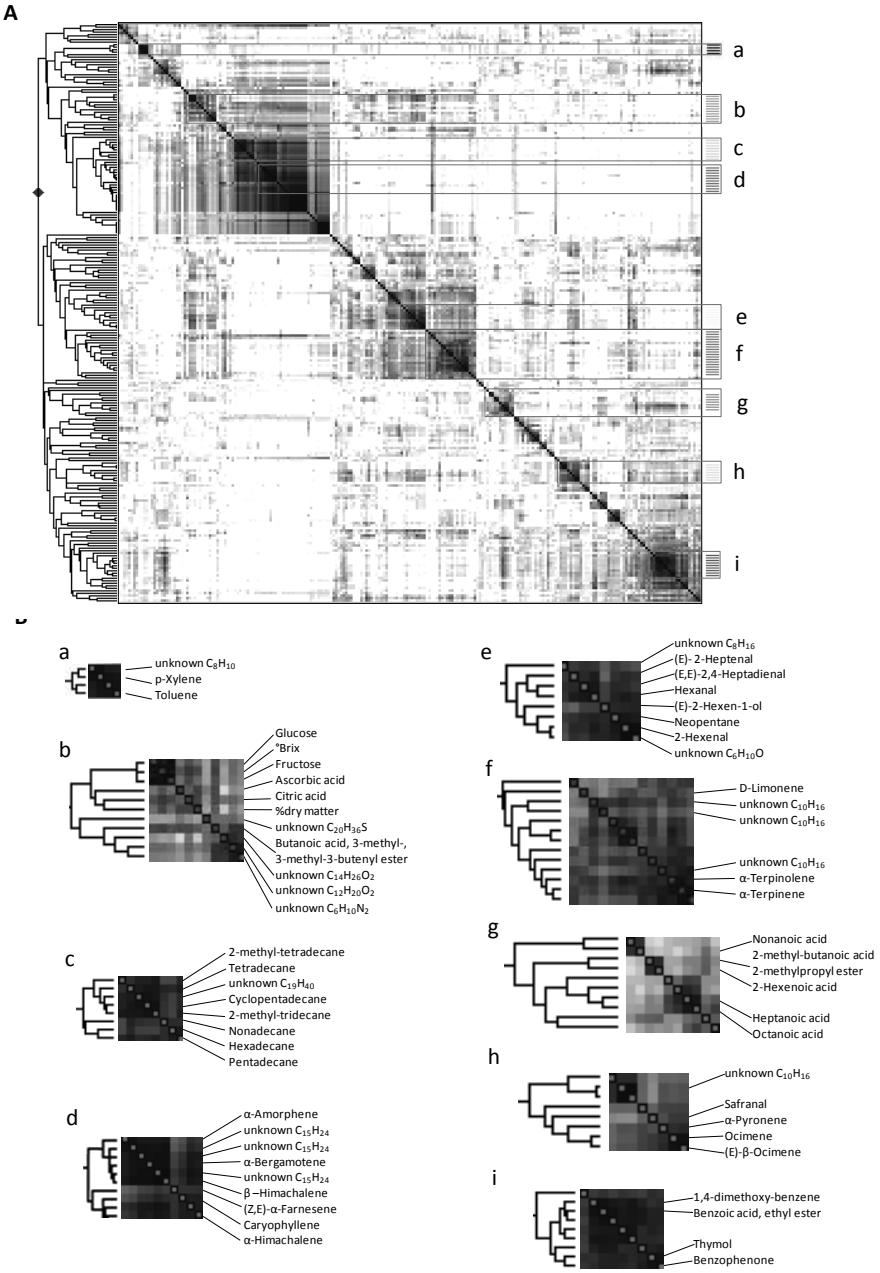

Fig. 1. Metabolite-metabolite correlation matrix based on intensity patterns of 254 volatiles, concentrations of 5 primary metabolites plus brix and dry matter content from the first harvest. **(A)** The main compound clusters are situated along the diagonal axis (clusters a-i). Correlations between metabolites are shade colored: the darker the color the higher the percentage of similarity between metabolite expression patterns. **(B)** Detailed dendrograms of each compound cluster with putative compound identity. Compound clusters: a and i, phenolics; b, non-volatiles; c, higher alkanes; d, sesquiterpenes; e, lipid derivatives; f and h, terpenoids; g, saturated fatty acids.

Hybrid 2, 3 and M were characterized by high sweetness scores and were separated along vector 2 from the gene bank accession PBC1405 with sour and grassy fruits (Fig. 2D). The positioning of genotypes F, Piquillo and PBC1405 in the extremes of the sensory PCA plot (Fig. 2C) was also reflected by their extreme positions in the PCA plot showing the metabolic differences (Fig. 2A).

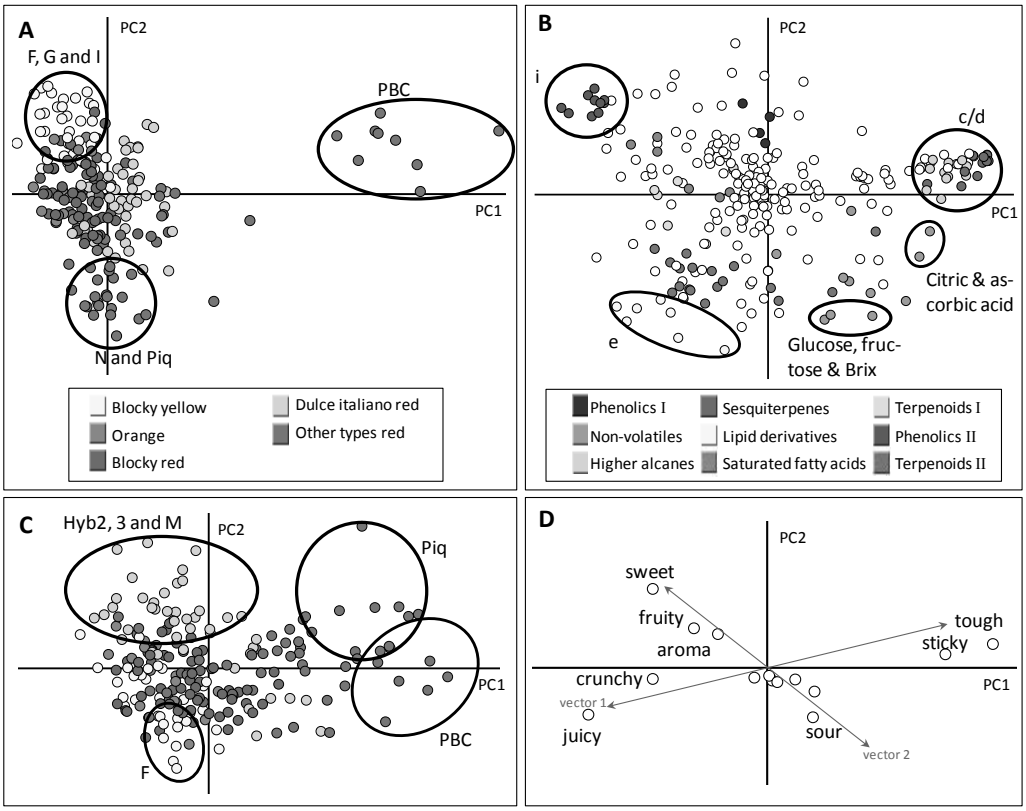

Fig. 2. Principal components analysis on metabolic (**A,B**) and sensory (**C,D**) data of 24 pepper genotypes in 3 replicates of 3 harvests. (**A,C**) PCA scores plots showing the major variation between the genotypes based on (**B**) intensity patterns of 254 volatiles, concentrations of 5 primary metabolites, brix, and dry matter content (PC1=16.2%), PC2=9.5%) and on (**D**) loadings of the 14 sensory attributes (PC1=50.4%), PC2=16.1%). Letters of the metabolite clusters correspond with Fig. 1. (For a color version of this figure, the reader is referred to Eggink et al. 2012b.)

Relation between biochemical compounds and attributes

A Random Forest (Breiman 2001) regression approach was used on individual harvests and on the three harvests together to relate each sensory attribute to the volatiles and non-volatiles and to determine importance of the individual compounds. Several common attribute predictors for aroma, fruity/apple, sourness and sweetness were found between harvests, like (E)-2-hexen-1-ol, glucose, fructose, 1-methyl-1,4-cyclohexadiene and (Z)-β-ocimene for sweetness (Table 2). Compounds which were predictive in more than one harvest always had the same correlation direction between harvests, with the only exception of (Z)-β-ocimene versus sweetness, which had respectively a positive and negative correlation in the first and third harvest (data not shown). The flavors of all common compounds matched with the attributes aroma, fruity/apple and sweetness, as they had sweet, spicy, almond and/or fruity descriptions (Table 2). As physical properties of the fruits other

than dry matter content were not measured, results of the texture related attributes juiciness, toughness, crunchiness and stickiness are not included. For the less contrasting attributes grassiness, green bean, carrot, perfume, petrochemical and musty flavor, the only interesting finding was 2-pentyl-furan with a significant contribution to the prediction of green bean flavor (correlation 0.43) and which flavor is described as green bean/butter like (http://www.flavornet.org/d_odors.html).

Table 2. Key-metabolites with predictive value in multiple harvests

Compound	Attribute[a]	Flavor description	Reference
(E)-2-Hexen-1-ol	a/f/s	Almond, fruit, spicy	Luning et al. (1994)
Neopentane	a/f/s	-	
Fructose	a/f/s	Sweet	
p-Menth-1-en-9-al	f/s	Spicy, herbal	Good scents company[b]
Glucose	f/s	Sweet	
3-Hepten-2-one	f	Creamy, coconut, cheesy	Mosciano et al. (1993)
(Z)-β-Ocimene	s	Sweet, herbal	Good scents company
unknown $C_{15}H_{24}$	s	-	
(Z)-2-Penten-1-ol	s	Rubber, plastic, green	Flavornet[c]
1-methyl-1,4-Cyclohexadiene	s	Fruity	Good scents company
unknown $C_{13}H_{18}$	s	-	
unknown $C_6H_8O_2$	s/so	-	

[a] Involved attributes aroma (a), fruity/apple (f), sweetness (s) and sourness (so).
[b] http://www.thegoodscentscompany.com , [c] http://www.flavornet.org/d_odors.html

Discussion

Metabolic contrasts between genotypes are caused by clusters of volatile and non-volatile compounds

In accordance to the genetic diversity of our studied panel, the genotypes displayed a high degree of fruit type, organoleptic and metabolic variation. A total of 254 different volatile compounds could be distinguished using GC-MS in combination with multivariate mass spectral reconstruction. This number of volatiles was very comparable to the number of volatiles found in twelve *C. annuum* genotypes by Rodriguez-Burruezo and colleagues (2010). For both volatile and non-volatile compounds, highly correlated clusters were found by HCA, which could be related to metabolic pathways and common biochemical precursors. The specific grouping of the non-volatiles glucose, fructose, citrate and ascorbic acid with the volatile compound 3-methyl-butanoic acid 3-methyl-3-butenylester and several other volatiles of unknown identity (cluster b, Fig. 1) seemed rather caused by population structure than by functional relationship. Metabolic contrasts between genotypes were caused by both qualitative and quantitative differences in the metabolic clusters, with the phenolic derivatives, higher alkanes, sesquiterpenes and lipid derived volatiles forming the major determinants. Changes of genes (expression) in such pathways would probably change complete clusters of volatiles, thereby affecting individual attributes or even overall flavor (*e.g.* Lewinsohn et al. 2005, Tieman et al. 2006).

A large role for texture and sweetness/sourness in pepper flavor

The variation in taste could be reduced into two major contrasts, which were a texture related contrast and the basic sweet-sour contrast. In tomato a similar sweet/fruity versus sour/watery contrast has been found in both a study with 16 tomato cultivars (Sinesio et al. 2010) and a study with 94 tomato varieties (Hageman et al. 2010), with a texture related contrast in the second principal component, which for tomato described a firmness-mealiness contrast. Although we found similar sensory contrasts as in tomato, in pepper the texture contrast explained the largest part of

variation (52.6%), whereas in tomato the sweet-sour contrast was most discriminative (~45% explained variation).

Metabolites influencing the major sensory attributes

Expected relations between (non-)volatiles and attributes, like sweetness and sugars, were found but also some new relations. Neopentane was found to contribute to both aroma and fruity/apple taste as well as sweetness, however no flavor description could be found. The most likely explanation for neopentane being correlated was that it has a very similar expression as (E)-2-hexen-1-ol (Fig. 1, cluster d), which seemed truly predictive based on its almond, fruit, spicy odor description (Luning et al. 1994). For fruity/apple taste and sweetness new relations were found with the compounds p-menth-1-en-9-al, (E)-β-ocimene, (Z)-2-penten-1-ol and 1-methyl-1,4-cyclohexadiene. Taking both the flavor description of these compounds and the direction of their correlation (not shown) with either fruity/apple taste or sweetness into account, it seemed reasonable that all four compounds are really contributing to the involved attribute. The only compound with a significant contribution to sourness was an unknown $C_6H_8O_2$ compound (Table 2). From the organic acids, citrate showed the best relation with sourness with a correlation of 0.34, which was however not significant. Organic acids seem therefore not to play a role of importance in pepper sourness. In tomato, however, a correlation of 0.76 was found between titratable acids (mainly citric and malic acid) and sourness (Tandon et al. 2003), while the variation and concentration of organic acids in that study were similar as in our pepper collection. An explanation for this could be that in pepper the effect of sour related metabolites is masked by other volatile and non-volatile compounds or texture differences. In our analyses we did not find an effect on flavor attributes of the well known compound 2-isobuthyl-3-methoxypyrazine, which is commonly described in sniffing port analyses as characteristic (green) bell pepper aroma (Luning et al. 1994, Van Ruth et al. 1995, Rodriguez-Burruezo et al. 2010), indicating different sensitivity of sniffing versus taste evaluations.

Perspectives for flavor improvement by breeding

The flavor of pepper is a complex trait, which is influenced by many factors, like the environmental conditions in which the fruits were grown, the interaction between many flavor related metabolites and the flavor perception and/or preferences of consumers. In this study we tried to investigate some of these aspects to understand their behavior better. Key-metabolites were identified that influence the sensory attributes, making targeted improvement of flavor components (attributes) by breeding feasible. Still a complicating factor is that the effect and interaction of individual attributes on overall consumer liking is not completely clear. Verkerke and Janse, however, reported already in 1998, that a ‘good tasting’ pepper should be sweet and crunchy with a fruity aroma. New consumer preference studies are required to confirm this, but it suggests already that compounds like fructose, glucose, (E)-2-hexen-1-ol and p-menth-1-en-9-al, with a positive contribution to both sweetness and fruity/apple flavor, are interesting candidates to increase the concentration of by breeding.

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Pepper research and breeding at AVRDC – The World Vegetable Center

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Abstract

Peppers (*Capsicum* spp.) with pungent (chili, hot pepper) and non-pungent (sweet pepper) fruits are important spice and vegetable crops worldwide. There are five domesticated species of the genus *Capsicum*, *C. annuum* being the most widely cultivated. Over the past 25 years, the pepper breeding program at AVRDC– The World Vegetable Center has focused on improving this species (hot and sweet peppers) with respect to incorporation of pest resistance and developing male sterile lines. In this presentation, we briefly share the research activities of AVRDC's pepper breeding program.

Keywords: *Capsicum,* pepper, disease resistance, male sterility

Introduction

Hot pepper (*Capsicum* spp.), the pungent New World spice that intrigued Columbus in the XVth Century, was initially confused with black pepper (Eshbaugh, 1993). Today hot peppers dominate the world spice trade and sweet peppers have become indispensable vegetables worldwide. Among the five cultivated species of the genus *Capsicum* (*C. annuum, C. frutescens, C. chinense, C. baccatum, C. pubescens*), *C. annuum* is the most widely cultivated. Peppers are an important cash crop for smallholder farmers in developing countries such as Ethiopia, Nigeria, Ghana, China, India, Pakistan, Bhutan, Indonesia, Cambodia, and Thailand. Considering the role of this crop in improving the income of smallholders in developing countries, AVRDC – The World Vegetable Center (AVRDC) initiated a pepper improvement program during the mid-1980s.The major focus has been breeding for resistance to several economically important diseases and development of male sterility systems.

Disease resistance

To achieve the breeding objectives pertaining to host plant resistance against economically important diseases, the Center's pathologists have studied pathogen biology and developed seedling screening protocols.

Anthracnose

Pre- and post- harvest anthracnose fruit rot is a serious disease of hot pepper. The disease is caused by several *Colletotrichum* species, mainly *C. capsici, C. gloeosporioides*, and *C. acutatum* (Than et al. 2008). These three species can be identified based on the sequence variation of ITS rDNA. A protocol of restricted fragment length polymorphism of ITS rDNA was developed at AVRDC for rapid species identification (Sheu et. al. 2007). The predominant species causing the disease in Taiwan and Indonesia was found to be *C. simondsii* (A2 genetic group of *C. acutatum*) (Shivas and Than, 2009). In Taiwan, two pathotypes of *C. simondsii* were identified based on the differential reactions on green fruit of 0538-8525, a *C. annuum* resistant genotype, derived from PBC932 (*C. chinense*). Pathotype CA2 broke down the resistance of 0538-8525, whereas CA1 did not (Sheu et. al. 2006). Our survey results showed pathotype CA1 was predominant before 2000,

while pathotype CA2 became predominant in some parts of Taiwan. Hence both CA1 and CA2 isolates are now being used at AVRDC for screening segregating germplasm materials and advanced lines using either spray inoculation or micro-injection inoculation. During the late-1990s, the Center identified anthracnose resistance sources in two domesticated *Capsicum* species i.e. *C. baccatum* (PBC80, PBC81) and *C. chinense* (PBC932). PBC-932 was used to develop several improved lines at AVRDC (Table 1). These lines express varying degrees of resistance depending on prevalent isolates and some of them are currently being evaluated in national variety release systems of several countries.

Phytophthora blight

The disease is caused by *Phytophthora capsici* and has a wide geographic distribution. Disease outbreak is often associated with warm and wet periods in high rainfall areas as well as in irrigated system in arid regions. Progress on utilizing Phytophthora- blight-resistant sources has been slowed down due to the large variability in virulence among pathogen populations and the complex inheritance of resistance. Four pepper lines, i.e. Early Calwonder, PBC137, PBC602 and PI201234, were used as the differential varieties to group *P. capsici* isolates into three pathotypes. A total of three pathotypes were identified in Taiwan, pathotype 3 was predominant and showed the highest virulence (Chen, 2009). Thus, pathotype 3 isolates are used routinely to screen germplasm accessions and improved lines for disease resistance. Screening was conducted with the root drenching method using zoospore suspension (1×10^5 spores/ ml). Eight known resistant accessions (PI201232, PI201234, PI201238, PI188478, CM331, CM334, Fidel, and C05485) were evaluated using root drenching and foliar atomization methods. Single resistant plants of these accessions were selected, and seeds were saved for distribution.

Bacterial wilt

Bacterial wilt, caused by the soil-borne bacterium *Ralstonia solanacearum*, occurs under warm and humid conditions. Use of resistant varieties is the simplest and easiest mean to control this disease. Germplasm was screened by inoculating seedlings with strain Pss71 (race 1, biovar 4) at the 5- to 6-leaf stage using soil drenching with the root-severing method. Roots were injured with a knife by cutting the soil at 1.5 cm distance from the stem and pouring bacterial suspension (10^8 cfu/ml) (1/10 volume of potting mixture) into each pot. Resistance sources identified include MC4, MC5, Chili Langkap, Paris Minyak, IR, CA8, Chinda 23, R1-26 (17), PBC384, PBC385 and PBC473 (Wang and Berke, 1997).

Bacterial spot

Bacterial spot is one of the most devastating diseases of pepper under warm and humid environments. The causal agents include four species of *Xanthomonas*, i.e. *X. euvesicatoria*, *X. gardneri*, *X. perforans*, and *X. vesicatoria*. However, the vast majority of strains that infect pepper are *X. euvesicatoria* (Jones et al. 2004). Strains infecting peppers can be differentiated into 11 races (P0 to P10) using 5 differential varieties, i.e. Early Calwonder (ECW), ECW-10R, ECW-20R, ECW-30R and PI235047 (*C. pubescens*). Resistance sources with a single dominant resistant gene, e.g. *Bs2* as well as quantitative resistance, e.g. CNPH703 (PBC137) have been utilized in our breeding program. To select for both qualitative and quantitative resistance, disease screening was conducted using a dipping method. Seedlings with 4 fully expanded leaves were dipped in bacterial suspension (10^8 cfu/ml) with 0.05% Silwet L-77 for 30 seconds, then inoculated at 28°C with overhead irrigation.

Viral diseases

Hot and sweet peppers are particularly prone to virus infections and can be affected by more than 60 different species of viruses in different parts of the world. In South and Southeast Asia, the predominant viruses infecting *Capsicum* species belong to six genera (Table 2). The potyviruses probably remain the most prevalent of the viruses infecting peppers in this region; *Potato virus Y*

(PVY) and *Chili veinal mottle virus* (ChiVMV) have been present in Taiwan for a long time, while *Pepper veinal mottle virus* (PVMV) (Cheng et al. 2009) and *Pepper mottle virus* (PepMoV) appear to be more recent introductions (Cheng et al. 2011). *Cucumber mosaic virus* (CMV, Cucumovirus) and the tobamoviruses, *Pepper mild mottle virus* (PMMV) and *Tomato mosaic virus* (ToMV) are present in all pepper-growing areas of SE Asia, though their incidences vary from season to season. Based on their prevalence and ease of mechanical inoculation (which permits the production and maintenance of pure cultures), PVY, ChiVMV, CMV and ToMV have, until recent times received the most attention at AVRDC. Pepper germplasm and breeding lines resistant to CMV, ChiVMV, PVY and ToMV were individually tested by mechanical transmission. Test plants were mechanically inoculated at the 3-5 true leaf stage, and then again 5-7 days later to reduce the number of infection escapes. Resistance/susceptibility scores were based on symptom development in relation to known susceptible check plants, with confirmation by specific enzyme linked immuno sorbent assay (Tsai et al. 2008).

As in most other vegetable crops, the whitefly-transmitted Geminiviruses (Begomoviruses) have emerged over recent years as major constraints to pepper production in many countries of South and Southeast Asia, though the begomovirus species involved is generally different in each country. As In Taiwan, the only begomovirus commonly infecting peppers is *Tomato yellow leaf curl Thailand virus* (TYLCTHV) (Shih et al. 2010). Although TYLCTHV generally does not cause severe symptoms in peppers, it is being used at AVRDC as part of an initial screen to identify pepper germplasm with potential begomovirus resistance. Because TYLCTHV is not readily mechanically transmitted, this screening relies on the use of viruliferous *Bemisia tabaci* whiteflies in a dedicated nethouse. Pepper germplasm also has been screened for resistance or tolerance to begomovirus infection in the field in several countries of South and Southeast Asia, and crosses have been performed to start to move some of the identified resistance into agronomically improved breeding lines (Table 1). Until recently, tospovirus infection in peppers was relatively rare across the region and generally assumed to be *Tomato spotted wilt virus* (TSWV). However, infections by both TSWV and the more recently identified *Capsicum chlorosis virus* (CaCV) have been recognized as becoming more common in pepper in many countries of South and Southeast Asia, including Taiwan (Huang et al. 2010; Zheng et al. 2010). Although tospoviruses can be mechanically transmitted, this is not as reliable as for some of the other viruses, and tospovirus isolates tend to lose pathogenicity after relatively few mechanical transfers. Maintaining thrips cultures for tospovirus inoculation is not easy, and procedures for getting reliable infection through thrips inoculation are difficult to establish. Despite these difficulties, a start has been made to screen the AVRDC germplasm collection for resistance to CaCV and TSWV. A Polerovirus, *Pepper vein yellows virus* (PeVYV), has recently been shown to be widespread across South and Southeast Asia (Knierim et al. 2013). Because poleroviruses cannot be mechanically inoculated, screening for resistance to PeVYV will have to be based on use of viruliferous aphids, or Agrobacterium-mediated inoculation with full-length infectious PeVYV clones. A number of resistant sources have been identified and lines with improved horticultural attributs have been developed (Table 1).

Insect resistance

The Center has screened the *Capsicum* germplasm for its resistance to sucking insects and broad mite. Some initial screening programs focused on aphid resistance. While screening several hundred accessions, three accessions *viz.,* PBC081, PBC151 (*C. baccatum*) and PBC272 (*C. chinense*) had fewer cotton aphid (*Aphis gossypii*) infestations. Additional germplasm screening resulted in four other pepper accessions with reduced aphid infestation *viz*., PBC880 (*C. baccatum*), PBC18, PBC30, and PBC84 (all *C. annuum*). Recently, we screened more than 100 germplasm accessions against various insect and mite pests under natural field conditions. Two accessions, namely, PBC145 (from India) and C00069 (from Costa Rica) were found to be resistant to pests including broad mite (*Polyphagotarsonemus latus*), aphid (*Myzus persicae*), and thrips (*Thrips palmi*). These

resistant lines were used in breeding programs and a set of potential inbred lines have been developed. Currently we are revisiting these inbred populations by screening them under field conditions with high insect pressure.

Table 1. Disease resistant germplasm identified and improved resistant lines developed at AVRDC

Resistant germplasm	**Improved resistant lines**
Anthracnose	
PBC932, PBC80, PBC81, PI594137, PBC1791, PI49798	AVPP0205, AVPP0412, AVPP0706; AVPP0513, AVPP0514, AVPP9813, AVPP0805, AVPP0803, AVPP0903, AVPP0906, AVPP0908 (HP)
Bacterial wilt	
PBC66, PBC375, PBC535, PBC631A, PI 201234	AVPP9702, AVPP9703, AVPP9705; AVPP0102, AVPP0103, AVPP0104, AVPP0201, AVPP0205, AVPP0206, AVPP0307, AVPP0511 (HP)
Phytophthora blight	
PI201232, PI201234, PI201238, PI188478, CM334, PBC271	AVPP9703, AVPP9803, AVPP0302(HP); AVPP9809, AVPP0117, AVPP0504, AVPP0601, AVPP0407 (SP)
Cucumber mosaic virus (CMV)	
HAD 248	AVPP0105 (HP), AVPP0602 (SP)
Chili veinal mosaic virus (ChiVMV)	
CM 334, PBC602, PBC495, VC 241, 9656-08, PBC-145, PBC-142	AVPP0105 (HP); AVPP0602 (SP)
Potato virus Y (PVY)	
PBC066, PBC 385, PBC 67, PBC1347, PBC204, PBC384, PBC473, PBC743, PBC580, PBC 602, PBC714	AVPP9813, AVPP9905, AVPP0012, AVPP0105 (HP); AVPP9807, AVPP0006, AVPP0204, AVPP0408, AVPP0502 (SP)
Leaf curl begomoviruses	
PBC143, PBC144, PBC145, PBC-149, PBC456, PBC495, PBC518,	AVPP1127, AVPP1128, AVPP0715, AVPP0716, AVPP0906, AVPP0813, AVPP0717

HP = hot pepper; SP = sweet pepper

Table 2. Characteristics including mode of transmission of the main genera of virus infecting peppers in Southeast Asia

Virus genus	**Particle type (and genome composition)**	**Transmission mode**		
		Vector	**Seed**	**Mechanical**
Begomovirus	Geminate-spherical (circular ss DNA)	Whitefly (persistent)	No	No
Cucumovirus	Spherical-isometric (ss RNA)	Aphid (non-persistent)	Yes	Yes
Polerovirus	Spherical-isometric (ss RNA)	Aphid (persistent)	No	No
Potyvirus	Filamentous rod (ss RNA)	Aphid (non-persistent)	Yes[a]	Yes
Tobamovirus	Rod (ss RNA)	Contact (soil)	Yes[a]	Yes
Tospovirus	Spherical-enveloped (ss RNA)	Thrips (persistent)	No	Yes[b]

[a] Not all species are seed transmitted; [b]Mechanical transmission not reliable for some species

Cytoplasmic male sterile (CMS) lines

The Center initiated the development of hot pepper CMS lines in 1996. To date more than a dozen pairs (with different genetic backgrounds) of CMS (Peterson's cytoplasm), maintainer and restorer inbred lines have been developed and made available to public and private sector partners. We have also developed sweet pepper CMS and maintainer pairs. Our current focus is restorer breeding in sweet pepper, as most of the sweet pepper lines are maintainers or poor restorer (Lin et al. 2007). This has been a limitation for the successful use of CMS in sweet pepper hybrid development and seed production.

Markers

Up to now, amplified fragment length polymorphism (AFLP) and microsatellite (SSR) and sequence characterized amplified region (SCAR) markers have been used in attempting marker-assisted selection in pepper. Except in one case, markers developed elsewhere (e.g. Phyto 5.2 for *Phytophthora* resistance; AFLPs for anthracnose resistance) could not be validated in lines developed by AVRDC. A SCAR marker associated with the restorer-of-fertility locus (*Rf*) of male sterile cytoplasm (CRF-S; Gyulas et al. 2006), however, has successfully been used in marker assisted back-crossing to introgress the strong *Rf* allele from hot pepper into sweet pepper genotypes.

Markers such as AFLP and SSR are inferior to single nucleotiode polymorphic markers (SNPs), currently the preferred marker system. Microsatellite markers available in the public domain, or designed based on publically expressed sequence tags (ESTs), have been applied to *Capsicum* germplasm, but the polymorphism levels among accessions of the same *Capsicum* species were found to be very low. For example, from 278 SSR markers, on average 26% were polymorphic between AVRDC *C. annuum* breeding lines, while more than 60% of these markers were polymorphic between *C. annuum* and *C. baccatum* accessions (AVRDC, unpublished). In a larger SSR marker set (500 SSRs), the proportion of polymorphic SSR markers among *C. annuum* breeding lines dropped to below 20%. Hill et al. (2013) described the construction and use of a 30K SNP array based on EST sequences and a 20K pepper SNP array is under development (Allen van Deynze, personal communication). These SNP arrays will greatly facilitate access to polymorphic markers. In parallel, genotyping by sequencing, for example restriction enzyme associated DNA (RAD) sequencing methods have the potential to yield large numbers of population - specific SNP markers. These marker resources are expected to greatly improve the application of molecular methods.

Seed dissemination

The Center disseminates seed of its improved pepper lines to public and private sector breeders, most of them working in developing countries. AVRDC-developed lines have been: (i) directly released as open-pollinated varieties through national varietal release procedures, (ii) subjected to selection (in cases of segregating germplasm) according to local trait preferences and subsequently released as new varieties, (iii) used (possibly after further selection) as parental lines in hybrid development, or (iv) used as sources of traits in crosses to develop new breeding lines.

A detailed search of our *Capsicum* germplasm distribution database from 2001 to 2012 was conducted (Fig. 1).

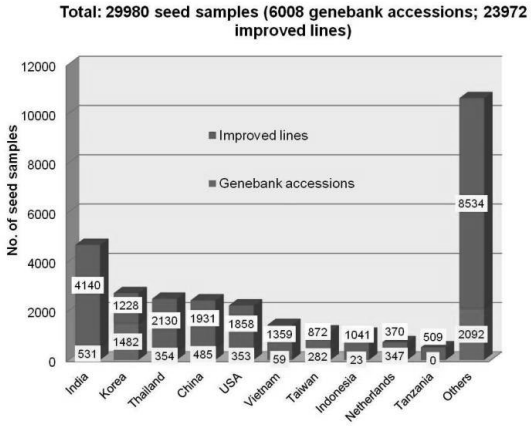

Figure 1. Seed distribution by recipient country (2001 to 2012)

A total of 29,980 germplasm materials have been distributed, comprising 6,008 genebank accessions (20%) and 23,972 improved breeding lines (80%). The top ten recipient countries were: India (15.6%), Republic of Korea (9.0%), Thailand (8.3%), China (8.1%), USA (7.4%), Vietnam (4.7%), Taiwan (3.8%), Indonesia (3.5%), Netherlands (2.4%), and Tanzania (1.7%) (Fig. 1). A total of 10,613 accessions (35.4%) were shipped to another 113 countries. More than 200 sets of CMS and maintainer lines were distributed to public and private institutions in 35 countries. Exploitation of these CMS lines has been much higher in the private seed sector, especially in Asian countries like India, where the vegetable hybrid seed industry is better developed, compared with other developing countries. Seed companies or public institutions have either directly used AVRDC's CMS lines to develop and produce hybrid seeds or used them to convert into locally adapted and preferred market types with better combining abilities. Selected examples will be presented.

Current focus

We are currently revisiting our anthracnose and begomovirus resistance sources and some of the breeding populations derived from previously identified resistant sources. Progress on incorporation of a strong *Rf* gene in sweet pepper inbred lines has been rather faster. We are expecting to accomplish this in few years, including seed increase of sweet pepper restorer lines for international distribution. We have learned from seed distribution experiences in the past that a major multiplying impact of our breeding and germplasm materials has been achieved in developing countries, when people working in both public and private sectors have enhanced skills. Capacity building of human resources will remain a corner stone of our strategy to harness a larger impact of the Center's bred pepper lines.

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Differential resistances to chili anthracnose as affected by fruit maturity, *Colletotrichum* pathotypes and inoculation methods

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Abstract

Chili anthracnose, caused by Colletotrichum spp., is one of the major diseases to chili production in Asia and the tropical areas of the world. Three Colletotrichum species including C. acutatum, C. gloeosporioides, and C. truncatum (syn. capsici) are the key causal pathogens, which have been grouped into several pathotypes based on differential reactions on a set of differential chili host genotypes. Breeding for durable anthracnose resistance requires a good understanding of the resistance mechanisms, which involve three significant factors including fruit maturity, Colletotrichum pathotypes and inoculation methods.

Correlation between physiological and biochemical changes during chili fruit ripening and anthracnose fruit infection was studied. The chili fruit of Capsicum baccatum PBC80 was susceptible to Colletotrichum acutatum pathotype 1 (PCa-1), but the fruit became fully resistant at the late mature green stage, prior to the color change.

The study suggested that fruit maturity played an important role in the expressions of resistance to anthracnose. Differential resistances to anthracnose as responding to two different Colletotrichum pathotypes, PCa-2 and PCa-3; and as by two different inoculation methods, microinjection (MI) and high pressure spray (HP), were studied. PBC80, highly resistant to PCa-2 and PCa-3, was crossed with a susceptible C. baccatum CA1316 to produce segregating populations for the genetic study. Detached ripe fruit of F2 and BC1s was assessed for anthracnose resistance.

The genetic study suggested that two new dominant genes namely Co6 and Co7 were identified. Co6 was responsible for the resistance to PCa-2 and PCa-3 by MI, and Co7 was responsible for the resistance to PCa-3 by HP. Co6 and Co7 were linked with 16.7 cM distance.

Biochemical basis of plant defence for leaf curl virus of chilli (*Capsicum annuum*)

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Abstract

The present research programme was framed to identify the source of resistance to chilli leaf curl virus, a white fly vectored Gemini virus and determine the association between different biochemical composition of leaf and manifestation of resistance. The disease reaction in the37 chilli genotypes evaluated in natural condition without any insecticidal cover which was ascertained on the basis of three parameters viz., per cent disease incidence , per cent disease index and coefficient of infection varied widely among the genotypes. Total phenol content and activity of the enzymes, "Peroxidase" and "Polyphenol oxidase" in the leaves also varied widely among the genotypes indicating ample scope for selection. The 37 genotypes were categorized into six groups according to coefficient of infection (CI). Only one genotype CUCH-4 (CI - 8.75) came under 'Resistant' group while the 'Moderately Resistant' group with CI ranging from 11.25 to 18.75 comprised of eight genotypes viz. CUCH-1, CUCH-5, CUCH-6, CUCH-7, CUCH-29, CUCH-31, CUCH-34 and CUCH-35. The remaining 28 genotypes with coefficient of infection ranging in between 20 to 100 fell in three susceptible categories. Coefficient of infection, the most reliable disease reaction parameter was significantly and negatively correlated with leaf phenol content, peroxidase activity, poly-phenol oxidase activity hence, these three biochemical parameters need to be considered for early identification of resistant genotypes during population screening.

Keywords: Leaf curl virus, plant defense, biochemical basis, breeding, bhilli

Introduction

Chilli (*Capsicum annuum* L.), both green and dry, is one of the important vegetable and spice crop in India and the country stands 11th among the chilli producing nations in the world. Chilli leaf curl virus, a white fly vectored Gemini virus is a serious production constraint of chilli in India and it assumes very serious proportion with the concomitant infestation of thrips and yellow mite. Application of insecticides to control the insect vector (*Bemisia tabaci*) does not always provide good control of the disease hence, breeding chilli for resistance is more rational approach to protect the interest of chilli farmers. The present research programme was thus framed to identify the source of resistance to chilli leaf curl virus through repetitive screening of 37 diverse chilli genotypes and determine the association between different biochemical compositions of leaf and manifestation of resistance.

Materials and Methods

Three field screenings of 37 chilli genotypes in natural condition without any insecticidal cover were done during 2007-08 to 2010-11 period in randomized block design with 3 replications at the Instructional Farm of Ramkrishna Ashram Krishi Vigyan Kendra, Nimpith, South 24 Parganas, West Bengal, India situated 88o28' East longitude, 22o22' North Latitude and altitude of 3.75 m

above mean sea level. 45 day old seedlings were transplanted by 2nd fortnight of December each year with a spacing of 45cm x 45cm keeping 40 plants in each replication.

Chilli leaf curl virus disease reaction in the genotypes was ascertained based on three disease reaction parameters viz., per cent disease incidence (number of diseased plants/total number of plants x 100), per cent disease index (sum of numerical ratings/ (highest grade of ratings x total number of plants) x 100 and coefficient of infection. The coefficient of infection was calculated by multiplying the percentage disease index by the response value assigned to each severity grade. Disease reaction data was recorded during 60 – 70 days after transplanting. The percent disease severity and disease intensity grade was calculated as per standard formula of Banerjee and Kaloo (1987) where, Percent Disease Intensity (PDI) = Number of diseased plant / Total number of plants observed x 100. The coefficient of infection was calculated by multiplying the PDI with the response value assigned to each severity grade (Table 1).

Table 1: Scale of classifying disease reaction of Chilli to leaf curl complex

Symptom	Symptom severity grade	Response value	Coefficient of infestation	Reaction
Symptom absent	0	0.00	0-4	HR
Very mild curling upto 25% leaves	1	0.25	5-9	R
Curling and puckering of 26-50% leaves	2	0.50	10-19	MR
Curling and puckering of 51-75% leaves	3	0.75	20-39	MS
Severe curling and puckering of >75% leaves	4	1.00	40-69	S
			70-100	HS

Note: HR = Highly resistant; R = Resistant; MR = Moderately resistant; MS = Moderately susceptible; S = Susceptible; HS = Highly susceptible

Random samples of the disease free 2nd to 3rd leaves from top of 60 days old plant were taken for the estimation of the contents of total phenol and two enzymes, “Peroxidase” and “Polyphenol oxidase” following standard methods. Leaf phenol content was estimated according to Sadasivam and Manickam (1996) though preparation of standard curve using different concentration of catechol. Peroxidase activity of leaf was estimated according to Hammerschmidt et al. (1982) in which absorbance was measured at 470 nm wavelength at every second for 60 second and inactivated enzyme with substrate served as the blank. The activity of polyphenol oxidase of the leaf was estimated according to the method of Serradell et al. (2000) in which absorbance was monitored at 410 nm wavelength at every second for 60 second and inactivated enzyme with substrate served as the blank. The data on the enzyme activity was expressed as enzyme units /min/g fresh tissue. Statistical analysis was done using the standard package of SPSS (16.1).

Results and Discussion

Mean percent disease index and coefficient of infection (CI) for leaf curl virus disease of the 37 genotypes showed wide variation among the genotypes (Table 2). Coefficient of infection appeared to be the most reliable disease reaction parameter because it considered both disease incidence and its severity. For this reason, percent disease index of CUCH-1 (33.25) and CUCH-4 (28.76) was less variable than their coefficient of infection being 18.75 and 8.75, respectively because of high severity of disease incidence in CUCH-1. Hence, screening of the chilli genotypes for leaf curl virus should be based on the parameter which considers both disease incidence and its severity.

According to coefficient of infection, the genotypes could be categorized into six groups (Table 3). The 'Resistant' group comprised of only one genotype CUCH-4 (CI - 8.75) and 'Moderately Resistant' group had eight genotypes viz. CUCH-1, CUCH-5, CUCH-6, CUCH-7, CUCH-29, CUCH-31, CUCH-34 and CUCH-35 with CI ranging from 11.25 to 18.75. The remaining 28 genotypes fell in three susceptible category groups, with CI ranging between 20 to 100 which amply suggested the severity and wide prevalence of this disease.

Total phenol content and activity of both the enzymes in the leaves varied widely among the genotypes indicating ample scope for selection of the genotypes based on these three biochemical characters (Table 4). Average phenol content of the nine genotypes under "Resistant" and "Moderately resistant" was markedly higher (3.45 mg/100 g fresh; range: 3.19 to 4.17 mg/100 g fresh) than that of the 28 genotypes under susceptible category (2.59 mg/100 g fresh; range: 1.91 to 3.32 mg/100 g fresh).

Peroxidase enzyme activity was highest (12.50 unit/minute/g fresh tissue) in the resistant genotype CUCH-4 and lowest (1.14 unit/minute/g fresh tissue) in the highly susceptible genotype CUCH-23.Polyphenol oxidase enzyme activity was also the highest (0.18 unit/minute/g fresh tissue) in the resistant genotype CUCH-4 and lowest (0.03 unit/minute/g fresh tissue) in the highly susceptible genotype CUCH-23. Coefficient of infection was significantly and negatively correlated with phenol content, peroxidase activity, poly-phenol oxidase activity in the leaves (Table 5) suggesting that least susceptible genotypes had high phenol content and enhanced peroxidase and poly-phenol oxidase activity in the leaves. Reports of earlier workers suggested that the resistance to different diseases caused by pathogen was attributed to the presence of high amount of phenol in the leaf (Jain and Yadav, 2003; Kushawaha and Narain, 2005; Parashar and Lodha, 2007). A positive correlation between host resistance and the amount of phenols and increased activity of peroxidase and polyphenol oxidase has been recorded in chilli by Jabeen et al. (2009).

Higher total phenol content and higher activity of peroxidase and poly-phenol oxidase enzymes in the leaves of 60 days old plant emerged as the dependable biochemical determinant of resistance in the host plant for chilli leaf curl virus disease which can be used for early identification of resistant genotypes during population screening.

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Table 2. Average disease reaction of 37 Chilli genotypes to leaf curl virus

Genotype	Disease reaction parameters		Disease reaction
	Percent disease index	Coefficient of Infection	
CUCH-1	33.25	18.75	MR
CUCH-2	25.18	37.53	MS
CUCH-3	25.38	33.75	MS
CUCH-4	28.76	8.75	R
CUCH-5	25.34	17.54	MR
CUCH-6	26.25	15.63	MR
CUCH-7	34.68	18.75	MR
CUCH-8	32.5	32.55	MS
CUCH-9	26.88	28.75	MS
CUCH-10	25.55	26.25	MS
CUCH-11	49.76	30.63	MS
CUCH-12	36.82	51.25	S
CUCH-13	36.77	52.53	S
CUCH-14	35.66	48.75	S
CUCH-15	37.83	22.52	MS
CUCH-16	36.93	23.75	MS
CUCH-17	38.75	70.63	HS
CUCH-18	38.37	77.51	HS
CUCH-19	39.26	71.25	HS
CUCH-20	39.57	43.75	S
CUCH-21	45.23	81.25	HS
CUCH-22	60.15	86.25	HS
CUCH-23	55.28	93.75	HS
CUCH-24	37.88	81.25	HS
CUCH-25	41.37	77.56	HS
CUCH-26	37.52	80.63	HS
CUCH-27	45.43	83.75	HS
CUCH-28	55.19	88.75	HS
CUCH-29	55.43	18.75	MR
CUCH-30	65.19	91.25	HS
CUCH-31	27.28	16.25	MR
CUCH-32	70.26	92.57	HS
CUCH-33	50.44	46.25	S
CUCH-34	31.55	13.75	MR
CUCH-35	29.47	11.25	MR
CUCH-36	50.55	73.75	HS
CUCH-37	45.53	86.25	HS

Table 3. Grouping of Chilli genotypes as per coefficient of infection (CI) to leaf curl virus

Grouping according to CI	No. of Genotypes	Name of the genotypes
Highly Resistant (0-4)	-	-
Resistant (5-9)	1	CUCH-4
Moderately Resistant (10-19)	8	CUCH-1, CUCH-5, CUCH-6, CUCH-7, CUCH-29, CUCH-31, CUCH-34, CUCH-35
Moderately Susceptible (20-39)	8	CUCH-2, CUCH-3, CUCH-8, CUCH-9, CUCH-10, CUCH-11, CUCH-15, CUCH-16
Susceptible (40-69)	5	CUCH-12, CUCH-13, CUCH-14, CUCH-20, CUCH-33
Highly Susceptible (70-100)	15	CUCH-17, CUCH-18, CUCH-19, CUCH-21, CUCH-22, CUCH-23, CUCH-24, CUCH-25, CUCH-26, CUCH-27, CUCH-28, CUCH-30, CUCH-32, CUCH-36, CUCH-37

Table 4. Average total phenol content and activity of Peroxidase and Polyphenol oxidase (PPO) enzymes in the leaves of the chilli genotypes

Genotype	Total phenol content (mg/ 100 g fresh)	Peroxidase enzyme units /min/g fresh tissue	PPO enzyme units / min/g fresh tissue
CUCH-1	3.26	6.25	0.14
CUCH-2	1.91	4.55	0.10
CUCH-3	3.13	4.17	0.11
CUCH-4	3.19	12.50	0.18
CUCH-5	3.23	6.25	0.16
CUCH-6	3.44	7.14	0.15
CUCH-7	4.17	5.00	0.11
CUCH-8	3.02	5.00	0.11
CUCH-9	3.31	5.00	0.11
CUCH-10	2.97	5.56	0.11
CUCH-11	3.26	5.56	0.13
CUCH-12	2.91	3.85	0.09
CUCH-13	2.96	4.17	0.09
CUCH-14	2.75	3.57	0.09
CUCH-15	1.98	6.25	0.13
CUCH-16	2.47	6.25	0.14
CUCH-17	2.79	2.94	0.09
CUCH-18	1.71	2.78	0.07
CUCH-19	1.98	2.78	0.08
CUCH-20	2.89	4.17	0.06
CUCH-21	2.38	2.50	0.07
CUCH-22	1.53	2.38	0.06
CUCH-23	2.13	1.14	0.03
CUCH-24	2.32	3.33	0.08
CUCH-25	3.32	3.13	0.08
CUCH-26	3.28	3.13	0.08
CUCH-27	3.05	3.13	0.08
CUCH-28	2.53	2.17	0.04
CUCH-29	3.41	7.14	0.14
CUCH-30	2.72	1.22	0.04
CUCH-31	3.38	8.33	0.19
CUCH-32	1.43	1.39	0.02
CUCH-33	2.93	4.17	0.10
CUCH-34	3.32	10.00	0.16
CUCH-35	3.65	8.33	0.18
CUCH-36	2.13	2.94	0.08
CUCH-37	2.88	2.50	0.05

Table 5. Correlation between coefficient of infection and biochemical parameters

	Peroxidase activity	Polyphenol oxidase activity	Coefficient of infection
Phenol content	0.340**	0.375**	-0.448**
Peroxidase activity		0.897**	-0.866**
Polyphenol oxidase activity			-0.888**

** Correlation is significant at the 0.01 level

Peppers and Potyviruses, a pathosystem teaches how to breed for durable resistance in plants.

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Abstract

The combination of major resistance genes with quantitative resistance factors is hypothesized as a promising breeding strategy to preserve the durability of resistant cultivar, as recently observed in three different pathosystems. Using the pepper (*Capsicum annuum*)/*Potato virus Y* (PVY, genus *Potyvirus*) pathosystem, we aimed at identifying plant genetic factors directly affecting the frequency of virus adaptation to the major resistance gene *pvr2*^{3} and at comparing them with genetic factors affecting quantitative resistance. The resistance breakdown frequency was a highly heritable trait (h^2=0.87). Four loci including additive quantitative trait loci (QTLs) and epistatic interactions explained together 70% of the variance of *pvr2*^{3} breakdown frequency. Three of the four QTLs controlling *pvr2*^{3} breakdown frequency were also involved in quantitative resistance, strongly suggesting that QTLs controlling quantitative resistance have a pleiotropic effect on the durability of the major resistance gene. With the first mapping of QTLs directly affecting resistance durability, this study provides a rationale for sustainable resistance breeding. Surprisingly, a genetic trade-off was observed between the durability of PVY resistance controlled by *pvr2*^{3} and the spectrum of the resistance against different potyviruses. This trade-off seemed to have been resolved by the accumulation of minor-effect durability QTLs under field selection.

Keywords: resistance durability, quantitative trait locus, resistance breakdown, *Capsicum spp*., PVY, eukaryotic translation initiation factor 4E, potyviruses, resistance spectrum

Introduction

When available in genetic resources, major resistance genes are very attractive for resistance breeding in crops because of their simple inheritance that makes rapid and cheap their introgression through backcrosses to high-yielding but susceptible cultivars, and because they are expected to confer a nearly-complete resistance against the targeted pathogen. The widespread deployment of such major resistance genes in many elite cultivars imposes a strong selection pressure on the pathogen population leading to the appearance and/or the increase in frequency of pathogen variants overcoming the resistance (or resistance breaking (RB) variants) (McDonald & Linde 2002). In order to preserve the durability of cultivar resistance, and when several major resistance genes are available, pyramiding of the distinct genes into a same cultivar, alternation of resistance genes across cultivation cycles or association of cultivars with distinct resistance genes were proposed. More recently, experimental data from 3 distinct pathosystems (plant-virus/fungus/nematode) showed that the emergence of pathogen variants breaking down major resistance genes strongly decreased in the cultivars which combined the major resistance gene in a quantitatively resistant genetic background, (Palloix et al. 2009; Brun et al. 2010; Fournet et al. 2013). This suggests that combining qualitative and quantitative resistance increases the durability of plant protection.

However, none of the studies cited above formally demonstrated that the increase in durability resulted from the action of quantitative resistance factors, since the durability of the major resistance was compared between pairs of genotypes with fully different genetic backgrounds. Consequently the decrease in resistance breakdown frequency might be due to any other genes from those genetic backgrounds. Contrarily, if the partial resistance factors are responsible for the

decrease in resistance breakdown, this would provide rationale tools for breeders to enhanced durability of major resistance genes, particularly when few genes are available.

In our study, we (i) examined whether resistance breakdown is a heritable trait, (ii) described the genetic architecture underlying the genetic background which affect the breakdown frequency of a major resistance gene and (iii) discussed how breeders can exploit these results to preserve the durability of major resistance genes. Using the pepper (*Capsicum annuum*)/*Potato virus Y* (PVY) system, our strategy consisted in (i) detecting QTLs affecting the breakdown frequency of the *pvr2*^{3} major resistance allele, which is a primary component of the resistance durability and (ii) comparative mapping between these QTLs and QTLs affecting the quantitative resistance to PVY.

Materials and Methods

Mapping population and linkage map.

A segregating doubled-haploid (DH) population comprising 350 lines was obtained from the F_1 hybrid between two *Capsicum annuum* inbred lines: 'Perennial' carrying the PVY major resistance allele *pvr2*^{3} in a partially resistant genetic background and 'Yolo Wonder' carrying the PVY susceptibility allele *pvr2*$^+$. All DH lines were genotyped with a tetra-primer ARMS-PCR which targets SNP signatures differentiating *pvr2*$^+$ from *pvr2*^{3} (Rubio et al. 2008).

The 350 DH progeny was an extension of the former 'PY' mapping population (Lefebvre et al. 2002). It was genotyped with 236 molecular markers including SNPs from Nicolaï et al.(2012) and Jung et al.(2010), SSRs (Alimi et al. this issue) and AFLPs (Lefebvre et al. 2002). Two known genes were also mapped: *pvr2* and *pvr6* coding for the eIF(iso)4E (Rubio et al. 2009). The genetic linkage map was constructed using the Mapmaker software version 3.0b. The total length of the map was 2457.7 cM (Kosambi) with an average length interval between markers of 12.3 ± 12.4 cM.

Measure of resistance breakdown frequency.

The breakdown frequency of the *pvr2*^{3} allele was tested after inoculation of a PVY clone "CI chimera" to the 153 DH lines carrying *pvr2*^{3}. This PVY clone is not virulent toward *pvr2*^{3} but has to generate a mutation in its VPg cistron to breakdown the resistance and infect *pvr2*^{3} carrying peppers (Montarry et al. 2011). Inoculum was obtained as in Ayme et al. 2006. Thirty pepper seedlings per DH line grown in a climate-controlled room at 20-22 °C, 12h light /day, with two expanded cotyledons were inoculated mechanically on their cotyledons. Thirty-eight days post-inoculation (dpi), plants were submitted to virus detection by DAS-ELISA. In these conditions, in each plant infected systemically, the virus population was shown to be composed of one or several VPg mutants carrying a *pvr2*^{3} RB mutation (Ayme et al. 2006). For each pepper line, the RB frequency of *pvr2*^{3} was assessed by the ratio of the number of systemically infected plants over the total number of inoculated plants. Two independent tests of 30 plants per DH line were performed.

Measure of quantitative resistance.

To evaluate the level of the quantitative resistance due to the genetic background, we used the "CI chimera VPg-N" PVY mutant which overcomes the resistance conferred by *pvr2*^{3} (infects all the plants carrying *pvr2*^{3}) and reveals the quantitative resistance due to the genetic background. Two different traits were assessed: the area under the disease progress curve (AUDPC) and the virus accumulation. The PVY "CI chimera VPg-N", which breaks down the *pvr2*^{3} resistance, was mechanically inoculated on 20 plants of each DH lines carrying *pvr2*^{3}. The plants were grown in a climate-controlled room as previously described. The AUDPC assessment combined both intensity of symptoms and latency period was calculated as in Palloix et al. 2009. At 36 dpi, the virus accumulation was independently evaluated on 10 individual plants per DH line by semi-quantitative DAS-ELISA, using a dilution range of extracts of PVY infected plants, and expressed relatively to a common reference sample incorporated in each ELISA plate, as in Ayme et al. (2006).

Statistical and QTL analyses.

Narrow-sense heritabilities (h^2) were estimated using the formula $h^2=\sigma^2_G/(\sigma^2_G+\sigma^2_E/n)$ (σ^2_G genotypic variance, σ^2_E phenotypic variance and n number of replicates). Because of the non-normal distribution of the RB trait, QTL detection was performed with a regression-based interval mapping approach, with threshold values calculated by permutations. QTL analyses used iterative composite interval mapping (iQTLm) methods implemented in the MCQTL v.5.2.4 software (Jourjon et al. 2005). The QTL confidence intervals (CI) were defined using a MCQTL test unit fall of 2. QTLs for AUDPC and virus accumulation (log (VA) +1) were also detected using multiple QTL mapping (MQM) methods implemented in the R/qtl package.

Results

Characterization of the phenotypic traits.

To evaluate the frequency of *pvr2*^{3} resistance breakdown, the PVY clone "CI chimera", which is not infectious *per se* toward *pvr2*^{3} resistant plants, was inoculated on the set of 153 DH carrying *pvr2*^{3}. In these plants, only new mutants of the CI chimera possessing non-synonymous substitutions in the VPg factor conferring virulence towards the *pvr2*^{3} allele were detected (Ayme et al. 2006; Palloix et al. 2009; Montarry et al. 2011.). In our tests, mosaic symptoms appeared in 100% of the susceptible (*pvr2*$^+$) control Yolo Wonder 2 weeks after inoculation, but mosaic or necrotic symptoms appeared 4 to 5 weeks after inoculation in some of the plants of some DH lines. Thirty-eight days after inoculation, the ELISA tests showed that RB frequencies varied from 0% to 93.2% between DH lines, with a mean of 14.7% (± 23.8). The correlation between the two independent tests was high ($\rho_{pearson}=0.77$; $p<0.0001$), and the heritability was estimated at 0.87. The distribution of the trait was strongly skewed towards low values, with 70 DH lines displaying no resistance breakdown and 83 HD lines displaying a RB frequency between 1.7% and 93.2%.

The quantitative resistance was assessed using a *pvr2*^{3}-breaking mutant of the PVY clone, the "CI Chimera VPg-N". This PVY clone differs from the "CI Chimera" by a single nucleotide substitution in the VPg cistron conferring the capacity to infect *pvr2*^{3} resistant plants. With this virus, the *pvr2*^{3} resistant lines displayed symptoms as soon as 14 dpi and 100% of the plants were infected at 35 dpi. Highly significant variations for virus accumulation (VA) and AUDPC were observed between DH lines with a heritability of 0.64 and 0.98, respectively. For statistical and QTL analyses, VA values were log transformed [ln(VA+1)] to approximate a normal distribution. The ln(VA+1) varied from 0 to 1.43 with a mean equal to 0.67±0.35 and AUDPC varied from 29.75 to 63 with a mean equal to 46.6±7.9.

Significant correlations ($p<0.05$) were observed between the three traits with a Pearson's coefficients equal to 0.40, 0.33 and 0.32 for RB/VA, RB/AUDPC and VA/AUDPC, respectively.

Mapping QTLs for the quantitative resistance traits.

QTL analyses of the quantitative resistance traits AUDPC and log(VA+1) were performed using iQTLm and MQM methods. Two significant QTLs for VA and three for AUDPC were detected by both methods. These QTLs were named VA-3, VA-6, A-1, A-3 and A-9, according to the trait considered and the chromosome location. The position, significance and effect of each QTL are detailed in table 1 and figure 1. VA-3 and VA-6 explained 34.5% and 15.7% of the VA variation, respectively. Epistasis tests did not reveal any interaction between QTLs and the genetic background. The final model combining the effects of the two significant QTLs explained 49.2% of VA phenotypic variation corresponding to 76% of the trait heritability ($h^2=0.64$). A-1, A-3 and A-6 explained from 14.6% to 16% of the AUDPC variation. Epistasis tests did not reveal any interaction between these QTLs and the genetic background. The part of the AUDPC phenotypic variation explained by A-1, A-3 and A-9 was equal to 33.8% corresponding to 34% of the trait heritability ($h^2=0.98$).

Mapping QTLs for the frequency of pvr2^3-resistance breakdown (RB).

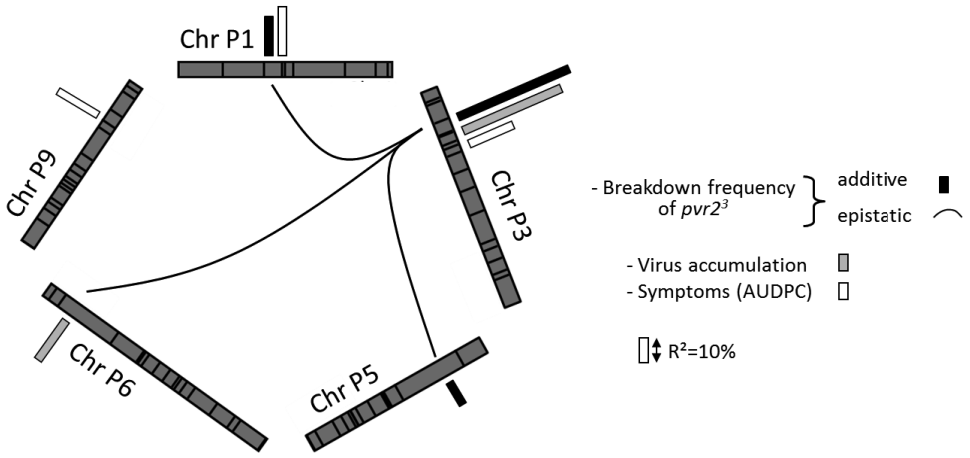
Due to the skewed distribution of the RB frequency, QTL detection was performed with the standard regression-based interval mapping approach implemented in MCQTL software (Jourjon et al. 2005). Three significant QTLs, explaining 12.8%, 39.8% and 8.6% of the *pvr2*^{3} RB frequency variation, were detected on chromosomes 1, 3 and 5, respectively. QTLs were named RB-1, RB-3 and RB-5 according to the trait and the chromosome location. Table 1 and figure 1 detail position, significance and effect of each QTL. Epistasis tests revealed that RB-3 interacts with three distinct positions in the pepper genome, on chromosomes 1, 5 and 6. The positions on chromosomes 1 and 5 interacting with RB-3 are included in the confidence intervals of RB-1 and RB-5, respectively, suggesting interaction effects between RB-1 and RB-3 and between RB-3 and RB-5 (figure 1). The final model combining additive and epistasic effects of the significant QTLs explained 68.9% of the variation of *pvr2*^{3} RB frequency, which corresponds to a great part (79%) of the trait heritability (h^2=0.87). The major QTL affecting the RB frequency of *pvr2*^{3} (RB-3) co-localizes with the major QTLs VA-3 and A-3 affecting VA and AUDPC, respectively. The QTL RB-1 co-localizes with QTL A-1. The position on chromosome 6 that interacts with RB-3 was involved in VA (figure 1).

Table 1: QTLs detected with the iterative QTL mapping (iQTLm) method.

Trait (heritability)	QTL	Location (chromosome@position)	Closest marker	Allele decreasing trait value	MCQTL Test[a]	Variation explained	
						locus	trait
	QTLs with additive effect :						
Resistance breakdown (0.87)	**Rb-1**	**1@87**	Gpms_178	**Per**	**5.2**	**12.8**	**68.9**
	Rb-3	**3@43.1**	Pvr6	**YW**	**17.4**	**39.8**	
	Rb-5	**5@57.1**	TG437	**Per**	**3.6**	**8.6**	
	QTLs with epistatic effect :						
	eRb-1-3	**1@87 – 3@43.1**	Gpms_178/ Pvr6		**7.4**	**18.9**	
	eRb-3-5	**3@43.1 – 5@57.1**	Pvr6 /TG437		**6.6**	**16.9**	
	eRb-3-6	**3@43.1 – 6@188.3**	Pvr6 / SNP11391		**4.2**	**10.6**	
Virus accumulation (0.64)	***QTLs with additive effect :***						
	Va-3	**3@49.1**	SNP23714	**YW**	**14.6**	**34.5**	**43.9**
	Va-6	**6@190.3**	SNP11391	**Per**	**6.3**	**15.7**	
	QTLs with additive effect :						
AUDPC (0.98)	**A-1**	**1@87**	Gpms_178	**Per**	**6.3**	**16.1**	**34.1**
	A-3	**3@40.8**	SNPISO_2	**YW**	**6.2**	**15.5**	
	A-9	**9@132**	SSCP_MP5	**YW**	**5.9**	**14.9**	

[a] The MCQTL test significance thresholds at P=0.05 are egal to 3.5 (RB), 3.4 (VA) and 3.5 (AUDPC)

Figure 1: Co-location between QTLs affecting resistance Breakdown, virus accumulation and AUDPC.



Effect of parental alleles on the resistance breakdown (RB) frequency.

All genotypes carrying the Yolo Wonder allele at QTL RB-3 showed a low resistance breakdown frequency with a mean equal to 0.31%, whatever the alleles at the other QTLs (table 2). Contrarily, the Perennial allele at QTL RB-3 increased the resistance breakdown frequency (mean 29%), but this increase depended on the alleles at the other QTLs. This explains the interaction (epistatic) effects between RB-3 and the 3 other QTLs observed in the QTL analysis: when combined to the Perennial allele at RB-3, the Perennial alleles at the 3 QTLs interacting with RB-3 (RB-1, RB-5 and RB-6) strongly decreased the RB frequencies (mean 5.3%), whereas the Yolo Wonder alleles at these 3 QTLs strongly increased the RB frequencies (mean 69.7%).

Table 2: Effect of QTLs interaction on the resistance breakdown frequency of *pvr2³*: average resistance breakdown (RB) frequency (in %) of the *pvr2³* for each allelic combination. Parentheses: number of lines in each allelic class.

		Rb-1		**Rb-5**		**Rb-6**		**combination 3 QTLs (Rb-1, 5, 6)**	
		YW	**Per**	**YW**	**Per**	**YW**	**Per**	**YW**	**Per**
Rb-3	**YW**	**0.37(34)**	**0.30(39)**	**0.17(39)**	**0.47(33)**	**0.18(37)**	**0.36(39)**	**0.18(9)**	**0.36(7)**
	Per	**42.0(37)**	**15.6(34)**	**41.8(32)**	**18.0(31)**	**36.2(40)**	**22.6(30)**	**69.7(7)**	**5.3(6)**

Discussion

The breakdown frequency (or durability) of a major resistance gene is a heritable trait

In this study, we measured the resistance breakdown (RB) frequency of a major resistance gene (*pvr2³*) in a set of pepper DH lines carrying *pvr2³* but segregating for the genetic background. The observed RB frequency directly resulted from the frequency of appearance and from the accumulation dynamics of RB variants in the resistant plants, two steps of the virus evolution toward RB that are considered as major components of the resistance durability (Fabre et al. 2009). Previous studies of this pathosystem showed that the RB mutation most probably occur in the resistant pepper host which allowed for weak PVY multiplication and that the genetic background acts at several levels, including the mutational pathways of the virus to overcome resistance and the speed of selection of the RB variants (Montarry et al. 2011, Quenouille et al. 2013). The variation

between DH lines for the RB frequency of *pvr2*^{3} (from 0% to 93.2%) and its high heritability (h^2=0.87) confirm the genetic control of this trait. This was not trivial since the RB processes involves factors with stochastic natures like the appearance of RB mutations in PVY genome, the genetic drift acting on virus during systemic invasion of plants and the fact that VPg mutations with contrasted effects on PVY competitiveness can lead to the *pvr2*^{3} breakdown (Ayme et al. 2006; Montarry et al. 2011). These may increase the heterogeneity of results between plants and experiments, decreasing the heritability of RB frequency. Measuring the resistance breakdown frequency with a reasonable number of plants (60 per genotype) compensated these factors of heterogeneity. The genetic background dependency of *pvr2*^{3} durability could be attributed to 4 distinct genetic factors with quantitative effects: one major-QTL (RB-3) and three additional QTLs (RB-1, RB-5 and RB-6) acting additively and/or in interaction with RB-3. This further indicate that direct selection for alleles increasing the durability of a major resistance is feasible but raises two questions : is the resistance breakdown frequency in experimental conditions representative of durability in fields ? and is it feasible to generate RB variants in all pathosystems ? Considering the first question, the RB frequency of different major resistance genes estimated by experimental evolution proved highly correlated with the durability of these genes in the field (Ayme et al. 2006; Janzac et al. 2009; Lacroix et al. 2011, Moury et Verdin, 2012). Moreover, the distributions of RB mutations in viral genomes are usually similar in field and laboratory observations (Hajimorad et al. 2010). Considering the second question, generating RB variants by direct inoculation may be difficult for resistance genes which proved very durable in the field. In such cases, alternative protocols imposing strong inoculum pressures like graft-inoculation or inoculation through agroinfiltration, were shown to increase the RB frequency (Bruun-Rasmussen et al. 2007; Janzac et al. 2009). This makes feasible to measure the breakdown frequencies of major resistance genes in most plant-virus pathosystems and provide powerful tools for the genetic improvement of the durability of virus resistance in plants. Considering other pathogens, experimental evolution may not be as much relevant, due to their longer generation time, smaller population size and smaller mutation rate. However, the relationship between RB QTLs and QTLs controlling the level of quantitative resistance may provide alternative selection criteria.

Increasing the resistance durability through breeding for quantitative resistance?

In the same pepper DH progeny, the QTL analysis of quantitative resistance was performed using a PVY variant which overcome the *pvr2*^{3} resistance and reveal the quantitative resistance conferred by the genetic background. This variant differs from the previous PVY by one single nucleotide substitution which was shown to restore the interaction between the PVY VPg and the pepper eukaryotic translation initiation factor 4E1 (eIF4E1) encoded by *pvr2*^{3}, causing the breakdown of the *pvr2*^{3}-mediated resistance (Charron et al. 2008). Five QTLs affecting virus accumulation and/or symptom expression were detected. Three of the four QTLs affecting the RB frequency of *pvr2*^{3} colocate with QTLs affecting quantitative resistance. These colocations may result from genetic linkage between the different traits or from a pleiotropic effect of quantitative resistance factors on the RB frequency of *pvr2*^{3}. Looking at the effect of parental alleles that colocate on chromosomes P1, P3 and P6, all the alleles that increased the quantitative resistance also decreased the breakdown frequency. Moreover, the QTL on chromosome P3 displayed the major effect on both virus accumulation and RB frequency. This concordance between parental allele effects for the different traits strongly suggests that colocations are due to pleiotropic effects and that alleles increasing the resistance level also increase the resistance durability. This is also consistent with the initial observations, in 3 pathosystems, that the durability of major genes is enhanced in partially resistant cultivars (Palloix et al. 2009; Brun et al. 2010; Fournet et al. 2012). Dissecting the genetic background into QTLs indicate that breeding for quantitative resistance alleles will enhance the durability of major genes. Such a strategy is achievable for most pathosystems where breaking down variants or experimental evolution tests are not available.

A genetic trade-off between broad-spectrum resistance and resistance durability that can be solved by breeding strategy.

Among the 4 additive and/or epistatic QTLs affecting the RB frequency, the alleles from Perennial were shown to decrease the risk of *pvr2*^{3} breakdown at 3 of these QTLs (RB-1, RB-5 and RB-6), whereas the Perennial allele increased strongly this risk for the major QTL RB-3. This result looks surprising since the *pvr2*^{3} allele was previously shown to be highly durable in the Perennial landrace (Palloix et al. 2009). It is noteworthy that the peak of QTL RB-3 was localized at the *pvr6* locus which encodes the eIF(iso)4E, an isoform of eIF4E1 encoded by *pvr2*. It was previously shown that the Perennial allele at *pvr6* included a deletion of 82 nucleotides followed by a premature stop codon resulting in a non-functional *pvr6* allele (or a natural knock-out allele of *pvr6*$^{+}$) (Ruffel et al. 2006). When combined with different *pvr2* alleles, the Perennial *pvr6* allele was shown to enlarge the spectrum of resistance to additional potyviruses, including *Chilli veinal mottle virus* (ChiVMV) & *Pepper veinal mottle virus* (PepVMV) (Moury et al. 2005; Rubio et al. 2009). Hence, the Perennial allele at the *pvr6*/RB-3 locus contributes to a gain of resistance against PepVMV and ChiVMV but also to a decrease of the durability of the *pvr2*^{3} resistance to PVY. Whether *pvr6* is the functional gene for the RB-3 QTL remains to be determinated through functional validation. However, the tight colocation between *pvr6* and RB-3 already reveals a trade-off between a large resistance spectrum against potyviruses and the durability of the *pvr2*^{3} PVY resistance in the Perennial genotype. Such a trade-off looks unusual since resistance gene pyramiding and broad-spectrum resistance is often considered favourable to durability (McDonald & Linde, 2002; Kou & Wang, 2010). In pepper, the combination of *pvr2* resistance alleles with *pvr6* is already used in breeding programs to create resistant varieties against a large range of potyviruses (Rubio et al. 2009) and such cultivars may lead to a premature breakdown of *pvr2*-mediated resistance by PVY. Translation initiation factor-mediated virus resistance is widespread and combination between mutated or knock-out alleles at different genes from this family is expected to provide a way to breed for large spectrum resistance (Mazier et al. 2011). Our study indicates that the combination of mutant alleles from genes belonging to the same multigenic family have to be used carefully, since the trade-off observed in pepper between large-spectrum and durability of resistance, may also occur in other plants species. Our previous observations already showed that introgressing resistance alleles from the plant germplasm into new elite cultivars with susceptible genetic backgrounds can endanger the long-term use of these genes as well as provide an evolutionary springboard to the pathogen for resistance breakdown (Palloix et al. 2009). Investigating the background-dependence of the durability of *pvr2*^{3} improves our understanding of how selection acted on this gene/genetic background combination. In field selection of plant populations by farmers, only the seeds from the healthiest and most productive individuals participate to the next cultivated generation. In North-West India where Perennial originated, plants combining *pvr2*^{3} and *pvr6* certainly gain a selective advantage in presence of ChiVMV. However, the rapid adaptation of PVY or other potyviruses would have counterselected this allelic combination in a few cultivation cycles. Over a few plant generations, and in heterogeneous plant populations, this multi-pathogen selection pressure promoted the recombinant individuals carrying favorable alleles at additional QTLs (RB-1, RB-5 and RB-6) which, in interaction with RB-3, decreased the risk of *pvr2*^{3} resistance breakdown. Such a polygenic combination of co-adapted alleles contributed to increase resistance in efficiency, spectrum and durability.

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Ménage à trois: the intimate relationship between phenolics content, polyphenol oxidase activity, and flesh browning in eggplant

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Abstract

Development of cultivars of fruits and vegetables with improved content in bioactive phenolics may result in increased levels of fruit flesh browning. Eggplant (*Solanum melongena*) fruits present a high content in phenolics; in particular, the flesh of the fruit contains considerable amounts of chlorogenic acid (CGA). Eggplant phenolics present many properties of interest for human health, and genetic improvement of eggplant for higher content in phenolics is an important objective in modern breeding programs. However, a drawback of increasing the content in phenolics in eggplant fruit is that when the fruit is cut or chopped for being processed or cooked, the phenolic compounds are oxidized by polyphenol oxidases (PPO) giving raise to brown colored compounds that cause the fruit flesh browning. Therefore, it is important to study the diversity and relationships among content in phenolics, PPO activity, and fruit flesh browning, as these results will have an impact on the breeding strategies to be followed to develop eggplant cultivars with the desired ideotype (improved phenolics content and low or moderate browning). We evaluated the total phenolics (TP) content, CGA content, PPO activity, 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging activity, liquid extract browning (LEB) from lyophilized tissue, and fruit flesh browning (FFB) in 18 varieties of eggplant. Our results revealed that a considerable variation exists in the materials studied for these traits. Differences of up to 2.8-fold and 2.5-fold were found for TP and CGA respectively, although the greatest value was found for FFB (differences of up to 3.4-fold). These wide ranges of variation indicate that the materials studied are amenable to selection. The study of the correlations among traits in all the samples revealed a positive correlation among the three functional activity traits (TP, CGA, and DPPH), as well as a non-significant correlation between the two browning traits (LEB and FFB). FFB presented a moderate correlation (r=0.43) with TP and a low correlation (r=0.25) with CGA. Amazingly, the correlation between FFB and PPO activity was not significant. Overall, the results show that reaching the objective of developing new eggplant varieties with high content in bioactive phenolics, like CGA, and low FFB is a feasible objective.

Keywords: antioxidants, browning, chlorogenic acid, polyphenol oxidase, *S. melongena*.

Introduction

Enzymatic browning of the fruit flesh after a fruit has been cut or chopped is the result of the oxidation of phenolic compounds mediated by polyphenol oxidase (PPO) enzymes (Mayer, 2006; Toivonen and Brummell, 2008). The quinones resulting from the oxidation of phenolics react non-enzymatically with oxygen and other compounds present in the cells and give brown-coloured compounds. Browning is especially relevant in those fruits and vegetables which have white or clear-colored flesh or tissues (e.g., apple, pear, apricot, peach, artichoke, eggplant, potato, carrot, lettuce, etc.). Browning reduces the apparent quality and, therefore, is an important objective in breeding programs.

During the last years there has been an increasing interest in the development of new fruits and vegetables with bioactive properties (Kris-Etherton et al. 2002). In particular, the development of new cultivars with increased content in phenolics is drawing a lot of attention. This is because phenolic compounds, which are powerful antioxidants, have been reported to prevent a number of chronic and degenerative diseases (Scalbert et al. 2005). However, a drawback of increasing the content in phenolics in fruits and vegetables may be an enhanced tissue browning. Therefore, in order to develop successful varieties (i.e., with increased phenolics and reduced browning), breeding strategies aimed at reducing browning are necessary.

Eggplant (*Solanum melongena* L.) is a vegetable whose fruits contain high concentrations of phenolics (Stommel and Whitaker, 2003; Hanson et al. 2006; Prohens et al. 2007; Mennella et al. 2012), which confer this fruit many beneficial properties for human health (Kwon et al. 2008; Akanitapichat et al. 2010; Das et al. 2011; Plazas et al. 2013). The main phenolics in the flesh of the eggplant fruit are hydroxycinnamic acids, mostly chlorogenic acid (CGA) (Stommel and Whitaker, 2003; Luthria et al. 2010; Mennella et al. 2012). Eggplant PPOs quickly oxidize CGA and other phenolics in the presence of air (Todaro et al. 2011). In consequence, because of its white flesh, high contents and CGA, and intense PPO activity, the eggplant fruit is highly susceptible to browning (Barbagallo et al. 2012; Mishra et al. 2012).

Because of their importance for functional activity and browning, the study of the diversity and trilateral relationships between phenolics content, PPO activity, and browning is of interest. This information will be relevant for the development of new eggplant cultivars presenting the desired ideotype (high content in phenolics and low browning), which will result in new cultivars with high added value. Several works have been devoted to studying the diversity of one or two of the components of this trilateral relationship (Doğan et al. 2002; Stommel and Whitaker, 2003; Hanson et al. 2006; Prohens et al. 2007; Raigón et al. 2008; Mennella et al. 2010, 2012; Prohens et al. 2013). These works have shown that there is a high diversity for these traits within the eggplant germplasm. The works of Prohens et al. (2007, 2013) also revealed that total phenolics and CGA content accounted for a low percentage of the variation in fruit flesh browning in a collection of germplasm and in an interspecific family between cultivated eggplant and its wild ancestor *S. incanum* L., respectively. Only recently, a paper has been published (Mishra et al. 2013) in which the relationships between phenolics content, PPO activity, and browning in eight accessions of eggplant from India (Oriental type) have been studied during fruit storage. These authors found that when these eight eggplant cultivars are compared at the same date, total phenolics and PPO activity present a low or moderate correlation with browning.

In order to shed light on this important trilateral relationship for obtaining eggplant cultivars with improved functional and apparent quality, we undertook a study on the diversity and relationships between traits related to content in phenolics, PPO activity, and browning in a collection of eggplants from Spain (Occidental type). The results of this study will be of interest for understanding the relationships between these traits as well as to devise strategies for eggplant breeding.

Materials and Methods

This study involved 18 eggplant accessions from the germplasm bank of the Universitat Politècnica de València (Valencia, Spain). These accessions were collected in the region of Valencia, situated in the Mediterranean coast of Spain. Accessions were cultivated under open field conditions at the Agricultural Experimental Farm of Carcaixent (Valencia, Spain). Five samples per accession, each of which consisted of three commercially ripe fruits, were obtained. Fruits were cut transversally for the measurement of fruit flesh browning. Subsequently, a longitudinal section was taken, peeled, and immediately frozen in liquid N_2 and stored at -80 ºC until lyophilized.

Six traits were analyzed in the lyophilized samples: total phenolics (TP) content, chlorogenic acid (CGA) content, 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity (DPPH), polyphenol oxidase (PPO) activity, liquid extract browning (LEB), and fruit flesh browning (FFB). TP content was measured spectrophotometrically according to the Folin-Ciocalteu procedure (Singleton and Rossi, 1965) by measuring absorbance at 725 nm according to the protocol indicated in Prohens et al. (2007) and expressed as g/kg. CGA content was measured by high performance liquid chromatography (HPLC) basically according to Bellés et al. (2008) and Naranjo et al. (2007); measurement of CGA was made at a wavelength of 320 nm and results were expressed as g/kg. DPPH scavenging activity was evaluated according to Falchi et al. (2006), and consisted in measuring by spectrophotometry (517 nm) the ability to quench the stable radical $DPPH^{\cdot}$ after 10 min. DPPH scavenging activity was expressed in percentage and calculated as $S=100-[(A_x/A_o)\times100]$, where A_x is the optical density of $DPPH^{\cdot}$ solution in presence of eggplant extract and A_o the optical density of $DPPH^{\cdot}$ solution in absence of the sample. PPO activity was measured following the methodology described in Bellés et al. (2006). The PPO enzymatic reaction was followed spectrophotometrically at 420 nm during the first 1.5 min of the reaction and expressed as units/mg. One unit of PPO activity was defined as the increase in 0.1 absorbance units/min. LEB was determined spectrophotometrically at 420 nm (Sapers and Douglas, 1987) after incubating for 10 min a sample of lyophilized tissue homogenized with water and comparing with a control homogenized with a solution of metaphosphoric acid, which inhibits PPO activity. One unit of extract browning was defined as a difference in 0.01 absorbance units between the sample and the control. FFB was determined using a chromameter according to Prohens et al. (2007) by comparing the degree of whiteness ($DW=[(100-L^{*})^2+a^{*2}+b^{*2}]^{0.5}$) of the central part of a transversal section of the fruit at 0 and 10 min.

For the six traits measured, means and standard errors were obtained for each accession. Pearson pair-wise correlations among traits were obtained. All statistics were conducted using specific software (Statgraphics Centurion XVI, StatPoint Technologies, Warrenton, VA, USA).

Results and Discussion

The results obtained confirmed that eggplant contains high amount of phenolic compounds and of CGA (Stommel and Whitaker, 2003; Mennella et al. 2010, Plazas et al. 2013). In this respect, average values in the lyophilized tissue for TP content and CGA was of 16.9 g/kg and 3.6 g/kg, respectively. Although the collection studied comes from a geographically limited region, important differences, of up to 3.4-fold in the case of FFB, were observed for the traits studied in the eggplant collection evaluated (Figure 1). This is an additional indication of the high diversity that can be found in this region, which forms part of a secondary center of diversity (Prohens et al. 2005; Hurtado et al. 2012), and also an indication that these materials are amenable to selection in order to develop materials with improved characteristics.

The content in TP and CGA presented differences of 2.8-fold and 2.5-fold, respectively. For TP we found a continuous range of variation, with two accessions with contents below 10 g/kg and six accessions with values above 20 g/kg (Figure 1). For CGA, accession V17 (with a content of 6.3 g/kg) presented values much higher than the others. The V17 accession also ranked second for TP content, with a value of 22.0 g/kg (Figure 1). This indicates that this accession could be an interesting source of variation in breeding programs for increasing the content in phenolics in eggplant. Accession V17 presents the advantage over other sources of variation for breeding eggplant for high content in TP and CGA, like materials of pickling eggplants (Prohens et al. 2007) and the wild relative *Solanum incanum* (Stommel and Whitaker, 2003), that this is an accession for regular use (cooking and frying). Therefore, very likely, a smaller number of generations will be required to introgress this trait into commercial varieties from a regular eggplant variety than from a pickling eggplant or a wild species.

We have found that DPPH scavenging activity ranged between 27.5% and 50.3%. In this case, the two accessions that rank first for DPPH scavenging activity (V9 and V17) are also the ones with highest CGA (Figure 1). This is a confirmation that CGA has a main role in the antioxidant activity of eggplant (Plazas et al. 2013). PPO activity was also very variable in the collection, with differences of up to 2.9-fold. Other authors have found differences among cultivars in the PPO activity (Doğan et al. 2002, Mennella et al. 2012; Mishra et al. 2013). These latter authors (Mishra et al. 2013) could associate polymorphisms in the DNA sequence of a PPO gene with differences in the eggplant fruit flesh PPO activity. This suggests that functional markers could be developed for selection of genetic variants with lower PPO activity.

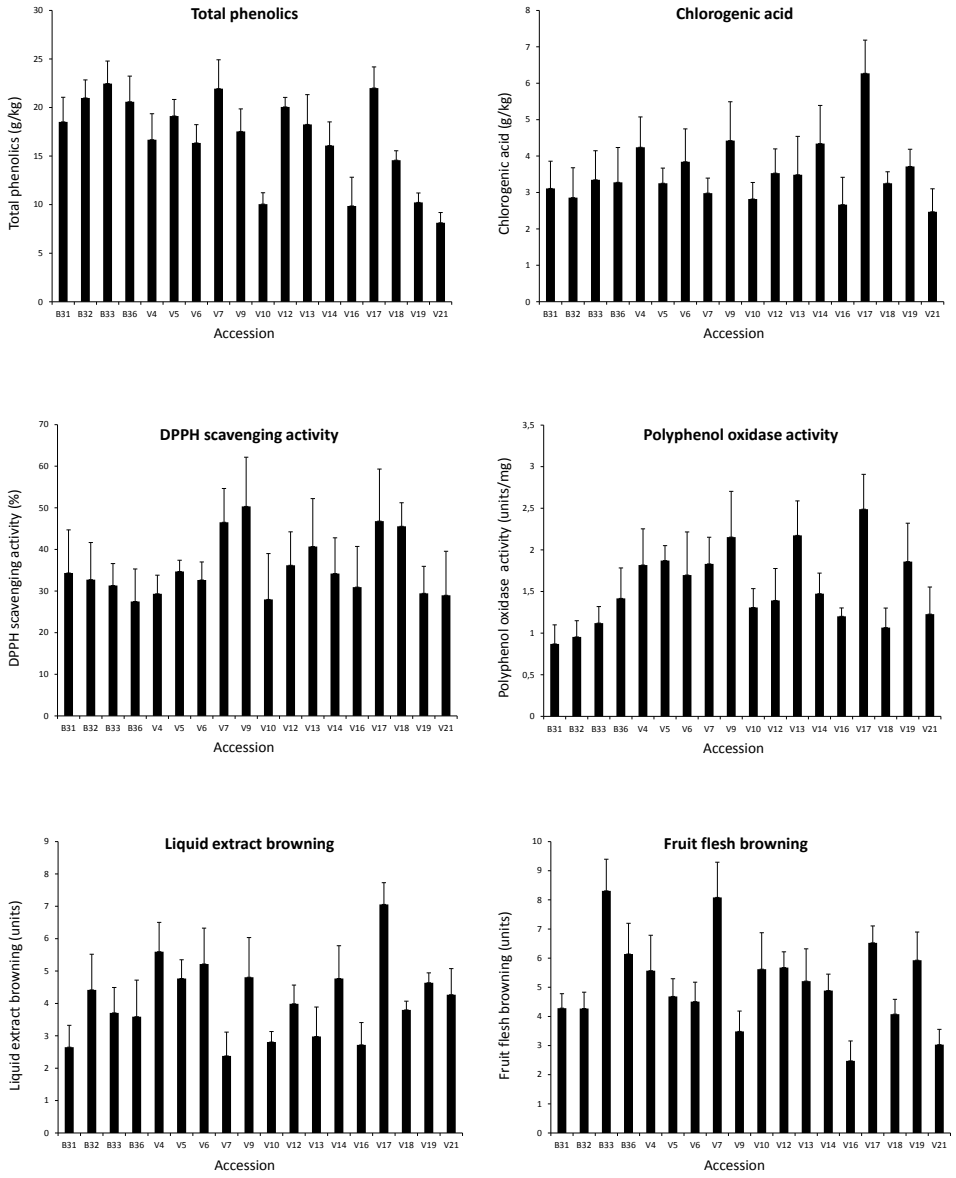

Figure 1. Mean values for the fruit traits measured in a collection of 18 accessions of eggplant. Bars represent standard error.

Both LEB and FFB browning traits were also were variable, with differences of up to 3.0-fold for LEB and of up to 3.4-fold for FFB (Figure 1). For both traits, a continuous range of variation was found, although accession V17 presented a remarkably high value for LEB and accessions B33 and V7 also had considerably high values for FFB when compared with the rest of varieties. High or low values of one of these browning traits were not associated with high or low values for the other trait.

All the correlations studied were positive. Also, with the exceptions of the correlations between TP and PPO, DPPH and FFB, PPO and FFB, and LEB and FFB, all the correlations were significant ($P<0.05$). Traits related to functional activity are positively correlated, with r values ranging from r=0.46 between TP and DPPH to r=0.63 between TP and CGA (Figure 2). These positive correlations were expected, as CGA forms part of TP, and eggplant phenolics, in particular CGA, are powerful antioxidants (Plazas et al. 2013). Amazingly, the two browning traits (LEB and FFB) were not significantly correlated. This may suggest that different factors affect browning in the extract obtained from lyophilized tissue and the browning measured directly in the fruit flesh. This is important, as it indicates that LEB may not be a good method to estimate FFB, which is the commercially important browning trait in eggplant (Mishra et al. 2012).

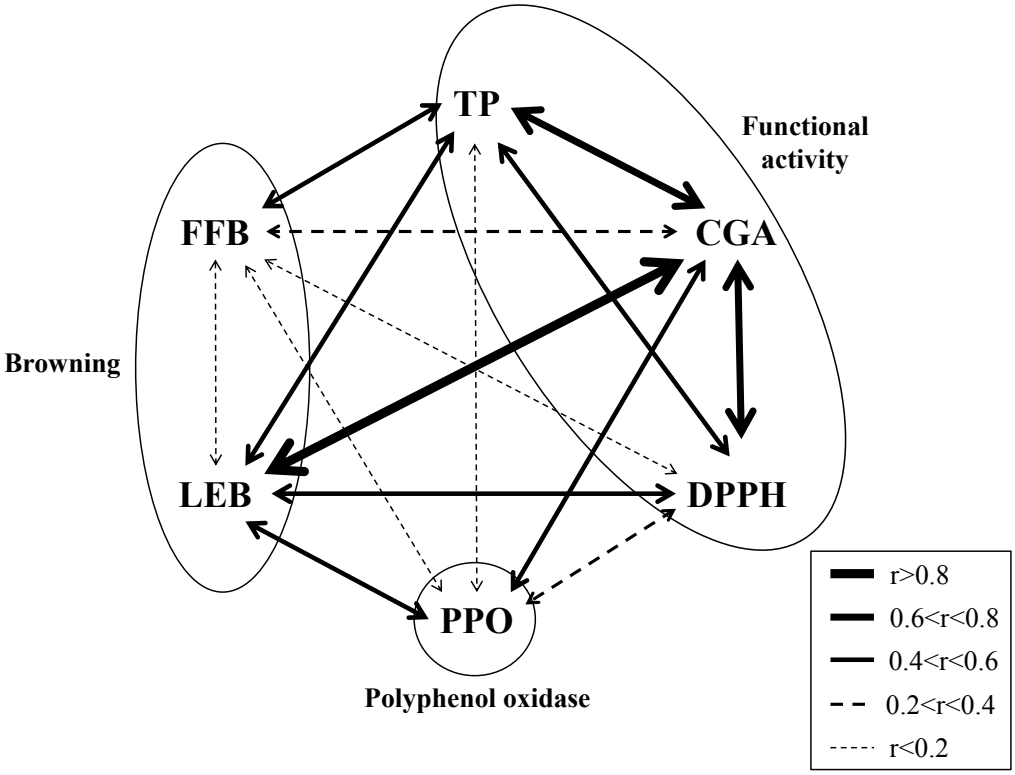

Figure 2. Schematic representation of Pearson correlations between the traits studied (TP=total phenolics, CGA=chlorogenic acid, DPPH=2,2-diphenyl-1-picrylhydrazyl scavenging activity, PPO=polyphenol oxidase activity, LEB=liquid extract browning, FFB=fruit flesh browning) in a collection of 18 accessions, with five samples per accession (n=90). All correlations were positive and the type of line indicates the value of the correlation. All correlations were significant ($P<0.05$) with the exception of those included in the category of $r<0.2$. Traits are grouped according to their association to functional activity, polyphenol oxidase activity, and browning.

Functional activity traits CGA and DPPH presented moderate (CGA) and low (DPPH) correlations with PPO activity (Figure 2). In this respect, in other crops it has been demonstrated that increases in CGA may activate expression of PPO genes (Mayer, 2006; López-Gresa et al. 2011), which may be the underlying cause of these correlations. When considering the relationships between the functional activity and browning traits, we found that CGA presented a very high correlation with LEB (r=0.85), while TP and DPPH also presented significant, although with lower values than CGA, for the correlation with LEB (r=0.51 and r=0.45, respectively). Eggplant PPOs present a great affinity for CGA (Todaro et al. 2011), which may explain this very high correlation value. Also, the results obtained for this correlation suggest that LEB could be used as a rough estimate of CGA content for preliminary screening of large eggplant populations. However, we found that correlations of functional traits with FFB were much lower in all cases, in particular the correlations with CGA (r=0.25) and DPPH (r=0.13; non-significant) (Figure 2). This suggests that it may be possible to select materials with high TP and CGA contents with moderate or low FFB. The correlations of PPO activity with the two browning traits were moderate in the case of LEB (r=0.46) and low in the case of FFB (r=0.18; non-significant). This is also important for breeding eggplants with low browning, as it suggests that PPO activity, at the levels found in the collection studied, does not play a main role in accounting for differences between cultivars in FFB.

Overall, the results obtained indicate that the collection studied presents a considerable diversity for the functional activity, PPO activity, and browning traits studied. Results also present evidence that it may be feasible to develop new eggplant cultivars with the desired ideotype, consisting of improved contents in TP and CGA with low or moderate FFB (Plazas et al. 2013). Further investigation is needed to elucidate what factors, in addition to TP, CGA, and PPO activity, are implicated in FFB in the eggplant fruit.

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Suppression of both phytophthora root rot and foliar blight resistance by a novel *Capsicum* gene.

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Abstract

A novel disease resistance inhibitor gene (*Ipcr*), found in *Capsicum annuum* accession, New Mexico Capsicum Accession 10399 (NMCA10399), suppresses resistance to *Phytophthora capsici*, but not to other species of *Phytophthora*. When resistant accessions were hybridized to NMCA10399, the resultant F_1 populations were completely susceptible. The F_2 population displayed a 3:13 resistant:susceptible (R:S) ratio. The testcross population displayed a 1:1(R:S) ratio, and a backcross population to NMCA10399 displayed complete susceptibility. These results demonstrate the presence of a single dominant gene suppressing the expression of *P. capsici* resistance in *C. annuum*. In addition, the *Ipcr* gene was tested for nonhost resistance, and the nonhost resistance was not overcome. Therefore, the *Ipcr* gene is repressing the expression of host specific resistance, but not the expression of the nonhost immunity. To better understand the function of the *Ipcr* gene, single nucleotide polymorphism (SNP) was used to find a molecular marker linked to the *Ipcr* gene. Putative SNP markers have been identified linked to the *Ipcr* gene that will help in cloning and sequencing the *Ipcr* gene. After sequencing, a Basic Local Alignment Search Tool (BLAST) will be accomplished to infer functional and evolutionary relationships of this sequence to other gene families.

Keywords: *Capsicum annuum*, dominant epistasis, host resistance, suppressor gene

Introduction

One of the most destructive plant pathogens of *Capsicum* is *Phytophthora capsici*, which is estimated to cause an annual damage in excess of $100 million world-wide (Bosland, 2008). In the *C. annuum-P. capsici* pathosystem, resistance against phytophthora blight is dominant (Walker and Bosland, 1999). A unique susceptible accession, NMCA10399 was discovered that is susceptible to all isolates of *P. capsici* evaluated. There are other accessions of *Capsicum* that are susceptible, but when NMCA 10399 is hybridized to the resistant accession, Criollo de Morelos-334 (CM-334), all the progeny are susceptible. Because CM-334 has *P. capsici* resistance that is dominant (Walker and Bosland, 1999), the presence of only susceptible individuals suggested the presence of a gene that inhibits resistance provided by CM-334. This gene has been given the name "inhibitor of *P. capsici* resistance" (*Ipcr*). Because the *Ipcr* gene causes susceptibility to all *P. capsici* isolates, it was evaluated for its effect on other *Phytophthora* species.

Materials and Methods

The accession, CM-334, is the most resistant accession known to *P. capsici* and was used as the resistant parent. This accession has not displayed susceptibility to any *P. capsici* isolate (Thabuis et al. 2004). For this study, two susceptible parents, NMCA10399 and 'Camelot,' were used. From the hybridizations among CM-334, NMCA10399 and Camelot, F_1 individuals, F_2 populations, and testcross population were developed. For root rot screening, the procedure of Bosland and Lindsey (1991) was followed. Foliar blight screening followed the procedure of Monroy-Barbosa and Bosland (2010). The six species of *Phytophthora* included *P. cinnamomi*, *P. citrophthora, P.*

infestans, P. nicotianae, P. palmivora, P. sojae, and *P. capsici* physiological race-*1*. The *P. capsici* race-*1* isolate was chosen because it has a well characterized virulence towards *C. annuum* accessions (Sy et al. 2008).

Inoculation and evaluation

In this study, the disease scale described by Bosland and Lindsey (1991), and Monroy-Barbosa and Bosland (2010) were used for phytophthora root rot and phytophthora foliar blight disease syndrome evaluations, respectively. The total number of resistant and susceptible plants presented as a resistant:susceptible (R:S) ratio.

Experimental design and statistical analysis

Single plants were used as the experimental unit in a completely randomized design. To characterize the inheritance of the *Ipcr* gene, at least 108 individuals were evaluated in each F_2 population in order to ensure 99.9% of observing a single segregate individual (Mainland, 1951). A chi-squared (χ^2) goodness-of-fit test ($\alpha = 0.05$) compared the expected versus the observed segregation (R:S) ratios in all the populations. Expected ratios were based on hypothetical Mendelian segregation ratios where the presence of a single dominant epistatic gene is involved. For genetic characterization of the *Ipcr* gene, two replications in two different years for root rot, and two replications in one year for foliar blight were evaluated. For nonhost resistance, 24 plants of each *C. annuum* population were screened. The experiment was repeated three times.

Results

Genetic characterization of a resistance inhibitor gene in NMCA10399

The CM-334 resistant parent always displayed phenotypic resistance. The susceptible parents (NMCA10399 and 'Camelot') were always dead for root rot, and complete leaf necrosis for foliar blight (Tables 1 and 2) as expected. The CM-334 X NMCA10399 (F_1) hybridization displayed complete susceptibility to phytophthora root rot and foliar blight disease. The CM-334 X 'Camelot' (F_1) hybridizations were completely resistant. The NMCA10399 X 'Camelot' (F_1) hybridizations were completely susceptibility. The CM-334 X NMCA10399 (F_2) populations displayed 3:13 (R:S) segregation ratio. This ratio confirmed the dominant epistatic effect of the *Ipcr* gene to the expression of the dominant R-genes for root rot and foliar blight (Tables 1 and 2). A segregation ratio of 3:1 (R:S) in the CM334 X 'Camelot' (F_2) populations demonstrated that 'Camelot' has one homozygous recessive gene, and does not have any R-alleles against *P. capsisi* race-1. Also, the 3:1 ratio emphasizes that CM-334 is carrying dominant R-alleles for the *P. capsici* isolate. Thus, neither one of the susceptible parents ('Camelot' and NMCA10399) have any R-genes against *P. capsici* race-1.

The testcross population, [(CM-334 X NMCA10399) X CM-334], fit the theoretical expected ratio of 1:1 (R:S). The backcross [(CM-334 X NMCA10399) X NMCA10399] population displayed complete susceptibility to foliar blight and root rot.

Nonhost resistance evaluation in NMCA10399

To ensure the virulence of the isolates, the six *Phytophthora* species were inoculated to their respective hosts. All the inoculated hosts showed disease symptoms. When CM-334, NMCA10399, and 'Camelot' were inoculated with the six *Phytophthora* species, none showed susceptibility in roots or leaves.

Discussion

These results indicate that the resistance gene action in CM-334 is dominant, and established CM-334 as homozygous for resistant to root rot (R_1) and foliar blight (R_2) (*$R_1R_1R_2R_2$ipcripcr*), while 'Camelot' is homozygous recessive (*$r_1r_1r_2r_2$ipcripcr*) for these traits. Conversely, complete susceptibility obtained in the NMCA10399 X 'Camelot' F_1 generation and a 3:13 ratio in the F_2 generation establishes that NMCA10399 does not have any R-genes for root rot or foliar blight resistance.

The results from the F_1 generation of the CM-334 X NMCA10399 hybridization show an epistatic dominant by dominant effect of the *Ipcr* gene over all host-specific resistance genes in CM-334. All susceptible individuals in the F_1 population from the CM-334 X NMCA10399 hybridization is clear evidence of the action of *Ipcr* affecting the expression of *P. capsici* resistance in *C. annuum*. The 3:13 (R:S) ratio in the CM-334 X NMCA10399 F_2 population confirms the presence of *Ipcr* and its epistatic nature to *P. capsici* resistance. This ratio establishes that NMCA10399 is homozygous recessive for the R-locus, while being homozygous dominant for the *Ipcr*-locus, suggesting the *$r_1r_1r_2r_2$IpcrIpcr* as the most appropriate genotype for NMCA10399.

The testcross population [(CM-334 X NMCA10399) X CM-334] displayed the expected segregation ratio of 1:1 (R:S) (Tables 1 and 2). The backcross population to NMCA10399 [(CM-334 X NMCA10399) X NMCA10399] displayed complete susceptibility due to *Ipcr* inherited from NMCA10399.

The CM-334 X NMCA10399 F_1 individuals have R-genes provided by the resistant parent, CM-334, however, the susceptibility of all the individuals in the F_1 is not explained by the absence of R-genes, but instead is explained by the action of an inhibitor gene interfering in the regulatory process of the host-specific defense mechanism.

When nonhost resistance was examined in NMCA10399, it displayed a resistant phenotype against all five *Phytophthora* species tested. This demonstrates that NMCA10399 has a robust and innate nonhost resistance defense to other *Phytophthora* species, despite the presence of the *Ipcr* gene that inhibits the expression of resistance to *P. capsici.* Based on these results, *Ipcr* interferes (inhibits) the expression of the host-specific resistance gene(s) against *P. capsici*, but not the nonhost resistance genes.

An interesting resistant reaction occurred when the two "susceptible" accessions, NMCA10399 and 'Camelot' were challenged for foliar blight resistance with *P. citrophthora* and *P. nicotianae*. A hypersensitive reaction (HR) in the leaves was observed, which is characteristic of type II nonhost resistance. Hypersensitive response is defined as a rapid plant cell defense response induced during penetration of the epidermal cell. It is usually localized to a few plant cells causing cell death, and then the pathogen is prevented from further growth (Vleeshouwers et al. 2000).

The discovery of the inhibitor gene, *Ipcr*, in the accession NMCA10399, provides a new genetic tool to study *Phytophthora* resistance in *Capsicum*. The action of *Ipcr* can provide new insights into the inheritance and defense mechanisms of the *C. annuum*-*P. capsici* pathosystem. The study of the *Ipcr* gene at a molecular level could provide new information to explain the resistance mechanism leading to a greater understanding of host resistance and new insights into the expression, inheritance, and number of genes involved in resistance against *P. capsici* in *C. annuum*.

Table 1 Phenotypic response to phytophthora root rot using *P. capsici* race-1 of the resistant parent Criollo de Morelos-334 (CM-334), the inhibitor parent NMCA10399, the susceptible parent 'Camelot;' the F_1, F_2 and backcross populations.

Entry	Expected genotype	Expected ratio (R:S)	Resistant plants	Susceptible Plants	Chi-square	*P*-value
CM-334 (P_1)	RRii[1]	1:0[2]	24[3]	0	N/A[4]	N/A
NMCA10399 (P_2)	rrII	0:1	0	24	N/A	N/A
Camelot (P_3)	rrii	0:1	0	24	N/A	N/A
CM-334 X NMCA10399 (F_1)	RrIi	0:1	0	127	N/A	N/A
CM-334 X 'Camelot' (F_1)	Rrii	1:0	79	0	N/A	N/A
NMCA10399 X Camelot (F_1)	rrIi	0:1	0	72	N/A	N/A
CM-334 X NMCA10399 (F_2)	R-ii/--I-/rrii	3:13	85	423	1.36	0.24[5]
CM-334 X Camelot (F_2)	R-ii/rrii	3:1	79	32	0.87	0.35
NMCA10399 X Camelot (F_2)	rr--	0:1	0	110	N/A	N/A
[(CM-334 X NMCA10399) X CM-334] (BC P_1)	R-Ii/R-ii	1:1	56	55	0.009	0.92
[(CM-334 X NMCA10399) X NMCA10399] (BC P_2)	-rI-	0:1	0	48	N/A	N/A

[1] R/r: dominant/recessive resistance gene, I/i: dominant/recessive inhibitor of *Phytophthora capsici* resistance gene (*Ipcr*).
[2] Resistant:Susceptible ratio.
[3] Plants were scored using the Bosland and Lindsey (1991) protocol.
[4] Not Applicable.
[5] α=0.05.

Table 2 Phenotypic response to phytophthora foliar blight using *P. capsici* race-*1* of the resistant parent Criollo de Morelos-334, the inhibitor parent NMCA10399, the susceptible parent 'Camelot;' the F_1, F_2 and backcross populations.

Entry	Expected genotype	Expected ratio (R:S)	Resistant plants	Susceptible Plants	Chi-square	*P*-value
CM-334 (P_1)	RRii[1]	1:0[2]	30[3]	0	N/A[4]	N/A
NMCA10399 (P_2)	rrII	0:1	0	30	N/A	N/A
Camelot (P_3)	rrii	0:1	0	34	N/A	N/A
CM-334 X NMCA10399 (F_1)	RrIi/Rrii	0:1	0	35	N/A	N/A
CM-334 X Camelot (F_1)	Rrii	1:0	37	0	N/A	N/A
NMCA10399 X Camelot (F_1)	rrIi	0:1	0	32	N/A	N/A
CM-334 X NMCA10399 (F_2)	R-ii/--I-/rrii	3:13	44	175	0.26	0.61[5]
CM-334 X Camelot (F_2)	R-ii/rrii	3:1	111	47	1.9	0.17
NMCA10399 X Camelot (F_2)	rr--	0:1	0	117	N/A	N/A
[(CM-334 X NMCA10399) X CM-334] (BC P_1)	R-Ii/R-ii	1:1	35	27	1.03	0.31
[(CM-334 X NMCA10399) X NMCA10399] (BC P_2)	-rI-	0:1	0	13	N/A	N/A

[1] R/r: dominant/recessive R-gene, I/i: dominant/recessive inhibitor of *Phytophthora capsici* resistance gene (*Ipcr*).
[2] Resistant:Susceptible ratio.
[3] Plants were scored using the Monroy-Barbosa and Bosland (2010) protocol.
[4] Not Applicable.
[5] α=0.05.

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POSTER PRESENTATIONS

SESSION I

Evaluation and release of breeding material/cultivars, and seed production

Efficiency of two methods to evaluate the pollen viability of eggplant (*Solanum melongena* L. cv. Lila criolla).

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Abstract

The Colombian humid Caribbean is characterized by extremely high rainfall, annual fluctuations in relative humidity and high temperature, factors which influence pollen viability at the time of its shedding and, accordingly, eggplant production. We assessed two methods for evaluating eggplant (*Solanum melongena* L. cv "Lila criolla") pollen viability of: *in vitro* germination and staining with a solution of the tetrazolium salt. We found out that for assessing pollen viability the best concentration of tetrazolium salt is 0.25%. On the other hand i*n vitro* pollen germination showed an highly significant linear trend up to eight hours of incubation. On the basis of our results *in vitro* germination appears more reliable in assessing pollen viability.

Keywords: *Solanum melongena* L, flowering, tetrazolium, germination *in vitro*.

Relationship between capsaicinoids content and peroxidase activity

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Abstract

The evolution of capsaicinoids content, evaluated by HPLC in three local varieties was studied in relation to the expression of peroxidase enzyme. During fruit development (10 to 60days after fruit setting), the magnitude of capsaicinoids content varied according to the varieties and fruit age. 'Rouge Long' and 'Msarreh' varieties showed the highest content of total capsaïcinoïds (1985.99 and 1446.23 µg.g-[1] dry weight, respectively) at 30days after fruit setting, while 'Chaabani' expressed the lowest value of capsaïcinoïdes content at 20 days after fruit setting (448,71µg.g-[1] dry weight). Peroxidase activity expressed on mmoles.mn^{-1}.mg of protein was in general inversely correlated to capsaicinoids synthesis. This activity increased when capsaicinoids content decreased and, vice versa, at 30 days after fruit setting peroxydase activity values were the lowest (70.5 mmoles.mn-1.mg of protein) in 'Rouge long', which had the highest capsaicinoides content at this stage of fruit development.

Keywords: Hot pepper, fruits, capsaicinoids, peroxidase activity

'IVR-338' – an excellent high yielding variety of chilli (C*apsicum annuum* L.) suitable for cultivation in NE India

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Abstract

Nine (9) elite varieties of chilli (*Capsicum annuum L*.) including LCA 206 and JCA 283 as national checks received under AICRP(VC) were tested along with Krishna Jalakia as local check for fresh fruit yield and component characters in the Deptt. of Horticulture, AAU, Jorhat consecutively for 3 years since 2008 - 2010. Among the tested entries IVR-338 exhibited highest average yield of 105.6 q/ha which was 36% higher than the best national check variety LCA 206 (77.7 q/ha). It had shown moderate plant height (40cm), fruits/plant (53.3) and fruit girth (4.3cm). In respect of single fruit weight (6.4 g) and fruit length (10.7cm), it exhibited highest performance. The fruits were getting ready for harvesting at 105-110 days after planting. The variety ranked first among all the tested entries in Bhubaneswar (Orissa), Srinagar (Jammu & Kashmir), Varanasi (UP) and Navsari (Gujrat) also. In view of the excellent yield performance, it had been identified in the AICRP (VC) workshop held at Junagadh, Anand Agril. University, Gujrat in January, 2011 for release in those states. Hence, the variety is suitable for commercial cultivation not only in Assam but in North East India also. However, the variety is moderate in pungency and susceptible to root rot and wilt which needs proper management practices. Appropriate breeding programme may also be taken up to improve these characters.

Keywords : IVR-338, HYV, Capsicum annuum, Variety, NE India

Introduction

Chilli (*Capsicum annuum L*) is an important vegetable crop which is used as condiment and for fresh consumption purpose. North Eastern region of India including Assam is rich in genetic resources of chilli but most of them are poor yielder. In order to earn more income from chilli cultivation, higher yield is an important criteria. Though India stands first in chilli cultivation in the world, the productivity of dry chilli is too low, i.e. 0.9 t/ha compared to world average of 2.0 t/ha (Pugalendhi et al. 2010). Therefore an extensive investigation was carried out at AAU, Jorhat under AICRP(VC) to identify a high yielding chilli variety which could be commercially grown by the farmers not only in Assam and North East but also throughout the country.

Materials and Methods

The field experiment was conducted at the Horticultural farm of the Assam Agricultural University, Jorhat during rabi seasons of 2008 - 2010 with 9 improved genotypes of chilli developed at different research centres of the country. They included LCA 206 and JCA 283 as national checks and in addition Krishna Jalakia as local check variety. The seeds were sown in the month of October and transplanted in November every year. The experiment was laid out in a randomized block design with 3 replications. The size of the plot was 4.5 m x 3.0 m with a spacing of 60 cm between rows and 45 cm between plants. The FYM and NPK were applied as per package of practices recommended for Assam. The observations were recorded on duration to picking (days), plant height (cm), fruit number per plant, fruit length(cm), fruit girth (cm), single fruit weight (g), and fruit yield (q/ha). The pungency was judged by organoleptic taste. The statistical analysis was done following Panse and Sukhatme (1978).

Results and discussion

The mean data pooled over different trials for fruit yield and other characters of the genotypes tested are presented in table 1. The results showed significant genotypic differences for all the characters studied. The variety exhibiting highest performance for fruit yield was IVR 338 (105.6 q/ha). It was followed by AKC 406 (99.7 q/ha) and PC 2062 (90.3 q/ha). The yield of IVR 338 was 36% higher than the best check variety LCA 206. It was having moderate plant height (40.0cm) and fruits/plant (53.3) and fruit girth (4.3cm). The fruit length (10.7cm) and single fruit weight (6.4g) of IVR 338 were the highest of all. Hence, the higher yield performance of IVR 338 was contributed by fruit length and single fruit weight. Bora et al.(2009) reported higher yield in chilli as contributed by either number of fruit or fruit weight or both. The genotypes viz., AKC 406, ACS 06-02 and LCA 206 © and Krishnajalakia © showed low incidence of root rot and wilt. However, IVR 338 showed comparatively higher incidence. It was ready for picking in 105-110 days. The yield data of the genotype IVR 338 and the best check variety either LCA 206 or local check are presented in table 2 over different locations of the country where it ranked first. Amongst the locations, it exhibited highest yield (217.2 q/ha) in IIHR (Karnataka) and lowest (81.2 q/ha) in Bhubaneswar (Orissa). In other locations it had shown moderate yield. This variation in yield might be due to genotype environment interaction. However, over the locations it has shown 39.3% yield increase over the best check variety. From the organoleptic taste, it was found to be low to moderate in pungency.

From these results, it could be concluded that the genotype IVR 338 was an excellent variety for fruit yield not only in Assam but also throughout the country particularly in Karnataka, UP, Gujrat, Orissa and Jammu & Kashmir. Because of excellent yield performance, it had been identified in the AICRP(VC) workshop held at Junagadh, AAU, Gujrat for recommendation in those states (Anon., 2011). The variety however needs to be studied thoroughly for root rot and wilt. The pungency has also to be improved where it determines the consumer preference. This may be done by using it as female parent and transferring the disease resistance and pungency conferring genes from the desirable donor male parent in the breeding programme (Anon.,2009).

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Table 1. Performance of chilli varieties for yield and other characters under AICRP(VC) at Jorhat Centre during 2008-09 to 2010-11

Varieties	Fruit Yield (q/ha)				(%) increase over best check	Plant height (cm)	Fruits /plant	Fruit length (cm)	Fruit girth (cm)	SFW (g)	Root rot & wilt incidence
	IET	AVT I	AVT II	Mean							
PC 2062	61.2	119.4	90.4	90.3	16.2	29.9	71.5	8.0	3.8	3.9	High
ACS 06-1	76.9	74.4	82.4	77.9	-	43.1	35.1	10.3	3.6	4.0	High
AKC 406	86.0	113.3	99.7	99.7	28.3	43.7	70.7	10.3	3.4	3.5	Low
BCC 1	64.4	62.6	60.7	62.6	-	49.0	63.7	4.6	5.1	4.8	-
IVR 338	82.2	120.3	114.4	105.6	36.0	40.0	53.3	10.7	4.3	6.4	High
CCH 05-01	75.3	69.9	72.6	72.6	-	35.2	58.4	5.1	3.5	2.3	-
ACS 06-02	62.4	61.9	61.4	61.9	-	44.3	38.6	10.2	3.9	3.7	Low
JCA 283 ©	53.1	58.5	55.8	55.8	-	41.5	52.1	6.7	3.4	2.3	-
LCA 206 ©	74.0	91.2	68.0	**77.7**	-	45.8	68.4	7.3	3.8	2.9	Low
Krishna (LC)	68.7	70.9	66.5	68.7	-	49.4	70.5	6.2	2.7	2.5	Low
CD (5%)	4.4	15.2	14.2	-	-	-	-	-	-	-	-
CV(%)	4.1	17.0	18.5	-	-	-	-	-	-	-	-
Duration to picking in IVR 338 = 105-110 days											

Table 2. Maximum yield (q/ha) of chilli variety IVR 338 in different centres of the AICRP (VC) in comparison to the best check variety

Variety /Centres	IIHR (Karna-taka)	Dharwar (Karna-taka)	Navsari (Gujrat)	Jammu (J&K)	Srinagar (J&K)	IIVR (UP)	Bhuba-neswar (Orissa)	Jorhat (Assam)	Mean	(%) increase over best check
IVR 338	217.2	155.0	88.4	121.3	173.4	195.2	81.2	120.3	144.0	39.3
LCA 206 /BC*	212.5	84.0	45.4	88.0	135.0	107.5	63.8	91.2	103.4	

*indicates best check.

Evaluation of drying and moistening methods on the physiological quality of seeds in chili pepper (*Capsicum chinense* Jacq.)

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Abstract

The interaction of genetic, physic and physiologic components of the seeds express their quality. The aim of work was to evaluate seed drying methods and moistening substrate forms in physiological quality of seeds in chili pepper (*Capsicum chinense* Jacq.). Seeds of the accession 12 were sown in polystyrene tray and transplanted after 60 days of sowing. Flowers in anthesis stage were daily labeled and fruits of them were harvested, for seed processing, 42 days after labeling, when were fully ripe. The experimental was in an entirely randomized design, in a factorial arrangement 2 x 2. The first factor was the drying methods: natural drying (environment temperature) and drying chamber with forced circulation of air (42°C). The second factor was the substrate moistening forms: water (H_2O) and potassium nitrate (KNO_3) at 0,02%. The analyzed variables were seed germination (%), GVI (germination velocity index), first germination (%) and seedling length (cm). There was significant interaction between the drying methods and substrate moistening forms for both evaluated variables. The seed germination was higher at nature drying and the substrate moistened with water. When the substrate was moistened with KNO_3 there were no significant differences between drying methods. Regarding vigor tests evaluated, there was emphasis on the seeds sown on moistened with water distilled, independent of the form of drying (natural or artificial) express more strongly their potential physiological.

In conclusion, the addition of chemicals or artificial drying is not needed to obtain a good seed germination of chili pepper.

Keywords: Seeds, germination, vigor

Introduction

The *Capsicum chinense* species is the most widespread in tropical America (Bosland, 1992; Pickersgill, 2007). This species is adapted to tropical and equatorial climates and has a great variability in fruit shape, color and pungency. They are the most consumed pepper in Brazil (Carvalho et al. 2003; Lannes et al. 2007).

Despite the high morphological diversity found within *Capsicum* species only few studies were done with Brazilian *Capsicum chinense* varieties (Lannes et al. 2007). Basic information of genetic variability in agronomical traits for improvement pepper quality is needed (Tavares et al. 1999; Marchesan et al. 2009).

Drying methods is a crucial step of the seed production industries. According to Faria et al. (2003) the initial drying affects the production of quality metabolic.

The aim of work was to evaluate seed drying methods and moistening substrate forms in physiological quality (germination and vigor) of seeds in chili pepper (*Capsicum chinense* Jacq.).

Material and Methods

Seeds of the accession 12 (*C. chinense*) were sown in polystyrene tray (128 cells) and transplanted after 60 days of sowing. Flowers in anthesis stage were daily labeled and fruits of them

were harvested, for seed processing, 42 days after labeling, when were fully ripe. The experimental was in an entirely randomized design with four replications, in a factorial arrangement 2 x 2. The first factor was the drying methods: natural drying (environment temperature) and drying chamber with forced circulation of air (42°C). The second factor was the substrate moistening forms: water (H_2O) and potassium nitrate (KNO_3) at 0,02%. Each replication was composed by 25 seeds. The analyzed variables were seed germination (%), GVI (germination velocity index), first germination (%) and seedling length (cm) according with (Nakagawa, 1999; Brasil, 2009). The obtained data were submitted to analysis of variance and Tukey's test ($p \leq 0.05$). All statistical analyzes were done using Genes software (Cruz, 2006).

Results and Discussion

There was significant interaction between the drying methods and substrate moistening forms for both evaluated variables (data not showed).

The seed germination and seed germination index were higher, at both drying methods, when the substrate was moistened with water (Table 1). Queiroz et al. (2011) tested natural and artificial drying methods in *Capsicum chinense* species (Habanero Yellow) and found 65 to 85% of germination. That authors showed a loss of germination in seeds submitted to 42°C, being natural drying and drying at 35°C the best germination percentage. In this work the dry temperature was 45 °C and the minor germination percentage was 65%, not differing of the data found by Queiroz et al. (2011).

The germination was better in natural drying method than the artificial one when the substrate was moistened by water. The seed germination index showed no significant differences between drying methods when the substrate was moistened with KNO_3 (Table 1). Marcos Filho (2005) showed natural drying at field the water loss is gradate leading them to create resistance mechanisms for dehydration. Faria et al. (2003) reported that the initial drying of seeds with high water content, high temperature affects the metabolic processes important in determining the quality of the seed. These reports found by these authors may explain the higher germination when natural drying was used in this paper.

Lima et al. (2011) with the aim of evaluating treatments to reduce or overcome dormancy in seeds of *Capsicum annuum*, observed an increase in germination with the use of KNO_3 where germination ranged 70-84%. In this work the use of KNO_3 in substrate wetting had a negative effect on germination when compared with the use of water.

Similar results were observed for both index of germination speed as for the other vigor tests (Table 2), especially for the test first germination count, as well as the length of seedlings (Table 2), which show greater vigor and for seeds sown on moistened with water, when subjected to both natural and artificial drying. The results indicate there is no significant difference between the drying methods evaluated.

The first count of seed germination is a routinely test utilized in the vigor evaluation, since the germination speed is reduced with the advance of deterioration of the seed. Thus, samples with higher values of the first count germination are considered vigorous. Although it is a simple test and easy to perform, it presents, in general, low sensitivity, not detecting small differences of vigor (Vieira and Carvalho, 1994; Barros et al. 2002).

Bhering et al. (2006) working with *Capsicum frutescens* L. reported values of the first germination ranged from 18.5 to 85% while, in this work we found values ranging from 34 to 63%. It is worth remembering that the mating system of the species, as well as their degree of domestication can influence different behaviors found within the same batch (Casali and Couto 1984; Borém and Miranda 2005; Rêgo et al. 2009; Rêgo et al. 2011).

As for vigor tests surveyed, it is observed that in general, the seeds were more vigorous when the substrate was moistened in water, regardless of the form of drying.

In conclusion, the addition of chemicals or artificial drying is not needed to obtain a good seed germination of chili pepper. Then, the costs of seed drying will be diminishing in production.

Table 1: Means of germination (%) and Velocity germination index of pepper (*Capsicum chinense*).

Germination (%)			**Germination velocity index**		
	Drying			**Drying**	
Moistening	**Natural**	**Artificial**	**Moistening**	**Natural**	**Artificial**
H_2O	85 aA	80 aB	H_2O	2,5 aA	2,3 aB
KNO_3	65 bB	79 bA	KNO_3	1,8 bA	1,9 bA

Means followed by the same lowercase letter (column) and capital letter (line) do not differ by Tukey's test ($p \leq 0.05$)

Table 2: Means of first germination (%) and seedling length (cm) of pepper (*Capsicum chinense*).

First Germination (%)			**seedling length (cm)**		
	Drying			**Drying**	
Moistening	**Natural**	**Artificial**	**Moistening**	**Natural**	**Artificial**
H_2O	63 aA	61 aA	H_2O	5,2 aA	5,3 aA
KNO_3	43 bA	34 bA	KNO_3	4,9 bA	4,6 bA

Means followed by the same lowercase letter (column) and capital letter (line) do not differ by Tukey's test ($p \leq 0.05$)

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Revisiting anthracnose-resistant *Capsicum* germplasm: preliminary field evaluation

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Abstract

Anthracnose fruit rot disease caused by *Colletotrichum* spp. is one of the most important diseases of pepper (*Capsicum* spp.). Forty-three putative anthracnose resistant lines and a susceptible line (9955-15) were evaluated in the field during 2012 at AVRDC – The World Vegetable Center in Taiwan. Three *C. annuum* (0204-4513-2-2, PBC550-a, and 0030-772-9-1), three *C. baccatum* (TC06498, PBC81, and PBC1791) and four *C. chinense* (PBC1776, 0030-770-18-6, PBC251, and PBC1755) lines were found to be resistant (less than 10% infected fruits) at green and red fruit stages with natural infection. The stability of the resistance of these 10 lines will be confirmed by screening with higher virulent isolates of *C. acutatum* (ca-2) and different inoculation methods on detached fruits.

Keywords : Capsicum, Anthracnose, Resistance

Introduction

Pre- and post-harvest anthracnose fruit rot disease is a serious production constraint of chili (*Capsicum annuum*) in Asian countries (Yoon et al. 2009). This disease is caused by a number of species of *Colletotrichum* (Than et al. 2008; Park et al. 2009). During the mid-1990s, AVRDC – The World Vegetable Center identified anthracnose resistance sources in two domesticated *Capsicum* species, *C. baccatum* (PBC-80, PBC-81) and *C. chinense* (PBC-932). These resistant sources have successfully been used in many countries as donor parents for improving anthracnose resistance in most widely cultivated *C. annuum* species. At AVRDC, PBC-932 was used to develop several improved lines (for example AVPP0205, AVPP0513, AVPP0514, AVPP0706, and AVPP0908) expressing varying degrees of resistance (Pae et al. 2005; Park et al. 2009). In 2012, we began to revisit available resistance sources against a newly identified, more virulent and prevalent pathotype of *C. acutatum* (Ca-2) in Taiwan. The objective of this study was to screen selected *Capsicum* lines under natural field infestation.

Materials and Methods

Forty-three putative anthracnose resistant lines and one susceptible check (9955-15) were screened under natural field infestation between 10 June and 15 October 2012 at AVRDC in Taiwan. The field was known to be mostly infested with *C. accutaum*. The entries consisted of 22 *C. annuum*, 8 *C. baccatum*, 11 *C. chinense* and 3 intra-specific hybrids (Table 1). The experiment was conducted following a randomized complete block design with two replications (each plot with 4 plants). At 2-week intervals, all fruits from each plot were inspected after harvesting. There were a total of 6 harvests. Infection rates for green and red fruit were calculated separately based on the

number of infected fruits over total fruit number. The number of anthracnose-infected and healthy fruits at green and red stages were counted and expressed as mean infection rate in percentage.

Results and Discussion

The average incidences of anthracnose in three species under field conditions are shown in Figure 1. The incidence of disease peaked 140 days after transplanting (in *C. annuum* and *C. chinense*) and 162 DAT (in *C. baccatum*). This was expected, as fruiting was delayed in all the *C. baccatum* lines compared with the other two species. Significant differences were observed among the genotypes in reaction to anthracnose infestation. In general, *C. annuum* lines were more susceptible to anthracnose compared with lines belonging to the two other species (Fig. 1). Among *C. annuum* lines, infection rates ranged from 0.7% in 0204-4513-2-2 to 61.3% in 9955-15 (susceptible check). Out of 22 lines, only three showed less than 10% infection. These lines were #1 (0030-772-09-1), #3 (0204-4513-2-2) and #13 (PBC 550a) (Fig. 2).

Table 1. List of putative resistant lines used for screening against anthracnose

Code	Line	Flowering (DAS)	Fruit position	Fruit color (Im.→Mat.)	Fruit length (cm)	Fruit width (cm)	Fruit weight (g)
	C. annuum						
1	0030-772-09-1	72	Erect	G→R	4.3 ±0.9	1.0 ±0.08	1.7 ±0.3
2	0038-9153-4	70	Pendent	DG→R	7.8 ±1.1	1.4 ±0.12	4.4 ±1.0
3	0204-4513-2-2	83	Erect	G→R	4.2 ±0.3	1.2 ±0.04	2.8 ±0.4
4	0338-8613-1	71	Pendent	DG→R	9.2 ±0.5	0.9± 0.33	3.3 ±0.5
5	0537-7558	64	Erect	G→R	4.2 ±0.6	0.7 ±0.07	1.1 ±0.3
6	0537-7559	69	Pendent	G→R	8.6 ±0.7	1.2 ±0.12	4.4 ±1.1
7	1037-7633-1	72	Pendent	WG→R	9.9 ±0.6	1.3 ±0.13	6.2 ±0.8
8	9955-15	68	Pendent	WG→R	10.0 ±1.4	1.5 ±0.28	8.6 ± 0.4
9	PBC1703	64	Pendent	G→R	1.9 ±0.2	0.9 ±0.06	0.7 ±0.1
10	PBC1713	68	Erect	G→R	3.9 ±0.3	0.8 ±0.06	0.9 ±0.1
11	PBC1714	66	Erect	WY→R	4.5 ±0.3	0.9 ±0.14	1.2 ±0.2
12	PBC271	65	Erect	G→R	2.7 ±0.4	2.9 ±0.20	5.7 ±1.5
13	PBC550-a	80	Pendent	DG→R	8.1 ±0.7	0.8 ±0.08	2.3 ±0.2
14	PP0537-7516	60	Pendent	DG→R	9.5 ±1.0	1.1 ±0.01	4.8 ±1.0
15	PP0737-7016	74	Pendent	WG→R	8.5 ±0.3	1.0 ±0.05	25.3 ±3.5
16	PP0737-7605	72	Erect	DG→R	4.7 ±0.4	0.9 ±0.11	1.7 ±0.3
17	PP0737-7631	69	Pendent	DG→R	1.3 ±0.2	5.6 ±0.33	4.0 ±0.3
18	PP9852-173	69	Pendent	G→R	7.5 ±0.7	1.3 ±0.11	4.8 ±1.0
19	PP9950-5197	62	Pendent	WG→R	7.0 ±0.4	1.4 ±0.20	4.4 ±1.1
20	TC5471a	69	Pendent	DG→R	2.1 ±0.5	2.4 ±0.31	4.7 ±1.2
21	TC5471b	71	Pendent	DG→R	9.2 ±2.2	1.7 ±0.24	10.3 ±3.4
22	TC6621	81	Pendent	WG→R	6.0 ±0.3	1.1 ±0.13	2.5 ±0.8
	C. baccatum						
23	PBC80	79	Pendent	WG→O	9.7 ±1.3	2.5 ±0.03	19.4 ±5.0
24	PBC81	79	Pendent	WY→Y or R	10.3 ±1.0	2.6 ±0.32	22.9 ±3.5
25	PBC880	70	Pendent	WG→R	2.3 ±0.2	2.7 ±0.23	3.5 ±0.5

Code	Line	Flowering (DAS)	Fruit position	Fruit color (Im.→Mat.)	Fruit length (cm)	Fruit width (cm)	Fruit weight (g)
26	PBC1752	78	Pendent	WY→Y	5.3 ±0.2	1.6 ±0.07	4.0 ±0.1
27	PBC1752	78	Pendent		6.2 ±1.2	1.8 ±0.11	4.5 ±0.7
28	PBC1791	68	Pendent	WG→Y	7.2 ±0.3	1.8 ±0.02	6.5 ±0.5
29	PBC1791	73	Pendent	WG→Y	8.7 ±0.6	1.0 ±0.09	3.8 ±1.1
30	TC06498	65	Pendent	G→R	3.5 ±0.4	1.0 ±0.10	1.0 ±0.1
	C. chinense						
31	0030-770-18-6	126	Intermediate	WY→R	1.5 ±0.2	2.6 ±0.23	2.2 ±0.3
32	PBC251	88	Pendent	DG→C	3.4 ±0.4	2.4 ±0.29	4.9 ±1.1
33	PBC272	63	Pendent	WG→R	3.0 ±0.6	1.6 ±0.11	4.7 ±1.6
34	PBC492a	73	Pendent	WY→R	3.5 ±0.2	2.5 ±0.02	5.2 ±0.4
35	PBC811	119	Pendent	WG→R	3.6 ±0.4	2.5 ±0.13	6.8 ±1.1
36	PBC879	82	Pendent	G→R	2.3 ±0.3	2.7 ±0.33	3.5 ±0.8
37	PBC911	83	Pendent	WG→R	3.3 ±0.1	2.3 ±0.17	3.0 ±0.7
38	PBC932-6-2	92	Pendent	G→O	2.3 ±0.2	2.7 ±0.02	3.5 ±0.3
39	PBC1755	88	Pendent	WGP→Y	3.1 ±0.3	1.0 ±0.08	1.6 ±0.5
40	PBC1776	81	Pendent	WG→R	3.4 ±0.2	2.9 ±0.02	7.4 ±0.4
41	PBC1794	79	Pendent	WYG→R	3.8 ±0.3	2.6 ±0.16	4.8 ±0.8
	Hybrid						
42	CCA11215 (C.a/C.a)	66	Pendent	G→R	9.2 ±1.1	1.4 ±0.15	6.5 ±1.6
43	CCA11362 (C.b/C.b)	74	Pendent	G→R	4.9 ±1.1	1.3 ±0.22	2.7 ±1.2
44	CCA11366 (C.c/C.c)	68	Pendent	DGY→R	4.1 ±0.4	2.3 ±0.37	5.4 ±0.8

DAS = days after sowing; DG= dark green, O = orange; R = red; W = white, Y = yellow

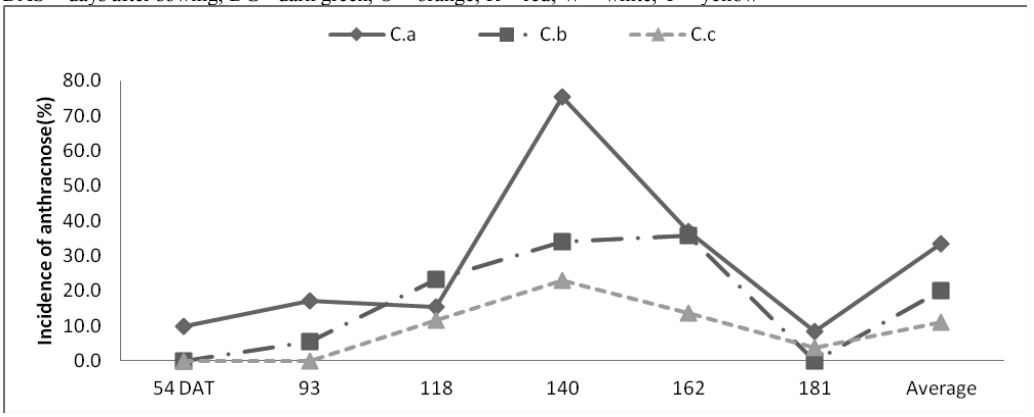

Figure 1. Incidence and progress of anthracnose disease in 44 genotypes of three *Capsicum* species (June to October 2012, Taiwan)

Among 8 *C. baccatum* lines, infection rates ranged from 0.7% in TC06498 to 35.8% in PBC1752. Three lines showed less than 10% infection. These lines were #24 (PBC 81), #28 (PBC1791) and #30 (TC06498) (Table 1, Fig. 3). Among 11 *C. chinense* lines, infection rates ranged from 2.1% in line PBC 1776 to 35.0% in line PBC 811. Four lines showed less than 10% infection and these were #40 (PBC 1776), #31 (0030-770-18-6), #32 (PBC 251) and #39 (PBC

1755) (Fig. 3). Among F_1 hybrids, infection rates ranged from 8.8% in PBC1755/PBC 272 to 52.4% in PP0537-7559/PP9950-5197 (Fig. 3).

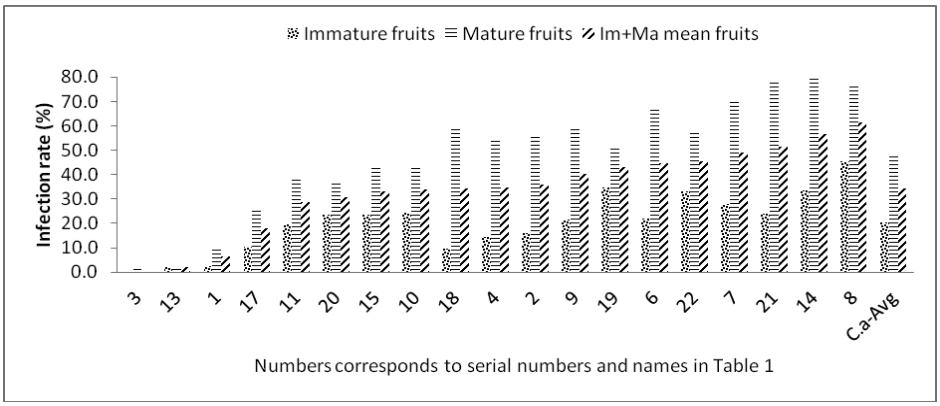

Figure 2. Natural field infection rate of 19 *C. annuum* lines

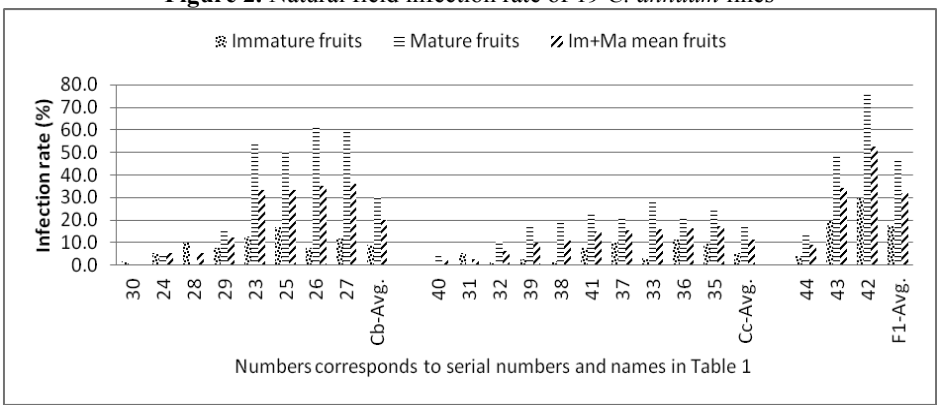

Figure 3. Natural field infection rate of 8 *C. baccatum,* 10 *C. chinense* and 3 F_1 hybrids

In conclusion, three *C. annuum* (0204-4513-2-2, PBC550-a, 0030-772-9-1), three *C. baccatum* (TC06498, PBC81, PBC1791) and four *C. chinense* (PBC1776, 0030-770-18-6, PBC251, PBC1755) were found to be resistant under natural field conditions with less than 10% infestation. We intend to challenge these 10 lines with a virulent isolate (Ca-2) using two different artificial inoculation methods to determine a future course of action for anthracnose resistance breeding.

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Phenotypic evaluation of pepper varieties at the Institute of Field and Vegetables Crops Novi Sad (Serbia)

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Abstract

Pepper is very spread vegetable species in many Balkan country as also in Serbia. The aim of this study was to phenotypic evaluate 11 pepper varieties from the Institute of Field and Vegetable Crops Novi Sad with a three domesticated pepper variety such as Kurtovska Kapia, California Wonder and Soroksari. Experiment were conducted in 2012 year in the field condition on chernozem soil. It was established in the randomized block design with five replications. Fruits from the second harvest were used for phenotypic evaluation. Varieties were characterized for 11 fruit traits. We used two multivariate analysis. Principal component analysis (PCA) was used to identify the most significant variables in the data set and to show geometrical distances between varieties in a scatter plot. Cluster analysis was used to connect varieties into groups. In a scatter plot bell peppers were very similar and they belonged to the largest group and they close related with tomato pepper type Novosadjanka, but other varieties were different from each other. Based on phenotypic data with cluster analysis all varieties were joined into three main groups. First and the biggest group was consist of bell peppers, second group were made of kapia types mainly and the third smallest group were consist of two varieties of shipka types. The longest was the fruit of Plamena (14.72 cm), while the shortest of Novosadjanka (3.48 cm) which had the biggest pericarp thickness (6,27 cm). The greatest fruit weight had variety Amfora (129 g). Our results showed that dominant type in Novi Sad assortment is bell pepper with yellow colour of immature fruit which is habit of local consumers.

Keywords: Pepper, evaluation, multivariate analysis

Anatomical investigation of brown seed pepper mutants (*Capsicum annuum* L.) of different origin

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Abstract

During the process of breeding different pepper cultivars (*Capsicum annuum* L.), certain lineages of so-called, 'brown seed' mutants were found. Due to their differing origin, these were termed on various genetic names: *gos*, *rb1*, *rb2*, *rb3*, *rb4*, *rb5, rb6, rb7*. A further observation of the breeders was that the stem of all the brown seed mutants was intensely pigmented under the epidermis. The two phenotypic traits were found to be linked to each other. Our aim was to examine the histological background of both observed characteristics.

In case of each cultivar, the surface colouring of the seeds was caused by the intensely yellow cell walls of the outer layer of the seed coats. However, no surrounding tissue elements (inner seed coat layers, endosperm, funicular or placentar tissues) bear similar features. When examining the shoot axes, we also observed yellow cell walls in the secondary xylem elements (tracheas, tracheids and fibers). Interestingly, the stem of the *rb3* and *rb4* mutants contained only dispersed yellow-walled tracheas, and the macroscopic colour was also much brighter. Besides, these mutants have lighter pigmented seed coats, as well. The genetic tests of the mutants proved that *gos, rb1, rb2* and *rb5* mutants share the same allele, whilst *rb3* and *rb4* traits have other genetic background.

As a consequence, we can state that the histological characteristics of the seeds are in close relation with the results of the hybridisation tests, in case of both the seed coat and the stem anatomy. Besides, the yellow-coloured secondary xylem elements of the stem seem to be a linked feature in case of these cultivars. The exact molecular background of the observed cell wall colour needs further clarification by appropriate analytical methods, in the future.

Keywords: brown seed, anatomy, seed coat, stem

Introduction

Although detailed anatomical investigations have been carried out on the vegetative organs of the pepper (*Capsicum annuum* L.) (e.g. Schuerger et al. 1997, Nwachukwu et al. 2007, Weryszko-Chmielewska and Michałojc 2009, Dias et al. 2013), the number of studies focusing on the generative organs is restricted. The most intensely examined objects is the fruit (Weryszko-Chmielewska and Michałojc 2011, Gersh et al. 1998, Sundberg et al. 2003), though the developing male gametophye was also studied from the anatomical aspect (Wang et al. 2004). The seed coat anatomy of certain cultivars is only sparsely documented and these works focus on the quantitative differences of the seeds (Krstić et al. 2001).

In case of the closely related tomato (*Solanum lycopersicum* L.) several brown-seeded mutants have been described (Soressi 1967, Yordanov & Stamova 1971, Downie et al. 2003). The darkened colour of these seeds is caused by the pigmented embryo (Downie et al. 2003). Nevertheless, similar observation has not been published up to now in case of the pepper fruits, according to our knowledge. During the genetic survey of more than 40 years on pepper plants, seven mutants of the Hungarian cultivar 'Fehérözön' have been found to possess dark pigmented seeds. The provisional genetic names of these mutants are *gos* (*gold seed*), *rb1* (*rust brown seed*), *rb2*, *rb3*, *rb4*, *rb5, rb6* and *rb7*. The genetic relationship of the mutants and their detailed characterisation is published by Csilléry (2013) in the present issue. According to the breeders' experiences, the broken stems of all the brown seed mutants have similar brownish tint under the epidermis. When comparing the

phenotypical traits of the mutants, obvious is the similarly dark colouration of *gos,* the *rb1*, *rb2*, *rb5* and mutant, while both the seed coat and the stem colour of *rb3* and *rb4* is much lighter (though discernibly yellowish compared to the wild-type plants). This is in correlation with the allelic background of the pigmentation in the different mutants.

Our aim was to examine the exact anatomical background of the observed phenomena on six of the brown seeded mutants (*gos*, *rb1*, *rb2*, *rb3*, *rb4*, *rb5*).

Materials and Methods

For comparing the histological structures of the different cultivars to that of the wild-type peppers, semi-thin sections were made by applying a cryostat (Leitz Wetzlar). In order not to conceal the difference in their pigmentation patterns, sections were examined unstained. Instead, they were contrasted by optical methods using phase-contrast and difference interference contrast (Nomarski-DIC) microscopy (beside the general bright-field method) (Zeiss, Axio Imager.A2). Photodocumentation was carried out with the Axio Vision software 4.8.

The following features were studied:

longitudinal and cross sections of the mature seeds, in case of longitudinal sections also of the funicle and the placenta;

cross sections of the stem, from the regions of secondary thickening.

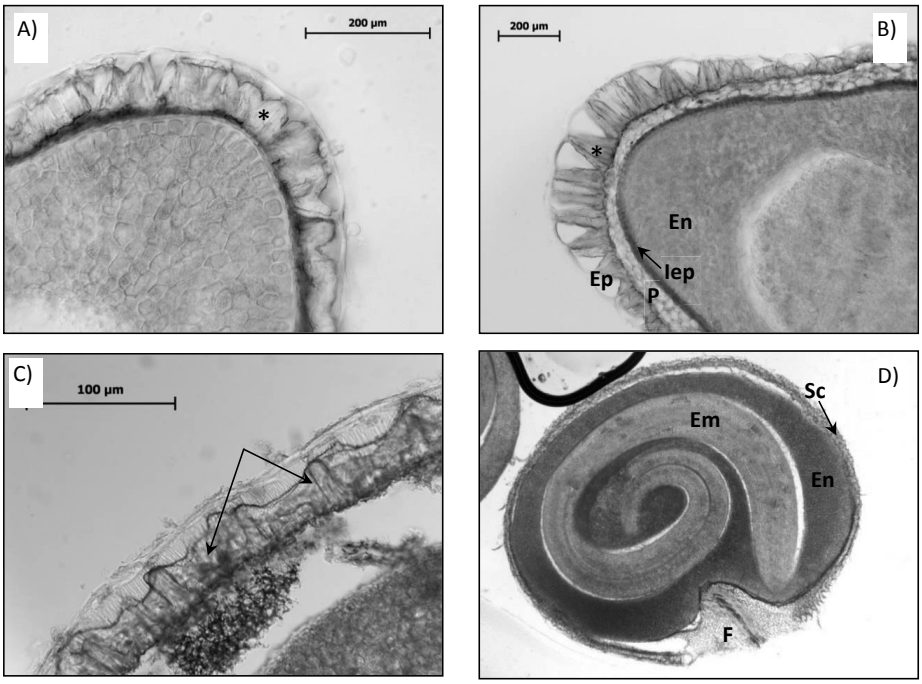

Figure 1. Seed anatomy of pepper cultivars. A) seed-coat of the wild-type pepper; B) seed-coat of the *gos* mutant. Asterisks indicate the pigmented cell-wall thickening of the epidermis being darker in the greyscale picture in case of the *gos* seed. C) the U-shaped thickened cell wall of the epidermis of the seed-coat, at higher magnification (cell wall thickening indicated by arrows). D) gross anatomy of the seed in longitudinal section (*rb4*). Abbrevations: Ep – outer epidermis, P – parenchyma layer, Iep – inner epidermis, En – endosperm, Em – embryo, Sc – seed coat, F – funicle. (A-B, D: bright-field images; C: differential interference contrast microscopic image.)

Results and Discussion

The anatomy of the seeds of each cultivar corresponds to previous descriptions (Augustin 1907, Krstić et al. 2001). The outer epidermis of the seed coat is composed of closely packed cells of U-shaped thickened cell walls. To the interior from this layer some parenchymatic cell rows lie, followed by the compressed cells of the inner epidermis (Figure 1). In case of all brown seed mutants, intense yellowish pigmentation was found in the thickened radial and inner tangential walls of the outer epidermis (Figure 1). This pigmentation was much more obvious in case of the *gos*, *rb1*, *rb2* and *rb5* mutants than in *rb3* and *rb4*. This observation corresponds to the consequences drawn from the genetic tests (see the results of Csillery in the present issue) *gos, rb1*, *rb2*, and *rb5* being caused by the same allele. The anatomical difference between the seed coats of pepper strains was also observed by Krstić et al. (2001), yet they found only quantitative dissimilarities of the epidermises of the cultivars, and not between the colours of this cell layer.

When examining the surrounding tissues, no similar pigment deposition was observed either in the placenta, or in the funicular tissues.

In the cross sections of the shoot axes, we also observed yellow cell walls in the secondary xylem elements (tracheas, tracheids and fibers) instead of white, brightly translucent secondary cell walls observable in the same cells of the control plants. As far as we know, such colour was never observed in previous anatomical studies on the stem (Schuerger et al. 1997, Wahua 2013). The stems of the *rb3* and *rb4* mutants contained only dispersed yellow-walled tracheas, and the macroscopic colour was also much brighter.

Consequently, the anatomical results showed the histological causes of the macroscopic features observed by the breeders. On the same time, the characteristic heritage of the seed colour (i.e. the F1 seeds are pigmented according to the genotype of the mother plants – see in Csilléry in the present issue) is also supported from the anatomical aspect, since the tissues of the seed coat are genetically identical with the mother plant, and not with the new sporophyte within.

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Evaluating *Capsicum* spp. root architecture under field conditions

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Abstract

Roots are responsible of many adaptive responses to abiotic stresses, such as drought, mineral starvation, etc. Despite breeding for improved root system is a need to face the challenges of the climate change, little is known about the diversity of root traits and their genetic control. Peppers (*Capsicum annuum* L.) and other cultivated *Capsicum* species (*C. frutescens*, *C. chinense*, and *C. baccatum*) are important commercial crops, which are grown in different ecological environments and display a great genetic diversity. However, there is still a lack of studies on the root architecture diversity in this crop. In this work, we compared the root architecture of 23 *Capsicum sp*. cultivars including commercial, traditional and wild varieties. Six plants per genotype were grown in the field during the 2012 summer season in Valencia. Root crowns were excavated from the soil, weighted and analyzed with WinRhizo Pro software. The results showed that there exist differences in the root architecture among genotypes, especially regarding the root length of a certain diameter. For example, 'Bola' and 'Chile de Arbol' had the same total root length but different distribution of diameters, the later had higher length of thick roots (more than 3 mm in diameter). Within *C. annuum* the best performers in terms of higher root weight and length, were 'Najerano' 'Serrano Criollo de Morelos' and 'Piquillo', differing among them in the distribution of diameters. The performance of the genotypes of *C. frutescens*, *C. chinense* assayed was similar to the best performers of *C. annuum*. The genotype of *C. baccatum*, outstood by its root length (the maximum of the experiment) and thin lateral roots. This experiment provides useful information about root contrasting genotypes for future stress resistance experiments, grafting experiments and QTL mapping.

Keywords: Diversity, grafting, abiotic stress, drought

Introduction

Roots are responsible of plant anchorage, water and mineral acquisition, hormonal supply and interactions with the soil. In addition to the biotic interactions, many adaptive responses to abiotic stresses, such as drought, mineral starvation, flooding, etc, occur at root level. Despite the information about the specific role of the root architecture in such stresses is increasing fast (Franco et al. 2011), little is known about the natural diversity of root traits and their genetic control. This knowledge is of paramount importance to breed for improved root system able to face the challenges of the climate change. Peppers (*Capsicum annuum* L.) and other cultivated Capsicum species (*C. frutescens*, *C. chinense*, and *C. baccatum*) are important commercial crops. Since they were domesticated they have been adapted to very different ecological environments: from wet lands in the Amazonian and tropical areas to very dry areas in Mexico (Bosland and Votava, 2000). Therefore peppers display a great genetic diversity. This indicates that probably *Capsicum spp*. also have root systems adapted to different ecological conditions. However, there is still a lack of studies on the root architecture diversity in this crop. The objective of this research was to screen the morphological differences at root level among several *Capsicum* accessions from different origins.

Material and Methods

Twenty-three *C. annuum* cultivars including commercial and traditional varieties of Spain, France, Italy, USA and Mexico, as well as one *C. chinense* from Ecuador, one *C. baccatum* and one *C. frutescens*, both from Bolivia (Table 1) were evaluated for their root system.

Table 1. *Capsicum* cultivars used in the study

Code	Common name	Origin	Species
Arn	Arnoia	Spain (Galicia)	*C. annuum*
Pad	Padrón	Spain (Galicia)	*C. annuum*
Piq	Piquillo	Spain (Navarra)	*C. annuum*
Guin	Guindilla de Ibarra	Spain (Basque Country)	*C. annuum*
Gue	Guernika	Spain (Basque Country)	*C. annuum*
Naj	Najerano	Spain (La Rioja)	*C. annuum*
Bol	Bola	Spain (Murcia)	*C. annuum*
Ca	California Wonder	Spain (Valencia)	*C. annuum*
Val	Valenciano	Spain (Valencia)	*C. annuum*
Mo	De Mojo	Spain (Canary Islands)	*C. annuum*
DiSe	Di Senise	Italy	*C. annuum*
Cu	Cuneo	Italy	*C. annuum*
DdL	Doux Longue des Landes	France	*C. annuum*
Num	Numex Big Jim	USA (New Mexico)	*C. annuum*
Chim	Chimayo	USA (New Mexico)	*C. annuum*
Ja	Jalapeno Candelaria	Mexico	*C. annuum*
ChiA	Chile de árbol	Mexico	*C. annuum*
Pas	Pasilla	Mexico	*C. annuum*
SCM	Serrano Criollo de Morelos	Mexico	*C. annuum*
An	Ancho	Mexico	*C. annuum*
Chin	Chinense2	Ecuador	*C. chinense*
Bac	Bol37R	Bolivia	*C. baccatum*
Fru	Bol144	Bolivia	*C. frutescens*

Six plants per genotype were grown in the field during the 2012 summer season in Valencia. At the end of the season (September) root crowns were excavated from the soil, washed, weighted and photographed (Fig 1). Then each root was divided into its lateral roots and they were scanned (Epson LA 1600+, Epson America Inc. Long Beach, CA, USA,). Then, images were analyzed with WinRhizo Pro software (WinRhizo Pro 2003b, Reagent Instruments Inc. Quebec Canada). WinRhizo provides the total length of the analyzed roots as well as the length of the roots of a certain diameter. For this experiment, we divided the lateral roots into 4 classes: i) fine roots, which consist of roots of diameter less than 1 mm, ii) lateral roots, which consists of roots between 1 mm and 3 mm in diameter, i) lateral thick roots, which consist of roots between 3 mm and 5 mm in diameter, i) structural roots, which consist of roots of diameter higher than 5 mm in diameter.

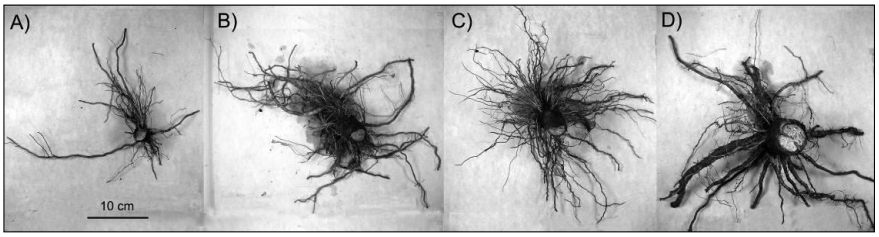

Fig 1. *Capsicum* spp. Crown roots excavated from the soil. A) Guernika, B) Piquillo, C) Bol37R and D) Serrano Criollo de Morelos

Results and discussion

There were differences in the root architecture among genotypes. *C. chinense*, *C. baccatum*, 'Serrano Criollo de Morelos', 'Najerano' and 'Piquillo' showed total root length values higher than 2000 cm, whereas 'Guernika', 'Guindilla', 'California' and 'Chimayo' showed values lower than 700 cm, showing the other fourteen genotypes intermediate values (Fig 2A). The results in terms of the root length by root type (diameter) resulted more interesting. For example, 'Bola' and 'Chile de Arbol' had the same total root length (around 1300 cm) but different distribution of diameters, the later had higher length of thick roots (more than 3 mm in diameter). Within *C. annuum* the best performers in terms of higher root weight and length, differed in the distribution of diameters. 'Serrano Criollo de Morelos' outstood by its great amount of structural and thick lateral roots (Fig 1D and 2C). This variety is well known by its resistance to *Phytophthora capsici* Leo. The performance of the genotypes of *C. frutescens*, *C. chinense* and *C. baccatum* in terms of total root length was similar to the best performers of *C. annuum*. *C. baccatum* outstood by its root length (the maximum of the experiment) and thin lateral roots, only a few proportion of the total length was due to thick roots (Fig 1C and 2B). On the contrary, *C. chinense* and *C. frutescens* showed in general greater proportion of thick lateral roots, similar to those of 'Serrano Criollo de Morelos'.

The results showed a great diversity in the root architecture within *C. annuum,* especially at the diameter distribution level. *C. frutescens* and *C. chinense* showed similar roots between them and to some *C. annuum* genotypes, whereas *C. baccatum* showed the most different root system, with long and thin lateral roots. These differences may be due to taxonomic and adaptative reasons. *C. frutescens* and *C. chinense* belong to the *C. annuum* complex, whereas *C. baccatum* belong to a distinct complex. Other studies have reported root differences at seedling level (Peláez et al. 2011) or in adult plants grown in pots among Capsicum genotypes (Kulkarni and Phalke, 2009), however this is, to our knowledge, the first attempt to evaluate *Capsicum* spp. root system diversity in the field. This experiment provides useful information about root contrasting genotypes for future stress resistance experiments, grafting experiments and QTL mapping.

Acknowledgements

This work has been partially financed by INIA projects RTA2010-00038-C03-03 and RF2010-00025-00-00. We also thank P.W. Bosland for providing us seed of Chimayo, Numex Big Jim and Jalapeno Candelaria, S. Lanteri for seeds of Cuneo and Pepperone di Senise, and NEIKER for seeds of Guernika and Guindilla Ibarra.

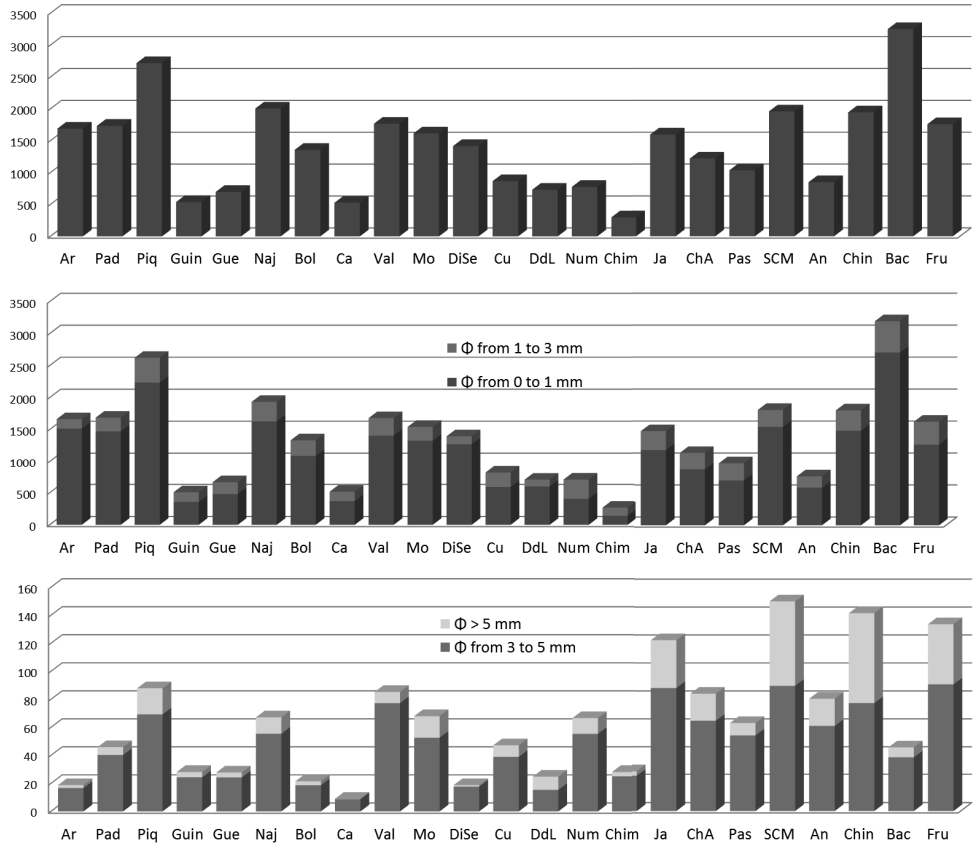

Figure 2. A) Length of lateral roots, cm; B) Length (cm) of lateral roots of diameter (Φ) less than 3 mm C) Length (cm) of lateral roots of diameter (Φ) over 3 mm.

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Resistance to root-knot nematode (*Meloidogyne enterolobii*) in *Capsicum* spp. accessions

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Abstract

The identification of sources of resistance to *Meloidogyne enterolobii* in the genus *Capsicum* has become a pressing need to develop cultivars resistant to this nematode, since genes conferring resistance to *Meloidogyne* sp. are not effective against *M. enterolobii*. The objective of this work was to identify accessions of *Capsicum* spp. resistant to the nematode *M. enterolobii* and to classify them according to their resistance degree. Thirty-nine accessions of the Universidade Estadual do Norte Fluminense Darcy Ribeiro (UENF) gene bank were evaluated. The collection included 12 accessions of *C. annuum*, 11 of *C. chinense*, ten of *C. baccatum* and six of *C. frutescens*. The experiment was carried out in a greenhouse in a completely randomized design with ten repetitions and one plant per plot. The inoculation was carried out 20 days after germination being inoculated 500 eggs per plant and evaluation conducted after 75 days. Reproduction factor and reproduction index were determined, and accessions were classified according to their resistance degree to the nematode. There was significant difference among the accessions tested in relation to the traits evaluated, indicating the existence of variability among and within species of *Capsicum*. Only UENF 1730 (*C. chinense*) accession was considered as resistant.

Keywords: chili pepper, sweet pepper, germplasm evaluation, disease resistance, pre-breeding.

Introduction

Genetic resistance is considered the most efficient way to control plant diseases and the search for resistant genotypes is fundamental for the development of breeding programs that aim to develop cultivars resistant to pathogens. An example of successful use of resistance genes in plants is the case of the interaction between plants of *Capsicum* (including chili and sweet peppers), and nematodes of the genus *Meloidogyne*. However, one *Meloidogyne* species, identified as *M. enterolobii* (syn. *M. mayaguensis*) (Yank and Eisenback, 1983) have become relevant in the areas of cultivation of *Capsicum* spp. because the sources of effective resistance against other nematodes are ineffective in its control, and the high virulence development causes small deformations in the root system and consequently a reduction in the quality and quantity of fruit (Carneiro et al. 2006; Brito et al. 2007).

This study aimed to screening a *Capsicum* gene bank to identify genotypes resistant to nematode *M. enterolobii*.

Materials and Methods

Thirty-nine accessions of germplasm collection were evaluated, of which 12 accessions were of *C. annuum* L., 11 of *C. chinense* Jacq., ten of *C. baccatum* L. and six of *C. frutescens* L. These accessions were characterized for morphological and agronomic traits by Sudré et al. (2005) and Bento et al. (2007), for resistance to yellow mosaic (Pepper yellow mosaic virus - PepYMV) by Bento et al. (2009) and to anthracnose (*Colletotrichum gloeosporioides*) by Silva (2012). The plantlets were grown in trays of 128 cells with commercial substrate and after the emergence of two pairs of true leaves, seedlings were individually transferred to plastic pots containing substrate

mixed and homogenized with the formulation Osmocote® 17-07-12. The experiment was conducted in a greenhouse in Campos dos Goytacazes, RJ, Brazil, in a completely randomized design with ten replicates and one plant per plot.

The inoculum source used was an isolate of *M. enterolobii* maintained in tomato plants in a greenhouse. This isolate was obtained from a commercial orchard of guava trees in São João da Barra, RJ, Brazil (lat. 21 º 41'22" S, long. 41 º 03'20" W). For inoculum preparation a modification of the methodology proposed by Cotter et al. (2003) was used: parasitized roots were put into 1L flasks filled with 500 mL of water and agitated in a shaker commercial (Tecnal model TE240) for four minutes. Subsequently, the eggs of the nematode were obtained by passing the resultant suspension through a sieve of 100 mesh and 500 mesh.

The *Capsicum* plants were inoculated 20 days after germination with the aid of pipettes, so that 500 viable eggs were inoculated per plant. Evaluations started 75 days after inoculation, when the root system of each plant was washed and processed individually according to Cotter et al. (2003) with modifications. The root systems were placed individually in glass jars of 500 ml filled with 300 ml of aqueous bleach 6%. Thereafter the bottles were agitated in a shaker commercial (Tecnal model TE240) for four minutes, 130 revolutions per minute. The nematode eggs were collected by passing the resulting slurry into sieves of 100 and 500 mesh and three aliquots 1mL were examined for counting the number of total egg population. The number of eggs per gram of root was calculated by dividing the number of eggs by fresh weight of root.

The reproduction factor (RF) was determined by dividing the number of viable eggs from the final population by the number of initial population. The value of the reproduction index (IR%) was calculated by the formula: 100 (number of eggs per gram of root of each repetition / average number of eggs per gram of root susceptible cultivar Ikeda). In accordance with the criterion established by Taylor (1967), the degree of resistance was rated as susceptible (S) - IR greater than 50% of the value obtained for the cultivar 'Ikeda'; slightly resistant (SR) - IR 26-50%; moderately resistant (MR) - IR with 11-25%; very resistant (VR) - IR with 1 to 10%; highly resistant (HR) - IR with less than 1% and immune (I) - when there was no reproduction.

In the statistical analysis, the resistance components were first tested for homogeneity of variances and normality of their errors, respectively, by testing Bartlett and Kolmogorov-Smirnov test at 5% probability. Since these assumptions were not met, the data were processed by the equation log (x + 1). After analysis of variance, the data were submitted to the Scott-Knott test. All analyzes were performed by the R program (www.r-project.org).

Results and Discussion

The differences among genotypes for reproduction factor (RF) and reproduction index (RI) was significant according to the F test, indicating the existence of wide variability among and within *Capsicum* species for resistance to *M. enterolobii*. RF values ranged from 0.30 (UENF 1730) to 38.42 (UENF 1627), with an average of 14.19, while RI ranged from 8.79 (UENF 1730) to 394.70 (UENF 1623), with a mean value of 97.47, indicating a high severity of the pathogen over accessions evaluated (Table 1). The value obtained for RF was superior to those obtained by Oliveira (2007) and Melo et al. (2011) who obtained values of 5.64 and 2.51, respectively.

For *C. annuum* accessions, the mean values of RF and IR were 17.95 and 124.46 respectively, and only UENF 1717 and UENF 1750 as slightly resistant (Table 1). The cultivar Ikeda presented a reproduction factor of 29.50, superior to that obtained by Melo et al. (2011) that was 2.6. UENF 1381 and Criollo de Morellos 334 (CM334) are used in *Capsicum* breeding programs, respectively, as resistance sources to bacterial spot (*Xanthomonas* sp.) (Riva-Souza et al. 2009) and to different nematodes (*M. incognita*, *M. arenaria* and *M. javanica*). Also, CM334 is resistance source for Potyvirus and also to *Phytophthora* (Oelke et al. 2003; Janzac et al. 2009). In the present study,

UENF 1381 and CM 334 showed, respectively, RF 17.44 and 12.72, and RI of 80.86 and 50.21, indicating high susceptibility to *M. enterolobii*. This result demonstrates the ineffectiveness of gene*Me7*, present in CM334, against the action of *M. enterolobbi*. Brito et al. (2007) also verified the inefficiency of genes *Mi-1*, *N* and *Tabasco* on infection and reproduction of *M. enterolobii* on tomato and pepper genotypes.

In relation to *C. chinense* accessions, the mean values RF and RI were 12.30 and 93.43, respectively, being UENF 1554, UENF 1706 and UENF 1780 accessions slightly resistant (RF: 3.14, 2.72 and 4.35, respectively); UENF 1706 was moderately resistant (FR: 3.68), and UENF 1730 was resistant (RF: 0.30) (Table 1). The UENF 1730 was also identified as resistant to PepYMV (Bento et al. 2009).

For *C. baccatum* accessions, mean values for RF and RI were 97.47 and 14.19, respectively. The UENF 1635 and UENF 1718 accessions were considered slightly resistant (FR: 4.24, RI 7.73, respectively) and UENF 1714 as moderately resistant (FR 2.80) (Table 1). Considering *C. frutescens* accessions, mean values for RF and RI were of 9.26 and 82.13, respectively, and only UENF 1747 was considered slightly resistant (RF: 7.05).

Genetic resistance to nematodes is one of the most efficient, economical and causes less environmental impact in controlling this endoparasite. The use of UENF 1730 accession in breeding programs is promising to develop new cultivars of chili and sweet pepper resistant to *M. enterolobii*. In addition, this *C. chinense* accession is also resistant to PepYMV, an important virus in the *Capsicum* producing regions in Brazil, and it belongs to the same gene pool complex that *C. annuum*, which facilitates possible introduction of this trait in *C. annuum*.

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Table 1. Reproduction factor (RF), reproduction index (RI) and degree of resistance (DR) of the *Meloidogyn eenterolobii* in 39 *Capsicum* spp. accessions.

Accessions	RF	RI(%)	DR
C. annuum			
UENF 1422 'Ikeda'	29.50 **a**	100.00 **b**	S[1/]
UENF 1381	17.44 **b**	80.86 **b**	S
'Criollo de Morellos'	12.72 **b**	50.21 **b**	S
UENF 1622	10.80 **b**	70.91 **b**	S
UENF 1623	19.96 **b**	394.70 **a**	S
UENF 1626	13.47 **b**	99.55 **b**	S
UENF 1627	38.42 **a**	190.96 **a**	S
UENF 1717	3.26 **c**	49.03 **b**	SR
UENF 1740	33.55 **a**	140.98 **a**	S
UENF 1741	8.00 **b**	67.61 **b**	S
UENF 1750	3.38 **c**	36.90 **c**	SR
UENF 1799	25.00 **a**	211.81 **a**	S
C. chinense			
UENF 1554	3.14 **c**	47.56 **c**	SR
UENF 1703	3.68 **c**	24.92 **c**	MoR
UENF 1706	2.72 **c**	34.09 **b**	SR
UENF 1730	0.30 **c**	8.79 **c**	R
UENF 1764	22.71 **a**	72.53 **b**	S
UENF 1765	38.35 **a**	388.30 **a**	S
UENF 1770	17.33 **b**	115.00 **b**	S
UENF 1772	29.05 **a**	101.12 **b**	S
UENF 1780	4.35 **c**	25.79 **c**	SR
UENF 1792	6.33 **b**	112.32 **b**	S
UENF 1798	6.31 **b**	97.38 **b**	S
C. baccatum			
UENF 1490	13.49 **b**	75.03 **b**	S
UENF 1624	7.50 **b**	80.03 **b**	S
UENF 1628	33.73 **a**	119.71 **b**	S
UENF 1635	4.24 **c**	39.43 **b**	SR
UENF 1714	2.80 **c**	24.69 **c**	MoR
UENF 1718	7.73 **b**	49.54 **b**	SR
UENF 1732	10.49 **b**	51.77 **b**	S
UENF 1733	24.19 **a**	113.99 **b**	S
UENF 1737	35.07 **a**	140.23 **a**	S
UENF 1797	8.89 **b**	92.69 **b**	S
C. frutescens			
UENF 1731	6.36 **b**	75.33 **b**	S
UENF 1747	7.05 **b**	37.74 **b**	SR
UENF 1766	22.24 **a**	220.98 **a**	S
UENF 1775	10.44 **b**	54.50 **b**	S
UENF 1776	5.12 **b**	51.52 **b**	S
UENF 1790	4.38 **c**	53.02 **b**	S

[1]S: Susceptible; SR: Slightly resistant; MR: Moderately resistant; and R: Resistant

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Variation for fruit shape morphology and candidate genes in eggplant materials

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Abstract

Cultivated eggplant (*Solanum melongena*) presents a wide phenotypic diversity for fruit morphology. New technologies have improved the quantity and quality of data that can be obtained for the morphological characterization of fruit traits. In this respect, the free software Tomato Analyzer allows the accurate and objective measurement of fruit shape and size of eggplant fruits. Tomato Analyzer characterization coupled with molecular characterization of candidate genes for fruit shape can be useful for elucidating the genetic control of fruit shape in eggplant, as well as for marker assisted selection. We crossed two eggplants with different fruit shapes: semi-round (Cristal) and long (Feng Yuang Purple) and obtained the F2 generation. A wide variability in fruit shape, which was characterized with Tomato Analyzer, was observed in the 53 individuals of the F2. These results, together from those of the F2 of another cross between a long and slender eggplant accession (MM1597) and a flat and fasciated accession (A0416) suggest that several genes affecting fruit shape are segregating in these populations. Conversely, a number of key genes have been described as responsible for controlling fruit shape in Solanaceae fruits. Among them, genes *SUN* and *OVATE* control fruit elongation in tomato fruits. In this respect, we are evaluating the allelic diversity of these genes in the Cristal and Feng Yuang Purple parentals. This will help us to develop molecular markers of the different genes and evaluate the potential role of the alleles in the control of fruit shape in eggplant. Also, other progenies developed by INRA which include different fruit shapes will be used for identifying possible allelic diversity of these genes within *S. melongena*. These materials may be useful for the identification of fruit shape QTLs and genes other than *SUN* and *OVATE*. These studies will help us to shed light on the genetics of fruit shape in eggplant as well as to identify the loci involved in creating the large and variably shaped fruit characteristic of the eggplant germplasm.

Keywords: *OVATE* gene, *Solanum melongena*, *SUN* gene, Tomato Analyzer.

Photosynthesis of two new genotypes of eggplant (*Solanum melongena* L.) in the Colombian tropic. I: effect of the water state and co_2 pressure.

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Abstract

The current situation of climate change, especially in the tropics, makes every day more important to know the functioning of the species on the basis of the changes in the energy flow. The 100% of the production of eggplant in Colombia is of small-scale farmers, but they do not know the response of new varieties towards the increases in CO_2 and the variation of the rainfall regime. In this work, we evaluated the photosynthesis (Pn) of two new genotypes of eggplant (CO-015, CO-029), subjected to low, normal and high irrigation levels (421, 842 and 1684 mm) and normal and high CO_2 concentrations (350 and 437 ppm). In the vegetative phase, the highest rates of photosynthesis ($p<0,01$) were reported by CO-029 (12.4 µmol CO_2 m^{-2} s^{-1}) when it was subjected to 1684 mm of water and 437 ppm of CO_2 pressure. In the flowering phase, photosynthesis rates were increased by 49.2 % compared to those observed in the vegetative phase and by 52.8 % compared to the fruiting stage, regardless of the watering level and CO_2 amount. At this stage, the highest values ($p<0.01$) were recorded in CO-029 (24.4 µmol CO_2 m^{-2} s^{-1}), with a watering level of 1684 mm of water and 437 ppm of CO_2 pressure; the lowest values were found in CO-015 (3.8 µmol CO_2 m^{-2} s^{-1}) with a watering level of 421 mm and 350 ppm of CO_2 pressure. In the fruiting phase, only the higher level of watering tested had significant effects on photosynthesis ($p<0,05$). Thus, the higher rates of photosynthesis may be obtained for CO-029 genotype during the flowering stage with no limitation to irrigation water.

Keywords: climatic change, crop physiology, water stress, carbon dioxide, eggplant.

Introduction

In Colombia 209 hectares are cultivated with eggplant, which are equivalent to a production of 1.791 tons. The Córdoba department is the largest domestic producer in Colombia, with a share of 42.95% equivalent to 111 has, a production of 769 tons and yield of 6.9 $ton.ha^{-1}$. However there is an annual reduction in yield of 1.6%, possibly because of changes in temperature and precipitation, as well as agronomic crop management (Agronet, 2013).

Vegetable production in Colombia is mainly of small producers, which use an inadequate technology that starts with a seed material obtained in their own plot or in the neighboring producers. Production takes place with little technological knowledge which influences the performance and stability in terms of production quality. Creole cultivars are characterized by a large non-uniformity in size and weight, seasonality of harvest, and variable coloration. These interconnected factors, contribute to reduce losses by up to 25% of the crop. The main technological limitations identified for cultivation of eggplant in the region are the production of good quality seed and the lack of varieties adapted to these environmental conditions with good features for the national and international market.

Climate change experts agree that periods of low and high rainfall will become more and more intense. It is also known that the interannual variability in global CO_2 increment shows strong spatial relationships to both tropical and temperate temperatures (Adams and Piovesan 2005). Thus, tropical crops are likely to be more frequently subjected to prolonged periods of drought and excessive rainfall in a single production cycle. As a consequence, moderate water stress in eggplant can significantly reduce the chlorophyll content, meaning a reduction in photosynthesis and hence

plant growth. This study evaluated the photosynthetic behavior of two new varieties of eggplant when subjected to three different watering supplies (low, average, high) and to any increases in atmospheric CO_2.

Materials and Methods

The research was conducted in the first half of 2012 in the greenhouse of the Faculty of Agricultural Sciences at the Córdoba University in Monteria-Cordoba (Colombia), geographically located at 8°52' north latitude and 76°58' west longitude, average temperature of 28 °C and relative humidity of 83%. This town is located in the transition zone of Tropical Dry Forest - Tropical Moist Forest (Holdridge, 1986). The genotypes evaluated were CO-015 and CO-029. Seedlings were established in a period of 30-45 days and then placed in plastic pots. We assessed the rates of photosynthesis in the vegetative, flowering and fruiting phases, under a completely randomized design with factorial arrangement (2 x 3 x 2) and 4 repetitions, where the factor A corresponded to genotypes, factor B at three levels of water supply: low, average and high (421, 842 and 1684 mm during the cycle), and the C factor to two CO_2 concentrations (350 and 440 ppm). The results were analyzed in the statistical package SAS 9.1.

Results

Vegetative stage: In terms of CO_2 levels, the results indicated highly significant differences in the photosynthesis for the main effect of water regime and significant for double interactions between CO_2 concentration and genotype and water status (Table 1).

Table 1. Analysis of variance for photosynthesis (µmol $CO_2.m^{-2}.s^{-1}$) in the vegetative, flowering and fruiting stage of two new eggplant genotypes.

	Vegetative		**Flowering**		**Fruiting**	
Source of variation	**Probability**	**Significance**	**Probability**	**Significance**	**Probability**	**Significance**
Model	0.0010	*	<.0001	**	0.0012	*
Genotype (A)	0.3164	NS	0.0767	NS	0.3026	NS
Water supply (B)	0.001	**	<.0001	**	<.0001	**
CO_2 concentration (C)	0.2282	NS	0.0332	*	0.9279	NS
A x B	0.3410	N.S	0.2792	NS	0.3908	NS
A x C	0.0104	*	0.6168	NS	0.7084	NS
B x C	0.0037	*	0.1361	NS	0.2339	NS
A x B x C	0.4838	NS	0.3333	NS	0.2818	NS
CV (%)	33.4		21.0		17.5	
R^2	0.62		0.87		0.65	

NS: No significant differences; *: Significant differences ($p<0.05$); **: High significant differences ($p<0.01$). CV: Coefficient of variation.

The interaction analysis of the water status x CO_2 concentration indicated that to increased water supply, CO_2 fixation is greater both environmental and enriched levels this gas. The interaction decomposition indicated that this was explained by the significant increase in photosynthesis Pn (57%) in high levels of environmental CO_2 (data not shown). Similarly, the interaction genotype x CO_2 concentration was observed that increasing CO_2 concentration of 350 to 440 ppm, CO-029 responded significantly increasing their photosynthetic rate in 47.6% ($p<0.05$) (Table 2).

Table 2. Double-entry analysis of average photosynthesis (μmol CO_2 $m^{-2}s^{-1}$), for the interaction genotype x CO_2 concentration in the vegetative stage of two new eggplant genotypes.

Genotype	CO_2 Concentration		Total unlike
	350	440	
CO-015	12.53	10.33	2.19 NS
CO-029	8.26	12.20	-3.93*

NS: No significant differences; *: Significant differences ($p<0.05$); **: High significant differences ($p< 0.01$).

Flowering Phase: Photosynthesis difference was significantly influenced by CO_2 concentrations and highly significant by water supply (Table 1). Regardless of the genotype and the CO_2 concentration, plants higly watered (1684 mm) achieved higher rates of photosynthesis with values of 22.5 mol CO_2 $m^{-2}s^{-1}$, followed by normal (842 mm) and reduced (421 mm) watering, with values of 15.07 and 6.08, respectively. The increasing of CO_2 concentrations enhanced the photosynthetic rate by 21.42%, regardless the genotype or the watering level.

Fruiting phase: Only water status exerted an effect on photosynthesis ($p<0.01$) (Table 1). The results indicate that, under controlled conditions and regardless the genotype or the concentration of CO_2, the higher level of watering tested increases the rate of net photosynthesis (11.53 μmol CO_2 $m^{-2}s^{-1}$), followed by normal (10.31 μmol CO_2 $m^{-2}s^{-1}$) and reduced watering (8.53 μmol CO_2 $m^{-2}s^{-1}$). The results are consistent striking with the two stages of development discussed above.

Discussion

The results observed in the vegetative phase suggest that it is possible to increase photosynthesis (mainly in CO-029), increasing the concentration of CO_2, provided that together with high levels of water supply. This could be very important because an increase in photosynthesis increases lead to similar synthesis in leaves and to increase leaf area in the early stages.

Physiological responses to elevated CO_2 are variable between crops: e.g. in cotton, by doubling the atmospheric concentration of CO_2, the rate of stem elongation increased by 20%, the leaf area by 25% and the dry mass of roots and fruits were higher by 30% and 26%, respectively (Reddy et al. 1998); on the contrary, the harvest index was the same at high concentrations of CO_2. Consistently, the behavior of photosynthesis in the flowering phase suggests that for any of the genotypes, photosynthesis rates are increased by having more CO_2 pressure and increased water availability. Wahid et al. (2007), indicated that light and the amount of water available are significantly involved in the physiological behavior of plants. Finally, the results of the reproductive phase confirm the importance of water supply in this species, suggesting that under controlled conditions of cultivation and CO_2 enriched environment, producers may choose to cultivate CO-029 genotype, providing a high level of irrigation

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Photosynthesis of two new genotypes of eggplant (*Solanum melongena* L.) in the Colombian tropic. II: effect of temperature and radiation.

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Abstract

The physiological plant responses to environmental changes are of great help to the inter-disciplinary work of plant physiologists and plant breeders. We evaluated the response of the net photosynthesis (Pn) in two new varieties of eggplant (CO-015 and CO-029) subjected to low, medium and high levels of photosynthetically active radiation PAR (500, 1000 and 1500 µmol photons.$m^{-2}.s^{-1}$), under two ranges of day/night temperature (33/24 and 28/21 °C). Results indicated that in the vegetative stage, high levels of PAR (1500 µmol photons.$m^{-2}.s^{-1}$), increased of 11.3 % the Pn rate of CO-029 compared to CO-015. However, as the day temperature increased from 28 to 33 °C, the Pn reduced by 7.05 % for CO-015 and 13.56 % for CO-029. In flowering stage, photosynthesis declined in both genotypes ($p<0.01$) in 31 and 18% at high light and temperatures respectively. Finally, in fruiting stage a significant effect of the radiation was not observed, but the Pn of CO-015 (8.7 µmol $CO_2.m^{-2}.s^{-1}$) and CO-029 (10.1 µmol $CO_2.m^{-2}.s^{-1}$) was reduced ($p<0.05$) of 17% and 39% respectively with the day/night higher temperature. These results suggest that the Pn of the two genotypes may vary according to their phenological status, with the PAR having more importance in early stages, while the increase in temperature negatively affects the Pn of eggplant at any phenological phase.

Keywords: Photosynthetically active radiation PAR, climate change, crop physiology, temperatures.

Introduction

The earth climate changes make really important to know the responses of plant species to any changes in energy flows. However, Colombia eggplant producers usually belong to low-income rural economy and do not know how species react to high temperatures and light levels. Eggplant is grown in tropical and temperate countries (Aramendiz et al. 2008; Concellón et al. 2007); For their production, the temperature of the day should be between 25 and 30 °C, accompanied by a good solar radiation to meet the needs of the plant and therefore greater efficiency in photosynthesis, so that carbohydrates can be translocate to their sinks (Aramendiz et al. 2008). Temperatures above 32 °C accelerate maturation and malformation of fruits while temperatures higher than 35°C for prolonged periods may cause low pollen viability and destabilization of pectic materials (Zhang and Chen 2006; Souza and Resende 2006). When water availability is adequate, the crop relatively well support elevated temperatures up to 40-45 °C (Infoagro 2003). The aim of our work was to evaluate the photosynthetic response of new varieties of eggplant with progressive increases in temperature and photosynthetically active radiation (PAR).

Materials and Methods

This research was conducted during the second half of 2011, in the Faculty of Agricultural Sciences of the University of Cordoba, located at Monteria - Colombia, 14 m.a.s.l., 8°52' north latitude and 75°58' west longitude from the meridian of Greenwich, 1346.1 mm average annual rainfall, relative humidity of 83%, annual average temperature of 28 º C, annual sunshine of 2108 hours and living area called the transition zone of Tropical Dry Forest - Tropical Moist Forest

(Holdridge 1986, cited by Palencia et al. 2006). Two new varieties of eggplant (CO-015 and CO-029) were evaluated, which were established in pots of 5 liters in volume, and a substrate made of a soil representative of the region.

Plants were arranged as a randomized complete block with 4 replications under 2x3x2 factorial arrangements. The factor A corresponded to new varieties (CO-015 and CO-029). Factor B were three levels of PAR (500 µmol above and below the reference value region PAR: 1.000 µmol photons.m^{-2}.s^{-1}). These levels of radiation were simulated by chlorophyll fluorescence module (CFM) of the infrared gas analyzer (IRGA) Ciras 2 of PP Systems Inc. The factor C corresponded to two temperature ranges, high (33 °C) and average (28 °C). To achieve high temperatures, the plants were placed in controlled greenhouse. Each plot was watered considering water requirements of the growth stages, the plants were managed according the techniques employed in the region. At each level of temperature and radiation and with a CO_2 concentration of about 350 ppm, the photosynthesis rates (Pn) were measured in the vegetative, flowering and fruiting stage, with a CO_2 concentration of about 350 ppm. The results were analyzed with the Statistical Analysis Sytem (SAS), by analysis of variance. When statistical differences were detected, we used the Tukey test ($p \leq 0.05$), and the interactions were broken through crosstabs.

Results

In general, we observed that genotype x radiation x temperature interactions had a significant effect at the vegetative stage, while at flowering and fruiting stages radiation and temperature had ignificant effect regardless of the genotype (Table 1).

Table 1. Mean squares and significance levels for photosynthesis rates (Pn) of two new eggplant genotypes under different radiation and temperature levels. Montería – Colombia (2011).

Source of variation	L.D.	Phenological stage		
		Vegetative	Flowering	Fruiting
Genotype (A)	1	3.63	13.54	0.16
Radiation level (B)	2	190.36**	205.98**	58.48**
Temperature level (C)	1	21.06	88.29**	92.20**
A x B	2	24.12	7.14	0.06
AxC	1	2.61	27.15	19.25*
B x C	2	63.42*	13.24	1.90
A x B x C	2	49.02*	4.76	7.63
Error	36	5.1797	12.5174	3.3555
R^2		0.65	0.44	0.59
CV (%)		18.90	25.58	22.74

*: Significant differences ($p < 0.05$); **: High significant differences ($p < 0.01$). CV: Coefficient of variation.

Vegetative phase. The results indicated that at this stage, photosynthesis rates are negatively affected by high (33 °C) temperature under non-saturating light, ie, about 500 µmol photons.m^{-2}.s^{-1} (Table 2).

Table 2. Analysis of temperature x radiation interaction in photosynthesis (µmol $CO_2.m^{-2}.s^{-1}$) at vegetative stage of two new eggplant genotypes.

Radiation (µmol photons.$m^{-2}.s^{-1}$)	Temperature		Difference
	Minimum (28°C)	Maximum (33°C)	
500	8.48	8.00	0.48*
1000	8.18	7.81	0.37 NS
1500	7.85	7.48	0.37 NS

NS: No significant differences; *: Significant differences (Pr < 0.05)

Flowering stage. At this stage, the radiation levels and temperatures influenced significantly the photosynthesis for both the two genotypes, photosynthesis declined by 31% when light levels reach 1500 µmol photons.$m^{-2}.s^{-1}$ and by 18% under high temperatures.

Fruiting stage. In fruiting stage, photosynthesis rates were affected by the combined effect of radiation and genotype, and the breakdown of this interaction indicated that, in accordance with previous phases, photosynthesis rates are significantly reduced under high radiation, but CO-029 is more affected compared to CO-015 (Table 3).

Tabla 3. Analysis of radiation x genotype interaction in the photosynthesis on fruiting stage of two new eggplant genotypes.

Radiation (µmol photons/$m^{-2}.s^{-1}$)	Genotypes		Difference
	CO-015	CO-029	
500	9,42	9,3	0,12 NS
1000	8,15	8,1	0,05 NS
1500	6,76	6,56	0,2 *

NS: No significant differences; *: Significant differences (Pr < 0.05)

Discussion

In the vegetative phase, the photosynthesis of the two genotypes was reduced under high light intensity (1000 and 1500 µmol photons.$m^{-2}.s^{-1}$), regardless of the temperature range. These results would be consistent with the saturation curves of light in most C_3 species (Taiz and Zeiger, 2006). In the flowering stage, high radiation and elevated temperatures reduce photosynthesis rates, indicating the limits of the two new genotypes to these environmental variables at this stage. Finally, in the fruiting stage, responses to high radiation confirm what discussed in the two previous stages, but this effect is more negative for CO-029. Presumably, reduction in photosynthetic activities may be due to the high radiation which could saturate the electron transport chain (Germ, 2005) and temperatures which may affect the efficiency of carboxylation of Rubisco (Waheed et al. 2007).

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Effect of irrigation water salinity levels on yield and drainage water quality of bell pepper cultivars in soilless media

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Abstract

Irrigation water quality in bell pepper (*Capsicum annuum* L.) production is one of the main yield reducing factor in Hungary. The critical elements in irrigation water are sodium, carbonates and chloride. A study was conducted to evaluate the effects of irrigation water derived salt on yield and drainage water quality of five bell pepper cultivars. The experiment was carried out under a non-heated high tunnel (5 x 20 m^{-1}) in coco coir filled buckets (7 litres media per bucket) using the following water treatments: 40 mg/l Na^{2+} (control), 80 mg/l Na^{2+} (S1) and 120 mg/l Na^{2+}(S2). Our water source naturally contains the sodium concentration of control treatment the other treatments received the control water with additional salts. Cultivars used in this experiment: 'Cinema', 'Celtic', 'Carma', 'Brendon', 'Zalkod'. Completely randomized block design was used to determine the effects of salt levels on different plants and leachate parameters. Results suggest that the changes of EC level (mS/cm^{-1}) of leachates had an increasing trend during the growing season and reached its maximum at the end of August in S1 and S2. The highest EC values were recorded in 'Brendon' cultivars regardless of salt treatment (12 mS/cm). In all cultivar the potassium release were high in control treatment. Although the pattern of potassium leachate loss were similar in all salt treatments. The highest losses were recorded in 'Celtic' cultivar (117 mg/l) in control. The early yields of bell pepper cultivars were not affected significantly by the saline water treatments. Although, 'Celtic' cultivar had a tendency to produce significantly higher number of Blossom End Rotted (BER) fruits. With the highest applied sodium level (S2) the marketable yields and root mass in all cultivars were not decreased as it was expected, moreover the highest nutrient use efficiency (NUE) was recorded in the S1 treatment.

Keywords: bell pepper cultivars, drainage water quality, saline water, yield

Introduction

Irrigation water source derives from formation water layer, which shows wide variation in water chemical composition. Hungarian growers in order to maximize their profit gradually shifted the production from soil to soilless media under unheated high tunnels. The water quality used in soil-based production may works in soil, but for soilless cultivation the standards are higher. Extensive works have been already done in semi-arid and arid regions, where saline irrigation water and high evapotranspiration led to salt accumulation in the top layers of soil (Maas and Hoffmann 1977, Kempkes and Stanghellini, 2003). Under protected cultivation even in higher latitudes salt accumulation in soil or in soilless media can be a potential yield-reducing factor.

Materials and Methods

Experimental details

The experimental set-up was conducted in the experimental field of SZIE, Szarvas, Hungary (46°52'N, 20°31'E, 81 m a.s.l.). The experiment carried out under a non-heated high tunnel (5 x 20 m^{-1}) in coco-coir filled buckets (7 litres media $bucket^{-1}$) using the following water treatments: 40 mg/l Na^{2+} (control), 80 mg/l Na^{2+} (S1) and 120 mg/l Na^{2+}(S2). Irrigation scheduling controlled by

time based drip irrigation system (K-Rain, RPS 469) fine-tuned manually for actual plant need. Our water source naturally contains the sodium concentration of control treatment, rest of the treatments were received the control water with additional salts. Cultivars used in this experiment were the following: 'Cinema', 'Celtic', 'Carma', 'Brendon', 'Zalkod'. Completely randomized block design was used to determine the effects of salt levels on different plant and leachate parameters. Air temperatures and relative humidity recorded along the growing season using a WS-8610 data logger (LaCrosse Tech.). Bell pepper fruit weight was recorded by using an analytical scale (Sartorius) and fruit diameter was recoded by a calliper. Number of Blossom End Rotted fruits, fruit diameter and weight were recorded after each harvest. A total of 10 harvests carried out from the beginning of July until the beginning of October. Leachate samples collected after drainage occurred and immediately transferred to the laboratory for determination of mineral N by nitroprusside method. Na and K measured by flame photometer (OE-85), while Ca and Mg determined by AAS (Unicam Scino 4).

Results and Discussion

Temperature and irrigation scheduling

Heat waves (Tmax≥35 °C) had negatively affected plant production during the growing season causing an increased number of unmarketable BER fruits. As an average the heat days only occur 2-3 times per year, during the season of 2012 summer we recorded 17 days when the daily maximum temperature exceeded 35°C. Applied feed solution quantity (130-180 ml per application) and irrigation frequency (2-10 times per day) for each irrigation event varied by plant growth stage and actual weather situation.

Cumulative yield, early yields, root dry weights and fruit quality

Cumulative yields exhibited wide variation over the experimental period (Table 1), but even in case of lowest recorded yields the cumulative quantity was in the range expected from unheated polytunnel. Yield response for increasing saline levels was not decreased significantly in case of 'Brendon', 'Celtic' and 'Zalkod'. This response was in-line with the findings of Reshef at al. (2004) where increasing EC and saline levels showed no yield reduction in cv. 'Mazurka'. However, 'Carma' and 'Cinema' showed some slight decline in cumulative yields, for elevated salt concentration. It is important to notice that the last two cultivars were the best performers in fruit quality.

Table 1. Cumulative yields (kg m^{-2}) over 10 harvest times of different bell pepper cultivars

Cumulative yields (kg m^2)					
	'Brendon'	'Carma'	'Celtic'	'Cinema'	'Zalkod'
Control	9.4a	11.1b	9.8a	9.1a	10.9b
S1	10.7b	11.7c	11.5c	11.0b	11.8c
S2	11.1b	10.1a	12.3c	9.4a	11.6c

The higher market price for early yields is important for farmers; therefore the cumulative yields of first four harvest events gave a better insight to evaluate each variety (Figure 1.). Cv. 'Celtic' achieved the highest early yields, but it is important to mention that the BER fruits reached 4-26% in the total early yield (data not shown). 'Cinema' was an outstanding cultivar in terms of early yields. Regardless of increasing saline levels, continuous high early yields produced even in S2 treatment in 'Cinema'. The cultivar was not just a heavy cropper, but the marketable yield was the highest compared to other cultivars. In case of harvest no. 4 the extra class fruits (fruit diameter 7

cm or greater) reached 71% of the total harvested yield and 1st class fruits in other early harvest times were always above 50%.

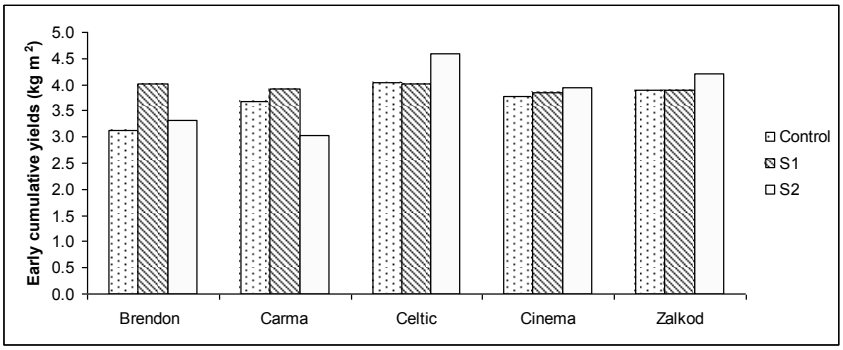

Figure 1. Cumulative early yields (kg m^{-2}) of different bell pepper cultivars

As for 'Zalkod' the high early yields (Figure 1.) were not coupled with such outstanding marketable yields as experienced in case of 'Cinema', and most of the fruits remained in 1st and 2nd class.

Increasing saline levels were not led on decreasing root mass in this experiment, while other authors found strong root mass decrease in soil (Pascale et al. 2003). Possible reason for that are the high cation exchange capacity (CEC) of coco-coir and large pore space resulting in easy leachate loss of cations or binding strongly the positively charged sodium ion. 'Cinema' exhibited reverse response for increasing sodium concentration and gave the highest root dry weight in S2 treatment.

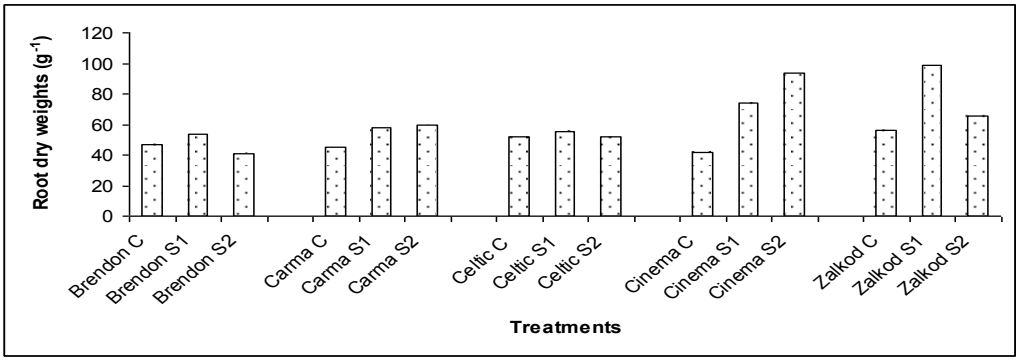

Figure 2. Root dry weight of bell pepper cultivars affected by saline irrigation water

Nutrient Use Efficiency (NUE)

In general, the NUE varies from 30 to 60% in horticulture. Under our experimental condition the main macro elements (NPK) utilization were the same as reported by many authors, although each nutrient has its own utilisation pattern changing with different salt treatments. By increasing saline levels in irrigation water the utilisation of N slightly increased up to S1 treatment, while in S2 the cultivars exhibited a lower NUE of N. Many N loss pathways existing not just in soil, but in soil less media as well (Vallejo et al. 2005). The water holding capacity (WHC) of coco coir need to be maintained at 70 % in order to keep continuous water and nutrient supply for bell pepper. Healthy rhizosphere environment under such condition claims 15-20% drainage for better leaching of salts from the limited volume of root balls. Therefore, highly mobile negatively charged nitrate (NO3-) N

can be easily lost through drainage water. The positively charged P and K bound for organic colloids strongly, moreover the concentration of K exceeded the concentration of other nutrients. Consequently, K uptake was the highest compared to other macro elements. The highest (59%) NUE of K exhibited by '*Celtic*' cultivar in S2 salt treatment. Phosphorous retention of coco-coir is well known, moreover the relative amount of this macro element was lower than each of N or K in nutrient solution. Perhaps this was one of the reasons for low NUE of P.

Drainage EC and pH

During the experiment the EC of drainage samples varied from 1.3 to 13.5 mS/cm. Highest changes of EC was observed in cv. Brendon irrigated with control feed solution. There was a continuous increase in drainage EC along the season, it was probably the saturation of coco coir through the growing season. Increasing saline levels in feed solutions also increased linearly the EC of drainage water but only in cv. Celtic. The other four cultivars responded differently. The high water demand concentrated the EC of drainage water, but with increasing saline level. The EC of drainage did not increased linearly.

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Effect of different colour of shading nets on spectral reflectance and SPAD values on 'Capia' type red sweet pepper leaves

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Abstract

Special 'Capia' type red sweet pepper varieties are consumed fresh or processed in fully ripe stage in the Hungarian diet. Varieties of this type, due to their excellent flavour, are processed by the processing food industry, as well as being sold for fresh consumption. Production of this vegetable has expanded in the last decades under unheated greenhouses in Hungary. To avoid the problem of too high temperature and high radiation during the summer period, growers reduce the incident radiation with the use of different coloured shading screens. Photoselective nets provide a new tool for light-quality manipulation in protected horticulture. An experiment was conducted in unheated greenhouses to evaluate the effect of coloured plastic net on spectral reflectance and chlorophyll meter (Minolta SPAD-502) values of red sweet pepper 'Capia' type hybrids T112 and K10 F1 in 2012. To determine the effects of shade, simultaneous comparisons were carried out among greenhouses that were either not shaded (control treatment) or shaded with red and green coloured plastic net positioned above the roof. The shading screens were kept until the end of the crop cycle and fruits were picked until August. We measured spectral transmittance of covering materials and reflectance of paprika leaves spectral range between 325-1075nm, by portable spectroradiometer. SPAD (Soil Plant Analysis Development) values of paprika leaves were also recorded by chlorophyll meter. The two paprika hybrids showed significantly different reflective spectral characteristics. Leaves of K10 variety showed higher reflectance under red net covered greenhouse than control, while under green net was lowest, but the reverse occurred in T112 hybrid. Results showed significant differences among covering materials in quantity and quality of transmitted light also. Shading ratio were 31%, 42% and 39% in control, red and green net covered greenhouses, respectively, for the reflectance spectra between 325-1075nm, against direct sunlight. Shading effects decreased continuously in relation to increasing wavelength at control and red net covered greenhouses. Seasonal evolution of SPAD values did not show significant differences among the three greenhouses, but we perceived varietal effect. Leaves of the same age and position were found to respond to different light quality the highest values at middle of July were obtained under the red net, followed by the control and green net.

Keywords: red sweet pepper, 'Capia', spectral reflectance, SPAD

Introduction

Capsicum annuum L. is the main cultivated Capsicum species, and has wide range of variability in quantitative and qualitative character of fruits (Bosland and Votava, 1996). Special 'Capia' type red sweet pepper varieties are consumed fresh or processed in fully ripened stage in the Hungarian diet. The 'Capia' varieties are the best-known type in the Balkans and have become popular in recent years in Western Europe (Csilléry, 2006). Varieties of this type, due to their excellent flavour, are processed by the processing food industry, as well as being sold for fresh consumption. As a consequence, production of this vegetable has expanded in the last decades under unheated greenhouse conditions in Hungary (Fruitveb, 2011). To avoid the problem of too high temperature and high radiation in low-roof greenhouses during summer period, growers reduce the incident radiation with the use of different coloured shading screens (Zhu et al. 2012).

Photoselective nets represent a new agro-technological concept, which aims at combining the physical protection, together with differential filtration of the solar radiation (Ilić et al. 2012; Legarrea et al. 2012). Shahak et al. (2008) have grown bell pepper under shade nets of 30-40% shading for producing high-quality fruit, avoiding sunburns, and saving on irrigation at the arid Besor area in Israel. Their measurements of average leaf chlorophyll content, photosynthetic rate of exposed leaves, and canopy fresh and dry weight did not reveal significant differences either.

Díaz-Pérez (2013) found that net photosynthesis and stomatal conductance decreased and leaf transpiration increased with increased shade level. High shade levels reduced leaf photosynthesis, but moderate shade levels (30% and 47%) are favourable for bell pepper plant growth and functions.

The aim of this study was to compare shading effects of different coloured screens on reflectance spectra and SPAD values of leaves of 'Capia' type pepper plants under unheated greenhouse conditions in Hungary.

Materials and Methods

The unheated greenhouse experiment was carried out to evaluate the effect of coloured plastic net on leaf spectral reflectance and chlorophyll values of red sweet pepper in the test site of Lajosmizse in Hungary. Conventional 'Capia' red, sweet pepper cultivars were study in this investigation, namely the T112 and K10 F1 hybrids in 2012. The experimental field, with a size of 100 m^2 per unheated greenhouse, was a brown forest soil, with mechanical composition of sandy-clay, the subsoil water cannot influence the water turnover. Seeds were sown on the 15th of March in heated greenhouse and planted out to unheated greenhouse on the 24th of April in 2012. The pepper seedlings were planted in twin rows, with 0.3 m spacing inside the row and 1 m between adjacent twin rows, the space between the plants in the row was 0.3 m, and irrigated water was given out through drip irrigation system.

To determine the effects of shade, simultaneous comparisons were carried out among greenhouses that were either not shaded (control treatment) or shaded with red or green coloured plastic screen positioned above the roof. The shading screens were kept until the end of the crop cycle and fruits were picked until August. We have measured shading of covering materials and reflectance of paprika leaves in the spectral range between 325-1075nm, by portable spectroradiometer (ASD FieldSpec® HandHeld 2). Shading effect of treatments was measured with white reference panel, simultaneously recorded white reference panel outside and inside per treatments in a minute. Reflectance spectra of leaves were recorded in four replicates per treatments and cultivars. SPAD values of paprika leaves of the same age and position were also recorded by chlorophyll meter (Minolta SPAD 502 Plus) in the same replicates.

Results

Results showed significant differences among covering materials in quantity and quality of transmitted light. Cumulative shading ratios were 31%, 42% and 39% in control, red and green net covered greenhouses, respectively, for teh reflectance spectra between 325-1075nm, against direct sunlight on 2nd of August. These were similar (38%, 48% and 46% in control, red or green net covered greenhouses, respectively) in photosynthetically active radiation (PAR) spectral range (400-700nm). Shading effects decreased continuously in relation to increasing wavelength in control and red net covered greenhouse. Green covered greenhouse showed higher shading ratio (47%) than other two between spectral range 600-700nm, which is not negligible regarding photosynthesis (Fig. 1).

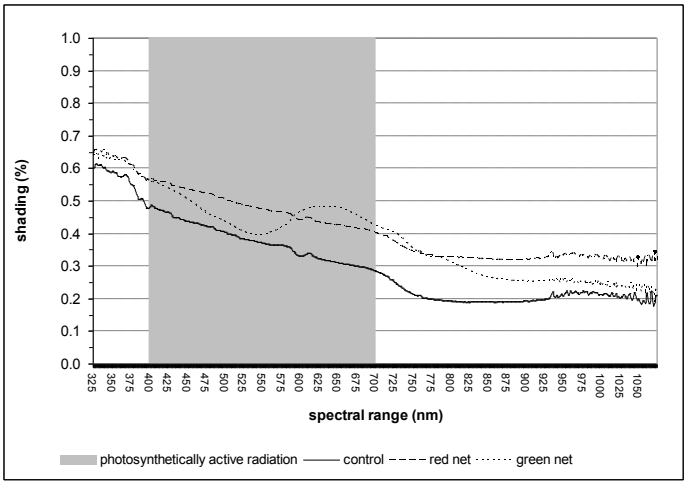

Fig. 1 Shading rates of different coloured plastic net covered greenhouses versus control in relation to reflectance spectra on 2nd of August in 2012.

Leaf reflectances were the lowest in the range of PAR in all of three greenhouses. The two paprika hybrids showed significantly different reflective spectral characteristics also. Plant leaves under red net covered greenhouse showed higher reflectance than control, while green was lowest above 400nm spectra in K10 crops (Fig. 2).

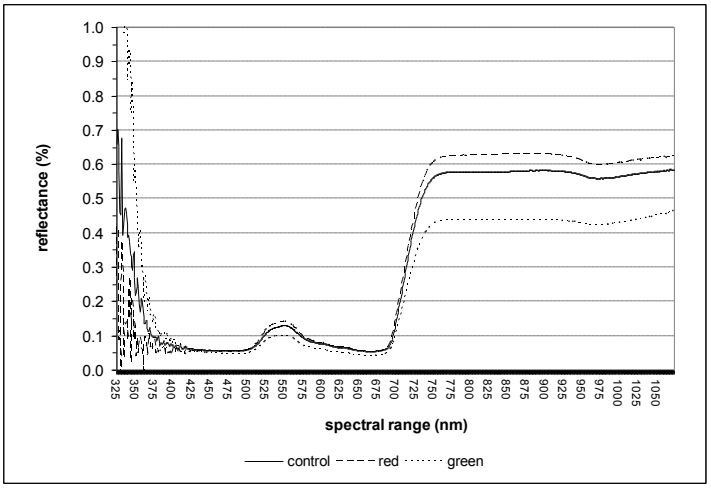

Fig. 2 Average spectral reflectance of K10 paprika leaves under different coloured plastic net covered greenhouses versus control on 2nd of August in 2012 (n=4).

In T112 hybrid plants, the results a reverse tend, in which green net covered greenhouse showed higher reflectance than control, while red was lowest in the whole spectra. The difference was significant above 700nm (Fig. 3).

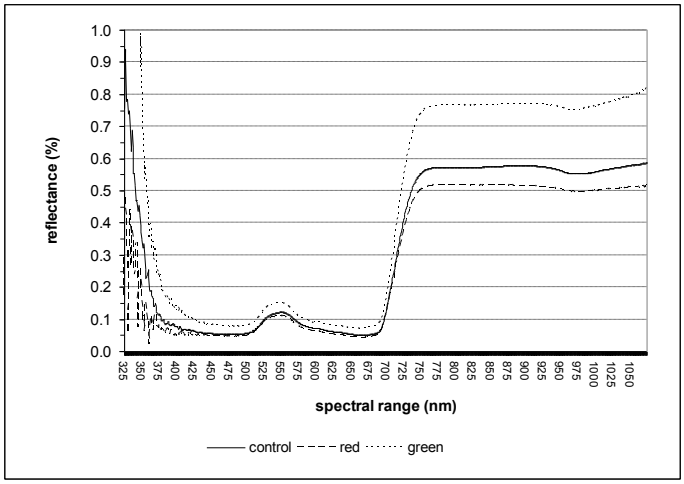

Fig. 3 Average spectral reflectance of T112 paprika leaves under different coloured plastic net covered greenhouses versus control on 2nd of August in 2012 (n=4).

Seasonal evolution of SPAD values did not show significant differences among the three greenhouses, but control plants produced higher values than red or green net shaded plants, respectively. Leaves of the same age and position were found to respond to different light quality. The highest values in the middle of July were obtained under the red net, followed by the control and green net in plants of K10 hybrid (Fig. 4).

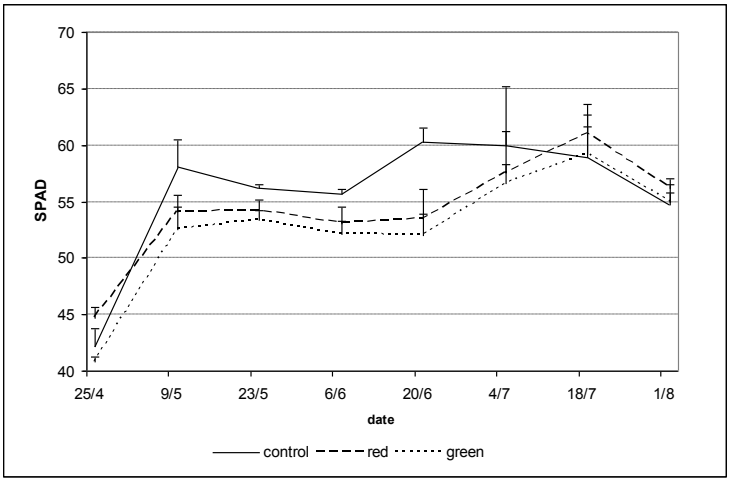

Fig. 4 Evolution of average SPAD values of K10 paprika leaves under different coloured plastic net covered greenhouses versus control in 2012 (n=20). Vertical bars represent the significant differences at P=0.05.

SPAD values were higher in the three greenhouses, but produced the same seasonal pattern in case of T112 hybrid plants (Fig. 5). Decreasing shading effect of aging coloured screens caused higher values in both treatments at the end of the season.

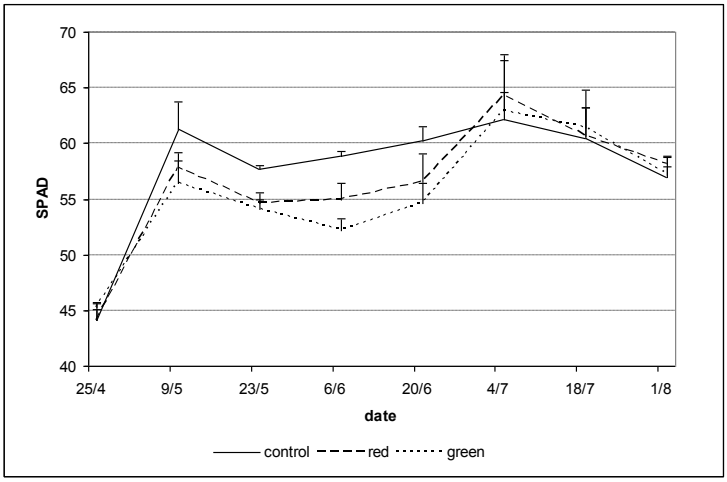

Fig. 5 Evolution of average SPAD values of T112 paprika leaves under different coloured plastic net covered greenhouses versus control in 2012 (n=20). Vertical bars represent the significant differences at P=0.05**.**

Our results are in agreement with previous studies, that indicate that moderate shade levels (30-40%) did not decrease average of leaf chlorophyll content significantly, which was favourable for bell pepper plant growth (Shahak et al. 2008; Díaz-Pérez, 2013). We did not observe any sunburn on fruit surface at all in both treatments compared with the control.

Conclusions

We can conclude that near-infrared spectral reflectance of leaves is more suitable to specify photo optical characteristic of paprika leaves. Shading screens decrease average leaf chlorophyll content, resulting in lower photosynthetic rate, but this is compensated by better fruit quality.

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Evaluation of Japanese *Capsicum* rootstock cultivars for resistance to Phytophthora blight and bacterial wilt, and for yield in grafted sweet pepper

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Abstract

Phytophthora blight and bacterial wilt are the most devastating soilborne diseases of sweet peppers (*Capsicum annuum*) in Japan. Since attempts at chemical, physical, and cultural control have had little success, grafting sweet peppers onto resistant rootstocks offers the only effective alternative. Therefore, in Japan, several rootstock cultivars that possess combined resistance to these diseases have been developed. We evaluated the resistance of five such cultivars ('Dai-Power', 'Buggy', 'Daisuke', 'Suketto C', and 'Bellmasari') to Phytophthora blight and bacterial wilt, and measured yields when they were used as rootstocks. 'Dai-Power' and 'Buggy' were highly resistant to Phytophthora blight, 'Bellmasari' was moderately resistant, and 'Daisuke' and 'Suketto C' were susceptible. 'Dai-Power' and 'Daisuke' were highly resistant to bacterial wilt, 'Buggy' and 'Suketto C' were moderately resistant, and 'Bellmasari' was susceptible. The yields when the cultivars were used as rootstocks were all higher than in non-grafted cultivation, and the difference was significant for 'Buggy' and 'Daisuke'. 'Dai-Power' appears to be the most promising rootstock cultivar to control Phytophthora blight and bacterial wilt in grafted cultivation of Japanese sweet peppers.

Keywords: bacterial wilt, grafting, Phytophthora blight, resistance, rootstock cultivars.

Introduction

Phytophthora blight caused by *Phytophthora capsici* and bacterial wilt caused by *Ralstonia solanacearum* are the most devastating soilborne diseases of sweet peppers (*Capsicum annuum* L.) in Japan. Since attempts at chemical, physical, and cultural control have had little success, the use of resistant cultivars offers an effective alternative. Previously, 'Bellmasari' was successful as the main rootstock cultivar in Japan. However, damage by both diseases has begun to increase in grafting cultivation using 'Bellmasari', so a new highly resistant rootstock is needed. For this purpose, several rootstock cultivars that possess combined resistance to both have been developed in Japan (Matsunaga et al. 2010).

The present study was conducted to clarify the differences in resistance to Phytophthora blight and bacterial wilt among the current Japanese rootstocks, using SCM334 as a control resistant to Phytophthora blight (Ortega et al. 1991) and LS2341 and 'Mie-Midori' as controls resistant to bacterial wilt (Mimura et al. 2009 and Matsunaga and Monma 1999). We also evaluated the fruit yields when the cultivars were used as rootstocks.

Material and Methods

Resistance to Phytophthora blight at the seedling stage

We used the current Japanese rootstock cultivars 'Dai-Power', 'Buggy', 'Daisuke', 'Suketto C', and 'Bellmasari' as test cultivars for resistance to Phytophthora blight, with SCM334 as a resistant control and 'Ace' as a susceptible control. We established a randomized block design with three

replications and 10 plants per replicate.

The *P. capsici* inoculum suspension was prepared according to the method described by Sugita et al. (2006) and Ueeda et al. (2006), with a minor modification. Strains of *P. capsici* maintained by NIVTS were grown on a V8 juice medium in 90-mm Petri dishes. The dishes were sealed with Parafilm and incubated in the dark at 28°C for 7 days. Thereafter, the Parafilm was removed and the strains were further incubated under fluorescent light at 28°C for 3 days. After the incubations, we poured 10 mL of distilled water into the Petri dishes and gently collected the zoosporangia with a paintbrush. The collected zoosporangia were incubated at 4°C for 1 h and then at 25°C for 3 h. The concentration of zoosporangia was adjusted to around 1.6×10^3/mL by adding distilled water.

On 8 May 2012, seeds were sown in sterilized soil in flats and germinated in a greenhouse. On 31 May, seedlings were excavated and their roots were gently washed and then dipped into the inoculum suspension for 1 min. After inoculation, the seedlings were transplanted into flats filled with sterilized soil and grown in a glasshouse. The soil temperature was maintained at about 28 °C and the minimum air temperature was kept above 15°C. On 15 June, we recorded the health or disease status of each plant and calculated the percentage of diseased plants in each accession.

Resistance to bacterial wilt in an infected field

We used the same rootstock cultivars in the resistance tests for bacterial wilt in an infected field, with LS2341 as a resistant control, 'Mie-Midori' as a moderately resistant control, and 'Ace' as a susceptible control. We established a randomized block design with three replications and 7 plants per replicate.

Strains of *R. solanacearum* isolated from an infected field at NIVTS were streaked on a tetrazolium chloride medium (Kelman, 1954) and incubated at 31°C for 48 h. Virulent colonies (fluidal white colonies with pink centers; Kelman, 1954) were transferred into Wakimoto liquid medium (Wakimoto, 1962) and cultured at 31°C with constant shaking for 48 h. The inoculum suspension was diluted with tap water to 10% of the original concentration, giving a bacterial concentration of 6.0×10^8 cells/mL.

On 16 April 2012, seeds were sown in sterilized soil in flats and germinated in a greenhouse. On 7 May, seedlings were transplanted into 10.5-cm-diameter plastic pots filled with sterilized soil and grown in a greenhouse. On 5 June, the seedlings were then transplanted into fields infected with *R. solanacearum* at NIVTS, at 30 cm between plants and 1.2 m between rows. On 26 July, part of the root system of each plant was cut with a sickle to facilitate infection, and 50 mL of the inoculum was poured into the soil around the base. On 20 September, we recorded the health or disease status of each plant and calculated the percentage of diseased plants in each accession.

Resistance to bacterial wilt at the seedling stage

We used the same accessions that we used in the infected field to establish a randomized block design with three replications and 10 plants per replicate.

Two strains of *R. solanacearum* (KP9547 and KP0779), supplied by the Kyoto Prefectural Agriculture, Forestry and Fisheries Technology Research Center, were used. Bacterial concentrations of both strains were prepared at 6.0×10^8 cells/mL using the same methods as used for the test in the infected field.

On 26 June 2012, seeds were sown in sterilized soil in flats and germinated in a greenhouse. On 17 July, seedlings were excavated, and their roots were gently washed and then dipped into an inoculum suspension for at least 5 min. After inoculation, the seedlings were transplanted into flats filled with sterilized soil and grown in a glasshouse. The soil temperature was maintained at about 30 °C. On 14 August, we recorded the health or disease status of each plant and calculated the percentage of diseased plants in each accession.

Yield as rootstocks in grafted cultivation

'Kyo-Suzu', a typical Japanese F_1 cultivar, was used as the scion and as the non-grafted control. On 6 March 2012, seeds of the rootstock cultivars were sown; on 12 March, seeds of the scion

Table 1. Percentage of diseased plants in the tests of inoculation with phytophthora blight or bacterial wilt.

Accessions	Phytophthora blight in seedlings	Bacterial wilt in an infected field	Bacterial wilt in seedlings	
			KP9547 isolate	KP0779 isolate
Dai-Power	0 a	19 a	13 a	30 a
Buggy	0 a	14 a	27 ab	70 b
Daisuke	100 c	5 a	17 a	23 a
Suketto C	100 c	24 a	87 cd	97 b
Bellmasari	17 b	81 b	63 c	97 b
[Controls]				
SCM334	0 a	-	-	-
LS2341	-	0 a	3 a	17 a
Mie-Midori	-	10 a	60 bc	83 b
Ace	100 c	100 b	100 d	100 b

Values within a column followed by the same letter do not differ significantly ($P<0.05$, Tukey's test).

cultivar were sown; and on 19 March, seeds of the non-grafted plants were sown. All seeds were sown in sterilized soil in flats and germinated in a greenhouse. On 11 April, grafting was conducted. On 20 April, the grafted seedlings were transplanted into 10.5-cm-diameter plastic pots filled with sterilized soil and grown in a greenhouse. On 11 May, the grafted seedlings were transplanted into open fields at NIVTS, at 40 cm between plants and 1.2 m between rows. We evaluated the yields of immature fruits from 18 June to 21 August.

Results and Discussion

Resistance to Phytophthora blight

In the seedling test, 'Dai-Power' and 'Buggy' both had a disease level of 0%, which was significantly lower ($P < 0.05$, Tukey's test) than those of any other accession except SCM334 (Table 1). 'Bellmasari' had a disease level of 17%, which was significantly higher ($P < 0.05$) than that of 'SCM334' but significantly lower than those of 'Ace', 'Daisuke', and 'Suketto C' (all 100%). These results suggest that 'Dai-Power' and 'Buggy' were highly resistant to *Phytophthora* blight, 'Bellmasari' was moderately resistant, and 'Daisuke' and 'Suketto C' were susceptible.

Resistance to bacterial wilt

In the infected field, 'Dai-Power' had a disease level of 19%, 'Buggy' of 14%, 'Daisuke' of 5%, and 'Suketto C' of 24%; these were significantly lower ($P < 0.05$, Tukey's test) than in 'Ace' (100%) and 'Bellmasari' (81%), but not significantly different from those of LS2341 (0%) or 'Mie-Midori' (10%) (Table 1). In the test with the KP9547 isolate, 'Dai-Power' had a disease level of 13%, 'Buggy' of 27%, and 'Daisuke' of 17%; these were significantly lower ($P < 0.05$) than in 'Ace' (100%), 'Suketto C' (87%), and 'Bellmasari' (63%), but not significantly different from that of LS2341 (3%). The disease incidence in 'Bellmasari' was significantly higher than that in LS2341 and significantly lower than that in 'Ace' (100%), and similar to that in 'Mie-Midori' (60%). In the test with the KP0779 isolate, 'Dai-Power' had a disease level of 30%, 'Daisuke' 23%; these were significantly lower ($P < 0.05$) than in all other accessions except LS2341 (17%). 'Buggy' had a disease level of 70%, 'Suketto C' of 97%, and 'Bellmasari' of 97%; these levels did not differ significantly from those of 'Mie-Midori' or 'Ace'.

These results suggest that 'Dai-Power' and 'Daisuke' were highly resistant to bacterial wilt, whereas 'Buggy', 'Suketto C', and 'Bellmasari' were moderately resistant.

*Combined resistance to Phytophthor*a *blight and bacterial wilt*

Summarizing the results of the tests against Phytophthora blight and bacterial wilt suggests that 'Dai-Power' had high resistance to both Phytophthora blight and bacterial wilt, 'Buggy' had high resistance to Phytophthora blight and moderately resistance to bacterial wilt, 'Daisuke' had high resistance to bacterial wilt, 'Suketto C' had moderate resistance to bacterial wilt, and 'Bellmasari' had moderately resistance to Phytophthora blight and bacterial wilt.

Fruit yield in grafted cultivation

There was no significant difference in the fruit yields of 'Kyo-Suzu' grafted onto the current Japanese rootstock cultivars (Table 2). However, the fruit yield of non-grafted 'Kyo-Suzu' was lower than that of all grafted cultivars, and the difference was significant ($P < 0.05$, Tukey's test) compared with 'Buggy' and 'Daisuke'. These results suggest that the fruit yield of the current Japanese rootstock cultivars was higher than or comparable to that of non-grafted cultivation.

Table 2. Yields of the cultivars in grafted cultivation.

Scion	Rootstock	Fruit yield ($kg \cdot m^{-2}$)	
Kyo-Suzu	Dai-Power	6.03	ab
	Buggy	6.84	a
	Daisuke	6.88	a
	Suketto C	6.34	ab
	Bellmasari	6.59	ab
	Ungrafted Kyo-Suzu	5.65	b

Values within a column followed by the same letter do not differ significantly ($P<0.05$, Tukey's test).

Conclusions

Our results suggest that 'Dai-Power' is the most promising rootstock cultivar to control both Phytophthora blight and bacterial wilt during grafted cultivation of Japanese sweet peppers.

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Heterosis estimation of the eight production characteristics in bell pepper genotypes

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Abstract

The aim of the present study was to identify the most promising varieties of bell pepper for inclusion in genetic improvement programs and to study the effects of heterosis for ten agronomic characteristics in 15 experimental hybrids (F_1) with respect to the mean of the parents, mean of the superior parent, a standard cultivar (All Big), and a standard hybrid (Atlantis F_1). The ratio between the coefficient of genetic variation and the coefficient of environmental variation (CVg/CVe) achieved values greater than 1.0 for all characteristics studied, demonstrating that genetic gains can be achieved with selection. For the heterosis, the highest values were found for early production and total weight of fruits. The hybrids HTV-2 x HTV-3, HTV-2 x HTV-4, HTV-5 x HTV-2, HTV-6 x HTV-2, HTV-2 x HTV-7 and HTV-6 x HTV-4 achieved positive heterosis values regarding early production in relation to the mean of the parents, the parent with the larger mean, the standard cultivar and the standard hybrid. The HTV-5 x HTV-2, HTV-2 x HTV-7 hybrids achieved positive heterosis values regarding weight of the fruit in relation to the standard hybrid.

Keywords: *Capsicum annuum*, hybrid vigor, variability.

Introduction

Bell Pepper (*C. annuum*), is an important crop as vegetable or for producing paprika in Brazil (Reifschneider and Ribeiro, 2004). Genus *Capsicum* shows a great diversity, and hybridization is a useful tool in breeding programs. Heterosis has been used in the improvement of this crop (Gomide et al. 2008).

The goal of this study was to identify the most promising varieties of bell pepper for inclusion in genetic improvement programs, and to study the effects of heterosis in 16 experimental hybrids (F_1) with respect to the mean of the parents, mean of the superior parent, a standard cultivar, and a standard hybrid.

Material and Methods

The experiment was conducted at Departamento de Agronomia, Área de Fitotecnia da Universidade Federal Rural de Pernambuco (UFRPE), em Recife, Pernambuco State, Brazil. Seven inbred lines (HTV-1, HTV-2, HTV-3, HTV-4, HTV-5, HTV-6, HTV-7), 15 F1 single cross hybrids (HTV-1 x HTV-2, HTV-1 x HTV-3, HTV-1 x HTV-4, HTV-2 x HTV-3, HTV-2 x HTV-4, HTV-3 x HTV-4, HTV-5 x HTV-2, HTV-6 x HTV-2, HTV-5 x HTV-3, HTV-6 x HTV-3, HTV-2 x HTV-7, HTV-3 x HTV-7, HTV-5 x HTV-4, HTV-6 x HTV-4, HTV-7 x HTV-4), one cross-pollinated commercial cultivar (All Big) and a commercial hybrid (Atlantis F_1) were evaluated in a greenhouse in soilless system. The substrate was coconut powder. Emasculation and pollination for obtaining the experimental hybrids were done by hand.

Seedlings of the 24 treatments were grown in a randomized complete block design with six replications. Each plot was consisted by a 5 L vase, with one plant/vase and a spacing of 1.0 m row to row and 0.5 m plant to plant within row.

The evaluated traits were: number of fruits, total fruit weight, average fruit weight, pericarp thickness, fruit length, fruit width, fruit length/width rate and locule number.

The data were subjected to analysis of variance and when significant differences were found, the treatments were grouped by Scott-Knott test criteria ($p \leq 0.01$). All statistical analysis were done using Genes Program (Cruz, 2006).

Mid-parent and high-parent heterosis was calculated. Also, the comparisons among experimental hybrids and of these with commercial hybrid and commercial cultivar were performed. The broad sense heritability and genetic coefficient of variation/ environmental coefficient of variation rate (CVg/CVe) was calculated for each evaluated trait.

Results and Discussions

All evaluated characters showed significant differences among treatments by F test ($p \leq 0.01$) (data not shown). The means of commercial hybrid "Atlantis" did not differ of the following experimental hybrids: (HTV-1 x HTV-2), (HTV-1 x HTV-3), (HTV-1 x HTV-4), (HTV-5 x HTV-2), (HTV-6 x HTV-2), (HTV-2 x HTV-7), (HTV-3 x HTV-7) (HTV-5 x HTV-4) (HTV-6 x HTV-4) and (HTV-7 x HTV-4), for total fruit weight (Table 1).

The genetic coefficient of variation/ environmental coefficient of variation rate was greater then one for all evaluated traits, and the broad sense heritability ranges 89.0 to 96.8 % (Table 1). According to Vencovsky (1987), this is a favorable situation to obtain gains in a breeding program. In this respect, the heritability is dependent of the character and genotypes evaluated (Ramalho et al. 2004; Cruz & Regazzi, 1997).

The hybrids with higher values of average fruit weight, fruit length and fruit length/width rate were HTV-5 x HTV-2 and HTV-2 x HTV-7. These ones showed the higher values of mid-parent heterosis and high-parent heterosis. They were also superior to the commercial hybrid and commercial cultivar (Table 2).

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Table 1. Means, genetic coefficient of variation/environmental coefficient of variation rate (CVg/CVe) and broad sense heritability (h^2_m) of eight traits in 24 bell pepper genotypes.

Genotypes	Trait[(1)]							
	NF	**TFW**	**AFW**	**PT**	**FL**	**FW**	**FL/FWR**	**LN**
HTV1	95.50c	9835.00b	86.62d	4.32a	7.87d	7.40c	1.08c	3.40[a]
HTV2	106.83b	10869.17a	105.19c	4.31a	9.56c	5.61d	1.71a	3.22[a]
HTV3	63.67e	7533.33b	87.83d	3.05c	7.99d	9.94a	0.81d	2.79b
HTV4	88.83d	9280.00b	69.18e	4.72a	6.29e	8.52b	0.74d	3.38[a]
HTV5	113.67b	12345.00a	100.20c	4.48a	9.11c	5.96d	1.55b	3.25[a]
HTV6	99.83c	10765.00a	97.35c	4.00b	8.85c	6.62c	1.35b	3.54[a]
HTV7	110.67b	11794.17a	80.07d	4.68a	7.28d	6.87c	1.07c	3.55[a]
HTV-1 x HTV-2	92.83c	10652.50a	107.35b	3.99b	9.76b	6.77c	1.46b	3.24[a]
HTV-1 x HTV-3	105.33b	11910.83a	96.98c	4.51a	8.82c	6.42d	1.40b	3.22[a]
HTV-1 x HTV-4	103.33c	11902.50a	90.29d	4.42a	8.21d	7.14c	1.16c	3.63[a]
HTV-2 x HTV-3	60.50e	8625.00b	101.71c	3.48c	9.25c	10.81a	0.87d	2.63b
HTV-2 x HTV-4	88.50d	9790.83b	72.61e	3.17c	6.60e	7.98c	0.83d	2.44b
HTV-3 x HTV-4	86.00d	9868.50b	100.69c	4.36a	9.15c	7.88c	1.19c	3.56[a]
HTV-5 x HTV-2	103.50c	12546.17a	111.29b	4.36a	10.12b	6.07d	1.67a	3.14[a]
HTV-6 x HTV-2	98.50c	11389.17a	95.79c	3.77b	8.71c	6.91c	1.27c	3.21[a]
HTV-5 x HTV-3	67.50e	9806.67b	108.55b	3.07c	9.87b	9.43b	1.06c	2.40b
HTV-6 x HTV-3	71.50e	8740.83b	94.12c	2.64c	8.56c	9.15b	0.94d	2.43b
HTV-2 x HTV-7	112.83b	13445.17a	115.68a	4.58a	10.52a	5.75d	1.85a	3.32[a]
HTV-3 x HTV-7	97.83c	11805.00a	119.65a	3.88b	10.88a	6.93c	1.62a	3.53[a]
HTV-5 x HTV-4	85.50d	12126.67a	102.31c	4.47a	9.30c	8.01c	1.17c	3.27[a]
HTV-6 x HTV-4	99.00c	11613.33a	82.03d	4.71a	7.46d	7.48c	1.00d	3.47[a]
HTV-7 x HTV-4	96.83c	11383.40a	97.25c	4.61a	8.84c	7.29c	1.26c	3.31[a]
All Big	139.00a	8780.00b	69.77e	3.93b	6.34e	4.55e	1.47b	3.59[a]
Atlantis F_1	100.17c	12207.50a	98.18c	4.33a	8.93c	7.37c	1.22c	3.23[a]
CV(%)	10.93	11.26	6.26	11.41	6.26	11.83	12.15	9.62
CVg/CVe	1.63	1.17	2.24	1.21	2.24	1.61	1.98	1.16
h^2_m (%)	94.11	89.09	96.78	89.82	96.78	93.95	95.93	89.01

[(1)] Number of fruits (NF). total fruit weight (TFW). average fruit weight (AFW). pericarp thickness (PT). fruit length (FL). fruit width (FW). fruit length/width rate (FL/FWR) and locule number (LN).
Means followed by the same letter at column belong to a same group by Scott-Knott criteria ($p \leq 0.01$)

Table 2. Mid parent heterosis (h_{MP}). superior parent heterosis (h_{PS}). commercial cultivar heterosis (h_{CP}) and commercial hybrid heterosis (h_{HP}). related with eight morphological traits in Bell Pepper.

Genotypes	Trait[1]															
	NF				TFW				FW				PT			
	$\%h_{MP}$	$\%h_{PS}$	$\%h_{CP}$	$\%h_{HP}$	$\%h_{MP}$	$\%h_{PS}$	$\%h_{CP}$	$\%h_{HP}$	$\%h_{MP}$	$\%h_{PS}$	$\%h_{CP}$	$\%h_{HP}$	$\%h_{MP}$	$\%h_{PS}$	$\%h_{CP}$	$\%h_{HP}$
HTV-1 x HTV-2	-8.24	-13.10	-33.22	-7.33	2.90	-1.99	21.33	-12.74	11.93	2.05	53.86	9.34	-7.53	-7.42	1.53	-7.85
HTV-1 x HTV-3	32.35	10.29	-24.22	5.15	37.16	21.11	35.66	-2.43	11.18	11.96	39.00	-1.22	22.39	4.40	14.76	4.16
HTV-1 x HTV-4	12.11	8.20	-25.66	3.15	24.54	21.02	35.56	-2.50	15.91	4.24	29.41	-8.04	-2.21	2.31	12.47	2.08
HTV-2 x HTV-3	-29.03	-43.37	-56.47	-39.60	-6.26	-20.65	-1.77	-29.35	5.39	-3.31	45.78	3.60	-5.43	-19.26	-11.45	-19.63
HTV-2 x HTV-4	-9.54	-17.16	-36.33	-11.65	-2.82	-9.92	11.51	-19.80	-16.72	-30.97	4.07	-26.04	-29.79	-26.45	-19.34	-26.79
HTV-3 x HTV-4	12.79	-3.19	-38.13	-14.15	17.39	6.34	12.40	-19.16	28.26	45.55	44.32	2.56	12.23	-7.63	10.94	0.69
HTV-5 x HTV-2	-6.12	-8.95	-25.54	3.32	8.09	1.63	42.89	2.77	8.37	11.07	59.51	13.35	-0.80	-2.68	10.94	0.69
HTV-6 x HTV-2	-4.67	-7.80	-29.14	-1.67	5.29	4.78	29.72	-6.7	-5.41	-8.94	37.29	-2.43	-9.27	-12.53	-4.07	-12.93
HTV-5 x HTV-3	-23.88	-40.62	-51.44	-32.61	-1.33	-20.56	11.69	-19.67	15.46	8.33	55.58	10.56	-18.46	-31.47	-21.88	-29.10
HTV-6 x HTV-3	-12.54	-28.38	-48.56	-28.62	-4.46	-18.80	-0.45	-28.40	1.65	-3.32	34.90	-4.14	-25.11	-34.00	-32.82	-39.03
HTV-2 x HTV-7	3.75	1.95	-18.83	12.64	18.65	14.00	53.13	10.14	24.88	44.47	65.80	17.82	1.89	-2.14	16.54	5.77
HTV-3 x HTV-7	12.23	-11.60	-29.62	-2.34	22.16	0.09	34.45	-3.30	42.53	49.43	71.49	21.87	0.39	-17.09	-1.27	-10.39
HTV-5 x HTV-4	-15.56	-24.78	-38.49	-14.65	12.15	-1.77	38.12	-0.66	20.81	2.11	46.64	4.21	-2.83	-0.22	13.74	3.23
HTV-6 x HTV-4	4.95	-0.83	-28.78	-1.17	15.87	7.88	32.27	-4.87	-1.48	-15.74	17.57	-16.45	8.03	17.75	19.85	8.78
HTV-7 x HTV-4	-2.93	-12.51	-30.34	-3.33	8.03	-3.48	29.65	-6.75	30.32	21.46	39.39	-0.95	-1.91	-1.50	17.30	6.47

Genotypes	Trait[1]															
	FL				FW				FL/FWR				LN			
	$\%h_{PS}$	$\%h_{CP}$	$\%h_{HP}$	$\%h_{MP}$	$\%h_{PS}$	$\%h_{CP}$	$\%h_{HP}$	$\%h_{MP}$	$\%h_{PS}$	$\%h_{CP}$	$\%h_{HP}$	$\%h_{MP}$	$\%h_{PS}$	$\%h_{CP}$	$\%h_{HP}$	$\%h_{MP}$
HTV-1 x HTV-2	11.99	2.09	53.94	9.29	4.07	20.68	48.79	-8.14	4.66	-14.62	-0.68	19.67	-2.11	0.62	-9.75	0.31
HTV-1 x HTV-3	11.22	12.07	39.12	-1.23	-25.95	-13.24	41.10	-12.89	48.15	29.63	-4.76	14.75	4.04	-5.29	-10.31	-0.31
HTV-1 x HTV-4	15.96	4.32	29.50	-8.06	-10.3	-3.51	56.92	-3.12	27.47	7.41	-21.09	-4.92	7.08	6.76	1.11	12.38
HTV-2 x HTV-3	5.41	-3.24	45.90	3.58	39.04	92.69	137.58	46.68	-30.95	-49.12	-40.82	-28.69	-12.48	-18.32	-26.74	-18.58
HTV-2 x HTV-4	-16.72	-30.96	4.10	-26.09	12.95	42.25	75.38	8.28	-32.24	-51.46	-43.54	-31.97	-26.06	-24.22	-32.03	-24.46
HTV-3 x HTV-4	28.15	45.47	44.32	2.46	-14.63	-7.51	73.19	6.92	53.55	60.81	-19.05	-2.46	15.4	5.33	-0.84	10.22
HTV-5 x HTV-2	8.41	11.09	59.62	13.33	4.93	1.85	33.41	-17.64	2.45	7.74	13.61	36.89	-2.94	-3.38	-12.53	-2.79
HTV-6 x HTV-2	-5.38	-8.89	37.38	-2.46	13.00	23.17	51.87	-6.24	-16.99	-25.73	-13.61	4.10	-5.03	-0.31	-10.58	-0.62
HTV-5 x HTV-3	15.44	8.34	55.68	10.53	18.62	58.22	107.25	27.95	-10.17	-31.61	-27.89	-13.11	-20.53	-26.15	-33.15	-25.70
HTV-6 x HTV-3	1.66	-3.28	35.02	-4.14	10.51	38.22	101.10	24.15	-12.96	-30.37	-36.05	-22.95	-23.22	-31.36	-32.31	-24.77
HTV-2 x HTV-7	24.94	44.51	65.93	17.81	-7.85	-16.30	26.37	-21.98	33.09	72.90	25.85	51.64	-1.92	-6.48	-7.52	2.79
HTV-3 x HTV-7	42.50	49.45	71.61	21.84	-17.55	0.87	52.31	-5.97	72.34	51.40	10.20	32.79	11.36	-0.56	-1.67	9.29
HTV-5 x HTV-4	20.78	2.09	46.69	4.14	10.64	34.40	76.04	8.68	2.18	-24.52	-20.41	-4.10	-1.36	0.62	-8.91	1.24
HTV-6 x HTV-4	-1.45	-15.71	17.67	-16.46	-1.19	12.99	64.40	1.49	-4.31	-25.93	-31.97	-18.03	0.29	-1.98	-3.34	7.43
HTV-7 x HTV-4	30.29	21.43	39.43	-1.01	-5.26	6.11	60.22	-1.09	39.23	17.76	-14.29	3.28	-4.47	-6.76	-7.80	2.48

[1]Number of fruits (NF). total fruit weight (TFW). average fruit weight (AFW). pericarp thickness (PT). fruit length (FL). fruit width (FW). fruit length/width rate (FL/FWR) and locule number (LN).

Behaviour of spice pepper varieties under different production technologies

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Abstract

In Hungary, use of intensive cropping methods are becoming increasingly popular at spice pepper growing. In a 2-year experiment, five Hungarian spice pepper cultivars (hybrids: 'Bolero', 'Délibáb', 'Sláger'& open-pollinated 'Szegedi 80', 'Kaldóm') were compared, under intensive field conditions, i.e., drip irrigation, foil mulching, using of well-developed transplant, and under foil tunnel. A horizontal cordon trellis system was used. Plant density was either 5.9 plant.m-^{2} for field or 4.5plant/m^{2} for foil tunnel Four replications were used with 12 plants/parcels evaluated. Vegetative growth of plants (plant height), yield (fresh fruit weight, powder yield), pigment content of pericarp (ASTA color), and the size of seeds (1000 seed weight) were measured.

The results indicated that plant height was significantly higher and in the case of indeterminate varieties had higher total volume and yield under plastic tunnel than in open-field. The varieties behaved the same with the two technologies. The varieties bred for forcing are also particularly were well adapted to intensive open-field conditions. In the examined hybrids, Délibáb had the highest production, with the yield of Sláger being second. Cultivation under unheated plastic cover resulted in better quality (content of pigments) than open-field technology. The 1000 seed weight was higher under forcing than in open-field production.

Keywords: spice pepper, varieties, intensive technology, ground, 1000 K weight

Introduction

Growing technology of spice pepper has changed significantly over the past few years in Hungary. In addition to the traditional open-field cultivation more intensive methods are being used. The intensive open-field, unheated foil tunnel technologies are most popular. This is because many farmers have forcing house or tunnel facilities that are unprofitable to growing other vegetables, but with a small effort, can be reused. Thus, there is a need to study new growing technologies (Somogyi, 2006).

With intensive cultivation (drip irrigation, foil mulching, using of well-developed transplants), in spring time a week before transplanting a ridge is formed, and the dropper ribbon and the casing foil are placed. Watering and fertigation are supplied by the dropper ribbon. The harvest can be started in middle of August (Red Pepper Research, 2012).

For the forcing technique, new hybrids are propagated at a commercial nursery. From 1st of April, the transplants (8-10 leaves) in soil-blocks are planted under unheated foil tunnel. Plant density is 4-5 plant.m^{-2}. Watering and fertigation supplied by dropper ribbon. Plants are growing using the trellis system described by Somogyi, 2006 and Somogyi et al. 2012. Highairspace is suited to forcing (Gyúrós, 2000). Nutritional values are influenced by some factors of the growing technology, e.g., nutrient supply until harvest (Irinyi – Slezák, 2006; Gyökös et al. 2009). In pepper, the carotenoid synthesis starts two weeks before red ripening (Márkus - Kapitány, 2001). In the course of cultivar selection, important traits such as early ripening, high pigment and dry matter content, good productivity, disease and insect resistance, easy picking and suitable flavour and aroma substances are needed (Kapitány, 2005).

Heterosis furnishes new possibilities for the production of spice pepper with higher quality and in better quantities (Luo et al. 2006).

A few years ago, spice pepper hybrids were not in use. The first heterosis variety (Sláger) was identified in 2008 (Somogyi, 2010). The best hybrids can have more than 7 kg.m-2 yield under plastic cover (Somogyi et al. 2012).

Material and Methods

The experiment was done at the Experimental and Training Farm of the Faculty of Horticulture, Corvinus University of Budapest, in 2011 and 2012. Two types of technology were compared an open-field and an under unheated plastic cover with different spice pepper cultivars. We examined five varieties of Spice Pepper Research-Development Nonprofit Ltd. ('Bolero', 'Délibáb', 'Sláger' hybrids; 'Szegedi 80', 'Kaldóm' constant varieties). Plant density for open-field was 5.9 plant.m^{-2}, while the forcing was 4.5 plant.m^{-2}. Four replications with 12 plants/parcels were evaluated.

Table 1. Main data of experiment

	foil tunnel	open-field
Date of planting	22.04.2011.	10.05.2011.
	24.04.2012.	08.05.2012.
Date of pickings	**2011:** 30.08; 27.09; 25.10.	**2011:** 30.08; 27.09; 25.10.
	2012: 27.08; 24.09; 08.10.	**2012:** 27.08; 24.09; 15.10.

*Evaluation of vegetative parts***:** During the experiment, plant height was measured at two weekly intervals. We used dip-stick (from top-soil, till the longest shoot tip). Every plant height was documented. In this publication the plant's vegetative growth was described by the results of the last measured date (in both years 8 of October).

Evaluation of fruit: After picking, the fruits were examined. At every harvest, a sample to determine dry matter content of fruits (20-20 fruits by treatments, air-dried at 70 °C in lab), and the pigment content of skin was obtained.

The results were analysed with Microsoft® Excel 2003 software and ROPstat statistic programme (one-way and two-way independent sample ANOVA).

Pigment content was measured in accordance with MSZ 9681-5:2002, by ASTA (American Spice Trade Association) method in laboratory of the Red Pepper Research-Development Nonprofit Public Company at Kalocsa.

Results

Vegetative growth

Growth tendency of the varieties correlated in both years, but it can be stated, that plants were higher in 2nd year than in 1st year (Figure 1). In both growing seasons, Delibáb foil tunnels had the highest average height. The highest stem's length was typical of Délibáb and Sláger varieties (176-212 cm in forcing, 91-112 cm in open-field), the lowest was Kaldóm variety (85-110 cm is forcing, 64-66 cm open-field). Comparing the two technologies, it showed that plant height in open-field cultivation was significantly lower, than foil tunnel plants.

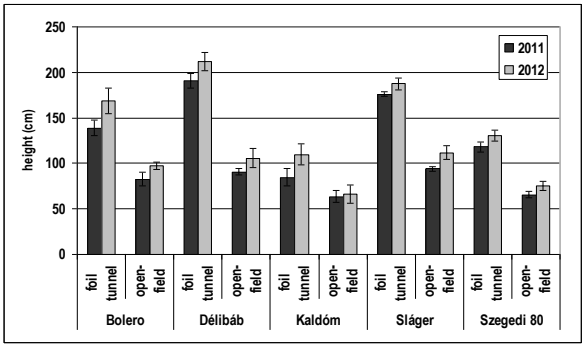

Figure 1. Plants height in October (2011-2012, Budapest)

Yiel

Average yield of ripened, healthy fruits from 1 square metre is shown in Figure 2. In the two years, Délibáb grown in foil tunnels had the highest volume, with Sláger and Bolero showing favourable results too. The Szegedi 80 and the Kaldóm cultivated in the open-field had the lowest yield, Kaldóm had low average-production with both technologies. Of the two years, the 2nd experimental year proved to be more favourable in most cases (variety, technology). Except for the results of Kaldóm in open filed and foil tunnels and foil tunnel grown Szegedi 80. The average of two years data had Délibáb with the highest yield (3.37 kg.m^{-2}) ($p<0.05$). When comparing the two types of technology, all of the spice pepper varieties (except Kaldóm) had higher production under the foil tunnels. According to two-way analysis of variance both the growing technology and the variety had an significant effect on yield.

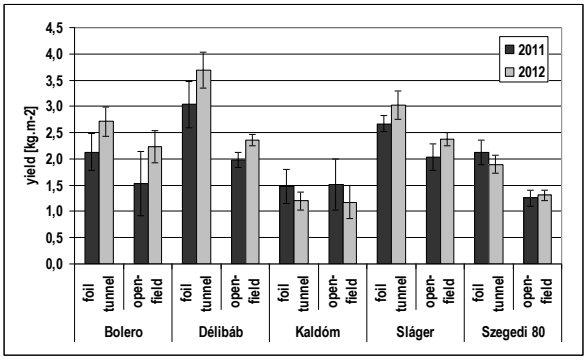

Figure 2. Yield (ripened fruits) (2011-2012, Budapest)

Ground Powder yield

Sláger grown under the foil tunnels had the highest amount of dry powder yield per square meter. The lowest paprika powder yield was produced Kaldóm in 2012 in the open field. The varieties, Délibáb and Sláger, had good results in foil tunnels and open-field too. The foil tunnel grown Délibáb in 2011 reached the highest yield of 0.58 kg.m-2, with Kaldóm producing the lowest in open field plots at 0.21 kg.m-2 in 2012. Within the foil tunnels, the powder yield was significantly higher than in the open-field, except for Kaldóm variety.

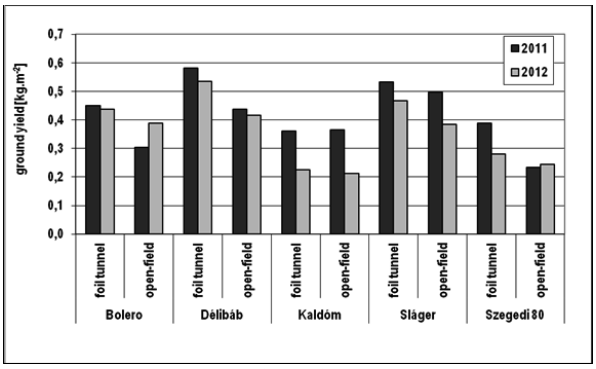

Figure 3. Ground yield (2011-2012, Budapest)

1000 seed weight:

The average 1000 seed weight showed significant differences in both years and among varieties. Significant differences were observed among the varieties with the same tendency in the two types of technologies. According to the results Bolero, Kaldóm and Szegedi 80 had a big seed size (6.6-6.8 g), while Délibáb and Sláger had smaller seed weight (6.0-6.1 g).

Pigment-content:

(It was examined in 2011 only). In 2011, the average pigment content of the varieties showed that following 3 weeks after-ripening of the fruits from the first and second picking, produced a "special" quality class of powder. Cultivation under foil tunnel resulted in better quality than open-field production. The best quality powder was produced by Bolero (344 ASTA), and Kaldóm's has the worst (230 ASTA).

Discussion

When comparing the two types of technology, the plant height of each variety in the foil tunnel was significantly higher, than in open-field cultivation. Indeterminate varieties had higher volume and yield of ground powder under foil tunnels than in open-fields. One of the advantages of growing in foil tunnels is that a smaller plant population is needed. Yield per plant in foil tunnels is far more greater than in open-field production.

In foil tunnels, indeterminate varieties grew to 1.8-2.1 meters high. Indeterminate hybrids had the best yields. Varieties bred for forcing also were well adapted to intensive open-field conditions.

From the examined hybrids, Délibáb had the highest yield, with Sláger showing favourable average yield too. Szegedi 80 had higher yield in forcing than in open-field, but in two type of technology, it yielded less than the other three hybrids. Kaldóm is also a variety for open-field, being half-determinate it had lower yield than indeterminate varieties. However based on the habit of plants, it can be planted higher plant density, than those used in this experiment, so probably the yields of Kaldóm could rise. Kaldóm vegetative growth was stronger under plastic cover, than in open-field cultivation, but the yield was not higher. Délibáb and Sláger had the most yield of ground powder in forcing and open-field too. Growing in foil tunnels results in significantly higher powder yield, than in the open-field, except in case of Kaldóm variety.

Cultivation in foil tunnels resulted in better quality (content of pigments) than in the open-field. The ground powder of Bolero had the best quality. Kaldóm's ground powder was substandard. (It was examined in 2011 only). The 1000 seed weight was higher in forcing than open-field.

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Determination of seed yield and traits of "Serademre 8" long pepper and "Doru 16" bell pepper (*C. annuum* L.) varieties over years

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Abstract

Peppers have an important share of agriculture in the types of vegetables in our country. Turkey ranks third in the world with a production by 1,837,003 tons pepper (*Capsicum annuum* L.) production. Turkey pepper production is done by using standard (non hybrid) varieties in open field. Certified foundation seeds of these standard varieties were produced by the breeder public Institutes and seed multiplication is carried out by the private seed producer companies. Serademre 8 (long type) and Doru 16 (bell type) pepper varieties which were developed by Batı Akdeniz Agricultural Research Institute (BATEM) are used widely in Turkey. In this study, seed yield and yield components, such as total fresh fruits weight, number of fruits, seed yield per plant, seed yield per fruit, seed index and the weight of 1000 seeds, number of seed in g^{-1}, seed yield, were determined during the autumn growing season of different three years of Serademre 8 and Doru 16 varieties. The seed traits of pepper types were investigated over years. As a result, relationships of seed traits were found positive-negative and significant.

Keywords: Pepper, *Capsicum annuum* L., seed yield on pepper, Serademre 8 , Doru 16, traits of seed, correlation

Introduction

Capsicum genus of Solanaceae family includes about 30 pepper species including *Capsicum annuum* L. in the culture (Esbaugh,1970). *C. annuum* L. has different types of peppers. Capia pepper type of the long peppers group in the classification by Bailey is named as *Capsicum annuum* var. *longum* (Vural and et al. 2000). Types of this species include chillis, red, green, yellow, sweet peppers and paprika. *Capsicum annuum* and *C. frutescens* are generally self-pollinated (George, 1985). Pepper's homeland is reported to be Mexico and Central America (Wien, 1997).

Seed can be seen as the most important part of the production of peppers (Bosland and Votava, 2000). Seeds are found inside the fruit on the central placenta. Thousand seed weight of pepper is 5 to 7 g. The seed is usually yellow in colour in cultivated *Capsicum* species (Somos, 1984, Bosland and Votava, 2000). Seed size is dependent on the variety and the growing conditions and generally larger fruits have larger seeds. Most seed falls in the range of 2.5-6.5 mm in length and 0.5-5 mm wide. Seed size can change the uniformity of pepper plants (Bosland and Votava, 2000). Seed yield ranges from 100 to 200 kg per hectare. The 1000-grain weight is 3,5 g for the small pungent types and 5 g in the sweet types (George, 1985).

Pepper growing for seed production is similar growing for fresh fruit production. The seed maturity time of fruits are easily realized completely red colour and some wrinkled fruits. Seed yield is related with variety, planted dimension and environmental conditions. Pepper average seed yield is 20 to 30 kg/da (Vural and et al. 2000).

Heavy and mature seeds give superior results than small and unripe seeds in germination and vigor. According to mostly researcher, the seed maturity is known as maximum dry weight stage (Şehirali, 2002).

Türkiye is in third place in pepper production and has an important potential in the world after China and Mexico (Anonymous, 2012a). Antalya province has the most pepper production in Türkiye with open field and greenhouse areas. The total produced greenhouse pepper production in

Antalya is currently 270127 tons (235947 tons protected cultivation and 34180 tons open field) (Anonymous, 2012b). Turkish pepper market comprises four main types: demre sivrisi (long), charliston (long yellowish), bell and capia (long conical) type.

"Serademre 8" long (demre type) and "Doru 16" bell pepper were improved and registered by Batı Akdeniz Agricultural Research Institute (BATEM). They are non-hybrid pepper (open pollinated) and are produced commonly in Turkey. Certified foundation seeds of varieties are produced by the Institute and are supplied to the private seed producer companies. In particular, Serademre 8 is the most cultivated variety in long-demre types of peppers. The seed production amount of Serademre 8 and Doru 16 have recorded 5235 kg and 650 kg respectively in 2011 (Anonymous, 2013).

The aim of this study was to determine pepper seed yield, seed traits and relationships of "Serademre 8" long (demre type) pepper and "Doru 16" bell pepper of Batı Akdeniz Agricultural Research Institute (BATEM) in unheated plastic greenhouse in autumn growing seasons of 2006-2013.

Materials and Methods

"Serademre 8" long (demre) type and "Doru 16" bell type pepper varieties which were developed by Batı Akdeniz Research Institute (BATEM) were used for this study.

Serademre 8 variety was grown in plastic greenhouse in autumn growing seasons of 2006, 2008, 2010 and Doru 16 variety was grown in plastic greenhouse in autumn growing season of 2007, 2009, 2012. Autumn growing season includes the period from September to April.

Greenhouse was heated only to protect plants from freezing by LPG heaters on certain days. Temperature and humidity values of production period are given in Figure 1. Soil nutrient content were analysed in every growing term and recommended fertilization programmes of pepper were supplied by drip irrigation system (Table 1). Pepper plants were planted as 90-50x50 cm (lines-beds x between plants) dimension. The branches were suspended with three ropes. Shading was applied by a white shading powder on plastic greenhouse in September and October. The door and side aeration windows of greenhouse were covered with fly net for isolation. The plant soil was mulched with poly ethylene (PE) to prevent weeds. Whenever required chemicals were used to protect against fungal diseases and insects such as *B.tabaci* and *H. armigera.*

Fruits were harvested as the maximum fruit number with red maturity colour. The elite plants were selected in every growing season. The seeds were extracted by hand, washed with water and dried in room at 24°C. The investigations of seed traits were performed after drying and packaging.

Figure 1. Average temperatures and humidity of growing period in greenhouse (2009-2010)

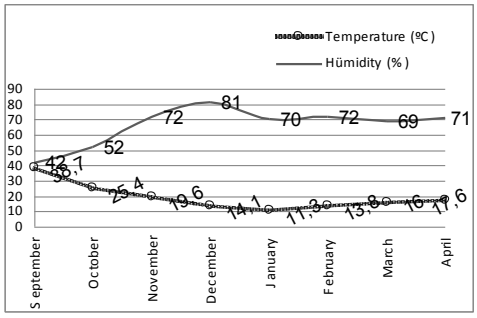

Table 1. Results of soil analysis (2012-2013)

pH (1:2,5)	8,3	Light alkaline
Lime (%)	12,6	Medium limy
EC micromhos/cm(25	858	Medium
Sand (%)	57	Sandy clay loam
Clay (%)	24	
Silt (%)	19	
Organic matter (%)	1,3	
P ppm (Olsen)	113	
K ppm	359	
Ca ppm	4174	
Mg ppm	144	

Seed yield and yield components such as total fresh fruits weight, number of fruits, seed yield per plant, seed yield per fruit, seed index, the weight of 1000 seeds and number of seed in g^{-1}, seed yield (g/da) were determined. Seed index was calculated as ratio of seed yield per fruit to fruit weight (Gözen and Yanmaz, 2008). Seed yield per da was calculated for 2857 plant in da as 90-50x50 cm (lines-beds x between plants) dimension. The relationship among seed traits were computed on JMP programme .

Results and discussion

Some cultural practices of growing periods and seed traits values of Serademre 8 and Doru 16 varieties are presented in Table 2. Average total fruit weight of Serademre 8 ranged from 1133 to 1514 g, average fruit number from 34,6 to 55,2, average seed yield per plant from 10,34 to 27,16 g, average seed number g^{-1} from 137 to 151, 1000 grain weight from 6,63 to 7,35 g, seed yield per fruit from 0,30 to 0,67 g, average fruit weight from 27,7 to 32,9 g, seed index from 49,9 to 123,5 and seed yield per da from 29546 to 77603 g over years.

Average total fruit weight of Doru 16 variety varied between 1009-1238 g, fruit number between 18,2-24, average seed yield per plant between 22,48-24,53 g, average seed number g^{-1} between 120-125, 1000 grain weight between 8,02-8,36 g, seed yield per fruit between 0,95-1,29 g, average fruit weight between 47,2-55,9 g, seed index between 44-58,3 and seed yield per da between 64211-70075 g over years. Doru 16 produces less seeds than Serademre 8 variety, but characterized by a higher weight.

Table 2. Growing acknowledges and values of seed characteristics of Serademre 8 and Doru 16

	SERADEMRE 8 (long type)			**DORU 16 (bell type)**		
	2006	2008	2010	2007	2009	2012
Sowing date	08.08.2006	01.08.2008	17.08.2010	01.08.2007	22.07.2009	31.07.2012
Transplanting date	24.08.2006	12.08.2008	31.08.2010	13.08.2007	04.08.2009	08.08.2012
Planting greenhouse date	13.09.2006	05.09.2008	21.09.2010	07.09.2007	27.08.2009	29.08.2012
% 50 Flowering	05.10.2006	30.09.2008	13.10.2010	27.09.2007	14.09.2009	15.09.2012
Harvesting date-mature	02.04.2007	07.05.2009	14.03.2011	02.04.2008	08.03.2010	04.03.2013
Days after sowing	238	280	210	246	230	217
Number of elite plant	24	16	15	16	29	16
Average fruit number	34,6±6,7	55,2±14,8	37,9±11,1	23±4,4	24±5,8	18,2±5,5
Average total fruit weight (fresh) (g)	1133±213	1514±363	1255±435	1238±255	1137±350	1009±288
Average fruit weight (g) (AFW)	32,9±3,8	27,7±2,9	32,9±3,2	53,9±4,5	47,2±7,1	55,9±5,02
Average seed yield per plant (g)	10.34±3,7	27,16±8,2	26±11,3	22,48±8,2	24,53±7,01	23,2±6,8
Seed yield per fruit (g) (SYF)	0,30±0,1	0,49±0,06	0,67±0,12	0,95±0,19	1,05±0,29	1,29±0,2
Average seeds number g^{-1}	137±8,8	137±5,6	151±5,7	125±6,8	121±9,2	120±5,5
1000 grain weight (g)	7,35±0,4	7,29±0,3	6,63±0,3	8,02±0,4	8,32±0,6	8,36±0,4
Average Seed İndex (AFW/SYF)	123,5±53	57,5±10,3	49,9±6,8	58,3±11,1	48,3±14,2	44±5,7
Seeds yield per da (g) (SYD)	29546± 10454	77603± 23417	74282± 32239	64211± 23431	70075± 20037	66282± 19536
Seed germination (%)	97	99	99	96	99	99

The seed trait correlations of Serademre 8 were given in Table 3. The positive and significant correlations were determined between total fruit weight and number of fruit, average seed yield per plant, seed yield per da. Relationships between number of fruit and average seed yield per plant and seed yield per da were found to be highly significant; relationships between average seed yield per

plant and seed yield per da; relationships between average seed yield per fruit and average fruit weight.

On the other hand; the negative and significant correlations were found between total fruit weight and average seed yield per fruit, average fruit weight; and also between number of fruit and average seed yield per fruit and average fruit weight; between average seed yield per plant and average seed index; between average seed number g^{-1} and 1000 grain weight; between average seed yield per fruit and average seed seed index; between seed İndex and seed yield per da.

Table 3. The correlations of seed traits of Serademre 8 long pepper

	TFW g	NF	ASYP g	ASN g^{-1}	1000 GW	ASYF g	AFW g	Sİ (AFW/ ASYF)
Total fruit weight (g) (TFW)	1,0000							
Number of fruit (NF)	0,8764***	1,0000						
Average seed yield per plant (g) (ASYP)	0,7593***	0,6750***	1,0000					
Average seed number /1 g (ASN)	0,0702	0,0864	0,1242	1,0000				
1000 grain weight (g) (1000 GW)	-0,0768	-0,0852	-0,1320	-0,9953***	1,0000			
Average seed yield per fruit (g) (ASYF)	-0,3617**	-0,5962***	0,0730	-0,1016	0,0972	1,0000		
Average fruit weight (g)(AFW)	-0,3095*	-0,6655**	-0,2709	-0,2039	0,2007	0,7744***	1,0000	
Average seed İndex (Sİ) (AFW/ASYF)	0,1242	0,1142	-0,5005*	0,0515	-0,0397	-0,6665***	-0,0843	1,0000
Seed yield per da (g) (SYD)	0,7593***	0,6750**	1,0000***	0,1242	-0,1320	0,0730	-0,2709	-0,5005**

*** significant at 0,001, ** significant at 0,01 and * significant at 0,05

Table 4. The correlations of seed traits of Doru 16 bell pepper

	TFW g	NF	ASYP g	ASN g^{-1}	1000 GW	ASYF g	AFW g	Sİ (AFW/ ASYF)
Total fruit weight (g) (TFW)	1,0000							
Number of fruit (NF)	0,8490***	1,0000						
Average seed yield per plant (g) (ASYP)	0,7107***	0,5707***	1,0000					
Average seed number /1 g (ASN)	-0,0934	-0,1116	-0,0257	1,0000				
1000 grain weight (g) (1000 GW)	0,0941	0,1233	0,0203	-0,9949***	1,0000			
Average seed yield per fruit (g)(ASYF)	-0,0590	-0,3396**	0,5537***	0,0433	-0,0611	1,0000		
Average fruit weight (g)(AFW)	0,3001**	-0,2309	0,2621*	-0,0068	-0,0140	0,5026***	1,0000	
Average seed İndex (Sİ) (AFW/ASYF)	0,1720	0,1903	-0,5261***	0,0836	-0,0659	-0,8309***	-0,0046	1,0000
Seed yield per da (g) (SYD)	0,7107***	0,5707***	1,0000***	-0,0257	0,0203	0,5537***	0,2621*	-0,5261***

*** significant at 0,001, ** significant at 0,01 and * significant at 0,05

The seed trait correlations of Doru 16 were given in Table 4. Compared with Serademre 8, relationship between total fruit weight and average fruit weight for Doru 16 was positive and significant, whereas it was negative and significant for Serademre 8. Relationship between average fruit weight and seed yield per da for Doru 16 was positive and significant, whereas it was negative and non-significant for Serademre 8. Relationships between average seed yield per plant and average seed yield per fruit; average seed yield per fruit and seed yield per da for Doru 16 were found positive and significant, whereas they were positive and non-significant for Serademre 8.

On the other hand, relationships between total fruit weight and average seed yield per fruit; number of fruit and average fruit weight for Doru 16 were found negative and non-significant, whereas they were negative and significant for Serademre 8.

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Capsaicinoid content and composition of Hungarian red pepper varieties and breeding lines

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Abstract

Red spicy pepper (*Capsicum annuum*) is a basic spice of the Hungarian cuisine. Univer Product Plc. as a market leader in Hungary in case of cold preserved red pepper cream products is performing a two-step pepper breeding project with its partners. In the first step we recover and develop the pungency, resistance and phenological features of existing Hungarian hot varieties *(Szegedi-178, Kalóz, Kalmár)*, in the second step we are breeding multiresistent hot red pepper hybrids using some of the selected lines from the first project and other lines as resistance sources. Both step's experimental work are performed under thermal water heated greenhouses, growing up two generation per year, and the greenhouse result will be validated by field experiments as well.

We determined the capsaicinoid content of the dried, ground pepper samples by HPLC applying modified hungarian standard method. While the initial total capsaicinoid level of the most pungent *Szegedi-178* variety was 836 mg/kg in average (from powder), after 4 generation selection in greenhouse it became 2.493 mg/kg.

The proportion of major capsaicinoids of *Capsicum annuum* varieties and lines can be considered typical in accordance with the literature data. Major components are capsaicin and dihydrocapsaicin; nordihydrocapsaicin is less than 10 % of total and homocapsaicin and homodihydrocapsaicin are less than 1-2 %. But concerning nordihydrocapsaicin we found 10-34 % ratio of total capsaicinoids in case of some *Kalóz* and *Kalmár* items and in case of a few crossed lines. Possible link between higher nordihydrocapsaicin proportion and some other features within these varieties is a topic of a further investigation.

Keywords: Capsaicinoids, Pepper, Nordihydrocapsaicin, Pungency, Capsicum annuum

Introduction

Red spicy pepper (*Capsicum annuum*) is a basic spice of the Hungarian cuisine. While the local powder products are mostly non-pungent, the cold preserved, crushed or fine-milled creamy pepper products are mostly pungent and that are used for post flavoring of soups and dishes. So the level of pungency is an important quality parameter of these kind of products. Univer Product Plc. is the category founder and market leader in Hungary in case of cold preserved red pepper cream products. Univer and its partners have started a two-step breeding project in 2010 in order to recover the competitiveness of red pepper product line. The first step includes a 3 years long selection project of 3 Hungarian hot varieties: *Szegedi-178, Kalóz* and *Kalmár* in order to recover and develop the pungency, resistance and phenological features. The aim of the second step is breeding multiresistent hot red pepper hybrids using some of the selected lines from the first step and other lines as resistance sources.

The five main capsaicinoids of *C. annuum* varieties grown in Hungary are capsaicin (C), dihydro-capsaicin (DHC), nordihydro-capsaicin (NDHC), homodihydro-capsaicin and homocapsaicin. The relative quantity of these components in pepper fruit and powder as well as

their contribution to the pungent flavour perception were topics of several researches (Bennett et al.1968; Reilly et al. 2001; (Zewdie and Bosland 2001; Wang et al. 2011).

Either in terms of proportion or contribution to pungency level the two key components are capsaicin and dihydrocapsaicin. The capsaicinoid composition of *C. annuum* species described in previous studies (C is dominant with about 2/3 proportion, DHC accounts for about 20-30 % meanwhile NDHC is present at less than 10 %, the other two are present at less than 1-2 %) resulted totally different in case of other (e.g. *C. pubescens*) species.

Materials and Methods

The experimental work of both steps of our breeding project is performed under thermal water heated greenhouses, growing up two generation per year, about 20.000 plants per generation, and the greenhouse results are validated on field as well.

Fruits of plant population underwent phenological selection and resistance tests will be examined for pungency from grounded and oven-dried (72°C, 24 h) form. Considering the huge amount of samples to be measured (3.000-5.000 fruits/generation) within a limited time we choosed the hungarian standard method (MSZ 9681-4,2002) for capsaicinoid analysis. The advantage of this method is the short extraction time with methanol and ultrasonic bath. The other time-factor of the HPLC-analysis is the run-time of each sample on the equipment; we shortened it significantly through the change of the column and some parameters. The results of these measurement regard normally C and DHC values and their sum; in presence of an atypical capsaicinoid profile the samples have been reinjected using NDHC-standard.

The standard method works with methanol extracion in ultrasonic bath for 2 min from the pepper powder samples, successively a filtration step by using 0,45 μm PTFE syringe filter cleanup, a HPLC separation step with 45% acetonitrile/55% KH_2PO_4-buffer (0.2 M, pH=4) as mobile phase (filtered and degassed), on Luna C18 column (5 μ, 250x4.6 mm) with 1.5 $ml \cdot min^{-1}$ flow rate and fluorescence detection (excitation at 288 nm, emission at 320 nm). A Kinetex 2,6 μ C18 column (100x4.6 mm) equipped with a C18 precolumn (4x3 mm) was also used. For this column the samples were filtered by 0,22 μm PTFE syringe filters and the flow rate was 1 $ml \cdot min^{-1}$. The used HPLC-equipment was Shimadzu LC-20 Prominence; capsaicin and dihydrocapsaicin standards were obtained from Sigma Aldrich, nordihydrocapsaicin standard from Phytolab.

Results and discussion

After 4 generations of our selection program we achieved significant increase of the total capsaicinoid content compared with initial values for all the 3 basic varieties (table 1.). The achieved ranges are significantly higher than level of pungency of existing hungarian Protected Designation of Origin (PDO) powder products categorized “hot” and “extra hot” in the PDO-specifications.

Field experiments were performed continuously in order to validate the greenhouse results: selected lines provided higher (10-30 %) total capsaicinoids levels than initial ones.

Table 1: Total capsaicinoids content of red pepper powder samples

Variety / line	Generation	Total capsaicinoids (mg/kg)	
		range	average
"Szegedi" (PDO) red pepper powder "hot"	n.a.	> 200 [a]	n.a.
"Kalocsai" (PDO) red pepper powder "hot"	n.a.	200 - 500 [a]	n.a.
"Kalocsai" (PDO) red pepper powder "extra hot"	n.a.	> 500 [a]	n.a.
Szegedi-178	F1	250 - 2.000 [b]	836
	F4	1.000 - 4.000 [b]	2.493
Kalóz	F1	0 - 500 [b]	267
	F4	500 - 3.000 [b]	1.494
Kalmár	F1	0 - 1.000 [b]	599
	F4	500 - 4.000 [b]	1.827
C. chinense Jolokia [c]	n.a.	40.000 - 60.000	n.a.

[a] specification of the given PDO-product
[b] more than 90 % of results are within the reported range
[c] grown and measured by ourselves in the greenhouse for "benchmark"

Concerning the proportion of capsaicinoids compared with the literature data (Bennett et al.1968; Reilly et al. 2001) it is conspicuous that in several cases NDHC is present in higher than 15 %, in a few cases in higher than 20 % (the highest proportion was 34 %) in Kalóz and Kalmár samples and crossings between Szegedi-178 and Kalóz or Kalmár meanwhile practically any kind of „atypical" NDHC-proportion can be found in case of Szegedi-178 variety (table 2.).

Table 2: Relative frequency of occurrence of higher nordihydrocapsaicin (NDHC) proportion in examined varieties and lines

Variety / line	Percentage of samples containing a given NDHC-proportion (of total capsaicinoids) within the given variety/line					
	Generation 4. (autumn 2011)		Generation 5. (springtime 2012)		Generation 6. (autumn 2012)	
	> 20 % NDHC	15-20 % NDHC	> 20 % NDHC	15-20 % NDHC	> 20 % NDHC	15-20 % NDHC
Szegedi-178	0,47	0,78	0	0	0,19	0,19
Kalóz	7,69	19,78	0	9,91	4,38	17,52
Kalmár	6,40	16,40	1,85	1,85	2,05	6,67
Szegedi-178 x Kalóz or Kalmár crossings	3,49	10,71	0	2,18	3,93	7,36

This NDHC-proportion is higher than that found in the literature. The only one known common feature of these higher NDHC-lines (Kalóz, Kalmár and crossings) is the Xanthomonas Bs-2 resistance, which is not a feature of Szegedi-178.

Usually the higher proportion of NDHC implied lower level of capsaicin; due to this fact in case of some samples DHC became the capsaicinoid component with the highest proportion.

Although well known (Zewdie and Bosland 2001) that the capsaicinoid profile is not suitable for chemotaxonomic indicator of *Capsicum* species, in case of the present experiments we could observe some differencies among varieties (within species) which seem to be genetically determined.

This phenomenon and possible link with other features will be an object of further investigations.

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Qualitative traits in chilli (*Capsicum annuum* L.)

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Abstract

Chilli (*Capsicum annuum* L) a member of Solanaceae family is one of the most widely grown cash crop throughout the world. It is cultivated for its fruits valued for colour, flavour, spice, vegetable and nutrition that it provides to the several food items. Chilli fruits are also used as food flavouring, colouring agent, pharmaceutical ingredient and in many other innovative ways. Therefore the objective of this study was to screen chilli germplasm lines including improved populations and inbreeds for quality parameters. Forty-six chilli (*Capsicum annuum* L.) genotypes of diverse origin were analysed for dry matter, vitamin C, capsaicin, oleoresin, extractable colour and colour value. Vitamin C was quantified by 2, 6-dichlorophenolindophenol method, whereas capsaicin, oleoresin, extractable colour and colour value by spectrophotometrically. Significant variations were recorded for all the parameters in the test genotypes. The dry matter ranged from 15.61 to 67.14% while vitamin C content ranged from 13.28-225.76 mg/100g fresh weights. The capsaicin content ranged from 0.1 to 1.47 %, whereas the oleoresin content ranged from 7.20 to 17.40%. Extractable colour (the standard measurement of colour in the spice industry) ranged from 53.30-294.38 ASTA. The genotype BS-78 had maximum colour value (116160 c.u.) and maximum extractable colour (294.38 ASTA). Multivariate Cluster analysis based on Ward's method showed that the genotypes were mainly divided at the first node into 2 clusters with 1 and 45 genotypes which were further sub divided into two groups.

Keywords: Chilli, capsaicin, oleoresin, vitamin C and extractable colour

Introduction

Chilli (Capsicum annuum L.), a species with new world origin, is one of the most important spice crops of the world. Chilli fruits are well known for their flavour, pungency and red ripe fruits are considered as one of the richest source of vitamin. The pungency in fruit is due to the capsaicin (8-methyl-N-vanillyl-6-enamide) and seven closely related alkyl vanillyl amides, collectively referred as "Capsaicinoids". More than 15 different alkaloids are found in chilli fruits. Among these, capsaicin and dihydrocapsaicin accounts for more than 80% of the capsaicinoids that determine the pungency. The degree of pungency varies widely within the genotypes of the five *Capsicum* cultivated species, from less than 0.05% in the mildly pungent types to as high as 1.3% in the hottest chillies. More than 30 different pigments have been identified in the chilli fruits. These pigments include chlorophyll, lutein, Zeaxanthin, violaxanthin, β-cryptoxanthin and β-carotene; and the red pigments capsanthin, capsorubin and cryptocapsaicin exclusively produced in chilli fruits. The capsanthin and capsorubin collectively known as oleoresin (Colouring agent) constitute more than 60% of the total carotenoids present in the fruits.

Besides vegetable and spice uses, chilli fruits are used as food flavouring as well as colouring agent, pharmaceutical ingredients and in other innovative ways (Kumar et al. 2006); as an example, in order to impart red colour to the egg yolk, the oleoresin is also mixed with chicken feed.

Demand for high quality oleoresin is increasing in the international market. The most highly valued characteristic of chilli genotype for oleoresin production is a high content of carotenoids as the commercial value of paprika (non-pungent oleoresin) depends on its colouring capacity, directly linked to relative pigment richness. Other Capsicum traits of interest are the very low content of capsaicinoids, low moisture content and a relatively thin pericarp. Chilli is the richest source of vitamin C.Vitamin C is an important antioxidative vitamin that is essential for maintaining a healthy immune system, building healthy connective tissue, bones and teeth, healing wounds and fractures. Thus, the major objective of this study was to screen chilli (hot pepper) and sweet pepper germplasm lines, including improved populations and inbreds, for vitamin C, capsaicin and oleoresin contents.

Materials and Methods

The germplasm included inbred lines, paprika lines (fruits with high colour and low pungency) and landraces. Seeds were sown in nursery beds during the month of July. Thirty days old seedlings were transplanted on raised bed at the distance of 60 x 45 cm. Recommended agronomic and plant protection practices were exercised in order to raise healthy crop. For most of the genotypes, red ripe fruits were collected from the single plant. However, for morphologically non-uniform genotypes, fruits were collected from 3-5 plants and bulked before the analysis. For the estimation of the dry matter, 200g red ripe fruits were randomlycollected from different replications at red ripe stage, dried to constant weight in an oven at 60 ± 2^{o}C and the dry weight measured, as well as the dry matter percentage.Ascorbic acid content (mg/100g fresh sample)was determined by 2, 6-dichlorophenolindophenol method, as described by Sadasivam and Theymoli (1987), wherein oxalic acid was used as titrating medium. Capsaicin content (percentage) in chilli powder was extracted and estimated spectrophotometrically by the method of Thimmaiah (1991). The absorbance of the sample was recorded at 650 nm using UV-visible double beam (Shimadzu UV-1601) spectrophotometer. Oleoresin was estimated as suggested by Mathew et al. (1971). Oleoresin percentage was calculated based on recovery of the residue. Extractable colour and colour value were determined according to the procedure described in AOAC. The absorbance was recorded at 460 and 462 nm using a UV-visible double beam spectrophotometer (Shimadzu uv-1601). All the above analyses were performed in triplicate. The range and means were calculated to assess the variability. Data were subjected to one-way analysis of variance (ANOVA) using standard statistical methods. Means were tested with Duncan's multiple range test (p□ 0.05 confidence level).Multivariate hierarchical clustering was carried for different quality characters. Distance between all pairs of genotypes was calculated using squared Euclidean distance method and genotypes were clustered based on Ward's method.

Results

The analysis of variance revealed significant differences ($p<0.05$) between chilli genotypes for the quality components of the fruits. Data reveal significant variation among the 46 test genotypes for fruit dry matter percentage. The maximum dry matter was recorded in JCA-9 (67.14%) followed by BS-27 (64.41%) and CCH-100 (53.32%), while minimum dry matter was recorded in NIC-268216 (15.61%) followed by Taiwan-1 (18.82%) and IC-119310B (19.01%). The genotypes also differed significantly for vitamin C content and values ranged from 13.28-225.76 mg/100g fresh weight. Maximum vitamin C was recorded in 9771-26 (225.76 mg/100g) followed by Taiwan-1 (193.20 mg/100g) and BC-4 (152.72 mg/100g) where as minimum vitamin C was recorded in 92-1206 (13.28 mg/100gm) followed by DC-3 (16.6 mg/100 gm)Capsaicin content varied from 0.1 to 1.47 %.The maximum capsaicin content (1.47%) was recorded in BS-35 followed by DC-16 (0.70%) and IC-9771-26 (0.68%), whereas minimum capsaicin content was recorded in IR-8 (0.10%) followed by JCA-9 (0.17%) and PBC-535 (0.18%). Oleoresin is also an important

processing product of pepper.In the present study the range of measured oleoresin varied from 7.2 to 17.40% : the maximum oleoresin was identified in IR-8 (17.40%), followed by JCA-9 (17.20%), while the minimum oleoresin was recorded in 92-1203 (7.20%) followed by DC-5 (9.40%) and DC-24 (9.80%). The genotype BS78 had maximum colour value (116160 cu) and maximum extractable colour also (294.38 ASTA-) followed by PBC-535 (115500cu and 287.82 ASTA for extractable colour). The minimum colour value and extractable colour was observed in IC-119361 (20790cuand 53.30ASTA).

The Cluster analysis showed mainly 2 clusters. From the dendrogram, it can be concluded that the genotypes were mainly divided at the first node into 2 clusters with 1 and 45 genotypes. First cluster again divided into 2 groups at the second node with 27 and 18 genotypes.

Discussion:

High dry matter for JCA-9 (67.14%) and BS-27 (64.41 %) might be a consequence of the thin skin and more seeds in smaller fruits. In earlier studieson dry matter in various genotypes of chilli, Shukla et al. (1957) reported a variation of 23.5 to 25 %, while Saimbhi et al. (1997) identified a range from 15.69 to 30.18 %. Kumar et al. (2003), reported a range of 78.30-188.30 mg /100g vitamin C in various genotypes of chilli. It has also been reported that the Vitamin C content diminishes to about 30% in canned and cooked pepper and nearly vanishes from dried pepper Lantz (1946).

Bajaj et al. (1980), reported a range of 0.34 to 0.95 % capsaicin, whereas, Kumar et al. (2003) reported a range of 0.33-0.49 %Singh et al. (2003) ,reported a variation of 0.33-0.70% capsaicin in various genotypes of chilli. Mathur et al. (2000), analyzed various varieties of chillies from different states of India through HPLC procedures and found that Tezpur chilli contained maximum capsaicin (4.28% w/w), while in other varieties, the capsaicin content ranged from 0.18 to 0.47%.

The lines identified for high capsaicin in this study can meet the demand of chilli varieties suitable for capsaicin extraction to be used mainly in medicine industry. The high capsaicin in cultivars may be due to smaller size of fruit and thin skin. Sharma and Saini (1977), reported that there is negative correlation of capsaicinoid content with size and thickness of fruit. Govinda, and Murthy (1999) reported maximum oleoresin (13.8 %) in chilli variety LCA-250. Singh et al. (2003), reported a range of 12.04 -17.06 %. oleoresin

The Extractable colour is a measurement of total pigment content in chilies. Extractable colour analyses are useful when pepper is added as an ingredient or colorant in oil based foods cosmetic or pharmaceuticals. Extractable colour measurement is standard tool of evaluation in the spice industry. Generally the higher the ASTA value the greater is the effect on brightness of the final product. Mathew et al. reported the range of colour value from 145000 cu to 170000 cu. Hosamini (2000), reported a variation of extractable colour from 173-213 ASTA with maximum values in Kt-Pl-19 (213 ASTA). The higher or lower carotenoid content for a given cultivar depends on various factors i.e. greater or lesser expression of genes governing carotenogenesis, physiological and morphological characteristics intrinsic to the particular cultivar, and growth conditions. Depending on the cultivar, certain biosynthetic pathways will be more important than others, and the fruit composition will differ. Hence, wide colour variation in the genotypes recorded in this study is not very surprising as the test genotypes comprised of diverse genetic background.

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Table 1. Quality performance of red ripe fruits of 46 chilli genotypes

Genotype	Dry matter (%)	VitaminC (mg/100g)	Capsaicin (%)	Oleoresin (%)	Extractable colour (ASTA)	Colour value (c.u.)
LCA-357	21.30	46.48	0.24	15.20	95.12	35970
PBC-535	25.58	49.80	0.18	14.00	287.82	115500
9852-173	25.96	19.92	0.19	14.00	188.60	749100
KA-2	50.25	25.76	0.38	10.20	280.44	111540
Taiwan-1	18.82	193.20	0.33	10.20	200.90	80850
DC-16	35.88	23.24	0.70	12.00	111.52	52470
9771-16	19.84	29.88	0.30	14.20	147.60	59070
92-1206	21.72	13.28	0.27	13.20	104.14	41910
9771-26	22.29	225.76	0.68	16.00	256.66	104280
92-1203	26.70	106.24	0.55	7.20	216.48	90750
977125-2	19.78	36.52	0.27	10.00	285.36	115500
IC-119310B	19.01	53.12	0.31	12.80	205.00	81840
IC-119368	32.43	23.24	0.28	10.60	210.74	84150
IC-119361	20.27	26.56	0.27	10.40	53.30	20790
IC-1402	35.25	36.52	0.39	12.60	213.20	80850
IC-119368A	27.45	36.52	0.63	12.80	110.74	47520
IC-119321	25.27	99.60	0.27	12.20	228.78	93720
IC-119474	23.09	109.56	0.26	15.00	195.98	77880
DC-3	38.26	16.60	0.41	10.20	243.54	99000
DC-24	39.00	16.60	0.41	9.80	227.14	91080
DC-7	32.20	29.88	0.26	11.40	171.38	67650
DC-5	36.73	122.84	0.39	9.40	90.20	34320
DC-4	29.11	16.60	0.34	16.80	134.48	53130
DC-28	27.47	23.24	0.35	11.40	185.32	78870
LCA-206	32.53	16.60	0.44	13.20	227.14	94380
PDAC-54B	20.26	16.60	0.83	10.60	189.42	74250
PDG-1	32.77	36.52	0.32	13.00	156.62	61380
NIC-268216	15.61	23.24	0.46	14.40	69.70	28050
LLS	22.77	19.92	0.53	10.20	161.54	62370
B. Kaddi-B	22.66	26.56	0.56	7.80	67.24	27390
B. Kaddi-A	25.18	19.92	0.29	13.00	208.28	78540
B. Dabbi	27.11	89.64	0.23	17.00	209.10	83160
AKC89/38	26.27	29.88	0.61	12.80	246.00	99990
BC-4	21.48	152.72	0.48	14.60	190.24	77550
LCFKM	29.90	53.12	0.46	13.60	260.76	103290
VNS-4	37.47	26.56	0.45	11.60	267.32	105930
No 476	27.26	16.60	0.35	8.80	134.48	52140
CCH-100	53.32	79.68	0.50	15.60	273.88	108240
IR-8	40.35	50.20	0.10	17.40	205.82	88440
BS-38	44.05	69.72	0.27	13.80	232.88	90750
BS-35	22.80	106.24	1.47	14.00	136.94	54780
BS-27	64.41	13.28	0.27	16.60	104.14	40920
BS-78	23.83	29.88	0.27	12.40	294.38	116160
Perennial	30.10	126.16	0.40	17.00	145.96	58080
EC-119457B	30.29	19.92	0.20	12.60	259.94	104280
JCA-9	67.14	19.92	0.17	17.20	127.10	49830
Mean	30.24	52.69	0.40	12.80	187.25	89731.30
Range	15.61-67.14	13.28-225.76	0.10-1.47	7.20-17.40	53.30-294.38	20790-116160
CD (0.05 %)	2.62	7.01	0.03	1.26	22.66	9759.76

CD – Critical differenceASTA- American Spice Trade Association, c.u- Colour Unit

Table 2. Analysis of variance for quality traits in chilli

Source of variance	D.F.	Mean sum of square					
		Dry matter	Vitamin-C	Capsaicin	Oleoresin	Extractable colour	Colour value
Replication	2	1.530	19.880	8.064	1.050	73.695	229616820.44
Treatment	45	377.490	7383.386	0.149	19.925	12829.051	2076385410
Error	90	2.618	18.693	5.119	0.603	195.183	36200662.998

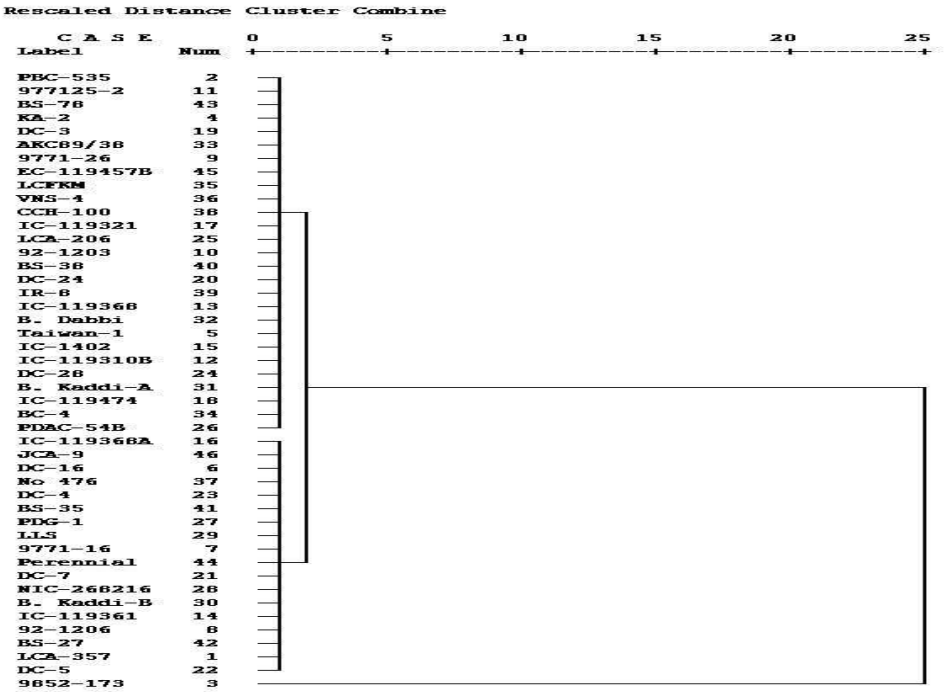

Fig. 1: Multivariate clustering pattern of chilli genotypes based on Ward's method

Brazilian ornamental pepper breeding program: a consortium among universities, small farmers and government agencies

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Abstract

The Federal University of Paraíba (UFPB) in cooperation with Federal University of Viçosa (UFV), small farmers, Ministry of Education and Brazilian Council for research and technologic development (CNPq) has been developing a breeding program for ornamental peppers, with the goal to evaluate and select breeding lines and promote the hybridization among the selected lines in order to distribute to small farmers in Brazilian states. The pepper program is composed of three stages: 1) searching, evaluation and selection of cultivars for growing in vase and longer post production life; 2) mass selection with progenitor's selection and 3) hybridization and evaluation of the single, triple and double hybrids and segregating generations. Parallel to the development of new cultivars by mass selection, it has been developed intra- and interspecific hybrids from the species *Capsicum annuum*, which are in evaluation with commercial cultivars. It was possible to select lines with increased longevity to 59 days. The segregate generations showed variability to ethylene sensitivity making possible selection for resistant lines to leaf and fruit abscission.

Keywords: *Capsicum* spp.; mass selection, hybridization; news cultivars

Introduction

The market of ornamental plants in pots is increasing at a higher rate than the cut flower market. Among the potted ornamental plants, peppers (*Capsicum* spp.) are very popular in the retail markets due to consumers looking for new products (Vieira, 2002; Rêgo et al. 2009). Despite the importance of potted peppers, only a few studies have been done in regards to plant height and precocity. In addition, other factors including capacity of aging during production and post production phases, action of ethylene, temperature and photosynthesis under low and high light intensity have not been addressed yet.

In general, the seeds for ornamental pepper varieties available in the Brazilian market are hybrids or traded cultivars like Gion Red, Pirâmide, Espaguetinho Ornamental and Grisu f-1 (Fabri, 2008). Despite the fact that the *Capsicum* genus has a large genetic variability to improve production, fruit quality, nutritional value, and resistance to diseases and insects (Casali and Couto, 1984), few studies have been conducted, in Brazil , regarding the improvement as of ornamental plants.

Because of that, the Federal University of Paraíba (UFPB) is developing a breeding program jointly with the Federal University of Viçosa (UFV) and the farmers' – Association for the Development of Communities of Macacos and Furnas (ADESMAF), with the financial support from CNPq and MEC –SESu. The goals of this program are to evaluate and select breeding lines of

pepper in order to produce hybrids with ornamental characteristics for small farmers in the state of Paraíba - Brazil.

We chose pepper as the subject of this breeding program because it is an easily grown species, development of a fast growing market, and an option to substitute the extraction of native plants from the wild. In addition ornamental peppers could be a new option for small farmers.

The steps of the program were to (1) produce hybrids and purelines of ornamental peppers, (2) training of small farmers, (3) implementation of greenhouses for the production of potted peppers, (4) production and commercialization of plantlets by the ADESMAF, (5) diversify the local production in the farmers' community, (6) release a book about the growth and processing of peppers, and (7) develop local meetings on the growth of ornamental peppers in the Brejo Paraibano – Areia City, Brazil. The results obtained in these steps, during the year of 2009 to 2010 will be described in the results section.

Today, the program has developed a more sustainable exploration of the natural resources by the farmers, generation of new jobs and less exploration of the natural biome and a progressive breeding program to develop ornamental pepper varieties including selection of fast growing genotypes that are resistant to aging and improved post production pot shelf life.

Material and Methods

The community of Macacos and Furnas are located around the ecological reservation of Mata do Pau de Ferro, composed by 30 families that survive exploring the native ornamental plants, in particular orchids, which are sold in the Areia City, Paraíba State (PB), Brazil and other neighboring cities.

The breeding program between UFPB and UFV is divided into three basic steps: (1) collection, evaluation, and selection of cultivars adapted to be grown in pots, with long post production durability; (2) mass selection with progeny testing; and (3) hybridization and evaluation of single and triple, and segregating offsprings. Promising accessions are tested for ethylene sensitivity at post production phase. New cultivar and hybrids are tested in the experimental field at UFPB.

Morphological characterization is done according to the descriptors for *Capsicum* genus (IPGRI, 1995). For the analysis 59 descriptors (qualitative and quantitative traits) were utilized. The chemical analysis followed the methods described by the AOAC (1990). Sensitivity to ethylene is done according to the methods described by Segatto et al. (2013).

The transfer of technology and training of the farmers were done in the years of 2008 and 2010.

Results and Discussion

Below are the main results obtained since the start of this program.

Development of breeding line and hybrids

Elizas Rainbow (UFPB 1): a new potted pepper ornamental cultivar

This cultivar was obtained after five cycles of mass selection with progeny testing during three consecutive years from a basic population of cherry like fruit of *Capsicum baccatum* chili pepper, from the "Accession 72" belonging to the germplasm bank of UFPB. The experiment was conducted in the experimental field and greenhouse at the Biotechnology Laboratory at CCA-UFPB. The plants within "Accession 72" had anthocyanin on the stems; canopy intensely branched, with an intermediate density of green leaves. The fruits are erect, pale yellow containing spots of anthocyanin, changing from purple, orange and red from the immature to ripe stages, respectively. The flowers are erect and white color with pale green spots and a greenish yellow corolla. The

values presented for the characteristics confirmed the potential use of "Accession 72" as an ornamental plant due to its short height (49 cm), similar to the traditional commercial ornamental cultivar Calypso. Also the plant produces many small fruits, an average of 30 per plant. The fruits are 3.1 cm in length and 3.1 in diameter, weighting 12 g each. The fruits have adequate quality for *in natura* consumption and for processing. The fruits have a pericarp thickness = 0.42 cm; fresh matter = 1.3 g; dry matter = 0.12 g; content of dry matter = 8.6%; vitamin C = 119 mg/100 g of fresh matter; total acidity = 0.79 g of citric acid/ 100 g of fresh matter; total soluble solids = 4.6 ^{o}Brix. Based on these data, the Elizas Rainbow (UFPB 1) has double use, as potted ornamental plants as well as a garden plant. Seeds of this cultivar were distributed to farmers in the communities of Macacos and Furnas, PB (Rêgo et al. 2012).

Ornamental hybrids

In parallel to the development of new cultivars by mass selection, intraspecific hybrids from *Capsicum annuum* were developed, and were compared to commercial cultivars. The program evaluated 36 hybrids as potted ornamental plants as well as generating segregating populations. These plants will be used as a base population to improve the resistance to deleterious effects of ethylene.

Post production

The response of ornamental peppers to ethylene was studied by Segatto et al. (2013). The authors found that after 48 hours in the presence of 10 μL/L ethylene, there was decrease in the chlorophyll content of the leaves in some genotypes of *Capsicum annuum*. The commercial cultivar Calypso was the most sensitive, dropping 100% of its leaves in the presence of ethylene. But the accession BGH 1039 was much less affected by ethylene, losing only 25% of its leaves when exposed to ethylene. Nevertheless this accession had abscission of fruit, which did not occur with the cultivar Calypso. Rêgo et al. (2009) and Silva et al. (2009) showed that the post production longevity of ornamental peppers varied from 13 to 72 days, after a simulated transport of 48 hours. Santos et al.(2013) working with an F_2 generation of ornamental peppers (*Capsicum annuum*), found segregation for ethylene resistance. The authors determined the presence of susceptible and resistant genotypes in the same F_2 family. This fact indicates a presence of a genetic component involved in the resistance to ethylene.

Construction of greenhouses

Three three greenhouses at the communities of Macacos and Furnas and one at CCA-UFPB were built. The greenhouses increased the income of small farmers. The greenhouses, which are being used for the production of ornamental plants, were funded by Ministry of Agricultural Development (MDA), Conselho Nacional de Pesquisa (CNPq) and Brazilian Ministry of Education

The greenhouse at CCA-UFPB improved the capacity of researchers and offered classes to the communities. The construction allowed for scientific publications.

This program was a unique opportunity to give better training to the undergraduate and graduate students in the programs of Genetics and Plant Breeding, Postharvest Physiology and Rural Extension. Furthermore, it developed partnerships with Federal University of Viçosa and small farmers from the region, and it build a better involvement of the CCA-UFPB with the research, extension and education.

It is important to mention that before the program developed by UFPB the farmers from ADESMAF use to sell 100 pots/month generating US$ 147. Now it is possible to produce 250 pots/month, generating a net profit of US$2,353, mainly with potted ornamental peppers. This result was better than expected, since the initial goal was to double the production and have a net income of US$1,412.

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New Jalapeño-type cultivars developed by EMBRAPA, Brazil

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Abstract

Capsicum agribusiness in Brazil is worth about US$ 50 to 60 million a year. Chile peppers are cultivated in all Brazilian states, with expressive areas of Jalapeño-type in Goiás, Minas Gerais, and São Paulo. *Capsicum* agribusiness has demanded the generation of new chile pepper cultivars adapted to Brazil, due to increased demands from small and large processing companies. Since 1980, the Brazilian Agricultural Research Corporation (Embrapa) has conducted projects on *Capsicum*, from germplasm collection in the Amazon to participatory breeding with farmers' communities. Embrapa´s main *Capsicum* breeding program is conducted by its National Research Center for Vegetable Crops, Embrapa Hortaliças. The program has been partly financed by the private sector. Two Jalapeño-type cultivars were released in 2009 that were the result of a joint research project between Embrapa and Sakura-Nakaya Alimentos Ltda. The main objective of this work was to develop high-yielding Jalapeño-type cultivars with high pungency, concentrated fruit set and agronomically adapted to Central Brazil. These new cultivars were derived from a varietal mixture cultivated by the private sector, with predominance of Jalapeño-like plants. Two superior open-pollinated Jalapeño-type lines were selected and released, 'BRS Sarakura' and 'BRS Garça.' Selection was based on plant and fruit characteristics such as plant architecture and height (compact plants), concentrated fruit ripening and easy picking, shape and size of fruit, immature and mature fruit color, pungency, field and industrial yield. Both cultivars are early yielding and highly uniform. 'BRS Garça' has yielded around 55 t/ha and its pungency is *circa* 50,000 SHU. 'BRS Sarakura' plants are compact with concentrated fruit ripening, yielding up to 60-65 t/ha with a pungency of about 58,000 SHU. Their resistance to virus diseases and nematodes are noteworthy; and in comparison to the original population, yields have increased by ca. 90-100%.

Keywords: *Capsicum annuum,* breeding, yield.

Introduction

Jalapeño peppers (*Capsicum annuum* L.) are originally from Mexico and were so named because they are traditionally grown in the city of Jalapa (Xalapa), Vera Cruz (Hernández, 1994). The jalapeño pepper is very popular in Mexico and in the United States of America, and it is becoming increasingly popular also in Brazil. Jalapeños are very versatile and green fruit can be consumed fresh, canned, pickled, dried and smoked (chipotle) and in sauces. The Jalapeño red fruit is commonly used by sauce processing industries in Brazil. This type of chile pepper is around 7 cm long and 3 cm wide, mildly pungent (8,000 to 30,000 SHU), and with a flavor similar to bell peppers (Casseres, 1981; Hernández, 1994). The standard Jalapeño pod is smooth, glossy, with suberized longitudinal cracks, thick walls, and taper to a rounded tip (Andrews, 1985).

The area cultivated with Jalapeño in Brazil is still small, restricted to the states of Minas Gerais, Goiás, Paraná, Bahia, and São Paulo, but the interest by processing industries in this type of hot pepper is increasing (Ribeiro et al. 2008). Most of the Jalapeño cultivars available in the Brazilian market have been imported from the U.S.A. and are not adapted to the agroecological conditions of central Brazil. Moreover, some farmers cultivate their own variety or population that presents high heterogeneity, segregating for important traits such as pungency. These genotypes generally present low yield, small fruit, with a pungency level below that desired by the processing industry, and indeterminate habit that hinders mechanical harvest.

Since 1980, Embrapa Vegetables has conducted projects on *Capsicum*, from germplasm collection to participatory breeding with farmers' communities; these programs have been partly financed by the private sector, and the main objective of this joint research project was to develop high-yielding Jalapeño-type cultivars, with good industrial traits (high pungency), and adapted to Central Brazil.

Materials and Methods

Individual Jalapeño-like plants were selected from a varietal mixture cultivated by the private sector. From each plant selected in the field F_2 families were developed, and single plant selection was accomplished within each family. Five generations of single plant selection and selfing were performed until the progenies showed no segregation. During each generation, selection for agronomic and processing traits relevant to the industry was undertaken. Selection was based on plant and fruit characteristics such as plant architecture and height (compact plants), concentrated fruit ripening and easy picking, shape and size of fruit, immature and mature fruit color, pungency, field and marketable yield.

Two superior lines were selected and seeds were increased to be used in subsequent field trials.

Replicated trials were conducted at Embrapa in Brasília, DF. Data for plant and fruit traits were based on a randomized complete block design with 8 replications containing 36 plants each over 2 years. Plants were uniformly spaced at 0.40 x 0.75 m for a plant population of 33,333 plants per hectare. Plants were grown using standard agronomic practices for pepper that are commonly used in central Brazil. From each plot, 10 randomly selected fruit were analyzed for quality traits. Pungency was determined by high-performance liquid chromatography and liquid chromatography-mass spectrometry (Parrish, 1996).

Both lines were evaluated for resistance to bacterial wilt (*Ralstonia solanacearum*), bacterial spot (*Xanthomonas campestris* pv. *vesicatoria*), phytophthora root rot (*Phytophthora capsici*), powdery mildew (*Oidiopsis taurica*), and root-knot nematodes (*Meloidogyne incognita* race 1, *M. javanica* and *M.mayaguensis*).

Results and Discussion

The new open-pollinated Jalapeño-type cultivars named 'BRS Sarakura' and 'BRS Garça' were released in 2009 (Figure 1). Both cultivars are early yielding and highly uniform. 'BRS Garça' yielded 55 t/ha and its pungency is circa 50,000 SHU. Plant height (≈ 80 cm) and width (≈ 75 cm) are higher than 'BRS Sarakura.' Plants of 'BRS Sarakura' are compact (plant height ≈ 40 cm and width ≈ 65 cm), fruit ripening is concentrated, and yields up to 60-65 t/ha with a pungency of about 58,000 SHU. In comparison to the original population (≈ 30 t/ha), yields have increased by 90-100% (Table 1).

Their resistance to bacterial wilt, bacterial spot and root-knot nematodes are noteworthy (Table 2) and virus diseases have not been detected in commercial fields of 'BRS Sarakura'.

Table 1. Fruit and plant characteristics for 'BRS Sarakura' and 'BRS Garça' evaluated over the years 2007 and 2008.

	Yield (T/ha)	Fruit width (cm)	Fruit length (cm)	Fruit weight (g)	Wall thickness (mm)	Heat (SHU)
BRS Sarakura	60	3.2	10	40	5	58,000
BRS Garça	55	3.0	11	40	5	50,000

Table 2. Evaluation of 'BRS Sarakura' and 'BRS Garça' for multiple disease resistance.

	BW	BS	PM	Nematodes			Phy
				Mi R1	Mj	Mg	
BRS Sarakura	IR	R	S	R	R	S	S
BRS Garça	R	R	S	S	R	S	S

BW= bacterial wilt; BS= bacterial spot; PM= powdery mildew; Mi R1 = *Meloidogyne incognita* race 1; Mj= *M. javanica*; Mg= *M.mayaguensis*; Phy= Phytophthora root rot.
R=Resistant; IR= Intermediate resistance; S=Susceptible.

'BRS Sarakura' and 'BRS Garça' produce excellent yields that have been superior to hybrids and open-pollinated cultivars available in the Brazilian market, and to the original population. The heat levels of both cultivars are considered high by industry standards, and are superior to the hot cultivar 'Early Jalapeño', with about 48,000 SHU (Bosland and Votava, 1998).

Both cultivars have a per-pod weight greater than 'NuMex Jalmundo' (34 g), an open-pollinated large-sized jalapeño cultivar recently released by New Mexico State University (Bosland, 2010).

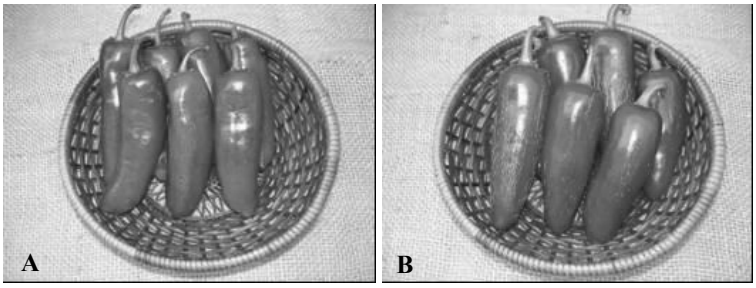

Figure 1. 'BRS Garça'(A) and 'BRS Sarakura'(B).

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Effect of conventional and organic conditions on the levels of ascorbic acid of *Capsicum* peppers

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Abstract

To assess the effect of organic cultivation on the nutritional value of peppers, we have evaluated the ascorbic acid content (AAC) in the fruits of a collection of 6 *C. annuum* accessions, the most common *Capsicum* species in Europe, and 1 *C. baccatum* accession. Plants were grown under conventional (control) and organic conditions and fruits were analyzed at both unripe and fully ripe stages. The ripening stage contributed the most to AAC and, in fact, all accessions showed higher levels at the fully ripe stage in both growing systems. Because of that, we consider separately each stage to study the accession (genotype, G) and growing system (environment, E) effects. Regarding unripe peppers, G, E, and G×E interaction contributed significantly to the observed variation. At this stage AAC was comprised between 198 $mg \cdot kg^{-1}$ of cv. Numex 6-4 and 823 $mg \cdot kg^{-1}$ of the *C. baccatum* accession under conventional cultivation (478 $mg \cdot kg^{-1}$ on average) and between 224 $mg \cdot kg^{-1}$ of cv. Valenciano and 723 $mg \cdot kg^{-1}$ of cv. Piquillo under organic cultivation (420 $mg \cdot kg^{-1}$ on average). Accessions showed different responses under each growing system, which explains the significant effect of G×E interaction. Thus, accessions like Numex 6-4 Heritage or Piquillo showed higher AAC under organic cultivation, while the contrary was found in *C. baccatum*, Bierzo or Valenciano. By contrast, only G and E contributed significantly to AAC at the fully ripe stage, and no significant contribution was found for G×E interaction. Thus AAC ranged from 881 to 1485 $mg \cdot kg^{-1}$ in *C. baccatum* and Najerano and between 941 and 1638 $mg \cdot kg^{-1}$ under conventional and organic systems, respectively. Moreover, organic cultivation increased AAC in the fully ripe fruits from all accessions, which caused the lack of significant G×E interaction. These findings suggest that there are ample opportunities for selecting *Capsicum* peppers with high AAC under organic cultivation, particularly at the fully ripe stage.

Keywords: *Capsicum* peppers, diversity, organic cultivation, ascorbic acid, genotype×environment interaction

Introduction

The interest for organic products is increasing in the last years and they are also recognized for contributing to sustainable agriculture (Raigón et al. 2010). However, there is still a broad discussion about the nutritional properties of organic vegetables provided by cultivation under these growing systems (Smith-Spangler et al. 2012). In fact, many comparisons between both conventional and organic cultivation have been limited to one cultivar/varietal type and, therefore, the knowledge of the effect of the genotype and the genotype-by-environment interaction is still scarce. In this regard, *Capsicum* peppers, both sweet and hot ones, are known for their levels of ascorbic acid, one of the most powerful antioxidants on nature (Bosland and Votava, 2000). Consequently, peppers can be considered as suitable materials to carry out comprehensive studies about the response of different genotypes to the cultivation under organic conditions and, then, to exploit the GxE interaction for selecting materials with the highest nutritional levels and added value for marketing.

In this regard, peppers are probably one of the most diverse vegetables. Indeed, a complex of five cultivated species and many wild relatives of genus *Capsicum* are used by humans in different regions of the world. *C. annuum* is the most economically important and genetically diverse species among the cultivated taxons of *Capsicum* and it is also the most common in Europe. *C. frutescens* and *C. chinense* are phylogenetically close to *C. annuum* (Lanteri and Pickersgill 1993; Bosland and Votava 2000), although their importance and adaptation to Europe, particularly the latter, is relatively low. Alternatively, *C. baccatum*, which has been limited to the Andean region until recent dates, has shown a satisfactory adaptation to the agroclimatic conditions of the Mediterranean region of Spain (Rodriguez-Burruezo et al. 2009), appearing as an alternative to diversify the production sector in Spain. Furthermore, the fruits of *Capsicum* are used in a plethora of dishes and recipes (CITA). Their double use, as a vegetable and/or as a spice, as well as the possibility of using them unripe or fully ripe has contributed to such diversity of culinary uses (DeWitt and Bosland 2009; Moreno et al. 2012). Consequently, breeders must take into account the ripening stage in their evaluations.

Materials and Methods

A collection of 6 *C. annuum* accessions (Bierzo, Cuneo, Najerano, Numex 6-4, Piquillo, and Valenciano) and 1 *C. baccatum* accession (BOL-37), selected by its good performance under Mediterranean agroclimatic conditions, were evaluated in the present experiment. Plants were grown open field under conventional (control) and organic cultivation systems during the 2012 Spring-Summer growing season in Sagunto (Valencia, Spain). The organic trial was carried out in the Marjal dels Moros protected area, while the control trial was carried out in a close field (distance less than 500 m). Therefore, water, soil and temperature conditions were the same in both cases. A total of 10 plants per accession-growing system combination were transplanted randomly on April 2012 at the 4-leaf stage. Fruits were harvested and analyzed at both unripe (green ripe) and fully ripe stages. Ascorbic acid content (AAC) was determined by potentiometric titration by selective electrode, using a Titrino 702 automatic titrater and a 0.05 M chloramine T solution as a standard. Data were submitted to analysis of variance (ANOVA) by means of Statgraphics 5.0 Software.

Results and Discussion

A preliminary ANOVA, considering simultaneously all the factors involved in the AAC, revealed that only the ripening stage contributed significantly to the observed variation (Table 1), suggesting that both the accession and the growing system were nonsignificant for this trait. In this regard, it is known that the ripening process increases AAC, although it has been also reported that Capsicums show a wide diversity for the levels in vitamin C and also that growing conditions may have a remarkable effect on this trait (Bosland and Votava, 2000). Therefore, we considered that the effect of ripening (and interactions) biased the real effect of the other main factors. For that reason, we performed new analyses of variation considering separately each ripening stage. Then, the ANOVA revealed highly significant effects ($P<0.01$) of the accession and the growing system at both ripening stages, while the interaction of both factors (G×E) only contributed significantly at the unripe stage (Table 2).

Descriptive results confirmed the ANOVA preliminary study and were in agreement with other previous reports. Thus, on average, AAC increased dramatically at the fully ripe stage (2-3-fold): from 400-500 $mg \cdot kg^{-1}$ at the unripe stage to 1100-1400 $mg \cdot kg^{-1}$ and, furthermore, all the studied accessions showed higher AAC values at this stage under both conventional and organic systems (Table 3). Nevertheless, accessions differed considerably in these increases, which was due to accession×ripening stage interaction. Thus, AAC of *C. baccatum* BOL-37 slightly increased with

the ripening process under conventional cultivation, while increases higher than 1000 mg·kg^{-1} were found in several accession×growing system combinations (e.g. most accessions under organic cultivation).

The comparison between growing systems at the unripe stage confirmed the G×E interaction detected by the ANOVA. As can be observed on Table 3, conventional cultivation provided higher AAC levels in Bierzo, Valenciano or BOL-37, while the contrary was true for Numex 6-4 or Piquillo. Due to this cross interaction, mean values were very similar between both growing systems and, therefore, we could not conclude which one provides higher AAC on average. Thus, G×E interaction must be exploited to select the best genotypes for organic production when unripe fruits are the target.

By contrast, all the accessions showed higher AAC values at the fully ripe stage under organic cultivation, which indicates the lack of G×E interaction for this ripening stage (Table 2). The increases due to organic production was 250 mg·kg^{-1} on average, and ranged among accessions from 50 to 500 mg·kg^{-1} of Piquillo and Bierzo, respectively (Table 3). Probably, the longer exposure of fruits to each growing system for covering the ripening process, and the AAC higher levels at this stage, contributed to reinforce these differences between organic and conventional cultivation in comparison to the unripe stage. Also, it has been suggested that organic cultivation provides more stressing conditions than conventional cultivation, which could explain the higher levels of this antioxidant in the former as a response of the plant to these conditions (Oliveira et al. 2013). In conclusion, we consider that the fully ripe stage offer breeders more opportunities for selecting cultivars adapted to organic cultivation with high AAC, providing an added value to peppers produced under these sustainable systems.

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Table 1. ANOVA table considering simultaneously the effects accession, growing system and ripening stage.

Effects	D.F.	Mean square	P-value	
Accession	6	120910	0.160	NS
Growing system	1	233503	0.084	NS
Ripening stage	1	28030900	0.000	***
Error	158	12175700		

Table 2. ANOVA tables for the effects accession (G), growing system (E) and its interaction (G×E) considering each ripening stage separately.

Effects	D.F.	Mean square	P-value	
	Unripe			
Accession (G)	6	373118	0.000	***
Growing system (E)	1	111232	0.008	**
G×E	6	267402	0.000	***
Error	76	19450		
	Fully ripe			
Accession (G)	6	388252	0.000	***
Growing system (E)	1	1088490	0.000	***
G×E	6	68309	0.336	NS
Error	76	58631		

Table 3. Ascorbic acid content (mg·kg-1 fresh weight) in the unripe and fully ripe fruits of the studied Capsicum accessions under conventional and organic cultivation.

Cultivar/accession	Unripe		Fully ripe	
	Conventional	Organic	Conventional	Organic
Bierzo	668±13	267±59	1060±28	1565±189
Cuneo	330±55	283±16	1115±41	1418±112
Najerano	244±37	247±37	1485±29	1638±117
Numex 6-4	198±2	653±39	1222±120	1598±107
Piquillo	557±95	723±87	1093±46	1147±65
Valenciano	524±11	224±22	1060±88	1394±102
BOL-37	823±28	545±25	881±66	941±58
Mean	478±23	420±20	1131±44	1386±37

Development of the parthenocarpic eggplant line 'Nasu Anou Kou 9 Gou'

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Abstract

The set and growth of eggplant fruits can be improved by using pollinator insects or by treating flowers with phytohormones. These techniques can be costly and labor-intensive. Parthenocarpic cultivars offer the most cost-effective solution to improving fruit set and growth under suboptimal conditions. Nasu Anou Kou 9 Gou, a parthenocarpic eggplant line developed at the NARO Institute of Vegetable and Tea Science in 2011, is an F_1 hybrid between two parthenocarpic inbred lines, AE-P01 and AE-P24. AE-P01 was selected from a cross between Talina (a commercial parthenocarpic F_1 hybrid that was widely cultivated in Italy) and Nasu Chuukanbohon Nou 1 Gou (a Japanese parental line). AE-P24 was developed from selective crossing of Nakate Shinkuro (a Japanese traditional cultivar), Talina, Nasu Chuukanbohon Nou 1 Gou, and Senryou Nigou (a commercial F_1 hybrid that is widely cultivated in Japan). Nasu Anou Kou 9 Gou produces commercial yields without phytohormone treatment; yields are higher than those of Anominori, another parthenocarpic cultivar developed at our institute.

Keywords: breeding, parthenocarpy, *Solanum melongena*.

Introduction

Using pollinator insects (Fernandez-Munoz et al. 1995) or treating flowers with phytohormones (Sarma & Barman, 1997) is necessary for stable fruit set in forcing culture of eggplant in plastic houses. However, these means are costly, labor-intensive, or both; it is necessary to keep a plastic house warm for pollinator insects to be active, and treating flowers with phytohormones takes about 25%–30% of total working hours required for eggplant forcing culture in Japan. Use of parthenocarpic cultivars is the most cost-effective solution for stable fruit set under suboptimal environmental conditions such as lower temperatures in forcing culture.

Several parthenocarpic eggplant cultivars have been bred in Europe, including transgenic cultivars (Rotino et al. 1997; Donzella et al. 2000; Acciarri et al. 2002; Maestrelli et al. 2003). However, these cultivars are unpopular in Japan because of their low yields and features that Japanese consumers don't like, such as green calyces and reddish purple fruits.

Japanese researchers started a breeding program in 1994 to develop a parthenocarpic eggplant cultivar acceptable to Japanese consumers by using a European parthenocarpic cultivar, Talina. At the NARO Institute of Vegetable and Tea Science (NIVTS) in 2006 we developed Anominori, a parthenocarpic cultivar that has a purple-black calyx and fruit of the type preferred by Japanese consumers (Saito et al. 2009a). Since then, we have developed a new F_1 hybrid, Nasu Anou Kou 9 Gou, with higher yield than Anominori. Here we describe the development and characteristics of Nasu Anou Kou 9 Gou.

Material and Methods

Development of Nasu Anou Kou 9 Gou

As a breeding material for parthenocarpy, we used Talina, a commercial parthenocarpic F1 hybrid released by Sluis & Groot Co., Ltd. (The Netherlands), and widely cultivated in Italy. Talina

was crossed to the Japanese traditional cultivar Nakate Shinkuro and to Nasu Chuukanbohon Nou 1, a parental line bred by NIVTS in 1994 (Fig. 1). A descendant of the cross between these two crosses, EP97B1-10-14-1, was crossed with the Japanese cultivar Senryou Nigou (Takii & Co., Ltd.), yielding AE-P24. The second cross also yielded AE-P01. From the progeny, we selected individuals with high parthenocarpic ability and fruit characteristics favorable for Japanese consumers, such as glossy, purple-black calyces and fruits. The F_1 hybrid between AE-P01 and AE-P24 was named Nasu Anou Kou 9 Gou.

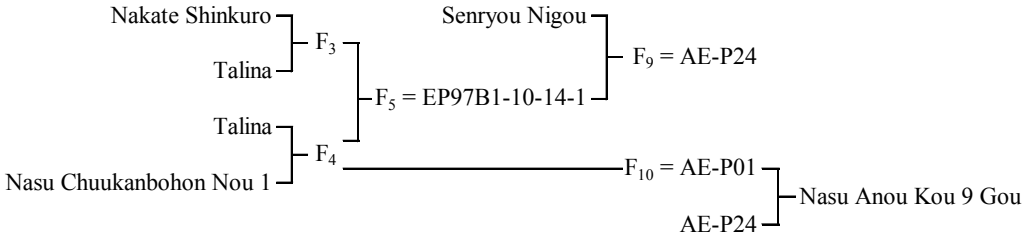

Fig. 1. The pedigree of Nasu Anou Kou 9 Gou

Characteristics of Nasu Anou Kou 9 Gou

The performance of Nasu Anou Kou 9 Gou was tested at NIVTS in open field culture in 2008 and in forcing culture in 2009 in comparison with Anominori and a leading Japanese cultivar, Senryou Nigou. Yield of marketable fruits and characteristics of plant and fruit were evaluated.

(1) Open field culture

Nine plants each of the three cultivars were tested. Seeds were sown on 21 March 2008. On 16 May, plantlets were transplanted to the field 0.6 m apart in rows separated by 1.2 m. Three main branches were trained. Phytohormones were not applied.

(2) Forcing culture

Six plants each of the three cultivars were tested. Seeds were sown on 3 August 2009. On 28 September, plantlets were transplanted into soil in a polyhouse 0.6 m apart in rows separated by 1.2 m. Three main branches were trained. Phytohormones were not applied to Nasu Anou Kou 9 Gou and Anominori. The opened flowers of Senryou Nigou were daily sprayed with a 50-fold diluted solution of the commercial phytohormone formulation Nissan Tomatotone (Nissan Chemical Industries, Ltd., Japan) which contains 0.15% (w/v) 4-CPA (2-methyl-4-chlorophenoxyacetic acid).

Parthenocarpy in Nasu Anou Kou 9 Gou

Six or seven plants each of the three cultivars were tested. Seeds were sown on 2 August 2011. On 27 September, plantlets were transplanted into soil in a polyhouse 0.6 m apart in rows separated by 1.2 m. Three main branches were trained. The opened flowers of half of the Senryou Nigou plants were daily sprayed with a 50-fold diluted solution of the commercial phytohormone formulation Nissan Tomatotone (Nissan Chemical Industries, Ltd., Japan) which contains 0.15% (w/v) 4-CPA (2-methyl-4-chlorophenoxyacetic acid). On each plant, we observed the fates of at least ten flowers that blossomed between 25 November 2011 and 8 January 2012. Parthenocarpy was calculated as the percentage of fruits grown to marketable size relative to the number of flowers.

Results and Discussion

Nasu Anou Kou 9 Gou produced oblong, glossy, purple-black fruits midway in shape between its immediate parents (Table 1, Fig. 2). The plant is tall, with long internodes, thick stems, and long petioles. The yields of marketable fruits were higher than those of Anominori in open field culture in 2008 and in forcing culture in 2009 (Table 2). The yield without phytohormone treatment was adequate for profitable use in forcing culture, comparable to that of Senryou Nigou treated with phytohormone (Table 2).

To compare parthenocarpy among the three cultivars, we quantified three possible fates of flowers: abscission, malformed fruits, and parthenocarpic fruits. The percentage of parthenocarpic fruits was almost as high in Nasu Anou Kou 9 Gou (91.5%) as in Senryou Nigou treated with phytohormones (96.7%), and much higher than in Anominori (64.5%). In contrast, Senryou Nigou did not set fruit without phytohormone (Table 3). Conversely, more flowers abscised and more malformed fruits were set in Senryou Nigou without phytohormone treatment than in Nasu Anou Kou 9 Gou and Anominori.

Table 1. Marketable fruit of evaluated eggplant cultivars and line in forcing culture in 2009.

Cultivars and line	Length (mm)		Diameter (mm)		Weight (g)		Shape	Calyx color
Nasu Anou Kou 9 Gou	159.7	5.6	47.9	0.9	115.7	6.3	oblong	purple-black
Anominori	166.0	4.0	46.1	1.0	118.1	5.8	slightly elongated	purple-black
Senryou Nigou	-	-	-	-	-	-	oblong	purple-black

Values are means for 15 fruits followed by standard error.

Table 2. Yield of of evaluated eggplant cultivars and line

Cultivars and line	Yield of marketable fruit (fruits/plant)	
	Open field culture	Forcing culture
Nasu Anou Kou 9 Gou	63.0 b	157.0 b
Anominori	54.6 a	125.8 a
Senryou Nigou	69.2 b	129.5 a

Fruits were harvested from 16 June to 6 October 2008 in open field culture, and from 15 October 2009 to 28 June 2010 in forcing culture. Values are means for 9 plants in open field culture and 6 plants in forcing culture, respectively. Senryou Nigou was treated with phytohormone in forcing culture. Values within a column labeled with different letters differ significantly among cultivars (Tukey's test, $P < 0.05$).

Table 3. Abscission, malformed fruits, and parthenocarpic fruits grown to marketable size as percentages of the initial number of flowers in evaluated eggplant cultivars and line during the parthenocarpy test

Cultivars and line	Treatment with phytohormone	Abscised flowers (%)	Malformed fruits (%)	Parthenocarpic fruits (%)
Nasu Anou Kou 9 Gou	–	7.0	1.4	91.5
Anominori	–	30.8	4.7	64.5
Senryou Nigou	–	74.4	25.6	0.0
Senryou Nigou	+	3.3	0.0	96.7

The parthenocarpic eggplant cultivars so far available in Europe need phytohormone applications to produce fruits of marketable size (Donzella et al. 2000). Also, the European parthenocarpic cultivars, Talina and Mileda did not produce commercial fruits in forcing culture without phytohormones, whereas Nasu Anou Kou 9 Gou produced profitable yields (data not shown). Nasu Anou Kou 9 Gou might produce profitable yields even under European winter conditions. Although the European parthenocarpic cultivars seem to readily elongate fruits once set without pollination, their ability to set fruits is low. By contrast, Japanese cultivars readily set fruits without pollination but their ability to elongate the fruits is low. Nasu Anou Kou 9 Gou seems to both set and elongate fruit more readily. Now we are trying to breed a new parthenocarpic and perfectly seedless eggplant cultivar by using cytoplasmic male sterility (Saito et al. 2009b).

Fig. 2. Fruits of Nasu Anou Kou 9 Gou (upper left), AE-P01 (upper right), AE-P24 (lower right), and plant of Nasu Anou Kou 9 Gou (lower left). Each black bar is 10 cm.

Conclusions

Nasu Anou Kou 9 Gou, a parthenocarpic eggplant line developed at NIVTS in 2011, is an F_1 hybrid between parthenocarpic inbred lines AE-P01 and AE-P24. Nasu Anou Kou 9 Gou produces commercial yields without phytohormone treatment. Yields are higher than those of Anominori, another parthenocarpic cultivar developed at our institute.

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Pepper rootstocks: agronomic evaluation and behaviour against *Meloidogyne incognita* and P*hytophthora* spp. in greenhouses of Murcia (Spain)

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Abstract

In the greenhouses of Region of Murcia, pepper (*Capsicum annuum*) is a monoculture since more two decades. *Meloidogyne incognita* and *Phytophthora spp*. are the main soil-borne pathogens. The agronomic and resistance characteristics to soil pathogens in twenty-one genotypes (varieties and rootstocks) were evaluated. Four independent trials, with some genotypes coincident, were carried out in two greenhouses (named greenhouse K and greenhouse AT), with the soil infested by *Phytophthora spp*. and *M. incognita*, characterized as virulent population in the greenhouse K. In all cases, grafted plants were equally or more productive than varieties non-grafted. Differences were observed between rootstocks, and also between varieties non-grafted in where those carrying resistance were equal or less productive. All rootstocks behaved as *Phytophthora* resistant, unlike the non-grafted varieties. Varieties susceptible to *Meloidogyne* were affected in all greenhouses. The other varieties and rootstocks, except one, behaved as resistant in the greenhouse AT, but some were susceptible in the greenhouse K.

Keywords: Soil-borne pathogens, crop yield, grafting, *Capsicum annuum*, resistant plant

Introduction

Pepper (*Capsicum annuum*) is a monoculture in the greenhouses of Region of Murcia (Southeast Spain) since more twenty five years. *Phytophthora* spp (*capsici* and *nicotianae*) and *Meloidogyne incognita* are the main soil-borne pathogens (Núñez-Zofio et al. 2013). *Phytophthora* is a limiting factor of this crop due to the importance of the losses it causes (Lacasa and Guirao, 1997). *M. incognita* became an emerging problem after the phase-out of methyl bromide (Guerrero et al. 2013). Disinfection with chemical fumigants has been the most widely used form of control to mitigate the effects of the two pathogens (Lacasa and Guirao, 1997). Currently most of the disinfectants are being reviewed for inclusion in Directive 91/414/EEC or are under exceptional use. The use of resistant cultivars is regarded as a strategy for soil disease control (Foster and Hausbeck, 2010; Djian-Caporalino et al. 2011; Colla et al. 2012). There are no commercial varieties of pepper with resistance to the two major pathogens, adapted to crop conditions and that meet market requirements. However, there are available many rootstocks which are a non-chemical alternative to control pathogens. In this study the behaviour against *M. incognita* and *P. nicotianae* and the marketable yield of several rootstocks has been evaluated and compared with susceptible and resistant commercial varieties grown in disinfected or not disinfected soil.

Materials and Methods

Twenty-one genotypes were used. Five were commercial varieties, including two with resistance to *M. incognita*. The other seventeen, which were used as rootstocks, belong to 13 F1 hybrids and 4 inbred lines with differential resistances to *M. incognita* and *Phytophthora spp.* (Table 1).

Four independent trials were conducted in two greenhouses (named greenhouse K and greenhouse AT) with soil naturally infected by *P. nicotianae* and *M. incognita* and non-disinfested or disinfested with the mixture of 1,3-dichloropropene + chloropicrin (1,3-D + Pic., in the greenhouse K for the reference variety). In the greenhouse K trials were repeated in three consecutive years. The experimental design was of randomized blocks with three replication and 50 plants per plot in greenhouse K and 45 plants per plot in greenhouse AT. In the middle of December was conducted the transplant, to frame 1 x 0.40 m (25000 plants / ha) and in early September finalized the crop. The reference variety was ´Traviata´ for greenhouse K and ´Gacela´ for AT.

Incidence of *Phytophthora* was measured expressing the results as percentage of affected plants. The *Meloidogyne* incidence was evaluated at the end of the growing season; results are expressed as percentage of plants with galls on the roots and the average of galls index, with values 0 to 10 according to scale of Bridge and Page (1980). The marketable yield was measured.

Table 1. Plant material features and distribution in greenhouses.

Variety or Rootstock	Resistances	Greenhouse K			Greenhouse AT
		2009-10	2010-11	2011-12	2001-12
Traviata F1 RZ (V)	No	--/DS	ND/DS	ND/DS	-----
Gacela F1 SG (V)	No	-----	-----	-----	ND
Atlante F1 RM (RS)	*Phytoph M. incog*	ND/---	-----	-----	-----
Brutus F1 GS (RS)	*Phytoph M. incog*	ND/---	-----	-----	-----
Creonte F1 (DR) (RS)	*Phytoph M. incog*	ND/---	ND/---	ND/---	ND
Costal IL (IMIDA) (RS)	No	-----	ND/---	-----	ND
P 4 F1 (IMIDA) (RS)	*Phytoph M. incog*	-----	ND/---	-----	ND
PP 10062 F1 (SK) (RS)	*Phytoph M. incog*	-----	ND/---	-----	-----
35144 RZ F1 (RZ) (V)	*M. incog*	-----	ND/---	-----	----
Avante F1 (RZ) (V)	*M. incog*	-----	ND/---	ND^p/--	ND
PG 5738 F1 (VL) (RS)	*Phytoph M. incog*	-----	-----	ND^p/---	ND
D7 F1 (IMIDA) (RS)	*Phytoph M. incog*	-----	-----	ND/---	-----
P18 F1 (IMIDA) (RS)	*Phytoph M. incog*	-----	-----	ND/---	ND
11S1820 (RZ) (V)	No	-----	-----	ND^p/---	ND
SCM334 IL (RS)	*Phytoph M. incog*	-----	-----	-----	ND
R1 IL (RS)	*M. incog*	-----	-----	-----	ND
C1 F1 (IMIDA) (RS)	*M. incog*	-----	-----	-----	ND
R3 IL (P)	*M. incog*	-----	-----	-----	ND^p
C3 F1 (IMIDA) (RS)	*M. incog*	-----	-----	-----	ND
2259 RZ F1 (RZ) (RS)	*Phytoph M. incog*	-----	-----	-----	ND
2260 RZ F1 (RZ) (RS)	*Phytoph M. incog*	-----	-----	-----	ND

(RS)= rootstock, (V)= variety; IL= imbred line; (IMIDA)= developed by IMIDA; GT= Gautier Semances; SG= Syngenta Seeds; DR= De Ruiter Seeds; RA= Ramiro Arnedo; RZ = Rijk Zwaan; VL= Vilmorin; SK= Sakata Seeds; DS= disinfected soil with 1,3-dichloropropene + chloropicrin (1,3-D +Pic); ND^p= Marketable yield no measured; X= no-disinfested soil; ----= no essayed.

Results

The F1 hybrids rootstocks with resistance to *Phytophthtora* spp. provided good control of the disease in both greenhouses. In all trials ´Creonte´ showed resistance to *P. parasitica*. ´SCM 334´ showed resistance, and ´R1´ and ´R3´ lines were not affected in the greenhouse AT. ´Costal´ was less susceptible than commercial varieties in greenhouse K (Table 2) and was not affected in greenhouse AT (Table 3). The reference varieties were more affected in greenhouse K than in greenhouse AT.

In greenhouse K, on the first and second year, F1 rootstocks, ´35144´ and ´Avante´ varieties, and ´Costal´ had low infection levels by *M. incognita* (Table 2). However, on the third year, the infection level for some rootstocks (´Creonte´, ´Atlante´, ´D7´) was similar to non-grafted susceptible variety. ´PG 5738´ and ´Avante´ had lower infection level than ´Traviata´, and similar or lower than ´Traviata´ grown on 1,3D + Pic disinfected soil. ´P18´ showed a very low index galls.

In greenhouse K, yields of grafted plants in the first and third years were higher than those of the commercial variety grown on disinfected soil (Table 2). In the second year, only plants grafted onto ´PP10060´ had similar yield to the non-graft variety grown on disinfected soil (Table 2). In greenhouse AT, all rootstocks had similar or higher yield than non-grafted varieties (Table 3). The yield of 'C1', 'C3', '2259RZ' and 'Creonte' were higher than 'Gacela' and only yields of rootstocks 'PG5738' and 'CM334' were similar to '11S1820' which was the lowest yield variety.

Grafting can be considered an alternative to soil disinfection to control *P. nicotianae*. When cultivation of grafted plants is reiterated, the *M. incognita* resistance in some rootstocks was overcome (´Creonte´), so it should establish integrated strategies for appropriate management of these resistances in order to maintain its effectiveness in controlling nematodes. It is suspected that nematode population of the greenhouse K overcome the resistance conferred by *Me7* gene, carried by the ´D7´ rootstock (hybrid of ´SCM334´), but not conferred by *Me1* gene, carried by the ´P18´ rootstock (hybrid ´HDA330´).

Table 2. Percentage affected plants by *Phytophthora nicotianae* (%Pp), index galls in roots (IG), percentage of plants with galls on the roots (%PG) and marketable yield (MY kg m^{-2}). Greenhouse K.

Variety/rootstock	**% Pp** [b]	**IG**[a]	**% PG** [b]	MY kg m^{-2}
2009-2010				
Traviata/Brutus	0.0a	0.0a	0.0a	10.2 a
Traviata/Atlante	0.0a	1.0a	26.7a	10.0 a
Traviata/Creonte	0.0a	1.4a	46.7b	10.8 a
Traviata 1,3-D+Pic	17.3 b	0.0a	0.00 a	8.5 b
2010-2011				
Traviata/Creonte	3.3a	0.0a	0.0a	7.15cd
Traviata/Costal	16.0b	1.7a	33.3b	7.26cd
Traviata/P4	2.0a	1.2a	33.3b	8.13d
Traviata/PP10062	2.0a	0.2a	10.0a	8.17de
Avante	75.33c	1.27a	40.0b	2.94 a
35144	84.67c	1.27a	40.0b	3.33 ab
Traviata	76.6c	6.0c	96.0c	4.55 b
Traviata 1,3-D+Pic	23.3b	4.4b	80.0c	9.5e
2011-2012				
Traviata/Creonte	1.33a	5.3d	100.00b	15.5d
Traviata/Atlante	0.7a	4.6cd	100.0b	13.8cd
Traviata/D7	1.33a	5.26d	100.0b	13.5c
Traviata/P18	1.33a	0.04a	4.17a	12.4c
Traviata/PG5738	0.0a	2.50b	100.0b	---
Traviata	60.0c	6.0d	100.0b	4.5 a
Avante	72.0c	3.6bc	100.0b	---
Traviata 1,3-D+ Pic	36.0b	3.8c	100.0b	9.7b

[a] Test LSD at 95% data were transformed with Log_{10} (x+1). [b] Test LSD at 95% data were transformed with Arcsin(√x). Values in the same column and year followed by the same letter are not significantly different ($P<0.05$).

Table 3. Percentage affected plants by *Phytophthora nicotianae* (%Pp), index galls in roots (IG), percentage of plants with galls on the roots (%PG) and marketable yield (MY kg m^{-2}). Greenhouse AT.

Variety/rootstock	**% Pp** [b]	**IG**[a]	**% PG** [b]	MY kg m^{-2}
Gacela	3.04b	4.87c	100.0	4.76bc
Gacela/Atlante	0.43a	0.00a	0.00	5.65ab
Gacela/Creonte	0.87a	0.00a	0.00	6.16a
Gacela/PG5738	0.00a	0.33a	14.29	4.04c
Gacela/SCM334	0.43a	0.00a	0.00	4.78bc
Gacela/Costal	0.00a	3.52b	100.00	5.03b
Gacela/2259RZ	0.43a	0.24a	19.50	6.67a
Gacela/2260RZ	0.00a	0.07a	7.14	5.87ab
Gacela/P18	0.43a	0.14a	9.52	5.57ab
Gacela/P4	0.87a	0.10a	4.67	5.54b
Gacela/R1	0.00a	0.00a	0.00	5.51b
Gacela/C1	0.00a	0.05a	4.67	6.34a
Gacela/R3	0.00a	0.00a	0.00	---
Gacela/C3	1.30a	0.00a	0.00	6.79a
Avante	4.78b	0.10a	4.67	4.43bc
11S1820	6.96b	5.48c	100.0	4.14c

[a] Test LSD at 95% data were transformed with Log_{10} (x+1). [b] Test LSD at 95% data were transformed with Arcsin(√x). Values in the same column followed by the same letter are not significantly different (P<0.05).

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Genetic background affects the expression of resistance to *Meloidogyne incognita* in pepper

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Abstract

In pepper *(Capsicum annuum*) four major resistance genes against *Meloidogyne incognita* (Me1, Me3, Me7 and N) were described. Under laboratory conditions, virulent populations were obtained against some of these. Likewise, in some soils where lines carrying the resistance was cultivated repeatedly, a selection of populations able to overcome them was observed, although showing differences in the level of infection among varieties carrying similar resistance gene. The objective is to study the behaviour against to *M. incognita* of different pepper lines in order to know the effect of genetic background on the expression of resistance to the nematode. Twenty three *C. annuum* lines were used. Three are carrying (in homozygosity) a resistance gene (Me1, Me3 or Me7). Five are from local varieties without known resistance genes, but well adapted to the environmental conditions and soil conditions of Region of Murcia (Spain), where the study was conducted. The other fifteen are F1 hybrids constructed from crosses between lines carriers and non-carriers of resistance. Parental and hybrids were tested under controlled conditions, with an avirulent and two virulent *M. incognita* isolates, and also in field conditions as rootstocks in a greenhouse with the soil contaminated by *M. incognita*, using as parameters of evaluation the number of eggs-mass for each plant and index galls. Results showed that, under controlled conditions, the Me1 homozygous line was not infected (no egg-masses in root) by any *M. incognita* isolates. However, the lines carrying Me3 and Me7 were infected by virulent isolates, although it was not so with the avirulent isolate. Some lines from local varieties showed high level of resistance to virulent and avirulent isolates, with a similar behaviour in greenhouse like lines carrying Me3 or Me7. In lines F1 differences were observed in the level of infection between lines with similar resistant gene, but with a different genetic background. Based on these results, genetic background in some varieties must be considered in breeding processes for durable resistance to *M. incognita*, and it is suggested the need for more detailed studies about the genetic characteristics responsible for this behaviour.

Keywords: Genetic resistance, Capsicum annuum, breeding line, nematodes, rootstocks

Heterosis of bacterial wilt (*Pseudomonas solanacearum*) resistance, yield and related traits in bell pepper (*Capsicum annuum* L. var. *grossum* Sendt.)

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Abstract

Forty bell pepper lines were screened for bacterial wilt resistance and resistant ones were selfed successively from 2004-2007 in the natural sick plots maintained at the vegetable research farm. Out of these, eleven inbreds were chosen as parents for the present study. Eight genetically diverse inbreds (lines) were crossed with three well adapted varieties (testers) in line x tester mating design during spring-summer, 2007 to obtain twenty four F_1 hybrids. Thirty five genotypes (eleven parents and twenty four crosses) were planted in a complete randomized block design with three replications during summer-rainy seasons, 2008 and 2009 and data were recorded for ten quantitative andfruit yield traits. Bacterial wilt disease observed in parents and hybrids confirmed the dominant nature of disease resistance, which can be exploited in combination with heterosis for yield. Correlation analysis indicated that selection for earliness, more fruits, longer harvest duration and high average fruit weight could be criteria for simultaneously increasing fruit yield in bell pepper. Therefore, due attention should be paid to improve these traits while making selection of high yielding genotypes or for choosing parents for heterosis breeding in cultivars/varietal improvement programme. Bacterial wilt incidence had negative correlation with days to 50 per cent flowering, plant height, harvest duration and fruit length at both genotypic and phenotypic levels suggesting that delay in flowering will decrease the incidence of the disease. Similarly, with decrease in plant height and harvest duration, level of disease incidence will increase and vice-versa.Bacterial wilt incidence had non-significant negative correlation with fruit yield at genotypic, phenotypic and environmental levels suggesting their combined effect in the expression of bacterial wilt.

Keywords: Disease, Heterosis, Correlations, Bell pepper, Bacterial wilt, Resistance

Introduction

Bell pepper (*Capsicum annuum* L. var. *grossum*Sendt.) is one of the few vegetable crops in which F_1 hybrids are being cultivated at commercial scale over world. Bacterial wilt caused by *Ralstonia solanacearum* (*Pseudomonas solanacearum*)is widely observed in Southeast Asia and is a serious problem in the cultivation of pepper crops (Mimura and Yoshikawa 2009). This disease seems to be a major hurdle in commercial cultivation of this crop and assumed an alarming proportion in some specific pockets of zone-I and zone-II of Himachal Pradesh. The objective of the study was to determine the heterosis and correlations for yield, related traits and to generate diversity for resistance to bacterial wilt disease in bell pepper hybrids

Materials and Methods

Forty bell pepper inbreds were screened for bacterial wilt resistance and resistant ones selfed successively from 2004-2007. Out of these, eleven inbreds were chosen as parents.

Plant materials

Lines: Kandaghat Selection (L_1), EC-464115 (L_2), EC-464107 (L_3), HC-201 (L_4), AC-48 (L_5), SKAU-SP-613-1 (L_6), PBC-631 (L_7) and SKAU-SP-633-1 (L_8) Testers: California Wonder (T_1),

Yolo Wonder(T_2) and SolanBharpoor (T_3). Method: Hand pollination using a standard procedure of emasculation. Mating Design: line x tester Year: 2004-09. Materials for evaluation trial:11parents and their 24 F_1 hybrids. Design: completely randomized block design. Replications: Three Year: summer-rainy seasons, 2008 and 2009. Traits Studied: days to 50 per cent flowering, days to first picking, plant height, harvest duration, fruit yield per plant, marketable fruits per plant, fruit length, fruit width, average fruit weight and bacterial wilt incidence.

Statsitcal analysis

Line x tester analysis (Kempthorne1957). Heterosis = increase or decrease in the mean F_1 performance over the better parent. The phenotypic, genotypic and environmental coefficients of correlation were also computed.

Results and Discussion

The results of field experiment revealed that there was sufficient genetic diversity among the genotypes (parents and F_1 hybrids) for all the traits studied (Table 1). The lines EC-464115 and EC-464107 and F_1 hybrids EC-464107 x Yolo Wonder and EC-464115 x California Wonder had high fruit yield alongwith resistance to bacterial wilt. The disease reaction in parents and respective hybrids (Table 1) confirmed the dominance of disease resistance, suggesting the possibility of exploiting heterosis in combination with bacterial wilt resistance. The significant negative better parent heterosis (BPH) was recorded in six cross combinations for days to 50 per cent flowering (Table 1). For days to first picking, the number of hybrid revealing hybrid vigour over better parent were higher (seven). compared to days to 50 per cent flowering. Introgression of earliness will serve as escape mechanism to the disease. For plant height, fourteen hybrids surpassed the better parent. Increased harvest duration contributes towards highest marketable yield in bell pepper. Out of twenty four hybrids, two expressed hybrid vigour over better parent. Mamedov and Pyshnaja (2001) also reported similar positive effectfor heterosis. Higher yield is basic objective for all the crop improvement programmes. The significant positive heterosis for fruit yield was recorded in fourteen F_1 hybrids. Heterosis for this trait has also been recorded earlier by Geleta and Labuschagne (2004), Khalil et al. (2004) and Sood and Kaul (2006) for other bell pepper genotypes. Nine F_1 hybrids reported significant heterosis for marketable fruits per plant. In case of fruit length and fruit width, heterosis was observed, respectively, in four and two hybrids,while it was showed in only four F_1's for average fruit weight. None of the hybrids expressed heterosis for bacterial wilt.

The genotypic correlation coefficients, in general, were higher than the corresponding phenotypic correlation coefficients, suggesting a strong inherent association among traits at genotypic level (Table 2). Correlation analysis indicated that selection for earliness, more fruits, longer harvest duration and high average fruit weight could be criteria for simultaneously increasing fruit yield in bell pepper. Therefore, due attention should be paid to improve these traits while making selection of high yielding genotypes or for choosing parents for heterosis breeding incultivars/ improvement programme. Bacterial wilt incidence had non-significant negative correlation with fruit yield both at genotypic and phenotypic levels suggesting their combined effect in the expression of bacterial wilt. The research has indicated that bell pepper hybrids resistant to bacterial wilt disease have been developed. Therefore, hybrids that are resistant and high yielding can be developed within the Indian ecosystem after further testing.

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The morphological factors determining the capsaicinoid content of pepper

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Abstract

Our observations indicate that flesh thickness of pepper fruit (1-8 mm), the rate of flesh and vein quantity, the number of veins on the surface of the fruit (2-4 veins), the length of veins, the number of glands on the veins, the size of the fruit substantially influence the capsaicinoid content of pepper.

Examination of these traits confirms that the total capsaicinoid content of thick fleshed fresh consumption pepper varieties is relatively lower than the capsaicinoid content of the thin fleshed spice pepper varieties. In case of species with outstandingly high capsaicinoid content (e.g. *C. chinense*) and certain varieties belonging to *C. annuum* species it is observed that the weight of their fruits is low, the fruit flesh is very thin, but the rate of the surface of the vein with capsaicinoid producing glands is relatively high. In case of fresh consumption pepper varieties with big fruits (e.g. California Wonder) the veins may contain as much capsaicinoid–as a pungent spice pepper variety with smaller fruit, but their pungency content concerning all the fruit is lower because of the bigger fruit weight than that of the pungent spice pepper varieties.

Therefore it is concluded the rate of components constituting pepper's fruit basically determines the order of magnitude of the capsaicinoid content of pepper types.

Keywords: Pepper, capsaicinoid, capsicum, pungency

Introduction

A common feature of species belonging to *Capsicum* genus is that their fruits contain capsaicin alkaloid. The consumption of pepper species containing capsaicinoid and certain varieties of *Capsicum annuum* is an important element in our eating culture. Consumers of fresh produce require peppers of different pungencies. To produce pungent pepper processing units, if possible, demand basic material with high capsaicinoid content, which they may dilute, as required. In addition to high capsaicinoid content, the fundamental demand of processing units is that the basic material could be produced and procured cheaply.

The presence of capsaicinoid is determined by dominant gene *C* (or *Pun*). The environmental and technological production impacts influencing capsaicinoid content are well known, but less is known about genetic traits influencing capsaicinoid content. Several methods can be used for measuring capsaicinoid content (Kirschbaum, 2002), the most widespread method in practice is the HPLC method making accurate measurement possible.

Materials and Methods

In the course of genetic improvement of pepper with high capsaicinoids content the measurement practice of capsaicinoids raised a fundamental problem. Big sample numbers must be compared during selection. There are significant differences in the water content of fruits; therefore only capsaicinoid content in relating dry material can be compared.

In the course of our activity of genetic improvement the capsaicinoid content of samples was determined by HPLC equipment using a modified hungarian standard method. The capsaicinoid evaluation was carried out through the following steps: methanol extraction in ultrasonic bath for 2

min. from oven-dried (72 °C, 24 hrs.) and grounded pepper powder, filtration through 0,22 μm PTFE syringe filter cleanup, HPLC separation with 45% acetonitrile/55% KH_2PO_4-buffer (0.2 M, pH=4) as mobile phase (filtered and degassed), on Kinetex C18 column (2,6 μ, 100x4.6 mm with C18 4x3 mm precolumn) with 1 $ml.min^{-1}$ flow rate and fluorescence detection (excitation at 288 nm, emission at 320 nm). The used HPLC-equipment was Shimadzu LC-20 Prominence; capsaicin and dihydrocapsaicin standards were obtained from Sigma.

Results and discussion

The capsaicinoid content was measured in all parts of pepper (flesh, placenta, seeds) by using dry material. We have observed that the pungency content of the bigger fruits and with thicker flesh was lower than that of spice pepper of traditional shape and size. Different types of fruits were analysed and compared to clarify the relationship between the morphological data and capsaicinoid content of pepper fruit.

The measurements were carried out on *Habanero* variety belonging to *C. chinense*, fresh, conical, white fleshed, edible sweet Cecei belonging to *C. annuum* species, pointed, pungent banana type and Hungarian, pungent spice pepper fruits. Average fruit weight, average seed amount, average fruit flesh weight, average vein weight, the length and shoulder thickness of the fruits, the thickness of flesh, the thickness, height, width and length of vein were measured in the several fruit types. The number of veins per fruits was examined separately for all pepper types. (Figure 1).

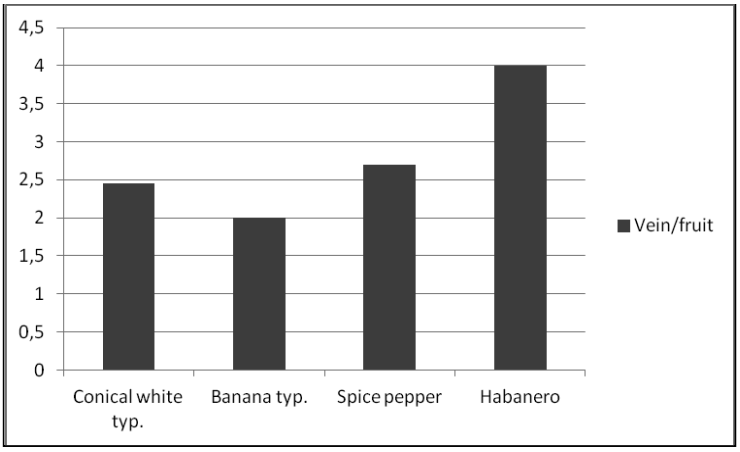
The percentage of parts constituting the fruits was determined from weights measured in dried condition (Figure 2).

The surface of the veins, where glands producing capsaicinoid are, was calculated from the measured data. In case of fruits of different types of peppers the vein surface by 1 g raw quantity and 1 g dry material was determined (Figures 3 and 4) Capsaicinoid content was determined from dried fruits with HPLC (Figure 5).

The rate of constituting parts of different pepper types, developed as a result of long selection processes, markedly differs. *C. chinense cv. Habanero* has the highest vein number per fruit among the examined types. The measurements indicate that the seed content of the Hungarian spice pepper is the highest. High seed content is an important selection criterion in case of types suitable for making ground pepper.

The examined quality traits do not show close relationship with the measured capsaicinoid content. The explanation is that there are significant differences in the number of glands producing capsaicinoids on the surface of the veins. Measurements prove that certain pepper types determine the achievable capsaicinoid content.

The selection for higher vein number per fruit can increase capsaicinoid content, what is morphologically determinated. This is an important improvement criterion.



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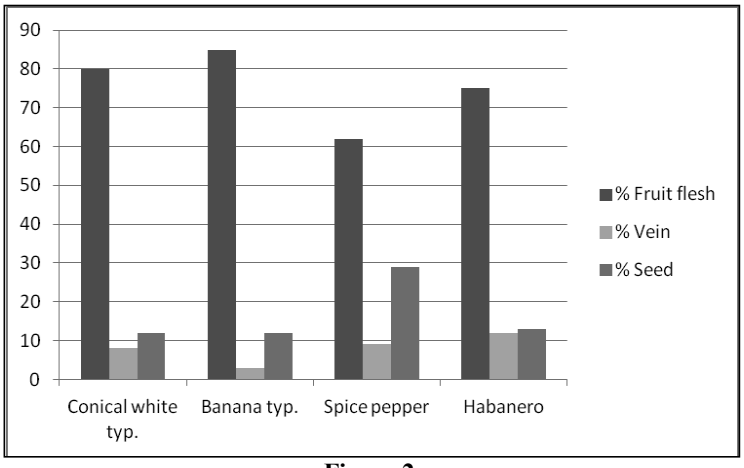

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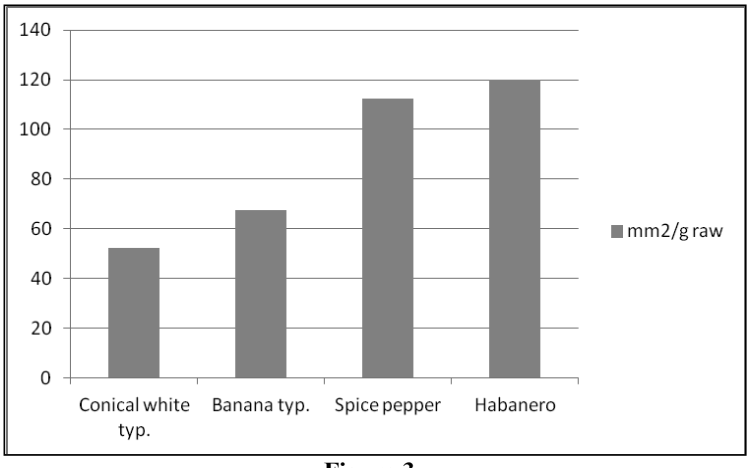

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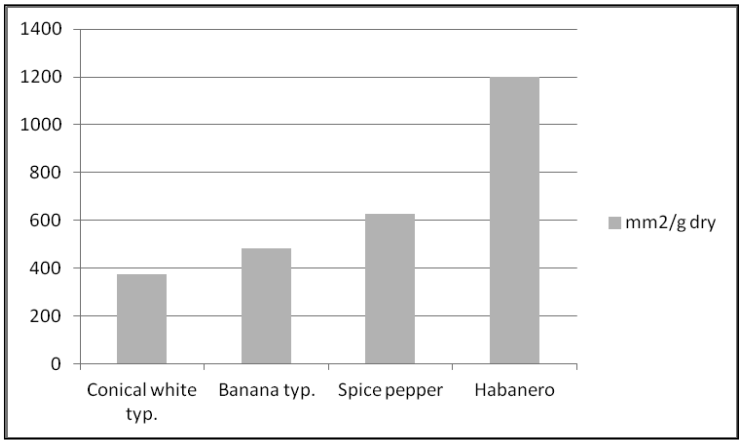

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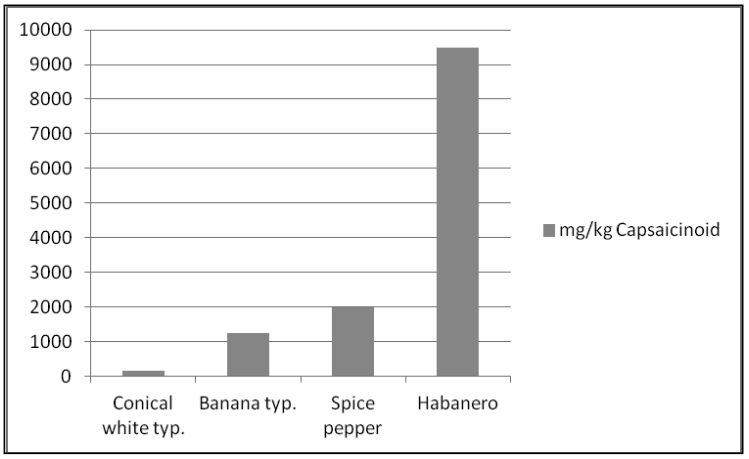

Figure 5.

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Monitoring of the stolbur wilting disease on the vegetable pepper in the transnistrian region

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Abstract

Stolbur or Big bud disease was diagnosed in plants of peppers with typical symptoms (yellowing, leaf roll, deformation and fruit's mummification, wilting), confirmed by tetracycline test and qRT-PCR. The pathogen which was detected in the affected plants of pepper and eggplant and tomatoes, as also in dodder and bindweed (*Convolvulus arvensis*), according to the fulfilled analysis belongs to the class of Mollicutes, a group of candidates, Stolbur Phytoplasma-kind type of 16SRXII-A Stolbur Phytoplasma,: Ca. Phytoplasma solani.

Study of the possible vector's population in model experiments on planting pepper helped to find besides leafhopper *Hyalesthes obsoletus* 8 new potential vectors of phytoplasma: Anaceratagallia, Eupteryx, Euscelis, Empoasca, Macrosteles, Psammotettix, Zyginidia, Zyginella, definitive systematic position of which is still being refined. Using the chemical method to control vectors of infection did not reduced the disease development in the open field, which to the end of the season became higher than 50% on the model variety Lumina. The agronomic control methods and breeding of resistant varieties and hybrids as well as using early ripening variety type Fasciculatum Sturt are promising methods of research which reduce affection by stolbur disease.

Keywords: *Capsicum annuum var. annuum* L., phytoplasma, disease monitoring, qRT-PCR, integrated struggle, chemical protection, breeding for resistance, breeding for early ripening.

Introduction

Stolbur of vegetable pepper was for the first officially registered in Moldova, since the end of the forties of the last century (Sukhov, Vovk, 1947). This disease has been marked in several countries, and also on many other cultures: tomatoes, eggplant, celery, carrot, lettuce, corn, rice, fruit, grapes, etc., and its role as an economically significant disease has increased over the past decade. (Har'kova, 1994; Nakashima, Hayashi, 1995; Moya-Raygoza, Nault, 1998; Boguutdinova et al. 2004; Gorkovenko, 2004; Bertaccini, 2007; Fominich et al. 2010; Konup, 2011; Lu, Xia, 2012). High damage and cyclicity are the main characters for this type of disease and usually epiphytoty is replaced by gradual decrease of the percentage and degree development of the disease on the industrial crops. But even during the years of remission, there is always the occasional sporadic manifestation of that disease which attributes it to natural - nidal type. The challenge to solve remained, including mapping of possible natural foci of pathogen reservation throughout the region and adjoining border areas and the development of an integrated control system above vector and pathogen. Although the pepper's Stolbur and other Solanaceae are well known disease in Transnistria, however, the pathogen had not been yet identified. In addition, it became necessary to

clarify information about the modern population of vector's infection thanks to the climate variation and changes of species composition of industrial plantings cultivars. The purpose of this study were the taxonomic identification of the phytoplasma pathogen of vegetable pepper, the analysis of the composition and dynamics of the population of the vector's infection during the growing season and the possibility of the regulating the plant protection against peppers phytoplasma by chemical and agro-technical measures, as well as evaluation of disease resistance of pepper.

Materials and Methods

The test plot was carried out the lower terrace of the Dniester River, surrounded by plantings of annual and perennial plants, including the bindweed (*Convolvulus arvensis*), nettle (*Urtica dioica*), etc. potential plant reservation of infection. Constant availability of reserves and vectors of infection around the test plot actually helped to create the provocative background with a high level of development of Stolbur disease. Study on the taxonomic identity of the pathogen were based on combined visual symptoms in the affected plants, tetracycline test and quantitative real time PCR (qRT-PCR). Susceptibility of plants under natural conditions was determined by visual estimation in an open field in stationary experiments on 4-point scale with 0 according typical symptoms to the generally accepted practice of phytopathology in determining the average score and percentage of sick plants. Test revealing the sensitivity of sick plants to tetracycline was conducted with the plants at the beginning of the disease when leaves of the cultivar Lumina plants were only chlorotic. In stationary experiments, plants were twice sprinkled by 1% solution of tetracycline. For qRT-PCR, material was prepared according previously described method (Konup, 2011) and later spent in reaction in thermal cycler Rotor-Gin using universal couple's primers fU5/fU3 specific for phytoplasma. In experiments on small plots entomological surveys were conducted in the dynamics, and the vector population caught on host-plants was identified with a identification guide with counting of the number of individuals according to taxonomy membership which was installed in the All-Russian Institute of Plant Protection (Russia). Insecticide spraying (concentrated emulsion) for multiplication factor 2, 4, 6, 8 and control (without spraying) was conducted by the readout dynamics of vectors flight every 7 days by one of the following insecticide: new Bi-58, Sherpa, and Kinfos. Standard insecticide input was appropriately 1, 0.3, 0.5 l/ha. The repeatability in experience was 4-th multiple. Planting of the surrounding perennials were not processed. The experiments started on 31 of July 2012 and ended on 4 of September 2012.

Results and discussion

Affection of model cultivar Lumina plants with stolbur symptoms and related viral diseases in stationary experiments under natural infection of the provocative background run up to over 70%. More than 50% of all diseased one was due to stolbur plants. When inspecting weeds on vegetable plots and pepper seasoned paired cultures (tomatoes, eggplants, cabbage, and potatoes and plants on lands adjacent to plastic greenhouses plants were found with obvious signs of yellowing only on bindweed. It may be due to asymptotically persistence of the most pathogen in reservation. At the same time the yellowing in crop plants on fields of Slobodzeya district was marked not only on peppers but also on eggplant and tomatoes too, may be thanks to more early contamination. Samples of the Solanaceae family were removed for detailed identification with typical visually manifested symptoms of Stolbur: chlorotic or yellowing leaves, their characteristic twisting, warping and changing of calyx colour, wilting. Test with tetracycline on chlorotic plants of Lumina in stationary experiments confirmed the phytoplasma etiology of the disease. After two months of antibiotic spray pepper plants visually recovered: growing young leaves of the upper tier were intensely green and evenly colored, without the typical twisting. The preliminary data for identification of the pathogen were confirmed by the most accurate method of quantitative PCR-diagnostics in real time. According to the analysis all tested samples of infected pepper plants,

eggplant and tomatoes growing next to the experimental plot, found bindweed and sample a dodder on it contained a pathogen which belong to the family of Mollicutes, a group Stolbur, type-A, 16SRXII Stolbur phytoplasma: Ca. Phytoplasma solani.

Study of vectors populations in model experiments on planting pepper found more 8 polyphages and potential vectors carrying the Stolbur, which belong to the following genera: Anaceratagallia, Eupteryx, Euscelis, Empoasca, Macrosteles, Psammotettix, Ziginidia, Ziginella, in addition to Hyalesthes obsoletus leafhopper, which did not become prevalent in Entomological collections when mowing. The collection of leafhoppers at direct planting pepper revealed the dynamics density of vector populations: in late May/early June the occurrence of caught in the net was represented by single individuals, in mid-June and July- by 30-40 pieces, at the end of July and August – up to 25 pieces and continued to meet at mowing until October. The tomato and pepper plants this year were intensively inhabited by cruciferous flea beetle in addition to leafhoppers due to the prevailing climatic conditions (increased average daily temperature and dry air). However, the role of the cruciferous flea beetles in transfer of stolbur remained unclear.

Vector's density population dynamics was the substantiation for carrying out systematic spraying against the carriers. Spraying was conducted from the end of May on a weekly basis. However, data about the sick plants on the provocative background showed the inefficacy of the chosen strategy of spraying and the use of conventional insecticides that do not impair the development of disease in open field on model cultivar Lumina in comparison with control in the case of continuous surroundings by plant reservation of phytoplasma and permanent arrival of new vector infection (Fig. 1).

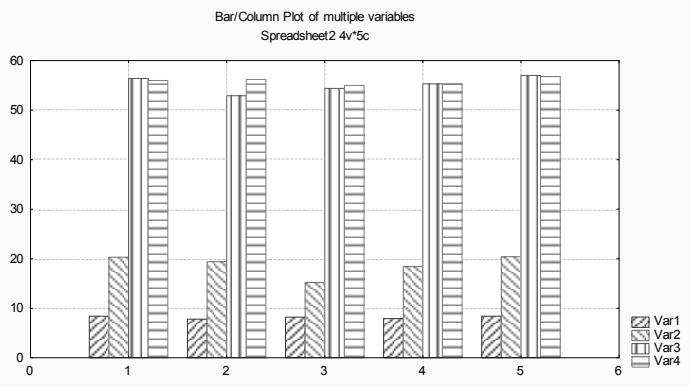

Figure 1 Dynamics of the stolbur disease development of the pepper cultivar Lumina planted in direct sowing culture on provocative background depending on the quantity of spraying. Note: testing date: Var1 - 31. 07; Var 2 - 15.08; Var 3 - 28.08; Var 4 - 4.09. The spraying number: 1 – control without processing, 2-2, 3-4, 4-6, 5-8 times.

Apparently, for effective plant infection vector of phytoplasma is not require more time than in the case of aphis transfer of a viral infection. At the same time, the results show, first, the necessity of the earlier preventive spraying for preventing infection of plants, and secondly, the need to eradicate plant reservation of phytoplasma (weeding, herbicide processing). In addition, since there has been a steady flow of polyphages vectors from plant reservation of infection, spraying them also are required. Insecticides used on pepper were not effective against phytoplasmas, which suggests a need to seek new effective one with directional action. Such drugs are found, for example, and it's recommended to use on grapes (Romanazzi et al. 2008). Because Stolbur in Solanaceae, and

Blackening of wood in grapes have the same causative agent and it is possible the free circulation of it in the population, the spatial isolation is recommended between these cultures.

The multi-year evaluation of sick plants of various cultivars of *C. annuum var. annuum* in open field evidenced the advantage of early ripening variety type *Fasciculatum* Sturt., which is less susceptible to a pathogen and yield before symptoms of the disease became visible (Table 1).

Table 1
Dynamics of disease development of the *C. annuum var. annuum* population under natural infection

Years of research	Studied		Disease development on variety type					
			Grossum		*Fasciculatum*		*Pomifera*	
	Samples	Plants	Mosaic	Wilting	Mosaic	Wilting	Mosaic	Wilting
1978	83	1660	2,2	3,3	-	-	1,7	2,2
1998	168	2660	1,2	0,5	0,8	1,1	1,1	1,8
2009	20	268	1,7	2,2	1,1	1,8	1,5	2,4
$LSD_{0.05}$=0,4								

Unheated plastic greenhouse ensure a mechanical barrier for spread of vectors on plants, even in the years of Stolbur epiphytoty, that prove the use of coulisse planting barriers in open field, as well as the creation of new varieties and hybrids adapted for growing in narrow-row planting technology under agricultural special material on drip irrigation.

Thus, monitoring of Stolbur on vegetable pepper refined taxonomic position of the causative agent of disease, revealed the components and dynamics of possible vectors population and indicated the system of effective measures of integrated control of the pathogen in the specific ecological and geographic cultivation area.

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Evaluation of different concentrations of tetrazolium to measure eggplant (*Solanum melongena* L.) seed viability

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Abstract

The objective was to evaluate different concentrations of tetrazolium to measure viability of seeds of eggplant. The study was conducted at the Laboratory of Genetics and Plant Breeding of the Universidad de Córdoba (Montería - Colombia), using eggplant seed of the commercial cultivar CO 029. We used the completely randomized design in factorial arrangement of 2 x 3 x 3, where the first factor represents soaking time, the second half of stain and the third the concentration of tetrazolium. The seeds after pre-conditioning were bisected lengthwise, discarding half of each seed. Soaking of each group (12 and 18 hours), were taken replicates of 25 seeds which were subjected to concentrations of 0.5%, 1.0% and 1.5% and within these different staining time of 6 , 12 and 18 hours in the dark. For a total of 18 treatments with four replicates each. Staining time has been standardized in order to make the observations and determine the outcome of each of the treatments. The results highlight that the soaking time is irrelevant in the quantification of the viability of the seed. Likewise, staining time and concentration accused significant differences. 0,5% concentration, coupled with a tetrazolium staining time of 6 hours is sufficient for proper staining and determining the physiological quality of the seed.

Keywords: *Solanum melongena*, seed quality, seed staining

Introduction

TTC (2, 3, 5-triphenyl-2H-tetrazolium chloride) test is a chemical analysis based on the oxidation-reduction reaction carried out by the living cells of the embryo or other seed tissues. Such cells contain enzymes, called dehydrogenases, involved in cellular respiration and they are able to react with the tetrazolium solution. In the reaction, hydrogen protons released in the respiration process, reduce the tetrazolium salt to formazan. TTC is used to differentiate between metabolically active and inactive tissues. The white compound is enzymatically reduced to red TPF (1, 3, 5-triphenyl formazan) in living tissues due to the activity of various dehydrogenases (enzymes important in oxidation of organic compounds and thus cellular metabolism), while it remains as white TTC in areas of necrosis since these enzymes have been either denatured or degraded (ISTA 1993).

The use of TTC is a relatively fast method that allows evaluating seed viability in 24h or in 4h if the seeds are incubated to high temperature (ISTA 1993).

For the farmer it is important to know the quality of the seed for planting because it will determine optimal germination, seedling emergence and growth and development of crop plants. The physiological quality of seeds of eggplant during production, maturation and storage is affected by abiotic factors such as temperature and relative humidity, which can decrease their viability, so it is necessary to evaluate their quality before planting, quickly and timely, particularly when they are produced and / or stored under particular ambient conditions such as those of Colombian humid Caribbean regions. Under this perspective, the aim of the study was to characterize the response of these seeds to a topographic test of 2, 3, 5 tetrazolium chloride, in order to identify healthy tissues and alterations in the same internal parts as well as the intensity of the coloration, and the contact time with the tetrazolium salt necessary to ensure its viability.

Materials and Methods

The experiment was carried out at the laboratory of Genetics and Plant Breeding of the Universidad de Córdoba, Monteria - Colombia, using commercial cultivar seeds CO 029, extracted from fruit at the optimum maturity in the second half of 2010. The conventional germination test showed a value of 90% germination.

To evaluate the viability of the embryos of eggplant seeds were considered two soaking times (12 and 18 hours), three staining times (6, 12 and 18 hours) and three concentrations of 2, 3, 5 tetrazolium chloride (0.5%, 1.0% and 1.5%), under a completely randomized design with factorial arrangement of 2 x 3 x 3, for a total of 18 treatments with four replications, consisting of 25 seeds each, temperature of 25 ° C.

The seeds were subjected to soaking in distilled water in Petri dishes at 12 and 18 hours, respectively, then were bisected longitudinally through the embryo, discarding half of each seed and then were treated with TTC at concentrations of 0, 5%, 1.0% and 1.5% and staining times of 6, 12 and 18 hours in absence of light.

After cutting, the seeds were placed in test tubes 120 mm x 25 mm containing 5 ml of 2, 3, 5, tetrazolium chloride to imbibition in the solution in the shortest possible time.

Variables evaluated responses correspond to those used in the methodology of Santos et al. (2007): 1. Seeds with viable embryos: the presence of pink to red coloration in the embryo and endosperm, with turgid tissue, firm and no visible lesions 2. Seeds with embryos partially dyed: characterized by staining absent or weak tissue by 50% in the embryo and endosperm, which fluctuates depending on the degree of deterioration from almost completely healthy until dead tissue unstained. 3. Seeds totally free of coloring embryos: presence of dead tissue over 50% in embryo and endosperm, generally flaccid, blurred, like white chalk-like or dull grayish-white.

After application of the treatments, seeds were washed several times with distilled water to remove excess dye. The viability was evaluated using a stereoscope (Vista Vision) 2.5x to improve the visualization of structures internal. Photographic records were made from tissues using a digital camera (Samsung 7.8 MP).

Results and Discussion

Lack of significance was recorded for soaking time (Table 1), so the 12-hour soaking is sufficient for a delicate operation and wasteful as longitudinal cuts without damaging seeds and enable direct contact between the embryo and endosperm with the tetrazolium salt for proper evaluation (Oliveira et al. 2009).

Highly significant differences were obtained for staining time and concentration of the tetrazolium salt in all response variables, indicating that the living tissues of the seeds are affected by salt staining at different times (6, 12 and 18 h) and concentration (0.5%, 1.0% and 1.5%). Interaction staining time by concentration presented highly significant differences for the variables completely dyed and undyed, while the variable dyed partially recorded significance 8%, indicating that the staining time is affected by the concentration of salt, they are not independent, these results are agree with those reported by Azeredo et al. (2011).

Test comparing means shows that the treatment of seeds, staining for six hours, was sufficient to identify living tissues and allowed identification of the highest number of embryos seed completely dyed, 10, 37 as well as the lowest number of seeds with partially dyed tissue and dyed, which is an advantage in obtaining reliable results in less time and is consistent with that reported by Gagliardi and Marcos Filho (2011) in chili.

Seed response to different levels of concentration of the tetrazolium salt was significantly different, indicating that a concentration of 0.5% is sufficient to identify healthy living tissues. Apparently higher concentrations are not suitable, since they exhibit dark colorations which make difficult to observe the damaged tissue, especially when they are associated with staining time.

Table 1: Mean squares and statistical significance level for variables completely stained, partially stained and unstained.

SOURCE	D.F.	COMPLETELY STAINED	PARTIALLY STAINED	UNSTAINED
ST	1	8,000 ***ns***	22,2222 ***ns***	2,000 ***ns***
TT	2	121,6252 **	49,8472 **	24,5416 **
CONC	2	801,8750 **	330,4305 **	127,1666 **
ST*TT	2	10,0416 ***ns***	6,4305 ***ns***	2,7916 ***ns***
ST*CONC	2	5,2916 ***ns***	2,1805 ***ns***	1,1666 ***ns***
TT*CONC	4	59,1875 **	14,4305 ***ns***	27,4583 **
ST*TT*CONC	4	3,1458 ***ns***	3,1388 ***ns***	1,9583 ***ns***
ERROR	54	4,97	6,50	2,09
TOTAL	71	33,96	17,20	7,66
MEDIA		8,08	13,9	3,0
CV %		27,58	18,29	48,21

** Highly significant differences 1%, * Significant differences 5%, NS Not significant

ST = Soak time, TT = Stain time; CONC = Concentration

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Evaluating the heat tolerance of sweet pepper by chlorophyll fluorescence assessment and effective pollination techniques

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Abstract

Chlorophyll fluorescence is a subtle reflection of primary photosynthetic (PS) reactions, and its measurement provides a non-invasive method of estimating PS performance. This study examined the PS activities of 12 lines of sweet pepper at standard temperatures (day/night (D/N) = 27/22 °C) and in high-temperature (D/N = 33/22 °C) environments. We examined six heat-tolerant lines of the bell type of pepper, and three lines of the paprika type, as well as two heat-susceptible lines of the bell type, and one commercial cultivar. Under standard temperature regimes, the heat-tolerant lines of C02080 and RB102 showed the highest photosystem II (PSII) efficiency among the 12 lines, based on the chlorophyll fluorescence parameters of Fv/Fm (variable fluorescence/maximum fluorescence). At high temperatures, the heat-tolerant lines of C02080 and C00611A demonstrated higher PSII efficiency within the Fv/Fm parameters. Furthermore, observations using a fluorescence microscope revealed stunted pollen tube growth at high temperatures in all lines except the heat-tolerant lines C02080 and C00611A, whereas all stigmatic receptivity remained unaffected. In summary, both chlorophyll fluorescence analysis and effective pollination showed that C02080 and C00611A demonstrated enhanced performance under heat stress. The results suggest that chlorophyll fluorescence analysis is a convenient method for evaluating the heat tolerance of sweet peppers.

Keywords： Chlorophyll fluorescence, Pollen tube growth, Heat tolerance, Sweet pepper.

Introduction

Capsicum annuum is native to the tropics of Central and South America, and most modern bell pepper cultivars are adapted to subtropical region. However, when these are grown in a tropical climate they tend to be short-lived and less productive. Recently, chlorophyll fluorescence has been widely used to evaluate the physiological effects of plant stress, including heat stress. Increasing the stress on plants will result in changes in fluorescence. Under stressful conditions, the Fv/Fm parameter decreases because of an increase in minimum chlorophyll fluorescence (Fo). In this paper we describe a non-destructive, relatively rapid screening method for sweet peppers using changes in chlorophyll fluorescence to measure heat tolerance. We also determined the effect of high temperature on pollination and pollen tuber behavior.

Materials and Methods

We selected 12 genotypes of sweet pepper in the field, including six genotypes of bell pepper and three of paprika pepper that might have had heat tolerance potential, and two additional

genotypes of bell pepper that had produced a lower yield during the summer. We also selected one commercial variety to evaluate the PS efficiency as well as the pollen viability test.

Modulated chlorophyll fluorescence measurements were carried out with a Walz PAM 2000 fluorimeter (Heinz Walz, Effeltrich, Germany). Differences among the various genotypes for a number of parameters and conditions, as well as the variance and the least significant difference (LSD), were evaluated using SAS software (SAS Enterprise Guide 4.1).

Results and Discussion

At D/N temperatures of 27/22 °C, 'C02080' and 'RB102' showed superior PSII efficiency among the 12 genotypes, as estimated by the chlorophyll fluorescence parameters Fo and Fv/Fm (Table1). For the heat stress experiment (D/N: 33/22 °C), from the flower differentiation stage to anthesis (42 to 62 DAS), we used the fluorescence parameters Fm, Fo and Fv/Fm to evaluate the PSII efficiency among the 12 genotypes (Table2). The results indicated that C02080 and C00611A showed superior performance, and Fv/Fm increased because of a decrease in Fo (Table 3).

Table1. Comparison of the chlorophyll fluorescence parameters Fo and Fv/Fm in the leaves of 12 genotypes of sweet pepper plants grown under 27/22 °C (day/night).

Genotype	Fo		Fv/Fm	
C01184	0.340 ± 0.007	a [z]	0.789 ± 0.004	cd
C02080	0.285 ± 0.005	d	0.804 ± 0.004	a
PS38	0.295 ± 0.010	cd	0.788 ± 0.007	de
C00611A	0.317 ± 0.005	b	0.792 ± 0.003	bcd
C00947	0.309 ± 0.005	b	0.787 ± 0.005	de
C03338B	0.307 ± 0.004	bc	0.790 ± 0.005	cd
C01336	0.294 ± 0.004	d	0.789 ± 0.004	cd
C05464A	0.288 ± 0.004	d	0.796 ± 0.004	bc
TC06979	0.287 ± 0.002	d	0.789 ± 0.004	cde
RB102	0.290 ± 0.005	d	0.800 ± 0.003	ab
KC104	0.296 ± 0.003	cd	0.789 ± 0.005	cd
Beauty Star	0.314 ± 0.003	b	0.782 ± 0.005	e

[z] Values are Mean ± standard error (n = 12). Mean values followed by the same letters within each column are not significantly different at the 5% level.

Table 2. ANOVA of the chlorophyll fluorescence parameters of four growth days of the leaves of 6 sweet pepper genotypes plants grown under 33/22 °C (day/night).

Source of Variation	Mean squares of different parameters [z]					
	df	Fo [z]	Fv/Fm	Fm	ΦPS Ⅱ	qN	Fm'
Replication	2	0.00003	0.00011*	0.00408	0.00307*	0.00088	0.00084*
Growing time (T)	3	0.00144**	0.00088**	0.08876**	0.05511**	0.00707**	0.02961**
Genotype (G)	5	0.00087**	0.00013**	0.02279**	0.00993**	0.00010	0.00418**
T × G	15	0.00011	0.00003	0.00104	0.00315**	0.00086*	0.00065**
Error	48	0.00006	0.00003	0.00218	0.00080	0.00040	0.00022

[z] Fo: Minimum fluorescence ; Fv/Fm: Maximal quantum efficiency of PSII photochemistry ; Fm: Maximum fluorescence ; ΦPSII: Effective quantum yield of PSII reaction centers ; qN: non-photochemical quenching coefficient ; Fm': Maximal fluorescence (light-adapted). *, ** significant at 5% and 1% level, respectively.

Table 3. Comparison of the chlorophyll fluorescence parameters Fo, Fv/Fm and Fm in the leaves of 6 genotypes of sweet pepper plants grown under 33/22 °C (day/night).

Genotype	Fo		Fv/Fm		Fm	
C01184	0.297 ± 0.003	a [z]	0.801 ± 0.002	c	1.493 ± 0.023	a
C02080	0.276 ± 0.003	cd	0.808 ± 0.001	a	1.440 ± 0.021	bc
C00611A	0.284 ± 0.004	b	0.806 ± 0.002	ab	1.465 ± 0.021	ab
C00947	0.283 ± 0.002	bc	0.803 ± 0.002	bc	1.433 ± 0.021	c
TC06979	0.272 ± 0.003	d	0.801 ± 0.003	c	1.365 ± 0.022	d
Beauty Star	0.283 ± 0.004	b	0.800 ± 0.003	c	1.417 ± 0.024	c

[z] Values are Mean ± standard error (n = 12). Mean values followed by the same letters within each column are not significantly different at the 5% level.

In addition, the influence of the high temperatures (33/27 ^{o}C) on the growth of pollen tubes were observed under fluorescent microscope. The results revealed that high temperature stunted pollen tube growth except in the heat-tolerant lines C02080 and C00611A, whereas all stigmatic receptivity remained unaffected as compared with the heat-sensitive line C00947 (Fig.1). In summary, both chlorophyll fluorescence analysis and effective pollination showed that C02080and C00611A demonstrated superior performance under heat stress (Table 3; Fig1).

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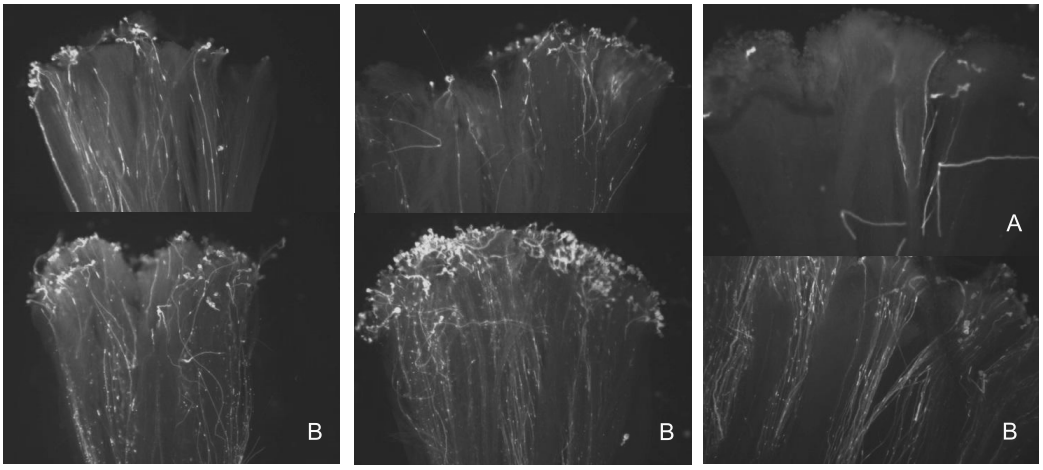

Fig.1. Pollen germination and pollen tube growth were investigated by fluorescent microscopy after 24 h pollination in 5 lines of bell pepper. (A: The pistil and pollen grains were derived from plants growing at 27/22 ° and 33/22 °C, respectively. B: The pistil and pollen grains were derived from plants growing at 33/22 °C and 27/22 °C, respectively.)

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SESSION II

Molecular genetics and biotechnology tools

Identification of DArT markers associated with male sterility *ms8* gene in sweet pepper (*Capsicum annuum* L.)

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Abstract

The male sterility *ms8* gene is a recessive nuclear gene that may be useful in the development of genic male sterility systems for breeding sweet pepper hybrid lines. The usefulness of such a system depends on the availability of molecular markers of the *ms8* gene. Although we detected several SCAR and COSII/CAPS markers that were linked to the *ms8* locus, tightly linked, reliable markers remain elusive. To find better molecular markers of the *ms8* locus, we used diversity arrays technology (DArT) combined with the bulk segregant analysis method (BSA; DArT-BSA). A total of 2888 pepper DArT markers were tested on DNA samples representing parental genotypes, bulked samples, and F2 individuals of a mapping population of 'line 320 x variety Elf' included in bulked samples. Seven DArT markers associated with the *ms8* gene were selected and DArT clones were sequenced. Interestingly, most of the DArT sequences showed homology to pepper cDNAs/expressed sequence tags. An attempt was undertaken to convert DArT markers into the polymerase chain reaction-based markers for further linkage mapping. Identified DArT markers could be useful for fine mapping and cloning of the *ms8* gene.

Keywords: genic male sterility, molecular markers, hybrid breeding

Introduction

More than 20 nuclear genes responsible for genic male sterility have been described in pepper (*Capsicum annuum* L.) to date (Shifriss, 1997; Wang and Bosland 2006). One of these is the *ms8* gene described by Daskaloff in the Bulgarian variety 'Zlaten Medal' (Daskaloff 1973). The expression of male sterility determined by this recessive gene is stable under field and plastic tunnel conditions in Poland, leading to attempts to use it in sweet pepper hybrid breeding programs (Korzeniewska and Niemirowicz-Szczytt, 1998; Sztangret 1998). Effective use of the gene in breeding programs requires molecular markers that would allow the selection of homozygous male-sterile plants at the seedling stage. The *ms8* gene is localized on the lower arm of chromosome P4 and several polymerase chain reaction (PCR)-based markers linked to the *ms8* locus have been identified, including SCAR_P2 and RAPD Z05-760. The latter are the closest markers identified and are linked to the *ms8* at the distance of 4.6cM (Bartoszewski et al. 2012). More useful in breeding programs would be markers that are codominant, closer to the *ms8* locus, and that could be introduced at reasonable cost.

Diversity arrays technology (DArT) is a high-throughput, cost effective method of molecular mapping and diversity assessment (Jaccoud et al. 2001). It is based on hybridization and is especially useful for plant species possessing complex genomes for which genomics resources are limited (Jing et al. 2009; Bolibok-Brągoszewska et al. 2009). DArT can be combined with bulk segregant analysis (DArT-BSA) to identify gene-specific molecular markers (Wenzl et al. 2007). This approach was successfully applied to map the gene conferring resistance to leaf rust in wheat

and the leaf rust resistance gene *Rph14* in barley (Czembor et al. 2008; Golegaonkar et al. 2009). In this study, we used DArT-BSA to search for molecular markers for the *ms8* locus. We used the F2 sweet pepper mapping population '320 × Elf' that we had developed previously.

Materials and Methods

Plant material and DNA preparation

A sweet pepper F2 mapping population '320 × Elf' obtained from a cross between the male-sterile line 320 and male-fertile variety 'Elf' was used in this study (Bartoszewski et al. 2012). DNA from parental lines and F2 individuals was extracted using the cetyl trimethyl ammonium bromide method. The concentration of DNA was determined using spectrophotometry (SmartSpec, Bio-Rad) and adjusted to 5 ng/µL. Two bulk DNA samples were constructed by mixing equal aliquots of DNA from F2 individuals. Bulk sample "Sterile" (S) was constructed by mixing DNA from 29 homozygous male-sterile F2 plants (genotype *ms8/ms8*) and bulk sample "Fertile" (F) was constructed by mixing DNA samples from 30 homozygous male-fertile F2 plants (genotype *Ms8/Ms8*).

DArT-BSA analysis and DArT marker sequencing

DArT-BSA analysis of DNA from the variety Elf, line 320, bulk sample S, bulk sample F, and the F2 individuals included in bulk F and S (total 63 samples) was performed as described by Wenzl et al. (2007) at the Diversity Arrays Technology Pty Ltd. (Yarralumla, Australia). A diversity panel representing 379 *C. annuum* genotypes was used in the analysis. DArT marker signals were scored and analyzed using MS Excel software. Inserts of selected DArT clones were amplified with PCR and directly sequenced. To edit sequencing reads and to build contigs, Sequencher 5.1 (GeneCodes, Ann Arbor, MI) software was used. Consensus sequences of DArT markers were used for the sequence similarity search, conducted with the Basic Local Alignment Search Tool (BLAST) (e-value cutoff = 5e-10).

PCR analysis and sequence comparisons

A set of PCR primers was designed based on DArT marker sequences using Oligo 7.51 (MBI, Cascade, CO). Primers were tested using standard PCR with a gradient-annealing temperature of 50-65°C and DNA samples of line 320 and the 'Elf' variety. PCR amplicons of the expected size were gel-purified and sequenced. Consensus sequences for line 320 and 'Elf' variety were obtained and compared using Sequencher 5.1.

Results and Discussion

The DNA of the mapping population from the parental lines, the bulk samples S and F, the F2 male-sterile individuals (*ms8/ms8*), and the F2 male-fertile individuals (*Ms8/Ms8*) included in the bulked samples was used for DArT genotyping. Based on the intensity of the hybridization signal, DArT markers, which are dominant, were scored either as present or absent. In total, 2888 DArT markers were informative and analyzed on all 63 DNA samples. Seven DArT markers distinguishing male-sterile and male-fertile plants in different ways were detected. Five DArT markers were present in bulk S and male-sterile F2 individuals. Two markers were predominantly present in male-fertile F2 individuals; however, they were present in both bulk samples and parents (Table 1).

Table 1. DArT markers associated to male sterility locus *ms8*.

DArT marker number	Parental lines		Bulk		Number of F2 individuals:	
	Elf	320	F	S	homozygous fertile	homozygous male sterile
672708	0	1	0	1	0	26
673090	0	1	0	1	0	23
673809	0	1	0	1	0	22
673153	0	1	0	1	1	21
674345	0	0	0	d.n.	1	17
705751	1	1	1	1	19	2
705205	1	1	1	1	15	1

0 – lack of the signal, 1 – presence of the signal, d.n. – data not available

All seven DArT markers were sequenced and nine sequences of lengths between 162 and 661 bp were obtained. Sequences of two markers, 673090 and 673153, were overlapping. For three markers, there were some inconsistences in separate sequencing experiments and, finally, two different sequences were shown to be representative of them. Using a BLAST search, DArT sequences were compared with sequences that had been deposited at the National Center for Biotechnology Information (NCBI), Solanaceae Genomics Network (SGN), and the University of California at Davis pepper GeneChip/IGA databases. Six out of nine sequences showed significant similarities to cDNAs of pepper, including three sequences (673090 /673153, 673809, and 705751) that showed identity to GeneChip MGMT contigs. One of the sequences representing DArT 673809 was similar to long terminal repeat (LTR)-retroelements. Remaining sequences were less similar. PCR amplification was successful for eight out of the nine DArT marker sequences for line 320 and the variety 'Elf': however, within the range of the sequences obtained, no polymorphisms were detected. Further studies including genome-walking and transcript-based PCR amplification are underway to develop PCR-based markers and map them in F2 population '320 × Elf'. The DArT markers that were identified in this study may prove useful in further fine-mapping and cloning of the *ms8* gene.

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Activity and characterization of a lipid-transfer protein (LTP) from *Capsicum annuum* L. seeds with novel α-amylase inhibitory properties

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Abstract

Plants lipid transfer proteins (LTPs) are basic peptides that are involved in the defense mechanisms against attack of microorganisms. They were first discovered, and thus named, for theirs ability to transport lipids between membranes *in vitro*. Recently, theirs antimicrobial activity has been better studied. However theirs mechanism of action is unclear and needs to be elucidated. In this work we report the characterization and immunolocalization of a purified LTP from *Capsicum annuum* seeds (*Ca*-LTP1), as well as its antifungal activity against the yeast *Candida tropicalis* and inhibitory activity against mammalian insect α-amylases *in vitro*. We have used a new method of purification, which change acetonitrile for propanol, in order to preserve the antimicrobial activity of *Ca*-LTP1. The purified *Ca*-LTP1 present 9 kDa in tricine-SDS-PAGE. The subcellular localization of *Ca*-LTP1 in *C. annuum* seeds, by confocal laser microscopy and by isolation of microsomal membranes and oil bodies for biochemistry characterization, was demonstrated in intracellular vesicles. The purified *Ca*-LTP1 presented inhibitory activity against the filamentous fungus *Colletothricum lindemunthianum* and the yeast *Candida tropicalis*. Membrane permeabilization assay demonstrated that *Ca*-LTP1 causes permeabilization of the treated fungal plasma membrane. In order to elucidate the action mechanism of *Ca*-LTP1 against the yeast *C. tropicalis*, *Ca*-LTP1 was conjugated to FITC dye and a co-localization assay with the marker of nuclei, DAPI, was done. The result indicated that the *Ca*-LTP1 enter in the cytoplasm and is localized in the nuclei. Besides these results morphological alterations, such as cellular agglomeration and difficulty in bud liberation, were shown in the treated yeast through scanning and transmission electron microscopes. The purified *Ca*-LTP1 also presented the ability to inhibit the enzymatic activity of the human salivary and of the insect *Callosobruchus maculatus* gut α-amylases. We have also showed that the purified *Ca*-LTP1 coupled with FITC has binding affinity to dissected tissues from *C. maculatus* larvae. Concomitantly to these experiments, it was done an attempt of cloning this LTP. In conclusion, our work shows that the LTP from *C. annuum* inhibits the growth of the yeast *C. tropicalis* and the activity of *C. maculates* gut α-amylase.

Keywords: *Capsicum annuum*, lipid transfer proteins, *Candida tropicalis*, *Callosobruchus maculatus*, α-amylase.

Introduction

Plant lipid transfer proteins (LTPs) can facilitate the transfer of lipids in *in vitro* assays and were thus named due to this ability. They were divided into two families based on their molecular masses: the LTP1 family, which consists of 9- to 10-kDa peptides and the LTP2 family, which present approximately 7 kDa (Kader 1996; Carvalho and Gomes 2007). The LTP_1 members share biochemical properties such as masses of 9-10 kDa, high isoelectric point (pI), eight cysteine residues engaged in four disulfide bridges and a hydrophobic cavity, that enable the peptide to bind

fatty acids, embedded in a three-dimensional structure formed by four α-helices and a long carboxyl terminal tail (Kader 1996; Carvalho and Gomes 2007). LTP1 members fulfill several activities in plants such as wax and cutin assembly (Cameron et al. 2006; Hollenbach et al. 1997), pollen tube adhesion (Park et al. 2000), mobilization of seed storage lipids (Tsuboi et al. 1992; Edqvist and Farbos 2002) and cell wall extension (Nieuwland et al. 2005). Additionally they are implicated in adaptation of plants to environmental stresses such as drought (Cameron et al. 2006) and salt (Jung et al. 2003, 2005). Among these activities are included the antimicrobial defense through the inhibition of fungal and bacterial growth (Molina et al. 1993; Terras et al. 1992). Recently our research group has characterized a new biological activity of LTPs, the α-amylase inhibitory activity (Zottich et al. 2011). In this work we report the characterization and immunolocalization of a purified LTP from *Capsicum annuum* seeds, designated *Ca*-LTP1, as well as its antifungal activity against the yeast *Candida tropicalis* and inhibitory activity against mammalian insect α-amylases *in vitro*.

Materials and Methods

For purification, proteins were extracted from seed flour and submitted to chromatographic methods according to Diz et al. (2006). Tricine-sodium dodecyl sulfate polyacrylamide gel electrophoresis (Tricine-SDS-PAGE) was done according to the method of Schägger and Von Jagow (1987). Tissue and subcellular localization of *Ca*-LTP1 in *C. annuum* seeds was done according to Diz et al. (2011). The antimicrobial assay for fungal growth inhibition was done following the protocol developed by Broekaert et al. (1990, for detail of the assay please refer to Diz et al. (2011). Ca-LTP1 was coupled with FITC as described in Diz et al. 2011, and incubated with *C. tropicalis* cells. The same cells were incubated with DAPI for nuclei labelling. Human salivary α-amylase inhibition assay was done following the method of Bernfeld (1955) and details are described in Diz et al. (2011). The localization of Ca-LTP1 in the gut of the *C. maculatus* (cowpea weevil) larvae was done with gut dissected from larvaes and the coupling of Ca-LTP1 to FITC was done as described before.

Results and Discussion

Immunohistochemical analysis of thin sections from imbibed chili pepper seeds treated with the anti-LTP antibodies revealed the presence of *Ca*-LTP1, preferentially in intracellular vesicles (Fig. 1). The purification of the *Ca*-LTP1 was accomplished by the combination of a cation and reversed-phase chromatographies. The purified *Ca*-LTP1 causes inhibitory effect on the growth of *C. tropicalis* (Fig. 2A) at a concentration of 400 µg/mL. The membrane permeabilization assay demonstrated the cells of *C. tropicalis* had the membrane permeabilized by the treatment with *Ca*-LTP1 (Fig. 2B-E). The scanning electron microscopy analysis demonstrated that the cell of *C. tropicalis* presented cellular agglomeration and difficulties in bud liberation (Fig. 2F and G). The conjugation of the *Ca*-LTP1 with FITC indicated that the peptide is internalized and photo comparison with DAPI indicates that the *Ca*-LTP1 has a nuclear localization in *C. tropicalis* (Fig. 3). *Ca*-LTP1 was able to inhibit the activity of the activity of the α-amylases from human saliva and *C. maculatus* extract gut. The *Ca*-LTP1 coupled to FITC revealed that the *Ca*-LTP1 interacts with gut structures of the larvae of *C. maculatus* (Fig. 4).

Acknowledgements

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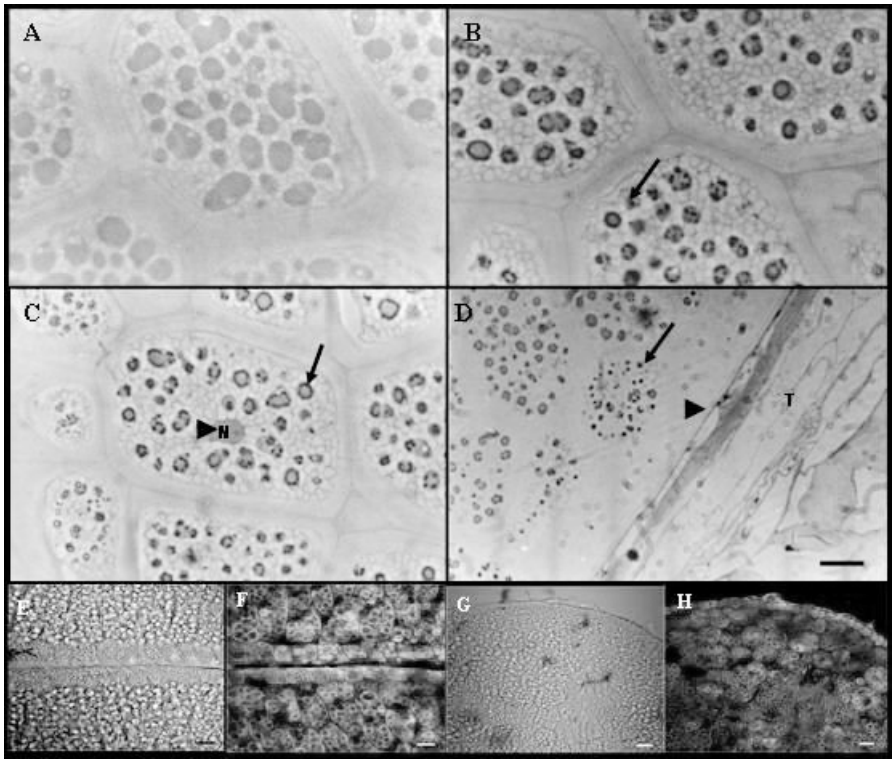

Fig 1 - Immunolocalization of *Ca*-LTP1 in *Capsicum annuum* seed tissues by optical microscopy (A–D). Control section was treated with pre-immune serum (A); cotyledon sections treated with anti-LTP serum (B, C); cotyledon and tegument sections treated with anti-LTP serum (D). N, nucleus; T, tegument; →, marked; not marked. Bars: A, B and C = 5 µm, D = 10 µm. Laser confocal microscopy of cotyledons cells of dry seeds of *Capsicum anuum* labelled with primary antibody anti-LTP and secondary antibody conjugated with Alexa fluor 488 (E-H). E and G - bright field, F and H - fluorescence.

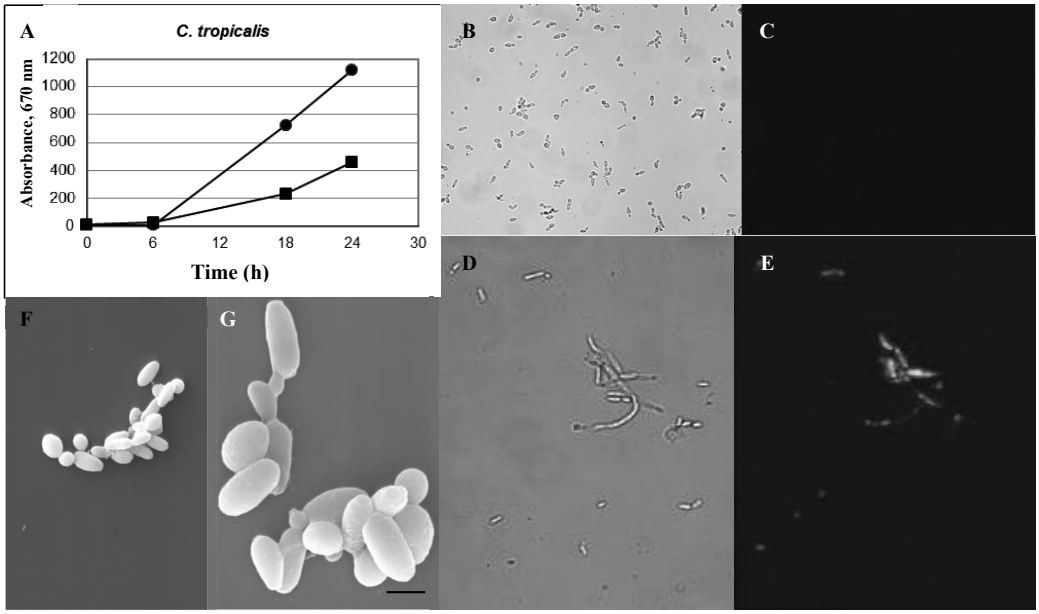

Fig. 2. Effect of purified *Ca*-LTP1 on the growth of pathogenic yeast *Candida tropicalis* growth (A), (-●-) Control; (-■-) Ca-LTP1 at 400 μg/mL. The absorbance at 670 nm was used to measure growth. Experiments were run in triplicate. Growth was monitored for 24 h. Membrane permeabilization assay of *C. tropicalis* (B-E). B and C, control, bright field and fluorescence, respectively; D and E, cells after the inhibition growth assay with *Ca*-LTP1, bright field and fluorescence, respectively. Enlargment = 400X. Scanning electron microscopy of *C. tropicalis* cells after the inhibition growth assay with Ca-LTP1. (F) control, (G) cells in the presence of 400 μg/ml of Ca-LTP1. Scale bars: F = 6,9 μm, G = 1,8 μm.

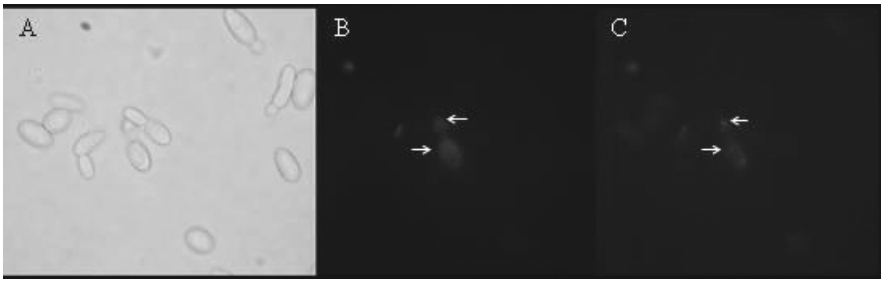

Fig. 3. Co-localization of *Ca*-LTP1 coupled to FITC (green fluourescence, B) and nuclei stained with DAPI (blue fluorescence, C) in *Candida tropicalis*. The arrows indicate the nuclei. A, brigh field.

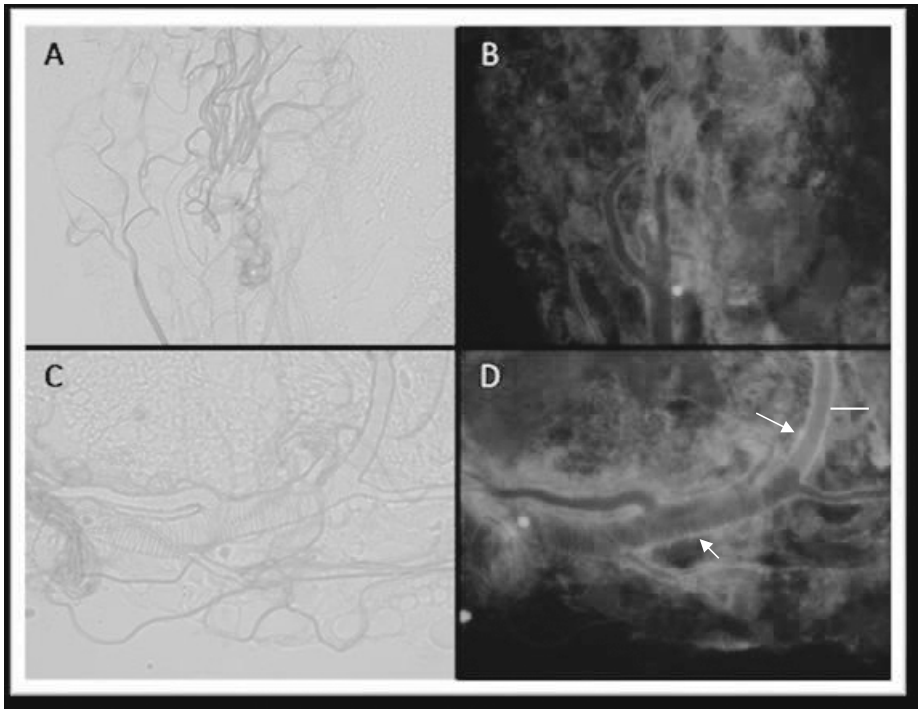

Fig. 4. Localization of *Ca*-LTP1 coupled to FITC in the internal organs of the *C. maculatus* larvae. A and B, control, bright field and fluorescence, respectively; C and D, *Ca*-LTP1 coupled to FITC labeling, bright field and fluorescence, respectively. The arrows indicate the labeling. Bar = 10 μm.

Exploring the eggplant genome: a high quality draft sequence

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Abstract

Eggplant (*Solanum melongena* L. 2n = 2x = 24, projected genome size 1.1Gbp) is the third most important Solanaceae crop after potato and tomato, with an estimated world production of about 46 million tons in 2012 (FAOSTAT), and Italy is the first European producer. Despite its economic and nutritional importance, the knowledge of eggplant genome organization is rather limited and it has been investigated merely by inspecting reduced-complexity libraries. On the other hand, the genome sequences of potato and tomato have been already published, representing a breakthrough towards genomic assisted breeding. Unlike tomato and potato, which belong to the subgenus Potatoe, eggplant belongs to the subgenus *Leptostemonum* and thus represents a unique member for comparative genomic analyses within the genus Solanum.

The inbred eggplant line '67/3', which is the parent of a RIL mapping population composed of 167 F6 RILs was used for genome and transcriptome sequencing. Size-selected Illumina libraries with different insert sizes (from 270bp to 10kb) were sequenced using Illumina technology, producing approximately a 155 X coverage, and assembled with SOAPDENOVO 2. The draft eggplant genome includes 1,721,543 contigs (1.39 Gbp, N50= 16,262) and 35,799 scaffolds (1.21 Gbp, N50=405,352).

From 19 tissues, strand-specific RNA-Seq data were produced, for a total of 74 Gb, and annotation is underway by means of the Maker software. The on-going low coverage re-sequencing of the RIL mapping population will be used to generate a dense genetic map, and to orient the genomic assembly, which will be organised in scaffold-based pseudomolecules. Our efforts will extend the current knowledge of the genome organization of eggplant, producing new tools and markers for accelerating eggplant breeding, as well as comparative genomic analyses.

Keywords: Eggplant, Solanaceae, genome and transcriptome draft

Structural and molecular characterization of the seeds, leaves and fruits of *Capsicum* species and its relation with defense mechanism

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Abstract

The genus *Capsicum* belongs to the Solanaceae family and has a great social, economic and agronomic significance. The species of this genus are described in terms of genetic divergence, considering morphological, agronomic and molecular databases. However, descriptions of genetic differences based on anatomical features are rare. This study aimed to characterize four species of *Capsicum* L. considering the anatomy and micromorphology of vegetative and reproductive organs, and discriminate the accessions using inter-simple sequence repeat molecular markers. The analyzed *Capsicum* species: (*C. annuum*, *C. baccatum* var. *pendulum*, *C. chinense* and *C. frutescens*) were grown in greenhouse. The leaves, fruits and seeds were sampled and analyzed by light microscopy and also scanning electron microscopy to determine possible polymorphism on anatomy, micromorphology which could be helpful to discriminate the species of *Capsicum*. The anatomy of leaves, fruits, seeds of *Capsicum* showed the following results: leaves with uniseriate epidermis covered with cuticle, slightly thicker in the adaxial surface; presence of anomocytic stomata on both surfaces; presence of tector and glandular trichomes on both surfaces; dorsiventral mesophyll; palisade parenchyma with a layer of elongate spongy parenchyma cells and 4-5 cell layers with different shapes and large intercellular spaces; bicolateral type vascular beams. Seeds are campylotropous, horseshoe-shaped, ellipsoid, albuminous, with abundant, semi-transparent and albescent endosperm. All four species have circinate embryo and reduced tegument. Polymorphism was observed in tector trichomes and also for fruit colors and shapes. The high variability between accessions was detected by ISSR markers. Despite the wide morphological and molecular variability shown in the studied species, this was not reflected by the anatomical features. Due to the diversity of possible applications in areas ranging from the protection of plants against pests, pathogens to medicine usage, such as cancer and inhibition of virus replication, the discovery of new IPs with properties are of great interest.

Keywords: Genetic diversity; Sweet and chili pepper; Micromorphology; Anatomy; ISSR markers

Introduction

Capsicum is native to Central and South America (Perry et al. 2007), where this genus is believed to have been selected in two areas of origin, one called the primary center and then introduced to other regions called secondary centers (Mongkolporn and Taylor, 2011). Brazil is considered a secondary center of diversity of this genus. Due to the selection process, varieties with new morphological characters arose in these new areas (Clement et al. 2010), and their genetic variability is poorly understood. Many varieties have overlapping morphological character states, potentially leading to unresolved or erroneous species identification. The great importance of correct species identification can be exemplified by the knowledge of the anatomical and

morphological characteristics that are necessary for studies on the interactions between plants and herbivores and other natural enemies (Price 1997).

The genus *Capsicum* has a very complex taxonomy, and its circumscription into one species or another can vary considerably based on the characteristics of the leaves, flowers and fruits, and these variations are often factors related to the geographic and weather conditions where the plants grow (Petters, 2002). In general, the identification of this genus and species is carried out by morphological features observed mainly in flowers (Sudré et al. 2010). However, flower characteristics are not enough and, in general, a combination of diagnostic characters associated with genetic characteristics is usually required to identify and differentiate *Capsicum* species.

Thus, this study aimed to evaluate the anatomy and micromorphology of vegetative and reproductive organs of four species of *Capsicum*, and to detect their special chemical constituents, providing data to assist in the understanding of these species, for ecological and medicinal studies. In addition, we determined the genetic divergence between the four accessions of *Capsicum* spp. based on morphological and molecular data and estimated the relation between genetic distances obtained based on morphological characteristics by inter-simple sequence repeat (ISSR) markers.

Materials and Methods

The analyzed *Capsicum* species (*C. annuum* var. *annuum*, *C. baccatum* var. *pendulum*, *C. chinense* and *C. frutescens*) were grown in greenhouse. The leaves, fruits and seeds were sampled and analyzed by light microscopy and also scanning electron microscopy to determine possible polymorphism on anatomy and micromorphology which could be helpful to discriminate the species of *Capsicum*.

Results and Discussion

The anatomy of leaves, fruits and seeds of *Capsicum* showed the following results: leaves with uniseriate epidermis covered with cuticle, slightly thicker in the adaxial surface; presence of anomocytic stomata on both surfaces of the leaves; dorsiventral mesophyll; palisade parenchyma with a layer of elongated spongy parenchyma cells and 4-5 cell layers with different shapes and large intercellular spaces; (Figure 1A); presence of tector (Figure 1B) and glandular trichomes (Figure 1C) on both surfaces; bicolateral type vascular beams (Figure 1A). Seeds are campylotropous, horseshoe-shaped, ellipsoid, albuminous, with abundant, semi-transparent and albescent endosperm (Figure 1D). All four species have circinate embryo and reduced tegument (Figure 1E). The exocarp of the fruit of the species *C. annuum*, *C. baccatum*, *C. chinense* and *C. frutescens* showed uniseriate epidermis, without overlap and tabular cells with dense texture and cellulosic walls typically found in berries. The cuticle was quite variable and usually shows how thick the fruit of the species *C. chinense* (Figure 1F). Polymorphism was observed in tector trichomes and also for fruit colors and shapes. The high variability between accessions was detected by ISSR markers.

The dendrogram generated based on the molecular data indicated the formation of two main groups (Figure 2), the first one containing accessions of *C. annuum*, *C. baccatum* and *C. frutescens* and the second group containing only the accessions of *C. chinense*. In the first group, although it had been separated by species, the accessions formed two subgroups, one gathering accessions of *C. frutescens* and the other gathering the species *C. baccatum* and *C. annuum*. The dendrogram generated by the molecular characters also showed a cluster pattern different from the proposed division of the *Capsicum* genetic complex (Pickersgill, 1991). In this proposal, the genetic complex of *C. annuum* encompasses the domesticated species of *C. annuum* var. *annuum*, *C. chinense* and *C. frutescens*, which demonstrates the existence of a great proximity and higher possibility of gene

exchange between these species, which also happens in the genetic complex of *C. baccatum*, which includes the *C. baccatum* varieties *pendulum*, *baccatum* and *praetermissum* and also *C. tovarii* (Tong and Bosland, 1999).

In our study, molecular analyses clustered *C. baccatum* with *C. annuum* and *C. frutescens*, separating the latter two species from *C. chinense*. The same result was observed by Costa et al. (2009) working with RAPD markers and morphoagronomic descriptors to estimate the genetic diversity between *Capsicum* accessions. In analyzing only morphoagronomic descriptors, these authors found accessions from *C. baccatum*, *C. annuum* and *C. frutescens* in the same cluster, while *C. chinense* accessions were placed in a different cluster. Also, the authors hypothesized that this clustering could indicate some closeness and the possibility of gene exchange between *C. baccatum*, *C. annuum* and *C. frutescens.* The results obtained by Monteiro et al. (2011) support this hypothesis, since fertile hybrids were obtained between the species *C. annuum* var. *annuum* (sweet or hot pepper) and *C. baccatum* var. *pendulum* with pollen viability exceeding 90%. Moreover, Potnis et al. (2012) transferred one gene that controls resistance to bacterial spot from *C. baccatum* to *C. annuum,* showing that gene exchange between different *Capsicum* species is quite feasible.

Despite the extensive polymorphism observed for ISSR markers, along with polymorphism for fruit and other agronomic traits, these differences were not reflected in the large variability for anatomical descriptors. Some studies concluded that the association between morphological, agronomic and molecular data is the most suitable approach to estimate *Capsicum* genetic divergence (Costa et al. 2009) or that joint analysis of quantitative and qualitative data resulted in greater efficiency in the determination of genetic divergence among the *Capsicum* accessions (Moura et al. 2010). Multivariate strategies such as the Ward-MLM methodology in data analysis for morphoagronomic characterization of accessions have allowed, with some level of efficiency, the separation of *Capsicum* species with the simultaneous use of morphological and agronomic variables (Sudré et al. 2010). However, Sudré et al (2010) observed that only morphological descriptors can efficiently discriminate between *Capsicum* species and their botanical varieties.

Acknowledgements

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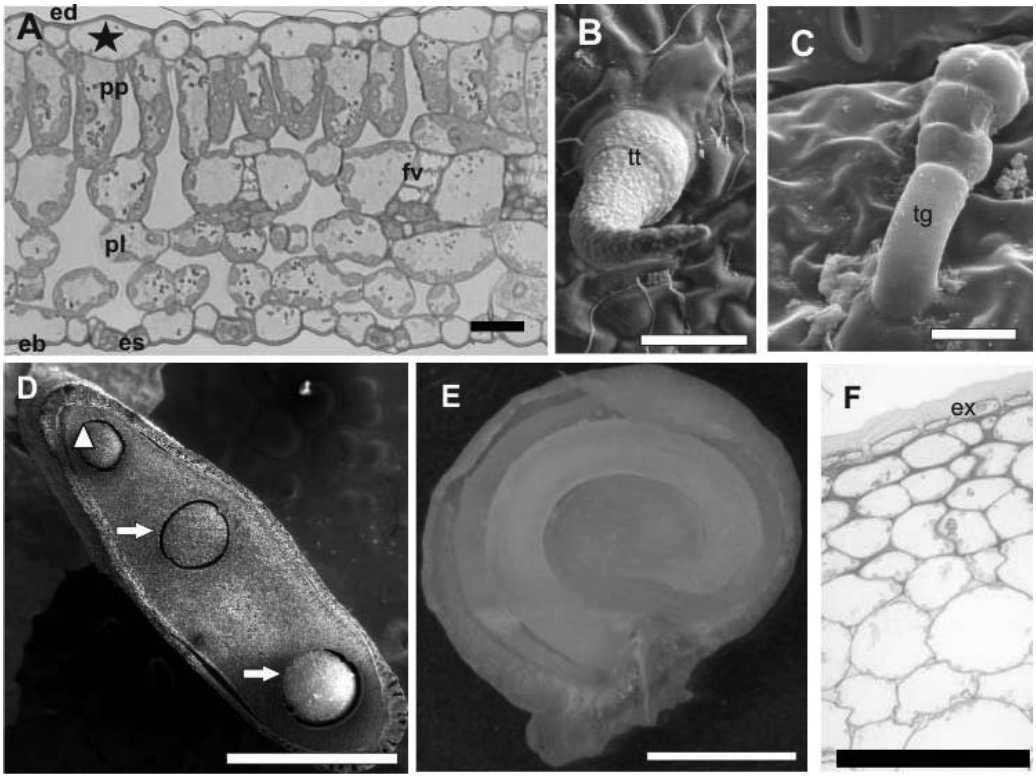

Figure 1 – A. Cross section of leaf from *Capsicum chinense*. B. Trichomes tector ornamented surfaces of the leaf the *C. chinense*. Cuticular ornamentation on the adaxial surface the *C. frutescens*. C. Trichomes glandular ornamented surfaces of the leaf. D. Cross section of seed visualizing the embryonic axis (triangle) and a double cotyledon (arrow). E. Longitudinal section of entire seed showing its ellipsoids, long, broad oval shape. F. Detail of the exocarp of the fruit. Bars: A= 50 µM; B and C= 20 µM; D= 1 mm; E= 1,2X (1 mm); F= 20 µM. Leg: pp = palisade parenchyma; ed = adaxial epidermis; pl = spongy parenchyma; eb = abaxial epidermis; es = stomata; fv = vascular system; ex = exocarpo.

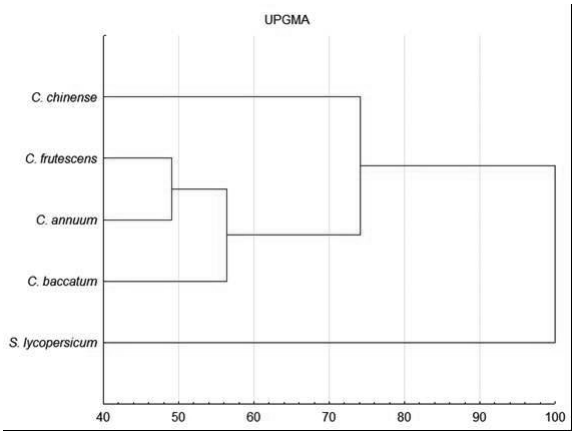

Figure 2 - Dendrogram obtained by the UPGMA method based on molecular markers among the 4 accession of *Capsicum* spp belonging to the germplasm bank collection of Universidade Estadual do Norte Fluminense.

Genome-wide microsatellite marker development using whole genome assembly in eggplant

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Abstract

Recently, an integrated linkage map (LWA2010) was developed in eggplant by using Solanum orthologous (SOL) gene sets and genomic SSR markers (Fukuoka et al. 2012). The overall syntenic relationships between eggplant and tomato genome were deduced by 326 common markers. On this eggplant map, it was found that genomic SSR markers were not evenly distributed throughout the eggplant genome and a considerable part of the genome could be surveyed by gene-based SNPs. This is not surprising since genomic SSRs are known to be unusually abundant in the pericentromeric heterochromatin regions (Shirasawa et al. 2010). Genomic SSR markers are, however, more flexible and convenient than functional gene-based markers (SSR/SNP), since they are generally multi-allelic and more polymorphic among cultivars. Especially for rough gene/QTL mapping using arbitrary and various parental combinations, a compact and versatile DNA landmark set covering the whole genome would be quite useful.

In order to build a marker set for such purposes, we tried to isolate genomic SSRs from targeted euchromatic regions using a draft eggplant genome assembly (cv. Nakate Shinkuro). The estimated genome size of eggplant is ~1.1Gb. Mate-paired (approx. 2kb insert) and paired-end libraries (200-300bp insert) were sequenced using an Illumina HiSeq 2000 sequencer (130-fold in total). In addition, a euchromatin-enriched library prepared by using EST-based oligo-microarray was sequenced using Roche GS-FLX sequencer (0.45-fold). The Illumina and the 454 reads were separately assembled using SOAPdenovo and MIRA3.2.1, respectively. The resulting scaffolds and contigs were hybrid-assembled by PCAP.rep assembler. The final assembly consisted of 96,579 scaffolds (N50 length = 40,857) spanning 945Mb, in which more than 95% of eggplant unigenes were represented. In the draft genome data, more than 36,000 SSRs were identified of which 13,112 were found in the scaffold/contigs that had good alignment to eggplant ESTs. Based on the associated EST sequences these SSRs were anchored to the tomato genome, and then, corresponding bins in the eggplant linkage map were inferred by using the tomato-eggplant syntenic information. Out of the 13,112 SSRs, 360 were tested for polymorphisms among cultivars. The most polymorphic ones (240 SSRs) were chosen for genetic mapping and most of them were successfully mapped into expected bins. Finally, at least one genomic SSR was mapped to more than 90% of the 10cM bins of eggplant genome. Among cultivars, the average SSR marker PIC was 0.61, a value much higher than that observed for SNP markers (0.11). The genomic SSR marker set, built by utilizing genome-wide information, is going to provide a convenient tool for eggplant breeding and genetic resource evaluation.

Keywords: eggplant, SSR, whole genome assembly, linkage map

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Molecular characterization of peppers genotypes based on SSR and SRAP markers

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Abstract

Simple Sequence Repeats (SSR) and sequence-related amplified polymorphism (SRAP) analysis were applied to 16 pepper genetic resources, which were different levels of resistance to *Phytophthora capsici* L. The results showed that the results of SRAP and SSR markers evaluated together may be more informative, more efficient and effective for studying genetic diversity of pepper than the results of only one technique. The ranges of the genetic similarity coefficient were 0.685-0.914 and 0.685-1.000 for pepper genotypes, with SRAP and SSR data, respectively. These genetic indices indicated that these genotypes are closely related genetically. The dendrogram generated with the SRAP markers was topologically different from the dendrogram based on SSR markers. The SRAP technique clearly distinguished all pepper genotypes from each other. Evaluation of genetic variation levels of resistant, moderately resistant and susceptible genotypes to *P.capsici* showed that the genotypes did not take place in different groups of the dendrogram. However, CMM 334, PM 702 and PBC 178 (resistant to *P.capsici*) were genetically separated to each others.

Keywords: *Capsicum annuum,* genetic similarity

Antimicrobial peptides from *Capsicum annuum* L. fruits as a template scaffold for new drug development against human pathogenic bacteria and yeasts

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Abstract

Among the set of strategies evolved by plants to defend themselves against pathogens, is an important armament, the production of antimicrobial peptides (AMPs). These peptides are characterized by the presence of large numbers of cysteines (4, 6 or 8), which are interconnected in pairs, forming disulfide bonds that confer high stability. In plants, several AMPs, including lipid transfer proteins, plant defensins, hevein-like peptides, knottin-like peptides, glycine-rich peptides, snakins, cyclotides some proteinase inhibitors and thionins, have already been characterized. In this work, we describe the extraction and fractionation of peptides with antimicrobial activity of *Capsicum annuum* fruits. Initially we performed the fractionation of peptides present in a crude extract from *C. annuum* fruits. Seven different fractions, (named F1 to F7) were obtained from it. All fractions were tested against pathogenic bacteria and yeasts. The fractions referred to as F1, which is composed of a single peptide of 6 kDa, and fraction F3, which was composed of two peptides of 6 and 8 kDa, were toxic to the cells of *Saccharomyces cerevisiae*, *Candida albicans* and *Candida tropicalis*. The 6 kDa peptide present in fraction F3, was sequenced and its comparative analysis in the database showed sequence similarity to an AMP of plants, known as thionins. In tests against bacteria, only F1 caused growth reduction of the species *Escherichia coli* and *Pseudomonas aeruginosa*. In order to verify the potential of F1 and F3 fractions in permeabilize the plasma membrane of yeasts cells, *S. cerevisiae*, *C. albicans* and *C. tropicalis* cells were incubated for 24 h with the different fractions at 50 $\mu g.mL^{-1}$. This experiment demonstrates that the peptides present in the F1 and F3 fractions induced changes in the membranes of all yeast cells, leading to their permeabilization. F1 was also capable of inhibiting acidification of the medium of glucose-induced cells of *S. cerevisiae* in approximately 78%, the opposite result was obtained for cells of *C. albicans* where there was 50% stimulation of acidification of the medium. Thus, the identification of molecular targets and physiological responses to pepper peptides in model eukaryotes that are similar to the yeast tested may provide new insights into molecular processes that are relevant to the development of new drugs.

Keywords: *Capsicum annuum*, antimicrobial peptides, thionins, yeast, bacteria.

Introduction

Plants have evolved many strategies to defend themselves against pathogens, including the ability to produce antimicrobial peptides (AMPs). They are important components of the innate immunity of living organisms, constituting an ancient immune defense mechanism that is found in a wide variety of eukaryotic organisms such as mammals, plants and insects and even in prokaryotes (Brogden and Brogden, 2011). These peptides share some common characteristics, such as small size, consisting of approximately 100 amino acid residues, amphipathicity (molecules that present the characteristic of having both a hydrophilic region and a hydrophobic region) and net positive charge at physiological pH (Nakatsuji and Gallo, 2012). They are characterized by the presence of a large numbers of cysteines (4, 6 or 8), which are interconnected in pairs, forming disulfide bonds that confer high stability to these peptides (Benko-Iseppon et al. 2010).

Among plant AMPs, there are a few that are better characterized as defensins, lipid transfer proteins (LTPs) and thionins (Benko-Iseppon et al. 2010). Thionins constitute a family of basic peptides with a low molecular weight and with a primary structure composed of 45 to 47 amino acid residues. Thionins have been isolated from a wide range of plant species, including monocotyledonous and dicotyledonous plants. The main feature of thionins is their toxic effect on biological systems, including many pathogenic fungi and bacteria (Stec, 2006).

In this study, we investigate antimicrobial and other biological activities of peptides from *C. annuum* fruits against yeast and pathogenic bacteria. Ours results indicate that pepper peptides possess ability to induce microbial inhibition and may, therefore, be targets for the design of new antifungal drugs.

Materials and Methods

The peptide extraction and fractionation from *C. annuum* fruit was done as described by Agizzio et al. (2003) and SDS-tricine-gel electrophoresis was performed according to the method described by Schägger and Von Jagow (1987). For amino acid sequence, F3 fraction was transferred to PVDF membrane according to Towbin et al. (1979). The protein band of 6 kDa was excised and submitted to amino acid sequencing according to Edman (1950) in an automated sequencer (Shimadzu). Antifungal assays were done according to Broekaert et al. (1990) and antibacterial assays were done according to Araújo et al. (2004). The membrane permeabilization assay was done according to Thevissen et al. (1999). The inhibition of the glucose-stimulated acidification of the medium assay was done according to Gomes et al. (1998). Experiments were performed in triplicate, and the values are the average of them.

Results and Discussion

The electrophoretic profile of the crude extract (CE) show several proteins with a molecular weight above 16.9 kDa. This CE was subjected to reversed-phase chromatography in a C2/C18 column on HPLC and it was separated into seven fractions referred to as F1, F2, F3, F4, F5, F6, and F7. The F1 fraction showed a single band with a molecular mass between the 6 and 8 kDa markers. The F3 fraction showed a profile with two bands, the lighter with a molecular mass of approximately 6 kDa and the heavier with a molecular mass of 10 kDa.

The analysis of the N-terminal amino acid sequence (APGCYKELTKDVATSSEPRLL) of the peptide from the F1 fraction revealed low sequence similarity with chitinases. However, further analyses to better characterize this peptide are under way. The deduced sequence of the 6 kDa band from F3 fraction was compared with protein databases, and it showed 100, 63 and 63% identity with the primary structure of plant AMPs belonging to the thionin family, *C. annuum* thionin-like peptide (1) (gi: 164698852), *C. annuum* thionin-like peptide (2) (gi: 6552502) and *C. annuum* γ-thionin 1 (gi: 6601331), respectively (Fig. 1).

F1 fraction caused 98, 100 and 96% inhibition of growth of the yeasts *S. cerevisiae*, *C. tropicalis* and *C. albicans* respectively, at a concentration of 100 $\mu g.mL^{-1}$. F3 fraction caused 0, 100 and 100% inhibition of growth of *S. cerevisiae*, *C. tropicalis* and *C. albicans* respectively, at a concentration of 100 $\mu g.mL^{-1}$ (Fig. 2). F1 fraction was able to cause a reduction of the growth of *E. coli* by 18, 34 and 34% at the concentrations of 100, 200 and 300 $\mu g.mL^{-1}$, respectively. *P. aeruginosa* was inhibited 10, 18 and 27% at concentrations of 100, 200 and 300 $\mu g.mL^{-1}$, respectively (Fig. 3). Other peptides that were isolated from pepper plants, particularly from seeds, showed some antimicrobial activity in vitro. Among these, we can cite the LTPs and protease inhibitors (Cruz et al. 2010; Diz et al. 2011; Ribeiro et al. 2012)

F1 and F3 fractions cause membrane permeabilization of all yeasts tested at the concentration 50 $\mu g.mL^{-1}$. Examples of well-characterized AMPs that cause membrane permeabilization are the defensins Dm-AMP1 (Thevissen et al. 1999) and Pvd1 (Mello et al. 2011), the viscotoxin A3 (viscotoxins are cationic proteins, isolated from different *Viscum* species, that belong to the group of thionins.), (Giudici et al. 2006). This activity is related to the fungus growth inhibition.

F1 fraction is able to inhibit acidification in *S. cerevisiae* by 78% when pre-incubated for 30 min and opposite result was observed to *C. albicans*. This result indicates that this fraction can alter the function of yeast metabolism and the difference between inhibition and stimulation for different yeasts may be related to differences in the composition of membrane proteins of different species.

Acknowledgements

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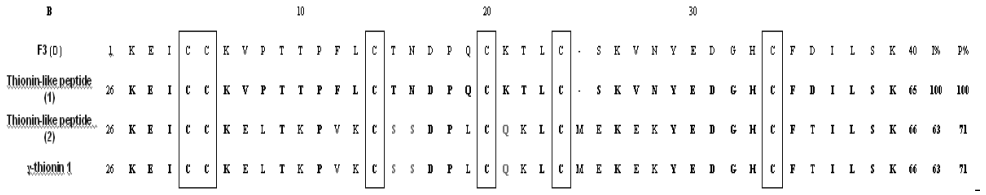

Fig. 1. Alignment of the deduced amino acids residues of the 6 kDa peptide from F3 fraction with the sequences of the thionins as follows: *C. annuum* thionin-like peptide (1) (gi: 164698852); *C. annuum* thionin-like peptide (2) (gi: 6552502); *C. annuum* γ-thionin 1 (gi: 6601331). Cys residues conserved among the sequences are boxed. I% indicates the percentage of identical residues (Cys residues included) and are written in bold. Gaps (-) were introduced for better alignment. P% indicates the percentage of positive residues (that present the same physic-biochemical features) and are written in gray.

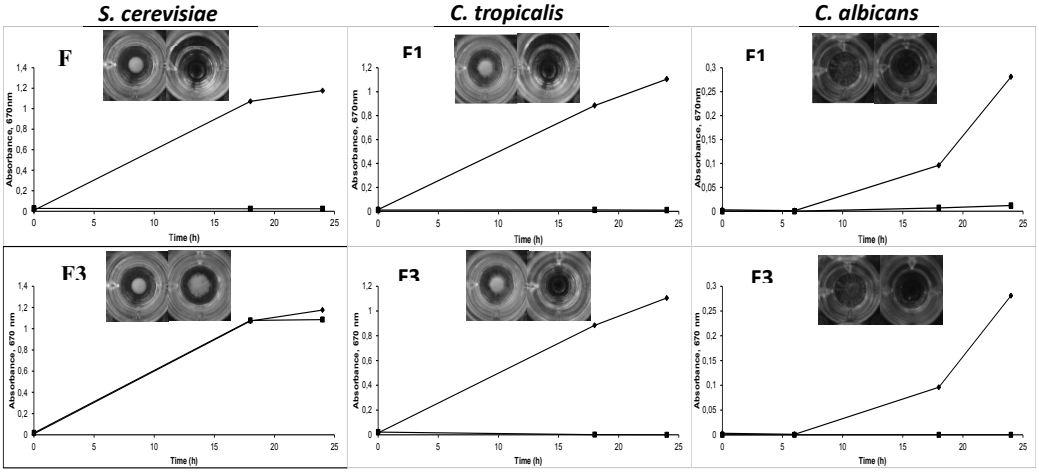

Fig. 2. The effect of fractions 1 and 3 on the growth of the yeasts. (♦) control; (■) 100 μg.mL^{-1} of each fraction. (Insert): photographs of the microplate wells after 24 hours incubation with 100 μg.mL^{-1} of the fractions.

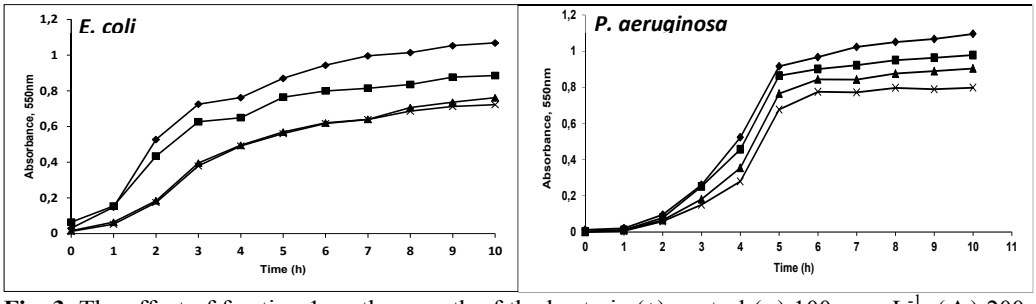

Fig. 3. The effect of fraction 1 on the growth of the bacteria (♦) control (■) 100 μg.mL^{-1}; (▲) 200 μg.mL^{-1}; (×) 300 μg.mL^{-1}.

Development of an ethyl methane sulfonate (EMS) mutant lines in *C. annuum.*

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Abstract

Mutant lines induced by ethyl methane sulfonate (EMS) have been used for crop improvement and functional genomics. Since pepper is very recalcitrant to be transformed, EMS mutagenesis could be an alternative method to generate useful mutant lines and to characterize the function of genes. We have developed mutant lines consisting of about 5,000 M_2 mutant lines using Korean local landrace, *C. annuum* 'Yuwolcho'. Yuwolcho has suitable traits for mutagenesis such as early flowering and maturation, large number of seeds per fruit, and susceptible to various diseases. Up to now 525 M_2 mutant lines were evaluated to confirm the effect of EMS. Each mutant line was characterized according to 4 classes and 14 subclasses. Mutant lines have shown variations in plant stature (small size, dwarfism, and early death), leaf development (light color, variegation, and morphological change), flower (inflorescence, morphological change, and organ color) and fruit (shape and color). We observed the largest morphological variation in leaf development. Most of these mutant phenotypes were inherited recessively. In addition, we will apply cel1-based TILLING to identify useful mutant lines. We are expecting that these mutant lines will be very useful to study the function of genes in *C. annuum*.

Keywords: EMS, induced mutagenesis, phenotypic categories, mutant screening, TILLING.

Introduction

Pepper (*Capsicum annuum* L.) is an economically important vegetable and cultivated all over the world. To date, a large amount of genetic resources have been collected in *Capsicum* spp. (Bosland. 1992), but the application of diverse germplasm for breeding has been limited because of the lack of genetic resources and sexual incompatibility among species. Recently, genetic transformation of the plant has become an important alternative to overcome limitations of conventional breeding methods (Ko et al. 2007). Pepper is known to be highly recalcitrant for *Agrobacterium*-mediated transformation, and current transformation methods are inefficient (Heidmann et al. 2011). Identification of the novel alleles by mutation can be a powerful tool to expand genetic variability. Among the mutagens, ethyl methane sulfonate (EMS) has been most successfully applied for development of mutant lines in many crops including wheat (Dong et al. 2009), tomato (Menda et al. 2004; Minoia et al. 2010), potato (Elias et al. 2009). We hereby report our results about development of EMS mutant lines in *C. annuum*.

Materials and Methods

Mutagenesis methods

Korean local landrace, *C. annuum* 'Yuwolcho', was treated with the chemical mutagen EMS. Seeds were presoaked in distilled water at 24℃ and shaken in an incubator for 18 h. In order to induce a proper mutation load, seeds were drenched in 1.5% EMS (Sigma–Aldrich, St. Louis, MO, USA) solution in 0.1 M phosphate buffer, pH 7.0 and then incubating the 20℃ for 12 h. EMS-

treated seeds were washed with 0.5% (v/v) ethyl acetate (Sigma–Aldrich) in 0.1 M phosphate buffer (pH 7.0) for 50 min (Jeong et al. 2010).

Construction of mutant lines

Mutant line derived from Yuwolcho have been achieved since 2009. To date, out of 6,812 M_1 mutant lines, 5,023 M_2 mutant lines were successfully obtained (73.7%). To assess whether our mutant lines were properly constructed, we tested the phenotypic variation of our 525 M_2 mutant lines (around 10% of a total population) in 2012.

M_2 mutant lines phenotyping

We characterized and categorized each mutant line according to 4 classes and 14 subclasses as previously reported (Menda et al. 2004; Minoia et al. 2010). These phenotypes include morphological variation in plant stature (small size, dwarfism, and early death), leaf development (light color, variegation, and morphological change), flower (inflorescence, morphological change, and organ color), and fruit (shape and color).

Table 1. Construction of M_1 mutant line.

Year	EMS treatment (%)	M_1 size	Germination rate (%)	No. of M_2 lines obtained (%)
	1.0	200	85.4	168 (83.2)
2009	1.5	990	83.0	735 (74.2)
	2.0	72	74.1	25 (36.7)
2010	1.5	950	80.2	720 (75.8)
2011	1.5	3,600	81.0	2,700 (75)
2012	1.5	1,000	78.1	675 (67.5)
Total		6,812		5,023

Results and Discussion

Phenotype observation in M_2 mutant lines

We constructed 5,023 M_2 mutant lines for studying the phenotypic variations of mutagenized plants. Among them, 525 M_2 mutant lines were grown to evaluate the mutagenized traits. The mutant phenotype categories were classified by morphological traits compared to untreated plant. The 525 M_2 mutant lines showed diverse mutant phenotypes. These traits were categorized 4 classes and subsequently into 14 subclasses that describe the phenotype in more detail (Table 2). Although the number of individuals in each mutant line (10 individuals per M_2 mutant line) was small, normal and mutant phenotypes were segregated in a 3:1 indicating that most of these mutant phenotypes were inherited recessively.

Out of 525 M_2 mutant lines, phenotype variations were observed in 133 M_2 mutant lines (25.3%). In the category for plant stature, we observed small plants that are smaller than the untreated plant in 36 M_2 mutant lines. Moreover, we observed dwarf plants that revealed retarded growth in 5 M_2 mutant lines and early death in 4 M_2 mutant lines. Leaf color changes such as light green color and variegated color were observed in 27 M_2 mutant lines. The largest number of morphological variation was observed for leaf morphology. 42 M_2 mutant lines showed wider or narrower leaves compared to untreated plant, curling up and down leaves, hair-like leaves, compact leaves, and long branched leaves.

When studying flower characteristics, three distinct inflorescence changes including the number of flowers on one branch, and floral organ changes like sunflower flower shape and altered stamen color mutant were observed. In fruit morphology, short and rounded fruit were observed in 8 M_2 mutant lines. Orange color fruits were also observed. While evaluating the M_2 mutant lines, we

observed hair-like leaves and curling up leaf. *Wiry* type phenotypic variation was already reported by *Yifhar* et al. (2012) in tomato using EMS mutant lines and *Flaccid* mutant (curling up leaf) reported by *Bosland* (2002) in pepper using EMS mutant lines. In addition, out of 525 M_2 mutant lines, we observed 123 mutant lines showed pleotropic phenotypes, which were affected more than one phenotypic trait per each line (Menda et al. 2004; Figure 1).

We evaluated EMS-induced mutagenesis in 525 M_2 mutant lines (around 10% of the total population). We are currently in the progress to develop a phenotype database based on morphological phenotypes observed in M_2 mutant lines. In addition, we are setting up cel1-based TILLING and RNA transcriptome analysis to identify genes controlling the observed phenotypes.

Table 2. List of phenotype categories and mutant characteristics.

Classes	Subclasses	No. of mutant lines	Rate (%)
Plant stature	Small plant	36	27
	Dwarfism	5	4
	Early death	4	3
Leaf	Variegation	18	14
	Light color	9	7
	Abnormal traits	16	12
	Compact traits	12	9
	Hair like traits	8	6
	long branch traits	6	5
Flower	Inflorescence	3	2
	Morphology	1	1
	Organ color	1	1
Fruit	Morphology	8	6
	Color	6	5
Total		133	100

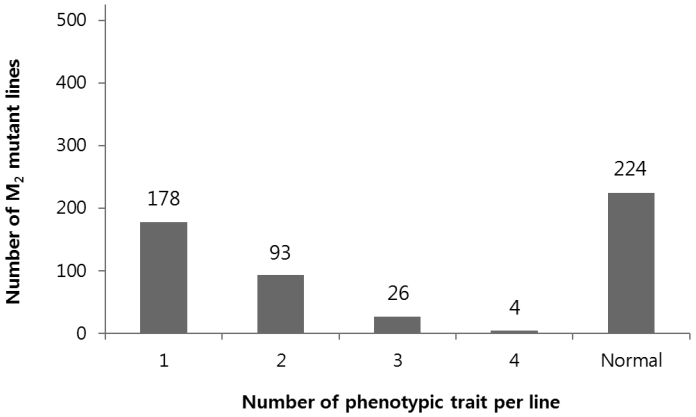

Figure 1. The number of mutant phenotypes and pleiotropic phenotypes within each mutant line. The Y-axis shows the number of M_2 mutant lines and the X-axis shows the number of morphological traits changed per line. The numbers on the bar indicates how often multiple mutant phenotypes were observed in M_2 mutant lines.

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Investigation of genetic factors related to quantitative control of capsiate biosynthesis

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Abstract

Capsinoid which were found recently in non-pungent pepper show the same biological effects as capsaicinoid including anticancer and anti-obesity. A precursor of capsaicinoid, vanillyl alcohol, is known to be produced by mutations in the *p-aminotransferase* (*pAMT*) gene. In the previous study, we showed that capsinoid production is also controlled by the *capsaicin synthase* (*CS*) gene. However correlation between the *CS* gene expression and capsinoid contents has not been fully understood. This study was conducted to elucidate correlation between the expression level of *CS* gene and capsinoid contents. Through germplasm screening, we identified one *C. chinense* pepper accession, SNU11-001, which contains capsinoid as much as *C. annuum* 'CH-19 Sweet'. SNU11-001 was crossed with five *Capsicum* accessions containing different levels of capsaicin, 'ECW' is non-pungent pepper line, and 'Takanotsume' and 'Yuwolcho' have mild pungency, and 'Habanero' and 'Jolokia' is known to be included in the most pungent pepper lines. When we analyzed the expression of *CS* and *pAMT* genes using the six *Capsicum* accessions, the expression levels of *CS* were higher in pungent *Capsicum* accessions. To test whether the expression levels of *CS* also control capsinoid contents, we analyzed several F_2 populations derived from crosses between SNU11-001 and *Capsicum* accessions containing different levels of capsaicin.

Keywords : Non-pungent capsaicinoid analogue, capsinoid, capsiate, *pAMT*, *CS*

Introduction

The unique characteristic of pepper is pungency, which is caused by capsaicinoid in fruits (Nelson and Dawson. 1923). Capsaicinoid is an alkaloid derived from pepper's placenta and have many biomedical functions such as cancer prevention, weight reduction, and cardiovascular (Thiele et al. 2008; Xiu-Ju et al. 2011). Capsaicin(Figure 1. (b)), one of the capsaicinoid analogs, is synthesized from phenylalanine. Vanillin is changed to vanillylamine by *pAMT*. Capsaicin is produced by condensation of branched-chain fatty acids and vanillylamine (Curry et al. 1999; Del Rosario Abraham-Juarez et al. 2008).

Nonpungent capsinoid is more palatable than capsaicinoid. Pepper cultivar 'CH-19 sweet' containing capsaicinoid-like substance was first reported by Yazawa in 1989 (Yazawa et al. 1989). Biosynthesis of capsiate(Figure 1.), one of the non-pungent capsaicinoid analogs, is caused by *pAMT* mutation by suppression of the formation vanillylamine from vanillin (Lang et al. 2009; Tanaka et al. 2010a). Instead, vanillyl alcohol is produced and the *CS* gene is responsible for biosynthesis of capsiate using vanillyl alcohol as one of substrates (Tanaka et al. 2010b; Han et al. 2013).

Capsiate is used in the production of an easy to-swallow vegetarian soft gel, 'Capsiate Natura'. However, pepper varieties producing capsinoid-rich fruits have not been developed yet. Besides basic research for breeding of cultivars containing high level of capsiate has not been elucidated clearly. In this report, we investigated factors affecting in capsiate production.

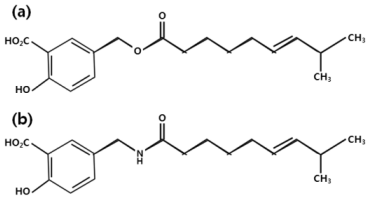

Figure 1. Structure of capsiate(a) and capsaicin(b). Capsiate is same with capsaicin except for replacement of NH by O. Capsaicin is a major member in capsaicinoid family and capsiate is a major member in capsinoid family.

Materials and Methods

Plant materials

A total of 20 *Capsicum* cultivars were used. Sixteen accessions were used for screening lines containing high level of capsinoid. These lines were selected by screening for mutations in PLP domain of the *pAMT* gene in previous study(Han et al. 2013). Among 16 lines, SNU11-001 and Habanero were included. ECW, Yuwolcho, Takanotsume and Jolokia were used to obtain F_2 populations and determine quantitative control of capsiate by *CS* activity. F_1 and F_2 populations are grown in the university farm, CALS, SNU.

HPLC analysis of capsaicinoid and capsinoid

Three to five fruits per a plant were harvested from all *Capsicum* accessions and placenta was carefully extracted from fruits and stored at -20℃. HPLC analysis was performed according to the method described by Han et al. (2013).

Isolation RNA and RT-PCR

Total RNA was obtained from the placenta using the Hybrid-R^{TM} RNA extraction kit(GeneAll Biotechnology, Seoul, Korea) according to the manufacturer's instructions. To test *pAMT* expression, (primers *pAMT* F-1 and *pAMT* R) were designed from *pAMT* cDNA sequence. *CS* F and *CS* R were developed from *CS* cDNA sequence to test expression pattern of *CS*.

Results and Discussion

Germplasm screening and Selection by HPLC analysis

We analyzed capsaicinoid and capsinoid contents of sixteen pepper cultivars by HPLC methods. Among accessions, Habanero contains the highest level of capsaicinoid (106.2 mg/placenta gDW). SNU11-001 has the highest capsinoid content, 12.7 mg/placenta gDW. We selected SNU11-001 for development of cultivar containing high level of capsaicinoid. Habanero contained high level of capsinoid, while capsiate levels were minimal.

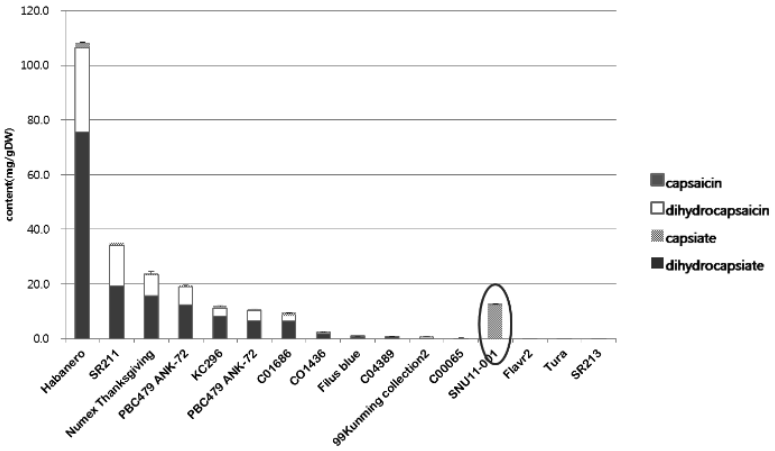

Figure 2. Capsaicinoid and capsinoid contents (mg/placenta gDW) in 16 *Capsicum* accessions.

Identification of pAMT and CS expression pattern.

We tested *pAMT* and *CS* expression pattern in five cultivars including SNU11-001 and the other four cultivars showing different levels of pungency. *pAMT* transcript with a size of 1455bp was amplified in four accessions except SNU11-001. Non-pungent bell pepper, ECW, also transcribed the *pAMT* gene. The transcription was detected in 'SNU11-001' but the two kinds of transcripts which were larger than transcripts in other cultivars were detected in SNU11-001. Similar amount of *CS* transcript was expressed in four cultivars. *CS* mutant cultivar, ECW, did not transcribe the *CS* gene as expected. *CS* expression was detected in the other cultivars from non-pungent pepper SNU11-001 to Jolokia. We predict that Jolokia have the highest *CS* activity among these accessions.

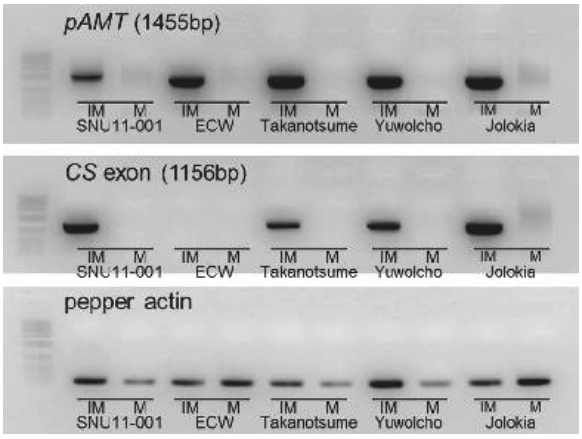

Figure 3. The *pAMT* and *CS* expression patterns in 5 cultivars by RT-PCR. (IM : RNA from mature green fruit, M : RNA from mature fruit)

Genotype analysis of pAMT in SNU11-001 x Habanero F_2 populations

The *pAMT* genotype was determined for F_2 plants of SNU11-001 x Habanero population. The homozygous *pamt*/*pamt* plants are 49, heterozygous and homozygous plants were 160 in a total of 215 individuals. Therefore we can select *pAMT* mutant lines after analysis of capsaicinoids and capsinoids contents among 49 plants for further study.

Table 1. Segregation analysis in SNU11-001 x Habanero F_2 populations

Number of plants	Total	*pamt*/*pamt*	*pAMT*/*pamt* *pAMT*/*pAMT*	Undetermined
	215	49	160	6

We screened sixteen cultivars containing various levels of capsaicinoid and capsinoid and selected SNU11-001 for breeding of capsinoid-rich cultivar. We are constructing F_2 populations to confirm that *CS* transcriptional level is concerned with quantitative control of capsiate biosynthesis and *pAMT* mutant progenies producing large amount of capsiate in F_2 populations can be valuable materials for breeding of non-pungent *Capsicum* cultivar containing high level of capsiate.

Acknowledgements

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ISSR evaluation of sweet pepper accessions for selection

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Abstract

This study was initiated by obtaining sweet pepper lines derived from 30 founder accessions originated from different geographical regions with a few details of their breeding history, which were adapted to growing under Belarusian conditions. To select divergent genotypes for mating design we assessed the diversity in this collection with a set ISSR markers. Besides, lines were grown in an unheated greenhouse during two consecutive years and appropriate quantitative traits estimation was performed. Both approaches were used to dissect similar lines and determine ones which have the best compound of the traits.

Analysis of ISSR markers yielded a total of 121 amplification products (average of 8.1 fragments per primer), of which 52 (42.9%) were polymorphic.

Maximum number of polymorphic fragments were obtained by $(CTC)_6AA$, $(AC)_8AA$ markers (76.9% and 62.3% respectively), which were the most informative for the analysis of sweet pepper divergence. For other markers polymorphism did not exceed 50%.

Molecular and biometric data were used for making branching architecture of phylogenetic trees, which showed different clustering of the lines into groups with different levels of similarity. For selection of the divergent genotypes we based on the result of ISSR approach, since variability of quantitative traits substantially depends on environmental conditions and can't be objective criterion of genetic heterogeneity. Five highly similar lines were excluded, while the lines of other relatively dispersed groups were considered in the context of the mating design.

Based on the result we selected five genotypes with good compound of the trait and wide genetic distances, which were included in designs-full diallelic experiment. Further investigation will lead to the conclusion about the effectiveness of this approach for the sweet pepper selection.

Keywords: *Capsicum annuum* L., diversity, ISSR

Construction and analysis of eggplant parthenocarpy SSH cDNA library

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Abstract

Cloning of eggplant parthenocarpy related gene had good significance for researching the molecular mechanism of parthenocarpic fruit development and guiding parthenocapic cultivar breeding. Four SSH-cDNA libraries were constructed with the parthenocarpic eggplant line D-10 and unparthenocarpic eggplant line 03-2. The four libraries include 2943 clones. After sequenced and assembled, 1632 Unique ESTs were gotten. Blast analyses revealed that 647 of these unique ESTs were homologous to the genes their function are available, and 275 could be new genes. In the isolated ESTs, some matched with products involved in fruit growth and development, such as auxin growth promoter protein and expansin protein. Some ESTs are related to the development of flower organ, such as transcription factor MADS-box gene family and zinc finger protein. Some ESTs are related to signal transduction, such as MAPKK gene and WRKY transcription factor Other ESTs are related to environment response factor, such as histone.Using the Gene Ontology nomenclature to cluster and analysis the ESTs, results show that the difference between parthenocarpy and unparthenocarpy almost existed in cell. Parthenocarpy may be regulated by some binding and catalytic factors, and differentially expressed genes were involved in many biological processes.The ESTs which may be related to eggplant parthenocarpy are chosen to research their expression in parthenocarpic and unparthenocarpic eggplant fruits by real time PCR experiment. The result show that Methionine sulfoxide reductase gene，Auxin Growth Promotor Protein gene and MADS-box *SEPALLATA3* gene are up regulated in parthenocarpy eggplant. The expression trend indicated that the 3 genes may be related with parthenocarpy. The 3 genes were finally cloned from parthenocarpy eggplant fruit by RACE technology.

Keywards: Eggplant, parthenocarpy, SSH cDNA-library, cloning

Introduction

Fruit development in higher plants normally requires pollination and fertilization to stimulate cell division of specific floral tissues. In some cases, fruit development proceeds without either pollination or fertilization (parthenocarpy). Parthenocarpic fruit without seed has higher commercial value and environment adaption than seeded fruit. The eggplant varity D10 produced parthenocarpic fruits under low temperature and regular seeded fruits were generated when the temperature was appropriate. Notwithstanding, the molecular mechanism of the eggplant parthenocarpic fruit development is unknown. A powerful method for exploring this mechanism is the isolation of full length cDNA genes encoding proteins that are associated with parthenocarpic character. First, by means of SSH (Diatchenko et al.1996) techonogy, four libraries of differentially expressed clones were obtained. Then, these clones were sequenced, their expression patterns were identified by Real-time PCR, and the selected candidate parthenocarpic genes were cloned. This research has good significance for understanding the molecular mechanism of parthenocarpy and guiding parthenocapic cultivar breeding.

Materials and Methods

Plant material and tissue sampling

The eggplant parthenocarpic D-10 line exhibits cold resistance. Normal growth and development of parthenocarpic fruit occurs when the daily minimum temperature ranges from 7 °C to 15 °C during anthesis and fruiting periods. Fruits produce seeds when the temperature is appropriate. The non-parthenocarpic 03-2 line does not grow normally when the daily minimum temperature ranges from 7 °C to 15 °C during anthesis and fruiting periods (Liu et al. 2005).

Plant materials were planted in the open field in the spring 2009. The fruit of line D-10 expressed parthenocarpic characteristics and produced seedless fruit when temperature was low (daily minimum temperature ranged from 9 °C to 15 °C), while non-parthenocarpic fruit did not inflated. Regular seeded fruit was generated by the two lines when the temperature was appropriate (daily minimum temperature ranged from 16 °C to 21 °C). Ovaries and fruit from the two lines were harvested, snap-frozen using liquid nitrogen and stored at -80 °C prior to analysis.

RNA isolation and cDNA synthesis

Total RNA was extracted from D-10 and 03-2 samples with Plant RNA kit (Omega, USA). For SSH, each 125ng of total RNA from four time points (7 days before anthesis, the point of anthesis, 7 days post-anthesis and 20 days post-anthesis) of D-10 at low temperature were mixed, and each 125ng of total RNA from four time points of D-10 at appropriate temperature were mixed. The two kinds of mixed total RNA, total RNA extracted for anthesis day from D-10 and total RNA extracted for anthesis day from 03-2 were seperately reverse transcribed to cDNA by using the SMART^{TM} PCR cDNA Synthesis kit (Clontech , USA), following the manufacturer's instruction.

Construction of subtracted cDNA library

SSH was conducted by using PCR-Select^{TM} cDNA Subtraction Kit (Clontech, USA). Forward 1 library (D-10 mix in low temperature as tester and D-10 mix in approriate temperature as driver), reverse 1 library (D-10 mix in approriate temperature as tester and D-10 mix in low temperature as driver), forward 2 library (D-10 in anthesis day as tester and 03-2 in anthesis day as driver) and reverse 2 library (03-2 in anthesis day as tester and D-10 in anthesis day as driver) were constructed following the manufacturer's instructions. The cDNA clones were randomly picked from each substracted SSH library and comfirmed by PCR with nested PCR primers provided in the PCR-Select^{TM} cDNA Substraction Kit (Clontech, USA).

Sequence analysis

Nucleotide sequences of the inserted cDNA fragments were sequenced by Beijing Genomics Institute. All sequences were compared to the NCBI database using BLAST. The Blast2Go (Conesa et al.2005) program was used for gene ontology (GO).

Real-time PCR verification

To comfirm the EST sequence expression and select pathenocarpy related gene, total RNA was extracted from the ovaries and fruits of the two lines (D-10 and 03-2) in low temperature with the Plant Kit(Omega). First-strand cDNA was synthesized from each total RNA (5 μg) using the SuperScript® III First-Strand Synthesis System for RT-PCR Kit (Invitrogen, USA). Primers sequences were designed with Primer 5 from the cDNA sequence. Ubiquitin served as a reference gene (Cooper et al. 2004). Real-time PCRs verification was performed according to Zhang et al. (2011).

The full-length cDNA cloning of differentially expressed genes

Total RNA extracted from the seedless fruit of the first inflorescence of the D-10 at 7 days post-anthesis was used as initial template. The primer pairs were designed according to the differentially expressed ESTs. The full-length cDNA of differentially expressed ESTs were cloned using the SMARTer^{TM} RACE cDNA Amplification Kit (Clontech, USA).

Results and Discussion

Construction of SSH libraries

To isolate genes differentially expressed in parthenocarpic variety "D-10" compared with variety "03-2" during fruit development, four SSH libraries were constructed. In forward 1library and reverse 1 library, 2347 clones were comfirmed to be recombinant by using PCR with nested primers. In forward 2 library and reverse 2 library, 596 clones were comfirmed to be recombinant. The four libraries include total 2943 recombinant clones.

Sequence analysis

All the 2943 recombinant clones in the four libraries were sequenced by Beijing Genomics Institute, and 2505 high quality EST were obtained. After removing vector sequences and assembly, 1632 unique ESTs were obtained. BLASTX analysis showed that 1366 unique ESTs exhibited high sequence homology with known proteins in the NCBI non-redundant protein sequences database (E-value $<e^{-10}$) and that 275 were classified as no hits (E-value $>e^{-10}$). Table 1 showed a selected list of genes with putative functions that could be important for parthenocary mutant.

Table 1. Selected list of relative candidate genes for parthenocarpic eggplant in SSH libraries

Unique EST No.	Putative homology gene	Accession No. of homology gene	E-value
Fan1	putative auxin growth promotor protein	AAK84479.1	0
Fan23	MAPKK	NP_001234591.1	9e-62
Fan743	WRKY transcription factor 3	BAE46417.2	2e-62
Zheng191	cytochrome P450 NADPH-reductase	AAZ39649.1	3e-57
Fan11	SEPALLATA3-like MADS-box	AAP83377.1	8e-84
F1	MADS-box protein	AJ302015.1	9e-28
Z351	120 kDa pistil extension-like protein	AAX82552.1	2e-11
Z569	Peptide methionine sulfoxide reductase	P54153.1	7e-36
Z599	3-ketoacyl-CoA synthase	ACT21783.1	3e-81
Z603	Histone H3.2 precursor	AAB36496.1	3e-26
Z613	Cryptochrome 1b gene	AF348462.1	2e-13
Z622	Ribosomal protein PETRP	AAR83848.1	2e-31
Z626	Prf interactor 30137	AAV80420.1	3e-106
Z707	AP2/ERF domain-containing transcription factor	XP_002307390.1	3e-17

Go categories were assigned to 1632 unique ESTs with BLASTX hit using Blast2GO (Conesa et al. 2005). 275 unique ESTs were found to be potentially new genes having no similarity in the public database, while 710 unique ESTs having hypothetical proteins but no defined biological functional annotation. The number of unique ESTs annotated for the biological function was 647. Figure 1 shows the percentage distribution of GO terms (2^{nd} level GO terms). Since a gene product could be assigned to more than one GO term, the percentage in each main category will add up to ＞100%. The classification results show that the difference between parthenocarpy and unparthenocarpy almost existed in cell. Parthenocarpy may be regulated by some binding and catalytic factors, and differentially expressed genes were involved in many biological processes.

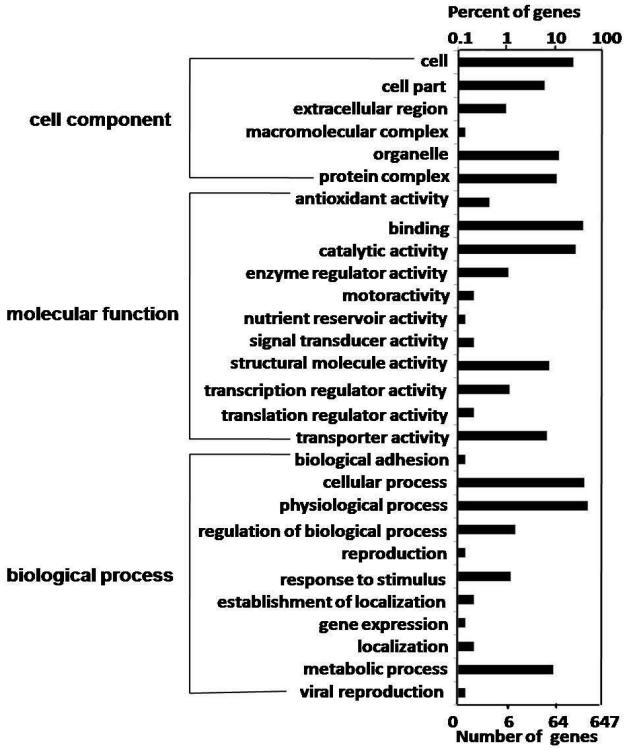

Fig. 1. Distributions of differentially expressed ESTs according to GO (2nd level GO terms) consortium

Screening of differential expression sequence

Real time PCR was performed to test the expression quantity of several unique ESTs selected in SSH libraries. Several tested unique ESTs are differentially expressed in some stage between line D-10 and 03-2. Among these, Z569, Fan1 and Fan 11 are significantly up-regulated ain line D-10 parthenocarpic fruit, especially in the early stage of fruit development. Figure 2 depict the expression of the three unique ESTs.

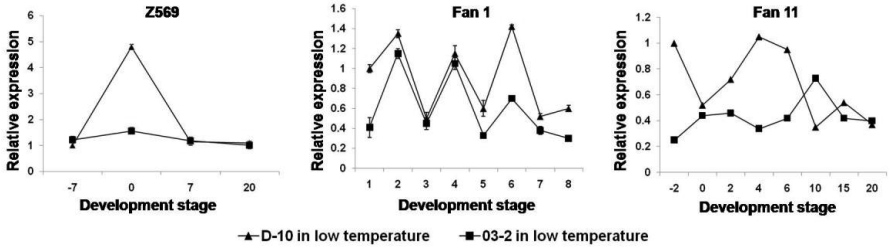

Fig. 2. Relative expression of three unique ESTs in D-10 and 03-2 fruit. -7: days before anthesis ; -2: 2 days before anthesis; 0: anthesis day, 2, 4, 6, 7, 10, 15, 20: 2, 4, 6, 7, 10, 15, 20 days past anthesis.

cDNA cloning of candidate parthenocarpic genes

Based on the unique ESTs Z569, Fan 1 and Fan11, three full-length cDNA sequences were cloned from parthenocarpic eggplant D-10 utilizing RACE technology. The full-length gene of Z569, named *SmMsrA* gene, was 934bp with a 600 bp open reading frame (ORF), 63 bp 5'-noncoding region, and 271 bp 3'- noncoding region. *SmMsrA* gene was similar to methionine sulfoxide to methionine A (MsrA) proteins identified in several other species with the function of restoring oxidized methionine sulfoxide to methionine A. The full-length gene of Fan1, named *SmAGPP* gene, was 2064bp with a 1521bp ORF, 223 bp 5'-noncoding region and 319 bp 3'-noncoding region. *SmAGPP* gene has 87% identity to putative auxin growth promotor protein (accession number: AF275345) in tomato. The full-length gene of fan11, named *SmSEP3* gene, was 966 bp with a 726 bp ORF, 51 bp 5'-non coding region and 189bp 3'-non coding region. *SmSEP3* gene has 96% identity to SEPALLATA3-like MADS-box protein (accession number: AAP83377.1) in tomato.

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Unraveling the resistance mechanism to thrips in pepper (*Capsicum* spp.)

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Abstract

Production of pepper both in the greenhouse and in the field is constrained by the high infestation of thrips. Thrips can cause both direct and indirect damage. Therefore resistance to thrips is a desirable trait in pepper. However, while resistant accessions have been found, not much is known about the mechanism of resistance. The objective of our study was to unravel possible mechanisms of thrips resistance in pepper. Preference, adult and pre-adult survival, developmental time, and reproduction rate were assessed. We also explored the relationship between some morphological characters and metabolite content of the leaves to thrips resistance.

Our study showed that resistance in pepper had no effect on adult survival, but that oviposition rate and larval mortality are significantly affected. In the resistant accessions the development of thrips larvae was completely blocked. We found no evidence for a role of morphological characters in resistance. Using GC-MS we identified several compounds that correlate with level of resistance to thrips. Since the resistant accessions significantly affect the biology of the thrips and do not support the development of thrips, it is likely that antibiosis plays a more important role in resistance of pepper to thrips than tolerance.

Keyword: antibiosis, larval mortality, oviposition, Solanaceae, secondary metabolites

Introduction

Pepper (*Capsicum* spp.) is one of the most produced vegetables in the world based on data released by the World Food and Agriculture Organization (FAO, 2012). Unfortunately the production of pepper both in the greenhouse and in the field is still constrained by the high infestation of insect pest in which thrips (*Frankliniella occidentalis*) are among the most damaging, both in greenhouse and field cultivation (Siemonsma & Piluek, 1994). *F. occidentalis* can cause large losses in pepper production through direct damage by feeding on leaves and fruit and indirect damage by transferring viruses (Jones, 2005). Thrips control is difficult because of their polyphagous nature, high reproductive rate, their facultative parthenogenic mode of reproduction, their ability to develop resistance to pesticides, and their cryptic habit: larvae hide in closed bud and pupate in soil (Jensen, 2000; Bielza, 2008).

Therefore resistance to thrips is a desirable trait in pepper. However, while resistant accessions have been found (Fery & Schalk, 1991; Maris et al. 2004; Maharijaya et al. 2011), not much is known about the mechanism of resistance to thrips in pepper. The objective of our study was to unravel possible mechanisms of thrips resistance in pepper. Preference, adult and pre-adult survival, developmental time, and reproduction rate were assessed. We also explored the relationship between some morphological characters and metabolites content of the leaves to thrips resistance.

Materials and Methods

Plant and thrips

Three highly resistant, three medium resistant and three susceptible *Capsicum chinense* accessions (PI281428, PI315023, 4661) were chosen based on the results of a previous screening for thrips resistance (Maharijaya et al. 2011). An F_2 population consisting of 196 F_2 plants was developed from a cross between *C. annuum* AC 1979 as female parent and *C. chinense* 4661 as male parent. Thrips was reared on the susceptible *Chrysanthemum* cultivar Spoetnik® (Fides, De Lier, the Netherlands) in a growth chamber at 25^{o}C, 16/8 hr day/night, and 70% relative humidity. Thrips larvae (L1 stage) were obtained by allowing female thrips to lay eggs in small cucumber fruits for one day, after which the adult thrips were brushed off and fruits were kept at 25^{o}C for four days, when the new larvae emerged.

Resistance test

Five newly emerged *F. occidentalis* L1 larvae were placed on a single fresh fully opened leaf that was placed with the abaxial side downwards in a sterile 50 x 9 mm petridish with lid (BD Falcon®). Leaves and larvae were incubated in a climate chamber at 25^{o}C, 16 h light, 70% RH. Damage caused by larvae was scored after two days using a visual scale ranging from 0 (no damage) to 3 (severe damage) as described in Maharijaya (2011). Survival of L1 larvae into the L2 stage was assessed by counting the number of L2 larvae and dividing this by the total number of larvae placed on the leaf.

Preference test

A preference test was conducted through choice test in a petridish system. 2 leaf discs (∅ 4cm) of resistant and 2 leaf disc of susceptible accessions were placed in opposite direction with abaxial side upward in a petridish with agar (15 g/l agar). Five female thrips were placed in the middle of the petridish covered with air permeable plastic (Fresh Cling®). Number of female thrips stay on each leaf was counted after 15 and 30 minutes carefully.

Developmental and reproduction test

The effects of resistance factor in pepper to the developmental stages of thrips were assessed by adult survival test and developmental study. Adult survival was studied by placing 10 females on a single leaf disc taken from new fully opened leaf using a leaf punch (∅ 4cm) that was placed with the abaxial side downwards on 1.5 % agar in a Petri dish, covered with air permeable plastic (Fresh Cling®). After four days the numbers of living and dead females were counted. Thrips development was studied by placing one individual synchronized L1 (first instar) larva on a leaf disc (∅ 4cm). Sixty leaf discs were used for each accession. The number of individuals developing through successive developmental stages was determined by daily observation. For reproduction rate ten females were placed on a single leaf disc (∅ 4cm). After allowing 24 hours for oviposition all females were removed. Every day the newly emerged larvae were counted under a stereo microscope and removed.

Morphological characters test

Leaf color, trichome density (hairiness), cuticula thickness, and leaf toughness were assessed in F_2 population. New fully opened leaves were used and subjective scoring from 0 to 3 was applied for each character except for cuticula thickness. For cuticula thickness the leaf blade was inserted in polystyrene foam and excised as thin as possible with a razor. The sections were put on a glass slide and coloured with 0.1% safranin in glycerin-water (1:1) and then covered. The sections were observed and cuticle thickness was measured under a binocular microscope equipped with a micrometer. Correlation analysis was done for each morphological character versus resistance scores.

Metabolites test

Metabolite content of the pepper leaves were measured using Gas-chromatography-mass-spectrometry (GC-MS). To select candidate metabolite compounds related to thrips resistance, Pearson correlation analysis followed by False Discovery Rate correction were applied.

Results and Discussion

Antibiosis is the dominant resistance mechanism in pepper.

In this study we found no significant difference between the number of female adult of *F. occidentalis* on leaves of resistant and susceptible accessions. This indicates that preference (antixenosis) might not influence much in resistance mechanism against thrips in pepper. This is supported by previous study showing that there is highly significant correlation between damage score of thrips on pepper leaves in a non-choice (leaf assay) versus choice situation (greenhouse test) (Maharijaya et al. 2011).

Our study showed that resistance factor in pepper strongly affected the development of thrips from larvae to pupae as well as reproduction rate. There was a clear and significant difference in survival of larva reared on leaves of resistant, medium resistant and susceptible accessions. The F2 population also showed a high correlation between damage caused by larvae with the survival of larva. The resistance factor in pepper also affect the oviposition rate as shown as the less number of egg produced by female adult thrips reared on resistant accessions. Because the resistance factors in leaves can affect strongly the biology of thrips i.e. survival of the larvae and suppress reproduction rate; it is more likely that the resistance mechanism is antibiosis (Smith, 1989).

No effect of leaf color, hairiness, cuticula thickness and toughness to resistance against thrips in pepper.

Any leaf characters that interfere with suppression to thrips life-cycle and reproduction are potential resistance factors which may contribute to mechanism of defense against thrips. Some mechanisms of defense against insect in pepper have been reported such as tolerance to *F. occidentalis* (Fery & Schalk, 1991), trichomes against *Scritothrips dorsalis* (Yadwad et al. 2008), against whitefly (Firdaus et al. 2011) and against aphid(Bosland & Ellington, 1996). However, we found no evidence for a role of morphological characters in resistance since we found no significant correlation for those morphological characters with resistance level to thrips in this study.

Metabolites correlated with resistance in pepper.

In our study, we detected several metabolites that significantly correlated with level of resistance in pepper. Unfortunately, some of these metabolites could not be tentatively annotated. Some of these detected metabolites have been reported earlier to be involved in insect resistance in several crop species (Maharijaya et al. 2012). This finding suggested the possible role of metabolites in the resistance mechanism against thrips in pepper. The identification of metabolic compounds with a relationship to thrips resistance in pepper may help to elucidate the resistance mechanism and to identify the genes involved.

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Development and analysis of interspecific hybrids among three species in the genus of *Solanum*

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Abstract

The related wild species of eggplant (*Solanum melongena* L.) are resistant to various pests and pathogens and thereby are a source of desirable traits for crop improvement. Reciprocal crosses were made among three species, *S. melongena* L. (cv. Almaz and cv. Brilliant), *S. aethiopicum* L., and *S. integrifolium* L. Embryo rescue technique was applied to overcome incompatibility. ISSR (Inter-Simple Sequence Repeat) and IRAP (Inter-Retroelement Amplified Polymorphism) fingerprinting was used for purity testing of interspecific F_1 hybrids.

Keywords: eggplant, interspecific hybrids, embryo rescue technique**.**

Introduction

Interspecific hybridization is one of the tools for inducing variability and incorporation of agronomically important traits from wild and related species into cultivated varieties. Wild species *S. aethiopicum* L. and *S. integrifolium* L. are known to have useful characters for improving the cultivated eggplant such as cluster bearing habit, resistance to the soil born pathogens *Fusarium oxysporum* f. sp. *melongenae*, *Ralstonia solanacearum*, and resistance to root-knot nematodes *Meloidogyne* spp. (Nasrallah and Hopp, 1963; Yamakawa and Mochizuki, 1979; Hebert, 1985; Collonnier et al. 2001; Kashyap et al. 2003). Interspecific hybridization is limited by various pre-zygotic and post-zygotic barriers. Biotechnological tools like protoplast culture, somatic hybridization, and embryo rescue are useful techniques in overcoming of genetic incompatibility. The first report of successful application of embryo rescue technique for obtaining interspecific fertile hybrids between *S. melongena* and *S. khasianum* Clarke dates back to 1980 (Sharma et al. 1980). Since then, the successful interspecific crosses have been obtained with only few wild species when *in vitro* embryo rescue was employed. Such hybrids have either been sterile or have had very low pollen fertility (Sharma et al. 1980; Ano et al. 1991; Daunay et al. 1998; Blestsos et al. 1998; McCammon et al. 1983; Kumchai et. al, 2013).

The aim of the present study was the production of interspecific hybrids among three species, *S. melongena* L. (cv. Almaz and cv. Brilliant), *S. aethiopicum* L., and *S. integrifolium* L by using *in vitro* embryo rescue technique. In addition, the hybridity of the seedlings obtained was tested using ISSR and IRAP analysis.

Materials and Methods

Reciprocal crosses among three species, *S. melongena* L. (cv. Almaz and cv. Brilliant), *S. aethiopicum* L., and *S. integrifolium* L. were carried out in a greenhouse. The wild species, *S. aethiopicum* L. (Cat. Num 2937) and *S. integrifolium* L. (Cat. Num. 845), were obtained from the collection of N.I. Vavilov Research Institute of Plant Industry (St.Petersburg, Russia).

Ovaries from each cross were harvested at 20, 25, 30, 35, 40 and 45 days after pollination (DAP) and disinfected. Excised embryos were placed on MS basal medium supplemented with casein hydrolysate and two growth regulators, TDZ (0.1 mg/L) and NAA (10 mg/L) (Rotino et. al.

1987; Mariani et. al. 1992). The embryo cultures were incubated at 20-22°C under diurnal photoperiod 14h/10h (light/dark). Regenerated seedlings were subcultured on ½ MS medium without growth regulators. Plantlets thus obtained were subsequently potted in soil and transferred to a greenhouse.

The test of interspecific hybrid purity was done using ISSR primers ($(CAG)_5$ and $(GT)_8A$) and IRAP primer (AACGAGGGGTTCGAGGCC). DNA was extracted using Genomic DNA Purification Kit (Fermentas). PCR reaction mixtures (25 µl) consisted of 50 ng DNA, 100 µM of each dNTP, 1.5 mM $MgCl_2$, 10 pmole primer, and 0.5 unit Taq DNA polymerase (Fermentas). The reaction conditions were: 94°C for 5 min; 35 cycles of 94°C for 1 min, 46°C - 55°C (depending on primer) for 1 min, and 72°C for 1 min; 72°C for 5 min. PCR products were visualized on 1.5% agarose gel stained with ethidium bromide.

Results and Discussion

To determine the optimal time of embryo excising, the following developmental stages of zygotic embryo of eggplant were considered: globular, heart, torpedo, cotyledon, and mature embryo (Fig.1). The embryos of *S. aethiopicum* L. and *S. integrifolium* L. were the most fast-developing and the most slow-developing, respectively (Tab.1). The culturing of eggplant embryos on the medium MS supplemented with 0.1 mg/L TDZ showed the best results (Fig.2). The optimal age for development of isolated embryos was 30-40 days after pollination. Abnormal development was observed when embryos were cultured on MS with 10 mg/L NAA (Tab.2; Fig.2). The 69,0 % and 9,6 % of embryos cultured on MS with 0.1 mg/L TDZ and 10 mg/L NAA, respectively, survived and regenerated into mature hybrid plants.

The hybridity of the putative interspecific F_1 hybrids was identified through comparison of morphological traits (data not shown) and DNA banding patterns resulting from PCR with ISSR and IRAP primers (Fig.3). On the basis of the banding patterns, the parents could be distinguished from each other as could interspecific hybrids from the parents. For example, when IRAP primer was used (Fig.3 c), the hybrids patterns contained two parental bands (330 bp and 480 bp) specific for *S. aethiopicum* L. and two parental bands (750 bp and 1000 bp) specific for *S. melongena* L. Thus, the obtained plants possessed both male and female parent-specific bands, and they could be considered interspecific hybrids.

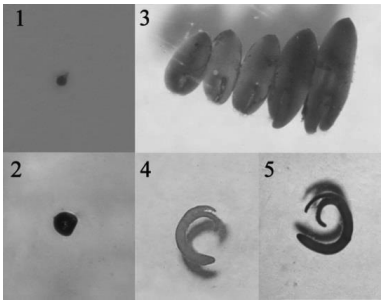

Figure 1. Stages of embryo development of eggplant. 1 - globular, 2 - heart, 3 - torpedo, 4 - cotyledon, 5 - mature embryo.

Table 1. Comparison of three species of *Solanum* L. by rate of embryo development

Species	Day after pollination	Length of embryo, mm	Developmental stage of embryo
S. melongena L.	20	0,032±0,001	Globular embryo
	23	0,1±0,01	Heart embryo
	27	0,6±0,06	Torpedo embryo
	31	1,2±0,06	Torpedo embryo
	35	3,8±0,09	Cotyledon embryo
	40	4,3±0,09	Mature embryo
S. aethiopicum L.	20	0,08±0,007	Globular and heart embryo
	25	2,2±0,3	Torpedo and cotyledon embryo
	30	4,1±0,05	Mature embryo
S. integrifolium L.	20	0,07±0,005	Globular embryo
	25	0,15±0,03	Heart embryo
	30	1,1±0,1	Torpedo embryo
	35	1,8±0,2	Torpedo embryo
	40	3,9±0,2	Cotyledon embryo
	45	4,5±0,1	Mature embryo

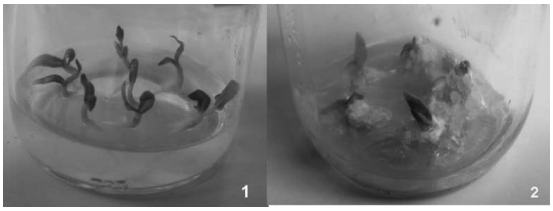

Figure 2. Embryo development obtained from cross *S. melongena* L. x *S. aethiopicum* L. on the MS medium with 0.1 mg/L TDZ (1) and 10 mg/L NAA (2).

Table 2. Characteristic of embryo development of interspecific hybrids depending on age of embryos (DAP) and growth regulators.

Hybrid combination	Day after pollination	Growth regulators	
		TDZ (0.1 mg/L)	NAA (10 mg/L)
S. melongena (cv.Brilliant) × *S. integrifolium*	25	No development	No development
	30	Abnormal cotyledon and root	White friable callus
	35	Normal seedlings	White friable callus
	40	Normal seedlings	White friable and green callus
S. melongena (cv.Brilliant) × *S. aethiopicum*	20	No development	No development
	25	No development	No development
	30	Normal seedlings	White friable callus
	35	Normal seedlings	White friable callus
S. melongena (cv. Almaz) × *S. integrifolium*	25	No development	No development
	30	Abnormal cotyledon and root	White friable callus
	35	Normal seedlings	White friable callus
	40	Normal seedlings	White friable callus
S. melongena (cv. Almaz) × *S. aethiopicum*	20	No development	No development
	25	No development	No development
	30	Normal seedlings	White friable callus
	35	Normal seedlings	White friable callus

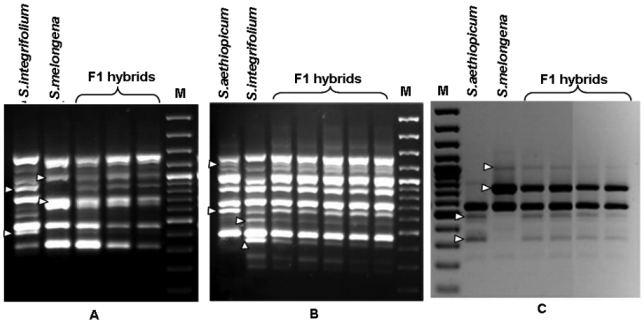

Figure 3. Purity test for F_1 hybrids using the ISSR primers (A and B) and IRAP primer (C). Arrows indicate the fragments that were transmitted from each parent to the hybrids. M – GeneRuler, 100 bp DNA Ladder (Fermentas).

Pollen fertility of obtained interspecific hybrids ranged from 23% to 33%. Sterility and very low pollen fertility of eggplant interspecific hybrids have been reported earlier (Sharma et al. 1980; Ano et al. 1991; Daunay et al. 1998; Blestsos et al. 1998; McCammon et al. 1983). The F_1 plants were selfpollinated and backcrossed to both parents. Seeds, however, were produced only when the F_1 hybrids were backcrossed as female with the cultivated eggplant *S. melongena* (cv.Brilliant and cv. Almaz).

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The effects of phenylacetic acid (PAA) on haploid embryo induction in eggplant (*Solanum melongena* L.) anther culture

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Abstract

The study was conducted to determine the factors, especially growth hormone concentrations, affecting anther culture response traits in eggplant. A major problem with anther culture in eggplant is the low numbers of plants that are regenerated. The beneficial effects of an auxin, phenylacetic acid (PAA), on gametic and somatic embryogenesis have been reported for cereals. PAA is a naturally occurring plant hormone that has auxin-like activity. In the present study two eggplant cultivars were used for anther culture technique. The effects of PAA and 2,4D, kinetin and their combinations on induction of haploid embryo in eggplant cultivars were investigated. Different concentrations of PAA, 2,4D and kinetin (0.1, 1.0, 2.5 and 5.0 mg/L) were used on solid Gamborg (B5) medium. Anthers at the mid or late uninucleate microspore stage were excised from sterilized buds and placed on induction media. The possible mechanisms of PAA on improving in a comparison with 2, 4 D and kinetin regeneration are discussed.

Keywords*:* Eggplant, anther culture, PAA, 2,4D, kinetin

Effect of active charcoal on *in vitro* rooting and growth of *S. melongena* x *S. aethiopicum* hybrids

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Abstract

Micropropagation of interspecific hybrids of eggplant (*Solanum melongena*) with related species is of great interest, in particular when crosses have a low degree of success. In this respect, hybrids between *S. melongena* and *S. aethiopicum* (scarlet eggplant) could be useful not only for their mass propagation to be used as rootstocks, but also for the breeding of both crops by means of biotechnological approaches. Rooting ability and *in vitro* development of four *S. melongena* x *S. aethiopicum* hybrids and the eggplant cv. Black Beauty were tested in a standard culture medium (BM) supplemented or not with active charcoal (CA). At thirty days of culture 100% of plants rooted, although callus formation was observed in some treatments, mainly in plants cultured in BM. When considering fresh weight (FW) and dry weight (DW) values, the highest root development was obtained in the hybrid SM27 X SA83 cultured in BM followed by the hybrid SM27 X SA84, both of which share the same maternal parent. For both hybrids, a higher FW was observed in the BM medium when compared to the CA medium. No differences among roots of plants cultured in BM or CA were observed in other genotypes. The number of vegetative nodes also differed among genotypes and culture media. It was higher in for Black Beauty, PI470273xPI413783 and PI470273xPI413784 cultured in CA medium with respect to those cultured in BM medium. Thus, the rate of micropropagation could be increased using the CA medium in these genotypes. Also, the number of explants that could be obtained for different purposes will be higher using the CA medium. In SM27 X SA83 and SM27 X SA84 hybrids the number of nodes was similar in CA and BM media. As in these genotypes rooting was better in the latter, BM medium is selected for micropropagation of these hybrids.

Keywords: micropropagation, biotechnology, eggplant

Introduction

Micropropagation protocols are developed to obtain efficient methods for commercial exploitation of valuable plant-derived pharmaceuticals, ornamental plants, trees and hybrids of interest selected from natural populations or obtained in breeding programs (Hossain et al. 2013; Vieitez et al. 2012; Ascough et al. 2009; Castellanos et al. 2008). Micropropagation of interspecific hybrids of eggplant (*Solanum melongena*) with related species is of great interest, in particular when crosses have a low degree of success as it is the case when *S. aethiopicum* is used in the cross (Behera and Singh, 2002).

Hybrids between *S. melongena* and the scarlet eggplant (*S. aethiopicum*) could be useful as rootstocks, but also for the breeding of both crops by means of biotechnological approaches. Interspecific hybrids have several advantages, including pathogens resistance from both parents (Daunay, 2008; Lee and Oda, 2003), heterosis for vigor (Bassett, 1986) and, in the cases where one of the parents is from the same scion's species, a greater degree of rootstock-scion compatibility (Miguel et al. 2007). Eggplant scions grafted onto interspecific hybrids *S. melongena* x *S. aethiopicum* displayed earliness, good vigour, excellent survival and high yield despite nematode

soil infestation (Gisbert et al. 2011a, 2011b). These results, together with the lack of deleterious effects on fruit quality traits or fruit composition of eggplant cultivars 'Black Beauty' (Gisbert et al. 2011a) and 'Cristal F1' (Gisbert et al. 2011b), indicates that these hybrids are promising materials for developing new rootstocks for eggplant production.

In order to develop a micropropagation protocol for *S. melongena* x *S. aethiopicum* interspecific hybrids, adventitious rooting and growth development were studied in a standard culture medium (BM) supplemented or not with active charcoal (CA). Rooting is an essential step in the vegetative propagation of plants and CA alone or in combination with auxin may induce rooting. CA may affect also development and regeneration as previously reported in several plants (Thomas, 2008).

Materials and Methods

Four interspecific hybrids from the cross *S. melongena* x *S. aethiopicum* together with the eggplant commercial cultivar 'Black Beauty' (BB) were used (Table 1). Seeds of all these materials were disinfested and germinated in vitro as described by Gisbert et al. (2006). Shoots were grown in culture medium (BM: MS including vitamins, DUCHEFA, The Netherlands, 1.5% sucrose and 7.0% plant agar) supplemented or not with CA (at 0.25%). At thirty days of culture the percentage of rooting, FW and DW of roots and the number of nodes were noted.

Table 1. Plant materials used for the rooting experiments, type of material and origin.

Plant material	Code	Species	Type of material	Origin[a]
PI263727 X PI413783	SM27 X SA83	*S. melongena x S. aethiopicum*	Interspecific hybrid	Local landraces, Puerto Rico x Burkina Faso
PI263727 X PI413784	SM27 X SA84	*S. melongena x S. aethiopicum*	Interspecific hybrid	Local landraces, Puerto Rico x Burkina Faso
PI470273 X PI413783	SM73 X SA83	*S. melongena x S. aethiopicum*	Interspecific hybrid	Local landraces, Indonesia x Burkina Faso
PI470273 X PI413784	SM73 X SA84	*S. melongena x S. aethiopicum*	Interspecific hybrid	Local landraces, Indonesia x Burkina Faso
Black Beauty	BB	*S. melongena*	Commercial variety	B and T World Seeds, Aiguesvives, France

[a] For commercial seed the Seed company and headquarters location is indicated; for interspecific hybrids country of origin are indicated. All these accessions (both from *S. melongena* and *S. aethiopicum*) are cultivated landraces.

Results and Discussion

Charcoal has the characteristic property of high absorptive power for colloidal solids, gases and vapors. It plays a critical role in several protocols of *in vitro* culture techniques such as micropropagation, seed germination, regeneration, rooting and stem elongation (Thomas, 2008). In our work, rooting ability and *in vitro* development of four *S. melongena* x *S. aethiopicum* hybrids and the *S. melongena* cv. Black Beauty were tested in a standard culture medium (BM) supplemented or not with CA in order to test the effect of this compound.

At 30 days of culture 100% of plants rooted, although callus formation was observed in some treatments, mainly in plants cultured in BM (Table 2). A drastic reduction of callus formation in plants of BB is observed in plants cultured in CA containing medium with respect to those cultured in BM. Lower callus formation in CA containing media has also been previously reported by Teng

and Ngai (1999) *Oxalis triangularis* ssp. When considering fresh weight (FW) and dry weight (DW) values, the highest root development was obtained in the hybrid SM27xSA83 cultured in BM followed by the hybrid SM27xSA84, both of which share the same maternal parent. For both hybrids, a higher FW was observed in the BM medium when compared to the CA medium. No differences among roots of plants cultured in BM or CA were observed in the remaining genotypes. Despite several works reported the positive effect of CA in root induction (Thomas, 2008), in our materials 100% of rooting was achieved in media with and without CA. Thus, no effect of root induction could be observed. On the other hand, similar or lower FW and DW of roots were obtained in the plants cultured in medium with CA respect those cultured in BM.

The number of vegetative nodes at 30 days of culture also differed among genotypes and culture media. Vegetative nodes number was higher in plants of BB, SM73xSA83 and SM73xSA84 cultured in CA medium with respect to those cultured in BM medium. This effect has also been observed in *Gossypium hirsutum* (Hazra et al. 2002) in CA containing media. Thus, the rate of micropropagation could be increased using the CA medium in these genotypes. In addition, the number of explants that could be obtained for different biotechnological purposes will be higher using the CA medium. In SM27xSA83 and SM27xSA84 hybrids the number of nodes was similar in CA and BM media. As in these genotypes rooting was better in the latter, BM medium is selected for micropropagation of these hybrids.

Table 2. Effect of active charcoal on root and growth of plantlets derived from germinated seeds. Roots plantlets were cut and shoots (1 cm) transferred to tubes with BM or BM supplemented with active charcoal (CA) for 30 days.

Hybrid genotype	Medium	Frequency of rooting	% of plants with callus	FW	DW	Number of nodes
SM27 X SA84	MB	100	6,66	0.16 b	0.013 b	2.8 bc
SM27 X SA84	CA	100	0	0.09 de	0.007 d	3.0 b
SM27 X SA83	MB	100	37,5	0.30 a	0.023 a	2.2 cde
SM27 X SA83	CA	100	20	0.16 bc	0.013 bc	2.6 bcd
SM73 X SA84	MB	100	4	0.12 cd	0.010 cd	1.8 e
SM73 X SA84	CA	100	4	0.10 de	0.007 d	2.8 bc
SM73 X SA83	MB	100	0	0.11 de	0.008 d	2.0 de
SM73 X SA83	CA	100	9	0.08 e	0.006 d	3.0 b
BB	MB	100	100	0.12 d	0.009 d	2.6 bcd
BB	CA	100	0	0.10 de	0.009 d	4.2 a
ANOVA						
Genotype				***	***	***
Medium				***	***	***
Genotype x medium				***	**	*

Means with different letters are significantly different by Duncan test ($P<0.05$).

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Micropropagation of the wild eggplant relative *Solanum incanum*

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Abstract

Solanum incanum is the wild ancestor of the cultivated eggplant (*S. melongena*). The use of *S. incanum* as parental for breeding programs, for screening for resistance to pests and diseases, as well as to be used as rootstock, is being limited by the low and irregular germination and high seed dormancy. Therefore, it is difficult to have available large numbers of plants for breeding programmes and for grafting scions. In this sense, it would be very useful to have a micropropagation protocol which allows obtaining a large number of plants when needed. In order to develop a protocol of micropropagation in *S. incanum*, nodal segments of young shoots of an adult plant of *S. incanum* were surface sterilised and cultured. A medium used routinely for pepino (*S. muricatum*) was initially used but explants produced callus at the base. Therefore, we used four new alternative media: medium 1: ¼MS + 2% sucrose; medium 2: ½MS + 2% sucrose; medium 3: ½MS + 2% sucrose + 0.1 µM IBA; medium 4: ¼MS + induction with 10 µM IBA during 5 hours. Explants cultured in media with higher salts concentration (2 and 3) developed a callus in the base of the explants without development of roots, and explants eventually died. Media with low salts concentration (1 and 4) did not induce callus formation and allowed rooting, in particular medium 4. However, even in media 4, the success rate was still low (38%) for mass propagation. Interestingly, increasing the number of subcultures improved the rooting rates. This report represents the first step in developing an efficient micropropagation protocol for *S. incanum*.

Keywords: Cloning, growth regulators, *in vitro* culture, protocols, *S. incanum.*

Introduction

Cultivated eggplant (*Solanum melongena* L.) was domesticated in India and Southeast of China from its wild ancestor *S. incanum* L. (Daunay et al. 1991). We previously used this species to produce a genetic map of eggplant (Vilanova et al. 2010) and are also using it to develop a set of introgression lines (ILs) in the genetic background of eggplant (Prohens et al. 2012). These ILs which will be of great utility in unraveling the genetic control of different agronomic traits. *Solanum incanum* is of great interest as a source of variation for developing interspecific hybrids with eggplant, which are very vigorous and can be used as eggplant rootstocks (Gisbert et al. 2011). In addition, this wild species has been reported as resistant to *F. oxysporium* sp. *melongenae* (Yamakawa and Mochizuki, 1979), it could provide tolerance to abiotic stresses such as drought and low or high temperatures (Knapp et al. 2013), and presents a high content in phenolics (Prohens et al. 2013), which are important objectives in eggplant breeding (Daunay, 2008).

However, the use of *S. incanum* as parental for breeding programs, for testing in resistance screenings as well as a rootstock, is being limited by the low and irregular germination and high seed dormancy (Gisbert et al. 2011). Therefore, it is difficult to obtain a large number of plants available for breeding programmes and for grafting scions. In this respect, it would be very useful to develop a micropropagation protocol which can provide a large number of plants when needed. Although micropropagation protocols for eggplant are at present available (Sharma and Rajam, 1995; Mallaya and Ravishankar, 2012), to our knowledge no protocols have been adapted for *S. incanum*. The objective of the present work is to develop a protocol of micropropagation from nodal segments in *S. incanum*.

Materials and Methods

The plant material used consisted of nodal segments of young shoots of an adult plant of *S. incanum,* accession MM577 (Figure 1A). Nodal segments were surface sterilised and cultured. We firstly applied a routine protocol previously used to micropropagate Solanaceous species such as pepino (*S. muricatum*) (Cornejo et al. 1990) by means of Murashige and Skoog (MS) medium at 4.2 g/L. However, the explants developed a callus in the base of the explants without roots development (Figure 1B). In addition, after seven weeks the explants started to die. Therefore we tried to improve the rooting ability by lowering the concentration of mineral salts and sucrose content, and by adding rooting growth regulators (Chakravarthi et al, 2010). The four alternative media used were: medium 1: ¼MS + 2% sucrose; medium 2: ½MS + 2% sucrose; medium 3: ½MS + 2% sucrose + 0.1µM 3-indolebutyric acid (IBA); medium 4: ¼MS + induction with 10µM (IBA) during 5 hours. Fifteen nodal segments were used for each treatment. The development of the explants was assessed every week.

Figure 1. Explants from a nodal adult plant of *S. incanum* accession MM577 cultivated *in vitro*: A) Nodal explants, B) Explant with callus, C) Rooted explant

Results and Discusion

Media 2 and 3, which contained ½ of MS, produced callus at the base of the explant (Table 1; Figure 1B). The addition of IBA (medium 3) far from inducing rooting (Han et al. 2009) increased callus formation (Table 1). These explants did not induce roots development, and after seven weeks the explants started to die.

The two media with lower mineral salt content (media 1 and 4, with ¼ MS) did not induce the formation of callus in the base of the explant (Table 1) and allowed the formation of roots and the normal explant growth (Figure 1C). The best of the four media was medium 4 which included an induction process with IBA, with a rooting success of 38% of the explants. In this respect, IBA has been previously proved to be effective for eggplant micropropagation (Mallaya and Ravishankar, 2012). The rate obtained by us is still low for massive propagation but allowed the creation of an initial pool of explants of *S. incanum* able to survive *in vitro* and suitable for subsequent subcultures. Interestingly, the increase of the number of subcultures improved the rooting rates (De Klerk, 2002). From rooted explants it was possible to acclimatize some plants (Figure 2).

Table 1. Results from the experiment of micropropagation of *S. incanum* 8 weeks after the cultivation of the explants in the different media.

Treatment	Nodes with callus	Rooted nodes
Medium 1	0%	7%
Medium 2	33%	0%
Medium 3	50%	0%
Medium 4	0 %	38%

Figure 2. Acclimatized plant of *S. incanum* after micropropagation.

Therefore, we recommend the use of low rates of mineral salts, the use of juvenile explants when possible, and the use of auxines like IBA for induction, but not as part of the culture medium. This report represents the first step in developing an efficient micropropagation protocol for *S. incanum*, which has shown to be a more difficult species to micropropagate if compared to eggplant (Sharma and Rajam, 1995; Mallaya and Ravishankar, 2012). The development of a highly efficient protocol for micropropagation of *S. incanum* will be of great utility for eggplant breeding.

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Effects of TDZ on multiple shoots in chili pepper

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Abstract

C. chinense belong to Solanaceae family and has great genetic variability for fruit traits, especially Vitamin A and C. Micropropagation allow high quality plantlets. The thidiazuron (TDZ) has been used for tissue culture as cytokinin to stimulate plant growing. The goal of this work was to induce shooting in five *C. chinense* lines belonging to Germplasm Bank of Centro de Ciências Agrárias (BGH-CCA/UFPB) Areia, Brazil. The seeds were disinfested and inoculated in MS medium supplemented with different concentrations of TDZ (0, 1.0, 1.35, 1.7 and 2.0 $mg \cdot L^{-1}$). After 30 days were evaluated the following traits: fresh weight, rooting, presence of callus and number of shoots. The experiment was evaluated in a factorial scheme 5 x 5 (genotypes x TDZ concentrations) in an entirely random design with two replicates. The data were subjected to Analysis of variance and Duncan's test ($p \leq 0.05$) and regression analyses. The lines 02 and 13 showed more shooting. The Best TDZ concentrations were 1.0 and 2.0 $mg \cdot L^{-1}$, yielding most number of shoots.

Keywords: micropropagation, pepper, *Capsicum chinense*

Introduction

Capsicum chinense is considered the most Brasilian of the domesticated species of chili pepper, by having the center of diversity in the Amazonian region (Bosland, 1992). This species have a high genetic variability, as evidenced mainly in fruits rich in vitamins A and C, and also a source of β-carotene. Besides having different shapes, color, sizes and levels of pungency, *C. chinense* is one of the most produced and consumed pepper species in Brazil (Lannes et al, 2007).

The micropropagation enables large-scale clonal propagation of superior genotypes. This technique depends on factors associated with induction, control of morphogenesis and regeneration of shoots and roots in the process of organogenesis (George, 1996). In most species, the presence of cytokinins in nutrient medium enhances the formation of shoots (Thorpe, 1993).

The superior capability of TDZ to the other adenine-type cytokinins was confirmed in several bioassays, where the TDZ has been used for the multiplication of axillary buds for woody species during micropropagation (Mok et al, 1980).

Thus, this study aimed to evaluate the different concentrations of TDZ on *in vitro* morphogenesis and its effects on the micropropagation of *C. chinense.*

Materials and Methods

The experiment was conduced in Laboratory of Plant Biotechnology of Centro de Ciências Agrárias (CCA)/Universidade Federal da Paraíba (UFPB), Brazil. Five genotypes (lines 02, 10, 12, 13 and 14) of *C. chinense* conserved in the vegetable germplasm bank of CCA/UFPB were used as plant materials.

Seeds of the five genotypes from *C. chinense* were disinfested for 10 min using a sodium hypochlorite solution at 1% plus three drops of Tween 20 The seeds were then rinsed for four times with sterile and distilled water. After sterilization, seeds were pre-cultured in Petri dishes with filter-paper Whatman 20® moistened with 2 ml of sterile and distilled water for a week at 25±2ºC

temperature and 16h/8h photoperiod (light/dark) with 45μM·m^{-2}·s^{-1} light intensity and relative humidity at 60%.

After the first week, the seeds were subcultured in test tubes (25mm x125mm) containing 20 mL of the MS basal medium (Murashige and Skoog, 1962) supplemented with 30 g·L^{-1} sucrose, B5 vitamins (Gamborg, 1968), 100 mg·L^{-1} myo-inositol and TDZ (0, 1.0, 1.35, 1.7. and 2.0 mg·L^{-1}). pH was adjusted for 5.6 ± 0.1. After adding Agar (8 g·L^{-1}), the medium was autoclaved at 121ºC for 20 min. The test tubes were subjected at the same culture conditions of the pre-culture. After 30 days, were evaluated fresh weight, presence/absence of the roots, callus and shoots number.

The experimental design was completely randomized with two replicate for treatment. The experiment was repeated twice. The data were subjected the variance analysis and mean was compared by Duncan´s test at 5% of probability level. Quantitative data were subjected at regression analysis.

Results and discussion

The interaction genotype x TDZ was significant at 5% probability level in relation to content fresh weight and shoots number (Table 1 and Fig 1).

The culture medium supplemented with 1.0 and 2.0 mg·L^{-1} of TDZ presented the higher values for shoots number, principally at 1.0 mg·L^{-1}. The control (without TDZ) was the worse responding with regarding to shoots number. The genotypes more responsive were lines 02 and 14 (Table 2).

Binzel et al (1996) reported that in pepper, the *in vitro* induction of shoots depends mainly on genotype and also on the type of cytokinin, where shoot number ranged from 0% to 20%.

Conclusion

At concentrations of 1.0 and 2.0 mg·L^{-1} of TDZ in MS medium were most effective for shoot multiplication. The genotypes 02 and 14 showed more responsive to TDZ.

Acknowledgements

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Table 1. Analysis of variance of factorial and regression on genotypes x levels of TDZ for four variables from chili pepper (*C. chinense*) *in vitro* conduced.

S.V.	Mean Square			
	Germination (%)	Callus weight (g)	Callus number	Shoots number
Genotype (G)	0.107^{ns}	0.043^{ns}	0.779^{ns}	3.22^{ns}
TDZ (T)	0.0401^{ns}	0.006*	0.219^{ns}	0.411*
G x T	0.075^{ns}	0.103^{ns}	0.277^{ns}	1.16*
C.V. (%)	24.11%	15.33%	56.51%	45.25%

ns. no significant. *significant at 5 % probability level by F test.

Table 2. Mean comparison of shoot percentage with regarding to levels of TDZ and five genotypes from chili pepper (*Capsicum chinense*) *in vitro* conduced.

Levels of TDZ ($mg.L^{-1}$)	Genotypes				
	02	10	12	13	14
0.0	2.9988 abA	0.9659 aAB	0.7071 aB	1.4029 aAB	0.7071 aB
1.0	3.5327 aA	0.7071 aB	1.2890 aB	2.2483 aAB	1.4142 aAB
1.35	1.8708 abA	1.8113 aA	0.7071 aA	2.5184 aA	0.7071aA
1.7	1.2890 bA	0.7071 aA	1.6283 aA	2.3080 aA	0.7071 aA
2.0	1.2890 bA	0.7071 aA	0.9659 aA	1.7260 aA	2.8682 aA

Mean followed the same lowercase letter in collumn and the same capital letter in line. not differ statistically. at 5% level of probability by Duncan´s test.

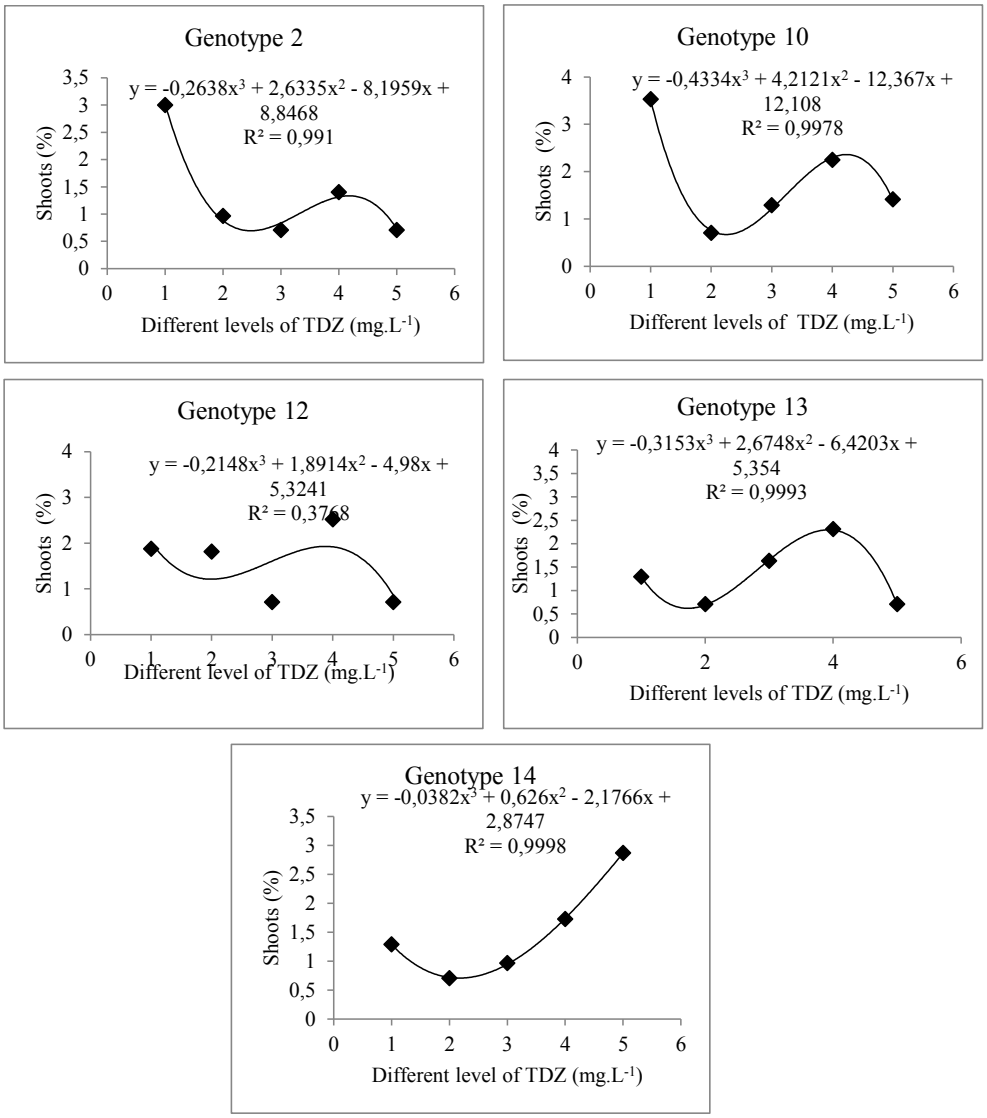

Figure 1. Influence of different levels of TDZ on the different genotypes of *C. chinense.*

Influence of the stage for anther excision in embryogenesis induction from eggplant anther cultures and isolated microspore cultures

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Abstract

In this work we address one aspect of eggplant flower biology potentially involved on the efficiency of anther culture: the selection of the best floral stage to excise anthers for culture. For two different accessions, we determined morphological criteria (length ranges) to identify buds and anthers enriched in vacuolate microspores and young bicellular pollen, the stages most responsive to embryogenesis induction. While these microspore/pollen stages were the most responsive when isolated and cultured in liquid medium, we observed that the culture of younger anthers provided better response. We analyzed eggplant anther walls and found that their particular thickness may be behind this apparent discrepancy, since they may delay the diffusion of inducing factors to the anther locule, reducing their effect over inducible microspores. Thus, the culture of younger anthers would allow for younger microspores to grow up to the inducible stages while nutrients and inducing factors are entering the locule. The practical application of these results may improve the efficiency of anther culture not only in these cultivars, but in others also presenting thick anther walls.

Keywords: Androgenesis, doubled haploid, haploid, *Solanum melongena*

Introduction

Production of androgenic doubled haploid (DH) lines from haploid microspores/pollen constitutes a promising alternative to classical breeding techniques for the generation of pure lines, essential for uniform hybrid seed production. Despite of its convenience, DH technology is still far from being a universal method for the routine production of pure lines.

In general, there are three main factors that prevent many species from being deviated to androgenesis: the genotype, the development stage of the microspore and the culture conditions. These three parameters must be optimized in order to obtain an efficient protocol. In many economically important crops, the lack of information on these three parameters represents a serious drawback to overcome in order to obtain a reliable and efficient method for androgenesis induction and DH generation. This is the case of eggplant (*Solanum melongena* L.).

Eggplant is one of the most important vegetables worldwide. Despite of its relevance, eggplant is already considered a recalcitrant species, since it has been possible to obtain DHs in some cases, but the efficiency is still moderate and the number of inducible cultivars still insufficient. With respect to the three factors above mentioned, it is well known that the genotype plays a key role in the androgenic response. In other hand, several culture conditions have been used to produce DHs through anther culture in different eggplant cultivars. It can also be concluded that among the different experimental conditions used up to now, the most useful in terms of DH yield and applicability to different genotypes are those initially described by Dumas de Vaulx and Chambonnet (1982) for anther culture, although with slight adaptations to particular genotypes. However, the establishment of criteria for the identification of the optimal anthers to be cultured has not been studied with the same level of detail. In this regard, there is a wide consensus about the best stage to deviate the microspore towards embryogenesis, which revolves around the first pollen mitosis. In other words, it is believed that the most responding stages are the vacuolate microspore (VM) and the young bicellular pollen (YBP). In the literature, buds with suitable anthers have been

traditionally identified using loosely defined criteria, mostly visual references that may vary among cultivars, or even among buds of the same individual. According to this, it seems evident the usefulness of defining morphological and easily quantifiable parameters to identify buds with anthers containing microspores at the optimal stage to be cultured and reprogrammed towards embryogenesis. This is the aim of the present work. These results may be directly applied to protocols currently used for anther cultures in virtually all eggplant cultivars, and have the potential to increase their yield in terms of increasing the amount of inducible microspores and of embryos produced from a given batch of donor plants.

Materials and Methods

Plant material

Two accessions of eggplant were used in this work, cv. Bandera and cv. Cristal. For each accession, 18-24 individual plants were grown at the greenhouses, at a minimum of 18ºC under natural light during spring months.

Correlation of bud and anther length with microspore/pollen developmental stages

Bud length was measured with a caliper, from pedicel insertion to the end of the corolla. The locular content of one anther of each bud was observed under a light microscope. Around 400 microspores per anther were counted and staged. The different microspore/pollen developmental stages were identified according to the morphological characterization of microsporogenesis and microgametogenesis described in Seguí-Simarro and Nuez (2005). Seven different stages (tetrads, young, mid and vacuolate microspores, and young, mid and mature pollen) were established based on morphological criteria such as cell size and shape, and number, type and position of the nucleus.

Anther culture and isolated microspore culture

Flower buds were harvested in the greenhouse and transported under melting ice. For anther culture, buds were surface sterilized and immediately dissected. Before plating the anthers, bud and anther lengths were measured. Anthers were cultured according to Dumas de Vaulx and Chambonnet (1982). Once embryos emerged from anthers, they were isolated and cultured individually on dishes or pots with V3 medium for germination and transformation into plantlets. For isolated microspore culture, microspores were isolated from the anther and cultured as described in Corral-Martínez and Seguí-Simarro (2012).

Light microscopy

Anthers of Bandera, Cristal, Ecavi and ANS-3 eggplant cultivars, as well as of the Herminio pepper cultivar were fixed in Karnovsky fixative solution (4% formaldehyde + 5% glutaraldehyde in 0.025 M cacodylate buffer, pH 7), post fixed in 2% OsO_4, dehydrated in ethanol series, embedded in EM-bed 812 resin (Electron Microcopy Sciences) and polymerized at 60ºC for two days. Thin (1 μm) sections were produced with a Leica UC6 ultramicrotome, mounted and observed under phase contrast in a Nikon Eclipse E1000 microscope.

Results and discussion

Determination of morphological parameters to identify the optimal stage for androgenesis induction

For nearly all species, it is known that bud length is directly related with anther length and with the developmental stage of their microspores or pollen grains. As reflected in Figure 1, the use of this morphological parameter may be useful in eggplant as well, since a clear correlation between

bud lengths and stages was evidenced. Then, we studied in a quantitative manner the correlation between bud/anther length and the developmental stage of the microspores/pollen contained in the anther locules. As seen in Figure 1, the developmental stage of the microspores or pollen changed as buds grew in size and matured. Anther length was the most useful parameter to find anthers clearly containing a high percentage of VM+YBP, as it gave more precise results than bud length. For cv. Bandera, anthers from 5.5 to 6.4 mm contained nearly 70% of VM + YBP, with little amount of microspores/pollen in not inducible stages. For cv. Cristal, the anthers from 4.5 to 5.9 mm were in similar stages.

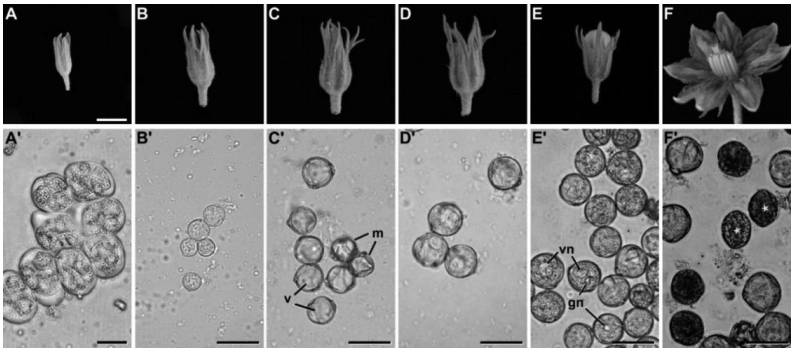

Figure 1. Changes in microspore/pollen contents of anthers with respect to bud size and length, in cv. Bandera. Figures A-F show buds/flowers representative of the ranges considered for this study. A: 8-9 mm; B: 10-11 mm; C: 12-13 mm; D: 14-15 mm; E: 16-17 mm; F: 19 mm and beyond. Figures A'-F' show the stages of microspore/pollen development corresponding to bud/flowers shown in A-F. A': tetrads. B': young microspores. C': mid (m) and vacuolate microspores; note the presence of a central vacuole (v). D': Young bicellular pollen grains. E': young and mid bicellular pollen, with a generative (gn) and a vegetative nucleus (vn). F': mid-late, mature pollen; note the progressive volume loss of maturing grains, becoming very dense, oval-shaped mature pollen grains (asterisks). Bars: A-F: 1 cm; A'-F': 30 µm.

Effect of the stage of anther excision on the embryogenic response in cvs. Bandera and Cristal

We tested the validity of these ranges by culturing anthers from long-styled buds at all developmental stages. For Bandera, nearly 90% of the embryos were produced from anthers between 4.5 and 5.9 mm, an interval characterized by a major presence of young (YM) and mid microspores (MM), being VM+YBP considerably less represented. For Cristal, anthers containing VM and YBP showed really low inductive response, while a high number of embryos arose from anthers containing YM and MM. These results indicated that the embryogenic response is mostly produced in anthers containing a majority of YM and MM at the moment of anther inoculation. Lately, we checked the androgenic competence of YM, MM, VM and YBP when isolated from the anther and cultured in liquid medium. We performed microspore cultures with isolated YM and MM, and in parallel, isolated microspore cultures with VM and YBP under the same experimental conditions. For isolated YM+MM, no response was observed in any of the culture dishes. However, in dishes containing VM and YBP embryogenic divisions, multicellular structures, globular embryos and elongated embryos evidenced the embryogenic response. From these results we deduced VM+YBP were the most responsive microspore/pollen stages, but there must be something that prevented them from showing their highest response when anthers containing them were selected for anther culture.

Histological analysis of eggplant anthers

In order to find an explanation as to why Bandera and Cristal anthers with YM + MM produced more embryos than anthers with VM + YBP, we processed anthers of these cultivars and observed them under the light microscope. The locule of these anthers was surrounded by approximately 10

cell layers (Figura 2A). Measurements of anther walls at the side proximal to the style in 14 Bandera and Cristal anthers containing VM+YBP gave an average total thickness of 248.7±14.08 μm. Similar measurements of anthers of other *S. melongena* cultivars (Ecavi and ANS3) gave similar results (data not shown). Thus, it appears that eggplant anthers are characterized by thick walls, which preclude inducing factors from immediately affecting VM and/or YBP within anthers. Therefore, it can be deduced that the use of anthers containing mostly YM and MM, would allow for these factors to reach the anther locule while YM and MM develop into VM. This hypothesis would be confirmed by the fact that when the physical barrier of anther walls is eliminated and VM+YBP are directly exposed to culture conditions from the very beginning (in isolated microspore culture), these stages are the most responsive. This hypothesis was also confirmed in pepper, whose anther walls are remarkably thinner than in eggplant (figure 2B), and the use of anthers containing VM and/or YBP has been reported to provide good results through both anther culture and isolated microspore culture (González-Melendi et al. 1995, Kim et al. 2004). Similar results involving anther culture in other species (Touraev et al. 2009) also with thinner anther walls, would additionally support that this feature is characteristic of eggplant.

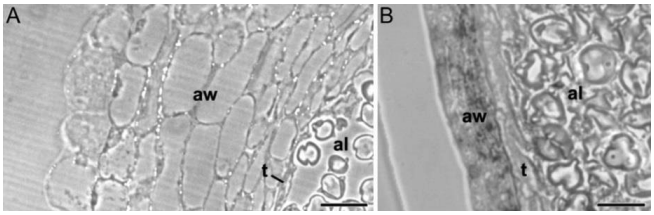

Figure 2. Histological analysis of anthers containing principally VM in (A) eggplant (cv. Bandera) and (B) pepper (cv. Herminio). Note that in eggplant, anther walls (aw) at this developmental stage are considerably thicker than in pepper. t: tapetum; al: anther locule. Bars: 50 μm.

We demonstrated that our hypothesis worked for two eggplant cultivars, increasing their embryo yield. The practical application of the main conclusion of this work for eggplant anther culture may improve the efficiency of this technique not only in the cultivars assayed, but possibly in others, also characterized by thick anther walls.

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Hybrid performance in sweet pepper relative to parental diversity detected by RAPD and ISSR markers

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Abstract

RAPD and ISSR divergence of sweet pepper lines of different ecogeographical origins was evaluated to investigate potentialities of DNA screening for breeding of the best hybrids. Based on biometric and molecular approaches, ten lines with appropriate compound of traits and genetic distances were selected for cyclic (scheme I) and test- (scheme II) crosses. The F_1 hybrids were significantly superior to the parents for the most traits. Heterosis was higher among hybrids of the scheme I. Analysis of the relationship between parental divergence and the F_1 productivity showed the hybrid productive potential of the scheme I was predetermined by ISSR genetic distance (GD) by 86% and by RAPD GD by 69%, whereas the F_1 yield of the scheme II was not related to the genetic distances. Since values of DNA divergence were closely associated with both mid-parent level and hybrid productivity, we assume that some parental DNA fragments of the scheme I belong to heterotic loci, which can be considered potential markers for breeding the best hybrids.

Keywords: sweet pepper, RAPDs, ISSRs, genetic distances, F_1 productivity

Introduction

In hybrid breeding of sweet pepper, as well as of other vegetables, the choice of the initial material is crucial. The selected parental form should have the desired traits and a sufficient level of divergence since, according to the genetic concept of heterosis, heterozygous loci make a major contribution to the heterotic effect (Birchler et al. 2003). However, identification of divergent genotypes among phenotypically similar forms is difficult even by pedigree, because breeding using particular strategies and a limited number of outstanding genotypes led to progressing reduction of the diversity of *Capsicum* genetic pool. Selection of individuals with a high genetic potential, i.e. capable to give a perfect offspring, is one the most expensive and labor-intensive stages of breeding, therefore prediction of the hybrid productivity is the most attractive aspect for improving the efficiency of breeding programs.

Since the 60s of the last century morphological indices, geographical origin information and later biochemical divergence were used for hybrid productivity and heterosis predictions, but their accuracy was low (Moll et al. 1965; Frei et al. 1986). With the development of molecular- genetic methods, DNA polymorphism was considered a measure of genetic diversity and a criterion for parental form selection. Despite the fact that DNA polymorphism evaluation proved useful in some studies (Selvaraj et al. 2010; Becker, Link, 2000), there were no consistent relations between parental diversity and the F_1 heterotic response in others.

Some researchers assume that the total divergence is not an adequate criterion for the mating design, since it shows general differences between individuals and is not associated with traits under selection (Renming et al. 2008). The results obtained by using other types of markers and QTL were also not conclusive (Garcia et al. 2008; Frascaroli et al. 2009).

In this study, we intended to evaluate prospects of using RAPD and ISSR divergence information for the sweet pepper mating design and prediction of the F_1 performance.

Materials and Methods

Plant material, characterization and growing conditions

We have performed a comparative study of 23 stable breeding lines of a different geographical origin. These lines were evaluated in unheated greenhouses for two successive (2010-2011) years after randomized block design (area per plant 35×50cm) in triplicate at the Research Station of the Institute of Genetics and Cytology (Minsk, Belarus). Pepper lines were characterized for some yield components: fruit weight per plant, fruit per plant, one fruit weight, length and thickness, pericarp thickness.

Molecular characterization

To assess genetic divergence and to calculate genetic distances, we used RAPD and ISSR methods, which provide screening of a great number of anonymous DNA fragments. The most effective 21 RAPD (Operon thech. A-, B-, C- sets) and 9 ISSR (UBC set) markers with reproducible patterns were chosen after the preliminary test for estimation of sweet pepper DNA polymorphisms. The genomic DNA was isolated using Fermentas DNA Purification Kit (Germany) from leaves of seedlings. Amplification was performed according to standard protocols.

Data analysis and mating design

The genetic distances (GD) were calculated for all pair combinations of the parents applying the method described by Nei and Li (1979) and then pepper lines were grouped into clusters using UPGMA. Euclidean distances (ED) were calculated through the main yield traits evaluation. Hybridization was performed by cyclic crossing – 9 pair combinations (scheme I) and test crossing – 1×9 (scheme II).

Results and discussions

Genetic characterizations sweet pepper lines and their hybrids

To assess pepper line divergence we considered polymorphisms of anonymous DNA fragments derived from RAPD and ISSR PCR. In total 206 fragments were obtained by 30 markers, with 6.9 fragments per marker. Both types of markers had similar efficiency, but the total quantity of polymorphic loci was low (26.8%), perhaps due to the reduced genetic pool of current sweet pepper diversity. Linkage of RAPD and ISSR data reached 55% (R^2 = 0.55 *), confirming their partial overlapping. ISSR GD significantly (r = 0.56*) correlated with ED, but no significant links were found between RAPD and biometric data.

Based on molecular approaches we have carried out line differentiation and selected 10 of them belonging to the different polymorphic classes. These lines were included in hybridization according to two schemes: I - cyclic crossing, II – test crossing.

The arithmetic mean of molecular (GD) and biometric (ED) distances of parental components of the scheme II exceeded that of the scheme I that was caused by using the most divergent line *L2771* as a tester (Table 1). As a result, GDs variation for the scheme II was lower than for the scheme I. Comparative investigations also demonstrated that there was a great variation among F_1 hybrids in their productivity and heterosis manifestation. The mid- and high- parent heterosis values widely varied for different traits. For the majority of the yield components, F_1 heterosis value of the scheme I was significantly higher that of the scheme II, where divergence was calculated relative to tester *L2771(♀).*

Relationship between GDs and F_1 performance

Correlation studies between genetic distances and hybrid performance have shown that 38% variation in F_1 for fruit weight per plant are the result of ISSR divergence, and 42% are due to

RAPD GD of the parental lines (Table 2). If one keeps in mind the limitations caused by using the best line as a tester and exclude the scheme II from the calculation, the relationships between F_1 productivity and GD increase to 0.83 and 0.93 for RAPD and ISSR GD respectively. Parental divergence also affected the main traits associated with yield (fruits per plant, one fruit weight, length and width), and led to high realization of the F_1 genetic potential also for scheme I.

The predicted ED potential calculated on the basis of morphological traits was low. Interestingly, despite the lack of link between ED and the F_1 performance, the mid-parent level of the main yield components was significantly correlated with that of the F_1 ($r>0.7$) and both DNA-divergence values for the scheme I (Table 2).

Relationship between GDs and F_1 heterosis

The total divergence was positively associated with F_1 heterosis for the main yield components only for the scheme I. The strongest link was found between GD and high- parent heterosis of the fruit weight per plant ($r = 0.51$) and the one fruit weight ($r = 0.57$). For the F_1 scheme II, we revealed only negative correlations between GD and mid-parent heterosis for fruit width ($r = -0.73$) and pericarp thickness ($r = -0.63$) that were interdependent and negatively associated with fruit weight per plant, fruit per plant and fruit length.

RAPD GD potential for predicting HPH for plant yield was higher ($r = 0,57$) in comparison with ISSR GD ($r = 0,3$). The level of the RAPD divergence was strongly and negatively associated with mid-parent heterosis of the pericarp thickness ($r = -0.83$). However, the influence of ISSR polymorphism on mid-parents heterosis of the fruit length reached 62% ($r = 0.79$).

Inconsistency of the results obtained from the two schemes can be explained by their features. Thus, a “divergent row” reproduced in the scheme II has a limited number of the polymorphic (potential heterotic) variant combinations relative to the common tester (L2771), which *per se* has a stable set of the improved genetic determinants. By contrast to this fact, we found closer positive correlations between GDs and heterosis for F_1 of the scheme I that was caused by great diversity of the polymorphic loci pair combinations.

Since GDs were closely associated with both mid-parent yield values and F_1 performance, we assume that some parental DNA fragments of the scheme I belong to heterotic loci, which can be considered as potential markers for breeding the best hybrids.

In general, our results show that for lack of information about the genetic nature of the initial collections, RAPD and ISSR techniques are good tools, which allow identification of the genetic pool diversity and selection of genetically heterogeneous individuals among phenotypically similar forms.

Consequently, the preliminary anonymous DNA screening is a useful addition to the classical genetic approaches, because it can increase the *Capsicum annuum L.* selection efficiency by better detection of the promising parental components.

Table 1. Hybrid characteristics: genetic distances assessed by three methods, high- parents (HPH) and mid- parents (MPH) heterosis

	F_1 combinations ♀ × ♂		*Total GD*	*ISSR GD*	*RAPD GD*	*ED*	Fruit weight per plant, %		Fruits per plant		Mean fruit weight	
							HPH	*MPH*	*HPH*	*MPH*	*HPH*	*MPH*
	L2771 ×	*L2772*	8,97	6,17	12,50	385	65*	88*	22	46*	17	24*
	L2772 ×	*L2316*	2,78	3,70	1,58	698	48*	107*	15	91*	-	-16
Scheme I	*L2316* ×	*L2773*	2,86	1,23	4,92	360	78*	124*	17	66*	16	32*
Scheme I	*L2773* ×	*L2774*	4,89	1,27	9,37	98	88*	98*	79*	85*	6	8
Scheme I	*L2774* ×	*L2319*	3,45	1,27	6,06	48	42	45	27	37*	1	6
Scheme I	*L2319* ×	*L2775*	6,29	2,56	10,77	159	67*	79*	38*	64*	-	-1
Scheme I	*L2775* ×	*L2777*	6,94	3,80	10,77	102	73*	74*	19	48*	-	6
Scheme I	*L2777* ×	*L2323*	1,49	0	3,57	616	26	70*	19	51*	6	16
Scheme I	*L2323* ×	*L2771*	10,08	6,33	14,75	1011	71*	152*	-	48*	32*	50*
	means						62,0*	93,0*	26.2*	59.6*	8.7*	13.9*
Scheme II	*L2771* ×	*L2772*	8,97	6,173	12,5	385	65*	88*	22	46*	17*	24*
Scheme II	*L2771* ×	*L2316*	9,09	7,50	11,1	1080	-	47	-	46*	-	-32*
Scheme II	*L2771* ×	*L2773*	9,22	6,33	12,90	720	26	63*	-	15	-	19*
Scheme II	*L2771* ×	*L2774*	9,59	7,50	12,12	624	58*	96*	3	56*	-	4
Scheme II	*L2771* ×	*L2319*	9,09	6,33	12,5	577	24	51*	-	24	-	8
Scheme II	*L2771* ×	*L2775*	8,33	6,33	10,77	419	51*	74*	-	27	9	25*
Scheme II	*L2771* ×	*L2776*	8,33	5,0	12,50	431	61*	85*	-	36	-	7
Scheme II	*L2771* ×	*L2777*	10,14	6,33	15,25	400	15	32	-	9	-	9
Scheme II	*L2771* ×	*L2323*	10,08	6,33	14,75	1011	49	119*	1	61*	6	21*
	means						38,8*	72,8*	2.9	35.6*	3.6	9.4

Table 2. Correlations between distances assessed by three methods, mid-parent values and F_1 performance (**P<0,05; **P<0,01)*

Method, Hybrid group \ Characters		fruits weight per plant	fruits per plant	mean fruit characters		
				weight	length	width
total GD	All hybrids	0.68**	0.75**	-0,54*	0,75**	-0,81**
	F_1 scheme I	0.95**	0.76*	-0,33	0,66	-0,67*
	F_1 scheme II	-0.29	-0,10	-0,30	0.40	-0,53
ISSR GD	All hybrids	0,62**	0,79**	-0,64**	0,79**	-0,86**
	F_1 scheme I	0,93**	0,87**	-0,46	0,87**	-0,78*
	F_1 scheme II	-0,43	0,00	-0,67*	-0,13	-0,45
RAPD GD	All hybrids	0,65**	0,63**	-0,40	0,63**	-0,68**
	F_1 scheme I	0,83**	0,60	-0,20	0,45	-0,51
	F_1 scheme II	-0,06	-0,14	0,11	0,47	-0,27
ED	All hybrids	0,19	0,35	-0,39	0,54*	-0,38
	F_1 scheme I	0,43	0,37	-0,20	0,56	-0,19
	F_1 scheme II	-0,47	-0,22	-0,43	0,17	-0,17
x (P_1;P_2)	All hybrids	0,67**	0,79**	0,67**	0,91**	0,78**
	F_1 scheme I	0,74*	0,74*	0,71*	0,97**	0,84*
	F_1 scheme II	0,47	0,56	0,15	0,52	-0,03

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Plant regeneration from isolated anther/microspore culture of sweet pepper (*Capsicum annuum* L. cv.'Zdorovie')

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Abstract

A protocol previously developed for hot pepper shed-microspore culture (Supena et al. 2006) was modified to produce embryos from sweet pepper (*C. annuum* L., cv.'Zdorovie'). Bud size, media constituents, incubation time and temperature of bud's pretreatment were examined. The medium contained Nitsch components, glutathione 30 mg/l and 2% maltose, with 1% activated charcoal in the solid under layer. Cultivar Zdorovie produced two to four plants per original flower bud.

Keywords: *Capsicum annuum* L., sweet pepper, anther/microspore culture, doubled haploid (DHs) plants

Introduction

Pepper (*Capsicum annuum* L.) becomes one of the popular crops in Russia**.** This raises a point of development of new varieties and hybrids suitable for growing in climate condition of Russia. Production of pure lines takes a few years when classical inbreeding and selection techniques are used. An alternative way is to develop homozygous (doubled haploid) lines through biotechnological *in vitro* methods such as anther, microspore culture and culture of unpollinated ovules.

The first reports of pepper haploid plant regeneration from anther culture date back to 1973 (Wang et al. 1973; Kuo et al. 1973; George and Narayanaswamy, 1973). At present, refined protocols have been developed to produce DHs from hot pepper anther and isolated microspores (Supena et al. 2006; Nowaczyk et al. 2006; Kim et al. 2008).

The anther/microspore culture is affected by numerous factors such as genotype, donor plant growing conditions, microspore development stage, flower buds or anther pretreatment, composition of culture medium, and incubation conditions. There is no universal protocol for induction of microspore embryogenesis in pepper because of differences among genotypes within the *Capsicum* species in terms of embryogenesis response.

In the present study, the basis protocol of Supena et al. (2006) developed for hot pepper was modified and adapted to particular cultivar 'Zdorovie' to produce doubled haploid plants of sweet pepper.

Materials and Methods

The cultivar 'Zdorovie' of sweet pepper *C. annuum* L. was grown in the climatic chamber with a 16 h photoperiod, 10.000 lux, and temperature 24-25^{o}C. Flower buds of the desired size (petals equal or slightly longer than sepals) were harvested in the morning, placed in Petri dishes on a wet filter paper and incubated at 10^{o}C for 1-7 days. The flower buds were then disinfected for 10 min in 5% NaOCl with 0.05% (v/v) Tween-20 added, 30 sec in 70% ethanol, and rinsed three times in

sterile water. Differential staining (Alexander, 1969) showed that these anthers contained more than 50% of microspores in the late unicellular stage.

Pepper anther culture was according to Supena et al. (2006) with modifications. A double-layered medium was used; both layers consisted of Nitsch components (Nitsch and Nitsch, 1969) with 2% maltose and 30mg/l glutathione. The under layer had 1% activated charcoal and was solidified with 0.3% Phytagel. Media for under layer was sterilized by autoclaving, prior to which the pH was adjusted to 5.8; liquid media for upper layer was sterilized by filtration. In the experiment with the growth regulators (Tab.1), sterilized aliquots were added in the liquid upper layer of medium. Cultures were performed in baby food culture jars with plastic caps with approximately 1.0 cm solid medium and 1.5 cm liquid top layer. Cultures were kept at 28^{o}C, always in continuous darkness during 2 months.

The embryos at globular to heart-shape and torpedo-shape stages were transferred into B-5 medium (Gamborg et al. 1968) contained 2% sucrose, 1.0 g/L activated charcoal, 0.1 mg/L GA, and 3.0 g/L Phytagel and maintained at 25^{o}C, 14 h photoperiod with about 2.500 lux. The seedlings showing cotyledons and a first true leaf were transferred into plastic jars with half strength MS medium supplemented with 2% sucrose, 1.0 g/L activated charcoal, and 3.0 g/L Phytagel. Seedlings that had formed four to six leaves and well developed roots were transferred to soil.

The ploidy level of regenerated plants was determined by indirect indicators such as pollen fertility, size of pollen grain, width of leaf blade, length of internodes, and chloroplasts number in the stomata guard cells (Pausheva, 1988).

For chromosome doubling, apical meristems of the haploid plants were treated by 0.5% colchicine with drop of Tween-20 added per each 100 mL of solution.

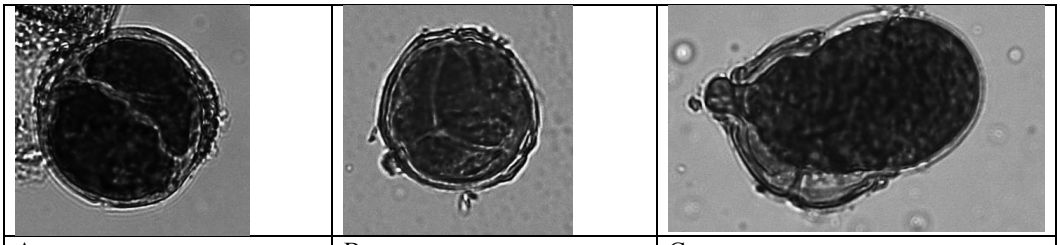
Results and Discussion

Selection of correct stage of microspore development is a one of the most critical factor for haploid production. In our experiments, embryos were formed only from late vacuolated microspores with a large diffuse nucleus situated laterally close to the wall. Age of donor plants was also important. Embryo yield was higher when flower buds were harvested from young (5-10 weeks) donor plants of pepper. Embryo production decreased with the age of donor plants. It was found that embryo yield did not depend on the duration (1, 2, and 3 days) of flower buds pretreatment at 10 ^{o}C, but an extended exposure for 5 days or more decreased the number of developed embryos. Cytological analysis showed that development of embryos occurs after equal (symmetrical) division of microspore into two cells as well as after unequal division when the one of the cells is smaller than another (Fig.1)

The effect of the addition of zeatin (2.5 μM), IAA (5 μM), TDZ (0.1 mg/L), BAP (1 mg/L), 2,4-D (1 mg/L), kinetin (2 mg/L), and epibrassinolide (0.01 μM) to the liquid upper layer was studied. Embryo formation was completely failed with addition of the most studied growth regulators with the exception of the combination of zeatin and IAA resulted in formation of mostly abnormal-looking embryos (Tab.1). Lack of a functional, zygotic-like shoot apical meristem in microspore-derived embryos was observed (Fig. 2B). The presence of anatomical and functional abnormalities in microspore embryogenesis is reported to be main problem in embryo development in pepper (Supena, 2006; Kim et al. 2008; Parra-Vega et al. 2010). So, in contrast to earlier findings (Supena et al. 2006), we found improved embryogenesis without exogenous growth regulators (Tab. 1, Fig. 2A).

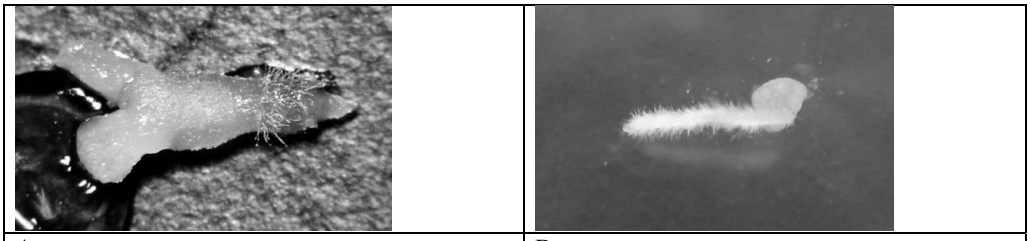
Table 1. Effect of the combined addition of growth regulators in the liquid upper layer medium on embryo yield in anther/microspore culture of sweet pepper *C. annuum* L.**,** cv. 'Zdorovie' with pretreatment of flower buds at 10°C for 2 days.

Combination of growth regulators	Total No of embryos/100 anthers	No of normal-looking embryos/100 anthers
Culture without exogenous growth regulators	58,6	49,5
2.5 µM zeatin + 5 µM IAA	30,5	2,7
0,01 µM epibrassinolide	0	0
5 µM IAA + 1 mg/L BAP	0	0
5 µM IAA + 2.5 µM zeatin	0	0
5 µM IAA + 2 mg/L kinetin	0	0
1 mg/L 2,4-D + 2.5 µM zeatin	0	0
0.1 mg/L TDZ	0	0



A B C

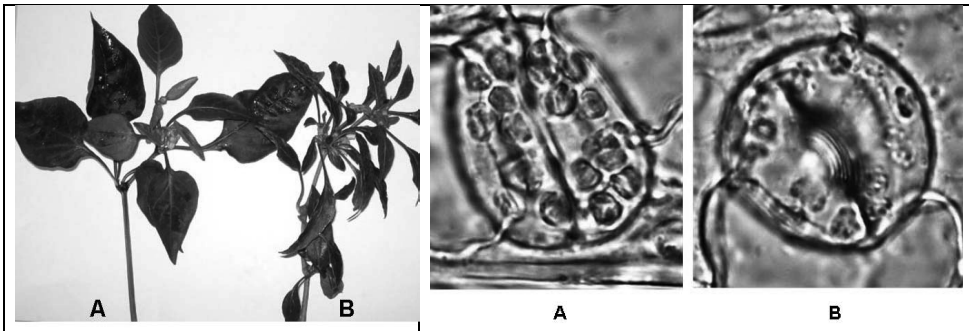
Figure 1. Dividing of embryogenic microspore of pepper. A - symmetrical division. B - asymmetrical division. C - release of embryo from microspore cell wall.



A B

Figure 2. Normal (A) and abnormal (B) microspore derived embryos in anther/microspore culture of sweet pepper cv. 'Zdorovie'.

Based on indirect traits (pollen fertility, size of pollen grain, width of leaf blade, length of internodes, and chloroplasts number in the stomata guard cells), the ploidy level of 80 pepper plants regenerated from microspore-derived embryos was determined. The majority of the plants were haploids (Fig. 3B). Two plants were spontaneous double haploids (Fig. 3A). One polyploid plant had unusually large fertile pollen and wider leaf blade. Treatment of apical meristems of regenerated plants by 0.5% colchicine increased the number of doubled haploid plants by 20%.



I II

Figure 3. Determination of ploidy level of plants regenerated from microspore derived embryos by morphological traits (I) and chloroplasts number in the stomata guard cells (II).
A - spontaneous double haploid plant. B – haploid plant.

In this study, the shed-microspore culture developed for Asian hot pepper (Supena et al. 2006) was modified and adapted to sweet pepper cv. 'Zdorovie' and so has proved efficient for European pepper types. Doubled haploid plants regenerated from microspore-derived embryos were obtained. The selfed progeny of these plants were included in breeding program of sweet pepper in Russia as an initial breeding material for development of new hybrids and cultivars.

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Preliminary analysis of genes related to heat stress in leaves of eggplant SSH-cDNA libraries

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Abstract

In this study, suppression subtractive hybridizations were carried out to study the gene expression of eggplants under heat-stressed treatment. A forward and a reverse suppression subtractive libraries were constructed. The forward library contained 2412 clones with 1793 positive clones related to heat stress (74.34%). The insert clones size were between 250-1500bp. The reverse library contained 1532 clones, including 492 false positive clones; the positive rate was 67.89%, quality parameters of the constructed libraries shows good for further sequence analysis. Through random sequencing, a total of 528 non-redundant genes showed to be putatively involved in the heat stress response reaction. A comprehensive database of biological information on heat resistant related sequences of eggplant has also been constructed. By means of biological information systems and multiple calibration correction, the function of sequenced genes were speculated, including the stress response, metabolic repair and membrane ion transport , which might be directly related to heat stress response. This provides the alternative reference information for the further validation of the functions of target genes. Furthermore, by fluorescence quantitative expression validation analysis, the expressions of *YP16, YP61, YP248, YP51, YP175* and *YP266* were confirmed to be up regulated in heat-stressed eggplant; while the expressions of *YN38, YN30* were confirmed to be down-regulated in heat-stressed eggplant. The analysis of the speculated proteins encoded by these candidate sequences, allow to preliminary inferred the heat resistant of eggplant related to enhancement of calcium signaling, nitrogen metabolism, abscisic acid, and ethylene pathway and accompanied by the generation of all kinds of heat shock protein. And inhibition of chitinase in carbohydrate metabolism and chlorophyll protein in the PS Ⅱ system were also involved in the reaction process.

Keywords: eggplant (*Solanum melongena* L.); heat-stress; SSH-cDNA; genes

Introduction

Plants adapted to abiotic stresses by multi-inducible gene expression. In a long evolutionary process, some anti-adversity plants formed an extremely complex set of resistance mechanism, and accumulated a wealth of anti-adversity genes. Therefore, building a plant cDNA library under abiotic stresses and analyzing stress resistance-related genes and their expression play an important role on the study of plant resistance related mechanisms.

In this study, eggplant heat stress related SSH-cDNA libraries were constructed. The ESTs were analyzed for redundancies and clustered and further quantitative expressions of partly heat stress related genes were analysed in *vivo*.

Materials and Methods

Plant Material and Heat Stress Treatment

Eggplant cv. 896 was selected from Chongqing Academy of Agricultural Sciences. At the 4-leaf stage, 80 seedlings were selected, moved to a growth chamber and grown under a 12h photoperiod with an irradiance of 400 μmol • m^{-2} • s^{-1} at 20/28℃ (day/night) for one day. The test seedlings were

then divided into heat-stressed treatment group and control group. The heat-stressed treatment group was grown under a 14h photoperiod at 43/38℃ (day/night) for periods of 0.5h, 1h, 3h, 6h, 12h, 24h, 48h, 96h. The relative humidity was kept about 70-80% in the growth chamber. For each treatment, three replications were used. The young leaves from each seedling were clipped and frozen in liquid nitrogen and then stored in a -70℃ ultrafreezer.

Construction of SSH Libraries

Total RNA was isolated from leaves from different heat-stressed treatment groups and the control group using Trizol kit (Invitrogen, Carlsbad, CA, USA). The heat stress SSH-cDNA library of eggplant was built by SMART PCR cDNA Synthesis Kit and PCR-select^{TM} cDNA Subtraction Kit (Clontech).

PCR Screening of cDNA Inserts

The second purified PCR products were ligated to the vector pMD18-T, and then transformed into *E.coli* DH5α. PCR primers from kit were used for detecting the inserted fragments. Clones showed either low or no differences in band intensities were considered as false-positive.

DNA Sequence Analysis

The positive clones were sequenced by the Beijing Ding Guo Biotechnology Co., Ltd. Repeated and too short sequences were removed from ESTs data. Then, cluster and pharp have been used to assemble the Unigenes. Using NCBI NT, NR, GO these Unigenes have been annotated and classified; the E value of unigenes less than the threshold 1e-5 were considered valid.

RT-qPCR and Quantification of RNA Levels

Eight unigenes were chosen for expression validation analysis. The quantitative expression analysis of YP16, YP61, YP248 were commissioned to Beijing Ding Guo Biotechnology Co. Lt, using the external standard curve on ABI 7700 real-time fluorescent quantitative PCR instrument. The quantitative expression analysis of remaining sequences were done in our laboratory, using Livak Method (2-ΔΔCt) on C1000 Thermal Cycler (Bio-Rad), primers were commissioned BGI synthesis. The amplification conditions were: 95 ° C for 3 min (1 cycle); 95 ° C, 20 s; the Tm 20 s; 72 ° C for 20 s (35 cycles).

Table1. The list of qPCR primers and information of selected unigenes

Clone ID	Homology sequence	Accession no.	TM (° C)	Primers（5'to 3')
YP16	Small heat shock protein, chloroplastic;	sp|Q95661.1|	64	F:TGTCTCTCCTTTTGGACTGTTA R:TCCTTCTTGTGTTCGCCTTTTA
YP61	carbonic anhydrase	emb|CAH60891.1|	63	F:GTGCCTGTGGAGGTATCA R:CCCTTCAATGCCAATGTT
YP248	Calcium-dependent protein kinase	sp|P28583|	64	F:TGAAAAGAGGAACAACCC R:CAGCCAGAACAATGAGAG
YP175	protein phosphatase 2C ABI2 homolog	dbj|BAI39595.1|	65	F:ATACTCTGCTGCTGCTTGTG R:ACGGTTTGTGGGATGTGATG
YP266	EIN3-like protein	dbj|BAC99307.1|	64	F:CCACCTCCCTTTGATCTATTT R:TTTGGCTTTTGTTTCCTTGTA
YP51	chloroplast heat shock protein 70-2	gb|ABZ04081.1|	63	F:TCAACCACCAAAACCTTCTT R:CTGGACCCTGCTGTCATTAC
YN30	Chlorophyll a-b binding protein 4, chloroplastic	sp|P14278.1|	65	F:CCTCCACCAACTCTGTATCCT R:AAACCCAAACCTTGTTCACGC
YN38	Endochitinase B	sp|P24091.1|	63	F:AATACAACATTGCCTACCTCC R:GTCCCTAACAAATTCTCGCTC
B-actin			63	F:CCCAAGGCCAACAGAGAGAA R:CCAGCAAGGTCCAAACGAAG

Results

Indentification the Differentially Expressed Gene Transcripts in Heat-Stressed Eggplant by SSH

Forward and reverse SSH libraries of heat-stressed eggplant leaves were constructed. The forward library contained 2412 clones, 1793 positive clones related to heat stress (positive rate = 74.34%). The insert clones size were between 250-1500bp. The reverse library contained 1532 clones, including 492 false positive clones (positive rate = 67.89%), quality parameters of the constructed libraries shows good for further sequence analysis.

Functional Analysis and Charaterization of the SSH Libraries

In total, 900 clones were randomly selected for sequencing. After clustering and assembly, 335 unigenes in the forward SSH library and 193 unigenes in the reverse SSH library were obtained.

In the forward SSH library, 41 unigenes showed 139 KO terms in KEGG analysis,146 unigenes showed 176 unrepeated GO terms in Gene ontology analysis and 23 unigenes have unknown functions. Fourteen, 100 and 62 unrepeated GO terms belonged respectively to "Cellular Component", "Molecular Function" and "Biological Process"; most unigenes involved in "Metabolic process", "Cellular process" and "Response to stimulus".

In the reverse SSH library, 31 unigenes showed 127 KO terms in KEGG analysis, 81 unigenes showed 114 unrepeated GO terms in Gene ontology analysis and 21 unigenes have unknown functions. Ten, 62 and 42 unrepeated GO terms belonged respectively to "Cellular Component", "Molecular Function" and "Biological Process"; most unigenes showed "Binding" function, "Catalytic activity", "Transporter activity".

Confirmation of Heat-stressed Differential Gene Expression

Eight unigenes were selected for RT-qPCR analysis. Two genes (YP16, YP51) for stress, three genes (YP61, YN30, YN38) for metabolism, three genes (YP175, YP266, YP248) for signal transduction. RT-qPCR analyses of 896 leaves subjected to heat treatment from 0 to 72h are shown (Fig1,2), all data are normalized to RNA levels of housekeeping gene β-actin.YP16, YP61, YP248, YP51, YP175, YP266 were selected from forward library and their expression were all up-regulated. YN38, YN30 were from reverse library and have shown to be down-regulated in the treatment.

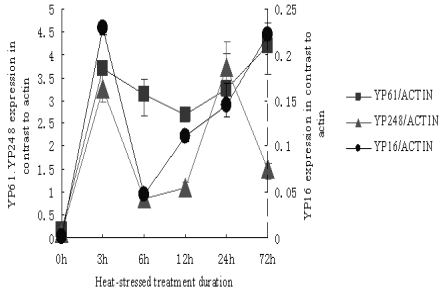

Fig 1 qPCR analysis of positive expression unigenes *Y16*、*Y61*、*Y248*

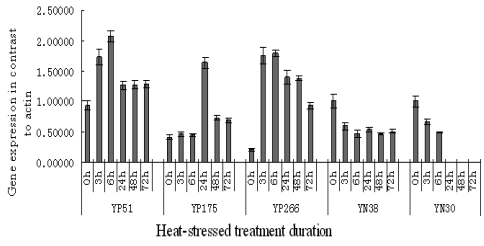

Fig. 2 qPCR analysis of positive expression unigenes *YP51*、*YP175*、*YP266*、*YN38*、*YN30*。

Discussion

The preliminary inference of this study, according to the speculated proteins encoding by selected unigenes, is that in eggplant the heat-stress might enhance the expression of genes in calcium signaling, nitrogen metabolism, abscisic acid signaling, ethylene signaling and accompanied by the generation of various kinds of heat-shocked proteins. While it also might effect the carbohydrate metabolism and PS Ⅱ system by inhibition the expression of chitinase and Chlorophyll a-b binding protein.

Evidences have been previously presented for links between calcium signaling and thermotolerance in maize (Gong et al. 1997). Ron et al. (2011) have presented a schematic model for temperature sensing in plants; in this model, the membrane fludity, lipid rafts, activation of ion channels (Ca^{2+}), signal transduction events and plant metabolism altered in a process called acclimation may serve as the primary heat sensing mechanism of plants (Ron et al., 2011). So the calcium signaling might play an important role in the heat resistance of plants. In this study, a Calcium-dependent protein kinase resulted to be involved in the reaction of heat-stressed eggplant; showing two expression peaks in the heat-stressed process, one of them detected in the early

treatment. This expression peak might originate by the inward flux of calcium or phosphorylation of the key transcriptional regulator of basal thermotolerance.

The heat stressed treatment seems to elicit a complex network of molecular sensors located in different cell compartments. The triggering of one pathway might require for the activation of the others. There might be connections among calcium signaling, abscisic acid signaling, ethylene signaling and other pathways in the heat stressed process of eggplant. More evidences need to be found to clear the mechanism of the thermotolerance of eggplant.

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Cloning and characterization of a methionine sulfoxide reductase-encoding gene from eggplant (*Solanum melongena* L.)

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Abstract

This study aimed to identify eggplant parthenocarpic genes and their molecular mechanism. A specific EST (expressed sequence tag) sequence (Z569) sharing high homology with the MsrA gene from an eggplant parthenocarpic suppression subtractive hybridization library (SSH library) was identified. A full length cDNA (934 bp) was cloned by rapid amplification of cDNA ends (RACE) using RNA extracted from parthenocarpic fruit of eggplant (*Solanum melongena* L.) line D-10. The cloned gene (SmMsrA) contained a 600 bp open reading frame (ORF) encoding a predicted protein (199 amino acids) exhibiting high homology with methionine sulfoxide reductase A (MsrA) proteins and containing a conserved GCFWG sequence. RT-qPCR analysis revealed SmMsrA expression throughout fruit development in parthenocarpic (line D-10) and non-parthenocarpic (line 03-2) eggplants. Highest gene expression levels were detected at anthesis in parthenocarpic ovaries under sub-optimal temperatures, indicating that high SmMsrA expression is linked to parthenocarpic fruit formation.

Keywords: *Solanum melongena* L.; parthenocarpy; gene cloning; methionine sulfoxide reductase; expression analysis.

Introduction

The phenomenon of parthenocarpy involves seedless fruit formation within the ovary in the absence of fertilization. This is a desirable agronomic trait in horticultural crops for improving fruit quality and environmental adaptation in addition to reducing cultivation costs (Yao et al. 2001). Consequently, cloning of parthenocarpic genes for investigation of the mechanism of action may be a significant focus of research into breeding of parthenocarpic plant varieties.

Methionine (Met) is an important sulfur-containing amino acid, which is the first residue to be translated during the process of protein synthesis in eukaryotes. Met is readily oxidized by reactive oxygen species (ROS) to form Met-R, S-sulfoxide (Weissbach et al. 2005), which can result in reduction or abolition of the biological activity of the protein. Met-R, S-sulfoxide is restored to Met by the activity of methionine sulfoxide reductase A and methionine sulfoxide reductase B, with concomitant recovery of protein function (Moskovitz et al. 1996). Furthermore, methionine sulfoxide reductase has been shown to exert effects on fruit development (DellaPenna et al. 1989) and abiotic stress tolerance (Romero et al. 2004).

In this study, a specific EST sequence (Z569) sharing high homology with the *MsrA* gene was identified from a SSH library, and full length cDNA of the methionine sulfoxide reductase A gene (*SmMsrA*) was cloned and characterized.

Materials and Methods

Plant material and tissue sampling

The eggplant parthenocarpic D-10 line exhibits cold resistance. Normal growth and development of parthenocarpic fruit occurs when the temperature daily minimum temperature ranges from 7 °C to 15 °C during anthesis and fruiting periods. Fruits produce seeds when the temperature is appropriate. The non-parthenocarpic 03-2 line does not grow normally when the daily minimum temperature ranges from 7 °C to 15 °C during anthesis and fruiting periods (Liu et al. 2005).

Materials were planted in open field in the spring 2009. The fruit of line D-10 expressed parthenocarpic characteristics and produced seedless fruit when temperature was low (daily minimum temperature ranging from 9 °C to 15 °C), while non-parthenocarpic fruit did not inflate. Regular seeded fruit was generated from the two lines when the temperature was appropriate (daily minimum temperature ranging from 16 °C to 21 °C). Ovaries and fruit from the two lines were harvested at four time points (7 days before anthesis, the point of anthesis, 7 days post-anthesis and 20 days post-anthesis), snap-frozen using liquid nitrogen and stored at -80 °C prior to analysis.

cDNA cloning of the SmMsrA gene and sequence analysis

The *SmMsrA* gene was cloned using the SMARTer™ RACE cDNA Amplification Kit (Clontech Laboratories Inc., Mountain View, CA, USA). Total RNA extracted from the seedless fruit of the first inflorescence of the D-10 at 7 days post-anthesis was used as initial template. Primers for amplification of the SmMsrA gene were designed according to the specific EST sequence Z569 obtained from an eggplant parthenocarpic SSH library as follows: forward:5'-CAGGCTCAACTAGCAAGGGAATC-3, reverse: 5'- CCGTAGCACCTTATTGGGTCATT-3'.

The forward primer and the universal primer A mix (UPM) supplied within the kit were used for amplification of the 3' sequence of the SmMsrA gene. The reverse primer and the UPM were used for amplification of the 5' end sequence. Amplifications were performed by running the following program: 94 °C for 30 s and 72 °C for 3 min, 5 cycles; 94 °C for 30 s, 70 °C for 30 s, and 72 °C for 3 min, 5 cycles; 94 °C for 30 s, 68 °C for 30 s, and 72 °C for 3 min, 25 cycles. The full-length SmMsrA gene cDNA was obtained by alignment and splicing of the 5' and 3' PCR sequences. The primers SmMsrA-5' (5'- ATGGCTTCCAAAGAAGAGG -3') and SmMsrA-3' (5'-TCAACCGTAGCACCTTATTG -3') were derived from the sequence of the full-length cDNA allowing the amplification of the open reading frame. Amplifications were performed under the following condition: 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 2 min, 30 cycles.

SmMsrA gene sequence was analyzed through NCBI ORF Finder for predicted protein sequence and by means of BLAST search of NCBI database for predicted protein sequence conserved protein domains。

SmMsrA gene expression analysis

First-strand cDNA was synthesized from total RNA (5 µg) extracted from the fruits of the two lines (D-10 and 03-2) in low and high temperature at four time points using the SuperScript® III First-Strand Synthesis System for RT-PCR Kit (Invitrogen Corp., Carlsbad, CA, USA). Primers qforward (5'- CTGAAGAGTATCACCAGCAA -3') and qreverse (5'-ATGTCAACCGTAGCACCT -3') were derived from the sequence of the full-length *SmMsrA* gene cDNA. Ubiquitin served as a reference gene (Cooper et al. 2004) and was amplified using the ubi-forward (5'- GTGTGGGCTCACCTACGTTT -3') and the ubi-reverse (5'-ACAATCCCAAGGGTTGTCAC -3') primers. All PCRs were performed in technical triplicate. The reaction system contained: 8 µl template, 1 µl forward primer, 1 µl reverse primer, 10 µl SYBR Green Master Mix. PCRs were performed under the following condition:, 95 °C for 10 s, 57 °C for 20 s, 72 °C for 30 s, 45 cycles.

Results and Discussion

cDNA cloning and analysis of SmMsrA gene

A 650 bp 5' and a 400 bp 3' RACE sequence of *SmMsrA* gene was amplified. The 5' and 3'RACE sequences were aligned to obtain a 934 bp contig containing a 63 bp 5'-UTR, a 600 bp open reading frame (ORF) and a 271 bp 3'-UTR (NCBI accession number JN663890). The ORF encoded a 199 amino acid polypeptide containing conserved amino acids (GCFWG), whose mutation influences the activity of *MsrA* (Moskovitz et al. 2000). Blast searches demonstrated that the protein encoded by this MsrA gene exhibited high homology with similar proteins derived from tomato (Cordes et al. 1989), cotton (Zhao et al. 2003), *Arabidopsis thaliana* (Bechtold et al. 2004) and poplar (Rouhier et al. 2007). Expression analysis of the *SmMsrA* gene in parthenocarpic and non-parthenocarpic fruit

Real-time PCR analysis indicated differences in *SmMsrA* gene expression in parthenocarpic D-10 line fruit and non-parthenocarpic 03-2 line fruit. (Figure 1). The highest expression level of the *SmMsrA* gene was observed at the point of anthesis in all kind fruits. High levels of ROS are produced at the point of anthesis, during ovary inflation, pollen germination and pollen tube elongation. We suggest that this period requires abundant MsrA for elimination of ROS.

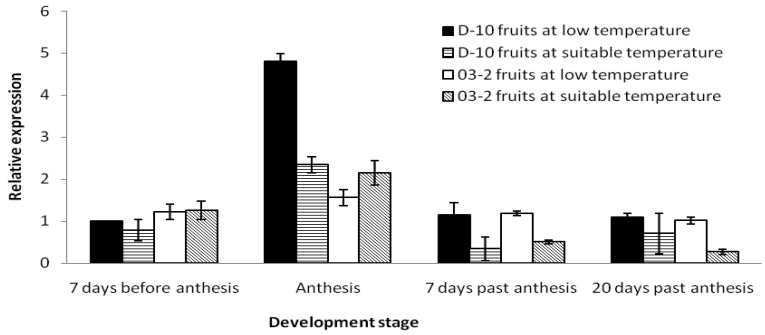

Figure 1. Relative gene expression of *SmMsrA* in parthenocarpic and non-parthenocarpic fruits.

On the day of anthesis, *SmMsrA* expression in fruits of D-10 line at low temperature, was 2-fold higher than in fruit of D-10 line and 03-2 line at suitable temperature, and 3.08-fold higher than in fruit of the 03-2 line at low temperature. It has been reported that abundant ROS and methionine sulfoxide are produced in inflating fruits (Sadanandom et al. 2000) and under conditions of low temperature stress (Berlett & Stadtman, 1997), resulting in cellular dysregulation and tissue damage. Oxidation resistance may be enhanced by increasing *SmMsrA* expression through elimination of the ROS generated in inflating fruits and in response to low temperature stress, thus protecting key proteins involved in the process of fruit inflation and allowing normal growth and development. Fruit development of the non-parthenocarpic line was inhibited by the inability to eliminate ROS induced by low temperature stress. These data indicate that *SmMsrA* is involved in fruit development of parthenocarpic eggplants.

Methionine is a precursor of S-adenosine-L-methionine (SAM) (Ravanel et al. 1998) which plays an important role in polyamine biosynthesis. Therefore, SmMsrA is a key enzyme involved this process. Studies have indicated that parthenocarpic characteristics in cucumber are related to the polyamine content (Chen et al. 2005). The content of endogenous polyamines was higher in parthenocarpic cucumber ovaries than in non-parthenocarpic lines (Chen et al. 2005). At the point of anthesis, *SmMsrA* expression was higher in the parthenocarpic fruit of D-10 line than that in the

non-parthenocarpic 03-2 line at low temperature(Figure1). Thus, it can be speculated that parthenocarpic characteristics in eggplant are related to polyamine content regulated by *SmMsrA*.

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SESSION III

Genetic resources

Evaluation and characterization of chilli (*Capsicum annuum* L.) germplasm lines of North East India

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Abstract

A total of 51 germplasm lines collected from different locations of Assam and North East India were evaluated and characterized during rabi seasons of 2008-09 and 2009-10 at the Horticulture Experimental Farm of the Assam Agricultural University, Jorhat. The germplasm materials included Krishna Jalakia, Suryyamukhi and LCA 334 as check varieties. The experiment was laid out in a randomized block design with 3 replications. All recommended package of practices were followed to raise the crop. The germplasm accessions were evaluated for yield and component characters. Characterization was done for most of the qualitative characters. Among the germplasm accessions, 10/KS-3 exhibited highest fruit yield of 370 g per plant. It was followed by 10/KS-4 with an average fruit yield of 360 g per plant. Ch 09/19-1 stood 3 rd position with an average fruit yield of 350 g per plant. All these 3 lines were significantly superior to the best check variety LCA 334. They were pure line derivatives of Krishna Jalakia with single or two gene differences. The former two lines were having short, medium thick, black fruits with pointed apex. 10/KS-3 had purple coloured leaves with upright fruits whereas 10/KS-4 had black leaves with hanging fruits. Ch 09/19-1 had heavy and large fruits. All these lines were having moderate pungency. They may be promoted to develop as new varieties or may be used as parent in the crossing programme

Keywords : Evaluation, Characterization, *Capsicum annuum,* Germplasm, NE India

Effects of chemical reagents on seed germination of two wild eggplant rootstocks

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Abstract

The effects of 15 chemical reagents on seed germination of two wild eggplant rootstocks (*Solanum Torvum* and *Solanum capsicoides Allioni*) were studied in cultured dishes. The results showed that the germination vigor and vigor index of *Solanum Torvum* were increased obviously by treating the seeds with 15 mg/ml potassium nitrate and 1mg/ml Gibberellin 3; by using 1 mg/ml Gibberellin 3 better results were obtained compared to 15 mg/ml potassium nitrate. While the germination vigor and vigor index of wild *Solanum capsicoides Allioni* were increased obviously by treating the seeds with 2 mg/ml Gibberellin 3, 5 mg/ml potassium nitrate, 2 mg/ml penicillin and 0.1 mg/ml ascorbic acid. The effect of 2 mg/ml Gibberellin 3 was ranked best, followed by 5 mg/ml potassium nitrate, 2 mg/ml penicillin and 0.1 mg/ml ascorbic acid. It subjects two eggplant rootstocks have different physiological responses to the different chemical reagents, even they have different suitable concentration on the same chemical reagent. We presumed that different species eggplant rootstocks have different mechanism of germination inhibition. And it is necessary to find the suitable germination method for every eggplant rootstock.

Keywords: Eggplant Rootstock, Germination Vigor, Vigor Index

Morphological and molecular diversity of an eggplant (*Solanum melongena* L.) germplasm collection.

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Abstract

A germplasm collection consisting of 238 eggplant breeding lines, heritage varieties and selections within local landraces from Asia and the Mediterranean Basin was characterized for 19 fruit and plant traits and fingerprinted with 24 microsatellite markers (SSRs) uniformly distributed throughout the genome. With the goal to identify stable, highly homozygous genotypes, 47 accessions were discarded on the basis of the SSR-based estimate of residual heterozygosity or observed phenotypic variation. Based on phenotypic data, three main clusters which grouped accessions mostly in relation to their fruit shape, regardless their provenance, were identified. On the other hand, a relationship between molecular data and provenance was found. The relevance of our results for eggplant evolutionary studies and future breeding programmes of the species are discussed.

Keywords: *Solanum melongena*, SSRs, germplams, evolution

Introduction

Eggplant (*Solanum melongena* L.) belongs to the *Solanaceae* family but, unlike other family crop species like tomato, pepper and tobacco, is native to the Old World. Lester et al. (1991) have suggested that the eggplant's ancestor was the subtropical species *S. incanum*, others have postulated that it was *S. undatum* (Meyer 2012). Recent morphological and molecular work has shown that species-level differences exist between *S. incanum* and *S. melongena* while, on the basis of a new nomenclature, *S. undatum* and *S.cumingii* have been re-classified as *S. insanum* (Knapp et al. 2013). The latter is fully inter-fertile with *S. melongena* and almost certainly its wild progenitor.

Archaeological records, suggest that Rajasthan may have been an area of domestication (Kashyap et al. 2010), on the other hand the use of eggplant as a vegetable crop was described in Chinese literature dating to 59 BCE (Wang et al. 2008). Eggplant spread westwards to Persia, was unknown by the ancient Greeks and Romans, and was introduced to the Mediterranean Basin by Arabs from the 7th century CE (Daunay, 2008).

The germplasm grown worldwide shows high variation in plant and fruit morphology and is conventionally grouped in “Occidental” eggplants, cultivated in North Africa, Europe and America, and “Oriental” or “Asian” eggplants, cultivated in Eastern and South-eastern Asia. This germplasm is at risk of genetic erosion since only a few popular cultivars and F1 hybrids are represented in the global trade (Rodriguez-Burrruezo et al. 2008; Munoz-Falcon et al. 2009). The maintenance and characterization of germplasm collections is therefore becoming a priority in providing a source of ‘useful genes’ for gaining genetic advances in breeding programs and address future breeding challenges of the species (Hurtado et al. 2012).

The phenotypic and genetic diversity of local Spanish (Prohens et al. 2005; Muñoz-Falcón et al. 2011), Indian (Behera et al. 2006), Chinese (Ali et al. 2011) and Turkish (Demir et al. 2010) germplasm have been previously explored. More recently the morphological and molecular

diversity among 52 accessions from three geographically well separated centres of diversity has been assessed (Hurtado et al. 2012) while, thorough historic, morphologic and molecular data, Meyer et al. (2012) made assumptions on phylo-geographic relationships among candidate progenitors and Asian landraces.

We report a molecular and morphological characterization of a wide eggplant collection including "Occidental" and "Oriental" breeding lines, heritage varieties and selections within local landraces. The objectives were to assess the extent of genetic diversity that they contain, to illuminate on their genetic relationship, and to provide criteria for the identification of a core germplasm collection.

Materials and Methods

Plant material and morphological characterization.

A collection of 238 *S.melongena* accessions, of which 94 'Oriental' (Eastern – EA) types, hailing from China, Indo-China, Indonesia, India and Japan, and 139 'Occidental' (Western - WE) ones, from Italy, France, Spain, Turkey and North Africa, was set up. Each entry was scored for 19 plant, leaf, flower and fruit traits (Table 1), in two locations in Italy (Montanaso Lombardo - ML, 45 20'N, 9 26'E and Monsampolo del Tronto - MT: 42 53'N; 13 47'E) in each of 2010 and 2011. For each trial, six plants per accession were planted in two completely randomized blocks. A principal component analysis (PCA) was carried out to identify the most discriminating traits. A Hierarchical Clustering on Principal Components (HCPC) was applied to define a cluster subdivision based on phenotypic traits. Statistical analyses were implemented by means of the software R (R Core Team, 2009).

Molecular characterization.

Total genomic DNA was extracted from young leaves (3 plants per accessions) and a set of 24 microsatellite (SSR) markers, uniformly distributed across the genome (Table 2), was applied for molecular fingerprinting (Barchi et al. 2010). The SSR products were scored as band presence (1) and absence (0), thus generating a binary matrix imported into Past 2.08 software (Hammer et al. 2001) and used to compute pair-wise similarity coefficients (Dice, 1945). Alleles with a frequency ≥ than 1% were considered as rare alleles. Principal co-ordinate analysis (PCO) was carried out to display the multi-dimensional relationship between accessions. The polymorphic information content (PIC) of each SSR locus was evaluated as reported by Anderson et al. (1992). In order to identify the minimum number of accession retaining 100% of SSR diversity the M (maximization) method was applied as implemented in the MSTRAT software (Gouesnard et al. 2001)**.** A cophenetic correlation between matrices based on morphological and molecular data was calculated and Mantel's test, including 5,000 permutation as implemented in Past 2.08, performed.

Trait	Code	Evaluation method
Peel color	pcol	L*a*b* color coordinates
Peel glossiness	pglo	Scale from 0 (high opacity) to 3 (high glossiness)
Fruit curvature	fcur	Scale: 1 (no curvature), 5 (curved), 9 (U shaped)
Fruit weight	fw	Grams
Fruit length	fl	Centimeters (from the base of the calyx to the tip of the fruit)
Fruit diameter max	fdmax	Centimeters
Fruit diameter max position	fdmaxp	Scale from 1 (close to the calyx) to 8 (close to the apex)
Fruit shape	fs	fl / frdmax
Flesh fruit firmness	firm	Scale from 1 (very loose) to 9 (very dense)
Leaf hairiness	lha	Scale from 0 (no hairiness) to 5 (highly hairiness)
Adaxial leaf lamina anthocyanin	adlan	Scale from 0 (green) to 5 (complete purple coloration)
Stem anthocyanin	stean	Scale from 0 (green) to 5 (complete purple coloration)
Calyx coverage of the fruit	cacov	Scale from 1 (<10% of the fruit length) to 5 (>50%)
Fruit calix prickliness	fcpri	Scale from 0 (no prickles) to 9 (high prickliness)
Leaf prickliness	lepri	Scale from 0 (no prickles) to 5 (high prickliness)
Plant growth habit	hab	Scale from 1 (upright) to 9 (prostrate)
Inflorescence flowers	inflw	Number of flowers for inflorescence
Flowering abundance	flwab	Number of flowers on the plant, scale from 1 (very few) to 5 (many)
Flowering time	flwt	Number of days from seedling emergence after sowing when at least 50% of the plants have its till first flower opened

Tab le 1 - List of the traits scored. For each trait the evaluation method and unit of measurement are reported.

Marker	Chromosome	Position (cM)	Alleles	Rare alleles	PIC
CSM 31	E01	107.4	12	2	0.83
ecm001	E01	77.7	7	2	0.73
emh21J12	E01	91.8	11	5	0.76
emf01G17	E02	35.4	10	5	0.65
EM 133	E02	11.6	6	4	0.24
emg11I03	E03	6.0	6	2	0.77
emj03A17	E03	34.3	3	1	0.38
emf01K16	E04	0.0	4	0	0.63
EM 117	E04	47.6	6	1	0.76
emf01A06	E05	64.3	4	1	0.45
EM 146	E05	50.6	7	2	0.68
CSM 7	E06	35.6	3	0	0.48
CSM 19	E07	0.0	4	1	0.55
CSM 69	E07	73.4	2	0	0.48
ecm023	E08	13.9	2	0	0.35
emi03M03	E08	13.5	3	0	0.45
CSM 54	E09	16.1	7	1	0.66
eme03B08	E10	46.5	6	0	0.74
emf11F07	E10	53.7	7	1	0.75
emf21K08	E11	0.0	9	3	0.63
EM 080	E11	25.6	2	0	0.35
CSM 29	E12	82.3	6	1	0.67
CSM 73	E12	0.0	5	1	0.69
emb01O01	E12	43.9	8	1	0.77
total			140	34	0.60

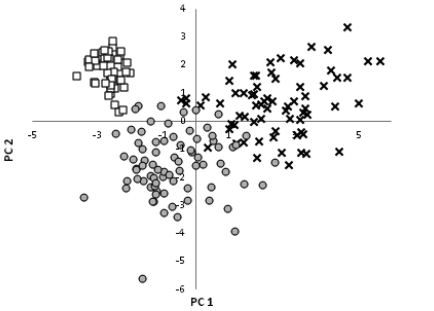
Table 2 – List of the 24 SSR markers. The chromosomal location, number of detected and rare alleles and PIC are reported.

Results and Discussion

With the goal to identify stable, highly homozygous genotypes, 47 accessions were discarded on the basis of the SSR-based estimate of residual heterozygosity or observed phenotypic segregation.

In both WE and EA accessions a wide variation for most of the plant and fruit traits was displayed. The first six components of the PCA (75.43% of the total variance) were used for HCPC analysis and identified three main groups including genotypes from both Asia and the Mediterranean Basin (Fig 1). The first one included genotypes producing elongated fruits, with a mean ratio of fruit length/fruit maximum diameter (fs) around 5. The second and the third morphological groups included accessions producing oblong-shaped fruits (mean fs of 1.95) and round shaped fruit (mean fs ~1) respectively. The high morphological variation observed matched with high levels of molecular diversity. However according to a Mantel test, only a weak correlation between the phenotypic and the genotypic data sets was found.

The 24 SSR loci amplified 140 alleles (average 5.8 per locus) (Tab. 2) and each accession was uniquely fingerprinted. The average PIC value was 0.60. The number of rare alleles was 34 (24%) of which 14 exclusive to the ‘Oriental’ and 20 to the ‘Occidental’ accessions. Based on SSR fingerprinting 38 the accessions showed a residual heterozygosity higher than 10% and were discarded. Among the remaining 200 accessions an high level of genetic differentiation was found (average Dice similarity coefficient of 0.32). Based on the M-method, the minimal set sufficient to capture all 106 non-rare alleles was 16, while the size of set required to capture all 140 alleles was 48, including the rare alleles.



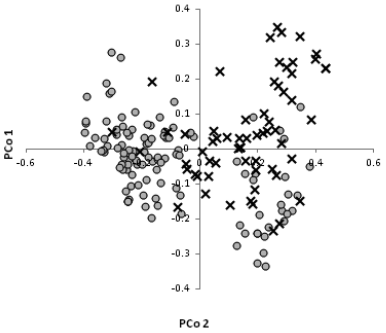


Figure 1 The first two PCA components plot, displaying accessions belonging to different morphological groups (cross – group 1 elongated fruits; dots - group 2 semi-elongated fruits; square - group 3 round fruits).

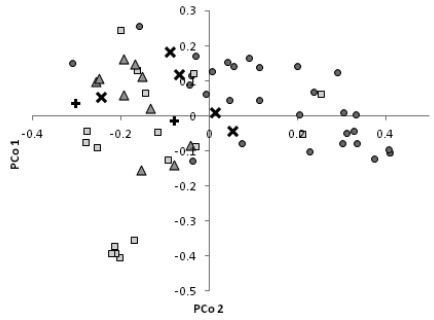


Figure 2 First two PCoA coordinates with different symbols representing different geographical areas (dots - WE; crosses - EA).

Figure 3 First two PCoA coordinates showing the Asian accessions clustering according with the country of provenance (dot - China; square - India, triangle - Indochinese region, crosses - Indonesia; vertical crosses - Japan)

The PCO analysis (Figure 2) showed a clear picture of differentiation between provenances not detected for morphological traits. The first two coordinates grouped together most of the EA and WE accessions. When PCO analysis included only WE accessions (data not reported), no evidence of direct correlation between provenance and genetic distance was detected, suggesting that this gene pool has experienced extensive exchange of breeding materials. On the other hand within EA accessions a trend of clustering was detected and most of the genotypes from the Indian, Indo-Chinese and Indonesian regions grouped together and separately from the Chinese ones (Figure 3). Recent studies highlight that the modern eggplant evolved from the species *S. insanum* (Knapp et al. 2013), and it has been generally assumed that it was domesticated in Indian subcontinent (Weese et al. 2010; Wu et al. 2009; Sękara et al. 2007), possibly in Rajasthan region (Kashyap et al. 2010). However, the genetic differentiation of Chinese accessions we found might support what reported byAli et al. (2011) that China is also a candidate site of domestication. Indeed, recently Meyer et al. (2012) delineated the hypothesis that eggplant have been domesticated independently in these two areas, and this trend of multiple rather than single domestication events is increasingly braced for many crops (Olsen & Gross, 2008). The introduction in the Mediterranean area by the Arabs in the early Middle Ages presumably represented a bottleneck in eggplant genetic diversity which was alleviated by subsequent selection, *de novo* mutations and recombination events as well as adaptation to different environments. This, despite some movement of germplasm across the Asian and Mediterranean countries occurred over time, justify the genetic differentiation we detected between genotypes from the two geographical areas.

The data set as a whole contributes significantly to the knowledge base regarding the level and distribution of genetic diversity in the WE and EA eggplant gene pool, and sets the scene for a well-founded association mapping exercise to derive genotype-phenotype relationships

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Genetic diversity in eggplant germplasm resources as inferred from SSR and SRAP fingerprints

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Abstract

Simple Sequence Repeat (SSR) and Sequence-related Amplified Polymorphism (SRAP) markers were employed to assess the genetic diversity amongst 222 eggplant accessions originating from 35 countries including 13 regions in China. In total 5 SSR and 25 SRAP primer combinations were selected to fingerprint the used accessions. A high polymorphism was observed, with level of 65% for SSR and 75% for SRAP. The program STRUCTURE was used to analyze the genetic diversity and population structure. Five subpopulations, S1, S2, S3, S4 and S5 in the collection were revealed and this classification is often more related to their geographical origins than to fruit traits. The accessions of subpopulation S1, S2, S3, S4 and S5 originated mainly from Africa-Nigeria, Africa-Togo, Asia-India and China, Asia-Turkey and Asia-China, respectively. The fruits of eggplant varied widely in shape and color, these characteristics could differentiate between groups but not completely associated with the classifications.

Keywords: eggplant, genetic diversity, population structure, SSR, SRAP

Introduction

Eggplant (*Solanum melongena* L.) belongs to Solanaceae family together with the important species tomato (*Solanum lycopersicum* L.), potato (*Solanum tuberosum* L.), bell pepper (*Capsicum annuum* L.) (Fukuoka et al. 2012). Eggplant is one of the most important vegetables in many countries in Asia, the Middle and Near East, Southern Europe, and Africa. It originated in the south Asian tropical region, and it was at first domesticated in the Indian region. China had a long cultivation history and was recognized as secondary origin center of eggplant, which is rich in an abundant germplasm resource. Study on the genetic diversity of eggplant germplasm resources has an important significance for the collection, conservation, identification, innovation and breeding work of eggplant germplasm resources.

The development of molecular marker technology has been useful for analyzing genetic diversity in many plant species and has considerable potential for generating a large number of polymorphic loci. Compared with the other three species, eggplant has been used less often in molecular genetics research because of limited sequence information. A limited number of Random Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP) and Simple Sequence Repeat (SSR) markers have been used in genetic map construction in eggplant (Nunome et al. 2001, 2003, 2009; Barchi et al. 2010).

In this investigation reported here, we used SSR and SRAP markers to analyze the relationships among 222 eggplant accessions derived from different parts of the world. The software STRUCTURE was used to identify different groups in the population.

Materials and Methods

Plant materials

A collection of 222 eggplant accessions (*S. melongena, S. Aethiopicum*, *S. macrocarpon,* and

wild type *S. torvum* and *S. sisymbriifolium*) including various morphological types and geographical origins was used in this study. One hundred forty seven accessions were obtained from the Dutch Crop Genetic Resource Center (CGN) in Wageningen, and seventy five accessions were collected within China. The plants were grown in the tunnel in experimental farm of Heibei Agricultural University, China form April to July in 2012.

Genotyping

Leaf material from one plant per accession for DNA extraction was collected from seedlings and then grounded. DNA was isolated based on a modified CTAB method. The accessions were profiled with 5 microsatellite (SSR) primer pairs and 25 Sequence-related Amplified Polymorphism (SRAP) primer pairs. The SSR sequences information was obtained from public information of eggplant, tomato and pepper (Suliman-Pollatschek et al. 2002; He et al. 2003; Nunome et al. 2003; Minamiyama et al. 2006). The PCR was performed in 10 uL volumes with 1 unit of Taq DNA polymerase, 5 mmol/L dNTP, 10 X SuperTaq buffer, and 50 ng of each primer. DNA was present in the PCR at a concentration of 1 ng/uL. The PCR program was performed with the following cycling profile: denaturation at 94 °C for 3 min; 35 cycles of 94 °C for 0.5 min, 55 °C for 0.5 min, and 72 °C for 1 min; and a final extension at 72 °C for 7 min. The PCR products were separated on 6% denaturing polyacrylamide gels and visualized by silver staining.

Population structure analysis

The program STRUCTURE version 2.1 was used to identify groups in the population, using a Bayesian approach (Falush et al. 2003). The number of subpopulations was set to vary between 1 and 10, and for each fixed number of subpopulations, two independent Markov Chain Monte Carlo processes were run using 500 000 iterations for each with burn in of 100 000.

Results

Population structure

The model-based approach of STRUCTURE suggests the presence of 5 subpopulations S1, S2, S3, S4 and S5 (Fig. 1). The selection of five subgroups (K = 5) was done as the average likelihood value for a given K value increased gradually until K = 5. Most of African accessions (*S. Aethiopicum* and *S. macrocarpon*) are grouped into S1 and S2, most of Chinese accessions (*S. melongena*) are grouped into S5, and most of Asian and European accessions (*S. melongena*) are grouped into S3 and S4.

The S1 consisted of 31 accessions, 27 of which originating from Nigeria, Togo and Ghana in West Africa, had over 50% of their genetic polymorphism assigned to this group. Two wild accessions 7 (*S. torvum*) and 8 (species unknown) were also assigned to S1, but their profile is an admixture with S2, S3 and S5. Group S2 was small, with its 11 accessions, mainly from Togo and Ghana, having at least 50% of their genetic variation assigned to the group. One wild accession 6 (S. *sisymbriifolium*) was included into S2 with an admixture of S4. Sixty three accessions mostly from China, India, Turkey and Europe, out of 66, had at least 50% of their variation assigned to group S3. Group S4 consisted of 51 accessions, out of which 45, mostly from India, Turkey and Europe, had over 50% of their variation assigned. Lastly, 59 accessions mostly from China, out of the 63 accessions of group S5, had over 50% of their genetic variation assigned to this group.

Genetic variation

In total, 23 scorable amplification products were generated from 5 SSR pairs, 22 of them were polymorphic, with an average of 4.6 polymorphic bands per primer combination. Wild accessions (6, 7, 8) displayed multiple mono-morphic bands that contributed considerably to the SSR polymorphism rate. If these mono-morphic bands were excluded from the analysis, the degree of

SSR polymorphism was 65.2% instead of 96%. The allelic diversity was also investigated to see whether different STRUCTURE groups were more diverse as reflected by total allele numbers per group of accessions. The S1 subpopulation had a mean of 2.4 alleles per marker, the S2, S3, S4 and S5 subpopulations had a mean of 1.8, 1.8, 2.2 and 2.2 alleles per marker, respectively.

In SRAP analysis, 239 amplification products were generated; all of them were polymorphic, with average of 9.6 polymorphic bands per primer combination. The number of bands per primer combination in the collection varied between 7 (ME3EM6, PM36EM7 and OD3SA1) and 14 (ME7EM3 and SA1OD3).The S1, S2, S3, S4 and S5 subpopulations had a mean of 4.2, 4.5, 3.9, 4.3 and 5.0 bands per primer combination, respectively.

Variation in fruit color and shape

In Table 1, fruit shape and color are listed for the different subgroups. Fruits vary widely in shape and color; these characteristics could partly differentiate between subpopulations. Most of variation for fruit shape was found in the S3, S4 and S5 subpopulations. Long-conical shapes are characteristic for the S3 and S4. Fruit color varies greatly among subpopulation S3 and S5. Dark color fruits are found in the S5. White color fruits are characteristic for the S1 and S3.

Discussion

The software STRUCTURE was successfully applied to identify groups in many species. In this paper, the accessions used include several species such as *S. melongena, S. Aethiopicum*, *S. macrocarpon,* and wild type *S. torvum* and *S. sisymbriifolium*. One strategy to deal with substructure is to identify relevant genetic group on the basis of neutral markers. In this paper, 5 subpopulations are identified using STRUCTURE analysis; this will help in understanding the genetic relationships between different accessions.

The interesting information revealed by the population structure analysis in this investigation is that classification of accessions is more related to their country of origin and species identity than to fruit traits. This may explain why the different morphotypes could emerge independently in the different geographic regions. Three wild accessions 6, 7 and 8 used are more related to accessions derived from Africa, with admixtures of different subpopulations. It will be interesting to further investigate the genetic diversity using more genetic markers.

Acknowledgement

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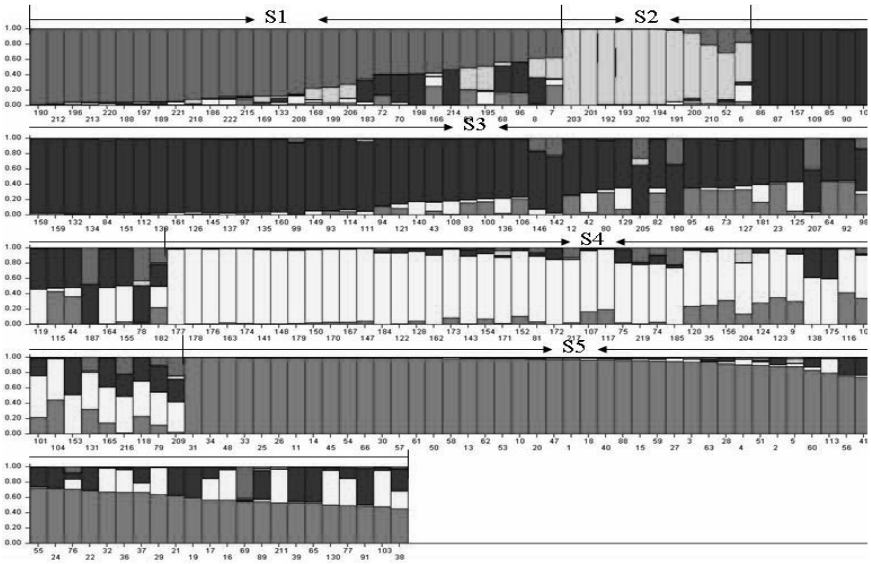

Fig. 1 Results from structure under the assumption of cluster number K=5.
Accessions are represented by a bar which is partitioned into several segments with different gray shade according to the individual's estimated membership fractions of the 5 clusters.

Table 1 Fruit shape and color for some accessions of different subpopulations

Traits	Characteristics	Number of accessions of subpopulation				
		S1	S2	S3	S4	S5
shape	Flat round	15	6	0	0	5
	Long round	0	0	12	8	6
	Oval	0	0	17	12	4
	Long oval	0	0	8	6	4
	High round	2	0	0	5	5
	Round	4	2	8	1	9
	Long	0	0	3	1	3
	Short conical	0	0	1	1	1
	Long conical	0	0	3	1	0
Color	Light green	12	2	4	0	0
	White	6	0	8	0	0
	Green	3	5	2	0	2
	Purple	0	0	10	5	4
	Dark purple	0	0	13	8	3
	Purple red	0	0	6	7	4
	Black purple	0	0	13	10	12
	Black	0	0	0	0	8
Number of accessions*		31(21)	11(8)	66(56)	51(35)	63(47)

*indicates the total accessions in 5 subpopulations and the number of accessions measured fruit traits.

Brown seed coat pepper mutants

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Abstract

Pepper seed colour is determined either by the colour of the embryo or by the colour of the seed coat. Seed coat tissues are originated from the mother plant. From the F_1 hybrid seed production point of view, only an endosperm mutation can be used as a phenotypic marker. The brown seed coat mutant can be used as a morphological marker, and to the best of our knowledge, such mutant has not been reported in pepper. During the period of 2010-2013, we found brown seed colour mutants in pepper varieties with different genetic backgrounds, such as Hungarian conical white fruit type, apple form type, spicy pepper type and the dark green blocky type. The provisional names of these mutants are *gold seed* (*gos*) and *rust brown seed* (*rb1, rb2, rb3, rb4, rb5, rb6, rb7*). We further noticed that all of these mutant pepper lines had not only brown seed, but the xylem of their stem was brown, too.

Crossing results are as follows. If the mother plant was of brown seed and the father plant was of yellow seed the F1 seeds were brown (mother characteristic) but the xylem of the F1 plant was white. All of the seeds in the F1 fruit (F2 seeds) were yellow, but the F2 plants segregated into 25 % brown xylem and 75 % white xylem. Every plant with brown xylem had brown seed and plant with white xylem had yellow seed. The results suggested it that the brown seed coat mutants determined by single recessive gene. According to the first result of the allele tests the gos, *rb1* and *rb2* genes are the same, but the *rb3* is another gene.

Keywords: pepper, brown seed, brown xylem, mutant

Introduction

Seed coat colour mutations are known in several plant genera (*Pisum, Phaseolus, Cucurbita, Citrullus, Lycopersicon, Arabidopsis*). Seed colour might be determined by either the colour of the embryo or seed coat colour. In tomato, the brownish colour of the embryo causes a slight colour difference between the seeds of the normal and the brown seeded mutant (Soressi 1967; 1972, Philouze 1970; Yordanov 1972). Monti (1972) described several brown-seeded mutants of tomato. Downie et al. (2003, 2004) studied the physiological effects of the genes causing the brown pigmentation of the seeds. Debeaujon et al. (2000) investigated the effects of the seed coat mutations of the *Arabidopsis* on the dormancy, germination characteristics and storability.

Theoretically, if the colour of the embryo in a mutant seed differs from the wild-type, this feature could be used as phenotypic marker for hybrid seed production. The embryo of F_1 seeds having derived from a cross between a recessive brown-embryonic (female) and a dominant normal seeded (male) parent has normal pigmentation. In spite of the possibility, phenotypic seed colour marker has never been applied successfully in the hybrid seed production. A recessive seed coat colour mutant cannot be used as a marker gene for the F_1 hybrid seed production, as the tissues composing the seed coat are of direct maternal origin. In a cross between brown seeded × normal seeded parents, the colour of the F_1 hybrid seeds will be always brown, irrespective of the fact that some seeds may develop as a result of self-pollination due to the chance (technical mistakes during the process of crossing). However, the brown seed coat as a unique feature of the individual can be used as a seed variety marker. If a company inserts the gene of the brown seed coat into the mother lines of all its hybrid cultivars, this characteristic would be a unique marker gene of the company that differentiates the seeds from the products of any other concurrent companies.

Materials and Methods

In the past three years, we found brown seeded pepper plants on several occasions. We assigned them genetic symbols and we carried out allelic tests to examine their genetic identity. All genes were crossed with normal plants and with each other, as well.

Results and Discussions

During our studies of more than 40 years on pepper genetics and breeding, we examined the offspring of several hundred thousands of plants, but did not find any brown seeded plant.

Although the nonviable seeds of poor quality are also brown, this feature does not have genetic background.

Between the years 2010 and 2013 we experienced unexpected observations in our pepper research field. In 2010, a pepper breeder colleague of us found a specimen with brown seeds from the Hungarian, conical white fruit cultivar Fehérözön, which is under commercial cultivation for more than 30 years. We named this mutation as *gold seed – gos*. In the same year, we found rust brown seeds in a Hungarian Cecei type, conical white fruited cultivar of foreign origin. This mutation was named *rust brown* (*rb1*). In the spring of 2011, in another foreign breeding material of green, Blocky-type peppers we found another brown-seeded specimen, which was found to be non-allelic to *rb1*, so we named this *rb2*. While evaluating our autumnal experiments, we observed yet another brown-seeded plant (*rb3*) in the F_3 hybrid generation of a cross between Hungarian Cecei and Mexican Jalapeno. The unusual series of observations continued in year 2012, when we found brown seeds in three parcels of Hungarian spice peppers of completely independent origins (*rb4*, *rb5* and *rb6*). Finally, in the spring of 2013, in a population of Hungarian apple-shaped, white-fruited pungent pepper, we found brown-seeded (*rb7*) again. The gene symbols are used only temporarily, applied for the allelic tests that are planned to examine the identity of the alleles.

When examining the brown-seeded plants, we found that the xylem of the stem of each cultivar is brown, in contrast with the whitish colour of the wild plants. This important observation has a great aid to distinguish the brown-seeded plants from the yellow-seeded ones at rather early stage of development (i.e. when the plants have only 6-8 leaves). In 2013, *rb7* mutant was found based on the colour of the stem tissues.

In 2011, plants bearing *gos* and *rb1* genes were crossed with normal plants of yellow seeds, and the two mutants were also crossed with each other. Based on our results gathered so far (and compiled in Table 1), we can state that the cause of each seven brown seeded mutants is the alteration of the colour of the seed coat. The tissues of the seed coat are of maternal origin. The F_1 seeds of the brown seed × yellow seed cross were brown, whilst the F_1 seeds of the reciprocal cross were yellow. However, the xylem of the F_1 plants was always whitish, thus the allele of the brown seed inherited as recessive trait. By evaluating the F_2 generation, we observed that mutations *gos, rb1, rb2, rb3, rb4, rb5* and *rb6* are all monogenic and recessive (Table 1). The stem of the brown-seeded mutant is always brownish. According to our allele tests accomplished so far, genes *gos*, *rb1* and *rb2* are identical despite their independent origin (Table 1), yet the gene *rb3* differs from this.

The results of the histological study on the brown seed mutants are discussed in the present issue by Erős-Honti & Csilléry (2013).

Table 1. Allelism test for different brown stem/brown seed pepper mutant genes

Cross type	White stem			Brown stem		
	Expected	Detected		Expected	Detected	
	%	Number of plants	%	%	Number of plants	%
gos x rb1 F1	100	0	0	0	162	100
gos x rb1 F2	0	0	0	100	86	100
rb1 x gos F1	100	0	0	0	52	100
rb1 x gos F2	0	0	0	100	69	100
gos x rb2 F1	100	0	0	0	220	100
gos x rb2 F2	0	0	0	100	62	100
rb2 x gos F1	100	0	0	0	85	100
rb2 x gos F2	0	0	0	100	53	100
gos x rb3 F1	100	58	100	0	0	0
gos x rb3 F2	56,25	106	53,3	43,75	93	46,7 *
rb1 x rb3 F1	100	72	100	0	0	0
rb1 x rb3 F2	56,25	71	59	43,75	49	41 **
gos x rb4 F1	100	84	100	0	0	0
rb1 x rb4 F1	100	36	100	0	0	0
rb2 x rb4 F1	100	58	100	0	0	0
	*chi2: 0,694, p-value: 0,4					
	**chi2: 0,432, p-value: 0,5					

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Exploring South-East Brazilian wild *Capsicum*.

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Abstract

A large number of little-known species of wild *Capsicum* grow in South-East Brazil. In 2011 and 2012 a group of Italian keens on *Capsicum*, including one of the authors, made three trips to verify the presence of populations of these species in known sites, with the purpose of documenting their characteristics and report about the current situation. The search for wild *Capsicum* in their habitat revealed interesting aspects. Few species are clearly differentiated on the basis of their morphology and habitat. Some populations found in different sites and classified as distinct species show minor differences and therefore could be ecotypes belonging to the same species, with differences caused by environmental conditions. In other cases there are significant differences between populations assigned to the same species. Some species are widespread, others restricted to limited areas, but represented by large populations. However, some species are represented by extremely small populations, sometimes only a few individuals; they could disappear in a short time. Our experience highlights the need to develop criteria for a more precise identification of the species. It seems also necessary to protect some populations before they are lost forever, also through their ex-situ cultivation. The possibility of using these wild species as a source of useful genes for cultivated species should be also evaluated, in order to add resistance to diseases and adverse weather conditions.

Keywords: Wild Capsicum South-East Brazil Classification Protection Breeding

Introduction

The wild species of the genus *Capsicum* grow in Center and South America. South-East Brazil hosts about 10 little-known species, concentrated in a relatively small area. Many species from the Central America and Andean region are well-known, since they are available through the seeds banks and are grown by chile peppers enthusiasts around the world.

The species of the South East Brazil were instead almost completely unknown; only few botanists had the chance to locate and study them in their natural habitat. These species are unknown also to Brazilian people that don't use them in any way.

Claudio Dal Zovo, one of the authors, wished to know more, so he decided to visit Brazil, together with other Italian keens on wild *Capsicum*, to locate populations, describe them through photographic documentation and morphological characterization and report about the current situation. Data here reported are based on the point of view of the authors and may differ from the ones of other participants to the mission.

Materials and Methods

A meticulous preparatory work was carried out by examining almost all the available literature, searching herbaria sheets of Embrapa [13] and exchanging information with Brazilian and Argentinian botanists. We chose the months of Brazilian late Summer/Autumn (late February-early June) in order to obtain the highest chance to find both open flowers and ripe berries.

Three explorations were performed; two people participated to the first and second ones, four to the third one. The Argentinian botanist Carolina Carrizo García joined the group in the last two

days of the third trip.

During the first trip (late February 2011) we visited the area North-East of São Paulo: Salesópolis (Casagrande), Caraguatatuba (Rodovia dos Tamoios), São Luís do Paraitinga, Cunha (Pedra da Macela, road to Paraty), Lima Duarte (Park of Ibitipoca), Bertioga, Cubatão.

During the second trip (early June 2011) we explored the area North of Rio de Janeiro: Nova Friburgo (Pico da Caledonia), Castelo (Caxixe), Viçosa, Teresópolis (Parque dos Orgãos).

During the third trip (late April-early May 2012) we expanded the search to the South and North, from Curitiba to Belo Horizonte, and visited Paranapiacaba (Reserva Biológica do Alto da Serra), Salesópolis (Estação Biológica de Boracéia), Campos do Jordão (Park and Lefévre Station), Monteiro Lobato, Maria da Fé, Piquete, Petrópolis, Caraça (Park of the Sanctuary), São Miguel Arcanjo (Parque Botelho), Morretes (Estrada da Graciosa).

Historical sites of findings and other promising areas, including many natural parks, were explored.

Results

During the trips we found out and documented populations or individuals of known species and yet unclassified accessions, which are identified by the collector codes and the provisional names (*Capsicum* sp. followed by a progressive number) assigned during an exploration conducted by Brazilian botanists in 1999 [5].

Capsicum villosum Sendtn.	
Capsicum villosum Sendtn. var. *muticum* Sendtn.	
Capsicum schottianum Sendtn.	
Capsicum cornutum (Hiern) Hunz.	LBB1542, LBB1546, LBB1547
Capsicum dusenii Bitter	
Capsicum pereirae Barboza & Bianchetti	
Capsicum friburgense Bianchetti & Barboza	
Capsicum hunzikerianum Barboza & Bianchetti	
Capsicum buforum Hunz.	LBB1550, LBB1551
Capsicum recurvatum Witas.	*Capsicum* sp.1, LBB1520, LBB1521
Capsicum sp.6	LBB1559, LBB1564, LBB1556
Capsicum sp.9	LBB1569

Prof. Carolina Carrizo García, with our help, discovered and documented two populations of uncertain classification, similar to *C. recurvatum* and *Capsicum flexuosum* Sendtn.

We also observed cultivated plants of *Capsicum parvifolium* Sendtn., now re-classified as *Capsicum caatingae* Barboza & Agra, and many plants of quite common species such as *Capsicum praetermissum* Heiser & Smith and *Capsicum baccatum* L. var. *baccatum.*

All the black-seeded South-Eastern species have common features (except *C.flexuosum*). The number of chromosomes is 26 (2n=2x) (not verified in *C. hunzikerianum*). The typical habitat of the wild *Capsicum* spp. is the "Mata Atlantica", the forest which covers the mountains along the South-East Atlantic coast of Brazil. All the species live at quite high altitudes, with a few exceptions, from a minimum of 500 m asl up to a maximum of almost 2000 m asl. The plants prefer the transition zones between light and shadow; they grow mainly at the edges of roads and trails or at the limits of clearings (with the exception of *C.pereirae*). The plants have shrub or small tree habit with long branches and vigorous suckers and sprouts. The height of adult plants varies from 50-60 cm up to

over 3 m, but we found out also plants with creeping stems several meters long and suckers up to 3 m in height. The leaves along the branches are often in pairs with one leaf larger than the other. Plants are covered by pluricellular trichomes; some species are almost glabrous, other very pubescent; populations characterized by the presence of glandular trichomes were observed. The flowers are usually multiple per node, erect and geniculate at anthesis. The corolla in most cases is stellate, white with greenish/yellowish spots in the throat and purplish red spots in the petals lobes. Fruits are small and roundish, as large as a pea, pendulous and deciduous when ripe. Fruits of all the species are pungent, except in *C.dusenii*; they are quite hot when immature, less hot, sweet and juicy after ripening; the final color is greenish yellow, almost translucent. Seeds are black, very coriaceous.

The morphological characters distinguishing the different species or botanical varieties are the position of the flower at anthesis, the corolla colours, the presence of teeth in the calyx, the pubescence and (in some cases) the trichomes type.

C. villosum is widespread and has homogeneus traits in all the identified populations; plant is wholly covered with trichomes; calyx has 5 long teeth; flower is erect and geniculate at anthesis; corolla is white with greenish/yellowish spots in the throat and purplish red spots in the lobes.

C. villosum var. *muticum* share the same characteristics of *C.villosum*, but calyx is toothless.

C. schottianum is also widespread, with significant morphological differences from area to area; plants often grow to huge size (over 3 m); trichomes are scarce; calyx is toothless or with tiny teeth; flower is erect and geniculate at anthesis; corolla is white with greenish or yellowish spots in the throat and the lobes, sometimes with purplish red spots in the distal part of the lobes.

C.cornutum shows a great variability in the corolla color, with greenish, yellowish or brownish spots; in some populations corolla is entirely white; flower is erect and geniculate at anthesis; calyx has 10 teeth (sometimes from 5 to 9) of different size; plants are scarcely pubescent.

C. dusenii is very pubescent; flower is erect and geniculate at anthesis; corolla is slightly campanulate with purplish/brownish spots; calyx has 10 long teeth of the same length. Fruits are not pungent. [3]

C. pereirae grows in a very special and narrow habitat in the relatively arid Park of Ibitipoca, two “grutas humidas” with scarce natural light and very high humidity. Plant is glabrous with coriaceous leaves; calyx is toothless; flower is pendulous; corolla has greenish or yellowish spots in the throat and purplish red spots in the lobes.

C. friburgense is undoubtly a unique species; plant is scarcely pubescent; flower is erect and geniculate at anthesis; calyx has 5 teeth, not always well-developed; corolla is unique among all *Capsicum*, campanulate urceolate, entirely lilac-fuchsia. The species grows in a very restricted area near Nova Friburgo, at high altitude (1750 m).

C. hunzikerianum is very distinct from the others; it grows in marshy places in a very misty habitat in a narrow area in the Estação Biológica de Boracéia. Plant is glabrous, with nodes and young branches violaceous and coriaceous leaves; calyx has 5 evident teeth; fruit is larger than in other species; flower is erect not geniculate; corolla is large, with greenish/yellowish spots in the throat and purplish red spots in the lobes.

C. buforum grows near Campos do Jordão, inside the Park and (few plants) near the railway station E.Lefévre. Plant is scarcely pubescent; calyx has 5 evident teeth; flower is erect and geniculate at anthesis; corolla has greenish/yellowish spots in the throat, purplish red spots in the lobes, visible in the back of petals.

Some populations growing South of São Paulo could be classified as *C.recurvatum,* e.g. *C*.sp.1 LBB1520 and LBB1521 in the Park Botelho and another population along the Estrada da Graciosa.

Flower has greenish/yellowish spots; calyx bears 5 teeth curved backward, sometimes reduced or barely visible. A population found at Paranapiacaba, inside the Reserve and in the neighbourhood, has similar features.

Capsicum sp.6 includes populations with similar traits, i.e. calyx with 5 teeth and corolla with clear purplish red spots in the lobes.

C. sp.6 LBB1559 is an accession found along the road to the Park of Ibitipoca; plant is scarcely pubescent; flower is erect and geniculate at anthesis; calyx has 5 teeth variable in length (sometimes reduced); corolla is characterized by purplish red spots very evident and sometimes spread throughout the petals.

C. sp.6 LBB1556 grows near Piquete; it is similar to the previous one, but with more marked purplish red spots which often are also visible in the back of the petals.

C. sp.6 LBB1564 grows in a narrow area at high altitude in the Parque dos Orgãos.

Plant is scarcely pubescent; flower is erect or intermediate, geniculate at anthesis; calyx has 5 fleshy teeth; corolla is slightly campanulate; the back of the petals and the buds are violaceous.

Capsicum sp.9 LBB1569 grows at Caraça and it is characterized by linear leaves, up to 20 cm long and less than 2 cm wide. Plant is quite pubescent; calyx has 5 well-formed teeth, sometimes with additional shorter teeth. Flower is variable in size, very large (up to twice the size of flowers of other wild species), erect or, more often, intermediate, geniculate at anthesis, with greenish/yellowish spots reduced and purplish red spots very evident in the lobes. Fruits are quite large and slightly irregularly shaped.

C. flexuosum is a species with 24 chromosomes spread in Paraguay and North Argentina, but some populations were also found near São Paulo.

The population of Monteiro Lobato is very similar to *C.flexuosum* (coriaceous leaves, red ripe fruits), but differs in the presence of purplish red spots in the corolla lobes.

C. caatingae is a species with 24 (2n=2x) chromosomes typical of Central Brazil arid biomes, but two plants are grown by Prof. Casali at the University of Viçosa. Plants are impressive, formed by a large array of stems; leaves are glabrous; flowers and fruits form fascicles up to 15-20; immature fruits are greenish, ripe fruits are yellowish; seeds are straw/brownish; flower is pendulous not geniculate; corolla shows a sequence of 5 colors, light green in the throat, yellow, dark violet, violet and finally white in the lobes.

C. praetermissum is widespread; corolla is stellate or rotate with various colors and shapes; four plants with four different corolla shapes were present in a flower-bed at Biritiba Mirim.

C. baccatum var. *baccatum* is also rather widespread; corolla is rotate with yellow spots in the lobes.

We didn't find any population of *Capsicum campylopodium* Sendtn., although this species is reported to be rather common.

Discussion

The search for wild *Capsicum* in their habitat revealed interesting aspects.

Species differentiation.

Few species are clearly differentiated on the basis of their morphology and habitat. *C.hunzikerianum* is very different from all the other species in its habit and habitat, its leaves, flowers, fruits and lack of pubescence.

C. friburgense is unique for the shape and color of its corolla.

C. dusenii is well-differentiated for its flower, the dense pubescence, the calyx with 10 teeth of the same length and above all for the lack of pungency.

C. villosum is immediately recognizable for the very dense pubescence.

The population of *C.pereirae* which grows at Ibitipoca is well-differentiated for its habitat, the flower pendulous not geniculate, the leaves coriaceus.

Capsicum sp.9 of Caraça is unique for the linear leaves and the size of its flowers.

This accession has no name yet; in some features it is similar to *Capsicum mirabile* Mart. described in Flora Brasiliensis [1], especially for the leaf shape, but it differs for other traits, such as pubescence and growing area. Possibly it represents a species not yet classified, also in consideration of its geographical isolation from other *Capsicum* populations.

Uncertainties in the classification (Groups of species or populations with classification issues).

Some populations found in different sites and classified as distinct species show minor differences. It is thus possible that these populations are actually ecotypes belonging to the same species with differences caused by environmental conditions.

In other cases there are significant differences between populations assigned to the same species; we could observe a great variability in morphological traits such as corolla color and shape, number, length and shape of calyx teeth. These traits vary even within the same population, especially when it consists of many individuals (e.g. *C.recurvatum* at Park Botelho) or when populations grow in different climatic and soil conditions. This peculiarity makes difficult in many cases to establish clear boundaries and determine to which species belongs a certain population. Notwithstanding it's possibile to identify some groups with common features.

The group of *C. cornutum* includes populations characterized by the calyx with 10 teeth (sometimes 5 inconspicuous), but different corolla colors, with green, yellow or golden spots (Serra do Mar), completely white (accessions LBB1547 at Cunha and plant LBB1546 along the road Cunha-Paraty) or with additional brownish/purplish spots (Paraty); corolla colors of accession LBB1542 at Casagrande are unknown. This is a heterogenous group which could include several species.

Morphological differences with *C. dusenii* are clear, especially the presence/absence of pungency, even if some botanists consider *C. cornutum* synonymous of *C. dusenii*.

Another group includes populations with 5 teeth in the calyx, corolla with greenish/yellowish spots in the throat and purplish red in the lobes, scarce pubescence; *C. buforum* and various populations identified as *Capsicum* sp.6 belong to this group. Some experts think that these populations match *C. mirabile* described in Flora Brasiliensis [1], but the recent literature contains conflicting indications on the name to use, *C. buforum* or *C. mirabile*. Despite the common features, there are obvious differences and a great variability, even in the same population.

The population of Lefévre and Campos do Jordão fit the definition of *C. buforum* given by Hunziker in 1969 [2] and grow very close to the original site.

The population of Orgãos is very similar to the previous one and well recognizable from others, but has some peculiarity, especially in the intensity of corolla and buds colors.

In the population of Piquete the purplish red color in the corolla fills almost entirely the lobes and is variable from plant to plant; it's often also well visibile on the back of the petals.

The population of Ibitipoca also shows a great variability in the intensity of the purplish red component of the corolla and in the teeth length; at first sight we thought that *C*.sp.6 shared the same area with a population of *C. schottianum*.

The group of *C. recurvatum* spread in the area at South and East of São Paulo (Parque Botelho, Morretes, Paranapiacaba) presents great variability in the shape, orientation and length of the teeth; sometimes they are well-formed and curved backwards, in other cases reduced or almost absent, even in plants growing side by side. Corolla has greenish/yellowish or pure green spots.

The group of *C. schottianum* includes populations with calyx toothless and corolla with greenish/yellowish spots, sometimes with more or less obvious purplish red component which may be absent or present even in flowers from the same plant at different times. Calyx has 5 nervatures which sometimes originate small teeth. The difference between *C. schottianum* with small teeth and *C.recurvatum* with reduced teeth is minimal; some populations, for example those at Paranapiacaba (in the Reserve and near the railway station) could be included interchangeably in both groups.

C. campylopodium could also be part of the *C. schottianum* group. The distinguishing characters of this species are corolla with yellow spots, androecium heterodynamous with 3 short stamens and 2 long stamens, fruit compressed laterally, toothless calyx. These differences are quite vague because one or more of the same characteristics may be detected even on *C. schottianum* or other species.

Stamens of different length were documented in *Capsicum lanceolatum* (Greenm.) Morton & Standley, *C. pereirae* and *C*. sp.9 of Caraça, but it's a temporary phenomenon caused by a different speed of maturation of the anthers after the flowering; it's possibile that this phenomenon occurs even on *C. schottianum*, although not observed. The lateral compression of the fruits is caused by a peculiar arrangement of a group of 4 seeds, 2 per locule; the same peculiarity is frequent in *C. schottianum* too, but not on all the fruits of the same plant.

It is interesting to highlight that the areas where *C. campylopodium* was found in the past are almost always overlapped to those of *C. schottianum* and that, despite our extensive exploration, we never found plants identifiable certainly as this species, even if it should be widespread.

A single small plant of *C. villosum* var *muticum*, morphologically very similar to *C. villosum* (especially for the dense pubescence) was found; however it showed two important differences, the lack of teeth in the calyx (hence the name *muticum* of the variety) and above all the corolla with greenish/yellowish spots, but purplish red component absent or very limited.

The peculiar color could be due to the growing conditions, but it should be noted that *C.villosum* presents a great homogeneity in the corolla colors, even in populations growing far apart each other; the purplish red spots are always present and intense.

Purplish red spots are described in literature for *C.villosum* var. *muticum* [13], so the plant we found could be an exception; further investigations are needed.

Two populations of *C. pereirae* in two far apart areas and different habitat share common features such as pendant and not geniculate flower and toothless calyx.

At Ibitipoca the species grows in two distinct sites, but shows homogeneous characteristics. The habitat ("gruta humida") is the most peculiar among all those visited, a kind of oasis in the middle of an arid Park, with very scarce natural light and high humidity.

The populations of Castelo live in a very different habitat, the typical Mata Atlantica. A careful observation of the few photographs in the paper where the species was at first described [8] highlights that the corolla colors and other features are variable; in one photograph a flower shows different colors and shape and in the background there is another flower clearly geniculate.

C. caatingae is a case unto itself. When we saw this species, it was still classified as C. *parvifolium*, but when we carefully observed its characteristics, it soon became clear that it didn't correspond to *C. parvifolium* described in Flora brasiliensis [1], especially for the absence of teeth in the calyx. We also noticed a feature yet not highlighted in literature, the annular constriction,

more evident in mature fruits. The classification was clarified in a paper published shortly after [12]; the plants cultivated at Viçosa are just a new species, *C. caatingae.* The paper accurately describes also the "true" *C. parvifolium* and a third, new species of arid biomes characterized by very long teeth in the calyx, trichomes of various type and absence of pungency: *Capsicum longidentatum* Agra & Barboza.

Features of fruits and seeds.

The features of fruits and seeds suggest that the main dispersors are small mammals which gather the fruits fallen on the ground. The higher pungency in the immature fruits could encourage the consumption of only ripe fruits and deter any attempts to collect immature ones. Tough seeds could be an adaptation to pass without damage through the digestive system of small mammals and rodents. However, there aren't studies on fruits predation and seeds dispersion for these species.

Distribution of species and risk of extinction.

Some species are widespread (*C. schottianum*, *C. villosum*). Others live in small areas, but with large populations; the population of *C.recurvatum* in the Parque Botelho extends along a dirt road for over 20 km. However, there are species with extremely small populations (in the visited sites), sometimes only a few individuals in a restricted area.

We found a dozen plants of *C. hunzikerianum,* only half of which were adult. Approximately 20 plants of *C. friburgense* are present in an area less than 1000 m^2 wide; only one of the three populations originally described at different altitudes is still present and the plants found near the road were recently cut and re-sprouted. Only a few plants of *C. pereirae* are present at Ibitipoca, fortunately in a well-preserved habitat. The species seems to have disappeared from one of its original sites, the area near Castelo, subject to a rapid development and intensification of agricolture.

C. villosum var. *muticum* was not found in one of its historical sites, only a single small plant was found nearby in a quite risky situation.

The situation of *Capsicum* sp.9 at Caraça is critical; we found out only one single adult plant and a few seedlings in a cut underbrush; other populations might grow in other sites, inside the Park, but so far there are no reports of other findings. Single adults plants can often be found at roadside, just outside the area cut for maintenance, but the small "mudas" are steadily cut. The destruction of the habitat is not the only risk; another critical factor is the difficulty of reproduction in some populations.

In some cases very old isolated plants were found, without "mudas" nearby. An emblematic case is the plant of *C. cornutum* with white corolla found along the road Cunha-Paraty; the plant grows in the exact place where a single plant with the same features was found in 1986 and looks old enough to be the same plant; there aren't other Capsicum plants for kilometers all around.

Even if there may be undetected populations or interesting areas still unexplored, some species could disappear in a short time; it seems necessary to protect some populations at risk, even growing them ex-situ.

Exploration of sites not mentioned in literature led in many cases to find out populations or single plants, but always belonging to the most common species (*C. villosum* and *C. schottianum*). A systematic exploration of promising areas could lead to find out new populations or perhaps new species; the recent classification of well-differentiated species (*C. pereirae*, *C. friburgense*, *C. hunzikerianum* [8]) demonstrates that there is still much to discover.

Classification criteria.

Our experience highlights the need to develop criteria to more precisely determine different

species. It would be necessary to define which morphological criteria are definitely relevant to differentiate the species as a great variability on the corolla colors and leaves shape can be observed, even in the same species or populations, while the presence and number of teeth and the pubescence seems to be relatively constant.

Field observations suggest that corolla colors and its shape (more or less open), the size of calyx teeth and the size and proportions of the leaves are strongly depending on the exposure to the sun and on the growing conditions and may vary from individual to individual in the same population; thus these characters aren't always useful parameters to differentiate these species.

Growing these species in a controlled environment could reduce environmental influence. Presumably DNA-based assessment might solve many doubts. Interspecific breeding could also give information on possible crossing and species differentiation.

In literature only one attempt to use South East Brazilian wild Capsicum in interspecific crosses has been documented [7], but not with other species from the same area. In nature interspecific crosses between these species are not known and we never found plants with intermediate features in sites where populations of different species grow side by side, e.g. *C. villosum* and *C. schottianum*.

Breeding.

The possibility of using these wild species as a source of useful genes for cultivated species should be evaluated, in order to add resistance to diseases and adverse weather conditions.

First step is the determination of potentially useful genetic traits. It is possible that these species are particularly resistant. For example, a simple experiment carried out by Claudio Dal Zovo in Italy on *C. flexuosum* (plants from seeds distributed by the USDA genebank) demontrate without any doubts that this species is frost resistant. Plants survived two winters outdoor (in pots), with temperatures down to -12°C.

The investigation of the possibile use in breeding should consider that all these wild species are 26n (except *C. flexuosum*) and therefore are not easily hybridizable with the cultivated species. Tong and Bosland reported [7] an attempt to hybridize *C. buforum* with 9 cultivated and wild species (*C. praetermissum*, *Capsicum cardenasii* Heiser & Smith, *Capsicum eximium* Hunz., *C. lanceolatum*, *Capsicum tovarii* Eshbaugh, Smith & Nickrent). The results of interspecific hybridizations showed varying degrees of compatibility, but no viable hybrid seeds were produced.

Gábor Csilléry, one of the authors, deeply studied interspecific crosses using wild species [4], but none from South-East Brazil; however, his experience could be very useful. A good starting point to investigate interspecific crosses could be *C. flexuosum* x *C. baccatum* L., because *C.flexuosum* is a 24n species and should be quite similar to *C. baccatum*.

In cooperation with Brazilian Institutions and in accordance with the International Conventions on Biodiversity Conservation, these species should be grown and studied, before they disappear!

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Gluey fruit pepper mutant

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Abstract

One of the Kapia K90 breeding lines segregated plants bearing normal and gluey fruits with unglazed appearance. The line with gluey fruits was self-pollinated and did not segregate in the next generation bearing all gluey fruits. The progenies of wild type × gluey fruit crosses had wild type fruit phenotype in the F1 generation. In the F_2 generation the gluey fruit character segregated as a monogenic recessive trait. The proposed name for the mutation is *gluey fruit (gf)*. In humid conditions gluey fruits were found more sensitive to infections of saprophytic fungi. Gluey fruits showed remarkably higher rate of postharvest water loss than wild type ones. This characteristic might be interesting from the perspective of spicy pepper production.

The anatomy of the *gf* mutant fruits remarkably differs from that of wild type bearing a much thinner cuticular layer on the epidermis, yet being covered by a thin, yellow, lipophilic extracellular matter (somewhere forming only scattered droplets). On the same time, the hypodermal collenchyma of the *gf* fruit is thicker, and consists of more cell layers and the cells have significantly thicker walls. The statistical comparison of the anatomical traits of the *gf* and wild-type fruits to those of F1 generation hybrid plants also presented here. Besides, we also monitored the ontogeny of the *gf* pericarp from the anatomical aspect.

Keywords: gluey fruits, *gf* gene, pericarp anatomy

Introduction

The common feature of three important vegetable plants of the family Solanaceae, tomato, pepper and eggplant is that all their fleshy fruits are covered with a thick epidermis and a supportive collenchyma of several cell layers. In case of the pepper (*Capsicum annuum* L.) detailed histological investigations dealt with the anatomy of the stem (Schuerger et al. 1997) and the leaf (Tal et al. 1974; Schuerger et al. 1997; Nwachukwu et al. 2007; Weryszko-Chmielewska and Michałojc 2009). Fruit anatomy was examined comparatively in certain cultivars (Weryszko-Chmielewska and Michałojc 2011) or focusing on the detachment area (Cochran and Cowart 1937; Gersh et al. 1998; Sundberg et al. 2003). Some studies related the anatomical characteristics of certain pepper species to their phylogenetic relationships (Dias et al. 2013; Wahua et al. 2013).

Nevertheless, no thorough examination has been carried out yet on the histology of the pericarp, focusing on the structure of the epidermis. The outer tissue layers of the fruit basically determine the outlook (glossy or matt) and further economically important characteristics (storability, shelf life) the health of the vegetable, as well as features determining its digestibility. The structure and thickness of the epidermis and the hypodermal collenchymas layers may differ admirably between the cultivars. For instance, a general observation of the breeders is the thin and longitudinally cracked epidermis of the Mexican cultivars 'Jalapeño', whilst the fruits of the Kapia types of Balkan origin have the most durable outer pericarp layers, what enables the frying of the flattened, 'two-veined' fruits. The amount of cutin deposited on the exocarp of the Hungarian cultivar 'Fehérözön' is minimal, so it is easily digested by the consumer (Fischer 1992) without causing any

health troubles. On the same time, spice peppers appropriate for milling has the thickest cuticle layer that prevents the decay of the fruit during the long process of drying on the sun. The thickness of the exocarp (i.e. the epidermis) is a dominant characteristic determined by few genes, but it is highly dependent on the environmental factors (e.g. glass house or field conditions, year of growing). During the process of breeding, we found two fruits amongst the offspring of a self-fertilised specimen of breed K90 that possessed matt surfaces being sticky by touch. The collected fruits began to wither admirably quickly. The characteristic got the genetic name '*gluey fruit' – gf* (Csillery 2013).

In our present work, we aim to evaluate quantitatively the intensity of the post-harvest water loss and the histological parameters of the *gf* mutant in comparison with the wild type (*gf*+) fruits and with those of the F1 generation of cross-breeds.

Materials and Methods

Water loss measures

For measuring the intensity of water-loss, biologically ripened (red) fruits were held at room temperature. In the summer of 2011, the wild (*gf*+) and gluey (*gf*) fruits of field-grown K90 plants were examined for 11 days, measured daily (results shown on Figure 1/A). During the autumn and winter of 2011, a comparative study was carried out on the post-harvest water loss of greenhouse-cultivated plants. We compared the withering of the fruits of different cultivars and the F_1 generation to those of the *gf* plants. Measurements were taken for 30 days with a sampling frequency of 3 days. The following study arrangements were applied:

Series 1: three hot, Hungarian spice pepper cultivars (Kalóz – Kal, Kalmár – Kam, Szegedi 178 – Sze) compared to K90 *gf* (results shown on Figure 1/B);

Series 2: the F_1 generation hybrid plants of K90*gf* and the three Hungarian spice pepper cultivars (Kal × K90*gf* F_1, Kam × K90*gf* F_1, Sze × K90*gf* F_1) compared to K90 *gf* (results shown on Figure 1/C);

Series 3: a Kapia-type breed (K31), a white, conic Cecei-type Hungarian cultivar (C487) and a large-fruited, green pepper of Blocky type (B350) compared to K90 *gf*+;

Series 4: the F_1 generation hybrid plants of K90*gf* and the wild-type plants of the Kapia-type breed (K31), a white, conic Cecei-type Hungarian cultivar (C487) and a large-fruited, green pepper of Blocky type (B350) (K31 × K90*gf* F_1, C487 × K90*gf* F_1, B350 × K90*gf* F_1) compared to K90 *gf* (results shown on Figure 1/D);

Series 5: back-crossed generation of the F_1 hybrid plants of K90gf+ (wild type) × K90gf (gluey) origin and the K90gf (gluey) parent plant, compared to the original K90gf fruits.

Anatomical studies

For examining the exocarp anatomy, semi-thin cross sections were made from the fruits using a cryostat (Leitz Wetzlar). No staining was applied for the bright-field illuminated microscopic examination (Zeiss, Axio Imager.A2). Photodocumentation and measurements were carried out with the Axio Vision software 4.8. Measured data were statistically analysed using the PAST software (Hammer et al. 2001).

In all cases the following histological parameters were measured: the thickness of the outer periclinal cell wall of the epidermis, the total thickness of the epidermal cell layer, the cell wall thickness of the collenchymal cells of the hypodermis, the total thickness of the hypodermal layers together with the epidermis. The following anatomical investigations were made:

Comparison of the wild-type (*gf*+) and the gluey (*gf*) fruits in three different regions of the capsules: one close to the stalk (bottom), one from the tip region (tip) and one equidistant between the previous ones (centre);

Monitoring the ontogeny of the *gf* type fruit in four distinct stages of maturity;

Comparison of wild-type (*gf*+) fruits, gluey (*gf*) fruits and fruit anatomy of the F_1 generation of hybrid (K90gf+ × K90gf) fruits both in the stages of economic maturity (ripen green fruits) and biological maturity (ripen red fruits).

Results and Discussion

Genetic background

Cross breeding tests were made between the K90*gf* mutant and several Hungarian red-fruited spice peppers as well as Kapia-type cultivars, Hungarian white-fruited strains and green blocky cultivars. Hybridisation tests were repeated in each case; the parental genotypes were used both as mother and pollinator plants, as well. The offspring analysis of the hybridisation test (K90*gf*+ x K90*gf*) supported our preliminary expectation, i.e. gluey fruit is determined by a single, recessive allele, since all F_1 plants possessed normal fruits. The same genetic background was evidenced by the analysis of the 512 F_2 offspring of 128 parental plants of different cultivars. Moreover, the back-cross of (*gf*+ x *gf* F_1) x *gf* BC1F1 resulted a phenotypic ratio 1:1 for the wild-type and the gluey fruits.

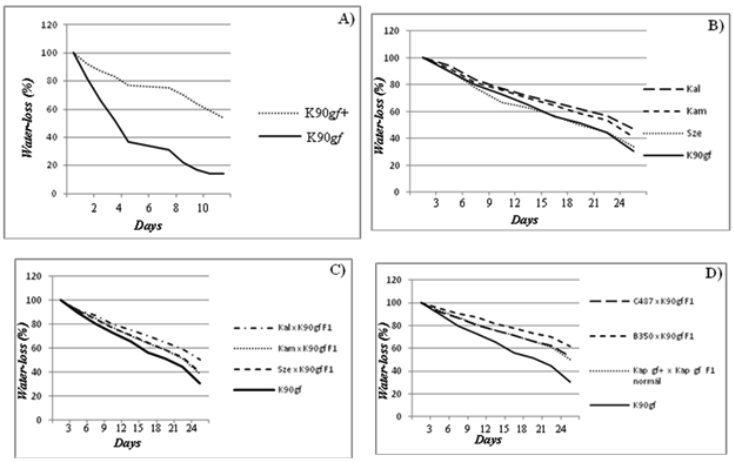

Figure 1. Results of the water-loss analyses. A) comparison of wild-type (K90*gf*+) and gluey fruited (K90*gf*) plants; B) red spice pepper cultivars in comparison with the K90*gf* mutant; C) F_1 hybrids compared to the *gf* mutant; D) hybrids of light sweet peppers compared to the *gf* plant. (For the abbreviations see the text!)

Water loss analysis

The fruit wall thickness of spice peppers is usually app. 2-3 mm, and the dry matter content of the fresh fruit is high, thus the peppers can be dried among continental climatic conditions without any remarkable loss. The cv. 'Szegedi 178' looses water more quickly than the average (Figure 1). The pericarp of the F1 hybrids of spice peppers × K90*gf* is thicker, the water-loss intensity is around the average, so the *gf* gene inherits recessively, and it has no effect in the heterozygotes. The thickness of the fruit wall of Kapia, Cecei and Blocky lines is even beyond that of the K90*gf*, thus their water-loss is even slower. A similar result was observed in case of the F1 hybrids of K90*gf* × Kapia, Cecei and blocky parents (Figure 1). When examining the BC_1F_1 offspring of the (K90*gf*+ x

K90*gf* F_1) x K90*gf* back-cross, we found that the water-loss intensity of *gf*+ specimens was rather slow, whilst *gf* fruits lost water in a speed similar to that of the original *gf* specimens.

The dry autumn of 2012 was appropriate for the drying out of the *gf* mutants amongst field conditions, yet several fruits rotted or were invaded by saprophytic fungi. In the greenhouse of high water vapour content these symptoms were more frequently observed. Probably pepper containing the *gf* gene cannot be cultivated on field amongst the climatic conditions of Hungary, yet in countries of a drier climate, plants of the *gf* gene can be cultivated safely.

An important and quite energy-consuming step of the process of spice pepper grain production is the drying of the fruits. According to the traditional technology, the stringed fruits have to be dried on the sun. Following this, prior to grinding, they are further desiccated in a chamber of 40-50 °C till the water content of the fruit reaches 6-8%. Decreasing the cost of this step would mean an admirable saving for the producers.

Pericarp anatomy

In each fruit, no significant difference was found between the measured anatomical parameters of the three regions of the fruits. Besides, the results of all statistical comparisons were similar in case of both the biologically and the economically ripened fruits.

The *gf* fruit is characterised by a relatively thin epidermis covered with a distinct, yellow-pigmented waxy layer sometimes forming oily droplets in the light microscopic images. On the same time, the epidermis of the wild (*gf*+) plants is significantly thicker owing to the remarkably thick outer periclinal cell walls (Figure 2). Interestingly enough, when analysing the soluble epicuticular wax content of the fruits, a higher amount of dissolved waxes was found in case of the *gf* plants, what may be an artefact caused by the dissolved lipid content of the thin-walled epidermal cells. Besides, the postharvest water loss results seemingly contradict the measured wax content data, yet they are explained by the histological traits of the epidermis. The intensity of water loss is reduced not by the epicuticular, soluble waxes of the *gf* plants, but the process might be hindered by the thick outer cell wall (i.e. the cuticle) of the wild-type (*gf*+) fruits. Similar mutants bearing deficient cuticle are described in the tomato (*cd* mutants) with similar characteristics (Isaacson et al. 2009; Nadakuduti et al. 2012).

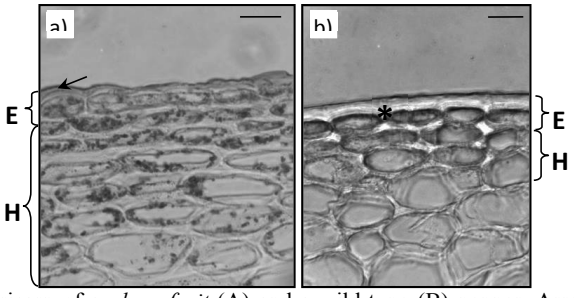

Figure 2. The outer pericarp of a *gluey fruit* (A) and a wild-type (B) pepper. Arrow indicates the distinct waxy layer, * stands for the thick cuticle of the wild-type fruits. Abbreviations: E-epidermis, H-hypodermal collenchyma. (Bars: 20 μm.)

In addition to the exocarp, the hypodermal collenchyma layers also differed significantly between the *gf* and the *gf*+ plants (Figure 2). This tissue layer was significantly thicker, composed of more cell rows and cells of significantly thicker wall in the gluey (*gf*) fruits. According to the observations of Fischer (1992), the tough hypodermis can be attributed to the decreased digestibility of the fruits, what may even cause health problems for the sensible consumer.

When monitoring the tissue development of the outer region of the gluey (*gf*) pericarp, we found gradual increase of the cell size both in the epidermis and the hypodermal collenchyma throughout

the process of ontogeny. However, the cell wall characteristics were found to be constant during the early stages, and increased thickening was observed only in the last stage (both in the epidermal and the hypodemral cells).

By comparing the anatomical traits of the F_1 hybrid generation (*gf*+ × *gf* F_1) to the parents, we found that epidermal characteristics are statistically identical with the wild-type parents, but differs from the other one. This supports the recessive inheritance of the *gluey fruit* allele. Nevertheless, the histological features of the hypodermal collenchyma resemble the *gf* parents, what indicates that thick-walled, multi-layered collenchyma is a dominant trait. Obviously, this observation needs further examination.

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Fruit phenotypic variability among *Capsicum* spp. accessions from southwest Mato Grosso state, Brazil.

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Abstract

Capsicum species are very important in Brazil because of economic, cultural and biological factors, and the country is considered a diversity center for this genus. Methods of multivariate analysis have been carried out to quantify the genetic divergence between accessions to, for example, define crossings to generate segregating populations for breeding programs. The state of Mato Grosso is a potential area for prospective action to find interesting genotypes that have been kept for Brazilian small farmers through the years. This specific region is near the border of Brazil-Bolivia. This study aimed to characterize 128 accessions collected in small farms in four distinct traditional communities in Southwest of the Mato Grosso state (Cáceres, Curvelândia, Mirassol d'Oeste, São José dos Quatro Marcos). The borderline region of Cáceres is the center of origin of *Capsicum*, it is bounded on the west by Brazil-Bolivia border, and has a connection with the floristic region Amazon. The *Capsicum* spp. accessions were characterized based on 17 fruit descriptors according to IPGRI (International Plant Genetic Resources Institute, renamed Bioversity International). Twelve distinct groups were formed by the Tocher procedure. By UPGMA method, four major groups were formed for *Capsicum* species, and results indicate that there are duplicated accessions. The well-defined dendrogram showed a cophenetic value of 0.86. Four main groups of genotypes were identified and could be further used in breeding programs. The results provided useful insights for better management of the germplasm collection, optimizing conservational and breeding efforts.

Keywords: Agrobiodiversity, fruit characterization, cluster analysis.

Introduction

Brazil is an important *Capsicum* diversity center, for having not only domesticated species, but also semidomesticated and wild species (Carvalho et al. 2003). In this genus, native of the Americas, more than 30 species have been described, but only five of them, *C. annuum* var. *annuum*, *C. chinense*, *C. frutescens*, *C. baccatum* var. *pendulum* and *C. pubescens* are considered domesticated (Pozzobon et al. 2006; Moscone et al. 2007).

The species and domesticated varieties and semidomesticated can be identified by morphological characteristics observed mainly in flowers and fruits. The great morphological variability displayed by fruit is highlighted by multiple shapes, sizes and colors. The format varies among and within species, and there are elongated fruits, rounded, triangular or conical campanulate, square or rectangular (Carvalho e Bianchetti 2007).

The morphological characterization, the assessment of genetic diversity and the documentation of a germplasm bank are essential for maintaining a base active for the exploitation of genetic variability in breeding programs (Carvalho et al. 2001; Viana et al. 2006; Arriel et al. 2007; Lannes et al. 2007).

This study aimed to characterize 128 accessions collected in small farms in four distinct traditional communities in Southwest of Mato Grosso state (Cáceres, Curvelândia, Mirassol d'Oeste, São José dos Quatro Marcos), Brazil.

Materials and Methods

In this study 128 accessions of *Capsicum* spp. from the germplasm collection of the Universidade Estadual de Mato Grosso (UNEMAT) were used. All the analyzed accessions are local varieties, which were donated by small farms of four traditional communities, from the southwest region of the state of Mato Grosso, Brazil.

For the characterization and evaluation of morphoagronomic accessions 17 descriptors proposed by Bioversity International were used: calyx margin; calyx annular constriction; fruit color at intermediate stage; fruit color at mature stage; fruit shape; fruit length; fruit width; fruit weight; fruit shape at pedicel attachment; neck at base of fruit; fruit shape at blossom end; fruit blossom end; fruit cross-sectional corrugation; number of locules; fruit surface; seed color; number of seeds per fruit.

The data were analysed considering the mode of each accession for each descriptor. The data were submitted to analysis of genetic divergence by multicategoric procedure. The divergence among the accessions was performed by Tocher's clustering method (Rao 1952) and UPGMA (unweighted pair group method with arithmetic mean), for the training of the dendrogram. The consistency of the grouping by hierarchical method was checked using Cophenetic Correlation Coefficient (CCC). Analyses were performed using Genes program (Cruz, 2006).

Results and Discussion

Considering Tocher's method, the 128 *Capsicum* accessions were clustered in twelve groups (Table 1).

Table 1. Grouping of the 128 accessions of *Capsicum* spp. formed by Tocher's method for fruits descriptors. Cáceres-MT, UNEMAT, 2012.

Group	Accessions
I	70 85 86 87 89 90 69 74 88 71 84 72 75 73 14 11 9
II	116 120 125 114 117 118 123 126 127 115 124 80 128 122 78 121 15 119 13 10 82 109 32 12 108 79 77 81 37 67
III	5 6 58 59 57 56 95 2 7 60 54 1 55 4 93 97 94 3 98 96 92 103
IV	16 17 22 21 19 18 20 40 23
V	110 112 51 107 113 106 111 38 46 49 83 64 65 34 105 104
VI	42 52 33 35
VII	50 100 43 44 102 101 99 41
VIII	25 29 24 26 30 27 28
IX	36 66 61 39 48 53 45 68
X	76 91
XI	8 47
XII	31 62 63

The UPGMA method clustered the accessions in four groups, according to their similarity, being grouped 57 accessions in GI (Group I); 21 accessions in GII; 46 accessions, in GIII and seven accessions, in GIV. The cophenetic correlation coefficients (CCC) of the dendrogram of 0.86

showed a good fit between the graphical representation of the distances and its original matrix (Rohlf, 2000), enabling the performance of inference by means of visual assessment (Figure 1).

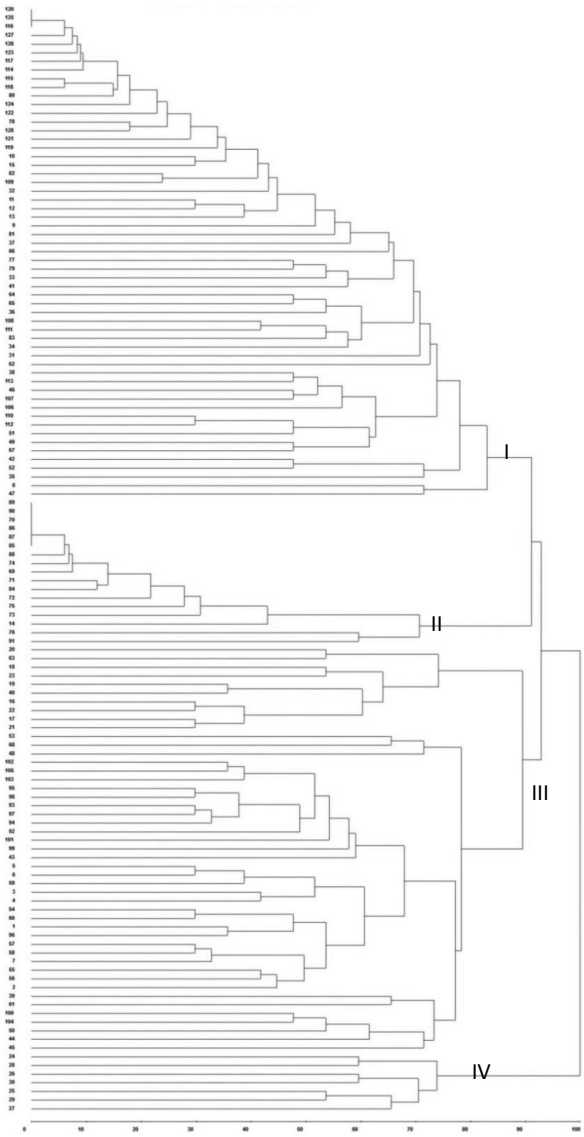

Figure 1. Hierarchical Clustering (UPGMA) of 128 accessions of *Capsicum* spp. from germplasm bank *Capsicum* of UNEMAT. Cáceres-MT (2012)

In the dendrogram generated on the basis of morphological and agronomic traits descriptors, a cut to 90% has led to the formation of four main groups. The GI corresponds to accessions from 47 to 120. GII corresponds to accessions from 89 to 91. The GIII of 20 to 45, and GIV of 24 to 27. The interpretation of the dendrogram is subjective and can generate difficulties in decision-making as to the number of groups generated. Therefore it is recommended the establishment of a visual

examination of points where occur high changes of levels, enabling the visualization of the groups (Cruz & Carneiro, 2006).

Most of the accessions were gathered in GI. In the first group, it was found that three accessions identified as UNEMAT 120, UNEMAT 125, UNEMAT 116) were considered similar. It was observed that, within the GII, the accessions UNEMAT 89, 90, 70, 86, 87 and 85 were similar, resulting in a greater number of possible duplicates. Characters such as the fruit neck, presence of appendix on the tip of the fruit, seed color, and number of seeds per fruit varied very little among the accessions.

We detected wide genetic variability among the accessions collected, as expected, since the accessions were collected near the region considered as the center of origin of *Capsicum*, especially *C. baccatum*. The value of the fruit characterization verified in this work was also described by Sudré et al. (2006) that obtained efficiency in taxonomic distinction among *Capsicum* accessions using qualitative morphological and agronomic descriptors.

The extensive genetic variability among *Capsicum* spp. accessions was demonstrated by analyzes carried out. Methods of grouping of Tocher and the hierarchical UPGMA were partially concordant with regard to the formation of groups.

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Chinese eggplant cultivars distribution：germplasm resources and research advancement

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Abstract

China is one of the most abundant eggplant germplasm resources country in the world, and eggplant was cultivated at least from Han dynasty. According to our data of 2005, there were 1601 cultivars, relatives and wild eggplant resources preserved in mid-term germplasm bank in the Institute of Vegetable and Flower of Chinese Academy of Agriculture Science (IVF, CAAS). Among all of these, 1468 different eggplant types embodied in "China vegetable resource catalogue" which includes 220 main eggplant germplasm resource filed in "Chinese vegetable cultivar record". We summarized and analysed the records. It showed that resources could be classified into 7 distribution districts according to their agricultural characters.

Based on the collected germplasm resources, research work has been done to select in situ resistant eggplant materials to the serious diseases in each production area. The results showed that there were not high resistant eggplant cultivars to *verticillium*. Resistant eggplant landraces to *phomopsis* wilt and eggplant *phytophthora* rot were found. Genetic analysis showed that both resistant traits are controlled by a single dominant gene. Moreover, chill tolerance in the Chinese Xian Luqie and Kuaiyuanqie landraces were found.

Keywords: germplasm resources; distribution; biotic and abiotic stress.

Introduction

Eggplant (*Solanum melongena* L.), is also named as Luosu, Kunlun Gua and Ai Gua. It is cultivated in most regions of the world, especially in Asia, around the Mediterranean Basin and in central Europe. Eggplant originated from southeast tropical regions of Asia. *Solanum insanum* L. originated in eastern India and *Solanum undatum* Lamarck in southern tropical regions of China, which may be its original wild species.

China is one of the most abundant eggplant germplasm resources countries in the world, and eggplant was cultivated at least from Han dynasty (Wang Jinxiu and Fu Dezhi, 2003; Zhen Weihua, 2003). According to our data of 2005, there were 1601 cultivars, relatives and wild eggplant resources preserved in mid-term germplasm bank in the Institute of Vegetable and Flower of Chinese Academy of Agriculture Science (IVF, CAAS, 1992, 1998, 2001). Among all of these, 1468 different eggplant types embodied in "China vegetable resource catalogue" which includes 220 main eggplant germplasm resources filed in "Chinese vegetable cultivar record".

Eggplant landraces distribution

During long-term domestication and cultivation processes, with different ecological environments and consumers habits, eggplant formed a number of relatively stable types of local varieties. The analysis of cultivated eggplant and wild resources in mid-term germplasm bank, showed that fruit colour and fruit shape are differently distributed among Chinese provinces（Table 1).

Considering both traits together, the germplasm can be classified into 7 fruit typologies.

Round fruit-shaped eggplants district

The regions include Beijing, Tianjin, Hebei, middle of Inner Mongolia, Henan, northern of Shandong and most of Shanxi province. Most of local varieties of eggplant are round fruit shape with purple peel from light to dark color for different consuming habits.

Black purple and long cylindrical fruit district

It includes Heilongjiang, Jilin, Liaoning and east of Inner Mongolia. In recent years, with the development of protection eggplant cultivation in winter and spring, due to the influence of low light and temperature, especially the purple peel varieties is not good in tinting, so green peel with long rod shape eggplant varieties had rapidly developed in early spring protected cultivation in Liaoning.

Red purple and long and slim fruit district

It includes the southern of Jiangsu, Zhejiang, Shanghai, Fujian, Taiwan,etc.

Red purple and long cylindrical or elliptical fruit district

It includes Anhui, Hubei, Hunan, Jiangxi, etc. .there are many local varieties, mainly red purple with long cylindrical or elliptical shape varieties.

Red Purple and long fruit district

It includes Guangdong, Hainan and Guangxi province. This region mainly cultivated long and red purple varieties, and there are a few of white and green peel varieties.

Purple elliptical fruit district

It includes Shanxi, Gansu, Ningxia, Xinjiang and Qinghai, etc. The region cultivated globular fruit varieties in purple, and it also had a few of green and white fruit peel varieties.

Purple cylindrical and elliptical fruit district

It includes Chongqing, Sichuan, Yunnan, Guizhou and Tibet. There are many local varieties with long or round fruit shape in purple.

Table1. Eggplant landraces distribution

Region	Peel colour					Fruit shape							Sum of
	P	BP	RP	G	W	R	O	E	LE	SC	LC	LS	
Beijing	2	12	—	—	1	6	5	—	1	—	3	—	15
Tianjing	—	6	6	1	—	8	1	—	3	1	—	—	13
Hebei	15	34	42	14	7	51	25	18	8	6	4	—	112
Shanxi	14	19	32	8	5	18	12	13	17	5	11	2	78
inner	—	5	15	2	—	6	—	4	9	—	1	2	22
Liaoning	11	43	7	32	1	10	2	14	14	9	24	21	94
Jilin	21	17	12	8	—	—	1	3	10	13	26	5	58
Heilongjia	23	30	15	2	—	—	—	1	10	4	49	6	70
Shanhai	1	2	1	1	1	—	—	—	2	—	1	3	6
Jiangsu	6	20	3	5	6	—	—	7	8	6	9	10	40
Zhejiang	1	7	9	4	—	—	—	1	6	2	5	7	21
Anhui	6	7	20	13	6	12	1	15	7	1	10	6	52
Fujian	1	7	8	1	6	—	—	—	2	—	7	14	23
Taiwan	—	3	3	—	—	—	—	1	—	1	1	3	6

Jiangxi	1	9	8	1	1	5	1	1	8	1	2	2	20
Shandong	29	30	41	7	2	21	12	20	41	—	3	12	109
Henan	17	3	8	51	—	20	1	30	22	—	5	1	79
Hubei	4	13	7	6	3	5	—	4	4	2	9	9	33
Hunan	4	13	24	3	5	4	—	13	17	6	4	5	49
Guandong	—	3	20	1	3	2	—	1	1	8	3	12	27
Hainan	—	8	12	6	—	6	1	4	1	4	5	5	26
Guangxi	—	3	6	1	—	—	—	—	—	1	6	3	10
Sichuan	14	38	76	11	7	6	1	24	39	14	42	20	146
Chongqing	1	3	2	1	—	1	—	2	—	—	4	—	7
Guizhou	5	15	19	—	—	6	—	5	8	—	3	17	39
Yunnan	11	26	47	14	8	20	3	18	8	12	36	9	106
Tibet	—	—	—	—	—	—	—	—	—	—	—	—	—
Shanxi	2	9	12	4	1	9	1	5	4	3	5	1	28
Gansu	—	9	8	—	—	6	2	1	4	1	—	3	17
Qinghai	1	—	—	1	—	—	—	2	—	—	—	—	2
Ningxia	2	4	4	—	2	4	3	3	—	—	—	2	12
Xinjiang	1	10	9	1	—	6	2	2	1	6	2	2	21

Note: P-purple; BP-black purple; RP- red purple; G-green; W-white; R-round;O-oblate; E-elliptical shape; LE-long and elliptial shape; SC-short and cylindrical; LC-long and cylindrical;LS-long and

R-round

O-oblate

E-elliptical shape

E-long and elliptial shape

SC-short and cylindrical

LC-long and cylindrical

LS-long and slim

Research on eggplant germplasm resources

During cultivation, biotic and abiotic stress affect eggplant growth. In china , the major diseases which effect the eggplant production are *Verticillium* wilt, bacterial wilt, eggplant *Phytophthora* rot and eggplant P*homopsis* rot etc, the main pests are *Leucinodes orbonalis,* aphids, mites and beetles,

and so on. Eggplant genetic resources have been screened for insect resistance in open field under natural conditions.

Biotic stress

Bacterial wilt: our institute screened the genetic resources by using artificial inoculation. Highly resistant (HR), resistant (R) and moderately resistant (MR) accessions. were identified (Table 2).

Table 2. Eggplant germplasm resistant to bacterial wilt

code	Type of resistance	code	Type of resistance
VO6B0093	HR	VO6B0095	HR
VO6B0096	MR	VO6B0099	HR
VO6B00105	HR	VO6B00118	HR
VO6B00131	HR	VO6B00134	HR
VO6B00140	MR	VO6B00147	R
VO6B00155	HR	VO6B00180	MR

Verticillium wilt: our institute also screened by artificial inoculation 1013 accessions kept in our national mid-term germplasm bank (Xiao Yunhua and Lin Baiqing,1995). The results show that there are not highly resistant eggplant cultivars. However, local cultivar "Chandingqie" was identified as moderately-resistant.

Eggplant *Phytophthora* rot and *Phomopsis* rot: "Fuxin"purple long eggplant No.-002 was identified as highly resistant to *Phytophthora* rot, and genetic analysis showed that the resistance was controlled by a single dominant gene (Zhao Guoyu and Tian Xinlin, 1995). Eggplant "83-02" was found resistant to *Phomopsis* rot (Zhang hanqing etc), and its resistance was also controlled by a dominant gene (Liu xuemin et al. 1998).

Abiotic stress

Eggplant wild relatives *S. grandiflorum* and *S. mammosum* expressed cold resistance, and *S. macrocarpon* L. was resistant to drought. In our country, there are also some local cultivars resistant to abiotic stresses, such as "Xian Luqie" and "Kuaiyuanqie" landraces, which tolerate chill (Bao Lanchun et al. 2004).

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Cooking influence on physico-chemical characteristics in three eggplant (*Solanum melongena* L.) genotypes

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Abstract

The changes in physico-chemical traits on raw fruits and after two domestic cooking treatments, such as grilling and boiling, have been evaluated in three eggplant genotypes. The local variety "Tunisina" (nasunin-containing), the breeding line "Buia" (D3R-containing) and the introgression line "L305" were used in this study. The physico-chemical traits comprised chlorogenic acid, anthocyaninidin glycosides, Folin-Ciocalteu assay, superoxide anion scavenging and NMR relaxation properties. The three genotypes showed differences both in terms of composition in raw fruits and changes after cooking. The chlorogenic acid, high in Tunisina and Buia with respect to L305, was depleted especially after boiling. The delphinidin pigments, nasunin and D3R, were mostly depleted after grilling. The Folin-Ciocalteu index and the superoxide anion radical scavenging capacity, were increased after cooking. The Tunisina and L305 genotypes had the best and worst performance, respectively. The NMR experiments clarified the changes in terms of decomposition of larger molecules and production of small ones after cooking.

Keywords: vegetables, cooking, phenols, antioxidants, NMR relaxometry

Introduction

The eggplant (*Solanum melongena* L.) fruit is an interesting plant system for its composition in terms of phytochemicals, belonging to phenolic acids (Whitaker and Stommel, 2003) and anthocyanins (Ichiyanagi et al. 2005), noted for their antioxidant properties (Kaneyuchi et al. 1999). Previous studies, made on different vegetables than eggplant, have demonstrated a cooking-related depletion in measured antioxidant compounds, followed by a simultaneous increase of antioxidant indexes (Miglio et al. 2008). In the present study, an approach based on the main eggplant phytochemical analysis and two different antioxidant assays together with a magnetic resonance technique for plant systems, such as the nuclear magnetic resonance (NMR) relaxometry, was carried out. Besides, most of the previous works on this topic have concerned the cooking induced changes studied on a single plant genotype, with scarce consideration for a comparative evaluation among different genotypes. The aim of the present work is an approach between the changes in some physico-chemical characters quality-describers before and after two commonly used cooking procedures, grilling and boiling, on three different eggplant genotypes.

Materials and Methods

Plant material and cooking.

Eggplant fruits, "Tunisina", "Buia", and "L305" were harvested in the full commercial ripening stage at the experimental field of the CRA-ORL Montanaso Lombardo (Lodi, Italy). About 50 fruits were visually selected on the basis of their homogeneous size, shape, colour and apparently absence of diseases, and randomly divided into two batches, each of which was further subdivided into three groups: one left untreated (RAW), and the others for grilling and boiling processing. The fruits in each group were cleaned and sliced into pieces of the same thickness (about 1 cm). RAW samples were immediately frozen in an air blast tunnel at -50°C, and then lyophilised. The aliquots for cooking were either grilled for 4-5 minutes on either side or boiled for 10 minutes in tap water at 1:10 ratio of slices and cooking water. The temperature was maintained at 100°C in the inner part of the slices during the cooking time in both cooking ways. After cooking, samples were allowed to stand at room temperature for 15 minutes, boiled samples being drained by water, then frozen in an air blast tunnel at -50°C, and lyophilised.

Chemical assays.

The vortexed and subsequently centrifuged extracts of 300 mg of eggplant powder with 20 ml of 30 mM HCl were analysed. The analyses have been made on the supernatant.

Phenolics.

Chlorogenic acid was quantified according to Whitaker and Stommel (2003) using a RP-HPLC-DAD separation. The results were expressed as mg/100 g of dry matter (DM). The anthocyanins contained in the peel were extracted from the whole fruit sample and analysed as described by Ichiyanagi et al. (2005). The results were expressed as mg/100g of dry weight.

Antioxidant capacity.

This trait was assayed in an extract (see above) of lyophilised tissue spectrophotometric analysis, using a modified Folin-Ciocalteu method (Singleton et al. 1974) and by the superoxide anion radical scavenging capacity, performed using electron spin resonance (ESR) spectrometry, as described by Kaneyuchi et al. (1999). The results of both assays were expressed as mg of chlorogenic acid equivalents per 100 g of dry weight.

FFC NMR relaxometry.

In order to deep knowledge about fast field cycling NMR relaxometry, the readers are addressed towards specific review papers (Kimmich and Anoardo, 2004; Ferrante and Sykora, 2005). All the samples were analysed in the solid state as reported in Conte et al. (2009). ^{1}H-NMRD profiles (i.e. relaxation rates R_1 or $1/T_1$ vs. proton Larmor frequencies) were acquired on a Stelar Spinmaster-FFC-2000 Fast-Field-Cycling Relaxometer (Stelar s.r.l., Mede, PV – Italy) at a constant temperature of 25°C. The proton spins were polarized at a polarization field (B_{POL}) corresponding to a proton Larmor frequency (ω_L) of 25 MHz for a period of polarization (T_{POL}) of 1 s. The longitudinal magnetization evolution were recorded at values of a relaxation magnetic field (B_{RLX}) corresponding to ω_L comprised in the range 0.01-20 MHz for a period of time (τ) arrayed with 128 values, chosen in an exponential progression from 2.3 to 233 ms. The exponential progression ensured the covering of the entire relaxation curve of interest. Finally, a ^{1}H 90° pulse was used at the starting of the acquisition period contemporarily to an acquisition magnetic field (B_{ACQ}) corresponding to a ω_L of 16.2 MHz. The observable magnetization was revealed as free induction decay (FID) with a time domain of 100 μs sampled with 512 points. 2 scans were accumulated. Data elaboration applied in this study has been already reported in Maccotta et al. (2013).

Results and Discussion

Phytochemicals

The amount of chlorogenic acid was generally reduced after grilling (-17 and -34 % for Buia and L305, respectively), except for the "Tunisina" sample, that showed an increase of 12% (Table 1). The boiling treatment of "Tunisina" and "Buia" resulted in a decrease of 19 and 27% respectively, while in "L305" it resulted in a diminution of 33 %. The other main important phenolic compounds in eggplant are related to the anthocyanins present in the peel: the grilling induces a diminution with respect to the RAW samples, with higher stability in "Tunisina" (-58 %), than "Buia" (-77 %) and "L 305" (-76 %). The boiling process induces a less reduction than grilling in anthocyanin content, with "Buia" and "L305" at -61 and -49 % content. "Tunisina", which synthetize nasunin, had a diminution of only 19 %, due to its higher stability for the higher protection of phenol OH with sugars and with *p*-coumaric acid, with respect to D3R.

Antioxidant capacity.

The Folin reducing index, whose values are shown in Table 1, resulted in a general increase in all samples after cooking, with the common trend of a difference between grilling and boiling. In fact, during grilling, TP were increased from 50 % in L305 to 91 % in Tunisina while the boiling process induced a lower increase than grilling, from 25 % in Tunisina to 36 % in L305. Concerning the superoxide anion scavenging (Table 1) the cooked samples have higher scavenging indexes than the raw ones. The largest increase (90 %) was found in grilled Tunisina. However, L305 showed no great variations in raw vs cooked superoxide scavenging (Table 1). On average, the increase in Folin index resulted of 49 %, conversely for superoxide scavenging it was of 33 %. It can be resumed in the fact that the main phenols of eggplant are subjected to a decrease with the cooking, while Folin and superoxide scavenging index have an increase.

Table 2 reports the NMR relaxometry parameters found by applying the model free analysis to the Bloembergen, Purcell and Pound (BPP) model (Kimmich and Anoardo, 2004) where α represents the high-field relaxation rate and β is a constant related to the dipolar interactions. τ_C is the correlation time which describes the random molecular motions of molecular systems either in solution or in porous media. Namely, correlation time is the time taken for a molecule to rotate one radian or to move a distance of the order of its own dimension. The longer the τ_C value, the slower the molecular motions, thereby revealing restrictions in the motional freedom degrees of spatially restrained molecular systems. Conversely, as a molecule encompasses faster motions due to higher degrees of freedom in larger spaces, shorter correlation time values are expected. As for the α index, the "Tunisina" and "Buia" show an increase in both cooked samples with respect to raw ones, with the strongest increases in "Buia". The same fact is in L305 only for GRILL, while the RAW and BOILED eggplant samples show practically the same values.

The cooked-induced decomposition of the eggplant structure causes a formation of little molecules that tend to a reciprocal estrangement, so decreasing the β values in cooked samples with respect to the raw ones. This fact is evident in "Tunisina" showing the main decreases, in "Buia" and only in the grilled samples of L305, thereby confirming the trend of the α indexes. The FFC NMR relaxation experiments have been made in solid phase. For this reason, the molecules present in the tissue are mainly subjected to rotational movements, being the traslational ones avoided by the physical status of the system. The cooking processes, yielding a significant number of molecules from the degradation of raw fruit tissues, gave a higher solvatation with the residual water, causing a strengthening and a subsequent hindrance in their movement, hence longer correlation times. This is exactly what happens in "Tunisina" and "Buia", with a higher increase in "Tunisina" for both cooking ways. In the L305 genotype, only the boiled sample shows the expected τ_C value increase with respect to raw one. It's evident a different behaviour of L305 in comparison with the other genotypes, also confirmed by previous chemical and antioxidant data.

The NMR relaxometry data confirmed that the cooking induces a process of decomposition of the eggplant tissue, resulting in an increase of extractable antioxidant compounds, not belonging to the measured eggplant phytochemicals. Besides the decomposition it is possible the presence of neo-formed antioxidants, such as the products of Maillard reaction: this fact can be possible in grilled samples, evaluated by a darken colour of fruit slices, but the boiling process can not sufficiently account for this fact. So, further studies are needed to better explain what happens in eggplant tissue after cooking.

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Table 1. Composition in chlorogenic acid, anthocyanin (ATH), Folin index and superoxide scavenging before (raw) and after two ways of cooking. Different letters in each column indicate significantly different values ($p<0.05$). The column "% variation" indicate for each genotype the loss (negative) and the increase (positive value) of cooked sample vs the corresponding raw one.

genotype	treatment	chlorogenic acid		% variation	ATH		% variation
		mg/100g dm			mg/100g dm		
Tunisina	raw	1376	abc		60.3	b	
Tunisina	grilled	1546	a	12	25.4	b	-58
Tunisina	boiled	1120	cd	-19	49.2	b	-19
Buia	raw	1540	ab		154.7	a	
Buia	grilled	1281	abc	-17	35.5	b	-77
Buia	boiled	1130	bcd	-27	60.0	b	-61
L305	raw	1265	abc		123.9	a	
L305	grilled	839	d	-34	30.3	b	-76
L305	boiled	852	d	-33	63.4	b	-49
Average				-20			-57
		Folin index		% variation	superox		% variation
		mg CA eq/100g dm			mg CA eq/100g dm		
Tunisina	raw	1574	c		1241	c	
Tunisina	grilled	3008	a	91	2360	a	90
Tunisina	boiled	1966	bc	25	1539	bc	24
Buia	raw	1578	c		1215	c	
Buia	grilled	2561	ab	62	1647	bc	36
Buia	boiled	2065	bc	31	1868	b	54
L305	raw	1345	c		1774	b	
L305	grilled	2015	bc	50	1671	bc	-6
L305	boiled	1826	bc	36	1783	b	1
Average				49			33

Table 2. NMR relaxation properties of raw and cooked tissues of eggplant. The column "% variation" indicate for each genotype the loss (negative) and the increase (positive value) of cooked sample vs the corresponding raw one.

		α	% variation	β	% variation	τ_c 10^{-8} (s)	% variation
Tunisina	raw	1.6		1463		0.395	
Tunisina	grilled	3.4	113	801	-45	1.000	153
Tunisina	boiled	4.4	175	788	-46	1.070	171
Buia	raw	0.4		1400		0.513	
Buia	grilled	14.9	3927	904	-35	0.881	72
Buia	boiled	4.0	981	1088	-22	0.718	40
L305	raw	4.3		773		0.604	
L305	grilled	14.8	244	587	-24	0.333	-45
L305	boiled	4.4	2	788	2	1.060	75

Establishing a core collection of chili germplasm using microsatellite analysis

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Abstract

Chili is a world economically important vegetable and spice. The Tropical Vegetable Research and Development Center, Kasetsart University, Kamphaeng Saen Campus has collected more than 2,000 chili germplasm accessions since 1989. High cost has been invested to maintain and evaluate such a large germplasm collection. For an efficient germplasm management and to reduce cost of germplasm maintenance, a core collection needs to be established. Therefore, the study was aimed to investigate the genetic diversity of the chili germplasm using microsatellites. Ten anchored microsatellites were used to evaluate 230 chili germplasm accessions. Forty two alleles were generated with size ranging from 87 to 323 base-pairs. The average Expected Heterozygosity (He) value was 0.62 ranging from 0.473-0.714, average Polymorphism Information Content (PIC) value was 0.57 ranging from 0.414-0.681, and Probability of Identity (PI) from 0.17-0.49. The combined PI of the ten microsatellite loci was 2.30×10-6. Similarity Index (SI) ranged from 0.18 to 1.00. The 230 chili accessions were divided into two major groups. Group I comprised mainly *C. annuum*, and group II comprised other *Capsicum* species including *C. frutescens*, *C. chinense* and *C. baccatum*. Matrix comparison showed that the cophenetic correlation of 0.81 indicated the best fit of the obtained dendrogram. PowerCore program selected 28 representative chili accessions to form a core collection, which maintained similar level of diversity as of the overall 230 chili accessions.

Keywords: *Capsicum*, chili pepper, diversity, SSR, simple sequence repeat

Agronomical and morphological characterization of a "Friariello" pepper ecotype collection

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Abstract

In the present study, the characterization of a "Friariello" pepper ecotype (*Capsicum annuum* L.) collection is described. In an open-field trial, carried out during spring-summer of 2012 in Battipaglia (Sele valley), a collection of 24 accessions were assessed for agronomic performances and morphological traits for a total of fifteen traits. All genotypes were grown in a completely randomized experimental design with three replicates and 10 plants for each plot. Results showed an average total yield of 1556.2 grams per plant, ranging from 705 (S1) up to 2724 grams (Torricello hybrid). The most late genotypes were generally the most productive. Average fruit weight was 14.9 grams, ranging from 7.4 up to 25.1 grams (S1 and N6 accessions, respectively). Four ecotypes (N3, N8, N11, T1) and two hybrids (Torre and Tenerello) resulted the most promising genotypes in terms of total yield/plant, marketable yield/total yield ratio (up to 93%), size and shape uniformity of fruits. The morphological characterization allowed to identify three different types of "Friariello" pepper, commonly called "Torrese or Napoletano" (8 accessions), "Nocerese or Frigitello" (13 accessions) and "Sigaretta" (3 accessions). In order to authenticate "Friariello" pepper and to combine molecular data with those of DUS test with the aim to release new varieties, biochemical and molecular characterization of the same genotypes is underway.

Keywords: Accessions, Field trials, Local varieties

Introduction

A large number of pepper ecotypes carrying peculiar organoleptic characteristics and highly appreciated by consumers, are widespread in Italy (Portis et al. 2006). In order to preserve this useful germplasm as source for breeding programs, previous studies have been conducted (Nervo 1997) with the aim to obtain novel varieties improved for resistance and quality traits.

The sweet pepper "Friariello" (*Capsicum annuum* L.) is one of the most interesting ecotypes developed in southern Italy. This vegetable is cultivated both in open field and in protected environment in Campania region, in a wide area extended from Salerno to Napoli.

"Friariello" is represented by three typologies of fruits well known as "Nocerese", "Torrese or Napoletano" and "Sigaretta". The distinction of these types is based on typical morphological traits including the presence of the lobes (from one to three) at blossom-end. "Friariello" pepper represents a specialty of Neapolitan cuisine and is well appreciated by consumers for the high digestibility and the strong flavour. Fruits are put on the market still unripe, when the color is bright green, and the shape changes from trapezoidal to horn-shaped. Consumer preferences include fresh consumption in salads or fried with tomatoes.

In the present work a collection of 24 "Friariello" pepper accessions were assessed for agronomical and morphological traits comparing local types to commercial varieties.

Materials and Methods

A selection of 19 traditional accessions and of 5 commercial varieties were analysed in an open field located in Battipaglia (Sele valley) during spring-summer 2012. Seeds were supplied by seed

companies and by the farms located in the Agro Nocerino-Sarnese area (Salerno province) (Parisi et al. 2013). These peppers, which represent a collection of the three common types ("Torrese" or "Napoletano", "Nocerese" and "Sigaretta") were compared to similar types originating in Italy ("Lombardo" and "Corno di capra" ecotypes) and Spain (Padròn F1 hybrid).

Seedlings were transplanted in single rows (110 cm between rows and 40 cm in the row) at the end of May. Irrigation, plant protection and weed control were carried out according to local practices and the cultivation techniques included stakes as support and galvanized wires. Field trials were conducted in a completely randomized experimental design with three replicates and 10 plants. Four harvests (August 3 and 27, September 20, October 24) were performed. Agronomic performance were analyzed in all accessions except the similar types; the traits scored included total yield and its components (commercial and unmarketable) (g/plant), earliness considering the first two harvests (%), fruit weight (g), shape and size uniformity of the fruits (score from 1 to 10). The last two measurements were also carried out on the three similar "Friariello" types.

In order to highlight the variability in the "Friariello" collection, a morphological characterization was carried out using the DUS-TEST protocol (CPVO-TP/076/2 Final). The fruit characteristics analyses were: intensity of color before maturity, anthocyanin coloration, shape in longitudinal and in cross section, sinuation of pericarp including and excluding the basal part, glossiness, number of locules, thickness of flesh, aspect of calyx, shape of apex. The last trait was assessed using an arbitrary scale (1= apex with one lobe; 2= apex with one and two lobes; 3= apex with two and three lobes).

All agronomic data were analysed using ANOVA adopting the statistical software GenStat, Version 12. Mean comparisons were performed by Duncan's test. Cluster analysis based on 15 morphological traits, including 3 quantitative and 12 qualitative and pseudo-qualitative characteristics (CPVO-TP/076/2 Final), was performed using the computer package XLSTAT 2012.1. Similarities between genotypes were estimated using Ward's coefficient.

Results

For morphological traits, dendrogram separated the genotype into two main clusters (Fig. 1). The first, subdivided into two more sub clusters, included all "Friariello Nocerese" types, two "Torrese" hybrids (Torricello F1, Torre F1) and the Italian similar type Corno di Capra. The second main cluster, which included the other typologies, was subdivided into two subgroups: the first one contained the similar types Padròn and Lombardo, the second one included the three "Sigaretta" (S3, S1, S2) and the "Torrese" types.

Results for productive traits (Tab. 1), showed the average total yield/plant of 1556.2 grams, ranging from 705 grams up to 2724 grams (N9 and "Torricello", respectively). All hybrids as well as 11 of the 19 tested accessions, showed values higher than average both for total yield than for commercial yield (Tab. 1). Earliness showed an average of 57.4% with the highest values for the accessions T3 and S1/S3 (respectively 64.0% and 62.7%). Overall results showed that late genotypes were generally the most productive ("Torricello" hybrid and the N2, N5, N8 and N10 accessions).

For merceological quality-traits, results showed an average in size homogeneity of the fruits of 8.0, ranging from 6.3 to 8.9 (N7 and T2, respectively) whereas shape homogeneity was on average 8.3, ranging from 5.9 to 9.5 (N9 and S1/S3, respectively). Other accessions (T5, N10, T4, S1, N8, N4, N2, T3 and T2) and the hybrid "Tenerello", showed good size and shape uniformity, with the highest values for these two traits. Fruit weight was on average 14.9 grams with the highest values for the "Nocerese" types (Tab. 1). In the "Torrese" type, hybrids showed greater fruit weight than the not improved accessions. On the other hand, for the "Nocerese" type, the accessions N1, N2, N5 and N6 had fruit weight similar or greater than Vesuvio F1 and Dolcetto F1.

Discussion

Morphological characterization allowed to well discriminate the three typologies under study (see also Parisi et al. 2013), including the similar typologies Padròn F1 and Lombardo.

Four ecotypes (N3, N8, N11 and T1) and two hybrids ("Torre" and "Tenerello") resulted to be the most promising genotypes in terms of total yield/plant, marketable yield/total yield ratio (up to 93%), size and shape uniformity of fruits. Several local genotypes resulted to be very competitive in comparison with commercial hybrids.

Despite the collection under study has interesting productivity and quality features, tested genotypes need however, the introgression of the resistance to the principal viruses (TSWV, CMV, PVY and *Tobamovirus*), which cause serious damage both in open field and protected environments. To our knowledge, few efforts have been done until now by seeds companies.

Such breeding program is underway in our Institute and will pursue the maintenance of the typical characteristics of the "Friariello" in terms of fruit morphology (weight and shape), organoleptic properties (volatile substance content) and quality (vitamins). Furthermore, in order to authenticate "Friariello" properties, biochemical and molecular characterizations is underway.

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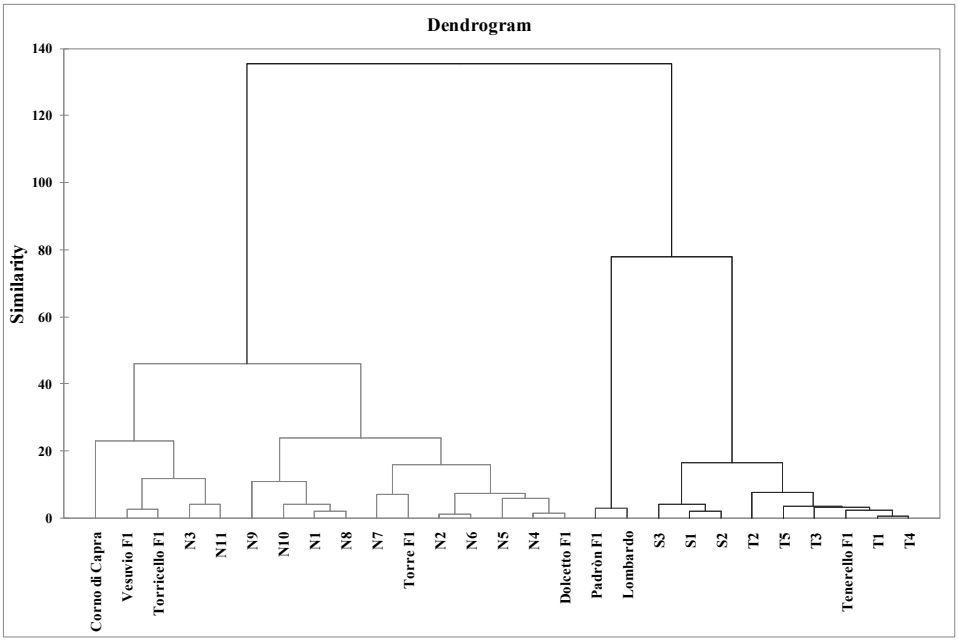

Figure 1: Dendrogram obtained for the morphological traits

Table 1 – Productive traits analyzed on "Friariello" pepper collection

Genotype	**Yield**						**Fruit**					
Friariello	**total** *g/plant (1)*		**commercial** *g/plant*		**earliness** *%* *(2)*		**homogenity** **size** *(3)*		**shape** *(3)*		**weight** *g*	
Nocerese-type												
N1	1625.0	cdef	1500.0	cdef	58.3	cde	7,8	c	8,3	df	18,5	ghi
N2	1656.0	cdef	1555.0	cdef	52.0	abcd	8,8	gh	8,5	fg	18,6	ghi
N3	1868.0	def	1753.0	def	55.8	bcde	8,2	cde	8,2	cdef	16,0	ef
N4	1655.0	cdef	1576.0	cdef	58.6	cde	8,7	fgh	8,3	def	15,6	e
N5	1856.0	def	1653.0	cdef	42.8	a	7,2	b	7,2	b	20,1	i
N6	1928.0	ef	1790.0	ef	57.1	cde	7,3	b	7,8	cd	25,1	j
N7	1022.0	ab	874.0	ab	59.0	cde	6,3	a	7,2	b	15,7	e
N8	2007.0	f	1877.0	f	51.7	abcd	8,5	efgh	8,5	fgh	16,7	efg
N9	705.0	a	612.0	a	58.6	cde	6,5	a	5,9	a	11,9	cd
N10	1782.0	def	1622.0	cdef	53.4	abcde	8,3	cdef	8,6	fghi	16,1	ef
N11	1719.0	cdef	1627.0	cdef	55.0	bcde	8,7	fgh	7,8	cd	17,5	efgh
Dolcetto F1	2062.0	f	1957.0	f	58.6	cde	8,4	defg	7,8	c	18,2	ghi
Vesuvio F1	1796.0	def	1593.0	cdef	58.7	cde	7,3	b	7,8	cde	19,0	hi
Mean	***1667.8***		***1538***		***55.4***		***7.8***		***7.8***		***17.6***	
Torrese-type												
T1	1598.0	cdef	1516.0	cdef	50.1	abc	8,5	efgh	8,2	cdef	10,6	bc
T2	1379.0	bcd	1271.0	bcd	60.7	cde	8,9	h	9,3	jklm	9,7	b
T3	1244.0	bc	1171.0	bc	64.0	e	8,8	gh	9,0	gij	9,5	b
T4	1408.0	bcde	1336.0	bcde	59.3	cde	8,3	def	9,0	ghij	11,2	bcd
T5	1080.0	ab	1024.0	ab	60.6	cde	8,2	cde	8,7	fghi	9,2	ab
Tenerello F1	1820.0	def	1688.0	def	57.6	cde	8,5	efgh	8,3	def	13,1	d
Torre F1	2047.0	f	1974.0	f	60.0	cde	8,0	cd	9,0	ghijkl	18,0	fgh
Torricello F1	2724.0	g	2564.0	g	45.5	ab	7,2	b	8,3	def	19,6	hi
Mean	***1662.5***		***1568.0***		***57.2***		***8.3***		***8.7***		***12.6***	
Sigaretta-type												
S1	926.0	ab	862.0	ab	62.7	de	8,3	def	9,5	jm	7,4	a
S2	1679.0	cdef	1600.0	cdef	53.7	bcde	7,3	b	9,5	jkm	10,8	bc
S3	1410.0	bcde	1315.0	bcde	62.7	de	8,0	cd	9,0	ghijk	9,3	ab
Mean	***1338.3***		***1259.0***		***59.7***		***7.9***		***9.3***		***9.2***	

Legend: (1) Genotypes with different letters (a-k) are statistically different (P <0.01) for the trait considered; (2) first two harvests / four total harvests ratio (%); (3) minimum value = 1; maximum value = 10

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Pre-fertilization and post-fertilization interspecific hybridization barriers between *Capsicum* L. species.

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Abstract

Capsicum annuum, *C. frutescens*, and *C. chinense* do not hybridize readily with *C. baccatum,* preventing the gene transfer and indicating the presence of barriers to fertilization. The objective of this study was to evaluate the type of barrier to fertilization, pre-fertilization or post-fertilization, by evaluating pollen grain germination, pollen tube growth *in vivo*, fruit set, seed set, and pollen grain viability. To verify the occurrence of pre-fertilization barrier, hand pollinated pistils were collected at 2, 4, 6, and 8 hours after pollination (HAP), fixed in FAA, softened with 8N NaOH, stained with 0.1% aniline blue in 0.1 M K_3PO_4, and observed under a fluorescent microscope. For post-fertilization barriers evaluation, reciprocal crosses were done by hand pollinations and it was verified the fruit set, the seed development, and the pollen grain viability of the hybrid. It was observed that in the combinations between *C. annuum*, *C. frutescens* and *C. baccatum*, the pollen grains germinated and pollen tube grown toward the ovule. However, in the combination *C. chinense* x *C. baccatum* most of the pollen grain did not germinated and the germinated ones had slow growth 8 HAP, suggesting that in this combination there is a pre-fertilization barrier. All reciprocal crosses had fruit set and seeds even though most of them were empty or plump seeds probably due to the endosperm degeneration. The pollen grain viability of hybrids varied from 15.5% to 50.1% indicating some degree of male sterility of the combination. These results suggest that in hybrids between *C. annuum, C. frutescens* and *C. baccatum* there are two post-fertilization barriers, the seed abortion and pollen sterility; but, in combinations *C. chinense* and *C. baccatum* there is also a pre-fertilization barrier. The results show that the better results were obtained when *C. baccatum* was used as female parent.

Keywords: Sterility, pollen grain viability, pollen grain germination**.**

Introduction

Interspecific hybridization is an important tool in plant breeding, because the breeder attempts to transfer genes from one genetic material, usually a wild species, to a cultivated form deficient in this gene (Hodgin and Hajjar 2007; Ito et al. 2011). However, the crossing between species may be incongruent or incompatible due to the existence of barriers, pre and post-fertilization. As pre-fertilization barriers we have the lack of pollen grain germination or delay/inhibition of pollen tube growth. After fertilization, the main barriers are the embryo abortion due to endosperm degeneration or partial sterility of hybrid plants (van der Valk et al. 1991; Satya 2012). These barriers have prevented the use of wild species carrying important genes in breeding programs by using interspecific hybridization.

The *Capsicum* domesticated species (2n = 2x = 24 chromosomes) can be grouped into gene pool based on the crossability or hybrid viability. The annuum Complex consists of the *C. annuum*, *C. frutescens*, *C. chinense*, *C. chacoense* and *C. galapagoense*; baccatum Complex consists of *C. baccatum* and *C. tovari*, and the *pubescens* Complex is formed by the *C. pubescens*, *C. cardenasii*, and *C. eximium* (Pickersgill 1997; Ibiza et al. 2011). Interspecific hybrids involving *Capsicum* species belonging to different gene pool are difficult to be obtained due to incongruity, unilateral incompatibility, embryo abortion, and male sterility (Pickersgill 1997). The species *C. baccatum* is considered important for breeding pepper because it has resistance genes absent in the pepper (Potnis et al. 2012). Considering the importance of the *C. baccatum* for pepper breeding, this study

was done to evaluate the occurrence of barriers pre-and post-fertilization in interspecific hybrid from crosses between *C. annuum*, *C. frutescens*, and *C. chinense* with *C. baccatum* by pollen grain germination, pollen tube growth *in vivo*, fruit set, seed development, and hybrid pollen viability.

Material and Methods

Five accessions from *C. annuum*, *C. chinense*, *C. frutescens* and *C. baccatum* were used. Seeds from them were put to germinate and then were transplanted to pots and cultivated under greenhouse. For pre-fertilization barrier study, crosses were done by hand pollination using pollen grains/anther from donor parent at anthesis and put on the stigma of the recurrent parent (female). The hand pollinated pistils were collected 2, 4, 6 and 8 hours after pollination (HAP) and were fixed in FAA solution, softened in 8n NaOH, stained with 0.1% aniline blue in 0.1 M K_3PO_4, and observed under fluorescent microscope. For post-fertilization evaluation, 24 to 48 hours after crosses, by hand pollination, the fruit set was verified by fruit fixation. It was registered the number of crosses by combination, the number of fruits set, the number of aborted or plump seeds per fruit. The hybrid seeds were manually extracted from the fruits, dried and stored at room temperature. Later the hybrid seeds were sown in pots at greenhouse. At flowering, the hybrid pollen grain viability was tested by triple solution. To do that, flower buds, at anthesis, were collected in 70% ethanol solution and anthers were squashed in drops of triple solution (malachite green, orange G and acid fuchsin). After that, slides were observed under an optical microscope and five slides were prepared for each hybrid and parents. Two hundred and fifty pollen grains, classified as viable pollen grains (purple) and non viable pollen grains (green), were counted per slide.

Results and Discussion

All hybrids showed pollen grain germinated, but there was a different response depending on the combination. Two hours after pollination, pollen grains germinated and pollen tube growth *in vivo* were observed in hybrids combinations between *C. annuum* and *C. baccatum*; in *C. frutescens* x *C. baccatum* pollen grain germination was observed 4 HAP and pollen tube were observed at the style, but in *C. chinense* x *C. baccatum* pollen grain germination and pollen tube growth were slow, suggesting some kind of pre-fertilization barrier in this combination. The self pollination of parent accesses showed normal pollen grain germination and pollen tube growth. The pre-fertilization barriers includes differences in pollination timing and duration, altered communication resulting from rejection of non self-pollen, malformed shapes, sizes and disturbed behavior of pollen tube growth, deposition of callose and non-recognition of pollen tube by female gametophyte (Satya 2012; Chapman and Goring 2010). In *C. chinense* x *C. baccatum* combination the most important feature of pre-fertilization barrier was the slow growing of pollen tube.

The fruit and seed set were observed for all hybrids (Table 1). Ninety-five crosses were done between *C. annuum* and *C. baccatum* and reciprocal and 22 out of 52 fruits had well formed seeds and the others were empty fruits or had plump seeds. Seven hybrid combinations reached the flowering season. In the *C. frutescens* x *C. baccatum* and reciprocal combination 117 crosses were done and 53 fruits were obtained, but only 15 had well formed seeds. Five hybrids were obtained from this combination. In the *C. chinense* x *C. baccatum* combination, 69 crosses resulted in seven fruits with seeds and thirty-two without seeds but only one hybrid combination reached the maturity. So, the setting rate varied from 23 to 10% indicating that fruits were obtained in the combination and better results were obtained when *C. baccatum* was the female parent (Table 1). The most remarkable feature in the hybrid combinations was the empty fruits or plump seeds; there was a high frequency of empty seeds or high aborted embryo due to endosperm degeneration. Yoon et al. (2006) also reported in *C. annuum* x *C. baccatum* hybrid a variation in the embryo development and they observed that embryo abortion occur before the globular stage.

Table 1. Number of crosses made (NC), number of fruit with seeds and setting rate (NFS and SR), number of fruits without seeds (NFWS), number of survived plants (NSP) and pollen grain viability (PGV) for crosses made between C. *annuum*, *C. frutescens*, and *C. chinense* with *C. baccatum.*

Crosses	NC	NF		NSP	PGV (%)
		NFS (SR)	NFWS		
C. annuum x *C. baccatum*	44	11	14	04	34,0
C. baccatum x C. annuum	51	11	16	03	35,0
	95	**22(23)**	**30**	**07**	
C. frutescens x *C. baccatum*	40	05	21	01	15,5
C. baccatum x C. frutescens	77	10	17	04	50,1
	117	**15(13)**	**38**	**05**	
C. chinense x *C. baccatum*	27	03	16	00	-
C. baccatum x *C. chinense*	42	04	16	02	25,0
	69	**07 (10)**	**32**	**02**	

Egawa & Tanaka (1986) observed 6.5% for pollen viability for *C. annuum* x *C. baccatum* hybrid; Kumar et al. (1987) observed 42.6% on the *C. baccatum* x *C. annuum* var. *cerasiformis* and Bapa Rao et al. (1992) observed, on average, 24.5% of pollen viability for *C. baccatum* x *C. frutescens* hybrid. The pollen grain viability for all combinations varied from 15.5% to 50.1% indicating some degree of male sterility. According to Shifriss (1997) the degree of male sterility in hybrids between *C. annuum* and *C. baccatum* is variable and depends on the parental forms used in the hybrid combination. These results can be explained by the lack of genomic affinity between parental genomes since the genomic similarity facilitates recombination during meiosis (Techio & Davide 2007).

Usually the low pollen grain viability reflects the meiosis of the hybrid. The metaphase I pairing is used for genome analysis or phylogenetic relationship among species based on the assumptions that only similar chromosomes pairing during meiosis, that similar chromosomes are homologous, and hence, that the extent of chromosome pairing in hybrids reflects the degree of relationships between parental species (Segerb & Petersen 1998). However, Lee et al. (2011) observed that the genome analysis and the interaction of genetic factors, but no chromosome number nor karyotype similarity, determine the pairing behavior in *Paphiopedilum* hybrids. In *Capsicum* the low pollen viability of the interspecific hybrids might be explained by lack of chromosome pairing, structural repartitioning of chromosomes, erratic meiotic behavior, genes for sterility, segregational imbalances following intergenomic recombination (Egawa & Tanaka, 1986; Kumar et al. 1987; Bapa Rao et al. 1992).

Based on the results, there are two barriers of fertilization in interspecific hybrids among *Capsicum*: in *C. annuum* and *C. frutescens* with *C. baccatum* it was observed a post-fertilization barrier, embryo abortion and pollen sterility; in combinations between *C. chinense* x *C. baccatum* besides these two barriers, it was also identified one pre-fertilization barrier due to slow growth of tube pollen grain in the pistil. Those kind of barriers are reported for *Capsicum* (Yoon et al. 2006) as well as to others species such as *Hibiscus cannabinus* (Satya, 2012) and *Allium* sp. (Van de Valk et al. 1991). However, the barriers are not complete, so the plant breeder might use different approaches, as embryo rescue and/ovule culture, to overcome the barriers.

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Morphological characterization of common (*Solanum melongena*), gboma (*S. macrocarpon*), and scarlet (*S. aethiopicum*) eggplants from Africa

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Abstract

Africa is home to many local varieties of common (*Solanum melongena*), gboma (*S. macrocarpon*), and scarlet (*S. aethiopicum*) eggplants. Here we present the results of a morphological characterization of 29 African eggplant accessions from the European Eggplant Genetic Resources Network (EGGNET) collection, of which 11 correspond to *S. melongena*, 12 to *S. aethiopicum* (seven of group Gilo, four of group Shum, and one of group Kumba) and six to *S. macrocarpon*. The *S. melongena* Spanish variety 'Listada de Gandía' and the French line LF3-24 were used as controls. All the African accessions of the three species presented a good adaptation to our conditions. Characterization with EGGNET descriptors showed that the three species can be easily distinguished by a number of morphological traits. *Solanum aethiopicum* Gilo, Shum, and Kumba groups also present considerable morphological differences. A wide diversity is found within each of the species (and also within *S. aethiopicum* groups) for vegetative and fruit traits indicating that African eggplant germplasm is highly variable. Some accessions of *S. melongena* and *S. aethiopicum* with high yield have been identified. This work provides valuable information regarding the diversity of traits of agronomic interest in African eggplants, which is of interest for the selection and breeding of the three eggplant crops.

Keywords: breeding, descriptors, EGGNET, genetic resources, landraces.

Introduction

Apart from the common eggplant (*Solanum melongena* L.), two other domesticated eggplant species, namely the scarlet (*S. aethiopicum* L.) and the gboma (*S. macrocarpon* L.) eggplants are grown in Africa (Daunay et al. 1997; Schippers 2000). The African local varieties of these three species represent genetic resources for their genetic improvement. Furthermore, as the three species can be intercrossed with different degrees of success (Daunay 2008; Oyelana and Ugborogho, 2008), genes from one species may be introgressed into the genetic background of the others.

African *S. melongena* accessions correspond to group H within the "eggplant complex", which is characterized by vigorous plants with large fruits and few prickles (Daunay 2008). Regarding *S. aethiopicum*, this is a highly variable crop native to Africa, and for which four groups (Gilo, Shum, Kumba and Aculeatum) have been recognized depending on their morphology and uses (fruit, leaves, ornamental) (Lester et al. 1986). *Solanum macrocarpon* is less variable than *S. aethiopicum* and is used for their fruits and also for the leaves, which are also edible (Schippers, 2000).

Here, we study the morphological diversity in a collection of African *S. melongena*, *S. aethiopicum* and *S. macrocarpon* from the European Eggplant Genetic Resources Network (EGGNET) collection. The results will provide information on the diversity of the three crops and will allow identifying accessions with desirable characteristics, which is relevance for selection and breeding programmes.

Materials and Methods

The plant material used corresponds to accessions from West Africa of the RNL (for Richard Neville Lester) series, which are part of the EGGNET collection (Daunay et al. 2006; van der Weerden and Barendse 2007). A total of 29 RNL accessions, of which 11 correspond to *S. melongena*, 12 to *S. aethiopicum* (seven of group Gilo, four of group Shum, and one of group Kumba), and six to *S. macrocarpon* were used (Table 1). As controls we used the *S. melongena* INRA line LF3-24 and the Spanish local variety 'Listada de Gandía' (Clemente Seeds, Vitoria, Spain).

Plants were grown in the open field during the summer season in an experimental plot of the Universitat Politècnica de València. Plants were spaced 1.2 m between the rows and 0.5 m apart in the rows and drip irrigated. The standard horticultural practices for eggplant cultivation in the area of Valencia were followed. For most accessions 10 plants were evaluated. However, due to poor germination, for a few of them less than 10 plants were available. Plants were distributed according to a completely randomized design.

Accessions were characterized using the EGGNET descriptors for primary characterization of eggplants (Prohens et al. 2005; van der Weerden et al. 2007). For each accession, seven vegetative traits and 29 inflorescence and fruit traits were measured.

Results and Discussion

As in other trials made under climatological conditions similar to ours (Polignano et al. 2010; Sunseri et al. 2010), under the Valencia conditions the African accessions of the three species grew successfully and produced fruits. Regarding the uniformity within accessions, it was quite high, corresponding to the mostly autogamous reproduction of the three species (Daunay et al. 1997). However, for one of the *S. melongena* accessions (RNL548) we found two types of plant and fruit, and therefore we subdivided it into two new accessions (RNL548A and RNL548B).

In general, *S. melongena* accessions were taller than those of *S. aethiopicum* and *S. macrocarpon*. Regarding other morphological traits of interest, *S. aethio*picum groups Shum and Kumba and *S. macrocarpon*, in which the leaves are frequently consumed (Schippers 2000), had low pilosity, in particular in the case of *S. macrocarpon*. The accessions of the three species presented no prickles in the leaves. Also, *S. melongena* presented longer fruit pedicels and larger fruits than *S. aethiopicum* or *S. macrocarpon*.

When considering flowering earliness, African accessions of *S. melongena*, and *S. aethiopicum* Gilo and Kumba presented earliness values similar to the *S. melongena* controls (±10 days of difference), which is in agreement with the results obtained by Polignano et al. (2010). However, *S. aethiopicum* Shum flowered earlier (up to 20 days) and *S. macrocarpon* flowered later (up to 20 days) than the controls. The evaluation performed allowed the identification of two *S. melongena* accessions (RNL019 and RNL580) with a yield higher than the control accessions. Also, *S. aethiopicum* Gilo accessions RNL255, RNL288 and RNL395 presented high yield, similar to that of the *S. melongena* controls. This shows that selection of high yielding *S. aethiopicum* may be feasible within the available germplasm (Sunseri et al. 2010). Hybrids between these high yield accessions may also be heterotic for yield (Lester and Thitai 1989).

A considerable diversity for fruit traits was found by Polignano et al. (2010) within the African *S. melongena* accessions. In our case, we also found a wide diversity, with fruit shapes from round to long and a wide diversity of colours at the commercial ripening stage, ranging from white to purple black, and including green types (Table 1). Some accessions presented uniform fruit colour, while others displayed additional colour, with a mottled or stripped distribution. Fruit flesh is white, green, or intermediate and, in general, the calyx either did not present prickles or present few and small prickles

(Table 1). In general, the variation for fruit traits was similar to that found for Spanish local varieties of *S. melongena* (Prohens el al. 2005).

Although *S. aethiopicum* fruits are smaller than those of *S. melongena* (Daunay et al. 1997), as has been found in other studies (Lester et al. 1986; Polignano et al. 2010; Sunseri et al. 2010), we found a wide variation for fruit size (Table 1). In this respect, fruits of group Shum were very small, which is in agreement for their exclusive use for the leaves (Lester et al. 1986; Scippers 2000). The fruits of the *S. aethiopicum* Gilo and Kumba accessions were considerably larger than those of group Shum. The fruits of the Kumba accession were flattened with many locules and with deep grooves, while those of the Gilo accessions presented different shapes, but with less locules and less flattened than those of the Kumba accession (Lester et al. 1986). One of the Gilo group accessions (RNL398) presented fruits considerably larger than those of the rest of Gilo group accessions (Table 1). Fruits of the *S. aethiopicum* accessions were green coloured at the commercially ripe stage, except for some Gilo accessions, in which it was white. Fruit flesh colour at the ripening stage was yellowish or white.

Fruits of *S. macrocarpon* were considerably smaller than those of *S. melongena* (Daunay et al. 1997; Polignano et al. 2010), although there were important differences in fruit size among accessions (Table 1). However, *S. macrocarpon* accessions were less diverse for fruit shape (flattened and without grooves) than the two other species. Fruit colour at the commercially ripe stage was green or white and the fruit flesh was white, with the exception of RNL367, in which it was green.

The results indicate that the collection of African accessions studied present a good adaptation to our growing conditions, and in the case of *S. aethiopicum* and *S. macrocarpon* they could be introduced successfully as new crops in our region. As already found in other studies (Polignano et al. 2010; Sunseri et al. 2010), a wide diversity has been found for the three species. We have also been able to identify some accessions with good agronomic and fruit characteristics, which is of interest for the selection and breeding of the three cultivated eggplant species.

Acknowledgements

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Table 1. Values for several fruit traits of interest in the African (RNL codes) accessions evaluated corresponding to the common (*S. melongena*), scarlet (*S. aethiopicum*), and gboma (*S. macrocarpon*) eggplants. Accessions LF3-24 and Listada de Gandía correspond to the controls.

	Pedicel length (cm)[a]	Length (cm)[a]	Breadth (cm)[a]	Predominant colour	Additional colour
Solanum melongena					
RNL019	6.2	18.5	6.3	Milk white	Yes (Yellowish)
RNL203	6.5	14.6	5.3	Green	Yes (Milk white)
RNL541	4.6	11.0	12.4	Purple	No
RNL543	3.4	10.8	10.1	Lilac grey	Yes (Green)
RNL545	4.2	11.0	12.4	Green	Yes (Lilac grey)
RNL547	3.2	12.3	7.6	Purple	Yes (White)
RNL548A	4.6	16.2	4.8	White	No
RNL548B	3.7	14.0	7.9	Purple	No
RNL577	5.0	11.5	11.5	Purple black	No
RNL578	5.5	12.0	7.2	Purple black	No
RNL579	7.7	18.7	5.0	Purple black	No
RNL580	8.0	14.1	8.2	Purple	Yes (Milk white)
LF3-24	8.5	17.7	5.8	Purple	No
Listada de Gandía	5.6	20.3	12.4	Purple	Yes (Milk white)
Average SE	*0.3*	*0.5*	*0.4*	---	---
Solanum aethiopicum Gilo group					
RNL252	2.3	2.8	2.6	Green	No
RNL255	1.7	4.8	2.3	Green	Yes (Greenish white)
RNL288	1.7	5.1	4.9	Milk white	No
RNL296	2.0	4.3	7.2	Milk white	No
RNL392	2.2	4.1	5.6	Milk white	No
RNL395	1.8	2.9	4.3	Green	No
RNL398	3.2	9.7	10.0	Milk white	No
Average SE	*0.1*	*0.3*	*0.3*	---	---
Solanum aethiopicum Shum group					
RNL022	1.3	1.1	1.6	Green	Yes (Dark green)
RNL023	0.9	1.2	1.5	Green	No
RNL340	1.2	1.4	2.0	Green	No
RNL343	1.0	1.2	1.3	Green	No
Average SE	*0.2*	*0.1*	*0.1*	---	---
Solanum aethiopicum Kumba group					
RNL187	1.8	2.3	4.4	Green	No
Average SE	*0.1*	*0.1*	*0.1*	---	---
Solanum macrocarpon					
RNL016	1.5	3.3	4.1	Green	Yes (Dark green)
RNL367	3.3	4.7	8.4	Milk white	No
RNL371	1.9	3.2	6.4	Green	Yes (Dark green)
RNL372	2.0	4.1	6.5	Milk white	No
RNL373	1.7	3.5	4.3	Green	Yes (Dark green)
RNL374	2.0	3.7	5.8	Milk white	No
Average SE	*0.1*	*0.1*	*0.2*	---	---

Morphological characterization of some local and commercial varieties of pepper (C*apsicum annuum* L.) threatened by genetic erosion [Campania]

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Abstract

In the "Vallo di Diano", a small district with a strong horticultural vocation in the province of Salerno, sixteen local varieties of peppers were rescued.

Although local farmers raised of a period of twenty years, they are highly threatened by genetic erosion. As they are plant genetic resources particularly important in the local context, in order to protect and preserve their distinctive features, the objective of this work was their entry in the register of conservation according to Italian Legislative Decree 149 of 29 October 2009.

For this purpose, a morphological characterization of these resources was made using the Bioversity International guidelines (Descriptors for *Capsicum*) evaluating the homogeneity of each variety according to the parameters of the European Directive 2003/90/EC.

At the experimental farm of the Research Centre for Vegetable and Ornamental Crops of Pontecagnano (SA), hereinafter referred to as CRA-ORT, the rescued material was grown in pots and placed in a greenhouse according to a randomized scheme with three replications.

The analyzed varieties were all devoid of capsaicin, they had a red color at maturity and as a function of the shape of the fruit grouped into three types: eight varieties of elongated shape, four triangular, and two with blocky-shaped fruits. All varieties were homogeneous except two that presented a mixture in the shape of the fruits.

Keywords: local varieties, pepper, morphological characterization, fruit.

Introduction

Local germplasm of pepper from researchers of CRA-ORT was rescued from a small area of the Region of Campania. To safeguard them by genetic erosion it was decided to collect and preserve their seeds in National seed bank of CRA-ORT. In the past year a research activity of genetic-morphological was undertaken to assess these lines in order to insert them in the Catalogue of Conservation of local varieties.

In this context we will show the work done on the morphological characterization of sixteen local varieties of *Caspicum annuum* L. threatened by genetic erosion.

Materials and Methods

Plant Material

Sixteen varieties of local germplasm of pepper (table 1) were examined morphologically in the spring 2012. During this activity these materials were compared with commercial varieties (table 2) to evaluate the grade similarity that existed between them.

Twelve plants per variety were transplanted during March 2012 in pots placed on metallic pillars distant from each other 0,5m and completely randomized. The experimental test was done in a greenhouse with irrigation and fertilization monitoring.

Morphological Observations

Observations were made on fruits of all the plants recording their traits (table 3) according to the rules of the Bioversity International guidelines (Descriptors for *Capsicum*). In every variety the assessment of the homogeneity was made in according to European Directive 2003/90/EC.

Data set of genotypes and traits were displayed by scatterplot matrix and then a principal component analyses was undertaken. Numerical analyses and graphs were performed using the R environment (R Core Team, 2012) and their contributed packages *lattice* (Cleveland W.S., 1993), *BiplotGui* (la Grange, 2013).

Table 1. List of local varieties rescued in Vallo di Diano and tested at the CRA-ORT

Code	Local variety	Locality	Farm
7	Cornetto di Teggiano	Teggiano (SA)	Granada Fabio
8	Pupanetto per aceto di Teggiano	Teggiano (SA)	Granada Fabio
9	Pupanetto per aceto di Teggiano tipo 1	Teggiano (SA)	Granada Fabio
11	Pupanetto G.Mazza	Teggiano (SA)	Mazza Giovanni
6	Cornetto buccinese corto	Buccino (SA)	Catone Donata
16	Cornetto dolce Sala	Sala Consilina (SA)	Le acacie
18	Cornetto dolce Sala 106	Sala Consilina (SA)	Le acacie
19	Cornetto in su tipologia 36	Teggiano (SA)	Le acacie
3	Sciuscillone eco 2 122	Teggiano (SA)	Mazza Giovanni
30	Sciuscillone Mazza	Teggiano (SA)	Mazza Giovanni
12	Corno di capra Controne Ferrante	Controne (SA)	Ferrante Michele
13	Corno di capra Controne 2009	Controne (SA)	Ferrante Michele
22	Friariello 2009	CRA-ORT	
13	Corno di Capra Paolo 2009	Polla (SA)	Maltempo Paolo
21	Papaccella	Buccino (SA)	Catone Donata
40	Peperone melanzana Grippo	Caggiano (SA)	Grippo Gerardo

Table 2. List of commercial variety as a test control

Code	Commercial variety	Society	Local variety to control with test
TR	Trottolino amoroso	La Semiorto	Cornetto buccinese corto
CDC	Corno di capra	Carlo Cupo	Corno di capra Controne Ferrante, Corno di capra Controne 2009
TOR	Friariello selection Torre	Nunhems	Friariello 2009
PA	Patroclo	ISI Sementi	Sciuscillone Mazza
VES	Friariello selection Vesuvio	Nunhems	Friariello 2009
SI	Sigaretta	La Semiorto	Cornetto dolce Sala, Cornetto dolce Sala 106, Cornetto di Teggiano
TOP	Topepo	La Semiorto	Papaccella
TYR	Tyrex f1	Nunhems	Pupanetto per aceto di Teggiano tipo 1

Table 3. Observed traits of fruits and their codes for figures

Code	Trait	Code	Trait
Len_fr	Fruit length [cm]	Wei_fr	Fruit weight [g]
Wid_fr	Fruit width [cm]	Wid_ped	Pedicel width [mm]
Len_Wid_fr	Fruit length/width ratio	Mat_st	Days to fruiting
Th_wa_fr	Fruit wall thickness [mm]	Sh_fr	Fruit shape
Col_fr	Fruit colour at mature stage	Cap_fr	Fruit capsaicin

Results and discussion

Four variables detected on fruits (Len_fr, Wid_fr, Wei_fr and Sh_fr), in a Scatterplot are displayed in figure 1. The varieties were separated along an axis that goes from top left to bottom right, on the upper part of the panel showed the correlations between the variables. Along this axis, the majority of population is divided in three groups by shape of fruit: 1) plants with elongated fruit, 2) plants with shaped triangular fruit, and 3) plants with fruits in the form of blocky. Few genotypes have a mixture of fruits with different shapes. The varieties with a series of off-type were insufficient to be defined heterogeneous, are: "Corno di Capra Paolo 2009", "Papaccella", "Cornetto buccinese corto", and "Pupanetto per Aceto di Teggiano". Instead, the varieties "Pupanetto per aceto di Teggiano tipo 1" and "Sciuscillone Mazza" were heterogeneous: the first is composed of plants with different types of fruit: campanulate-shaped, triangular-shaped and horn-shaped; the second has two types of plants, six with elongated fruit and six triangular ones.

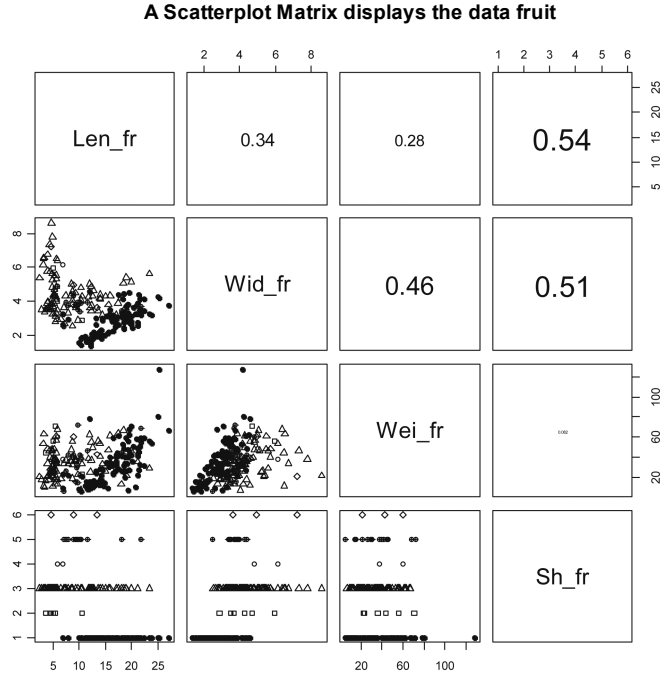

Figure 1: A scatter plot matrix displaying the twenty-four varieties and four variables. The varieties were separated along an axis that goes from top left to bottom right, the correlations between the variables are shown on the upper part of the panel.

The first four principal components of the elongated-shaped fruit traits accounted for 70% of variance: the first was correlated with length, width and weight of fruit; the second with days to fruiting and length/width ratio of fruit; the third with width of pedicel; the fourth with wall thickness of fruit. The first group is pictured from varieties positioned at the left side of the diagram: varieties “16”, “18” and “7” very similar to the control variety called “Sigaretta”. The second group is pictured from varieties more similar to "Corno di Capra" and they are positioned at the right side of the diagram.

Varieties similar to "Sigaretta" are characterized by fruits smaller than those of the second group, both in size and weight; furthermore they have no capsaicin, soft and a thin pericarp.

The "CDC" variety and its similar genotypes had fruits with a red dark colour, without capsaicin, a length between 15-25cm and a width from 2.5 to 4.5 cm (figure 2).

The first four principal components of triangular-shaped fruit traits accounted for 40% of variance: the first was correlated with length, width, length / width ratio and wall thickness of fruit, the second with width of pedicel and weight of fruit, the third with days to fruiting. Local genotypes with triangular-shaped fruits were divided into two typologies (Figure 3): 1. plants with elongated triangular fruits (30, 13, 15 and 11) and early fruiting and 2. plants with short triangular fruits and later fruiting.

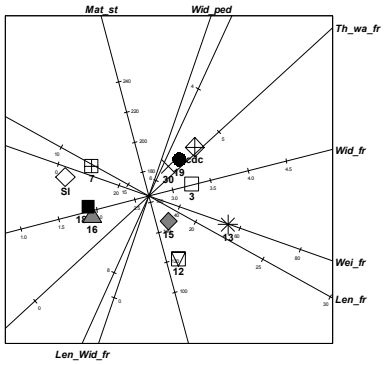

Figure 2: Biplot PCA sample group means, plants with elongate-shaped fruits

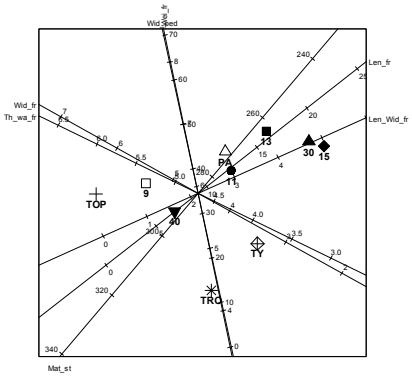

Figure 3: Biplot PCA sample group means, plants with triangular-shaped fruits

All varieties were homogeneous and therefore without off-type individual for characters related to the fruit. The examined varieties appeared to be well differentiated from each other. Morphologically, genotypes "Trottolino" and "Peperone melanzana Grippo" had similar fruits both size and color, but the main differences were recorded in the presence and absence of capsaicin: present in Trottolino and absent in "Peperone melanzana Grippo", in the average weight of each fruit, in fact the first variety had fruits with a lower weight than the second.

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Growth and fruit production in pepper grafted on *C. annuum*, *C. baccatum* and *C. pubescens* genotypes

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Abstract

Good compatibility was obtained when plants of sweet pepper cv. Filon were grafted on three putative rootstocks belonging to three domesticated species of *Capsicum* (*C. baccatum*, *C. pubescens* and *C. annuum*). Plant growth and fruit production were compared in plants grafted onto these rootstocks and those ungrafted, self-grafted or grafted onto three *C. annuum* known rootstocks. The lowest number of fruit per plant at the end of the culture was obtained in plants grafted on the *C. annuum* and *C. pubescens* genotypes. However, the number of fruit set per plant after 70 days of culture (8-9) as well as the fruit weight (around 180 g) was similar in plants grafted onto the *C. baccatum* genotype respect those obtained in the controls. These results indicated that the tested *C. baccatum* genotype could be of interest as rootstock for grafting pepper.

Keywords: rootstocks, growth, yield

Introduction

Grafting is a common technique that was developed in order to cope with soil borne pathogens, to enhance water or nutrient uptake (Lee 1994; King et al. 2010) and to combat abiotic stresses such as low temperatures or salinity (Schwarz et al. 2010). However, grafting may influence, positively or negatively on plant growth, yield, and the quality of scion fruit (Rouphael et al. 2010). Thus, an exhaustive characterization needs to be performed when breeders develop rootstocks.

Pepper is not typically grafted commercially, except in winter greenhouse production on the Korean peninsula to control *Phythophtora capsici* (Kwon et al. 2006). However, interest in pepper grafting worldwide has risen because of the increase in sustainable practices and the withdrawal of methyl bromide (Rivard and Louws, 2008). Some reports of pepper grafting to control *P. capsici* and/ or nematodes have been reported (Oka et al. 2004; Morra and Bilotto, 2004; Kokalis-Burelle et al. 2009; Gisbert et al. 2010). In these works, commercial rootstocks or materials resulting from breeding programs have been used.

Despite some interesting traits like tolerance to flooding, resistance to some soil borne pathogens or cold tolerance have been described in some accessions of the *C. annuum* related species, *C. frutescens*, *C. baccatum, C. chacoense, C. pubescens* or *C. chinense* (Sahin and Miller, 1987; Randle and Honma, 1980; Gisbert et al. 2013), scarce work was reported in relation to the use of these species as rootstocks.

In the present study, we have compared the behavior of ungrafted and self-grafted pepper plants with plants grafted onto three *C. annuum* tested rootstocks and three untested genotypes of *C. baccatum*, *C. pubescens* and *C. annuum*.

Materials and Methods

Sweet pepper cv. Filon (provided by Clause Vegetable Seeds, France) was grafted onto three rootstocks (Charlot (CH), Foc and RT) and three untested genotypes of *C. baccatum* (CB), *C. pubescens* (CP) and *C. annuum* (CA) as described in Gisbert et al. (2010). Charlot and Foc were

developed by our group and RT is a commercial rootstock from Syngenta (RT0012). Plants were transplanted to pots with coconut fiber and grown under hydroponic conditions. After transplanting, growth (plant height) was measured at 40 and 60 days of culture and the number of fruit set was noted at 50 and 70 days. Ripe fruits were harvested along a month.

Results and Discussion

Good compatibility was obtained when plants of pepper cv. Filon were grafted onto the *C. annuum*, *C. baccatum* and *C. pubescens*. At 40 days after culture under hydroponic conditions, growth was equivalent in all treatments (Table 1). However, at 60 days, growth was significantly lower in plants grafted on *C. pubescens* and *C. annuum* genotypes. On the contrary, plants grafted onto *C. baccatum* showed good or even higher development than the rest of graft combinations (Table 1).

The number of fruit set per plant at 50 days of culture was lower in plants grafted on *C. baccatum* and similar to the rest of treatments. However, at 70 days, the number of fruit per plant in *C. baccatum* grafted plants was equivalent to that observed in ungrafted and self-grafted plants (Table 1).

Table 1. Effect of grafting combination on plant height and number of fruit set per plant. In parentheses, mean of fruit per plant >5cm. Graft combinations: F (ungrafted pepper plants cv. Filon); F-F (self-grafted plants); CH-F, FOC-F, RT-F, CA-F, CB-F, CP-F (Pepper plants cv. Filon grafted onto Charlot, Foc, RT, *C. annuum*, *C. baccatum* and *C. pubescens* rootstocks, repectively).

	Plant height (cm)		Number of fruit set per plant (>5cm)	
Grafting combination	At 40 days	At 60 days	At 50 days	At 70 days
F	83.8 a	107.6 ab	5.8 a (1.6 abc)	9.2 a (8.0 a)
F-F	82.4 a	117.0 a	5.7 a (1.4 abc)	9.2 a (8.0 a)
CH-F	79.08 a	111.4 ab	6.1 a (2.0 ab)	8.4 ab (7.9 a)
FOC-F	77.0 a	113.6 ab	6.4 a (1.2 abc)	8.1 ab (6.3 ab)
RT-F	72.4 a	114.8 ab	5.7 a (0.8 bc)	6.9 ab (5.3 ab)
CA-F	71.6 a	92.8 c	4.6 ab (1.3 abc)	5.0 b (4.1 b)
CB-F	80.8 a	118.2 a	2.6 b (0.3 c)	9.5 a (7.0 ab)
CP-F	74.8 a	100.6 bc	6.1 a (2.2 a)	7.0 ab (4.8 ab)
ANOVA(P-value)	0.1956	0.0068	0.0297 (0.0363)	0.0729 (0.0838)

Means with different letters are significantly different by Duncan test (P<0.05).

The lowest number of fruit per plant at the end of the culture was obtained in plants grafted on the putative rootstocks of *C. annuum* and *C. pubescens* (Fig. 1A). Fruit weight was in the range of 165 to 195 g per fruit without differences among values obtained in one grafted combination respect those obtained in ungrafted or in self-grafted plants (Fig. 1B).

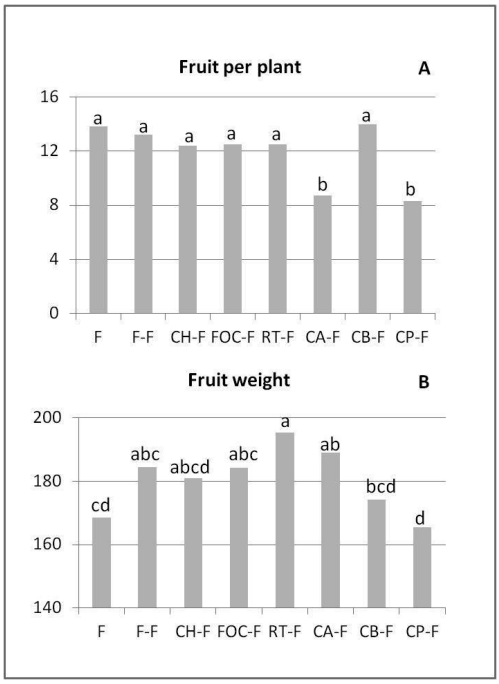

Figure 1. **A.** Mean number of fruit per plant after one moth of harvest **B.** Mean fruit weigh (g). Graft combinations: F (ungrafted pepper plants cv. Filon); F-F (self-grafted plants); CH-F, FOC-F, RT-F, CA-F, CB-F, CP-F (Pepper plants cv. Filon grafted onto Charlot, Foc, RT, *C. annuum*, *C. baccatum* and *C. pubescens* rootstocks, repectively).

As a reduction in growth and in the number of fruits was observed in CA-F and CP-F plants compared to ungrafted and self-grafted plants, both rootstocks were discarded as putative rootstocks of pepper cv. Filon. These adverse effects could be due to the scion-rootstocks interactions. In the case of plants grafted onto the CB genotype, similar growth and yield (number and weight of fruits) of those obtained in plants grafted onto Charlot, Foc or the commercial rootstock RT was observed. These results indicated that the tested *C. baccatum* genotype could be of interest as rootstock for grafting pepper.

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Peppers grown by urban farmers of Caceres, Mato Grosso, Brazil: Brazil-Bolivia border region, transition area Pantanal-Savana-Amazon biomes

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Abstract

The species of the genus *Capsicum* are part of the food culture of Brazilian populations, thus, its production contributes to income generation of farmers. Caceres, Mato Grosso, is a border town located on the border of Brazil-Bolivia. This town falls in a transition region between the Pantanal, Cerrado and Amazon biomes, with a *Capsicum* or human populations from different regions of Brazil. The objective of this study was to identify and characterize the properties of peppers cultivated in in urban and peri-urban areas of Caceres. The work began in December 2010 with initially identifying farmers. A list of farmers nominees was prepared through visits to centers of trade and the indication of the farmers themselves. After this, visits were carried out to interview them through a structured questionnaire and geographical points of production units were also obtained with the help of a GPS navigation to construct a map. Furthermore, the accessions of pepper were collected from the farmers' fields and also from local markets. Accessions were characterized using descriptors and we identified 29 farmers growing peppers, of which 17 were cultivating it for marketing. A total of 108 accessions were obtained being 6 C. *annuum* var. *annuum*, 1 *C. annuum* var. *glabriusculum*, 29 *C. baccatum* var. *pendulum*, 59 *C. chinense*, 12 *C. frutescens*, 1 *C. baccatum* var. *baccatum* and 35 accessions in the identification stage.

Keywords: Agrobiodiversity, *Capsicum*, Center of origin, Descriptors, Phenotypic characterization, Urban agriculture.

Introduction

Peppers (*Capsicum* spp.) are a valuable asset of Brazilian biodiversity. Its cultivation occurs throughout the national territory, from Rio Grande do Sul to Roraima, presenting a huge range of genotypes with diverse fruit sizes, colors, flavors and sours.

More than 30 species have been described in the genus Capsicum, which is native to the Americas. However only five of them are considered to be domesticated: *C. annuum* var. *annuum*, *C. chinense*, *C. frutescens*, *C. baccatum* var. *pendulum* and *C. pubescens* (Moscone et al. 2007).

In Brazil, the plants of *Capsicum* are of economic, cultural and biological importance. Fruits are used in preparing typical food, which is diversified across regions and sometimes unique in a particular locality of the country. The multiple and diverse ways for using these fruits are related with ethnic and multicultural aspects. It is probably the result of genetic diversity observed in each region of the country (Sudre et al. 2010), as Brazil is considered one of the centers of diversity of the genus *Capsicum*.

Brazil is the second largest producer of chili in the world and most of this production comes from family-based, which in some areas are characterized by use of local produced seeds from their own crops (Ribeiro et al. 2008). Yield of peppers in Brazil is important in family farming, a prominent example in integrating small-farmer agriculture industry, resulting on permanence of small farmers and their families in the countryside. The city of Caceres is well known for growing various vegetables mostly by small farmers and traditional communities (Milk et al. 2008). Seabra

Jr et al. (2012) pointed out that in this municipality family farming is prevalent, with garden areas ranging from 303.32 m² to 15,279 m².

Characterization of pepper cultivation and production, especially the cultivars and types in the region is very important. This helps to discover the demands and needs of growers and also to understand available genetic base in the form of old varieties or primitive populations.

Therefore, present study was conducted with two major objectives: (i) identify and characterize the properties that grow peppers in Caceres-MT and (ii) identify the commercial cultivation of peppers located in urban and peri-urban areas of the municipality of Caceres-MT, Brazil.

Materials and Methods

The study was performed in the production areas that cultivate peppers in Caceres, Mato Grosso – Brazil, starting December 2010. The municipality has an area of 24,398,399 km2, located in the southeastern region of Mato Grosso, with approximately 87,912 inhabitants. Caceres integrates mesoregion Mid-South Mato Grosso and microregion of Alto Pantanal, located 215 km from the capital. The climate, according to Köppen classification, is hot and humid tropical with dry winter (Awa).

The production units were identified and cataloged through visits to agricultural stores, fairs, supermarkets and through the appointment of pepper producers.

Thereafter, visits to farms were made, to formalize the invitation to participate in the research survey. Upon acceptance of the producer, by signing the consent and informed form, the producers were interviewed using a structured questionnaire for marketing characteristics of *Capsicum* spp., and socioeconomic status.

For the preparation of thematic maps, geographic points were recorded with the help of a GPS navigation and the Laboratory of Geo UNEMAT. The points were added in Geographic Information System (GIS) ArcGIS, version 9.2, Esri, and used to generate a map of local general cultivation of the species of peppers. The data obtained by the production units questionnaires were recorded and analyzed using descriptive statistics.

The accessions (108) were characterized and evaluated based on morphological characters. For this purpose, an experiment was conducted under field conditions in UENF in Campos, Rio de Janeiro, Brazil (21 ° 45'S, 41 ° 18'W) using a randomized block design with collected three replications (10 plants per plot).

Results and Discussion

We identified 29 areas of pepper cultivation in 11 districts. The locations of different producer, present in urban and peri-urban areas of the municipality of Caceres-MT is shown in figure 1. Majority of producers were lower-class families, who cultivate pepper in backyards.

Of the 29 areas and farmers identified, 17 were growing pepper as cash crop for marketing. The other production units were growing peppers only for their own consumption and for decorative purposes.

Growing *Capsicum* was more intensified in peri-urban areas. The occupation of land in the urban spaces by farmers causes the ruralization of urban space. This fact may be related to the low human development indices presented by the municipality. Generally, residents are seeking ways to use the space around it, converting it into livelihoods, income and means of conserving the diversity of plant species.

All of the 17 farmers that produce chili commercialize chili fruits *in natura*, but only ten properties commercialize in the pickled form, one of them shaped sauce and other in form of liquor. The financial return complements family income at all properties.

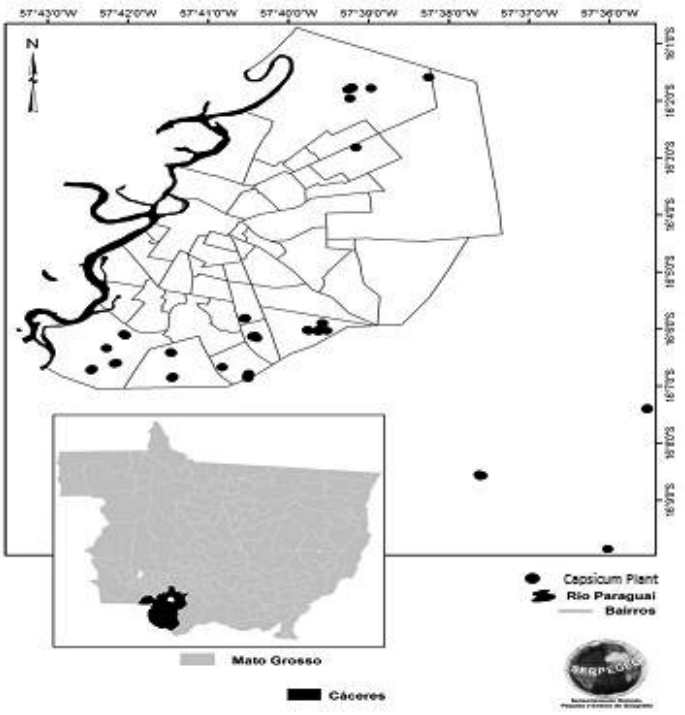

Figure 1: Location of pepper growing areas in Caceres / MT (LABGEO / UNEMAT) 2011.

It was observed that all producers commercialize in the county fair. These, three properties also traded in local markets, three producers possessed her petty trading, one inside the property sells.

It was observed that 96% of all of 29 farmers produce their own seeds for the production of peppers, and the majority of these seeds are from region where producer came. This explains the small amount of production area found in the production units and the ignorance of most of the producers on the species marketed.

The producers cultivate several varieties of peppers. Due to this great diversity of materials existing in subsistence agriculture, is frequent the occurrence of problems with the nomenclature of varieties.

During this study, we collected 108 accessions of *Capsicum* belonging to: *C. annuum* var. *annuum* (6), *C. annuum* var. *glabriusculum* (1), *C. baccatum* var. *pendulum* (29), *C. chinense* (59), *C. frutescens* (12), *C. baccatum* var. *baccatum* (1)

It can be concluded that the municipality of Caceres-MT features a vast diversity of peppers. The pepper production system in region is still incipient, but producers lack technical knowhow, which lead to poor productivity and not enough surplus for marketing peppers to supplement the family income.

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Microsatellite and morpho-agronomic polymorphism in eggplant and pepper: genotyping and identification of traditional cultivars

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Abstract

In the framework of a project for the conservation of local landraces threatened with extinction, we estimated genetic relationships and true cultivar identity of 7 and 25 accessions, respectively, of eggplant and pepper landraces from the Campania Region (Southern Italy). We used an integrated approach including SSR markers (15 for eggplant and 16 for pepper) and 9 morpho-agronomic biometric traits. Out of all the tested SSR markers, 5 and 7 markers showed polymorphism between landraces in eggplant and pepper, respectively, detecting 18 alleles in eggplant and 31 alleles in pepper. These markers generated the same genetic pattern for every accession of each investigated cultivar. Despite more markers may potentially detect polymorphism, these findings suggest an elevated genetic homogeneity between landraces. The morpho-agronomic traits investigated allowed only a partial discrimination of landraces and thus call for more detailed investigation.

Keywords: Genetic diversity, SSR, landrace, germplasm conservation

Introduction

The identification and characterization of crop varieties is becoming mandatory, as the assessment of diversity in different accessions is essential for efficient germplasm acquisition, conservation, classification, and utilization (Jackson et al. 1997; Dhillon et al. 2004) as well as for the economic exploitation and correct valorisation of traditional entities. Even more so, this is important in countries such as Italy where geographic and politic fragmentation together with the presence of many different climatic conditions has led to the selection of an astonishing number of local cultivars. In this context, we characterized genetic relationships and true cultivar identity of traditional cultivars of eggplant and pepper of the Campania Region (Southern Italy). Genetic diversity of crop varieties is often assessed through the characterization of morpho-agronomic traits because of their stability, ease of detection and relevance for cultivation. However, an approach exclusively based on morpho-agronomic traits has severe limitations in terms of resources and time required for the evaluation in the field of a large number of accessions (Dhillon et al. 2004). These problems are overcome by a different approach for the assessment of genetic diversity based on molecular markers. Among the various molecular markers available, microsatellites or simple sequence repeat (SSR) markers are widely used for diversity analysis (Gao et al. 2005; Thomson et al. 2009). In this study, as previously done by different authors (Kumar et al. 2009; Nunome et al. 2009; Ghalmi et al. 2010; Ince et al. 2010; Sharma et al. 2010), we used an integrated approach in which both morpho-agronomic and molecular analyses were carried out.

Materials and Methods

Plant Material

Eggplant (*Solanum melongena* L.) and pepper (*Capsicum annuum* L.) are diploid species with 12 chromosomes (2n=24) belonging to the family Solanaceae. Plant materials were traditional

cultivars or selections within traditional cultivars (Table 1). For each accession, three representative plants were chosen to represent the local diversity and were analysed.

Table 1 - Investigated landraces of eggplant and pepper.

Landrace: *Eggplant*	**Molecular analysis (n. of accessions)**	**Morpho-agronomic characterization (n. of accessions)**	**Landrace*: Pepper***	**Molecular analysis (n. of accessions)**	**Morpho-agronomic characterization (n. of accessions)**
A grappolo	1	-	Cazzone	2 red, 2 yellow	1 red, 1 yellow
Cima di viola	2	3	Cornetto di Acerra	1 red, 1 yellow	1 red, 1 yellow
Napoletana	2	4	Corno di capra	1 red, 1 yellow	1 red, 1 yellow
Violetta tonda	2	-	Friariello napoletano	1 green	1
			Friariello nocerese	2 green	-
			Friariello a sigaretta	1 green	-
			Marconi	1 red, 1 yellow	-
			Papaccella napoletana liscia	1 red, 1 yellow	1 red, 1 yellow
			Papaccella liscia	1 red	-
			Papaccella napoletana	2 red, 3 yellow	1 red, 1 yellow
			Sassaniello	1 red, 1 yellow	1 red, 1 yellow
			Peperone corno rosso	1 red	-

Morpho-agronomic and molecular analysis

Nine informative morpho-agronomic and biometric traits were measured on both eggplant and pepper accessions. These descriptors include two whole-plant traits (size, structure), and seven fruit traits (shape in longitudinal section, shape at the fruit apex, presence of locules, fruit length, fruit diameter, mesocarp thickness, fruit mean weight). All the measurements were performed at the same plant development stage and at the same fruit ripeness stage.

Genomic DNA was extracted from frozen young leaves of each accession (Table 1), using GenElute Plant Genomic DNA Miniprep Kit (Sigma). Quality and concentration of extracted DNA were examined by gel electrophoresis and spectrophotometry. Fifteen (for eggplant) and 16 (for pepper) SSR markers from previous studies (Stàgel et al. 2008; Minamiyama et al. 2006) were selected and preliminary tests for PCR amplification were conducted. All PCR reactions were performed in 15 µl final volume containing approximately 20-30 ng template DNA, 1xTaq buffer (Invitrogen), 200 µM of each dNTP, 2 pmol of each primer, 6-FAM or HEX end-labelled, and 1 U Taq polymerase (Invitrogen) and carried out according to the authors (Stàgel et al. 2008; Minamiyama et al. 2006). Specificity of PCR products was estimated by agarose gel electrophoresis. All loci displaying more than one band were excluded from further analysis. Allelic size was estimated with GENEMAPPER 3.7 (Applied Biosystems) and custom LIZ (500) labelled size standard. Each individual was genotyped at each locus by scoring the length of the amplified SSR band. As a descriptor of the overall morphological differences among investigated landraces, Principal Component Analyses (PCA) based on molecular data was performed using Genalex (Peakall and Smouse 2006). PCA based on morpho-agronomic traits was conducted using SPSS 14.0 statistical package. A combined PCA based on morpho-agronomic and molecular traits was also conducted using SPSS 14.0 statistical package.

Results

Out of 15 SSR markers, only 5 (EEMS-48; EEMS-18; EEMS-12; EEMS-37; EEMS-17) showed polymorphism between eggplant landraces, giving 1 to 5 alleles per locus. The allele number was higher for EEMS-48 and EEMS-18, and lower for EEMS-12 and EEMS-37 (data not shown). When analysed through PCA, our molecular data distinguished all the investigated

landraces, with 'Melanzana a grappolo' forming a remarkably different cluster from all the other landraces (Fig. 1A). On the contrary, the two landraces 'Napoletana' and 'Cima di viola' showed a similar molecular pattern (Fig. 1A).

In pepper, only 7 (CAMS-351; CAMS-456; CAMS- 117; CAMS-405; CAMS-644; CAMS-015; CAMS-864) out of 16 SSR markers showed polymorphism between landraces, giving a number of alleles per locus ranging from 2 to 10. Allele number was higher for CAMS-864 (10 alleles) and lower for CAMS-456 (2 alleles). Though molecular fingerprinting was not able to distinguish all landraces, molecular PCA analysis allowed a clear discrimination of most cultivars ('Cazzone', 'Cornetto di Acerra', 'Friariello napoletano', Friariello nocerese', 'Friariello a sigaretta', 'Marconi', 'Sassaniello', 'Peperone corno rosso') (Fig. 1B). Among these landraces, accessions with different fruit colour clusterized very close to each other ('Marconi' and 'Cornetto di Acerra') or were co-localized ('Cazzone' and 'Sassaniello'). The three landraces of the 'Papacella' type grouped together, but were not clearly distinct from each other, with accessions of different fruit colour within each landrace forming separate clusters (Fig. 1B). Only the traditional cultivar 'Corno di capra' could not be discriminated, since its yellow accession co-localized with the yellow accession of "Cornetto di Acerra", whilst the red accession localized independently (Fig. 1B).

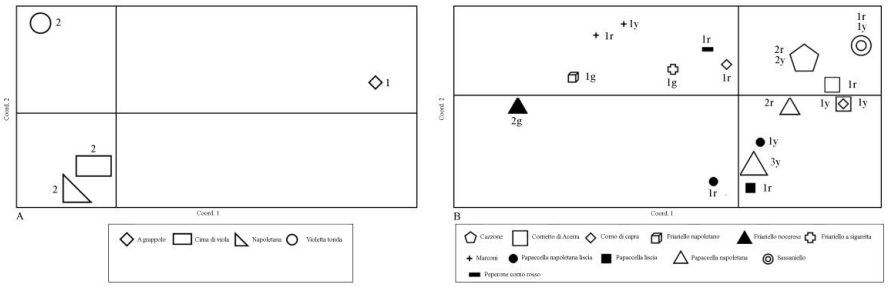

Figure 1. PCA based on SSR relationships among accessions; A) eggplant, B) pepper. All samples were evaluated using polymorphic SSR markers and were represented on the two first components (Coord. 1 and Coord. 2) of the principal components analysis. Size of symbols is proportional to the number of overlapping accessions, which is also indicated close to the corresponding symbol. For pepper accessions, fruit colour at commercial maturity is also indicated (g, green; r, red; y, yellow).

The PCA based only on morpho-agronomic traits did not allow a clear discrimination of all the pepper landraces (data not shown). To better discriminate pepper landraces, a PCA was carried out combining morpho-agronomic and SSR markers. Three groups were clearly distinguished: one includes all the 'Papaccella' peppers, another encompasses the closely related cultivars 'Cazzone' and 'Sassaniello', and the third comprises 'Corno di Capra' and 'Cornetto di Acerra' (Fig. 2). The last two landraces, which co-localized in the molecular based PCA, were clearly discriminated by combined morpho-agronomic and molecular PCA, though the analysis confirmed the two landraces to be remarkably similar.

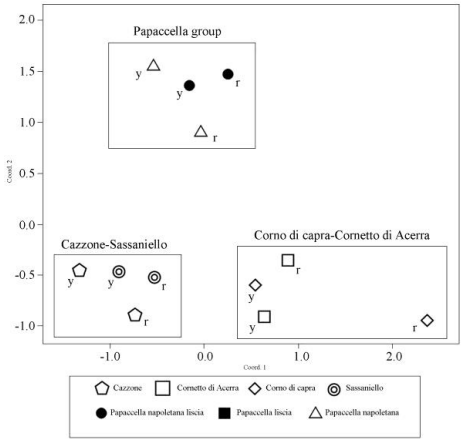

Figure 2. Combined PCA based on morpho-agronomic and SSR markers of a subset of pepper landraces (Table 1). All samples were evaluated using polymorphic SSR markers and were represented on the two first components of the principal components analysis. Fruit colour at commercial maturity is also indicated (r, red; y, yellow).

Discussion

In this study, utilizing an integrated approach with morpho-agronomic and molecular markers, we characterized 7 accessions of eggplant and 25 accessions of pepper from a Southern-Italian region (Campania). Overall, with a single exception, we found molecular differences even in very closely related landraces both of eggplant and pepper. Among all the investigated SSR markers, 5 and 7 showed polymorphy in eggplant and pepper, respectively. Even if based on few markers, this finding suggests a high level of relatedness between cultivars. A low or null level of observed heterozygosity was observed within each investigated landrace, likely as a consequence of intensive selection.

Preliminary analysis of morpho-agronomic traits detected differences between the two investigated eggplant cultivars, but was unable to discriminate all the pepper landraces. This calls for the use of further morpho-agronomic traits to allow thorough discrimination.

Combined morpho-agronomic and molecular traits PCA allowed a clearer discrimination of two very similar eggplant landraces (‘Napoletana’ and ‘Cima di viola’) (not shown), but was especially useful in pepper, since it distinguished ‘Corno di capra’ and ‘Cornetto di Acerra’, two landraces with identical molecular pattern.

These results support the idea that an integrated approach is an effective method for the discrimination of closely related cultivars. Comparison between landraces from Campania region or from other Southern-Italian regions with similar morpho-agronomic patterns may be of help in the resolution of synonymy and homonymy in cases of particular complexity. Finally, the assessment of true cultivar identity and the identification of synonymic cultivars could be used for the establishment of germplasm collections, with positive consequences on conservation of traditional crop species of the Campania Region.

Acknowledgements

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Morpho-phenological traits of parthenocarpic germplasm of *Solanum melongena* L. bred at the CRA Vegetable Research Center

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Abstract

A morpho-phenological examination of 23 eggplant lines showing partial to near-complete parthenocarpy, bred at CRA Research Centre for Vegetable and Ornamental Crops, was conducted in the spring 2011. Six plants per line were randomized over the trial plot on mulched soil in a plastic tunnel, spaced 1 m in a square arrangement and grown up to the physiological ripeness of the first fruit. Observations were made on all plants following the Community Plant Variety Office TP/117/1 03/2008 and the UPOV TG/117/4 protocols, recording also fruit yield and the seed yield of the first fruit. With hierarchical classification three groups of vegetative morphology were detected: 1) violet plantlet, semi-erect plant with small green narrow leaves and dark violet flowers; 2) big globose plant with violet flowers flowering late; 3) green plantlet, big prostrate plant with pale green leaves and pink flowers. Earliness was correlated with plant growth habit, yield with fruit length, though not with fruit size. With clustering on correspondence analysis seven fruit shapes were differentiated: ellipsoidal; spotted oboval without ribs; unstriped and unspotted oboval with ribs; piriform; cylindrical; ovoid; striped globular with prickly calyx. For fruit size five cluster were identified, differentiated mainly on the length/width ratio: 1) very small with the highest ratio; 2) small with above mean ratio; 3) medium-large with below mean ratio; 4) small with below mean ratio; 5) large and short with the smallest ratio.

Keywords: eggplant, *S. melongena*, germplasm, parthenocarpy, morpho-phenology

Introduction

Several eggplant lines have been bred at Vegetable Research Center of the Agricultural Research Council, including two registered cultivars (Partena and Partenone), whose reproduction and preservation is the Center's responsibility (Restaino et al. 2004). The breeding program was discontinued a few years ago with the retirement of the breeder, leaving most lines inadequately assessed. A recent occasion of conducting mandatory controls of varietal purity on the registered lines was used for beginning an assessment of the eggplant germplasm, starting from morphology and phenology.

Materials and Methods

Seed was available for 23 lines, pedigree and F1 hybrids, some of them (tagged with the characters PC) and the registered two capable of parthenocarpy in protected culture:

- registered cultivars (F1 hybrids) Partena (3DHx18PC), Partenone (6DHx3DH) and their parents 3DH, 6DH and 18PC (DH meaning di-haploid);
- other pedigree lines: 1PC, 16PC, 20PC, A63, A67, A79, AK2, 5E, 7E, 26E, 39E;
- other F1 hybrids: 39Ex3DH, 16PCx3DH, 3DHx26E, 1PCxA79, 18PCx6DH, 39ExAK2, 16PCx18PC.

Six plants per line (doubled for registered cultivars and their parentals) were transplanted at the beginning of May, completely randomized and spaced 1m in a square arrangement, on a mulched

plot under a plastic tunnel in the Center's farm at Pontecagnano (SA). Cultural practices common in the area and integrated pest management criteria were followed. Weekly observations were made on all plants for 31 traits, following the Community Plant Variety Office TP/117/1 03/2008 and the UPOV TG/117/4 protocols, recording also fruit yield and the seed yield of the first fruit (Table1). The criterion for assessing varietal purity was the proportion of plants falling in groups formed by ordinations of the observed traits. Genotype and trait ordinations were obtained by hierarchical clustering on Ward distances of the factors with significant contribution resulting from mixed factorial analysis (for vegetative traits), multiple correspondence analysis (for qualitative fruit traits) and principal component analysis (for quantitative fruit traits). An overall ordination of genotypes and traits (numerically coded and rank transformed) by row and column permutations of the Minkowski distance matrix was synthesized in a Bertin map (Bertin, 1977). Numerical analyses and graphs were performed with the *R* environment (R Core Team, 2012) and contributed packages *ade4* (Dray and Dufour, 2007) FactoMineR (Husson et al. 2010), and *seriation* (Hahsler et al. 2008).

Table 1. Observed traits and their codes for figures.

Code	Trait	Code	Trait	Code	Trait
Plwine	plantlet blue-reddish hue	Lrug	leaf surface rugosity	Fcol	flower color
Phab	plant growth habit	Lcol	leaf color	FrRib	fruit ribs
PH	plant height	d2F	days to flowering	Cwine	calyx blue-reddish hue
StH	stem height	d2M	days to maturity	Cpr	prickly calyx
Stwine	stem blue-reddish hue	FrPL	fruit peduncle length	Ccr	crenulate calyx
Stpub	stem pubescence	FrWine	blue-reddish hue under calyx	FrColR	fruit colour at maturity
LL	leaf length	CW	calyx width	FrL	fruit length
LW	leaf width	FrCol	fruit color	FrW	fruit width
LR	leaf length/width ratio	FrSp	fruit color spots	FrR	fruit length/width ratio
Lsin	leaf margin sinuation	FrStr	fruit color stripes	FrS	fruit shape
		FrFc	fruit flesh color	Yield	relative fruit yield

Results and Discussion

Plant traits

Plant colour was positively correlated with plantlet colour, and leaf surface (roughness) rugosity was frequent in pale-coloured plants with erect habit. Plant growth habit was erect for five genotypes (3DHx18PC, 7E, A79, AK2, 20PC), semi-erect for fourteen genotypes, including both the largest plants (18PCx6DH, 16PCx18PC, 16PCx3DH, 6DHx3DH) and the smallest (5E, A63, 26E, 39E), prostrate for A67 and 16PC, with some variation within the lines 3DH, 18PC and 16PC. Leaf dimensions showed a positive correlation. The first four principal components of vegetative plant traits accounted for 66% of variance (Figure 1 left and right): the first was correlated with plant size, leaf length and early flowering; the second with plantlet colour, leaf surface pattern and colour, plant growth habit the third with rugose leaves, deep violet flowers and prostrate habit; the fourth with plantlet blue-reddish hue and days to flowering. Small plants and dark leaves characterized 5E; erect habit and wide leaves 20PC; large plants and late flowering 18PCx6DH and 6DHx3DH; green plantlets and pale leaf colour 16PC, A67; narrow and rugose leaf and pale flower A63; rugose and dark leaf 7E and 26E; premature flowering and lack of blue-reddish hue 39Ex3DH, 16PCx18PC, A63 e AK2; large leaves of medium green colour 6DH, 39ExAK2 and 1PCxA79.

Genotypes could be grouped in three clusters of traits: 1) violet plantlet, semi-erect growth habit, small and narrow green leaf, dark violet flower, best represented by 5E, 39E, 26E, 39ExAK2, and 6DH; 2) large plant with long stems; large, smooth, roundish and dark green leaf; violet flower and late flowering, best represented by 20PC and 6DHx3DH; 3) green plantlet, large plant, long stem and prostrate habit, pale-green leaf, pale violet flower (AK2, 16PCx18PC, 16PC, A67, A63).

Lack of uniformity was displayed by plants of the lines 3DH, 18PC and their hybrids.

Fruit traits

Fruits had white skin for A63 but violet for the other lines and white flesh for all. The calyx was green with a blue-reddish pigmentation showed only by 6DH. Prickly calyx, associated with striped and ribbed fruits, characterized some lines with oboval and pyriform fruit and most of those with globular fruit. The largest fruits were the widest of medium length, with globular, oboval or ovoid shapes, while the smallest fruits were generally elongated, with cylindrical, ellipsoid or pyriform shapes. The first factor of the correspondence analysis for qualitative traits (Fig.2, left) contrasted ellipsoid and globular shapes; the second factor contrasted the presence/absence of blue-reddish pigment at the base and the presence of ribs or spots against prickly calyx and stripes, as well as the cylindrical shape against the ovoid and ellipsoid, and ribs against stripes.

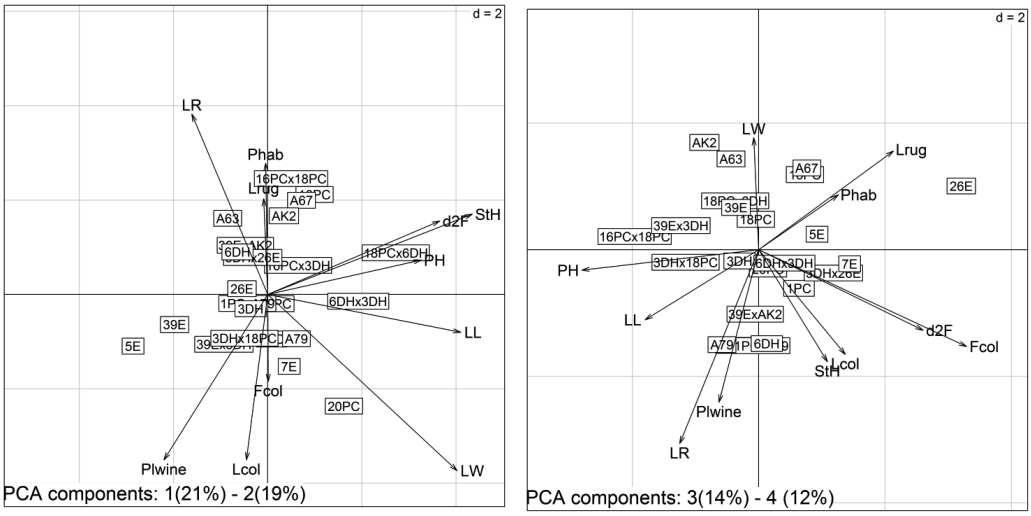

Figure 1. Projection of the genotypes on the plans of the first four principal components of plant traits (trait codes in Table 1).

Lines 26E is notable for prickly calyx; A63 and 39E for fruits with ribs and no spots, 7E and 3DHx26E for ovoid shape, 3DH and 1PCxA79 for spotted colour. The first principal component of the quantitative traits (Fig.2, right) showed obvious contrast between width and length/width ratio, the second association with fruit length and yield. AK2 stands out for the widest fruits, 1PCxA79 for the narrowest; 6DHx3DH, 39E and 39Ex3DH were the highest yielding, in contrast with 26E, 3DHx26E and 1PCxA79 at the lowest end of the yield scale. Hierarchical clustering of fruit traits separated seven qualitative and five quantitative traits. Qualitative, with representative lines: 1) ellipsoid (3DH); (2) oboval, spotted, without ribs (6DHx3DH, 3DHx18PC); (3) oboval, with ribs, but no spots and no stripes (1PC, 39E); (4) piriform (39Ex3DH); (5) cylindrical (A79, 39ExAK2); (6) ovoid (16PC, 3DHx26E, 5E); (7) globular, striped, with prickly calyx (26E, 7E). Quantitative, mainly based on the length/width ratio (LWR): 1) very small, with the highest LWR (1PCxA79); (2) small with high LWR (1PC, A79, 39Ex3DH, 39ExAK2); (3) medium-large, with below average LWR (39E, 6DHx3DH); (4) small, with below average LWR (26E); (5) very large, with very low LWR (AK2).

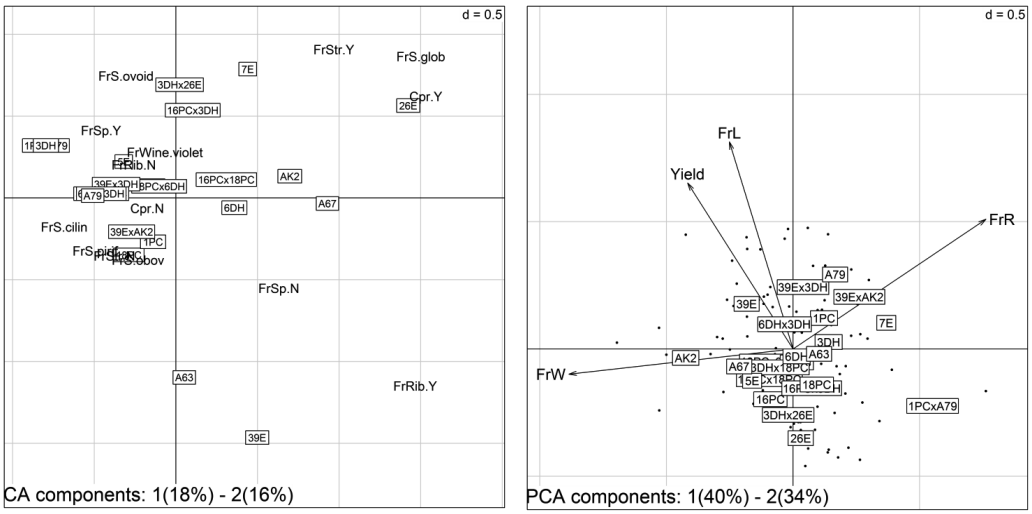

Figure 2. Projection of the lines on the plans of the first two dimensions of the correspondence analysis of qualitative fruit trait (left) and of the first two principal components of quantitative fruit traits and fruit yield (trait code in Table 1).

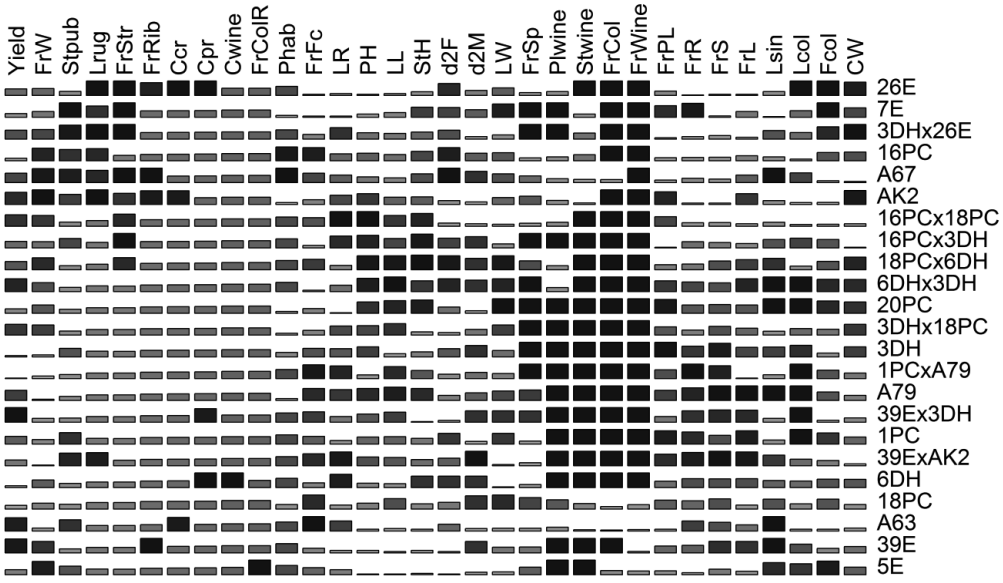

Figure 3. Bertinplot of the ordered Table genotype (rows) per trait (columns). Bar shade and size show trait intensity (trait code in Table 1). Both rows and columns are ordered by similarity of profiles.

The ordering of genotypes and traits by row and column permutation of the distance matrix of rank scores for all observed traits, with suitable numerical coding of qualitative ones, is represented in Figure 3. The ordering of lines seems to capture rather well genotypic relationships, as hybrids are positioned generally beside one of their parents. Genotypes A63 and A67 are the most distant ones. Other genotypes with distinguishable features are 26E, 7E, 1PC and 3DHx26E. A67, 16PC and AK2 may be contrasted with 3DH, 39ExAK2, 6DH, 39E and 5E. Close profiles are apparent

between plant growth habit and earliness; leaf surface rugosity and ribs on fruits; fruit length and yield; plant and stem height and leaf length.

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Molecular characterization of local varieties of eggplant from the region of Valencia, Spain

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Abstract

Eggplant (*Solanum melongena* L.) is the most important *Solanum* crop native to the Old World, and it ranks as one of the most important vegetable crops in the world. Despite the economic and nutritional importance of eggplant, breeding efforts in this crop have been limited. Assessment of the diversity and relationships of the cultivated species facilitates the establishment of conservation strategies, the use of genetic resources in breeding programmes, and the study of the crop evolution. The characterization of eggplant with both morphological descriptors and molecular markers has proved useful for the study of the diversity and relationships of different varietal groups of eggplant, as they sample different levels of diversity. The use of characterized germplasm in breeding programmes can be of great relevance for the improvement of the crop and for addressing future breeding challenges. In this respect, the genetic diversity of modern eggplant cultivars seems to be reduced, and incorporation of new materials from different origins could increase the genetic base and contribute to better exploitation of the heterosis resulting from the crosses of genetically distant materials. The region of Valencia (Spain) has a long tradition of cultivation of vegetable crops and is home to a high number of local varieties of eggplant. The aim of this work is to address the molecular diversity of a collection of local eggplant accessions from the region of Valencia stored in the COMAV germplasm bank. A set of 30 accessions were analysed with 19 selected SSRs based on the PIC values in order to assess the relationships among them. Different analyses have been performed including cluster analysis, principal coordinate analysis (PCoA) and Factorial Analysis of Correspondence. All the accessions could be separated unambiguously and all the analyses performed detected two main groups. The results are important for eggplant breeding, as it shows that sources of variation of interest can be found in the materials evaluated, and also suggests that crossing among selected individuals situated at high genetic distance may result in heterotic hybrids.

Keywords: Genetic distances, genetic resources, landraces, SSRs

Introduction

Evidence indicates that Arabs introduced eggplant (*Solanum melongena* L.) into Spain. Since then, a considerable diversity of Spanish eggplants has been accumulated and many different traditional varieties have arisen (Prohens and Nuez 2001). The evolution of these local varieties has been linked to open field cultivation. However, nowadays the economic importance of greenhouse cultivation is much greater than cultivation in the open field, and most of the varieties used in Spain, especially F1 hybrids, are specifically developed for greenhouse cultivation (Marín 2007). Hybrids have a low morphological diversity and most types have a uniform purple or purple black colour (Daunay et al. 2008). However, as has occurred in tomato and pepper there is an interest in the markets for a diversification of eggplant types. Spanish traditional varieties may be useful for obtaining hybrids or other types of commercial varieties, either for open field or greenhouse cultivation, for diversification of types, and also for the widening of the genetic base of the modern varieties. Molecular characterization of collections is of interest because it can lead to the discovery of materials of value, establish relationships among varieties and provide estimates of the genetic diversity.

SSR markers present several advantages, like their high reproducibility, co-dominance, hyper-variability, relative abundance, and high genome coverage (Morgante et al. 2002), for being used in the characterization of diversity and relationships of plant landraces. SSRs have been successfully applied to the characterization and to obtaining genetic fingerprints of eggplant (Demir et al. 2010, Muñoz-Falcón et al. 2009, Nunome et al. 2003 and Stàgel et al. 2008). In this respect, SSRs seem to be more suited than AFLPs to studying specific sets of genetically related materials, probably because of its sensitivity to neutrality and/or linkage disequilibrium (Tam et al. 2005).

Here we study the molecular diversity of local varieties of eggplant from the region of Valencia with microsatellite markers.

Material and Methods

Plant material and DNA extraction

Thirty accession, of which 28 correspond to *S. melongena* local accessions from the region of Valencia and two controls (a commercial variety 'Listada de gandia' and LF3-24 accession from INRA) were used for SSRs characterization (Table 1).

Genomic DNA was extracted from leaves according to CTAB method procedure (Doyle and Doyle, 1987). The quality of DNA was checked on 1% agarose gels and the DNA concentrations were measured with a Nanodrop ND-1000 spectrophotometer. Samples were adjusted to a DNA concentration of 20 ng/ul.

Microsatellite characterization

Nine-teen microsatellites, developed by Vilanova et al. (2012) were used to evaluate the genetic diversity of the accessions. Amplifications were performed as described by Vilanova et al. (2012). Microsatellite alleles were resolved on an ABI Prism 3100 DNA sequencer (Applied Biosystems) using GeneScan 3.7 software and precisely sized using GeneScan 500 LIZ molecular size standards with GenoTyper 3.7 software (Applied Biosystems).

Data analysis

Marker analysis was performed using the matrix of allele size and the program PowerMarker (Liu and Muse, 2005). The following parameters were calculated:

Number of alleles, allele frequency, major allele frequency, polymorphism information content (PIC), gene diversity and genetic distances according to Nei et al. (1983).

Neighbor-joining (NJ) tree was calculated with PowerMarker 3.25 and plotted with Treeview software (Page 1996). Principal co-ordinates analysis (PCoA) was performed with GenAlex ver.6 (Peakall and Smouse, 2006). A factorial correspondence analysis was performed using the computer program Genetix (Belkhir et al. 2004).

Num.	Code	Shape	Origin	Num.	Code	Shape	Origin
1	V-S-1	semi-long	COMAV	18	V-S-18	Long	COMAV
2	V-S-2	semi-long	COMAV	19	V-S-19	Long	COMAV
3	V-S-3	Long	COMAV	20	V-S-21	Long	COMAV
4	V-S-4	Long	COMAV	21	IVIA-025	semi-long	IVIA
5	V-S-5	Long	COMAV	22	IVIA-347	semi-long	IVIA
6	V-S-6	Long	COMAV				
7	V-S-7	semi-long	COMAV	23	07-A25-01	semi-long	COMAV
8	V-S-8	semi-long	COMAV				
9	V-S-9	semi-long	COMAV	24	Listada de Gandia	Long	Semillas Clemente
10	V-S-10	semi-long	COMAV				
11	V-S-11	semi-long	COMAV	25	LF3-24	semi-long	INRA
12	V-S-12	Long	COMAV	26	N/A	semi-long	IVIA
13	V-S-13	Round	COMAV	27	N/A	Round	IVIA
14	V-S-14	Long	COMAV	28	N/A	semi-long	IVIA
15	V-S-15	semi-long	COMAV	29	N/A	Long	IVIA
16	V-S-16	semi-long	COMAV	30	N/A	semi-long	IVIA
17	V-S-17	Long	COMAV				

Table 1. Group of eggplant accessions used in this study including the fruit shape and origin of each accession

Results and Discussion

SSR characterization

A total of 65 SSR alleles were detected, ranging from two to 11 per locus, with an average of 4.33 alleles per locus.The mean expected heterozygosity (H_e) was 0.57, and ranged from 0.79 in CSM31 to 0.06 in CSM74. Mean observed heterozygosity (H_o) was 0.01. All but two markers (CSM4 and CSM57) were homozygous. These results suggest a high level of inbreeding, probably due to the mostly autogamous nature of eggplant (Pessarakli and Dris, 2004). The average PIC was 0.50 ranging from 0.06 (CSM74) to 0.77 (CSM31). In general, the values obtained are similar to those observed in previous studies conducted with eggplant closely related materials.

Genetic relationship

The phenogram obtained by UPGMA cluster analysis clearly distinguished two main clusters (Fig. 1A). The accessions seem to be clustered based mainly on the fruit shape. The upper cluster show elongated fruit shapes; on the other hand most of the accessions located in the bottom cluster show round fruit shapes. We can also detect sub-clusters like the ones formed by the accessions V-S-17 and V-S-14 or V-S-9 and V-S-11.

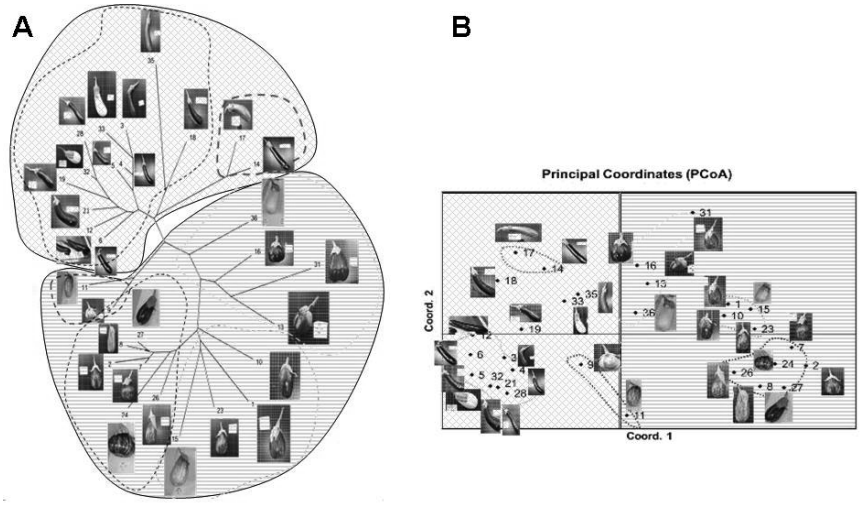

Fig 1. A. Unrooted-UPGMA phenogram of 30 accesions of eggplant based on SSR markers. **B**. Relationships between 30 eggplant accessions based on the first two principal coordinates of the principal coordinates analysis (PCoA).

Multivariate PCoA analysis was performed in order to complement the information obtained with the cluster analysis. The first and second coordinates of the PCoA analysis performed with SSR data account for 47% and 13% of the total variation, respectively (figure 1 B).

The PCoA graph shows also two different groups: in the left-hand part we have the accession located in the upper part of the UPGMA tree; the accessions located in the lower par of the UPGMA tree now appear in the right-hand. These results were also confirmed by factorial correspondence analysis.

In conclusion, all the accessions could be separated unambiguously and all the analyses performed detected two main groups. The evaluation of the SSR markers developed as potential tools for fingerprinting has been demonstrated, as all the accessions used have had a unique SSR fingerprint. In fact, SSRs have proved very useful for studying variation among closely related materials of eggplant (Muñoz-Falcón et al. *2009b*, *2011*).

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Race Distribution of Bacterial Spot Pathogen of Pepper in Korea and A New Source of Hypersensitive Resistance

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Introduction

Bacterial spot caused by *Xanthomonas campestris* pv. v*esicatoria* (*Xcv*) is one of the major limiting factor in pepper production in Korea and in many pepper production areas worldwide. The disease causes spots on the leaves, stems and fruits of pepper and reduces yield and market quality. When the environmental conditions are favorable, the disease can destroy the crop in the field. Use of pepper cultivars resistant to *Xcv* is considered economically and environmentally the most reliable method of control of this disease. Henceforth, identification of resistant cultivars and introgression of resistance genes into existing commercial cultivars and other economically acceptable cultivars is necessary. So far, both hypersensitive (*Bs1, Bs2, Bs3, Bs4*, *Bs1*) and quantitative (*bs5* and *bs6*) resistance genes have been reported in different pepper accessions, and transferred to varieties of economic potential. Near-isogenic lines (NILs) have been developed by transferring the resistance genes to susceptible cultivars such as Early Calwonder (ECW) (Cook and Stall, 1963; Cook and Guevara, 1984; Jones et al. 2002; Kim and Hartmann, 1985; Sahin and Miller, 1996 and 1998; Stall et al. 2009). Currently, Potnis et al. (2012) identified a new hypersensitive resistance (HR) gene, *Bs7* from a genotype of *Capsicum baccatum* var. *pendulum*, namely, 1556 and created ECW70R by transferring *Bs7* gene to ECW. In Korea, Kim, et al. 2007 created Chilseong NILs for race identification purpose. On the other hand, host-differentiated races have also been described and designated as races P0-P10 after finding *Bs4* gene in *Capsicum pubescens,* PI235047(Stall et al. 2009). The prevalence of many races makes controlling the disease by using resistant cultivars complicated. Therefore, identification of the pathological races distributed in a given area and understanding host pathogen relationship are important for breeding resistance cultivars. In Korea, only race 1 and race 3 have been previously reported by Nam et al. (1987), Kim, et al. (1990) and Pae et al. (1994). In this study, we differentiated the races of *Xcv* collected from various regions of Korea by using differential hosts of Chilseong NILS, ECW NILS, KC939 and PI235047. KC939 is a resistant accession which has very high resistance to bacterial spot disease and whenever it is inoculated by spraying method, it remains almost free from the disease or only one or two pin-point spot appears. So far, we considered KC939 as a quantitative resistance source, and we did not identify any hypersensitive reaction in this accession. We included it in this study in order to know its reactions to various isolates. In the process of race identification study, KC939 was found hypersensitively resistant to all bacterial spot isolate tested. Inheritance of the hypersensitive resistance of KC939 was analyzed in an F_2 population resulting from a cross between KC939 and a *Phytophthora* resistant breeding line.

Materials and Methods

Host materials

The differential host, ECW 10R, 20R, and 30R with *Bs1, Bs2, Bs3* hypersensitive resistance genes, respectively, and PI235047 with *Bs4* were kindly provided by USDA while others were at hand. In July 2012, all differential hosts were grown in 200-cell trays with Wonjo mix and 16 plants of each were transferred to 32 cell-trays one month after sowing. On September 2012, the plants

were again transferred to 13 cm diameter pots and inoculated with 63 isolates collected from various location of Korea (from years) to identify the races of each isolate. In January 2013, the confirmation study was again carried out only with ECW NILs and PI235047 after transplanting to 25 cm pots.

Inoculum preparation and infiltration

Xcv isolates to be tested were streaked from the stock cultures on Yeast Extract Dextrose Calcium Carbonate (YDC) media and incubated at 28-29°C. After 2-3 days, typical round, convex, mucoid and yellow colonies were again streaked on YDC media. Two days old pure cultures were taken and dispersed in distilled water by sterile cotton swab for inoculum preparation and the concentration of the bacterial cells was adjusted to approximately 10^8cfu/ml (0.2 OD at 470nm). Inoculation was carried out by hypodermic infiltration to the abaxial surface of the leaves. For each isolate, infiltration was done on each side of midrib and two leaves were used as replication. Races were differentiated by induction of hypersensitive (HR) and susceptible(S) reactions on the differential hosts.

Inheritance of resistance R gene from new source of HR

In the process of race identification, KC939 showed HR reaction to all isolates tested, as did ECW 20R (*Bs2* gene). Within the frame of another disease resistance breeding programme in 2011, Yanggang No.4, which is a *Phytophthora* resistant breeding line, was crossed with KC939 to combine both bacterial spot and *Phytophthora* resistances. In April 2012, the F_1 seeds were sown for producing F_2 seeds and F_2 plants were grown in August in 50 cell-trays with Wonjo mix. F2 segregation of bacterial spot resistance R gene derived from KC939 was examined by infiltration with Xcv 076 collected from KNU greenhouse in 2011. The HR reaction was identified and susceptible plants were discarded. After testing again for *Phytophthora* resistance, the plants resistant to both pathogens were transferred to 32-cells trays and grown for F_3 seeds production.

Results

Race determination

Hypersensitive necrotic lesions were visible after 48 hr while the susceptible phenotype appeared after 3 or 4 days. Out of the 63 isolates tested, only 45 were clear in their reaction with the differential hosts, while the rests were somewhat confusing, particularly with PI235047 (*C. pubescens*). So, confirmation study is underway. The reactions of the 45 isolates are given in Table 1. *Xcv* race P1, P3, P7 and P8 were distributed in 5 provinces of Korea. Among the 45 isolates, 27 (60.0%) were P1, 3 (6.7%) were P3, 9 (20.0%) were P7 and 6 (13.3%) were P8. P7 and P8 are new races, which were not previously reported. Race P7 and P8 are differentiated from P1 and P3, respectively, on the basis of reaction to PI235047 (Sahin and Miller, 1998). The distinct HR reaction of KC939 to all isolates tested resembled to that of ECW20R (Fig. 1).

Inheritance of hypersensitive resistance in KC939

Evidence of HR and susceptible reactions could be seen after 2-3 days in F_2 plants. Out of 500 plants tested, 376 plants showed HR to the isolate Xcv076 used. The 376 HR to 124 non-HR segregation well fitted the 3:1 ratio for single gene segregation with Chi Square value of 0.01. Now the question was whether the HR gene in KC939 was the same as *Bs2* or not. Seed samples of KC939 were sent to Kang in Seoul National University for testing the presence of *Bs2* gene by molecular marker, and also to Jones in the University of Florida for testing reaction of KC939 to race P4, P5, and P6 of *Xcv* which can induce susceptible reaction to the plants carrying Bs2 gene. Molecular results from Kang and reaction to races 4, 5 and 6 from Jones agreed that the HR gene carried by KC939 is the same as *Bs2*.

Discussion

Our results concerning race identification indicate that race P1 was predominant, but races P3, P7, and 8 were also found. Further, strains inducing susceptible reaction on ECW 20R and KC939 were not found. This indicates that races P4, P5, P6, P9 and P10 that can overcome *Bs2* are not present in Korea so far. Strains inducing HR on ECW10R were also not found. This also suggests that race P2 is not present in Korea. From several collections made in 1985 to 1986, Nam et al. (1987) reported that all isolates collected were race P1. In 1990, Kim et al. reported that race P3 was predominant in Korea as they found that 36 isolates out of 41 belonged to race P3. Among 10 isolates they collected from Gyeongbuk province, 8 were race P3. However, Pae et al. (1994) identified race P1 in 13 out of 19 isolates from Gyeongbuk and Gyeongnam province collections. In the present study, 14 out of 28 isolates were race P1 and no race P3 was found among Daegu and Gyeongbuk isolates, and 27 (60%) of all 45 countrywide isolates were race P1. The difference between the reports may be because of different collection site even in the same province. Pohronezny et al.(1992) described the sudden race shift of the predominating race of *Xcv* in Southern Florida. Kousik and Ritchie (1996) also reported that when ECW plants inoculated with P1 isolates were grown among ECW30R plants, race P1 infected all ECW30R plants by changing to race P3 within 10 weeks. Similarly, in 1994, as they grow race-P2-inoculated plants among ECW10R plants, it changed to race P3 and infected the plants within a growing season. By this evidence, race P1 and race P2 can easily change into race P3 within a short period of time. Similarly, the previous reports together with our findings suggest that there would be some possible race shifts in Korea. Moreover, race P0 to P10 were already reported throughout the world, and we report here the presence of races P7 and P8 in Korea. The above evidences suggest that resistance of cultivars with hypersensitive genes alone or combined can easily breakdown if new pathotypes resulting from race shifts occur. In summary, as suggested by Kim et al, 1990, the pyramiding of major genes with race non-specific quantitative resistance is essential to get cultivars with durable resistance to bacterial spot pathogen, and race determination of more isolates throughout Korea is also necessary.

KC939 was found to carry a *Bs2* gene. *Bs2* gene was originally found in PI260435 that belongs to small-fruited wild species *Capsicum chacoense* (Cook and Guevara, 1984) and it was introduced into Early California Wonder (ECW) to breed ECW20R. KC939 is an accession of *C. annuum* with typical chile-type characteristics, i.e., dark green leaves and pungent chile fruits 12cm or longer. KC939 shows clear and fast HR reaction when leaves are infiltrated with *Xcv* isolates. It exhibits almost no symptoms on the leaves when inoculated by spraying. Thus, this accession appears to be a rich source of resistance that may carry polygenic background in addition to *Bs2*. A confirmative experiment for allelic relationship between *Bs2* gene in ECW 20R and supposedly *Bs2* in KC939 is being conducted.

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Table 1. Distribution of *Xanthomonas campestris* pv. *vesicatoria* in 5 provinces of Korea

Location (Provinces)	Total No. of isolates	Races			
		P1	P3	P7	P8
Daegu and Gyeongbuk	28	14	-	8	6
Chungbuk	3	1	2	-	-
Gangwon	1	-	1	-	-
Jeonnam	8	7	-	1	-
Jeonbuk	5	5	-	-	-
Total	45	27	3	9	6
Total (%)	100	60.0	6.7	20.0	13.3

Fig. 1. Hypersensitive reaction on a leaf of KC939 (left). Fruits of KC939 (right).

SESSION IV

Breeding strategies

Reaction of some resistant and susceptible eggplant genotypes to different isolates of Fusarium wilt

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Abstract

Two experiments were carried out to investigate "genotypes x isolates" interaction for resistance to *Fusarium* wilt in eggplant. A total of fifteen genotypes including wild accessions and cultivated forms were tested against twelve *Fusarium oxysporum* Schlecht. f. sp. *melongenae* (*Fomg*) isolates (eleven from Turkey and one from Italy) in the study. Root-dip method was performed for artificial inoculation of the pathogen. Experiments were conducted according to randomized complete block design in split-plot using three replications. Infected plants were scored for the severity of the disease symptoms using a 1 to 5 scale after four weeks. The findings of the experiment showed that the isolates and genotypes were placed into two groups (low and high virulence) and three groups (resistant, partly resistant and susceptible), respectively. Two wild accessions belonging to *Solanum aethiopicum* species and two wild accessions belonging to *Solanum melongena* were completely resistant to all tested isolates. Some wild genotypes and one cultivar (Topan 374) were partly resistant, showing specific interactions with *Fomg* isolates, being more resistant to some isolates but less to some others. Other cultivated genotypes were highly susceptible to all isolates.

Keywords: *Solanum melongena* cultivated forms, wild *Solanum* species, artificial inoculation

Introduction

Eggplant is one of the most popular vegetable crops of the Mediterranean countries. It is also largely grown in Asia, Africa, some part of the Europe and the United States. The important problems encountered in cultivation of this crop are soil borne fungal diseases and one of them is a wilt disease caused by *Fusarium oxysporum* Schlecht. f.sp. *melongenae (Fomg*) (Altınok & Can, 2010). *Fusarium* wilt causes significant losses in yield by killing the infected plants (Singleton et al. 1992) and its damages occur both in open field and under cover cultivation. The important commercial eggplant cultivars and hybrids are susceptible to the disease (Cappelli et al. 1995, Boyaci et al. 2012). However, some resistance genotypes including wild and cultivated forms have been identified and breeding studies are in progress (Rotino et al. 2001; Rizza et al. 2002; Toppino et al. 2008, Boyaci et al. 2011 and 2012). In such breeding programs, knowledge on the virulence of the pathogen used in the tests is very important. It is also essential to know if there are the differences in the responses of the resistant genotypes against different isolates of the pathogen. Although non-pathogenic isolates of *Fomg* have been described, genetic variability of this group is not well known. Altınok and Can (2010) showed that the cultivar resistance and isolate pathogenecity of *Fomg* showed some variation due to location. In another study Baysal et al. (2010) tested the aggressiveness of ten isolates obtained from Turkey on a sensitive cultivar and found a big variation among them. The aim of the study presented here is to evaluate the response of resistant and sensitive cultivated and wild eggplant genotypes against various *Fomg* isolates and to investigate if there is a "genotypes x isolates" interaction for the resistance.

Material and Methods

In the study two experiments were conducted in a climate-controlled greenhouse of BATEM in Antalya. A total of 15 wild and cultivated eggplant genotypes and 12 *Fomg* isolates were used in the experiments. The tested genotypes were consisted of eight cultivated forms (one hybrid, four OP cultivars and one inbred line) and seven from wild *Solanum* species (*S. aethiopicum, S. integrifolium, S. sisymbrifolium and S. violaceum*). Of the tested twelve isolates, eleven were collected from the Mediterranean part of Turkey (FOM 28 and FOM 36 from Adana; FOM 10, FOM 16, FOM 20 from Mersin and E 5, E 6, E7, E 8, AF and 1934 PT from Antalya provinces) and one (TLY) provided by Prof. Dr. Rotino from Italy. In the first experiment all genotypes were tested against all isolates. In the second experiment only selected five genotypes belonging to *Solanum melongena* (LS 1934, LS 2436, KEMER, TOPAN 374 and NSFB 99) were tested against selected four isolates (FOM 10, FOM 36, E 6, AF). For inoculums preparation, first pure cultures of isolates were obtained by monospore isolation and than they were cultured on Potato Dextrose Agar (PDA) medium for a week in an incubator at 24°C in dark. The seedlings were grown in 180x165 mm disinfected plastic pots containing sterilized 1/1 (v/v) mixture of "peat moss - perlite". The root-dip method described by Cappelli et al. (1995) was used for resistance tests. The 3-4 fully expended true leaf stage seedlings were inoculated by immersing their roots into the conidial suspensions (Pitrat et al. 1991) of fungus for five minutes. Control plants were treated similarly and dipped in sterile distilated water. Inoculated plants were transplanted to a climate-controlled greenhouse (temperature of day/night 25±2 ^{0}C/20±2 ^{0}C, 60 % humidity). After four weeks, disease symptoms were evaluated according to the 1 to 5 scale descibed by Cappelli et al. (1995), where 1: no symptom, 2: plants lacking one or two cotyledons, 3: reduced growth plants with yellowed leaves, 4: heavily stunted plants, 5: died plants. Disease density was described as the percentage of diseased plants. The disease severity index was calculated with the formula $I=^5\Sigma$ n i/n, where n: number of plants and i: disease index. Plants with rating 1 to 3 were considered resistant while 4 to 5 susceptible. Experiments were conducted randomized block design in split-plot with three replications using 30 seedlings per plot.

Results

Statistical analysis of the data obtained from the first experiment has shown that there are significant differences both among the genotypes for their resistance to disease (F=120.11**) and among the isolates for their aggressiveness on eggplant plants (F=45.10**). "Genotype x isolate" interaction was also found significant (F= 3.77**). Among the isolates FOM 20, FOM 10, FOM 28 and E 8 were found more virulent than the others (Table 1). Most of the wild *Solanum* accessions were resistant to disease. Among them two *Solanum aethiopicum* (genotype numbers 3 and 4) were found completely resistant to all isolates. Other wild accessions showed a great variation; they were completely resistant to some isolates while partly resistant to some other ones. Among the genotypes belonging to *Solanum melongena,* two accessions (LS 1934 and LS 2436) were completely resistant to all isolates and one (Topan 374) was partly resistant. Topan 374 was found completely resistant to some isolates, partly resistant to some ones while susceptible against some others. In the second experiment only five selected *Solanum melongena* genotypes (two resistant, two susceptible and one partly resistant) were tested against four selected isolates. The results of this trial were very confirmative of the results of the first experiment (Table 2). There were very clear differences among the genotypes (F= 760.81**) for their resistance and isolates for their aggressiveness (F= 35.96**). Genotype X isolate interaction also was found highly significant (F= 8.59**). LS 1934 and LS 2436 were very healthy and did not show any disease symptoms. Kemer and NSFB-99 showed severe symptoms rating 4.43 and 4.06, respectively. However, the response of the partly resistant genotype Topan 374 varied among the isolates.

Discussion

The findings of our two experiments showed that there is a big variability in term of the response of the resistant genotypes to different isolates of the *Fomg.* Besides the genotypes which were either wholly sensitive or resistant to all isolates, there were some genotypes (i.e. Topan 374 or *S. aethiopicum* I) with varying resistance depending on the aggressiveness of the infecting isolates. Thus, in spite of the apparent statistically significant interactions between genotypes and isolates, the results do not permit to suggest the presence of physiological races or the pathogen that can affect breeding strategies.

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Table 1. Disease severity groups formed by *Fomg* isolates on different genotypes in the first experiment.

	ISOLATES												
Genotypes	**FOM 10**	**FOM 16**	**FOM 20**	**FOM 28**	**FOM 36**	**E5**	**E6-719**	**E7-738**	**E8-738**	**1934 PT**	**AF**	**TLY**	**Average**
S.aethiopicum I	4.27 ab	3.03 c	3.80 abc	3.80 ab	2.47 cdef	3.73 ab	2.27 bcde	1.47 e	3.77 a	2.97 bc	2.40 def	1.00 e	**2.96**
S.aethiopicum L.II	3.03 bcd	3.83 bc	4.20 ab	4.10 a	3.97 ab	4.23 a	3.80 a	4.00 a	4.70 a	4.20 ab	3.83 ab	3.33 ab	**3.95**
S. aethiopicum gr *Aculeatum* II	1.13 ef	1.13 e	1.13 e	1.27 d	1.27 e	1.00 g	1.00 g	1.00 e	1.00 b	1.07 d	1.10 g	1.00 e	**1.09**
S. aethiopicum gr *Gilo* III	1.83 def	1.70 de	2.57 cd	1.37 cd	2.03 def	2.00 defg	1.47 defg	1.73 de	1.13 b	1.47 d	2.53 cdef	1.73 cde	**1.78**
S. integrifolium II	2.73 cde	2.67 cd	2.57 cd	2.57 bc	2.60 cde	2.03 defg	1.40 efg	1.17 e	1.90 b	1.83 cd	2.73 bcde	1.67 cde	**2.16**
S. sisymbriifolium II	3.13 bc	1.67 de	3.00 bcd	2.03 cd	1.53 ef	1.67 efg	1.67 cdefg	1.67 de	2.20 b	2.20 cd	2.20 defg	3.10 ab	**2.17**
S. violeceum	4.20 ab	3.63 bc	4.47 a	4.23 a	2.33 cdef	2.30 cdef	1.70 cdefg	3.57 ab	4.27 a	3.00 bc	1.98 efg	2.10 bcde	**3.15**
LS 1934	1.77 ef	1.40 e	1.03 e	1.27 d	2.43 cdef	1.00 g	1.30 fg	1.00 e	1.50 b	1.23 d	1.43 fg	1.37 de	**1.39**
LS 2436	2.57 cdef	1.47 de	2.03 de	1.60 cd	1.40 ef	1.50 fg	1.50 cdegf	2.00 de	1.57 b	1.13 d	1.40 fg	1.47 de	**1.64**
Topan 374	4.73 a	3.53 bc	4.90 a	4.90 a	4.60 a	1.60 efg	1.93 cdefg	2.07 cde	4.70 a	4.03 ab	2.37 def	2.23 bcde	**3.47**
Aydın Siyahı	4.93a	5.00 a	5.00 a	5.00 a	2.90 bcd	2.80 bcde	2.73 abc	2.60 bcd	4.90 a	5.00 a	2.70 bcde	2.30 bcd	**3.82**
Long purple	5.00 a	4.70 ab	5.00 a	5.00 a	3.33 bc	2.80 bcde	2.67 abcd	2.67 bcd	5.00 a	5.00 a	3.98 a	2.20 bcde	**3.94**
Kemer	5.00 a	5.00 a	5.00 a	5.00 a	2.53 cde	3.33 abc	3.50 ab	3.30 abc	5.00 a	5.00 a	3.73 abc	2.53 bcd	**4.08**
NSFB-99	5.00 a	5.00 a	5.00 a	5.00 a	3.17 bcd	3.13 abcd	2.67 abcd	3.70 ab	5.00 a	5.00 a	3.27 abcd	3.97 a	**4.16**
Lima F_1	5.00 a	4.43 ab	5.00 a	5.00 a	3.13 bcd	2.70 bcdef	2.63 abcde	2.60 bcd	5.00 a	4.93 a	3.33 abcd	2.83 abd	**3.88**
Average	**3.62**	**3.21**	**3.67**	**3.48**	**2.65**	**2.39**	**2.15**	**2.30**	**3.44**	**3.20**	**2.60**	**2.19**	**2.91**

LSD = 1.259161 probability = 0.01

Table 2. Disease severity groups formed by *Fomg i*solates on different genotypes in the second experiment.

Genotype	**FOM 10**		**FOM 36**		**E6-719**		**AF**		**Average**	
LS1934	1.00	i	1.00	i	1.00	i	1.00	i	**1.00**	**D**
LS2436	1.00	i	1.00	i	1.00	i	1.00	i	**1.00**	**D**
Topan 374	3.10	fg	2.33	h	2.47	gh	3.47	def	**2.84**	**C**
Kemer	4.80	ab	4.33	abc	3.83	cde	4.77	ab	**4.43**	**A**
NSFB-99	4.13	bc	3.97	cd	3.20	ef	4.93	a	**4.06**	**B**
Average	**2.81**	**B**	**2.53**	**C**	**2.30**	**D**	**3.03**	**A**		

LSD = .493709 probability = 0.01

Homogeneity analysis of *Capsicum frutescens* with SSR primers developed for *Capsicum annuum*

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Abstract

In a breeding program, among several requirements for release and launch of a new cultivar, it is necessary to have uniformity and stability. Uniformity refers to the homogeneity of the plants in the same generation, while stability is the maintenance of the characteristics through successive generations. Until recently, homogeneity was assisted by phenotypic methods. This kind of evaluation occupies extensive areas, is time and labor consuming and usually dependent on subjective decisions. The evaluation based on molecular markers of DNA is conclusive, it does not suffer from the environment influences and the data obtained are reproducible and stable. Microsatellite markers are suitable for those analyses because they are highly polymorphic, reproducible, codominant and inexpensive. The aim of this study was to select microsatellite markers and use them to determine the homogeneity of 50 plants of six peppers lines in S6, of the breeding program. For that, 250 primers designed for *Capsicum annuum* were tested and optimized for *C. frutescens*. The analysis of allelic frequency showed high value of homogeneity (Observed Heterozygosity from 0.007 to 0.07). It was, therefore, concluded that some of the strains in question were probably in an appropriate genetic uniformity to cease self-pollination.

Keywords: Microsatellite markers, uniformity, *Capsicum* lines

Introduction

The increasing peppers (*Capsicum frutescens*, *C. chinense* and *C. baccatum*) demand for domestic and foreign markets caused the expansion of the cultivated area in several Brazilian states, mainly in family farming initiatives. Besides being consumed *in natura*, peppers supply agribusiness and can be processed and used in several product lines, standing out among the species most used as condiments, surpassed only by garlic and onion. According to Guidolin (2005), in Brazil, dozens of different varieties of these peppers are produced. Although the pepper cultivation is still rustic, it is a market that moves around 40 million dollars per year, including domestic consumption and exports. Only the marketing of seed is responsible for a market of more than 1.5 million dollars.

Pepper is a predominantly autogamous species capable of self-pollination, ensuring greater uniformity of species. To launch new cultivars it is desirable to determine their uniformity and stability to ensure that they are uniform in their generation and keep the characteristics in future generations. Testing for these characteristics is generally based on morphological and physiological characteristics, called descriptors, obtained by planting cultivars side by side. This type of evaluation occupies large areas, is time and labor consuming and often submitted to subjective decisions (Cooke, 1995). Additionally, as many of the features are quantitative or multigenic and their expression is affected by environmental factors, repetitions of observations are required. Thus, it is important to develop more rapid and cost-effective testing procedures to improve the current testing systems, and the use of molecular markers is a promissory approach (Vélez and Ibáñez, 2012). Molecular markers define differences in nucleotide sequences, which are not affected by the stage of growth, climate, location or agronomic practices (Ferreira and Grattapaglia, 1998). Simple

Repeated sequences (SSR) or microsatellite are genetic markers used for a detailed genomic analysis as they are multi-allelic, highly polymorphic, reproducible and inexpensive besides being based on PCR and have co-dominant inheritance with high information content (Ferreira and Grattapaglia, 1998).

In wheat, Wang et al. (2009) studied the feasibility and effectiveness of molecular markers in evaluating the uniformity and stability of wheat cultivars based on the ratio of homozygous DNA loci. They found that cultivars with higher ratios of homozygous DNA locus possessed better uniformity and stability. In the 375 cultivars from the National Regional Trials, most cultivars with the ratio of homozygous DNA loci higher than 95% and a few cultivars with the ratios ranging from 90% to 95% showed good performance on uniformity and stability, whereas, cultivars with the ratio of homozygous DNA loci smaller than 90% had obviously phenotypic segregation. They concluded, therefore, that the ratio of homozygous DNA locus can serve as a supplementary criterion in evaluating uniformity and stability of wheat cultivars. In addition, analysis of homogeneity of a species through the use of molecular markers are quite efficient (Wang et al. 2009), since they aim to save time and financial resources, obtaining conclusive results and free from environment influence. Considering this, this study aimed to examine the homogeneity of lines of *C. frutescens* from the breeding program using SSR markers developed for *C. annuum*.

Materials and Methods

The pepper plants used, originated from the Capsicum breeding program of Embrapa Vegetables (CNPH). Leaves were collected from 50 plants from each of six *C. frutescens* breeding lines, in S6. Samples were taken to the Laboratory of Plant Genetics at Embrapa Genetic Resources and Biotechnology (CENARGEN) for DNA extraction, following the protocol CTAB 2% (Ferreira & Grattapaglia, 1998). The DNA was quantified and diluted and 12 individuals of *C. frutescens* were randomly assigned to test SSR primers designed for *C. annuum*. After optimization of primers for amplification of *C. frutescens* DNA, they were used for analysis of all the material.

To carry out the PCR amplification, reactions were performed with 3µL DNA (3.0 ng/µL), 3µL of primer (0.9 mM) and a mixture of 7µL mix consisting of 2.65 µL of autoclaved Milli-Q water, 1.30 µL of dNTP (2.5 mM), 1.30 µL of BSA (2.5 mg/mL), 1.30 µL of 10x buffer, 0.25 µL of MgCl2 (50mM) and 0.20 µL of Taq DNA polymerase, in a total of 13µL per reaction. The amplification reactions were performed in thermal cyclers GeneAmp PCR system 9700 AB, using 30 cycles.

Amplifications were visualized by gel electrophoresis in 3.5% agarose gel, stained with ethidium bromide. The visualization of the bands in the gel was made directly through photo-analyzer. The primer pairs that produced good amplification were used to estimate the sizes of alleles and genotyping. The number of alleles, PIC (polymorphism information content) and observed heterozygosity were calculated through the program GDA (Genetic Data Analysis) (Lewis & Zaykin 2001).

Results and Discussion

In this study the homogeneity of *C. frutescens* breeding lines for a set of 20 microsatellite markers have been surveyed. These markers were developed in our laboratory for *C. annuum*. One hundred of these SSR primers developed for *C. annuum* were tested and 42 amplified *C. frutescens* DNA. Of these, 20 were optimized (Table 1) and used for the analysis of homogeneity of 50 plants of each of the six breeding lines, at S6, of the *C. frutescens* breeding program. The number of alleles per locus varied from 2 to 4, with average of 2.45, a little smaller than 3.3 found by Kwon et al (2005) for 27 SSR primers used to analyze *C. annuum* cultivars. In addition, in the present study

the PIC values for the 20 microsatellite markers ranged from 0.01 to 0.27, with an average of 0.0529. Our results were smaller than that found by Kwon et al (2005), probably because of the plant material analyzed.

Table 1. Number of alleles per locus, polymorphism information content (PIC) and observed heterozygosity (Ho) values for the SSR markers examined in 50 plants of six *C. frutescens* breeding lines.

Locus	No of alleles	PIC	Ho
BRCA01	2	0.030674	0.031128
BRCA16	3	0.023251	0.001957
BRCA17	3	0.278369	0.201550
BRCA24	2	0.036248	0.036893
BRCA26	2	0.005803	0.005814
BRCA29	2	0.015579	0.015686
BRCA38	2	0.017340	0.017476
BRCA47	2	0.013487	0.013566
BRCA98	2	0.017373	0.017510
BRCA100	2	0.164150	0.001938
BRCA102	4	0.168558	0.003899
BRCA105	3	0.036213	0.036822
BRCA107	2	0.030733	0.031189
BRCA116	4	0.047747	0.048544
BRCA129	3	0.080617	0.074219
BRCA130	3	0.023117	0.011673
BRCA131	2	0.019211	0.019380
BRCA195	2	0.030557	0.031008
BRCA198	2	0.001961	0.001961
BRCA227	2	0.001942	0.001942

Table 2. The observed heterozygosity (Ho) and homogeneity of 50 plants of each line of the breeding program, analized with SSR markers.

Lines code	Ho	Homogeneity=1,00-Ho
2744-3	0.0068	0.99
2744-4	0.0352	0.96
3257-1	0.0507	0.95
3257-3	0.0120	0.99
3448-1	0.0688	0.93
3448-2	0.0069	0.99
3462-3	0.0100	0.99
3462-4	0.0306	0.97
3746-3	0.0046	0.99
3746-4	0.0163	0.98
3835-1	0.0353	0.97
3835-3	0.0414	0.96

The homogeneity or ratio of homozygous DNA loci, obtained varied from 0.93 to 0.99 (Table 2). In wheat, an autogamous species as *Capsicum*, Wang et al. (2009) concluded that the ratio of homozygous DNA locus can serve as a supplementary criterion in evaluating uniformity and

stability of wheat cultivars. Cultivars with the ratios ranging from 90% to 95% showed good performance on uniformity and stability, whereas, cultivars with the ratio of homozygous DNA loci smaller than 90% had obviously phenotypic segregation. In the present study the majority of the lines analyzed had the ratio of homozygous DNA higher than 0.95 and only one with 0.93. Therefore, it could be concluded that all the lines in question were probably with suitable genetic homogeneity. This analysis can serve as a supplementary criterion in evaluating uniformity of *C. frutescens* lines helping in the decision to stop the self-crossing. Moreover, with additional studies, this method could be also effective for other Capsicum species breeding, which may help to monitor the progress of breeding on homozygosis and predict the breeding period.

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Studies on the cytological and biochemical basis of the male sterility in the pepper line 'K132A'

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Abstract

Pepper is one of important vegetable crops. It takes a lot of time and spends much money to produce hybrid seeds. In order to reduce cost, a pollenination control mechanism for the production of hybrid seed on a large scale is also required. The major object is to look insight the reason of male sterile, and do basal research for the usage of male sterile.

The following results have been gotten:

1. Bud paraffin transverse sections were studied in anthers of normal and male sterile to ascertain the stage at which degeneration of microspores. Sections of the anthers of normal buds showed normal meiosis leading to normal development of microspores till the tetrad stage, and the locules were full of fertile pollens. In comparison, tapetum of the CMS line showed some abnormalities (swelled, with liquid materials) . At post –tetrad stages cells of the tapetum layer expanded into the locules and made no space for microspores to develop. At last the locules collaped and fulled with completely degenerated microspores and formed the "L" type.

2.The activities of POD, PPO and SOD in male sterile line were extensively higher than that in maintainer line whereas the activity of CAT in male sterile line was lower than that in maintainer line, suggesting that the unbalance enzyme activity might be the cause of male sterile in hot pepper

Observations of bud paraffin transverse sections viewed with the light microscope showed the tapetum of CMS (K132A) swelled abnormally and pressed against pollen grains of the locule. Results showed that there was no difference between male sterile and male fertility lines in leptotene stage . then the abnormal tapetum phenomenon (increased size, full of liquid) happened in male sterile plant . The irregular tapetum make the microspore can not absorb the nutrition and lend to the collapse of anther lobule containing shrunken pollen grains. And the anther lobule became 'L' type at end of tetrad stage.

In conclusion, the lower content of protein and proline, unbalance enzyme activity, irregular tapetum development lend to the collapse of anther lobule and to male sterile. These studies have provided a wealth of important informatiom on the cause of male sterility

Keyword: Pepper Cytoplasmic male sterile, Male gamete abortion

Detection of capsinoids by ESI-mass analysis

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Abstract

The substances responsible for the pungency of *Capsicum* are a group of alkaloids known as capsaicinoid. Recent studies have also revealed the presence of a novel group of non-pungent capsaicinoid-like substances named capsinoids. These compounds have structures similar to capsaicinoids and produce the same biological effects but without the undesirable irritation caused by the pungency, turning into molecules with potential applications in areas such as medicine. The capsinoid biosynthesis pathway is being investigated to trying to clarify the genetic and metabolic mechanisms of biosynthesis. The development of a more sensitive and selective analytical method to identify these compounds may be useful to select pepper cultivars that produce these compounds and help gain increase knowledge about their biosynthesis and biological activity.

Keywords: *Capsicum*, capsinoid, pepper, mass spectrometry

Introduction

Capsaicinoids are compounds responsible for pungency in pepper (*Capsicum* spp) that are synthesized by condensation of a common vanillyl moiety and a variable fatty acid (Nelson et al. 1923). These secondary metabolites exhibit pharmacological effects such as analgesia, anticancer, antioxidant and antiobesity activities among other (Reyes-Escogido et al. 2011). However, their use as ingredients in certain foods and pharmaceuticals has been limited by pungency, the main feature that makes them popular as a spice. Recently, another capsaicinoid-like substances, named capsinoids, have been discovered in pepper fruit extracts from different cultivars, such as 'CH-19 Sweet'(*C. annuum*) (Yazawa et al. 1989), 'Zavory Hot', 'Aji Dulce', 'Belice Sweet' (all of them *C. chinense*) (Tanaka et al. 2010), and 'SR211'(*C. annuum*) (Koeun et al. 2013). So far, capsiate (4-hydroxy-3-methoxybenzyl (*E*)-8-methyl-6-nonenoate), dihydrocapsiate (4-hydroxy-3-methoxybenzyl 8-methylnonanoate) and nordihydrocapsiate (4-hydroxy-3-methoxybenzyl 7-methyloctanoate) (Kobata et al. 1998; Kobata et al. 1999) have been isolated in pepper fruit extracts. Fundamental chemical structure and the biologic activity of these compounds are very similar to the capsaicinoids. Unlike capsaicinoids, capsinoids are synthesized by the condensation of a variable fatty acid and a vanillyl alcohol moiety, in addition, are non-pungent, producing the same biological effects but without the undesirable irritation caused by the pungency, turning into molecules with potential applications in areas such as medicine (Sasahara et al. 2010). Many analytical methods have been developed to determine capsaicinoids using gas (GC) and high-performance liquid chromatography (HPLC) coupled to UV-Visible (UV) or mass spectrometry (MS) detection (Schweiggert et al. 2006; Garcés-Claver et al. 2006). However, a limit number of methods has been developed to determine the capsinoid compounds, using most of them UV detection. Significant structural information can be obtained from electrospray ionization (ESI)-(MS), quadrupole time-of-flight (QTOF) and ESI-ion trap-MS that provide molecular fragmentation patterns. These techniques can be used to identify unknown capsinoid-type molecules and also to confirm the identification of the three known capsinoids in vegetable matrices, which could be difficult due to the low concentrations present and the complexity of the matrix.

Therefore, the aim of this study has been the optimization of a mass detection method by high-resolution tandem mass spectrometry (QTOF and ion trap MS analyzer) that allows accurate *m/z* measurements of capsinoid ions and their product ions and the characterization of the fragmentation patterns of capsiate and dihydrocapsiate.

Material and Methods

Chemical and Reagents

Vanillin, anhydrous pyridine, *t*-butyl-dimethyl silyl chloride and di-isobutyl aluminum hydride (1M in toluene) were purchased from Sigma-Adrich (St. Louis, MO). *Trans*-8-methyl-6-nonenoyl chloride was purchased from Ambinter (Greenpharma S.A.S., Orleans, France) and 8-methyl-nonanoic acid was obtained from Acros Organics (New Jersey, USA). Ethyl acetate (≥ 99.8%, HPLC-grade) and n-hexane (≥ 95%, HPLC-grade) were obtained from VWR International L.L.C. (Radnor, Pensilvania, USA). Magnesium sulfate anhydrous (96%), Copper (II) sulfate pentahydrate (99.0-100.5%), hydrochloric acid (37%), and ethanol (99.5%) were purchased from Panreac Química (Barcelona, Spain).

Synthesis of capsiate and dihydrocapsiate standards

The synthesis of capsiate and dihydrocapsiate were accomplished at the University of Cádiz (UCA) (Cádiz, Spain). Capsiate was synthesized from the esterification of reduced and protected vanillin with the corresponding acyl chloride. Hydroxyl group of vanillin was protected with *t*-butyl-dimethyl silyl chloride, followed by a reduction of the aldehyde group with di-isobutyl aluminum hydride (1M in toluene). The silylated reduced vanillin was esterificated with the corresponding acyl chloride to obtain the silylated capsiate. Capsiate was desilyated with 0.25M HCl/ethanol mixture (1:5). Similar procedure was employed for the synthesis of dihydrocapiate standard. Proton and carbon nuclear magnetic resonance (^{1}H NMR and ^{13}C NMR) spectroscopy (UCA) was used for the structural elucidation of the synthesized capsiate and dihydrocapsiate.

ESI-MS^2(QTOF) and ESI- MS^n(Ion trap) analysis

Capsiate and dihydrocapsiate standard solutions (10 μM, 70% methanol and 0.1% formic acid) were detected by direct injection with a syringe pump (Cole-Parmer Instrument Co., Vernon Hills, IL, USA) operating at 4 μLmin^{-1}, in a QTOF and ion trap mass spectrometer equipped with an ESI source (MicroTof-Q and Esquire 3000 plus, Bruker Daltonics, Bremen, Germany). ESI-MS^2(QTOF) analysis was carried out in positive ion mode, with capillary and endplate offset voltages of 4800 and -500 V, respectively, and using N_2 as collision gas. Collision cell energy was set to 10 eV, with an isolation width for the precursor ion of 4 *m/z* units. The nebulizer (N_2) gas pressure, the drying (N_2) gas flow rate and the drying gas temperature were 0.7 bar, 4.0 Lmin^{-1} and 200ºC, respectively. The mass axis was calibrated using Na-Formate adducts [10 mM NaOH, 2.5% (v/v) formic acid and 50% (v/v) 2-propanol] that were introduced by direct injection.

ESI-MS^n(Ion trap) analysis was carried out in positive mode, with capillary and endplate offset voltages of 4000 and -500 V, respectively and using He as the collision gas. Spectra were acquired in the *m/z* 50–500 range at the Standard/Normal scan mode. For MS^2 spectra the $[M+Na]^+$ ions were chosen as precursors, while product ions with 159 and 137 *m/z* (characteristic of capsinoid fragmentation) were used as precursors for MS^3 analysis with an isolation width of 4 *m/z* and an amplitude voltage of 0.45 V. Bruker Daltonik software packages micrOTOF Control v.2.3, Esquire Control v.5.3 and Data Analysis v.4.0 were used to control the MS apparatus and process data, respectively.

Results and Discussion

The aim of the study was to optimize the mass detection of capsinoid compounds, for this purpose capsiate and dihydrocapsiate standards were synthesized. The procedure used for the synthesis was previously developed by Barbero et al. (2010). The structures of the capsiate and dihydrocapsiate synthesized were confirmed by NMR analysis. NMR data for the two standards matched the results obtained by Barbero et al. (2010).

ESI-MS^2 spectra of capsiate and dihydrocapsiate were obtained by direct injection of standard solutions (10µM) on the QTOF and the ion trap mass analyzers. For both mass analyzers, the major peaks observed in the ESI-MS spectra were the $[M + Na]^+$ molecular ions at the *m/z* 329 and 331, corresponding to sodiated capsiate and dihydrocapsite molecules, respectively. These ions were selected as precursor for the ESI-MS^2 experiments. Figure 1 shows the ESI-MS^2 spectra obtained for capsiate and dihydrocapsiate. For both capsinoids, the most intense product ion was observed at *m/z* 159.0 corresponding to the sodiated vanillyl ring shared by all capsinoids. Also, other minor product ions detected corresponding to the vanillyl ring were *m/z* 137.1 and 177.1, generated by the protonated vanillyl molecule and the different fragmentation of the sodiated vanillyl ring, respectively. Product ions at *m/z* 193.1 and 195.1, for capsiate and dihydrocapsiate, respectively, indicated the acyl chain residues. This is the first time that the fragmentation patterns of capsiate and dihydrocapsiate has been carried out.

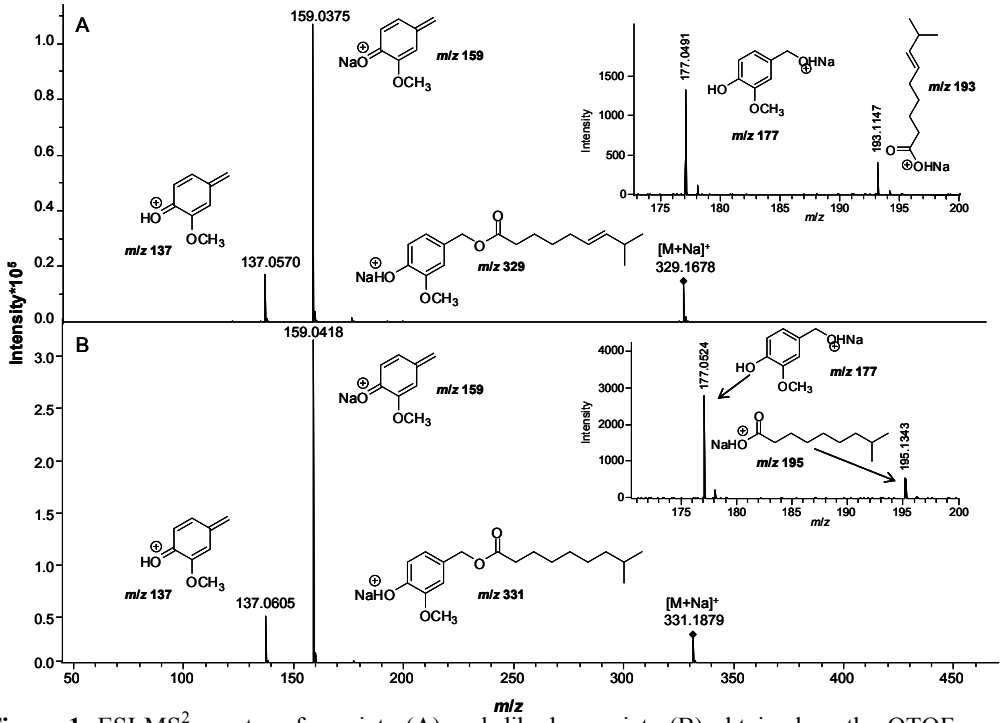

Figure 1. ESI-MS^2 spectra of capsiate (A) and dihydrocapsiate (B) obtained on the QTOF mass spectrometer in positive ion mode. Proposed fragmentation patterns for capsiate (A) and dihydrocapsiate (B).

The product ions with a 137 *m/z* obtained by the ESI-MS^2 analysis of capsiate and dihydrocapsiate standards were used as the precursors for MS^3(Ion trap) analysis. The major peaks observed in the MS^3 spectra were the molecular ions at the *m/z* 122.0, 79.2, and 107.0. Similar fragmentation patterns were observed for further fragmentation of the protonated ion at *m/z* 137

obtained of MS^2 experiment for capsaicin (Schweiggert et al. 2006), that corresponding to the common vanillyl ring.

The large number of capsaicinoids found in pepper fruit extracts (Schweiggert et al. 2006) can provide indications of possible occurrence of similar number of capsinoids in pepper fruit extracts. This study opens the possibility of applying ESI-MS(QTOF) analyses to identify unknown capsinoids in pepper fruit extracts. The fragmentation patterns obtained supply valuable information for further characterization of unknown capsinoid-type compounds.

Acknowledgements

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Action of ethylene and 1-methylcyclopropene on the post-production life of ornamental *Capsicum annuum* L.

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Abstract

Pepper accession registered as BGH-1039, belonging to the germoplasm bank from Universidade Federal de Viçosa (UFV) Brazil, is well adapted as ornamental plant to be grown in small volume (760 ml) pots. Nevertheless, at post-production stage under low light intensity, the plant shows symptoms of ethylene induced senescence, resulting in abscission of fruits and leaves. Mature plants were exposed to 10 μL L^{-1} ethylene for 48 hours, under fluorescent white light conditions (8 – 10 μmol m^{-2} s^{-1}). When the plants were treated with ethylene, significant drop on the contents of chlorophyll *a* and total chlorophyll occurred at the end of the treatment. But, no change on leaf carotenoid content was observed after the treatment with ethylene. When the plants were treated with 1-methylcyclopropene (1-MCP) at a concentration of 1 μL L^{-1} for six hours, followed or not by the ethylene treatment, the contents of chlorophyll a, b, total and carotenoid did not change. The control plants showed no changes on the leaf pigment contents after 48 hours under indoor conditions illuminated with low intensity fluorescent white light. Plants exposed to ethylene had the rate of fruit and leaf abscission increased by 7.5 and 2.0 fold, respectively. The 1-MCP treatment was able to block the abscission of fruit and leaves, even when the plants were exposed to ethylene after being treated with 1-MCP. Apparently, the treatment with 1-MCP completely blocked the ethylene action receptors in both fruits and leaves. In addition to that, no new sites of action were receptive or generated by the ethylene treatment if the plants were previously treated with 1-MCP.

Keywords: chlorophyll, carotenoids, leaf abscission, fruit abscission

Introduction

The interest for potted ornamental peppers has experienced a large increase in the past years at the Brazilian market of ornamental crops. Several cultivar *of Capsicum annuum* are well adapted as ornamental potted plants (Rêgo et al. 2011). In addition to that, the studies regarding the behavior of *Capsicum* species in pots and the influence of post- production factors on the longevity are still scarce.

The number of ornamental peppers sold on the Brazilian market is scarce. The Germoplasm Bank at Federal University of Viçosa (BGH/UFV) holds one of the largest collections of *Capsicum* species. The accession BGH-1039 has colorful fruits and compact canopy, considered optimal characteristics as ornamental pepper. Nevertheless, the post-production longevity is compromised due to relatively high rate of leaf abscission when exposed to ethylene (Finger et al. 2012). The authors found that relatively low concentration of ethylene in the atmosphere of storage was able to cause partial drop of leaves and fruits in less than 48 hours.

1-Methylcyclopropene (1-MCP) is effective to block the action of ethylene in fruits, vegetables and ornamental plants in general (Blankenship and Dole, 2003). In addition to that, the 1-MCP is a nontoxic plant growth regulator, which is naturally present in nature. The 1-MCP was able to prolong the vase life of potted mini roses and the ornamental pepper cultivar Calypso, by reducing the symptoms of premature senescence (Buanong et al. 2005; Segatto et al. 2013).

In this work, 1-MCP was applied to prevent the abscission of leaves and fruits and the yellowing of leaves in potted pepper BGH-1039 during the post-production shelf life.

Material and Methods

Seeds from accession BGH-1039 (*Capsicum annuum* L.) were sown in flats with single cells and transplanted to 760 ml pots when the plantlets had 4 to 5 true leaves. The pots were filled with soil mix and the plants grew in a greenhouse for four months until ready for commercial sale. The pots were fertilized weekly with a water diluted solution of 150 g L^{-1} of commercial Ouro Verde (15-15-20 NPK + Ca, S, Mg, Zn, B, Fe, and Mn). Commercial stage for sale was reached when the plants had 30% of fully ripened fruits. Afterwards, the pots were transferred to a room at 25^{o}C, illuminated with white fluorescent light with intensity of 8-10 µmol s^{-1} m^{-2}. Three individual pots were placed in a 60 L container for the 1-MCP or ethylene treatments.

The plants were treated for 6 hours with 1µL L^{-1} 1-MCP and/or with 10 µL L^{-1} ethylene for 48 hours. Concomitantly, control plants were kept inside the 60 L containers for 48 hours without the presence of 1-MCP or ethylene.

The controls, the 1-MCP and/or ethylene treated plants were evaluated for their leaf content of chlorophylls and total carotenoids. The pigments were extracted with 80% acetone from fully expanded leaves and the contents determined in a spectrophotometer according to method described by Lichtenthaler (1987).

The rate of leaf and fruits abscission was determined at every three days.

The vases were arranged in a complete randomized block design with five replicates of one pot each. The data were statistically analyzed using analysis of variance (ANOVA) and the significance among treatments was performed by Student's t-test at $P \leq 0.05$.

Results and Discussion

In absence of ethylene there is no decay on leaf chlorophyll *a*, *b* and total chlorophyll or change in carotenoids after 54 hours under low white light intensity illumination (Table 1). Similar behavior was observed when the plants were treated with 1-MCP for 6 hours or treated with 1-MCP for 6 hours followed by ethylene exposure for 48 hours. Nevertheless, when the plants were exposed to ethylene for 48 hours, significant degradation of chlorophyll took place (Table 1). But, ethylene and any of the remaining treatments did not affect the content of carotenoids on the leaves. Detached leaves of coriander had similar behavior found for the pepper leaves, where the 1-MCP inhibited chlorophyll degradation and promoted by exposing them to 10 µL L^{-1} ethylene for 24 hours (Jiang et al. 2002).

Table 1 – Content of chlorophyll *a, b*, total and carotenoids in leaves of the access BGH 1039 submitted to 1-MCP and/or ethylene treatments.

Treatments	1-MCP	Ethylene	1-MCP + Ethylene	Control
		Chlorophyll a		
Before	6.75	6.38	6.54	6.57
After	6.25^{ns}	4.76*	6.02^{ns}	6.14^{ns}
		Chlorophyll b		
Before	2.04	2.28	1.85	2.20
After	1.75^{ns}	1.95^{ns}	1.96^{ns}	1.93ns
		Total chlorophyll		
Antes	8.79	8.67	8.39	8.77
Após	8.00^{ns}	7.06*	7.48^{ns}	8.07^{ns}
		Total carotenoids		
Antes	1.13	1.03	0.96	0.92
Após	1.28^{ns}	1.23^{ns}	0.89^{ns}	1.09^{ns}

*Significant or ^{ns}non significant at 5% probability by the Student`s test.

After 18 days of post-production shelf life, the data of abscission showed that the fruits are more sensitive than the leaves to the ethylene action, which may reflect the more responsive receptors. When the plants were exposed to ethylene the accumulated fruit and leaf abscission during the post-production shelf life was increased by 7.5 and 2.0 fold, respectively (Figures 1A and 1B). The perception and sensitivity to ethylene is regulated spatially and temporally throughout the cycle of a plant (Ciardi and Klee, 2001). This might explain the differences of response on the rate of abscission between the fruit and leaves from the pepper plant.

The use of 1-MCP was able to block the ethylene action in inducing premature fruit and leaf abscission (Figures 1A and 1B). Furthermore, the 1-MCP treatment was able to extend the post-production shelf life compared to the control plants, by reducing both fruit and leaf abscission. By treating potted Regal Pelargonium with 1-MCP protected the flower from petal abscission in response to ethylene treatment, but did not improve the plant performance after simulated transport in any of the cultivars tested (Kim et al. 2007). In the case of the ornamental pepper BGH, the use of 1-MCP will improve the plant performance, suggesting that the pre-treatment before shipping the plants should be recommended. Similar to this work, Buanong et al. (2005) found that 1-MCP treatment improved the display life of miniature potted roses.

In conclusion our results demonstrated the beneficial effects of 1-MCP treatment as ethylene antagonist, resulting in improved display quality of ethylene sensitive ornamental *Capsicum* potted plants.

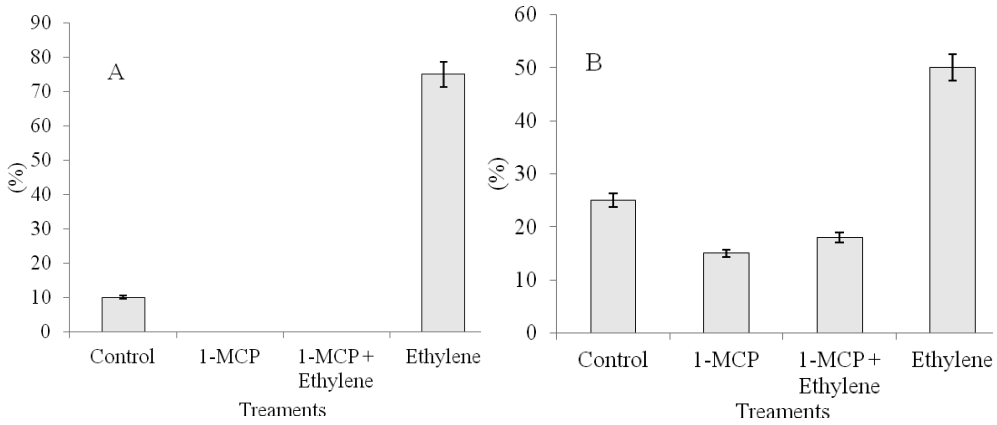

Figure 1 – Accumulated percentage of fruit (A) and leaf (B) abscission in plant peppers treated with ethylene and/or 1-MCP. Data recorded at 18th day of post-production shelf life. Values shown are means of number of florets for at least 5 plants ±S.D.

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Application of Doubled Haploid Production Technology (DHPT) in hybrid pepper breeding

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Abstract

The application of doubled haploid production technology is essential in pepper hybrid breeding programmes. Both growers and the market continuously require improved varieties. Using DHPT technology is not only important for developing new varieties that are higher-yielding, resistant to pests and diseases, but it is also a much faster method. For hybrid seed production, obtaining pure lines is a priority. Pure lines are traditionally generated by techniques based on classical breeding through successive rounds of selfing and selection. Biotechnological approaches to obtain homozygous lines are more efficient and sustainable than traditional methods. In the laboratory of Medimat Ltd. more than 3000 different genotypes originating from Hungarian sweet and spice pepper types (Cecei, tomato-shaped, apple-shaped, white blocky, light green and dark green blocky, green spice), Hungarian spice pepper genotypes, Dutch blocky types, Spanish types (Dolce Italiano, Lamuyo, red blocky) and Turkish types (e.g. Dolma, Charliston) have been tested and used to produce new breeding lines via *in vitro* anther culture and an improved (Lantos et al. 2009) microspore culture technique. Microspore culture is an attractive solution for production of many pure-lines and new paprika candidates for registration. Despite intensive efforts of Hungarian industry and academia, anther culture remains the most effective tool for pepper doubled haploid plant production. Improvement of the effectiveness of microspore culture requires the cooperation of all interested parties in Europe.

Keywords: pepper, doubled haploids, anther culture, microspore culture

Introduction

Obtaining pure lines is a priority for hybrid seed production. Pure lines are traditionally generated by techniques based on classical breeding through succesive rounds of selfing and selection. Doubled haploid production technology (DHPT) to obtain homozygous lines is more efficient and sustainable than traditional methods (Gémes Juhász et al. 1998, 2006; Mitykó and Gémes Juhász, 2006). The application of DHPT technology has numerous practical advantages. These can be summarised as follows:

- hybrids developed using DH parents are homogeneous, have uniform yield quality, and satisfy increasingly stringent market demands
- multiplying DH lines in isolation can preserve the purity of lines used in breeding programmes for decades
- it is an exceptionally efficient method for resistance breeding

- it can be used to ensure the rapid selection of economically valuable lines for use in hybrid combinations
- it provides excellent basic material for the breeding process if DH lines with good combining ability are used as stable testers
- the homozygous genetic material is an excellent basis for marker development and mapping

Not only pepper breeders but also researchers have attempted to develop an effective, profitable DH plant production method. As known there are some limiting factors of anther culture, for example, lots of manual work, strong genotype-dependent efficiency, obtaining a high percentage of haploids. These facts prompted the improvement of the anther culture method and the parallel development of other methods for DH plant production. Nowadays, many research groups focus on the improvement of different DH plant production methods like *in vivo* polyembryony (Jedrzejczyk and Nowaczyk, 2009), shed microspore culture (Supena et al. 2006) and isolated microspore culture (Lantos et al. 2009).

The microspore culture technique possesses some features which make it more promising than other methods. The isolated microspore culture system allows for better nutrient availability to the developing microspores and provides a superior method for tracking and studying microspore maturation and development of microspore derived embryos (Ferrie and Caswell, 2011).

Materials and Methods

Anther culture was carried out with the protocol of Mitykó and Gemes Juhász (2006). Microspore culture was performed for sweet and hot pepper (*Capsicum annuum* L.) genotypes (Lantos et al. 2009, 2012). The isolated uni-and binucleate microspores were co-cultured with foreign ovaries (barley, durum wheat, wheat and triticale). Four media (W14, B5, MS and NLN) were compared to test the effect of medium components. The effect of different ratios of 2,4-dichlorophenoxyacetic acid (0, 0.1, 0.2 and 0.5 mg l^{-1}) and kinetin (0, 0.2 and 0.5 mg l^{-1}) were investigated by determining the production of microspore-derived structures (embryo-like structures, calli and the ratio of these structures) and plant regeneration (number of roots and plantlets).

Results and Discussion

In the laboratory of Medimat Ltd., more than 3000 different genotypes originating from Hungarian sweet and spice pepper types have been tested in anther culture in the past years. The use of optimized anther culture has the advantage that it can be applied routinely and successfully for all types of varieties.

The DH plants produced by Medimat Ltd. are used in the Hungarian Wax pepper breeding program of Rijk Zwaan Budapest Ltd. and in the pepper breeding program of Rijk Zwaan Zaadteelt & Zaadhandel B.V. The Hungarian Wax pepper is the biggest and most important pepper type in Hungary. Growers and the market continuously require improved varieties. The breeders have to quickly react to these demands by creating new varieties with higher and higher quality. Until now, Rijk Zwaan Budapest Ltd. used DH lines as parent lines for some of the commercial varieties from their breeding program and future new varieties will be produced with this system. In past years, DH technology was combined with marker-based pre-selection and provided an exceptionally efficient method for resistance breeding (amongst others TMV and TSWV).

Tobamoviruses resistances (Tm2 or Tm3) are a basic requirement for Hungarian Wax varieties from the breeding program of Rijk Zwaan Zaadteelt & Zaadhandel B.V. *Tomato spotted wilt virus,*

TSWV, resistance is also a basic requirement since 2009 for the Hungarian market. This is because *Tomato spotted wilt virus* (TSWV) has become a very dangerous virus in pepper growing in Hungary with very large losses for growers. New sweet Hungarian Wax commercial varieties with TMV and TSWV resistance have been developed in the program.

Recently we have developed a new method of isolated microspore culture to increase the production of homozygote breeding material. The androgenic response of different sweet and hot pepper genotypes was tested in microspore culture. We found that in microspore culture the most promising combination of growth regulators was 0.1 mg l^{-1} 2,4-dichlorophenoxyacetic acid and 0.2 mg l^{-1} kinetin in B5 medium which produced the most plantlets in the tested genotypes. The most effective ovary donors were wheat and barley in terms of embryo production. Histology of microspore-derived structures was continuously monitored. In each tested genotype, androgenesis was induced, and embryo and embryo-like structures were dominantly produced from microspores. The number of embryo and embryo-like structures ranged from 20 to 100/petri dish (average 48.1 embryo-like structures/petri dish), while the number of green plantlets ranged from 0 to 8 (average 1.5 plantlets/petri dish) depending on genotype. The spontaneous rediploidization rate was 25% in isolated microspore culture based on the ratio of the fertile and total acclimatised sweet pepper plants (Lantos et al. 2012). The regenerated hot pepper DH plants originating from microspore culture have been integrated into the hybrid condiment paprika breeding program.

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Heterosis for yield and other yield contributing economic traits in eggplant (*Solanum melongena* L.).

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Abstract

Twenty-eight non-reciprocal F_1brinjalhybrids (*Solanum melongena* L.) obtained from eight diverse genotypes were evaluated to estimate heterosis over better parent, mid parent and standard checks for eighteen commercial traits. Four hybrids (Pusa Purple Long × Black Beauty, Gulabi Long ×Surati Ravaiya, Pusa Purple Long ×Gulabi Long and Pusa Purple Long × Green Long) in long fruited segment and one hybrid each in round fruited (Black Beauty ×Surati Ravaiya) and small fruited segment (BB-44 × Black Beauty)were excelled in economic heterosis and showed 7.82 to 112.87 % heterosis over various standard checks.These six hybrids could be evaluated in multi-location and may be released for commercial cultivation as per adaptability to various agro-climatic regions. The pink fruit colour is dominant over purple colour, nevertheless recessive with dark purple and black colour.

Keywords: Hybrid vigour, heterosis, eggplant (*Solanum melongena* L.), qualitative and economic traits.

Introduction

Eggplant (Solanum *melongena* L.), known also as brinjal, is an indigenous to India, with a cultivated areas of 0.68 m ha, being West Bengal, Odisha, Andhra Pradesh, Gujarat, Bihar major growing states. The worldwide cultivated areas is 1.73 m ha, mainly grown in South & South East Asian countries, China, Turkey, Egypt and China has the highest cultivated surface (over 0.73 m ha). Productivity of brinjal in India, China and world is 17.5t/ ha, 33.5 t/ ha and 25.0 t/ ha respectively (NHB,2011).Eggplant breeding work is carried out worldwide by several companies. It is one of the most important vegetables due to cheaper price, round the year availability and wide culinary use. This crop possesses rich genetic diversity for various qualitative and quantitative traits and it has a great potential for improvement on the basis of consumers 'preferences. Realizing the importance of eggplant worldwide it is realistic to develop productive and region specific hybrids to cater the varied consumers' preference and market segment requirement.

Materials and Methods

The experimental materials used for the present study comprised eight diverse parents (Pusa Purple Long, Green Long, BB-44, Gulabi Long, Manjari Gotya, Black Beauty, Pusa Purple Round and Surati Ravaiya, coded as P1 to P8 respectively),twenty-eight non-reciprocal hybrids, and four standard checks. The standard checks were selected on the basis of the preference of consumers, such as Ravaiya (small fruited)and Ajay (variegated, spiny fruited), both private seed company hybrids, and PH-5 (long fruited) and PH-6 (round fruited), both public hybrids. The parents were grown during monsoon season 2005-2006 and crossed in all possible combinations excluding reciprocals. The seedlings of eight parents, twenty-eight hybrids and four standard check parents were grown in nursery and transplanted in plots having twenty-one plants per plot during monsoon

season of 2006-07 in RBD with three replications. Data were recorded for economic parameters, *viz.* plant height at first flowering (cm), plant height at last picking (cm), number of primary branches per plant, number of secondary branches per plant, days to 50 % flowering, days to first fruit picking, fruit setting flowers (%), non-setting flowers (%), volume of fruit (ml), moisture content in fruit (%), length of fruit (cm), breadth of fruit (cm), seed to pulp ratio (%), number of marketable fruits per plant, number of unmarketable fruits per plant, yield of marketable fruits per plant (kg), yield of unmarketable fruits per plant (kg) and average fruit weight (g).The data were analyzed statistically for analysis of variance (ANOVA) using the method 2 and model-I of Griffing (1956). The mid parent value (MP), hybrid performance (hybrid effect), mid parent heterosis or relative heterosis (%), better parent heterosis or heterobeltiosis (%) and standard heterosis or economic heterosis(%) for each hybrid were calculated.

Results and Discussion

The mean square estimates were significant for all the traits in hybrids while in parents it was also significant for all the traits except plant height at first picking, number of primary branches per plant and number of marketable fruits/ plant. This indicates that the prevalence of sufficient variability among parents and hybrids could be exploited for improvement of potential yield, horticultural parameters and traits of consumers choice. The estimates of heterobeltiosis, relative heterosis and economic heterosis were calculated over their respective parent and commercial checks. Among eighteen traits under study, desired negative heterosis was observed for seven characters in days to 50% flowering, days to first fruit picking, non fruit setting flowers, volume of fruit(ml), seed to pulp ratio, number of unmarketable fruit per plant and yield of unmarketable fruit per plant.

Only one hybrid (P 3 × P 6) in small fruited segment, eleven hybrids (P 1 × P 6, P 4 × P 8, P 1 × P 7, P 1 × P 2, P 1 × P 8, P 2 × P 8, P 1 × P 4, P 2 × P 6, P 1 × P 3, P 1 × P 5 and P 2 × P 4)in long fruited segment and one in round fruited segment (P 6 × P 8) were found to be economically heterotic for yield of marketable fruits per plant. The findings of Sahaet al. (2005), Babu and Thirumurugon (2001),), Shafeeq (2005), Suneethaet al. (2006), Timmapur (2007), and Sao and Mehta (2010) have also supported present results. Out of twenty-eight hybrids, only one hybrid (P 7 × P 8) showed economic heterosis for earliness over PH-6. None of the hybrids expressed earliness over Ravaiya, PH-5 and Ajay. Heterosis for earliness has also been reported by Sousa et al. (1998) and Timmapur (2007).Eight hybrids were heterotic for average fruit weight (P 4 × P 8, P 1 × P 8, P 1 × P 6, P 2 × P 6, P 1 × P 7, P 4 × P 6, P 1 × P 5 and P 2 × P 4) in purple long over PH-5, one hybrid (P 6 × P 8) in purple round over PH-6 and one hybrid (P 3 × P 6) in small fruited over Ravaiya. This observation is in full agreement withothers studies (Sousa et al. (2010);Patilet al. (2001);Babu and Thirumurugon (2001);Timmapur (2007);Sao and Mehta (2010).)

Heterosis for number of marketable fruits per plant was found in six hybrids (P 1 × P 2, P 1 × P 6, P 1 × P 4, P 1 × P 8, P 1 × P 3 and P 1 × P 7) for long fruited segment and none of the hybrids for round and small fruited segment. Heterosis for number of marketable fruit has also been reported by Prasathet al.(2000), Babu and Thirumurugon (2001), Shafeeq (2005), and Sao and Mehta (2010).Economic heterosis for moisture content exhibited by four hybrids (P 4 × P 8, P 2 × P 4, P 1 × P 4 and P 1 × P 7) in long fruited segment, and no hybrids were found to be heterotic over round and variegated segment.Seven hybrids showed economic heterosis over PH-5, four over PH-6 and one over Ravaiya for seed to pulp ratio and plant height at last picking stage. Heterosis for seed to pulp ratio have also been reported by Patil *et al.* (2001). For fruit setting flowers, four hybrids (P 1 × P 2, P 1 × P 4, P 1 × P 3 and P 2 × P 4) expressed heterosis in desired direction over PH-5 and two hybrids (P 6 × P 7 and P 6 × P 8) over PH-6. Only one hybrid (P 3 × P 6) was found to be heterotic for nine traits over Ravaiya, nevertheless none of the hybrids were significantly better for variegatedfruit(Ajay).

Among these twenty-eight economic hybrids, four hybrids (P 1 × P 6, P 4 × P 8, P 1 × P 4 and P 1 × P 2) over long fruited segment, one hybrid each for round fruited and small fruited segment, i.e. P 6 × P 8 and P 3 × P 6, respectively were the best in economic heterosis. The quantitative and qualitative traits along with heterosis value for aforesaid hybrids were summarized in Table 1. The maximum economic heterosis for marketable yield per plant of long fruited segment was realized in P 1 × P 6 followed by P 4 × P 8, P 1 × P 2 and P 1 × P 4 (112.87, 102.97, 69.30 and 50.00 %, respectively). Although the hybrid P 1 × P 4 showed negative heterobeltiosis, yet its performance over standard check was recorded 50 % heterotic, showing the practical importance of economic heterosis. The hybrids P 1 × P 4 and P 1 × P 2 showed economic heterosis for ten traits and P 1 × P 6 and P 4 × P 8 had expressed for nine and seven traits, respectively. The hybrid P 3 × P 6 expressed 31.62 % economic heterosis while only 7.82 % was realized for P 6 × P 8.

The magnitude and direction of economic heterosis for various traits of six hybrids (P 1 × P 6, P 4 × P 8, P 1 × P 4, P 1 × P 2, P 6 × P 8 and P 3 × P 6) revealed that none of the combinations were excelled for earliness (days to 50 % flowering and days to first fruit picking) and less moisture content in fruit (Table 1). The magnitude of heterosis was found to be higher for number of marketable fruits per plant, yield of marketable fruits per plant, average fruit weight, fruit breadth, and fruit length.Only one hybrid (P 3 × P 5) was found to be morphologically similar to standard check Ajay (Green + Purple + White, i.e. variegated fruit colour) with negative heterosis(-43.45 %) for yield of marketable fruits per plant. This trait is showing co-dominance and requires more variability for improvement over all. The pink fruit colour is dominant over purple colour (P 1 × P 4 and P 4 × P 8), nevertheless it showed recessive with dark purple and black colour.

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Table 1: Performance of top heterotic hybrids and standard checks along with qualitative traits of consumers' choice.

S. No.	Particular		Hybrid and standard check								
			Oblong to Long fruited segment					Round fruited segment		Small fruit segment	
			P 1 × P 6	**P 4 × P 8**	**P 1 × P 4**	**P 1 × P 2**	**PH-5**	**P 6 × P 8**	**PH-6**	**P 3 × P 6**	**Ravaiya**
1	*Per se* performance (fruit yield per plant in kg)		4.3	4.1	3.03	3.42	2.02	5.1	4.73	3.33	2.53
2	Heterobeltiosis (%)		37.82	30.99	-2.78	10.04	-	56.55	-	67.79	-
3	Economic heterosis (%)		112.87	102.97	50.00	69.30	-	7.82	-	31.62	
4	Calyx character	Calyx colour	Green	Green	Purplish green	Green	Purple	Green	Greenish purple	Green	Purplish green
		Calyx spininess	Few spine	Non-spiny	Non spiny	Few	Non-spiny	Non-spiny	Non-spiny	Non-spiny	Non-spiny
5	Fruit character	Fruit colour	Black	Pink	Pink	Greenish purple	Purple	Dark purple	Purple	Purple	Purple
		Fruit shape	Long	Oblong	Long	Long	Long	Oval round	Round	Oval	Oval
		Fruit length (cm)	12.83	13.00	15.33	16.00	14.00	8.33	6.67	8.83	5.67
		Fruit diameter (cm)	4.83	4.83	3.00	3.33	3.87	7.80	6.00	5.97	3.67
		Avg. fruit weight (g)	120	90	70	100	130	100	80	100	60
6	Heterobeltiosis	Character name	3, 5, 6, 13, 14, 16	3, 4, 13, 16, 17	7, 8, 13, 14	2, 4, 5, 6, 7, 8, 13, 14, 16	-	1, 3, 4, 8, 14, 16	-	1, 6, 13, 14, 15, 16	-
		Number of characters	6	5	4	9	-	6	-	6	
7	Economic heterosis	Character name	2, 3, 4, 9, 12, 13, 14, 16, 18	3, 4, 12, 13, 14, 16, 18	2, 3, 4, 7, 9, 11, 13, 14, 16, 17	1, 2, 3, 4, 7, 8, 11, 13, 14, 16	-	1, 2, 3, 4, 7, 8, 9, 11, 12, 13, 15, 16, 18	-	1, 2, 9, 11, 12, 13, 16, 18	-
		Number of characters	9	7	10	10	-	13	-	8	-

Character:

1. Plant height at first flowering (cm)
2. Plant height at last picking (cm)
3. No. of primary branches/ plant
4. No. of secondary branches/ plant
5. Days to 50% flowering
6. Days to first fruit picking
7. Fruit setting flowers (%)
8. Non setting flowers (%)
9. Volume of fruits (ml)
10. Moisture content in fruit (%)
11. Length of fruit (cm)
12. Breadth of fruit (cm)
13. Seed to pulp ratio (%)
14. No. of marketable fruits/ plant
15. No. of unmarketable fruits/ plant
16. Yield of marketable fruits/ plant (kg)
17. Yield of unmarketable fruits/ plant (kg)
18. Average fruit weight (g)

Heterosis for fruit quality traits in ornamental peppers

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Abstract

Diallel analysis can provide a wealth of information, providing estimates of genetic parameters essential for use in breeding programs. The objective of this work was to determine the effects of heterosis of varieties and hybrid ability for fruit quality characters and evaluate the most promising crosses among six parents of *Capsicum annuum*. For this, six parents belonging to the Vegetable Germplasm Bank of the CCA/UFPB, Areia - PB were crossed according to a half diallel table. The data were evaluated in an entirely randomized design with three replicates. Each replicates was an average of five plants. Subsequently, the data were submitted to analysis of variance ($p \leq 0.01$). The diallel analysis was performed according to the Gardner and Eberhart fixed model. Estimated values of heterosis and heterobeltiosis were significant, positive and negative, for all traits. The parents that had good potential and genetic divergence were 01, 77.2, 134 and 132. The most promising hybrid combinations with the greatest heterosis were 77.2 x 01, 01 x 132 134 x 137 and 134 x 77.2. The variables evaluated had additive and non-additive effects. The results show that *C. annuum* varieties with fruit quality attributes for ornamental purposes can be developed either by mass selection, pedigree or "bulk" breeding or by production of hybrids.

Keywords: *Capsicum annuum*, plant breeding, varietal heterosis, specific heterosis, heterobeltiosis, chili.

Introduction

Chili pod quality attributes depend on their uses and destinations. Poulos (1994) separated all chili pod types into five market types based on quality traits: fresh market (multi-color whole fruit), fresh fruit processing (sauce, paste, canning, pickling), dried spice (whole fruit, powder), oleoresin extraction and ornamental (plants or fruits). In a general way, consumers choice is determined by pungency level, color, pericarp thickness, exocarp thickness, size, dry weight, smooth and glossy surface, firm stalk, fruit shelf life, shape, flavor and size (Poulos 1994; Rêgo et al. 2009a). *Capsicum* species are also valued for their high vitamin A and C content, although this information, in general, is not available to consumers. The objective of this work was to determine the effects of heterosis on varieties and hybrid ability for fruit quality characters and evaluate the most promising crosses among six parents of *Capsicum annuum*.

Materials and Methods

Six chili pepper inbred lines were crossed in a half-diallel scheme: UFPB 01, UFPB 77.1, UFPB 77.2, UFPB 132, UFPB 134 and UFPB 137. Seeds of the six parents and 15 hybrids were sown in the greenhouse at Universidade Federal da Paraíba, Areia, Paraíba State, Brazil. Each entry was sown in three replicates for each parent and hybrids, following an entirely randomized design. The evaluated traits were: pericarp thickness (PT), placenta length (PL), fresh matter (FM), dry matter (DM), total soluble solids (TSS), titrable acidity (TA), and vitamin C (Vit C) (IPGRI 1995). The

chemical analyses were made according to AOAC methods (1990). Subsequently, the data were submitted to analysis of variance ($p \leq 0.01$). The diallel analysis was performed according to the Gardner and Eberhart (1966) fixed model. The midparent and high parent heterosis were also calculated.

Results and Discussion

The mean square analysis indicated differences among the treatments ($p \leq 0.01$) for all evaluated traits. Differences were detected for variety and heterosis effects, except for vitamin C which showed no heterosis effects. The effects of heterosis were significant ($p \leq 0.01$) for the placenta length (PL) dry matter (DM), total soluble solids (TSS) and acidity (AC) and at 5% probability for pericarp thickness (PT) and fresh matter (FM) (data not shown). The average for the heterosis effects differed statistically at 1% probability for AC and at 5% probability for TSS. But, no significant differences were determined for PT, LCP, FM, DM and Vit C. The significance for heterosis mean indicated that the hybrid differed from the genitors. The characteristics PT, LCP, DM, TSS and AC were different for the effects of variety heterosis, based on an F-test at 1% probability. Nevertheless, the variables FM and Vit C showed no significant heterosis in the hybrids. The significance of varietal heterosis indicated the presence of differences among the genitors when crossed among themselves. The effects of specific heterosis were significant at 1% probability for DM, TSS and AC, at 5% probability for FM, and not significant for PT, LCP and Vit C. The significance of specific heterosis indicated differences among the means of genetic frequencies at least in some of the parents (data not shown). The contribution of the sum of squares from the specific heterosis components for the sum of squares of the treatments indicated the importance of non-additive genetic effects (dominance and epistasis) controlling the characters FM, TSS, AC and Vit C. These results are in agreement with those obtained by Rêgo et al. (2009a) working with a diallel analysis of *Capisicum baccatum* for the fruit characteristics FM and TSS.

In all hybrids and for all characteristics, both positive and negative values for heterosis and heterobeltiosis were found in relation to the average of both parents or the better parent, respectively. For the FM and DM values, hybrid 01 x 77.2 had the highest positive scores for heterosis, with averages superior to the parents and heterobeltiosis greater than the parent with the higher positive value (Table 1). FM and DM are important characteristics for consumers that wish to grow an ornamental plant at home that produces fruits for sauces, culinary and medicinal uses. For the variable PT and LCP, the maximum positive values for heterosis were obtained with hybrids 01 x 77.2 and 01 x 77.1, with averages higher than the parents (Table 1). For heterobeltiosis, the hybrid combinations 77.1 x 77.2 and 77.1 x 137 showed higher values compared to their parents' highest averages (Table 1). Similar data were found by Blat (2007) in work with *Capsicum annuum*, where there were positive and negative values for heterosis and heterobeltiosis for PT. For the characteristics AC and Vit C, the hybrid 77.1 x 77.1 had the best combination among all hybrids and parents. The maximum positive values for the variable TSS were obtained in the hybrid combination 01 x 132 for heterosis, with averages superior to the parents and heterobeltiosis indicating a mean superior to the parent with the higher mean. According to Lannes et al. (2007) and Rêgo et al. (2009b, 2011) DM, TSS and PT should be elevated, in order to maximize the final yield in processed products.

Based on varietal effects (Vj), the best parents were 132, 134 and 77.2, since they had the highest potential to yield peppers with desirable characteristics such as PT, LCP, FM, DM, TSS, AC and Vit C. In order to obtain more heterotic (Hj) combinations, it is recommended that parents 01 and 77.1 are used, in order to obtain fruits with more pulp and placental tissues, higher FM and DM and high contents of TSS, AC and Vit C. Based on the values of Sij, the most promising hybrid combinations for improvement of PT, LCP, FM, DM, TSS, AC and Vit C were 01 x 77.2, 01 x 132, 134 x 137, 77.2 x 134 and 77.1 x 77.2 (Table 2).

The results show that varieties with fruit quality attributes can be developed either by mass selection, pedigree or "bulk" breeding or by production of hybrid ornamental *C. annuum* cultivars.

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Table 1. Mid parent heterosis (PPH) and high parent heterosis (HPH) for fruit quality traits in six parents and 15 hybrids of pepper (*Capsicum annuum*) CCA-UFPB, Areia, 2009/2010.

Hybrids	PT		PL		FM		DM		SST		AT		VIT C	
	MPH	HPH	MPH	HPH	MPH	HPH	MPH	HPH	MPH	HPH	MPH	HPH	MPH	HPH
01 x 77.1	15.3	13.2	58.2	50.8	68.7	37.1	29.4	5.71	19.98	12.15	-1.23	-1.28	-1.04	-1.31
01 x 77.2	20.1	10.6	18.2	9.5	85.3	73.7	138.0	51.5	6.91	4.12	-0.16	-0.21	-0.41	-0.63
01 x 132	-6.1	-18.0	-9.1	-47.2	46.8	-16.6	61.4	-5.3	33.25	27.75	0.48	-43.0	0.58	-42.9
01 x 134	-17.8	-33.3	9.1	-21.8	-8.0	-45.2	-8.6	-48.1	12.42	10.66	0.45	-44.6	0.45	-44.6
01 x 137	10.1	5.6	37.7	8.4	53.3	-10.7	25.4	-26.1	20.56	16.97	5.94	-42.7	6.31	-42.4
77.1 x 77.2	15.7	13.6	12.5	9.2	16.6	-9.7	2.5	-41.4	-15.58	-21.08	11.0	10.8	12.4	11.8
77.1 x 132	-15.3	-26.6	-25.3	-56.0	-29.5	-57.1	-25.7	-48.9	-28.75	-28.75	1.58	-42.4	1.64	-42.3
77.1 x 134	0	-19.4	10.0	-18.0	-2.5	-36.3	-40.3	-61.7	8.0	2.0	0.33	-44.7	1.01	-44.3
77.1 x 137	17.2	10.5	61.4	31.8	9.6	-30.8	-36.0	-55.5	-2.46	-3.65	10.0	-40.4	11.4	-39.7
77.2 x 132	-4.0	-21.3	-29.4	-58.0	-13.1	-51.4	3.0	-45.6	3.95	-2.82	-71.4	-83.8	-71.5	-83.8
77.2 x 134	-25.3	-42.7	-3.8	-27.6	-27.9	-57.9	-69.8	-84.3	22.19	23.55	-0.92	-12.8	-74.5	-85.9
77.2 x 137	-2.7	-13.8	23.1	2.94	5.3	-39.7	-10.5	-52.7	11.72	5.65	-2.94	-21.8	-76.7	-87.4
132 x 134	-19.3	-26.1	-28.1	-50.5	0.89	-14.1	-20.0	-29.6	16.70	10.22	-0.56	-10.2	-0.93	-12.8
132 x 137	-11.7	-20.0	-31.2	-55.9	-4.4	-15.5	-15.3	-16.6	14.56	13.17	-74.4	-85.9	-2.94	-21.8
134 x 137	-7.2	-22.2	1.40	-11.2	25.8	16.7	-1.4	-14.4	-3.02	-7.33	-76.6	-87.3	-0.57	-10.2

PT (mm) - pericarp thickness , PL (mm) – placenta length, FM (g) – fresh matter, DM (g) - dry matter, TSS - total soluble solids, AT - acidity and VIT C - vitamin C.

Table 2 – Average estimates of the effect of varietal components (Vj), average heterosis (H), effects of heterosis (Hj) and specific heterosis (Sij) for fruit quality traits in pepper (*Capsicum annuum*). CCA-UFPB. Areia. 2009/2010.

Components means	PT	PL	FM	DM	SST	AT	VITC
Vj							
Genitor 01	-0.015*	-0.658*	-0.431*	-0.087*	0.166ns	8.573*	34.045*
Genitor 77.1	-0.018*	-0.605*	-0.335*	-0.050*	-0.433ns	8.553*	36.995*
Genitor 77.2	-0.032*	-0.568*	-0.451*	-0.110*	0.566ns	8.594*	11.537*
Genitor 132	0.021*	2.065*	0.595*	0.066*	-0.433ns	-8.035*	-26.290*
Genitor 134	0.051*	0.035ns	0.241*	0.122*	0.4ns	-8.654*	-28.357*
Genitor 137	-0.005*	-0.268*	0.381*	0.059*	-0.266ns	-9.031*	-27.930*
H	-0.004ns	-0.062ns	0.047ns	-0.012ns	0.567*	-1.325*	
Hj							
Genitor 01	0.009*	0.229*	0.152*	0.046*	0.886*	1.769*	
Genitor 77.1	0.015*	0.195*	-0.057*	-0.015*	-1.072*	2.437*	
Genitor 77.2	0.005*	0.015ns	-0.067*	-0.0008*	-0.155ns	-3.722*	
Genitor 132	-0.014*	-0.548*	-0.065*	0.006*	0.002ns	-0.250ns	
Genitor 134	-0.020*	-0.039ns	-0.046*	-0.037*	0.323ns	-0.296ns	
Genitor 137	0.005*	0.146*	0.084*	0.0004ns	0.015ns	0.063ns	
Sij							
01 x 77.1	-0.003*	-0.041*	0.0007ns	-0.003*	0.801*	-3.117*	-9.923*
01 x 77.2	0.010*	-0.079*	-0.004ns	-0.005*	-0.781*	3.248*	9.999*
01 x 132	0.001*	0.209*	0.18*	0.025*	0.86*	-0.140ns	-0.291ns
01 x 134	-0.010*	-0.047*	-0.193*	-0.008*	-0.860*	-0.099ns	-0.237ns
01 x 137	0.001*	-0.041*	0.017*	-0.008*	-0.019ns	0.109ns	0.452ns
77.1 x 77.2	0.005*	-0.074*	0.11*	0.030*	-0.456*	4.722*	15.217*
77.1 x 132	-0.016*	-0.070*	-0.137*	-0.011*	-1.415*	-0.688*	-2.394*
77.1 x 134	0.009*	0.005ns	0.042*	0.001*	0.747*	-0.779*	-2.518*
77.1 x 137	0.003*	0.180*	-0.015*	-0.017*	0.322*	-0.136ns	-0.381ns
77.2 x 132	0.009*	0.025ns	-0.001ns	0.009*	-0.131ns	-2.474*	-7.780*
77.2 x 134	-0.015*	0.050*	-0.07*	-0.038*	0.947*	-2.521*	-7.969*
77.2 x 137	-0.009*	0.077*	-0.034*	0.002*	0.422*	-2.974*	-9.465*
132 x 134	0.008*	0.021ns	0.073*	-0.0005*	0.289ns	1.851*	5.898*
132 x 137	-0.003*	-0.185*	-0.114*	-0.023*	0.397*	1.452*	4.568*
134 x 137	0.007*	-0.029ns	0.148*	0.045*	-1.123*	1.548*	4.827*

PT (mm) - pericarp thickness , PL (mm) – placenta length, FM (g) – fresh matter, DM (g) - dry matter, TSS - total soluble solids, AT - acidity and VIT C - vitamin C. ** significant at 1% level, * significant at 5% probability by t test, ns – no significant.

Breeding *Capsicum baccatum* var. *pendulum* for pepper Yellow Mosaic Virus resistance and fruit quality

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Abstract

A *C. baccatum* var. *pendulum* breeding program is in progress at Universidade Estadual do Norte Fluminense Darcy Ribeiro, Brazil. The aim of this program is to obtain new genotypes with resistance to pepper yellow mosaic virus (PepYMV) and high fruit quality, by biparental crosses between UENF 1616 (susceptible accession) and UENF 1732 (resistant accession). Two classical approaches, pedigree and single seed descent (SSD), are being used to advance generations in order to develop recombinant inbred lines (RILs). In the pedigree method, 45 $F_{2:3}$ lines from 250 F_2 individuals were selected based on Mulamba and Mock Index, considering the following characteristics: fruit length, pulp thickness, soluble solids and fruit dry mass. The $F_{2:3}$ lines were evaluated in field conditions with spacing of 1.0 m between plants and 1.2 m between rows, with seven plants per row. In this experiment the characteristics evaluated were plant height, canopy diameter, number of days to flowering and fruiting, number of fruits per plant, mean fruit weight, fruit length, fruit diameter, pulp thickness, soluble solids, and fruit dry mass. $F_{2:3}$ lines means were superior to those obtained from parents and a standard cultivar for the following traits: mean fruit weight, fruit length, fruit diameter, pulp thickness of fruit, and fruit dry mass which are important market characteristics. Broad genetic variability for various characters such as shape, color and fruit size was recorded. It is expected that *C. baccatum* recombined lines will be obtained from both methods. These lines will have resistance to PepYMV and superior agronomic characteristics, including fruit quality to meet the needs of farmers and consumers.

Keywords: Chili pepper, pedigree, $F_{2:3}$ lines evaluation, plant selection

Introduction

In Brazil, the economic interest in *Capsicum* plants has increased especially because of the fruit versatility, which can be used not only in salads and sauces but also in food conservation, and the pharmaceutical and cosmetic industries. Breeding programs play an important role in developing new cultivars to supply the needs of farmers, consumers and industries. Besides fruit market quality, new cultivars should have other important traits such as disease resistance. Although *Capsicum baccatum* var. *pendulum* has potential for production, genetic information about and breeding of this species is rare.

A *C. baccatum* var. *pendulum* breeding program is in progress at Universidade Estadual do Norte Fluminense Darcy Ribeiro, Brazil (Rodrigues et al. 2012; Bento et al. 2013). The aim of this program is to obtain new genotypes with resistance to pepper yellow mosaic virus (PepYMV) and high fruit quality.

Materials and Methods

We evaluated 45 $F_{2:3}$ lines from crosses between the parents UENF 1616 (P_1), which has elongated orange fruits at maturity and is susceptible to PepYMV, and UENF 1732 (P_2), which has bell-shaped red fruits at maturity and is resistant to PepYMV (Bento et al. 2007). These lines were selected from the F_2 generation, using Mulamba and Mock Index, considering fruit length, pulp thickness, soluble solids and fruit dry mass, according to Bento (2012).

The experiment was carried out in field conditions in Campos dos Goytacazes, RJ, Brazil, from May to December 2012. The seedlings were grown in trays of 128 cells with the commercial substrate Vivatto®, and plantlets were transplanted after the emergence of two pairs of true leaves. The experimental design was completely randomized with 48 treatments (45 $F_{2:3}$ lines, both parents, and a commercial genotype of *C. baccatum* var. *pendulum* 'BRS Mari') with seven plants per row.

Eleven quantitative agronomic characteristics were evaluated: plant height (HEI) in cm; canopy diameter (CAN) in cm; number of days to flowering (DFL); number of days until fruiting (DFR); number of fruits per plant (NFP); mean fruit weight (MFW) in grams; fruit length (FRL) in mm; fruit diameter (FRD) in mm; pulp thickness of fruit (PTF) in mm; soluble solids (SS), measured by refractometer, and dry fruit mass (DFM) in grams. Agronomic traits were evaluated according to *Capsicum* spp. descriptors proposed by IPGRI – International Plant Genetic Resources Institute (1995). Data were analyzed using the statistical Genes program (Cruz, 2006) and lines means were clustered using the Scott-Knott (1974) test.

Starting with the same 250 F_2 plants, the SSD method is being conducted according to the classical definition (Fehr, 1987), using one seed derived from each F_2 individual. The plants from these seeds are being cultivated in a greenhouse to advance generations and to obtain further generations. For this, five flowers per plant are protected with white glue before anthesis to ensure selfing. At the end of this process, the performance of pedigree and SSD methods in obtaining superior RILs will be compared.

Results and Discussion

The results of analysis of variance identified significant differences for most traits evaluated, indicating variability among treatments. The coefficients of variation (CV) for the traits ranged from 7.40% to 32.08%, and these values were considered low to very high (Gomes and Garcia, 2002). However, according to Silva et al. (2011), in the pepper crop, experimental precision based on CV should be specific to each variable.

Means observed for $F_{2:3}$ lines were superior to those obtained for parents and the cultivar BRS Mari for the following traits: MFW, FRL, FRD and PTF which are important characteristics for the market and also for DFM (Table 1). These results indicated that is possible to select superior recombined genotypes for these traits in the studied population.

We also identified $F_{2:3}$ lines for which means were inferior to those observed for the parents and 'BRS Mari' for the traits HEI, CAN, DFL, FRD, NFP and SS. Lower values for HEI, CAN, DFL could be interesting considering that plants with lower height and lower canopy diameter would be most suitable to manage in field conditions. According to Bento et al. (2007) plant height and canopy diameter are important traits in determining how much the farmer must spend for labor and also for pesticides.

The $F_{2:3}$ lines means clustered according to Scott-Knott (1974) resulted in five clusters for DFL, PTF and DFM; four clusters for CAN, NFP, MFW, FRL, FRD and SS; three groups for DFR and two groups for HEI. The formation of several clusters demonstrates the variability between the studied lines.

Effective pedigree selection can be carried out only for traits with adequate heritability for individual plants, progeny rows or both. Also, pedigree selection usually is associated with visual selection, but it can be used equally with traits such as solid soluble. In this case, the amount of error associated with measurement of the character will influence the heritability.

Table 1. Means of 11 characteristics in 48 lines (45 lines $F_{2:3}$, UENF 1616, UENF 1732 and 'BRS-Mari'), clustered according to Scott-Knott (1974). Campos dos Goytacazes, RJ, Brazil, 2012.

$F_{2:3}$Lines	HEI[1]	CAN	DFL	DFR	NFP	MFW	FRL	FRD	PTF	SS	DFM
CBP-3	69.1 b[2]	94.9 c	35.7 e	99 c	91 d	10.9 c	72.8 c	33.3 c	2.6 c	8.7 c	17.69 c
CBP-4	81.0 a	110.7 b	38.1 e	100 c	140 c	14.2 b	61.9 d	51.0 a	2.7 c	9.3 b	23.35 b
CBP-7	68.8 b	101.0 c	45.7 d	107 c	105 d	13.6 b	78.3 c	31.2 c	2.5 d	9.8 b	22.54 b
CBP-12	73.0 b	105.7 b	44.3 d	105 c	119 c	13.4 b	89.1 b	38.7 b	2.3 d	9.5 b	21.68 b
CBP-21	68.0 b	88.9 d	42.1 d	106 c	107 d	11.6 c	108.8 a	28.1 c	2.2 e	9.7 b	18.43 c
CBP-26	81.4 a	128.4 a	43.8 d	96 c	206 b	12.8 b	65.2 d	49.1 a	3.0 c	9.9 b	21.04 b
CBP-29	80.6 a	113.2 a	38.4 e	100 c	199 b	13.9 b	128.6 a	37.0 b	1.9 e	9.7 b	17.21 c
CBP-30	78.9 a	114.3 a	45.3 d	103 c	194 b	12.5 b	109.2 a	39.3 b	1.8 e	9.6 b	17.85 c
CBP-34	58.3 b	89.2 d	43.2 d	103 c	76 d	13.1 b	93.7 b	30.8 c	2.2 e	9.0 c	16.97 c
CBP-40	78.0 a	106.4 b	41.9 d	103c	139 c	13.0 b	45.2 d	56.3 a	3.2b	8.9 c	18.88 c
CBP-42	82.4 a	115.3 a	42.0 d	104 c	195 b	16.8 a	61.9 d	53.8 a	3.2 b	9.1 c	26.11 a
CBP-43	85.3 a	113.3 a	42.7 d	103 c	171 b	12.9 b	112.7 a	32.7 c	2.6 c	9.6 b	20.43 b
CBP-44	65.7 b	87.7 d	50.3 c	106 c	107 d	13.2 b	82.9 c	34.0 c	2.7 c	8.6 c	18.41 c
CBP-45	78.2 a	102.4 b	44.8 d	99 c	177 b	10.7 c	103.5 b	30.8 c	2.3 d	9.7 b	18.54 c
CBP-52	76.3 a	113.4 a	44.0 d	105 c	136 c	10.1 c	48.9 d	38.2 b	2.9 c	9.6 b	18.92 c
CBP-70	85.1 a	74.4 d	40.6 e	100 c	121 c	14.2 b	84.6 c	42.3 b	2.8 c	9.1 c	20.04 c
CBP-75	68.5 b	100.2 c	41.7 d	112 b	119 c	12.2 b	98.9 b	34.8 c	2.4 d	9.0 c	20.27 b
CBP-170	81.5 a	112.2 a	43.2 d	103 c	138 c	18.2 a	96.3 b	38.8 b	3.3 b	9.4 b	25.84 a
CBP-176	75.7 a	104.6 b	42.4 d	99 c	104 d	15.7 a	102.8 b	39.0 b	2.4 d	7.6 d	22.64 b
CBP-189	66.9 b	90.1 d	49.0 d	109 b	67 d	14.1 b	76.4 c	35.4 c	3.5 b	8.2 d	22.09 b
CBP-190	74.0 b	113.6 a	46.7 d	106 c	119 c	13.1 b	72.5 c	37.1 b	2.7 c	9.6 b	21.56 b
CBP-198	84.0 a	105.4 b	59.3 b	114 b	71 d	12.0 c	57.7 d	35.0 c	2.1 e	10.2 b	21.33 b
CBP-200	71.6 b	84.9 d	55.3 c	116 a	87 d	15.6 a	46.5 d	43.2 b	3.9 a	9.0 c	25.04 a
CBP-209	80.5 a	117.3 a	45.7 d	101 c	131 c	12.8 b	77.9 c	38.3 b	3.4 b	9.0 c	21.02 b
CBP-223	74.1 b	114.1 a	42.7 d	101 c	106 d	14.0 b	73.8 c	40.6 b	2.6 c	9.7 b	24.89 a
CBP-236	59.6 b	98.1 c	39.3 e	101 c	132 c	9.7 c	106.8 a	30.2 c	1.9 e	11.1 a	13.87 d
CBP-237	70.4 b	85.6 d	42.1 d	104 c	81 d	16.9 a	121.5 a	27.6 c	2.1 e	9.3 b	26.03 a
CBP-238	65.4 b	104.0 b	39.7 e	99 c	120 c	13.7 b	87.3 b	40.7 b	2.7 c	8.0 d	19.75 c
CBP-241	64.9 b	94.4 c	40.9 e	98 c	127 c	12.9 b	72.3 c	41.4 b	3.0 c	8.2 d	19.47 c
CBP-244	63.1 b	116.0 a	42.4 d	99 c	126 c	15.6 a	119.6 a	39.9 b	2.1 e	9.9 b	22.42 b
CBP-245	76.0 a	108.3 b	44.9 d	99 c	146 c	11.3 c	64.4 d	43.3 b	2.5 d	8.7 c	17.76 c
CBP-246	81.4 a	104.6 b	39.1 e	96 c	98 d	13.4 b	91.5 b	41.5 b	2.2 d	8.9 c	20.91 b
CBP-247	75.8 a	95.3 c	38.0 e	100 c	184 b	13.2 b	95.9 b	42.0 b	2.4 d	8.3 d	16.70 c
CBP-334	69.6 b	98.1 c	40.4 e	102 c	115 c	14.9 a	95.2 b	39.9 b	3.0 c	8.3 d	21.03 b
CBP-346	66.2 b	104.2 b	42.0 d	101 c	144 c	14.7 a	89.2 b	35.0 c	2.8 c	10.1 b	22.82 b
CBP-348	73.3 b	109.3 b	41.9 d	98 c	113 c	13.5 b	83.1 c	38.8 b	2.8 c	9.4 b	19.73 c
CBP-373	86.0 a	102.4 b	43.1 d	100 c	102 d	15.9 a	127.9 a	28.7 c	2.8 c	9.5 b	24.31 a
CBP-381	87.3 a	120.4 a	38.4 e	100 c	170 b	10.0 c	96.9 b	34.8 c	2.5 d	8.2 d	14.50 d
CBP-390	67.4 b	97.4 c	43.0 d	103 c	101 d	12.6 b	90.6 b	32.4 c	2.4 d	9.7 b	20.89 b
CBP-392	68.9 b	94.1 c	40.9 e	98 c	116 c	13.1 b	99.8 b	37.1 b	3.0 c	7.9 d	18.96 c
CBP-397	65.3 b	103.1 b	44.4 d	102 c	142 c	14.7 a	112.0 a	41.7 b	2.8 c	8.2 d	17.20 c
CBP-398	73.9 b	106.6 b	36.4 e	98 c	122 c	15.7 a	64.0 d	42.2 b	2.7 c	9.4 b	23.09 b
CBP-403	86.7 a	99.3 c	53.4 c	106 c	159 b	8.7 c	61.0 d	37.3 b	3.0 c	10.2 b	21.11 b
CBP-409	60.6 b	84.4 d	38.4 e	104 c	113 c	11.7 c	80.7 c	38.5 b	2.3 d	11.2 a	20.63 b
CBP-414	78.4 a	104.9 b	43.0 d	99 c	117 c	11.9 c	67.7 d	45.8 b	2.3 d	8.4 d	18.89 c
'BRS Mari'	86.9 a	123.9 a	66.9 a	123 a	302 a	3.9 d	58.2 d	15.0 d	1.7 e	9.5 b	9.23 e
UENF1616	64.6 b	103.3 b	42.4 d	109 b	117 c	14.4 b	103.8 b	30.7 c	2.6 c	10.3 b	26.65 a
UENF1732	85.4 a	99.1 c	46.6 d	105 c	149 c	9.4 c	43.1 d	45.5 b	2.5 d	9.0 c	17.45 c

[1/]number of days to flowering (DFL); number of days until fruiting (DFR); plant height (HEI) in cm; canopy diameter (CAN) in cm; number of fruits per plant (NFP); mean fruit weight (MFW), in grams; fruit length (FRL) in mm; fruit diameter (FRD), in mm; pulp thickness of fruit (PTF), in mm; soluble solids (SS), in ^{o}Brix, and dry fruit mass (DFM), in grams.

[2/]Means followed by the same letter do not differ by Scott-Knott (1974) test at 5% probability.

Narrow sense heritability was estimated for MFW (h^2_r = 0.64), FRD (h^2_r = 0.75), PTF (h^2_r = 0.56) by Bento (2012). Considering these aspects and also standing that we are interesting in recombinants with better performance in fruit quality along with disease resistance, at this point we should focus our selection on lines and individuals with superior values for those three traits. Finally, it must be considered that the number of characters under selection is an important factor in pedigree selection. Some strategies, as the use of BLUP/REML, could be helpful in selecting superior recombinants.

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Performance of hybrids and prediction of heterosis based on molecular and morphological diversity in eggplant *(Solanum melongena* L.*)*

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Abstract

Seed purity and trueness can be detected by GOT (Grow Out Test), but it is a long term process and takes an additional season and is therefore, often skipped by seed companies. Thus, the quality and hybridity of seed needs to be tested quickly and efficiently. It should also be accurate, cheap and faster. Therefore, looking to these facts present study was carried out during *Rabi*-2010 to *Rabi*-2011 at the Molecular Biology Laboratory , Department of Genetics and Plant Breeding, College of Agriculture, I.G.K.V., Raipur (C.G) India.The experimental materials used in the study comprised of seven parents *viz.*, Green Long (GL), Muktakeshi (MK), Pusa Purple long (PPL), IBWL-2007-1 (IBWL), Pusa Purple Cluster (PPC), Pant Rituraj (PR), Punjab Sadabahar (PS) and their eleven F_1's and standard check PH-6. Molecular markers *viz.,* RAPD was used to test the hybridity. Heterosis analysis and mean performance of parents and hybrids was carried out for fruit yield and its components and morphological characterization as per descriptors for eggplant IBPGR, Rome.

Results showed that parents GL, IBWL, MK, PR & PPL along with their heterotic hybrids IBWL X MK, PPL X PPC, MK X PS and PR X PPL for traits *viz*., earliness, total fruit yield per plant and marketable colour revealed desirable performance for Chhattisgarh plains of India.

Out of twenty-nine RAPD, only four primers showed polymorphism between the parents. These primers are OPA-14, OPA-08, OPH-01 and OPH-20. According to banding pattern for the primer OPH-1, hybrid between P_4 and P_2 (IBWL-2007-1 X Green Long) resembles to male parent *i.e.,* Green Long (P_2) and in same reciprocal cross P_2 and P_4 (Green Long X IBWL-2007-1), the banding patterns of F_1 hybrid resembles male parent *i.e.* IBWL-2007-1 (P_4). In another hybrid, between P_3 and P_4 (Punjab Sadabahar X IBWL-2007-1) banding pattern of F_1 hybrid resembles to male parent *i.e.,* IBWL-2007-1 (P_4). For the primer OPH-20, F_1 hybrid between P_1 and P_3 (Punjab Sadabahar X IBWL-2007-1) banding pattern of F_1 hybrid resembles to male parent *i.e.* IBWL-2007-1 (P_3).For the primer OPA-14, F_1 hybrid between P_3 and P_4 (IBWL-2007-1 X Pusa Purple Long) banding pattern of F_1 hybrid resembles to male parent *i.e*. Pusa Purple Long (P_4) and in the same reciprocal cross P_4 and P_3 (Pusa Purple Long X IBWL-2007-1) banding pattern of F_1 hybrid resembles to male parent *i.e.* IBWL-2007-1 (P_3). For the primer OPA-18, F_1 hybrid between P_1 and P_3 (Punjab Sadabahar X IBWL-2007-1) banding pattern of F_1 hybrid resembles to male parent *i.e.* IBWL-2007-1 (P_3).

Variations are observed for morphological traits of brinjal genotypes which are visually classified on the basis of morphological characterization as per descriptors for eggplant, IBPGR Rome. Fruit traits (shape, size and colour) were identified as important morphological marker traits that most effectively discriminated among the brinjal genotypes.

Keywords: Heterosis, brinjal, hybrid, RAPD markers

Heterosis and inbreeding depression in two chili pepper population (*Capsicum annuum* L.) with different level of natural cross pollination.

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Abstract

The degree natural cross pollination of chili pepper which had not been consistently led to allegation of high heterosis and inbreeding depression in chili pepper. The objectives of this research were to study of heterosis and inbreeding depression in two chili peppers population that had different degree of natural cross pollination. The experiment was conducted from September 2012 to December 2012 at Bogor Agricultural University experimental field, Leuwikopo, Dramaga. The first population was IPB C2 x IPB C5 population (had high natural cross pollination) consist of seven set generations (F1, F2, F3, F4, F5, F6, and F7). The second population was IPB C120 x IPB C5 (had the lower natural cross pollination) consist of five set generations (F1, F3, F4, F5, and F6). All of those set generations planted at the same time. Beside those population, were planted too the parent genotypes (IPB C2, IPB C5, and IPB C120). Heterosis was estimated by comparing the means of F1 and the mid parents. Inbreeding depression was predicted by linier regression between level generation and the means of each character. The first population had positive heterosis in each character. But the second population just had positive heterosis in one character. Heterosis was varied from 3.8% - 41%. Inbreeding depression was clear in the first population, when the regression coefficient of each character was negative, but it was different with the second population which had positive regression coefficient in all of characters.

Keywords: Heterosis, inbreeding depression, chili pepper

The effect of genotypes and spacing radius on percentages of natural cross-pollination in chili pepper

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Abstract

The objectives of this research were to study the effects of genotypes and spacing radius on percentage of natural cross-pollination in chili pepper. The experiment was conducted from Oktober 2011 to March 2012 at IPB experimental field, Leuwikopo, Dramaga. The experimental design refers to Aries and Riesberg design. This experimental was used four genotypes (IPB C2, IPB C5, IPB C20, and IPB C120) and five spacing radius (0.5, 1, 1.5, 2.5, and 3.5 m). Determination of natural cross-pollination based on purple colour on pepper hypocotyl. The result showed that there were variation percentages of natural cross-pollination between chili pepper genotypes. Two genotypes had percentage natural cross pollination higher than 10%. The closest spacing radius wasn't show the highest percentage natural cross pollination.

Keywords: chili pepper, cross-pollination, genotype, hypocotil, purple

Breeding peppers for resistance to viral diseases

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Abstract

A lot of viral diseases infect pepper plantations in Europe. A survey was carried out in the Czech Republic during the past seven years and in total 613 Capsicum samples were examined by ELISA for the presence of possible viral infection. It was found that the most damaging viruses were *Cucumber mosaic virus* (CMV) and *Potato virus Y* (PVY). They decreased pepper yield and fruit quality and were present in 19 and 27% of samples, respectively. Furthermore *Alfalfa mosaic virus* (AMV) and *Broad bean wilt virus-1* (BBWV-1) infected mostly field pepper plantations and were present in 3 and 10% of samples, respectively. Some new emerging viruses such as *Pepper mild mottle virus* (PMMoV) and *Tomato spotted wilt virus* (TSWV) were observed rarely in greenhouses with about 1% occurrence. Eleven percent of collected samples contained a complex infection of two viruses. Isolates of the detected viruses were deposited in the Virus Collection of the Crop Research Institute, Prague, and are available to breeders for performing resistance tests. Our Department of Virology offers resistance evaluations based on comparison of the relative virus concentrations in plants after artificial inoculation. Most of the detected viruses are easily transmitted by aphids in a non-persistent manner, thus protection against them in open fields is difficult. Only resistant cultivars can effectively solve the problem which is a future task for breeders . Sources of resistance against CMV and PVY were described in some pepper cultivars, rarely with combined resistance against both of them. Commercially available cultivars are exceptionally resistant to viruses, at least to CMV.

Keywords: AMV, BBWV, CMV, PVY, resistance.

Introduction

Peppers (*Capsicum annuum* L.) are an important vegetable crop. They are grown on 244 hectares in the Czech Republic with yearly production of about 11,000 tons (Buchtova, 2011). Many diseases can decrease their yield and fruit quality. Among these, viral infections have a high importance, because they cannot be cured. The only fruitful way of protection is breeding for resistance.

Many pepper viruses occur throughout Europe. Marchoux et al. (2000) reported that five viruses are common on peppers in France: *Cucumber mosaic virus* (CMV), *Pepper mild mottle virus* (PMMoV), *Potato Y virus* (PVY), *Tobacco mosaic virus* (TMV) and *Tomato spotted wilt virus* (TSWV). In addition to CMV and PVY *Broad bean wilt virus* 1 (BBWV-1) is frequent in Sicily, Italy, and results in heavy pepper yield losses (Davino et al. 1989). The highest loss of pepper production, nearly 100%, was caused by *Broad bean wilt virus* 1 (BBWV-1) in Slovenia (Mehle et al, 2008). AMV, CMV, PVY and TSWV were found on red pepper plants in Slovenia (Vozelj et al. 2003) and Serbia (Petrovic et al. 2010). Each year TMV, CMV, PVY and AMV infections were reported in Hungary (Kiss et al. 2003) where TSWV significantly reduced yields in tobacco, pepper and tomato plantations (Jenser et al. 1996). *Tomato mosaic virus* (ToMV), CMV, PMMoV (Kostova et al. 2003) and TSWV (Neshev, 2008) are the most widespread viruses on peppers in Bulgaria. The most devastating are early infections. Avilla et al. (1997) reported that CMV and PVY drastically decreased fruit weight per plant up to 70% and 80%, respectively, when inoculated on 'Yolo Wonder' bell pepper plants as early as one week after transplanting to the field.

The aim of the presented work is to show the frequency of viruses on Capsicum plants in the Czech Republic. Another purpose is to demonstrate a valuable tool for evaluating the resistance level of cultivars with partial resistance based on the comparison of virus concentration in pepper plants.

Material and Methods

Plant material

Both field and greenhouse pepper plants (*Capsicum annuum* L.) grown in the Czech Republic were investigated for presence of viral diseases. Leaves showing virus-like symptoms were collected and examined for the presence of viruses by ELISA.

For the resistance evaluation, recent sweet pepper lines No. EL503, EL504, OL228, OL235, TE315, TE327, TE371, UH173 and UH180 from the seed company Peppers Libera, Ostrava, the Czech Republic were used.

Mechanical inoculation

The inoculum was prepared using 1 g of *Nicotiana benthamiana* Domin leaves systemically infected with the highly virulent Czech PVY strain (Virus Collection, 2013). Leaves were homogenized with 3 ml 0.06M K_2HPO_4. The first pair of pepper true leaves was then rubbed with fingers wetted with the homogenate. Inoculated plants were grown in a greenhouse under a light and dark photoperiod of 14 and 10 hours at 25 and 20°C, respectively. Young true leaves were collected for resistance tests four weeks after the inoculation, and the virus titer was assessed.

For the inoculation experiments, ten plants per tested cultivar and two plants of susceptible control *Capsicum frutescens* 'Tabasco' were used.

ELISA

Double-antibody sandwich ELISA (DAS-ELISA), described by Clark and Adams (1977), was used for the detection of AMV, BBWV-1, CMV, PMMoV, PVY, TMV and TSWV in the collected pepper leaves. Samples for ELISA were prepared by grinding 0.2 g of leaf tissue in the sample buffer, at a 1:20 ratio. Specific polyclonal antibodies were used according to the manufacturer's manual (Loewe Biochemica, Sauerlach, Germany). Plates were incubated for one hour at 20°C after pipetting the substrate solution, and the absorbance value was read at 405 nm using a MR 5000 (Dynatech, Germany) reader.

Determination of virus titer and concentration

The titer of the virus was determined as the highest dilution resulting in a positive reaction by ELISA at four weeks after the mechanical inoculation. A dilution series of each sample was prepared using the sample buffer. ELISA was performed using mixed leaf samples of ten plants per cultivar to average the deviations among the individual plants. The relative virus concentration was calculated according to Svoboda and Polák (2010).

Electron microscopy

Leaf samples with symptoms of viral infection were ground in a mortar with a 0.01M HEPES buffer, pH 8.2, at a ratio of 1:2. The homogenate was filtered through a nylon sieve and negatively stained by phosphotungstenic acid, pH 6.9, at a 1:1 ratio. Then the mixture was used for preparation of an electron microscope mount. Electron microscope grids were observed with a Philips 208S transmission microscope (Philips, Eindhoven, The Netherlands).

Results and Discussion

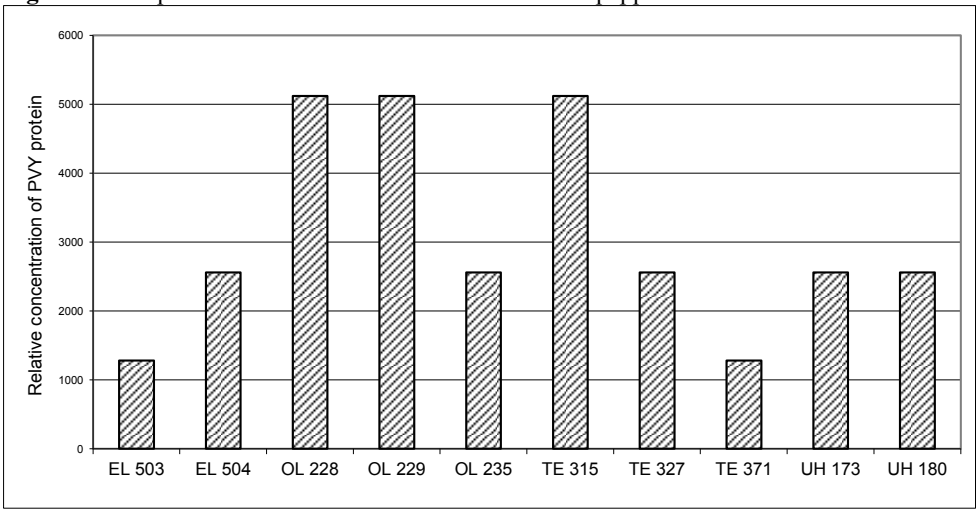
Viruses on Capsicum plants in the Czech Republic

In the last seven years a survey of selected viruses on peppers planted in the Czech Republic was carried out. Altogether, six hundred and thirteen leaf samples were examined by ELISA. Positive findings were confirmed by observation with the electron microscope. It was found that the most prevalent viruses were PVY and CMV followed by BBWV-1, AMV, TSWV and PMMoV. PVY was the most frequent virus on peppers with an average occurrence of 27% and PMMoV was the least common virus with an incidence below 1%. No other viruses were found. A complex infection of two viruses was discovered in sixty-five samples. PVY and CMV were both found in Moravia and Bohemia (east and west parts of the Czech Republic) whilst AMV, BBWV-1, PMMoV and TSWV were detected only in southern Moravia. With the exception of PMMoV and TSWV, all the detected viruses are easy transmissible by aphids in a non-persistent manner (Plant Viruses Online, 2013). In practice this means that these viruses are transmitted rapidly in several minutes without any long-time acquisition and inoculation by aphid feeding.

Resistance evaluation

The resistance tests were performed for the most frequent pepper-infecting virus in the Czech Republic, PVY. The results of evaluation of selected pepper lines for PVY resistance are shown in Figure 1. Comparison of the relative virus concentrations clearly demonstrated the high level of resistance of lines No. EL503 and TE371. Their relative virus concentrations were four times lower than in the susceptible lines No. OL228, OL229 and TE315 and two-fold lower than in the moderately resistant lines No. EL504, OL235, TE327, UH173 and UH180. Evaluation of resistance based on determining virus concentrations in plants is an essential tool for breeders. When compared to assessment based on the observation of symptoms, this method is quantitative and produces a more exact correlation among cultivars. Our Department of Virology offers the performance of such resistance tests to breeders.

Figure 1. Comparison of PVY concentrations in selected pepper lines.



Breeding peppers for resistance

Protection of pepper plants focused against aphid vectors by insecticide spraying may not be sufficient because aphids can successfully transmit viruses in a non-persistent way by a quick test feeding before they are killed. The only efficient method of protection seems to be using resistant

pepper cultivars. Mazourek et al. (2009) reported that they had developed a new tabasco pepper (*C. frutescens*) 'Peacework' with CMV resistance. Similarly Liang et al. (2005) reported a new chilli pepper hybrid F1 'Tianjiao No. 4', highly resistant to CMV. Efficient resistance to PVY (gene *Pvr4*) was identified in the wild hot pepper 'Criollo de Morelos 334' (Janzac et al. 2009). Another PVY-resistant jalapeno pepper is 'TAM Dulcito' (Crosby et al. 2007).

The resistance gene alleles are often found in chilli peppers. Only some cultivars possess complex resistance to CMV and PVY concurrently. For example 'Cecil F1' displays extreme resistance to both viruses (Horvath et al. 2000). Nevertheless, there is very rare evidence of combined resistance against CMV and PVY in sweet peppers. This is a task for Capsicum breeders for the future. The introduction of resistant cultivars would be a vital contribution for pepper growers worldwide.

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Introgression breeding in eggplant for agronomical and ornamental purposes

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Abstract

Allied species of eggplant are a valuable source for key fruit quality and plant breeding traits. Among them, *Solanum indicum* L. is a source of resistant traits to *Fusarium oxysporum* and *Verticilium dahliae,* and its plant architecture and fruit features made this species suitable for ornamental purposes as well. We performed crosses between this allied species and the two parental lines, 305E40 and 67/3, of our mapping population to take advance of all the genetic information available and to ease the molecular characterization of the newly introgressed progenies. The phenotypic, biochemical and molecular characterization of the parental lines, the interspecific hybrids, and BC1 and BC2 is described.

Introduction

Allied and wild relatives of eggplant (*S. melongena* L.) are an important source of potential genetic variability for many plant and fruit traits, as well as for nutritional and functional compounds and for resistance traits to diseases and pests (Rotino et al. 2013). This is reflected in great interest in introgressing genes of important useful traits from the allied species into cultivated eggplant through conventional and un-conventional methods like sexual and somatic hybridisation. The use of conventional breeding methods has been mainly performed through intraspecific crosses and only sporadically through interspecific crosses due to the presence of sexual barriers between *S. melongena* and its related wild species. In fact, many allied species were successfully employed in crossing with *S. melongena* in order to transfer useful traits, but only a limited number of them gave origin to fertile or partially fertile F_1 hybrids which were successfully backcrossed to the cultivated *S. melongena* (Bletsos et al. 1998), thus opening the possibility to be incorporated into a practical breeding program. Moreover, exploitation of allied species into breeding programs must cautiously be performed, in order to avoid to drag together with the useful traits also negative features such as undesirable agronomical traits, like susceptibility to (new) pests and diseases and/or a high level of unsafe compounds such as glycoalkaloids, harmful for human health and often present in great amount in the fruits of wild species of eggplant.

Solanum indicum L. is an allied species of eggplant which is known to carry traits of tolerance and resistance to important diseases and pests like *Verticillium* (*Verticillium dahliae* Kleb.), *Fusarium* (*Fusarium oxysporum* f. sp. *melongenae*) and nematodes. Moreover, its plant architecture, together with the small dimensions of its hair-velvety leaves and the bright red colour of its ripened fruits which last for long time on the plants, make this species also suitable for ornamental purposes. The potential of this allied species as a source of new major genes and alleles of quantitative trait loci (QTLs) with favourable effects on the plant architecture and physiology has not yet been investigated. With the purpose to introgress useful traits from this allied species into

cultivated eggplant and also to deepen the molecular characterization of the genetic regions underlying them, we employed *S. indicum* as male in sexual crosses with the two parental lines, 305E40 and 67/3, of our newly developed genetic map (Barchi et al. 2012). This facilitates the molecular characterization of the interspecific genetic materials through molecular markers already mapped. Few interspecific hybrids were obtained from cross between *S. indicum* and both the eggplant lines, and these were successfully backcrossed to the parental species. Here we report the first phenotypic, biochemical and molecular characterization of the resulting interspecific hybrid plants, the BC1 and BC2 plants.

Materials and Methods

Plants of the allied species *S. Indicum*, of the eggplant lines 305E40 and 67/3, of the F1 hybrids [305E40 x S.ind] and [67/3 x S.ind], and of 2 and 4 BC1 plants from crosses between the F1 plants and the corresponding recurrent parent 305E40 and 67/3, plus 41 BC2 plants from the cross with 305E40 were bred and evaluated under glasshouse conditions.

Resistance to *Fusarium oxysporum* and tolerance to *Verticillium dahliae* were assessed according to Toppino et al.(2010). Phenotypical characterization of fruits, flowers and other parts of plant was performed according to the ECPGR (2008) and the IBPGR (1990) eggplant descriptors guidelines.

Pollen viability was evaluated under light microscope after staining with acetic carmine. *In vitro* pollen germination assay was performed by collecting pollen grains from open flowers and germinating them in PEG 800 medium (Touarev and Heberle-Bors, 1999) overnight at 25°C in the dark and then observing pollen tube germination under a light microscope. After characterization, fruits at different ripening stages were collected from each plant, cut in slices and frozen in liquid nitrogen, then lyophilized in view of biochemical characterization. Analyses of total polyphenols, solamargine and solasonine glycoalkaloids, and soluble solids (°Brix) were performed according to Mennella et al., (2013). Genomic DNA samples were extracted from young leaves, using the GenElute™ Plant Genomic DNA Miniprep kit (Sigma, St. Louis, MO), following the manufacturer's protocol. Genotyping of parental lines, F1, BC1 and BC2 progenies was achieved by means of 267 different molecular markers, including SNPs, SSRs and COSII, all but 16 previously mapped (Barchi et al. 2012) in the mapping population (305E40 x 67/3). SNPs were screened using the GoldenGate assay (Illumina, San Diego, CA) or were converted into High Resolution Melting (HRM) markers and detected in a Rotor-Gene 6000 PCR machine (Corbett Research, Mortlake, NSW, Australia). The SSRs and COSII amplicons were separated on an AdvanCE™ FS96 capillary electrophoresis system (Advanced Analytical Technologies) or through electrophoresis on MetaPhor™ agarose (Lonza). Genotyping of the 41 BC2 plants was achieved by means of 118 markers selected from the first characterization of the parental lines as being polymorphic between 305E40 and *S. indicum*.

Results and Discussion

Plants were grown in the glasshouse and submitted to morphological characterization for fruit and plant traits, including presence of anthocyanins, hairs and prickles, plant habit and fruit quality traits. '305E40' produces long, average weighted, highly pigmented dark purple fruit characterised by the presence of a green ring in the flesh; the prickly plants have an upright growth habit, produce leaves without anthocyanins and pink flowers. '67/3' produces round, violet coloured fruits heavier than those of '305E40' and characterized by white flesh; the plants are anthocyanic and prickles-less, have a compact growth habit and produce big leaves and violet flowers. *S. indicum* produces a great number of light-violet flowers and small round orange coloured fruits with an average weight of three grams; the plants are bushy and produce small hair-velvety leaves without anthocyanins

and prickles. The allied species was confirmed as fully resistant to *Fusarium oxysporum* and highly tolerant to *Verticillium dahliae.* The flowers of all the parental lines produced viable pollen (> than 80% of coloured pollen grains after staining with acetic carmine and more than 60% of germinating pollen grains).

Biochemical analyses performed on fruits at different developmental stages showed that the wild species had increased content of both glycoalkaloids (solamargine and solasonine) and also a greater amount of total polyphenols, but a slightly lower (not significantly) total soluble solids (°Brix) content compared to the cultivated lines of eggplant. In the interspecific hybrids intermediated values between the parental lines were displayed for glycoalkaloids and for polyphenols content, while a lower total soluble solids (°Brix) content compared to the wild species and the cultivated lines of eggplant was detected. It has to be also noted that a general decrease in phenol content has been detected during the fruit ripening. As regard to plant morphology, all the interspecific hybrid plants resembled to the allied parent: the plants were bushy, without anthocyanins and prickles, but less hairy compared to *S. indicum*. All the interspecific hybrids set small green unripe fruits that at ripeness become violet and, then, orange, without spines on the calyx. Hybrid plants showed low pollen viability (averaging 12% of coloured and 20% of germinating pollen grains) and when crossed as female with the two eggplant recurrent parents gave origin to fruits containing no or few seeds. The 2 BC1 plants from the cross with '305E40' resembled more to the cultivated parent: the fruits were more elongated compared to the hybrid plants and characterized by purple colour and presence of a light green ring in the flesh. The plants (one plant upright with light violet flowers, while the other plant still more prostrated and producing pink flowers) presented prickles and low hairiness and produced bigger leaves. Also among the four BC1 plants from the cross with '67/3' the characteristic traits of the cultivated parent were more evident: in fact the plants were less hairy and produced bigger and more anthocyanic leaves compared to the hybrid plants. The fruits were bigger, round and violet or pale violet, sometimes ribbed and with white flesh. Pollen viability and germination resulted restored in all the BC1 plants, the correspondent values resembling the ones of the parental lines.

Genotyping of the parental lines, interspecific hybrids and BC1 was achieved by means of 267 markers, (169 SNPs, 58 microsatellites and 40 COSII markers), all but 16 previously mapped in the (305E40 x 67/3) mapping population (Barchi et al. 2012) and chosen as they amplified also in *S. indicum*. All the hybrid plants resulted, as expected, heterozygous for all the loci polymorphic between the parental lines. Genotyping of the two BC1 plants from the cross with '305E40' revealed the presence in all chromosomes, except E05, E07 and E10, of regions entirely belonging to the cultivated parent, with length ranging from few cM to the entire chromosome (E04). On the contrary, genotyping of the 4 BC1 plants from the crosses with '67/3' revealed only little regions on 4 chromosomes which do not contain fragments of allied species in at least one of the considered plant. Therefore, these BC1 plants result to be more suitable than those from '305E40' to generate introgression lines each one containing single small fragments of the whole genome of the allied species. Genotyping of the 41 BC2 plants from one 305E40 BC1 plant was performed by means of 118 markers (69 High Resolution Melting (HRM) SNPs, 40 microsatellites and 18 COSII), chosen from the previous screening on the parental lines as being polymorphic between *S. indicum* and 305E40 and also as being still present at heterozygous level in the BC1 plant from which they derived. Segregation analysis among the BC2 progeny of all markers will allow the positioning in the map of 16 new un-mapped markers. The molecular characterization of the BC2 progeny will also disclose the genetic composition of each plant, highlighting the presence and the position of the introgressed allied genomic fragments in the eggplant genome, and will, therefore, allow the selection of the most promising lines to be used in the next backcross cycles.

Moreover, the interspecific hybrids and some of the BC1 plants were successfully backcrossed to *S. indicum* to recovery the useful ornamental traits as overall foliage texture, plant architecture, flower and fruits colors in order to produce morphological novelty for plant ornamental market.

The prospects for the future activity are in a mid term scale: (i) the development of rootstock for eggplant cultivation especially if *S. indicum* will confirm to be also tolerant to nematodes, (ii) the incorporation in the breeding programs of the backcross lines displaying useful features as disease resistance, fruit biochemical composition and/or good plant architecture (ornamental and agronomical breeding); while in the long term scale the aim is to develop a collection of introgression lines, each containing a single independent homozygous insert of the allied genome. The entire representation of the allied genome could be employed as a living genomic library of the *S. indicum* into the genome background of the cultivated eggplant.

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