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Genetic Variation for Plant Breeding

Editors
Johann Vollmann
Heinrich Grausgruber
Peter Ruckenbauer

GENETIC VARIATION FOR PLANT BREEDING

Genetic variation for plant breeding

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Johann Vollmann, Heinrich Grausgruber
and Peter Ruckenbauer

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CURRENT AVAILABILITY AND USE OF GENETIC DIVERSITY

Part 1

Eds. J. Vollmann, H. Grausgruber & P. Ruckenbauer
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Changes over time in the genetic diversity of four major European crops – a report from the Gediflux Framework 5 project

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ABSTRACT: Genetic diversity in four major European crops was assessed over the latter half or more of the twentieth century using various DNA markers. Barley genetic diversity was quantitatively unchanged over the time period in question with more diversity found amongst varieties actively in commerce. Similarly wheat genetic diversity was greatest in varieties actively in commerce and an overall increase in diversity over time was noted. An initial increase in German maize genetic diversity was identified followed by a subsequent decrease. French maize genetic diversity showed qualitative rather than quantitative changes and no overall reduction was found. Potato genetic diversity was found not to decrease; indeed a slight increase was found when measured by NBS profiling.

Key words: Barley – maize – potato – wheat

Introduction

It has been claimed that plant breeding reduces genetic diversity in elite germplasm, risking future crop losses and prejudicing the continued ability to improve crops. The objective of this project was to determine any changes to genetic diversity over time in four widely grown agricultural crops: barley, wheat, maize, and potato. Any genetic erosion that might have occurred over the past 50 or more years in these crops was evaluated. This project was commissioned under the EC Framework 5, Quality of Life and Management of Living Resources Research Programme (Key Action 5 - Sustainable Agriculture).

Barley

Materials and methods

More than 500 European barley varieties bred over the past 50 years were selected, evenly representing material grown in each of the 5 decades. Breeders and countries of origin were considered, early material was included and representatives of widely available varieties (National List (NL)) and commercially important (widely grown) varieties such as UK Recommended List (RL) varieties were included. The diversity of these varieties was assessed using molecular markers including the retrotransposon based marker system S-SAP (sequence–specific amplification polymorphism). DNA was extracted from the flour of 30

seeds of each variety using the Qiagen DNeasy kit and genotyped as detailed in Leigh et al. (2003). Data from all time periods was assessed by Jaccard's similarity coefficient analysis. Convex hulls were constructed from axes 1 and 2 of the subsequent PCO analysis.

Results and discussion

101 alleles were identified from 4 S-SAP primer combinations. Material was classified into spring and winter, and 2- and 6-row varieties. The first two axes of the PCO analysis revealed two distinct clusters corresponding to the 2-row spring barley and the 6-row winter varieties. The clustering allowed the unknown habit of a variety to be predicted from the position of the variety within the PCO scatter-plot. The efficiency of this prediction was 97.5 % in the case of 2-row spring barley varieties over all time periods.

When compared to the base-line period 1900 - 1950, the convex hulls from each of the last 5 decades showed no contraction or expansion of genetic diversity in quantitative terms. This is consistent with other published work on the temporal flux of genetic diversity in UK barley – see for example Koebner et al. 2003 – but now of a European-wide set of varieties.

Preliminary analysis comparing the quantity of diversity available (NL) to that 'exploited' in commercial farming (RL) showed that the range of diversity in the RL varieties generally exceeded that of the NL material. This is over all decades with no partition into spring/winter or 2/6 row types. Studies of specific S-SAP alleles, over the 5 decades shows some losses and gains in a manner comparable with Donini et al. (2003). Very similar results were obtained using SSR analysis, showing qualitative rather than quantitative fluxes in genetic diversity. No genetic erosion was detected.

Wheat

Materials and methods

The winter wheat collection was divided into 'Euro-RL' and 'UK NL'. The former comprised of 281 entries, selected on the basis each entry occupied at least 5 % of the winter wheat acreage in a North European country in the period 1945-2000. The UK NL set was assembled from those varieties in commerce in the late 1990s and consisted of 229 entries.

Genomic DNA was extracted from 30 grains of each variety and purified in Qiagen columns. SSR typing was conducted using fluorescently labelled wgm primers on an ABI platform. We targeted 42 mapped loci (one per chromosome arm). S-SAP fingerprinting followed the methods outlined by Leigh et al. (2003), using ^{33}P labelled primers from the Sukkula 9900 LTR, on *TaqI* digests of genomic DNA, and employing 3 selective bases at the ligated end of the amplicon. The NBS fingerprinting method followed that of Van der Linden et al. (2004); we used three *R* gene domains (NBS2, NBS3, NBS5) and three restriction enzymes (MseI, HaeIII, PstI). Morphological data collected from field trials in 2003 included flowering time, awning, stem hollowness, ear glaucosity, mature ear colour, ear density, and glume hairs. In 2004, these were expanded to take in formal UPOV notes.

Results and discussion

Of the variety/ssr combinations, 94.1 % delivered a single allele, and 5 % displayed two or more alleles, though most of these showed a 'dominant' allele. There was no evidence of a decrease in overall genetic diversity and Euro-RL varieties showed more variation than UK NL, possibly because of the greater time and geographical span of the former. S-SAP profiling delivered 75 polymorphic bands with a frequency of > 2 % across the 510 varieties. NBS fingerprinting gave rise to 72 scorable products. These data await further analysis.

Maize

Materials and methods

A set of 85 hybrids representing a sample of the most important hybrids from Germany of the last four decades was chosen based on area sown. DNA was either extracted employing a modified CTAB procedure or by using GenElute Plant Genomic DNA miniprep kit. Primer sequences for 55 SSRs were obtained from the MaizeGDB (http://www.maizegdb.org) (Reif et al. 2004). Allele identity was assigned applying a modified stepwise mutation model. The modified stepwise mutation model allows for tetra- and penta-repeats beside the main classes and intermediate classes. Number of alleles, new alleles, and loss of alleles from one period to the next were determined. Gene diversity (*H*) was calculated according to Nei (1987). The Friedman rank sum test was used to investigate significance of gene diversity changes between the genotypes of the four time periods. All analyses were carried out with Version 2 of the Plabsim software (Frisch et al. 2000).

Results and discussion

Hybrid maize breeding was initiated in Germany in 1951 by crossing adapted European flint lines with high yielding US dents. In the first phase of hybrid breeding, dents were imported from the US and selected for early flowering, whereas the parental flint inbreds were commonly developed by selfing open-pollinating European populations. We observed a significant increase in gene diversity from period A to period B (Table 1).

Table 1. Number of individuals (N_i), alleles (N_a), new alleles (N_n) and lost alleles (N_l) from one time period to the next, as well as gene diversity (*H*) in the hybrids of the four time periods

Period	N_i	H^1	N_a	N_n	N_l
A (1961-1975)	20	0.59a	5.3	57	57
B (1976-1985)	21	0.63b	4.9	46	76
C (1986-1995)	22	0.63b	4.5	35	46
D (1996-2001)	22	0.61a	4.2	38	61

[1] Gene diversity values followed by the same letters are not different at the 0.05 significance level according to a Wilcoxon rank sum test.

The low gene diversity in the initial phase of hybrid breeding in Germany can be explained by the limited number of public lines used at this time for the generation of hybrids. The increase of diversity in Period B is most likely due to the continued introgression of dent germplasm from the US and the broadening of the flint pool with flint material from Europe. The diversity was narrowed from Period C to D, which could be due to the effects of second cycle breeding, the switch to single cross hybrids, and the increasing interchange of elite inbred lines between private breeding companies. Consequently, the slight decrease in the genetic diversity in the last decade indicates that breeders should be aware of a potential loss of genetic diversity. Complementary work in France showed no diminution of genetic diversity over time although qualitative fluxes in diversity were noted. Most genetic diversity was within rather than between time periods.

A set of 155 French cultivars representing the same 4 periods as for the German maize materials, were also analysed by GEVES using 51 SSRs. The pre 1975 period contained the highest number of alleles at 4.7 alleles per locus. The data were treated in both a dominant and co-dominant fashion. The general trends in terms of gene diversity stay the same, except the dominant data reduced the gene diversity in the same way for all periods. The gene diversity showed low differentiation between the timeframes, though it was slightly higher in

period 1. The allelic richness and diversity was slightly higher because of more unique alleles and fixed alleles in the early varieties when double and triple hybrids were prevalent. As time progressed and single hybrids became more common, gene diversity fell slightly, but there was no significant diminution of the genetic diversity. The changes revealed by the microsatellite analysis were qualitative rather than quantitative.

Potato

Materials and methods

We chose 500 potato varieties from three different time periods: 1946-50, 1971-75, and 1996-2000 to represent the diversity available in each time period. For each period, varieties from The Netherlands, United Kingdom and Germany were collected. Data such as pedigree, growth area, morphology, and major disease resistance were collated. Genetic diversity in each of the three time periods was assessed using three marker systems: microsatellites (SSRs), Nucleotide Binding Site profiling (NBS Profiling) and Single Nucleotide Polymorphism (SNP). NBS profiling was performed as described in Van der Linden et al. (2004) using three different primers and a single restriction enzyme (Mse I).

SNPs were detected using Pyrosequencing™ which allows accurate allele frequency determination. This also distinguishes the allelic states of tetraploids; for most SNPs, scores include allele distribution (0:4/1:3/2:2/3:1/4:0). Duplexes were developed for most SNPs. SSRs were taken from Milbourne et al. (1998) and profiles scored as patterns.

Results and discussion

77 NBS markers were used to assess the changes in biodiversity in the three time periods. No allele loss was observed, and several markers were present only in varieties that were introduced to the market after 1970. These results demonstrate that during the last 60 years there has been no genetic erosion, but a slight increase in overall genetic diversity has occurred. This may be due to introgression of disease resistance from wild potato into cultivars, particularly late blight and potato cyst nematode. Preliminary analyses with 30 SNPs indicate that biodiversity has not decreased over the last 60 years. No alleles were lost in the last 60 years, but shifts in the allelic distribution may have occurred. Preliminary results for 98 UK-grown potato varieties analysed with 10 SSRs again show that diversity is maintained over time and that the Recommended List system in the UK has not reduced diversity.

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Changes in the genetic diversity of the Hungarian wheat varieties registered over the last fifty years

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ABSTRACT: Pedigree analysis on the Hungarian wheat varieties registered over the last 50 years indicates a great increase in genetic diversity. Breeders cross a wide range of initial stock to develop varieties with increasingly complex pedigrees, as a result of which, diversity is also increasing at the production level. Some 18 - 20 wheat varieties now have substantial shares of the sowing area and the dominance of a single variety is a thing of the past. The age of the cultivated varieties, weighted with the sowing area, is 6 - 8 years and has remained practically unchanged for the last two decades. The genetic diversity in Hungarian wheat production, calculated on the basis of the number of varieties, the sowing area per variety and the coefficient of parentage of the varieties, has improved greatly over the last decade. There has been a reduction in genetic vulnerability, while the large number of genetically diverse varieties makes it possible to respond rapidly with a change of variety in the case of biotic stress or new market demands.

Key words: coefficient of parentage – genetic diversity – *Triticum aestivum*

Introduction

Nowadays, in addition to public concern about ‘gene erosion’ or ‘gene monoculture’ due to the real or imagined increase in similarity between cultivated varieties, farmers require assistance in distinguishing between the ever larger number of varieties available. It cannot be denied that modern varieties have very little genetic variability compared to the old landraces; nevertheless, the rust epidemic experienced in 1873 made it clear that genetic diversity within populations of cultivated landraces is not sufficient in itself to ensure safe production if the genetic variability within the population for certain traits, in this case rust resistance, is virtually non-existent, or if alleles responsible for resistance occur at very low frequency. This natural catastrophe decided the question of whether farmers should grow landraces with great heterogeneity or more homogeneous improved varieties before it was even asked; in Hungary, as in most parts of Europe, varieties resulting from the efforts of an increasing number of breeders had almost completely replaced the landrace populations by the first half of the twentieth century. The diversity desirable in wheat production could be achieved under such circumstances by growing varieties with different genetic backgrounds and by rational variety rotation. Due to the preferences of growers, however, only a small proportion of the available varieties are grown on large areas, even though it is common knowledge that it is safer to grow several varieties with similar properties but different origin, rather than a single variety. Due to the way in which farmers choose varieties, the extent of genetic diversity in wheat-fields does not depend entirely on the breeders. The task and responsibility facing breeders is to set up breeding programmes based on a wide range of genetic sources in order to develop varieties with very diverse genetic backgrounds, so that if crises arise (e.g. disease epidemics) they will be ready with recommendations for a rapid change of variety.

Materials and methods

Diversity studies were carried out on the pedigree and sowing area data of 187 registered winter wheat varieties grown in Hungary between 1957 and 2000. The coefficient of parentage (COP) was calculated using the International Crop Information System (ICIS) program developed by CIMMYT/IPGRI (Fox & Skovmand 1996, Skovmand et al. 1998).

When calculating the coefficient (r) it was assumed that: (1) varieties obtained by crossing obtained half their genes from each parent (r = 0.5), (2) all the parents were homozygous and homogeneous, (3) the most distant ancestors, with unknown parents, were not related (r = 0), (4) the r value between a variety and a new variety selected from it was 0.75, (5) the degree of relationship between two new varieties selected from the same variety was $r = (0.75)^2 = 0.56$, and (6) the relationship of the variety to itself was r = 1. The weighted genetic diversity of the wheat varieties cultivated in Hungary was characterised as suggested by Cox et al. (1986).

Results and discussion

Three periods can be distinguished in the choice of wheat varieties available in Hungary over the last 50 years. After the war the old, non-intensive Hungarian varieties continued to be grown until technological developments required the use of shorter-stemmed genotypes which could be mechanically harvested. Between 1963 and 1983 more than half the wheat-fields were sown to foreign varieties, which were then replaced by Hungarian-bred varieties on 70 - 90 % of the sowing area from the mid-eighties onwards.

Until the mid-seventies very few varieties (10 - 15) were registered. These originated from the isolated breeding programmes of various countries, so the degree of relationship between them was very low. The closely related Soviet varieties were the only exception to this.

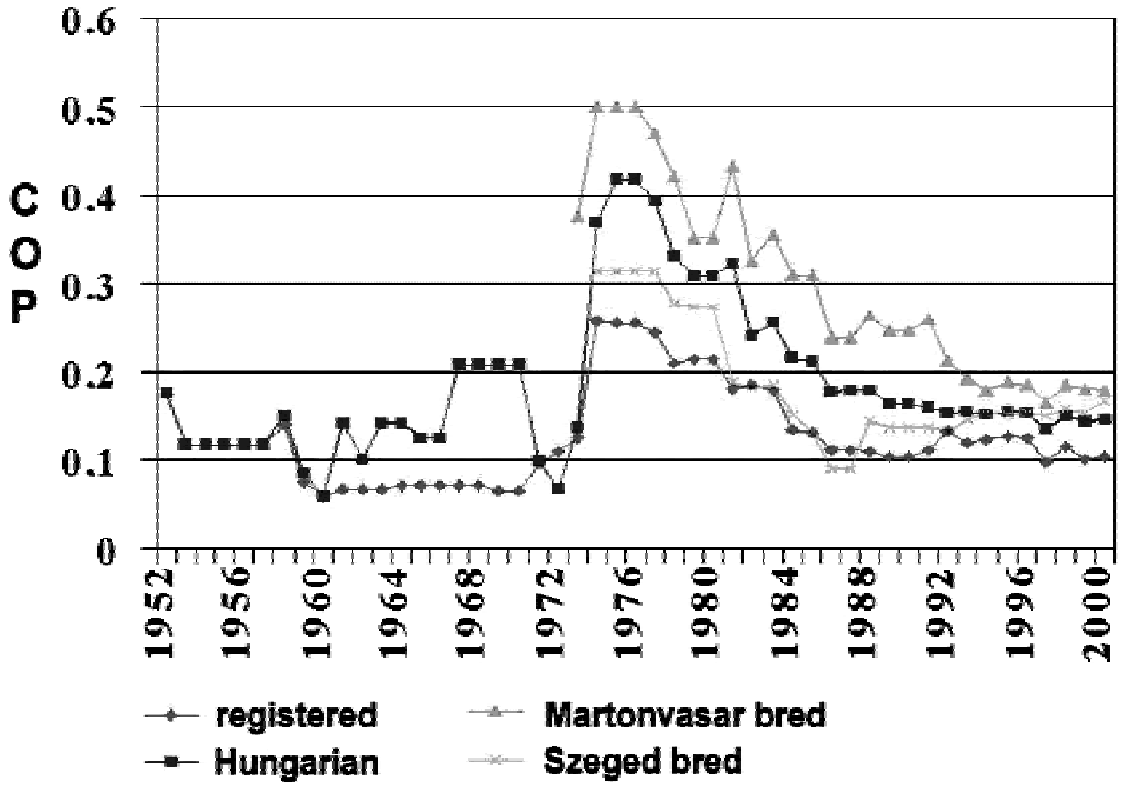

Figure 1. Average coefficient of parentage (COP) between wheat varieties registered in Hungary between 1952 and 2000

All the first series of varieties registered after 1971 arose from crosses involving 'Bezostaya 1', so they were all sibs or half-sibs (Figure 1). In the early years the varieties bred in Szeged exhibited greater genetic variability than those from Martonvásár, which had more east European ancestors, in an effort to preserve frost resistance and quality, but this difference has now disappeared. The pedigree of the new Hungarian wheat varieties is known for the last 15 - 20 generations; the 115 varieties originate from 264 landraces or varieties with unknown ancestors. The genetic sources which made the greatest contribution to the development of the Hungarian varieties were 'Ukrainka', 'Aka-komugi', 'Kanred', 'Rieti', 'Colonista' and 'Mironovskaya 808'.

For a long period, wheat production in Hungary was characterised by a low number of registered varieties and by the cultivation of very few varieties on large areas. By comparison, between 1995 and 2000 a total of 19 - 22 varieties were grown on more than 1 % each of the total sowing area, while the area sown to the leading varieties was 10 - 12 %. Even now, however, despite this more even distribution of varieties, only a small proportion of the registered varieties become widely grown.

Figure 2. Weighted diversity (calculated from COP, number of varieties and market share of varieties; range 0 to 1) in the Hungarian wheat production

In spite of the increase in the number of varieties, the rate of variety change has not accelerated but, apart from some slight fluctuation, has remained practically constant over the last three decades. The average age of registered varieties is 4 - 6 years, while the age of cultivated varieties weighted with the sowing area is even more, 6 - 8 years. Each year farmers choose to grow a new variety on around 20 % of the sowing area. Due to the relative area sown to each variety and to the decrease in their genetic relationship, a higher level of genetic diversity ('weighted diversity') than ever before has been achieved in wheat production (Figure 2), leading to a considerable reduction in the extent of genetic vulnerability.

The availability of a large number of varieties with similar agronomic value but different origin facilitates a rapid change of variety in response to changes in the pathogens or to new market demands. In spite of the favourable trends, breeders must continue their efforts to maintain and expand genetic diversity. This will be impossible without the use of detailed pedigree databases providing an accurate knowledge of the genetic background of the genotypes used in crosses. The 'Breeder' software developed in Martonvásár (Láng et al. 2001) not only stores variety pedigrees, but allows them to be analysed and the ancestors included in their genetic background to be identified, thus helping breeders to choose genetically diverse parents.

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Morphological and molecular characterization of hulled wheats

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ABSTRACT: The emmer wheat is one of the most cultivated hulled wheat in the Mediterranean basin. In Italy, it is cultivated mainly in marginal lands of Central and Southern Italy, where local varieties, adapted to the natural environments where they have originated, are used. Emmer cultivation was drastically reduced during the end of last century due to its low yield. Nevertheless, more recently its agronomic advantage, nutritive values and its use in dietary products, made its cultivation economically interesting in marginal lands with a parallel increasing of the cultivated area which overpass the 2000 ha. In the present paper, Italian accessions of *Triticum turgidum* L. spp. *dicoccum* Shrank ex Schübler (emmer wheat) have been evaluated for agro-morphological traits in field trials in central Italy and at the DNA level utilizing ESTs as molecular markers. The analysed materials have shown distinctive molecular traits and the existence of a huge amount of diversity between varieties. The molecular markers are able to cluster the accessions in agreement with some of their characteristics.

Key words: Ancient wheats – emmer – molecular markers – EST – *Triticum dicoccum*

Introduction

Hulled wheats are, at the three ploidy levels (2x, 4x and 6x), the firsts domesticated wheat species. Their spikes are not fragile, but are hulled. Therefore, they are the bridge between the modern cultivated wheat species (bread and durum) and the wild wheat species. At the diploid level *Triticum monococcum* L. ssp. *monococcum* (einkorn wheat) is the cultivated species derived from *T. monococcum* L. spp. *boeoticum* (Boiss). Einkorn wheat has a monophyletic origin being domesticated about 10000 years ago in the south-est of Turkey (Salamini et al. 2002). At the tetraploid level *Triticum turgidum* L. spp. *dicoccum* Schrank ex Schübler (emmer wheat) is the domesticated type from spp. *dicoccoides* (wild emmer wheat**)**, and from it the ssp. *durum* (Desf) Husn. (durum wheat) has originated. At the hexaploid level *Triticum aestivum* L. spp. *spelta* (spelt wheat) originated by polyploidization of a cross between *T. turgidum* spp. *dicoccoides* (4x) and *Aegilops squarrosa* (2x). The modern bread wheat (*T. aestivum* L. spp. *aestivum*) is the unhulled form derived from spelt.

Hulled wheats are still growing spontaneously in the centre of origin, the Middle East. Emmer is also widely spread in the Mediterranean basin, and it is present as a crop in marginal areas of central and southern Italy. *T. dicoccum* was the main cereal crop in the Mediterranean, but its cultivation has decreased during the 1960's due to its low productivity and hulled kernels. In the last decade, people's increasing interest in natural and organic products led to a 'rediscovery' of emmer for its healthy characteristics, associated to high content of resistant starch, its ability to grow on soils with limited fertility, utilizing low input techniques, and growing in cold climates. Moreover, it is a potential source of genes for breeding programs to improve durum and bread wheat. As a consequence, the cultivated area increased to about 2000 ha.

During the last decade field evaluations have been carried out to assess variation in agronomic and quality traits, to select strains among old landraces and to develop new genotypes through inter-specific crosses (Codianni et al. 2000). In recent times, several kinds of molecular markers (i.e. RAPD, RFLP, ISSR, SSR) have been utilised to assess genetic variation in emmer wheat accessions (Barcaccia et al. 2002, Large et al. 2003, Pagnotta et al. 2003a & 2003b, Figliuolo & Perrino 2004). In the present study Italian emmer wheat

accessions were evaluated for agro-morphological characteristics and their genetic variation at the DNA level was assessed by EST markers.

Materials and methods

Characters, reported in Table 1, have been evaluated in a randomised block design (RBD) with 3 replicates in Urbisaglia (43 °11' E, 13 °22' N). Six EST (DuPw4, DuPw23, DuPw38, DuPw124, DuPw167, DuPw254), chosen for their chromosome localization (Eujayl et al. 2002) have been used to evaluate the percentage of polymorphism (P), number of alleles per locus (A), number of polymorphic alleles per locus (Ap), and the observed heterozygosity (Ho) (Table 1). The genetic distance was utilized to construct the dendrogram (Figure 1) by GDA statistical software.

Results and discussion

The 2003 yield production ranged from 4.19 to 6.82 t ha^{-1} (Table 1); this is somewhat higher compared with normal field production, due to the small dimensions of the plots (2 m^2). In any case, a great diversity (CV = 10.8) between the 35 investigated emmer accessions was underlined. Moreover, there was no relationship between yield and accession origin. There were even no correlations between yield and plant height and yield and heading time, which was also found in earlier investigations carried out with other emmer accessions (Pagnotta et al. 2003b). The shortest accession was 'Mosè' which originated from a cross between emmer and durum (Codianni et al. 2000). It is important to report that the lodging score, which is correlated with nitrogen fertilization (data not shown), was not correlated with plant height, indicating different plant elasticity, a valuable character that could be utilized in breeding programs. There was a group of accessions from central Italy characterized by short plant and low hectolitre weight.

Fourteen out of 39 accessions were heterozygous (Table 1) and two out of six EST loci examined have revealed heterozygosity in at least one accession, indicating a great heterogeneity of the material analysed which had also a relevant (~36 %) variation within accessions (Pagnotta et al. 2003a). The observed heterozygosity is quite surprising considering that emmer is an autogamous species, hence a fair proportion of occasionally outcrossing can be suspected. The high proportion of variation within accessions could be due to both outcrossing and low selection pressure operated by farmers. It is interesting to note that these differences among accessions could not be assigned to a locality effect since accessions from the same geographic area had different heterozygosity and 'alleles polymorphic per locus'. This was the case, for example, for the accessions from Leonessa, a mountain area about 100 km north of Rome (Table 1). The heterozygosity was comparable with the data reported by Figliuolo and Perrino (2004) for Italian accessions, but only considering the accessions having heterozygosity; however, these authors had reported only the expected heterozygosity, even if they had utilized SSR markers, while here the observed heterozygosity is reported.

The genetic distance between accessions (Figure 1) clustered together the three derivatives from emmer x durum crosses, i.e. 'Mosè', 'Padre Pio' and 'Davide'). The second cluster includes the 9 accessions from central Italy, characterized by small plants. In the third cluster the accessions were not discriminated according to their region of origin. For example, *Potenza* (southern Italy) was close to *Garfagnana* (Tuscany). In present study *Ersa 4* was not more polymorphic, which is in contrast to the results obtained for the *Ersa* accessions by Barcaccia et al. (2002). Concluding, the investigated emmer accessions showed the existence of variation which could be utilized in breeding programs of both emmer and durum wheat.

Table 1. Agro-morphological characters and genetic data measured by EST markers

Accession	YLD[1]	YI	KW	HT	PH	LS	P	A	Ap[2]	Ho
Agnone Invernale	4.88	93	49.5	20	115	6	0.17	1.0	1.0	0.0
Agnone Primaverile	4.73	90	54.3	17	103	6	0.17	1.17	2.0	0.17
Amatrice	4.48	85	40.8	22	103	6	0.50	1.17	1.33	0.17
Bettini	5.24	100	54.9	20	102	9	0.33	1.17	1.50	0.17
Cagnano	4.06	77	40.1	21	101	6	0.50	1.17	1.33	0.17
Conero							0.17	1.0	1.0	0.0
Davide	5.15	98	66.9	7	93	6	0.67	1.0	1.0	0.0
Ersa 1	4.51	86	42.9	22	95	9	0.33	1.0	1.0	0.0
Ersa 2	4.45	85	40.6	22	101	9	0.33	1.17	1.50	0.17
Ersa 3	5.91	112	47.8	23	102	9	0.17	1.0	1.0	0.0
Ersa 4	4.65	88	46.8	22	111	9	0.17	1.17	2.0	0.17
Ersa 5	5.41	103	46.8	23	106	9	0.50	1.17	1.33	0.17
Ersa 6	5.28	100	48.8	24	104	9	0.17	1.0	1.0	0.0
Ersa 8	6.82	130	46.5	21	107	9	0.17	1.0	1.0	0.0
Farvento	5.63	107	40.3	25	113	4	0.17	1.0	1.0	0.0
Fiorani Chiaro	6.14	117	51.3	19	111	6	0.17	1.0	1.0	0.0
Fiorani Rosso	4.54	86	39.9	21	96	6	0.50	1.0	1.0	0.0
Filosini	4.76	90	46.4	20	97	9	0.17	1.0	1.0	0.0
Garf. Bra	5.59	106	54.7	21	109	9	0.0	1.0	n.c.	0.0
Garf Rene	6.45	123	48.7	20	108	9	0.17	1.0	1.0	0.0
Leonessa							0.17	1.17	2.0	0.17
Leonessa 1	4.38	83	39.6	21	95	9	0.50	1.17	1.33	0.17
Leonessa 2	4.19	80	44.2	21	107	6	0.50	1.0	1.0	0.0
Livesa	6.03	115	55.8	24	97	4	0.33	1.0	1.0	0.0
Lucania	5.21	99	50.1	22	113	6	0.50	1.0	1.0	0.0
Luzi	5.90	112	53.7	19	108	9	0.17	1.0	1.0	0.0
Monte Leone	5.00	95	45.6	20	98	6	0.50	1.17	1.33	0.17
Monte Leone Viterbo	5.62	107	49.5	22	103	6	0.17	1.0	1.0	0.0
Monte Leone Spoleto	5.14	98	49.4	22	100	6	0.17	1.0	1.0	0.0
Matteis	5.77	110	52.3	20	109	6	0.0	1.0	n.c.	0.0
Molise Colli	5.89	112	49.1	20	107	6	0.17	1.0	1.0	0.0
Molisano	5.93	113	50.8	20	103	9	0.0	1.0	n.c.	0.0
Mosè	4.43	84	53.1	10	69	1	0.83	1.17	1.20	0.17
Padre Pio	4.59	87	54.9	10	96	8	0.83	1.17	1.20	0.17
Potenza	6.41	122	50.9	21	103	6	0.17	1.0	1.0	0.0
Potenza 2	5.63	107	52.5	22	114	6	0.33	1.17	1.5	0.17
Promet							0.0	1.0	n.c.	0.0
Trevi							0.33	1.33	2.0	0.33
Trivento	6.55	124	53.8	21	111	9	0.17	1.0	1.0	0.0
Mean	5.33	100	49.2	20.2	103	7.1	0.30	1.06	1.21	0.06
LSD5%	0.93		3.6	2	7	4				

[1] YLD, yield (t ha^{-1}); YI, yield index = yield*100/mean yield; KW, kernel weight (g); HT, heading time (days after April 30); PH, plant height (cm); LS, lodging score (0-9; 9 = 100% lodging); P, polymorphism (0.95); A, alleles per locus; Ap, polymorphic alleles per locus; Ho, observed heterozygosity

[2] n.c., not calculated (since the polymorphism is equal to zero)

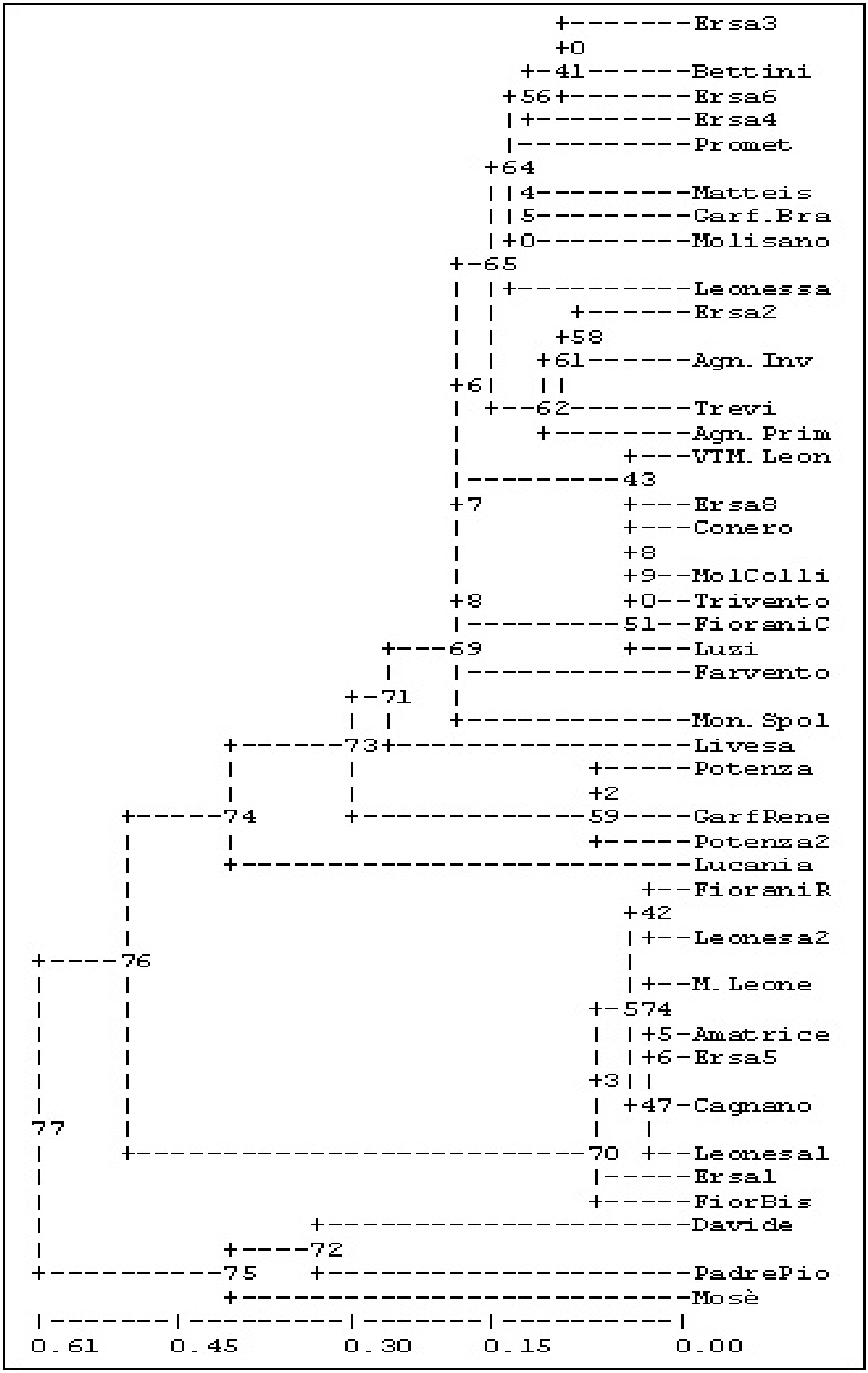

Figure 1. Dendrogram obtained with UPGMA function utilizing Nei genetic distance

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Genetic variation in agronomic and qualitative traits of ancient wheats

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ABSTRACT: The consumer's interest in natural, unconventional and nutritional foods led to the development of new specialty foods based on grain blends. Components of such foods are often so-called 'ancient wheats' or 'primitive wheats' which were never the subject of modern plant breeding programmes. Einkorn, emmer and Khorasan (syn. Oriental) wheat are such neglected and underutilized wheat species, which probably survived over the centuries in subsistence farming systems in Europe, the Near East and Central Asia. In the presented study agronomic and qualitative traits of genebank accessions of einkorn, emmer and Khorasan wheat were evaluated under eastern Austrian conditions. Some einkorn and winter emmer accessions were found to be adapted to the prevalent climatic conditions, resulting in yields above 4000 and 5000 kg of hulled grains ha^{-1}, respectively. Some Khorasan wheats, on the other side, reached acceptable yields above 3000 kg grain ha^{-1} only if sown in autumn. However, winter hardiness of these types is only moderate which can result in total yield losses in case of harsh winters. Considering technological quality (Farinograph, Extensograph, Alveograph) all accessions turned out to be significantly inferior compared to common wheat. However, einkorn was confirmed as source for high yellow pigment content, and all three wheats contained significantly higher protein contents. Hence, ancient wheats can represent sources for the production of specialty and/or health-promoting foods. However, they also represent a great challenge for the farmer and food technologist to find out the best suitable genetic material, and the best production and processing techniques.

Key words: Primitive wheats – *Triticum dicoccum* – *Triticum monococcum* – *Triticum turanicum*

Introduction

Currently, there is considerable interest in the consumption of ancient wheats, especially in organic, specialty, and health food markets. Einkorn (*Triticum monococcum*) and emmer (*T. dicoccum*) are the oldest cultivated wheats, originating ≈11,000 years ago. Together with barley these two hulled wheats formed the cereal founder crops of the Neolithic Revolution. Khorasan or Oriental wheat (*T. turanicum*) is a more modern free-threshing tetraploid wheat, first described in 1921 and probably endemic to the region south of the Caucasus and Caspian Sea that spans Anatolia in the west and Uzbekistan in the east. Khorasan wheat attracted unexpected attention by wheat scientists with the appearance of Kamut® products in health food outlets in the beginning of the 1990's. A common feature of all three wheats is that they are neglected and underutilized, and probably survived over the centuries in subsistence farming systems in Europe, the Near East and Central Asia. They were never the subject of modern plant breeding programmes.

In Austria the interest in primitive wheats was renewed with the revival of spelt wheat (*T. spelta*) cultivation in the 1980's. At that time, a few organic farmers began experimenting with various accessions of einkorn and emmer. While some farmers stopped their cultivation because of difficulties with sowing, unknown growth type, no possibilities and/or problems with dehulling, or at least low yields, a small group of enthusiastic organic farmers continued with the cultivation up to now. In the meantime diverse products are available, ranging from polished and pearled grain, and flour, to pasta-like products and bread and pastry.

Since the genetic diversity in the presently cultivated primitive wheats is low, we investigated genebank accessions of einkorn, emmer and Khorasan wheat under the low rainfall conditions of eastern Austria for selected agronomic and qualitative properties.

Materials and methods

Plant material

The accessions were provided by the genebanks of Arche Noah Schiltern, BAB Linz (both Austria), IPK Gatersleben (Germany) and NSGC Aberdeen (USA). In total, 25 einkorn, 33 emmer and 23 Khorasan wheat accessions were studied. The crops were grown from 2000 - 2003 in organic and conventional field trials at the experimental farm Groß-Enzersdorf, north-east of Vienna. Screening of the total einkorn nursery was carried out only in 2003. Einkorn was sown in autumn, while the emmer and Khorasan trials were sown both in autumn and spring.

Agronomic and qualitative traits

The field trials were evaluated for yield and tolerances/resistances to frost, lodging and fungal diseases. 10 - 20 g of the hulled einkorn and emmer grain were manually dehulled in order to determine the percentage of kernels. The rest was mechanically dehulled. Furthermore, 1000-kernel weight, protein content (Dumas combustion method) and wet gluten content (ICC Standard Method 137/1) were determined. From 2002 onwards, rheological properties (Farinograph, Extensograph, Alveograph) of some selected accessions were carried out according to ICC Standard Methods (Nos. 115/1, 114/1, 121). Yellow pigment content of all einkorn and Khorasan was done according to ICC Standard Method 152. In the following, the results are presented on a dry weight basis, and as minimum, maximum and mean values of the total variation over all environments analysed.

Results and discussion

Results for einkorn, emmer and Khorasan are presented in Tables 1, 2 and 3. Einkorn yields were in average projected 3000 kg hulled grain (gross yield) ha^{-1}, which would yield 2100 kg kernels (net yield) ha^{-1}, if a kernel percentage of 70 % is assumed. Unfortunately, mechanical dehulling devices have a loss of up to 20 % due to broken kernels. Hence, only about 50 - 60 % of the harvested material is available for further processing in practice after dehulling. Yield and yield-related data of our study are similar to those reported by Castagna et al. (1995) for German- and Italian-grown einkorn lines. However, minimum values for agronomic traits are lower in our study, probably due to a wild einkorn (*T. boeoticum*) accession included in the trials. Yellow pigment contents were similar (Borghi et al. 1996) or even higher (Abdel-Aal et al. 2002) to data reported earlier. However, preliminary carried out mixing and dough stickiness tests revealed no accession with promising technological quality, whereas Borghi et al. (1996) observed several einkorn lines with acceptable technological quality. Nevertheless, processing for baked products is possible, as it was recently demonstrated by the introduction of an einkorn pastry product by a major Viennese bakery.

Table 1. Variation in agronomic and qualitative properties of winter einkorn

Trait	Minimum	Maximum	Mean
Yield (g m^{-2})	42.2	430.6	300.3
1000-kernel weight (g)	12.7	28.0	21.3
Kernel fraction (%)	59.3	78.2	72.6
Protein content (%)	15.6	22.8	18.1
Yellow pigment (ppm) [1]	12.0	22.8	15.3

[1] expressed as β-carotene equivalent

For emmer variation in agronomic traits was considerable. Besides winter and spring types we observed also accessions of alternative growth type, which were not adapted to the prevalent climatic conditions. These types suffered either from frost or drought if sown in autumn and spring, respectively, and never reached acceptably high yields. Although protein and wet gluten contents were generally high in emmer, gluten quality was low. Although technological quality of emmer is well known to be inferior if determined by standard methods used for common wheat, a broad range of products is already available, especially in Italy (D'Antuono 1995) and Switzerland (Jenny 2000).

Table 2. Variation in agronomic and qualitative properties of winter and spring emmer

Trait	Minimum	Maximum	Mean
Winter Emmer			
Yield (g m^{-2})	149.7	653.0	383.2
1000-kernel weight (g)	25.0	57.6	37.7
Kernel fraction (%)	62.0	81.0	70.8
Protein content (%)	12.2	24.8	16.7
Wet gluten content (%)	30.5	62.5	43.2
Zeleny sedimentation value (ml)	11.5	17.0	14.7
Farinograph water absorption (%)	58.6	68.2	63.3
Farinograph dough development time (min)	2.0	2.5	2.2
Farinograph dough stability (min)	1.5	4.0	2.1
Farinograph dough softening (FU) [1]	200	100	156
Extensograph dough energy (cm^2)	8.5	73	24
Extensograph dough extensibility (mm)	75	185	125
Extensograph dough resistance (EU) [2]	80	245	115
Alveograph dough strength W (10^{-4} J)	59	209	109
Alveograph dough tenacity P (mm)	43	93	71
Alveograph dough extensibility L (mm)	33	94	56
Alveograph dough swelling G (cm^3)	13	22	16
Spring Emmer			
Yield (g m^{-2})	119.4	485.2	273.1
1000-kernel weight (g)	19.6	51.8	28.8
Kernel fraction (%)	66.0	81.0	75.2
Protein content (%)	15.7	22.8	19.2
Wet gluten content (%)	24.9	73.3	45.8
Zeleny sedimentation value (ml)	13	23	18
Farinograph water absorption (%)	60.0	64.4	62.8
Farinograph dough development time (min)	2.0	2.5	2.1
Farinograph dough stability (min)	1.0	2.5	1.7
Farinograph dough softening (FU)	180	90	136
Extensograph dough energy (cm^2)	10	24	18
Extensograph dough extensibility (mm)	92	177	124
Extensograph dough resistance (EU)	80	120	97
Alveograph dough strength W (10^{-4} J)	54	96	71
Alveograph dough tenacity P (mm)	46	61	53
Alveograph dough extensibility L (mm)	33	61	52
Alveograph dough swelling G (cm^3)	13	19	16

[1] Farinograph dough softening was measured 12 min after peak of the curve; FU, Farinograph units
[2] Extensograph dough resistance was measured at an extensibility distance of 5 cm; EU, Extensograph units

Table 3. Variation in agronomic and qualitative properties of Khorasan wheat

Trait	Minimum	Maximum	Mean
Yield (g m^{-2}) [1]	48.9	497.6	239.8
1000-kernel weight (g)	28.9	73.8	53.8
Hectolitre weight (kg hL^{-1})	48.1	79.4	72.4
Protein content (%)	9.8	19.7	18.0
Wet gluten content (%)	32	56	49
Zeleny sedimentation value (ml)	13	33	20
Yellow pigment (ppm)	3.5	6.3	4.0
Farinograph water absorption (%)	59.2	75.0	68.9
Farinograph dough development time (min)	2.5	3.5	2.9
Farinograph dough stability (min)	1.0	4.5	2.8
Farinograph dough softening (FU)	180	90	122
Extensograph dough energy (cm^2)	8	47	24
Extensograph dough extensibility (mm)	76	202	116
Extensograph dough resistance (EU)	60	210	136
Alveograph dough strength W (10^{-4} J)	177	458	273
Alveograph dough tenacity P (mm)	109	151	139
Alveograph dough extensibility L (mm)	29	67	48
Alveograph dough swelling G (cm^3)	12	18	15

[1] Minimum agronomic traits (yield, 1000 kernel weight, hectolitre weight) were observed for spring sown Khorasan, maximum values for autumn sown Khorasan

Khorasan wheat turned out to have only limited adaptation. Acceptable yields and high kernel quality (size, shape, vitreousness) was observed only in case of autumn sowing for a few genotypes. However, winter hardiness was only moderate which resulted in a total yield loss in 2003. Moreover, Khorasan was highly susceptible to fungal diseases. Considering mixing properties Khorasan showed no problems with dough stickiness and resulted in baked products with excellent yellow colour and flavour.

It can be concluded that primitive wheats represent a valuable genetic resource for the production of specialty and/or health-promoting foods. However, they also represent a great challenge for agronomists and food technologists to find out the best genetic material, and the best production and processing techniques. In addition, all einkorn and a few emmer accessions represent valuable resistance sources against powdery mildew (*Erysiphe graminis*), and yellow (*Puccinia striiformis*) and leaf rust (*P. recondita*).

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Variability in chemical composition and biologically active constituents of cereals

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ABSTRACT: Whole grain flours of diverse wheat species (*Triticum* sp.) and botanical varieties of barley (*Hordeum vulgare*), oat (*Avena sativa*) and rye (*Secale cereale*) were analysed for their chemical composition, with particular regard to dietary fibre and polyphenols. A considerable variation was observed between the cereal crops. Ancient wheats einkorn and emmer exhibited highest protein contents and lowest levels of dietary fibre. Rye showed highest contents of dietary fibre and intermediate ranges of total phenol content and reducing power. Barley revealed high contents of β-glucan, total phenol and reducing power. Oat contained the highest amount of total fat and represents also a valuable source of β-glucan. High contents of carotenoids were observed for einkorn, while blue and purple grain common wheats were significantly higher in their content of anthocyans. The results indicate that cereals represent a valuable source of biologically active constituents, which can profoundly affect the health-enhancing potential of a functional food. However, it is a challenge for food technologists that the physiological effects of these compounds are not altered significantly during certain processing techniques and that they bring them into palatable products, considering eventual negative effects on flavour and processing quality of single constituents.

Key words: Dietary fibre – functional food – β-glucan – health-benefit – secondary metabolites

Introduction

Apart from energy intake nutrition is taking on new meanings in the 21st century. Emphasis is now given on foods which can promote well-being and health, and help to reduce the risk of diseases. Responsible for these effects are certain food components that act on the body beyond or in addition to vitamins and minerals. Although these newly recognized food components have not been classified as essential compared to traditional nutrients, they are acting on the body to promote and improve health. The concept of 'functional foods' has become popular in recent years, first in Japan, and later in other developed countries. For foods with a specific health claim the physiological benefit must be well documented scientifically and approved by the respective administration.

Cereal based foods represent the bulk of all foods consumed and their contribution to human nutrition and health should be considered cumulative, immediate and significant. Cereals can and do contribute a significant amount of these new food ingredients in the diet. Biologically active constituents of cereals that promote beneficial physiological effects are dietary fibre, starch and polyphenols. Dietary fibre is defined as that portion of plant foods that can not be digested and absorped in the human small intestine, but are completely or partially fermented in the large intestine. Dietary fibres include poly- and oligosaccharides, lignin, and associated plant substances. The actions of cereal dietary fibre on the body are varied and highly beneficial, e.g. reduction of plasma cholesterol and postprandial glycaemic response, which decrease the risk of cancer, heart disease, hypertension and obesity in the long term. The relationship between consumption of foods rich in soluble fibre, especially β-glucan, and reduced risk of heart disease resulted in the first health claim for a specific food in

North America in 1997. Resistant starch resists enzymatic activity *in vivo* and *in vitro*, and therefore shows similar physiological effects as dietary fibre. Polyphenols constitute a large group of secondary plant metabolites (flavonoids, phenolic acids, lignins, etc.) found in the outer layers of cereal seeds. Phenolic compounds are effective antioxidants with the potential therapeutic value to reduce the risk for cardiovascular disease and cancer.

In the presented study the variability in the chemical composition of diverse cereals was investigated. Besides ash, starch, crude protein, total fat and crude fibre, special account was taken to dietary fibre and phenolic compounds.

Materials and methods

Plant material

The following cereals were grown in 2003 at the experimental farm Groß-Enzersdorf, north-east of Vienna: einkorn wheat (*Triticum monococcum*), emmer wheat (*T. dicoccum*), Oriental wheat (*T. turanicum*), durum wheat (*T. durum*), spelt wheat (*T. spelta*), common wheat (*T. aestivum*), barley (*Hordeum vulgare*), oat (*Avena sativa*), and rye (*Secale cereale*). Within some species diverse biotypes were analysed, i.e. red, blue, and purple grain coloured common wheats, hull-less, hulled and hulled black barley, hull-less and hulled oats, and common and semi-perennial rye (var. *multicaule*). Generally, grains with a kernel size between 2.2 and 2.5 mm were analysed.

Chemical analysis

Whole grain flour was milled using a ZM 100 ultra-centrifugal mill (Retsch Gmbh & Co KG, Haan, Germany) equipped with a 0.5 mm sieve. Afterwards the samples were analysed for moisture content (ICC Standard Method 110/1), ash content (ICC 104/1), crude protein content (ICC 105/1), total fat content (ICC 136), starch content (ICC 123/1), crude fibre content (Fibertec™ 2021/2023 Fibercap system; Foss Tecator AB, Höganäs, Sweden), total dietary fibre content (112979 Bioquant® Total Dietary Fiber Reagent Kit; Merck, Darmstadt, Germany), total β-glucan content (ICC 166; Beta-Glucan (Mixed-linkage) Assay Kit; Megazyme, Bray, Ireland), total yellow pigment content (ICC 152), total anthocyan content (modified after Abdel-Aal & Hucl 1999), total phenol content by means of the Folin-Ciocalteu reagent (Singleton et al. 1974), and reducing power according to the method of Oyaizu (1986) based on the chemical reaction of Fe(III) → Fe(II). The reported values are means of triplicate and/or quadruplicate measurements, and on a dry weight basis. Hulled wheats (einkorn, emmer, spelt) were dehulled before analyses, whereas hulled barleys and oats were not dehulled and, therefore, whole grain flours contained the husk part of the grain.

Results and discussion

The results of the chemical analyses are presented in Tables 1 and 2. Significantly higher contents of crude fibre and ash, and lower contents of starch were determined for hulled barleys and oats. This is not astonishing, since these cereals were analysed with the husk of the grain (oats: 25 - 30 %; barley: 6 - 15 %), which is known to contain more than two-thirds of the grain's cellulose. Very high protein contents (≥19 %) were determined for the 'ancient wheats' einkorn, emmer and Oriental, as well as for hulled black barley, whereas the lowest level (11 %) was observed for common rye. As for total fat content, oats contain about twice the amount than other cereals. All values reported in Table 1 are within the ranges known from literature, e.g. somewhat higher ash contents and significantly higher protein contents for ancient wheats compared to modern durum and/or common wheats were reported by D'Egidio et al. (1993) and Løje et al. (2003). In Table 2 mean values of some biologically active constituents are presented. Except for hulled barleys and oats, both forms of rye turned out to have highest contents of total dietary fibre (≈17 %), while grains of the hulled wheat

species einkorn, emmer and spelt showed the significantly lowest values (≤10 %). Similar results were obtained by Løje et al. (2003). In regard to β-glucan the lowest levels were observed for wheats (0.3 - 0.9 %), while rye contained about 2 %, and the highest levels were obtained for barley and oats (3 - 4.5 %). These results are very similar to those reported by Wagner and Kuhn (1996) for German-grown cereals. Variation in polyphenolic compounds was considerable. Einkorn, durum and purple grain common wheat contained highest values of yellow pigment (carotenoids). Abdel-Aal et al. (2002) report somewhat lower values of lutein, the major yellow pigment in wheat, for Canadian einkorn and durum genotypes, while D'Egidio and Vallega (1994) and Grausgruber et al. (2004) found contents of yellow pigments between 12 and 23 ppm. The high value for hulled oats must be neglected, since this value is most likely influenced by the co-analysed husk. Astonishing, however, is the high content for hulled black barley, which is similar to einkorn, durum and purple wheat, and nearly the double amount of hulled 'yellow' barley. Highest contents of anthocyans were observed for blue and purple grain common wheat. Somewhat higher contents than for the rest were also observed for black barley and rye. Total phenol content and reducing power were responsible for the significantly higher antioxidant activity of barley, especially of black barley. In addition, purple grain wheat and semi-perennial rye had somewhat higher contents than the other cereals.

Table 1. Composition of whole grain flours (%, db)

Sample	ASH[1]	TS	CP	TF	CF
Wheat					
Einkorn	2.21	50.46	20.03	2.44	1.32
Emmer	2.10	59.97	19.05	2.01	1.71
Oriental	2.38	57.96	19.68	1.40	2.22
Durum	1.93	61.12	16.79	2.26	2.97
Spelt	2.04	52.38	19.07	2.19	2.56
Red grain	1.90	59.73	15.74	1.83	3.05
Blue grain	1.61	61.97	14.95	2.07	3.13
Purple grain	1.77	60.66	14.14	1.52	3.38
Barley					
Hull-less	2.06	58.55	17.76	2.26	1.88
Hulled	2.44	54.66	15.03	2.20	4.02
Hulled black	2.54	49.55	18.83	2.07	5.20
Oats					
Hulled	2.75	33.61	13.19	3.88	12.77
Hull-less	2.15	56.61	17.59	4.82	2.01
Rye					
Common	1.81	59.71	10.84	1.52	2.17
Semi-perennial	1.68	55.51	15.76	1.62	2.41

[1] ASH, ash content; TS, total starch content; CP, crude protein content; TF, total fat content; CF, crude fibre content

Summarizing, there exists a considerable variabilitiy of biologically active constituents in cereals, and especially barley has great potential for healthy human food products. However, besides the potential therapeutic value, some compounds can negatively affect the flavour of products, e.g. phenolic acids, or the processing of the raw material, e.g. poor mixing characteristics of einkorn, barley and oat flour. Hence, it is a great challenge for food technology not to alter the active ingredients during certain processing techniques and to bring them into a palatable form.

Table 2. Contents of biologically active constituents of whole grain flours

Sample	DF[1]	BG	YP	CYAN	PHEN	RP
Wheat						
Einkorn	9.68	0.32	1.15	0.75	102.27	15.69
Emmer	8.89	0.31	0.66	0.78	105.85	20.45
Oriental	12.72	0.53	0.47	0.50	95.49	11.91
Durum	13.25	0.53	1.10	0.68	109.64	15.63
Spelt	10.16	0.74	0.51	1.23	110.77	13.46
Red grain	14.28	0.90	0.39	0.91	108.04	15.77
Blue grain	14.53	0.88	0.48	6.01	118.50	19.26
Purple grain	15.66	0.67	1.05	7.45	143.96	25.16
Barley						
Hull-less	12.32	3.51	0.57	0.49	171.35	44.47
Hulled	20.54	3.46	0.64	1.45	114.50	44.00
Hulled black	21.24	4.62	1.13	2.13	197.15	49.15
Oats						
Hulled	41.64	2.76	1.30	1.17	101.51	27.87
Hull-less	14.68	4.51	0.43	0.43	128.41	17.35
Rye						
Common	17.33	2.21	0.64	2.22	118.51	20.63
Semi-perennial	16.96	1.87	0.62	1.76	132.41	24.54

[1] DF, total dietary fibre (%); BG, total beta-glucan (%); YP, total yellow pigment (mg β-carotene equivalent/100 g, db); CYAN, total anthocyan (mg cyan-3-O-glucoside equivalent/100 g, db); PHEN, total phenol (mg ferulic acid equivalent/100 g, db); RP, reducing power (mg ascorbic acid equivalent/100 g, db).

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Responses of wheat genotypes to high temperature

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ABSTRACT: High temperature influences the quality of wheat and reduces the grain yield. Although the protein and gluten contents of the flour increase, the changes in baking characteristics may be disadvantageous. The present work presents the results of a phytotron study with two temperature regimes (one normal and one with 35°C in the grain filling period) and compares the data of different years in a long-term field study on the climatic conditions affecting yield quality characteristics. It was found that heat stress usually decreased the weight and increased the hardness of the grains both in the phytotron and in the field. High temperature usually increased the protein and gluten contents of the flour, but there was a difference in the direction of changes in SDS values, which were higher in heat-stressed plants than in the control and lower in years with more heat days than in that with fewer heat days.

Key words: Grain quality – heat stress – technological properties – *Triticum aestivum*

Introduction

A rise in temperature has been found to be unfavourable for plants (Delgado et al. 1994). Heat stress in wheat during anthesis and grain filling causes reductions in kernel size, kernels per spikelet, grain yield and harvest index (Blumenthal et al. 1995, Batts et al. 1998, Kafi & Stewart 1998, Stone & Nicolas 1998). High temperature may have negative effects on flour quality. Blumenthal et al. (1995) reported that despite the higher protein content, there was a decrease in the glutenin-gliadin ratio and in the percentage of very large glutenin polymers following heat stress. Szilágyi et al. (2002) found a contrasting result: high temperature increased the quantity of glutenin while the quantity of gliadins remained relatively constant. The present work deals with the effects of a high temperature regime during grain filling on the quality of the flour and grain in plants of three winter wheat varieties raised in a pot experiment. A comparison was also drawn between phytotron and field findings.

Materials and methods

Varieties

Three winter wheat (*Triticum aestivum* L.) varieties commonly cultivated in Hungary were chosen for the tests: 'Mv Martina', an early ripening variety with soft endosperm structure, medium breadmaking quality and record productivity, 'Mv Mezőföld', a mid-early, hard red variety with consistently good agronomic characteristics and good adaptability, and 'Mv Emma', a variety of the same type and maturity group as 'Mv Mezőföld', with excellent breadmaking quality, used to improve flour quality.

Growing conditions in the phytotron study

A pot experiment was carried out under controlled environmental conditions in Conviron PGV/36 growth chambers in the phytotron of the Agricultural Research Institute of the Hungarian Academy of Sciences, Martonvásár, Hungary. Seedlings grown individually in peat blocks were vernalized at +4°C in the one-leaf stage for 42 days. Sixty plants in each treatment and variety were then planted four to a pot (21 x 21 x 17 cm) in a 4:1 mixture of garden soil and sand. The pots were placed randomly in the growth chambers and rearranged regularly throughout the experiment to avoid possible heterogeneity of the growing conditions

and to achieve a closed canopy. The plants were watered daily and supplied with nutrients weekly in the first 8 weeks, and three times a week afterwards until ripening. On each occasion 3 g Volldünger solution (Linz, Austria) was added in water per pot (NPK 14-7-21 % and 2 % microelements). The temperature regime changed weekly beginning with a min / max / mean of 10 / 12 / 10.7°C during the first week and increasing until it reached 20 / 24 / 22.7°C (in the 11th week). Heat stress began 12 days after the average heading date (Zadoks 59), which was determined separately in each variety so that the groups should be in the same phenological stage during heat treatment. In the heat stress treatment the temperature was 20 / 35 / 25.2°C, the 35°C maximum temperature being maintained for 8 hours a day for 15 days. The maximum photosynthetic photon flux density increased from 280 to 400 □mol *m^{-2}*s^{-1} by the 9th week of the experiment.

Field trials

The small plot (6 m^2) field experiments were sown at the optimum date with 600 germs m^{-2}, following the normal wheat production practice in Hungary (single application of herbicide and insecticide, 60 kg ha^{-1} each of N, P and K in autumn, 60 kg ha^{-1} N in spring). The data of three years differing considerably in the number of days above 30°C were chosen for the tests: 1999 (15 heat days), 2002 (43) and 2003 (65), representing one cooler year (1999) and two hot years (2002 and 2003).

Measurements

The following instruments and methods were used to study the functional properties in each treatment and variety: Perten SKCS 4100 was used to determine the hardness and weight of grains on 300 seeds; Perten Inframatic 8611 for estimating the protein content, SDS sedimentation volumes and RMT values (ICC 202, 159); Kjeltec 1035 Analyzer to measure the protein content of the wholemeal (per dry weight), applying a factor of 5.7 (ICC105/2); and Glutomatic 2200 to characterize the gluten quality parameters of the flour (ICC 137/1, ICC 155). Statistical analyses were carried out on the collected data using two-way ANOVA to study the effects of the treatments on the three varieties.

Results and discussion

Phytotron study

15 days of heat stress during the grain filling period caused a decrease in the TKW in all the varieties compared to the control (Table 1), while the grains were harder in two of the three varieties due to the high temperature. The protein and gluten contents of the flour, which were very high in all the varieties, became even higher in response to high temperature during grain filling. The values of SDS and RMT increased at high temperature, too, while there was a weakening of gluten quality with increasing temperature in two varieties (the gluten index decreased). In ‘Mv Martina’, however, a variety with soft endosperm structure and medium breadmaking quality in field practice, no deterioration in flour quality could be proved under heat stress conditions. The direction of changes in the technological properties was similar in all the varieties, except for the gluten index in ‘Mv Martina’ mentioned above.

Table 1. Effect of high temperature on the quality of the grain and flour of three winter wheat varieties

		Phytotron study			Field trials			
		CON[1]	HEAT		1999	2002	2003	
Days >30°C (n)		0	15		15	43	65	
				$LSD_{5\%}$				$LSD_{5\%}$
TKW[2] (g)	Mv Martina	36.1	29.9 ↓	0.67	n.a.	33.7	33.4	2.06
	Mv Emma	35.5	29.8 ↓		n.a.	33.4	32.1	
	Mv Mezőföld	37.0	31.7 ↓		n.a.	35.4	32.4↓	
HARD	Mv Martina	24.9	28.8 ↑	3.09	23.7	25.1	24.8	4.05
	Mv Emma	72.6	73.3		53.4	62.4	59.5↑	
	Mv Mezőföld	64.8	69.4 ↑		52.4	59.9	59.1↑	
PROT (% db)	Mv Martina	18.09	20.43 ↑	0.15	13.18	n.a.	17.49↑	1.72
	Mv Emma	19.72	21.82 ↑		13.45	n.a.	16.09↑	
	Mv Mezőföld	20.23	21.65 ↑		12.06	n.a.	14.14↑	
NIRPROT (%)	Mv Martina	13.8	15.1 ↑	1.0	11.6	13.2	15.4↑	0.9
	Mv Emma	16.6	18.0 ↑		12.2	14.0	15.2↑	
	Mv Mezőföld	16.2	17.1 ↑		10.8	13.7	15.5↑	
SDS	Mv Martina	66.3	73.7 ↑	5.2	65.0	54.5	55.0↓	6.9
	Mv Emma	101.3	111.7 ↑		83.0	62.5	69.4↓	
	Mv Mezőföld	95.7	99.3		70.0	59.0	67.3↓	
RMT	Mv Martina	371.9	414.5 ↑	27.2	283.0	315.1	409.2↑	69.5
	Mv Emma	475.5	527.0 ↑		371.7	365.0	361.6	
	Mv Mezőföld	462.5	495.4 ↑		304.8	346.4	355.2	
GLUT (%)	Mv Martina	35.9	38.8 ↑	1.4	28.4	32.1	34.7↑	4.7
	Mv Emma	41.0	45.9 ↑		33.1	33.8	31.9	
	Mv Mezőföld	47.8	51.9 ↑		28.1	35.4	35.6↑	
GI	Mv Martina	95.7	94.9	6.0	77.4	76.4	61.4↓	16.0
	Mv Emma	99.0	92.8 ↓		91.4	97.5	98.0	
	Mv Mezőföld	73.1	59.4 ↓		67.4	54.4	67.7	

n.a.=not available; ↑↓ arrows show significant changes ($P<0.05$) between control and treated plants or the data of the cooler and hotter years

[1] CON, control; HEAT, heat stress

[2] TKW, 1000-kernel weight; HARD, hardness index PROT, protein content (Kjeltec); NIRPROT, NIR protein content; GLUT, wet gluten content; GI, gluten index

Field study

Field data showed larger variances than phytotron data, suggesting a more complex system of environmental factors affecting plant responses in the field than under controlled environmental conditions (Table 1).

Though data on the thousand-kernel weight were not available in 1999, this parameter was lower in one variety in the hottest year (2003) than in the slightly cooler one (2002). The hardness index of the seeds showed higher values in the hot years (significantly in two varieties). The flour of all the varieties contained more protein as the number of hot days increased. However, the gluten contents were only higher in two varieties, while in 'Mv Emma', a variety with excellent breadmaking quality, this parameter remained at a similar value irrespective of the number of hot days in the year. This was accompanied by a slight increase in the gluten index in this variety, while this parameter decreased in 'Mv Martina' and did not show a definite tendency in 'Mv Mezőföld'. The SDS sedimentation values were lower in the hot years (2002, 2003) than in the cooler year (1999), while the RMT increased with an increase in the number of hot days in two varieties and remained unchanged in 'Mv Emma'.

The difference observed between the phytotron and field data might be due to the fact that more environmental factors act simultaneously in the field than under controlled conditions. There was also a huge difference between the protein contents of the wholemeal

of plants grown in pots (with a relatively constant nutrient supply; high values) and in the field (with a more limited nutrient supply; lower values). This raises the possibility that the change in the flour protein composition due to heat stress depends on the grain protein level. Further studies will be needed to clarify this question.

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Variability and gene effects for boron concentration in wheat leaves

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ABSTRACT: The variability and gene effects for boron (B) concentration in wheat leaves at heading were evaluated on the basis of generation mean analysis. The epistatic gene effect was tested using the six-parameter model. Six basic generations (P_1, P_2, F_1, F_2, BC_1, BC_2) of three crosses were studied. The lowest B concentration was found in 'Rodna' (6.2 ppm), which was the most B-efficient genotype. The highest mean value for B concentration was found in the Russian genotype 'Bezostaya 1' (16.6 ppm). The mode of inheritance in the F_1 and F_2 generations was different (intermediate, dominant, superdominant) depending on the cross combination. B concentration in wheat leaves was under the control of the genes with additive, dominant and epistatic effects (a x a, a x d, d x d). Breeding B-efficient wheat genotypes could be interesting for the region of Vojvodina (the northernmost part of Serbia and Montenegro), where there are saline soils, as it is known that high B concentrations may cause problems, especially in interaction with drought stress problems.

Key words: Boron – gene effect – inheritance –*Triticum aestivum* – variability

Introduction

Boron (B) is a nutrient element required by plants in trace amounts. In arid and semi-arid regions toxicity symptoms may occur because of additions of B via irrigation water and lack of drainage. This element is often found in higher concentrations in association with saline soils, such as solonchaks and solonetz (Nable et al. 1997).

Genetic variation for tolerance of B toxicity exists within a number of crops, including wheat and barley (Cartwright et al. 1987, Moody et al. 1988, Paull et al. 1988, Yau 2002), lentil (Yau & Erskine 2000), field peas and pasture medies (Paul et al. 1992). Huang & Graham (1990) stated that the distinct and consistent differences among wheat genotypes in response to B toxicity both at the organ level and at the cellular level could serve as a basis for selection in a breeding program. The objective of the study was to get information on the variability and gene effects for B concentration in wheat leaves.

Materials and methods

In order to determine genetic variability and gene effects for B concentration six parents ('Yugoslavia', 'Rodna' and 'Stepa' from Serbia and Montenegro, 'Frontana' from Brazil, 'Bezostaya 1' from Russia) and their F_1, F_2, BC_1 and BC_2 generations were analyzed. A trial was conducted at the experimental field of the Institute of Field and Vegetable Crops in Novi Sad, located at Rimski Šančevi, using a RCBD with three replications. B concentration (ppm) in wheat leaves at heading was determined colorimetrically using 1.1.-dianthrimide.

Analysis of generation means for detection of additive and dominance effects and the presence of epistasis using A, B and C scaling tests was made according to Mather & Jinks (1982). A joint scaling test attributed to Cavalli (1952) was also conducted.

Results and discussion

Significant differences were found between the mean values for B concentration in the examined parents and hybrids. The B concentration in the leaves varied from 6.2 ppm in 'Rodna' to 16.6 ppm in 'Bezostaya-1'. In the F_1 the B concentration was the lowest in the

combination ‘Rodna’ x ‘Atlas’ 66 (13.7 ppm) and the highest in the cross ‘Frontana’ x ‘Yugoslavia’ (18.1 ppm). The mean values for B concentration in the F_2 were much lower than in the F_1 generation (Table 1).

Table 1. Mean values and standard errors for B concentration (ppm) in the parents and hybrids of wheat leaves

Generation	Frontana x Yugoslavia	Rodna x Atlas 66	Bezostaya 1 x Stepa
P_1	9.6 ± 0.10	6.2 ± 0.34	16.6 ± 0.33
P_2	13.4 ± 0.21	12.1 ± 0.38	11.4 ± 0.22
F_1	18.1 ± 0.13	13.7 ± 0.29	14.4 ± 0.51
F_2	14.3 ± 0.25	9.3 ± 0.15	9.4 ± 0.21
BC_1	15.3 ± 0.25	6.9 ± 0.14	7.6 ± 0.17
BC_2	15.7 ± 0.23	7.5 ± 0.14	7.2 ± 0.27

The mode of inheritance in the F_1 and F_2 generations was different (intermediate, dominant or superdominant) depending on the cross combination (Figure 1). As tolerance to high B concentration in wheat is expressed as a partially dominant trait with the response of heterozygotes being intermediate to the two parents, segregation in the F_2 generation appears to be continuous. Segregation ratios in the F_3 were consistent with tolerance to high concentrations of B being controlled by at least three major genes (Bo1, Bo2 and Bo3), as reported by Paull et al. (1991).

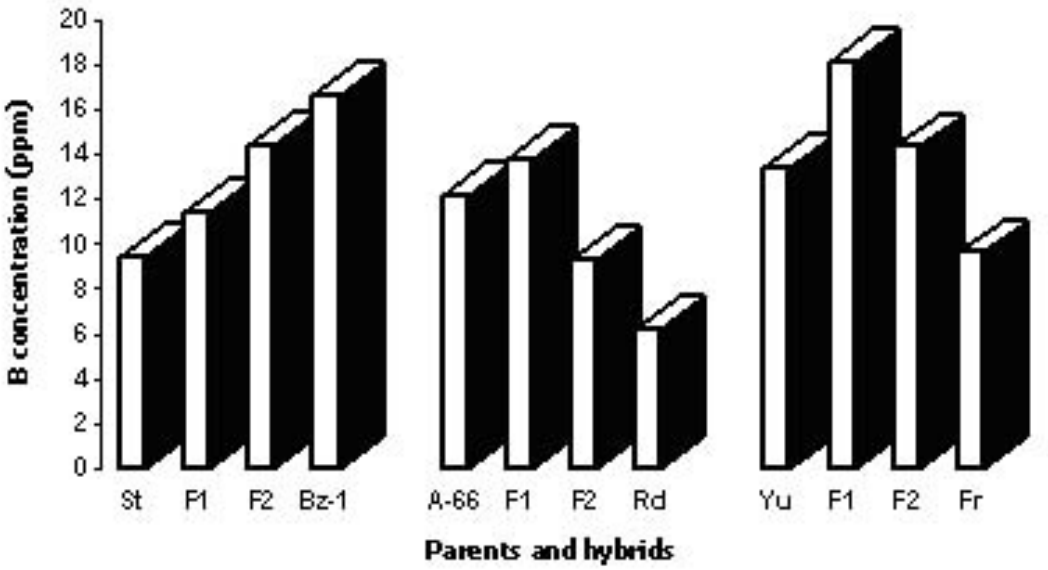

Figure 1. Inheritance of B concentration in wheat leaves (F_1 and F_2)

Additive (d) and dominance (h) gene effects were highly significant in all three crosses, with a prevalence of dominance. In all of the crosses epistasis was also involved in the inheritance of B concentration in wheat leaves, which can be seen from the significance of A, B or C in the scaling test (Table 2). The six-parameter model showed that B concentration was caused by all types of digenic epistasis, namely a x a, a x d and d x d (Table 2).

In all the crosses the duplicate type of gene interaction was observed [(h) and (l) had a different sign]. In the cross ‘Frontana’ x ‘Yugoslavia’ dominance had the positive sign and epistasis dominance x dominance (l) the negative one. In such cases, the epistatic effect

'reduces' the dominant effect, which, in turn, causes a decrease in the mean value of the trait (Table 2).

As reported by Jamjod et al. (2004), B efficiency in wheat was expressed as a partially dominant character, but the genotypes of F_1 hybrids, relative to parents, indicated genetic control varying from recessive to additive and to completely dominant with different cross combinations and B levels. Two genes, Bo1 and Bo2, could account for genetic variation for response to B.

Table 2. Gene effects for B concentration in three wheat crosses using the three-parameter, six-parameter and best-fit models

Gene effect	Frontana x Yugoslavia	Rodna x Atlas 66	Bezostaya-1 x Stepa
m	11.5 ± 0.11	6.4 ± 0.20	11.5 ± 0.18
[d]	-1.8 ± 0.11	-1.4 ± 0.16	0.5 ± 0.16
[h]	6.7 ± 0.18	4.1 ± 0.38	-5.2 ± 0.41
		Scaling test	
A	2.90 ± 0.49**	-6.2 ± 0.53**	-13.8 ± 0.70**
B	-0.06 ± 0.05	-10.8 ± 0.55**	-11.3 ± 0.77
C	-1.80 ± 0.06	8.5 ± 0.98**	-17.3 ± 1.37
x^2	40.9	46.5	43.4
P	<0.001	<0.01	<0.01
		Six-parameter model	
m	6.8 ± 1.21**	17.6 ± 0.77**	20.9 ± 1.07
[d]	-1.9 ± 0.12**	-3.0 ± 0.26**	1.6 ± 0.20**
[h]	18.9 ± 2.86**	-29.2 ± 1.89**	-39.4 ± 2.67**
[i]	4.7 ± 1.20**	-8.4 ± 0.72**	-7.8 ± 1.06**
[j]	3.0 ± 0.71**	4.6 ± 0.65**	-2.5 ± 0.75*
[l]	-7.6 ± 1.71**	25.4 ± 1.27**	33.0 ± 1.88**
		Best- fit model	
m	11.5 ± 0.12**	17.4 ± 0.77**	21.5 ± 1.06**
[d]	-1.9 ± 0.12**	-1.5 ± 0.16**	1.3 ± 2.60**
[h]	8.0 ± 0.67**	-28.7 ± 1.89**	-41.3 ± 2.60**
[i]	-	-8.4 ± 1.27**	-8.6 ± 1.03**
[j]	3.1 ± 0.71**	-	-
[l]	-1.4 ± 0.66**	25.1 ± 1.27**	34.2 ± 1.84**
x^2	15.3	50.6	10.6
P	<0.01	<0.01	<0.01

A useful variation in B tolerance exists in the examined material and breeding should be able to provide cultivars tolerant of higher levels of B. The most B-efficient cultivar in this experiment was 'Rodna', which had the lowest mean value for B concentration. This is in agreement with the statement that resistant genotypes accumulated less B in shoots than the sensitive ones (Paull et al. 1988). B-tolerant varieties generally have lower levels of B in leaf tissue, as reported by Moody and Rathjen (2003).

Breeding B-efficient wheat genotypes could be interesting for the region of Vojvodina, the northernmost part of Serbia and Montenegro, where there are saline soils, as it is known that high B concentrations may cause problems especially in interaction with drought stress conditions.

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Efficiency of different PCR-based marker systems in assessing genetic diversity among rye inbred lines

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ABSTRACT: The aim of this study was to investigate the efficiency of ISSR, SSR and SAMPL marker systems in detecting genetic polymorphism among 30 rye inbred lines. Each marker system was able to discriminate among the materials analysed with the lowest value of average genetic similarity (GS) obtained with ISSR markers (0.2888) and the highest with SAMPLs (0.5381). EST-derived SSRs turned out to be less efficient in detecting genetic diversity then those from genomic libraries (average GS values 0.3814 and 0.3221, respectively). The average GS value for combined SSR data was 0.3569. The lack of correlations between similarity and cophenetic matrices obtained with different marker systems suggests that different systems should be used simultaneously for a genetic diversity study to exploit as many sources of polymorphism as possible.

Key words: Genetic diversity – hybrid breeding – ISSR – SAMPL – *Secale cereale* – SSR

Introduction

Different marker systems have been routinely used in assessing genetic diversity in plant systems. SSR markers are known to be an attractive tool for a number of approaches including genetic diversity analysis, due to its multiallelic nature, high reproducibility and locus specificity. A previous sequence information is required for the development of SSR primers, which is a major drawback to their application in species studied less intensively. Number of SSR primers available for rye (*Secale cereale* L.) is still relatively small: 27 genomic (Saal & Wricke 1999) and 157 EST-based (Hackauf & Wehling 2002). ISSR technique in which polymorphisms result from the length differences between inversely oriented closely spaced microsatellites is a cost-effective alternative to SSR. Amplified fragments can be resolved using agarose gels and the reproducibility is higher then in any other marker system using single arbitary primers, namely RAPD. SAMPL is a relatively new molecular marker system basing on the AFLP methodology, technically more demanding than ISSR. Despite its many advantages, i.e. high multiplex ratio, SAMPL has not been widely used for analysis of plant genomes. For review of microsatellite sequence-based marker systems and their applications, see Rakoczy-Trojanowska and Bolibok (2004).

Rye is an important crop due to its nutrient efficiency, winter hardiness and tolerance to other environmental stresses such as drought; it is also a valuable genetic resource in wheat and triticale breeding. A better knowledge of genetic diversity and relationships between the existing rye inbred lines would aid in the development of breeding programs that efficiently utilize available rye germplasm. The objective of our work was to compare the efficiency of SSRs, ISSRs and SAMPLs in detecting genetic diversity among 30 inbred lines of rye. The genetic relationships of these inbreds have not been determined before. To our knowledge it is also the first study comparing the efficiency of SAMPLs in detecting genetic diversity with those of other microsatellite sequence-based marker systems - ISSR and SSR.

Materials and methods

Plant material and DNA isolation

Thirty rye inbred lines, mainly of Polish origin were used for genetic diversity study. The lines were chosen to represent a wide genotypic range and include components of mapping populations, male sterile forms, lines differing in tissue culture ability and resistance to powdery mildew and leaf rust. DNA was extracted from leaves of greenhouse-grown plants using CTAB method (Saghai Maroof et al. 1984).

ISSR assay

Fifty five ISSR primers were tested, of these 14 primers showing clear and polymorphic banding patters and were chosen for genetic diversity analysis. Amplifications were carried out in 15µl reaction mixtures each containing 50 ng of template DNA, 0.4 µM ISSR primer, 0.2 mM each of the dNTPs, 2.5 mM $MgCl_2$, 1x PCR buffer, and 0.3 unit *Taq* polymerase (Invitrogen) using the following PCR profile: 30 s at 94°C, 3 min at 50 or 55°C (depending on the primer), 60 s at 72°C, repeated 35 times. The amplification products were resolved by electrophoresis using 1 % agarose gels containing ethidium bromide.

SSR assay

A total of 31 SSR primers pairs were used for assessing genetic diversity: 18 EST-derived SSR: SCM0019, SCM0021, SCM0041, SCM0047, SCM0077, SCM0083, SCM0091, SCM0095, SCM0098, SCM0135, SCM0139, SCM0140, SCM0141, SCM0159, SCM0172, SCM0183, SCM0150 (Hackauf & Wehling 2002) and 14 genomic library-derived SSR: WSCM2, WSCM75, WSCM86, WSCM101, WSCM120, WSCM138, WSCM180, WSCM268, WSCM307 (Saal & Wricke 1999) and WRM206, WRM216, WRM220, WRM225 (Rakoczy-Trojanowska et al., unpublished). The cycling parameters for SSR markers developed by Saal and Wricke (1999) followed the recommendations of the authors. The remaining SSRs were amplified using PCR profile no. 20 described by Pillen at al. (2000). Amplification products were electrophoresed in 6 % denaturing polyacrylamide gels and silver-stained according to Pillen et al. (2000).

SAMPL assay

SAMPL procedure used in this study was based on the protocol of Singh et al. (2002) However, some modifications were introduced: 125 ng of genomic DNA were digested with restriction enzymes *Mse*I and *Hind*III. SAMPL primers used in the selective amplification included compound SSRs, 5'anchored SSRs and 3'anchored SSRs. In total 222 combinations of 37 SAMPL primers with 6 *Mse*I +3 were tested, of them 16 combinations of 10 SAMPL and 5 *Mse*I +3 primers were chosen for the assessment of genetic diversity. Amplification products were electrophoresed and visualised as described previously for SSRs.

Data analysis

For each marker system the presence or absence of bands was recorded 1 or 0, respectively to generate a binary matrix for calculating similarity coefficients. A similarity matrix was used for cluster analysis to construct a phenetic dendrogram using unweighted pair group method of averages (UPGMA). Principal component analysis (PCA) was also used to graphically display genetic relationships. Cophenetic values were computed for each dendrogram resulting in construction of a cophenetic matrix for each marker type. To assess the correspondence among the marker systems and to estimate the differences among dendrograms, the Mantel Z statistic was used to compare the similarity and the cophenetic matrices obtained with different marker systems. All statistical analyses were performed by NTSYS-pc, Version 2.1 (Rohlf 2001) for each marker type separately (ISSR, SAMPL, EST-

derived SRRs, genomic SSRs) and for combined SRR data and combined data from all marker types.

Results and discussion

The number of loci (alleles) analysed was 65 for ISSR, 101 for SSR (55 for EST-derived and 46 for genomic library-derived SSR) and 319 for SAMPL. Each marker system was able to discriminate among thirty inbred lines. However, when data from genomic SSR were analysed separately it was not possible to distinguish between two inbreds, namely M9 and M10. Conversely, the average value of similarity coefficient was higher for EST-derived SSRs (0.3814) then for those from genomic libraries (0.3221). This supports the results of Blair et al. (2003) and Cho at al. (2000) who found gene-based SSRs to be less polymorphic than anonymous genomic SSRs.

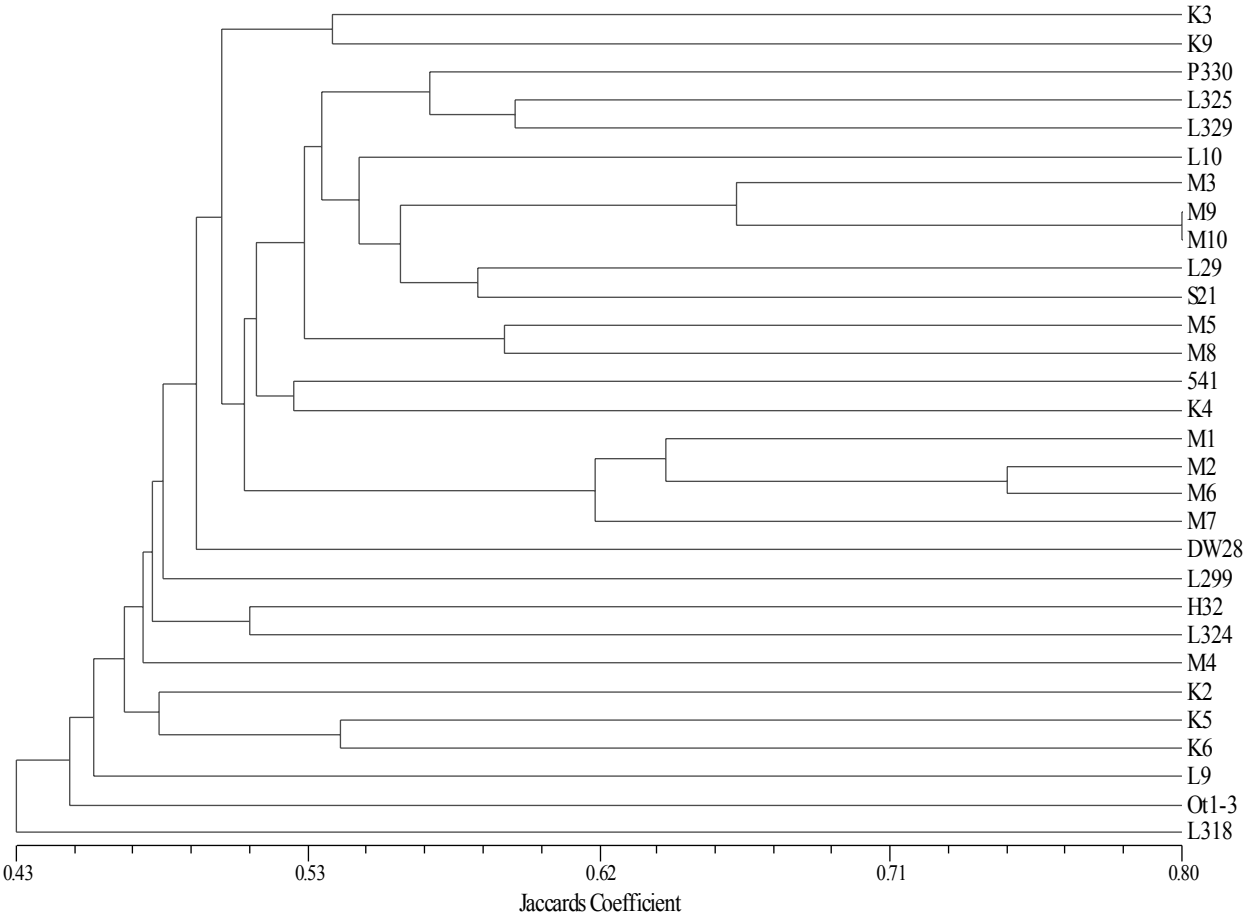

Figure 1. UPGMA dendrogram based on Jaccard's coefficient indicating genetic similarities among rye inbred lines derived from ISSR, SSR and AFLP data

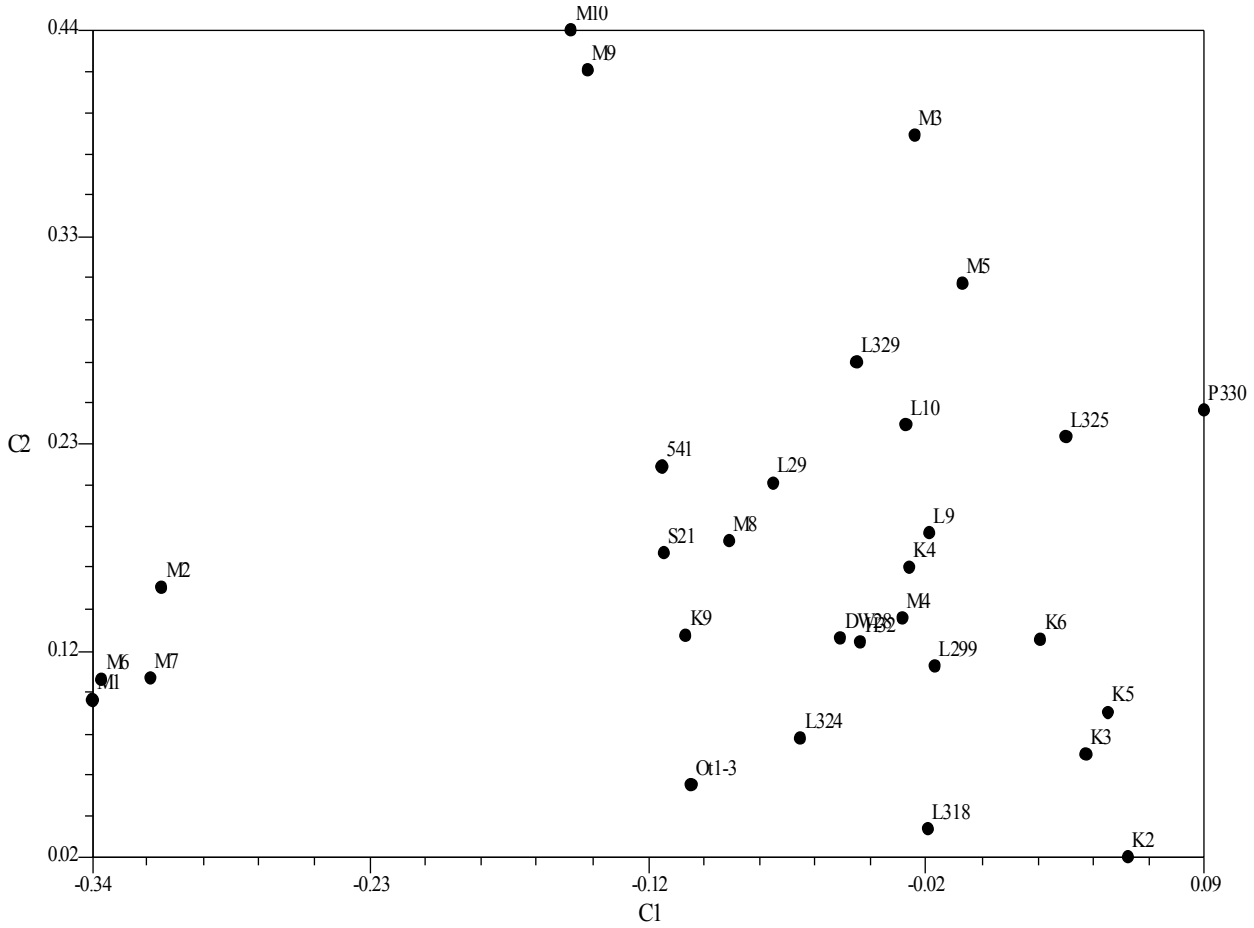

Figure 2. Two-dimensional principal component plot based on combined ISSR, SSR and SAMPL data

The average GS value for combined SSR data was 0.3569 which was intermediate to the values derived from ISSR and SAMPL datasets (0.2888 and 0.5381, respectively). The average GS value for combined data from all marker systems was 0.4902. The genetic-similarity dendrogram produced for the data from all the methods combined is presented in Figure 1. The PCA plot based on the same data is shown in Figure 2. Although certain patterns of groupings revealed by UPGMA were consistent across dendrograms for each marker type and for the combined data, Mantel's test showed a lack of significant correlations between similarity matrices and cophenetic matrices obtained with different marker systems. Such a result could be expected since the polymorphic fragments in the marker systems examined have different origin. It should however be noted that the results may be influenced by a relatively small number of loci analysed with ISSR and SSR markers, especially in comparison with a fairly large number of SAMPL loci. Concluding, we suggest to use different marker systems for assessing genetic diversity simultaneously in order to take the advantage of different sources of polymorphism.

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Genetic diversity and complexity of host resistance and pathogen virulence in the hybrid rye/leaf rust pathosystem

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ABSTRACT: Leaf rust is the most frequently occurring leaf disease in German winter rye. We wanted to test the effectiveness of race-specific resistance genes and to analyse the genetic composition of the leaf-rust population. A high diversity and complexity of pathotypes was found through three years by analysis of about 800 isolates with a differential set of 17 genotypes in the primary-leaf stage. In field experiments, we grew 30 synthetic populations segregating for one to four resistance sources along with their parental inbred lines in 17 environments in Germany under heavy natural infection. The different levels of host complexity and diversity were evaluated for their influence on the degree and variation of resistance that might be achievable in heterogeneous double-cross hybrids. Only two out of 30 synthetics and two Russian full-sib families were resistant across all environments, the remainder synthetics displayed various levels of resistance. High genotype x environment interaction was found caused by highly differing virulence patterns of the local rust populations as also demonstrated by Simpson indices around 0.9. Virulence complexity ranged from 3 to 15 across years with a median of 9. Observed resistance levels of the synthetics moderately corresponded to those predicted from the parental values, i.e. no advantage of host diversity occurred. In conclusion, highly effective race-specific resistances or new quantitative resistances are needed and should be combined to achieve a satisfactory level of leaf rust resistance in hybrid rye.

Key words: Gene deployment strategies – hybrid rye – *Puccinia recondita* – *Secale cereale*

Introduction

Leaf rust (*Puccinia recondita*) is a ubiquitous disease in rye (*Secale cereale* L.) growing areas. Both base populations of present hybrid rye breeding, 'Petkus' and 'Carsten', confer a low level of quantitative resistance that does not provide sufficient control of the disease. Race-specific, qualitative resistances from various materials are presently introgressed into the hybrid rye gene pools. Most of these are effective in the seedling stage, mono- or digenically inherited (Wehling et al. 2003) and easy to use in hybrid breeding (Miedaner et al. 2002). In view of the 'boom and bust' cycles known from pure-line cultivars, however, alternative strategies of resistance gene management should be developed. Our main goal was to (1) analyse the effect of increasing host complexity and diversity under multi-environmental field conditions and (2) determine the genetic diversity of the corresponding leaf-rust population by the detached leaf technique.

Theory

Commercial rye hybrids are commonly produced by pollinating a cytoplasmic male-sterile single cross with a restorer synthetic composed of at least two inbred lines. One possible strategy to achieve diversity and complexity of resistance in such hybrids would be to endow

each parent line with a different resistance gene. This approach is simulated in some of the synthetics produced for this project and compared with strategies leading to less diversity and/or complexity. The foregoing hybrids would combine the advantages of multi-line varieties and of resistance gene pyramiding in terms of host complexity and additionally hold up a high allele diversity by intermating during seed production. Assuming no linkage among resistance loci and full dominance, the expected number of different phenotypes in a randomly mated four-line synthetic in Hardy-Weinberg equilibrium equals 16 ranging from fully resistant (one to four resistance genes present in the homo- or heterozygous stage) to fully susceptible. The same phenotypes, however at slightly deviating frequencies, should occur in double-cross hybrids.

Materials and methods

Resistance tests

Eleven self-fertile rye inbred lines with race-specific resistances and three susceptible lines were used to develop 3 susceptible two-line synthetics, and 20 two- and ten four-line synthetics segregating for one to four resistance genes. They were built up from single and double crosses (=Syn-1 generation), respectively. The Syn-2 generation and two Russian full-sib families were grown at three northern and three southern German locations in three years (2000, 2001, 2002) with three replications resulting in 17 location by year combinations (environments, one environment missing). Additionally, a set of 17 differential lines and all parental lines were tested in one replication (the latter in 11 environments only). To assure maximum natural infection each entry plot was surrounded by four plots of a highly susceptible hybrid in a chess-cross design. From the segregating entries, a total of 120 single plants and from the inbred entries a total of 25 single plants per environment were rated for leaf rust severity on the F_1 leaf on a non-linear 1 - 9 scale (1 = no visual symptom, 9 = >65 % of leaf area affected).

Virulence survey

Leaf rust spores were randomly collected from highly susceptible rye material in the experimental fields and by a mobile spore trap mounted on a car from five main rye growing areas in Germany. A total of 827 single-pustule isolates was established and tested on a differential set of 17 inbred lines (Klocke 2004) by detached leaf technique with primary leaves. Parental lines of the synthetics were tested similarly with 55 isolates.

Results and discussion

All parent lines of the synthetics reacted race-specifically showing different reaction patterns in the detached leaf technique. Full resistance was observed when a line was inoculated by an isolate without matching virulence.

In the field experiments, only 2 out of 30 synthetics and the two full-sib families were fully resistant with ratings below 3. The other synthetics had mean disease severity ratings between 3.5 and 6.3, and the susceptible synthetics 8.4. All synthetics were segregating for leaf rust severity. The best synthetic had 60 % and the worst 14 % fully resistant plants, while the susceptible checks contained no resistant plants at all. No unambigous segregation ratios could be observed within synthetics, because none of the parental lines was fully resistant. Their mean resistance scores varied from 2.9 to 7.5, the two susceptible lines had 8.4 and 8.7, respectively. The resistance level of the synthetics moderately corresponded to that predicted from the performance of the parent lines ($r = 0.58$, $P = 0.01$).

Because the race-specific resistances were fully effective in the detached leaf test with appropriate isolates, their lower effectiveness in the field can only be explained by the occurrence of matching virulences in the field in varying frequencies although they were

never used in commercial varieties in Germany. Indeed, a high diversity of the leaf rust population in Germany revealing 265 different pathotypes among 827 isolates was detected. Virulence complexity ranged from 3 to 15 across years with a median of 9.

Large genotype x environment interactions occurred in the field experiments. Mean resistance of the four-line synthetic Syn33 with four different resistance genes, for instance, varied from 2.0 to 8.1 between environments of similar infection pressure, *i.e.* from 0.6 to 60 % leaf area affected. This was caused by different race spectra in the local pathogen populations as corroborated by the reaction of the differential lines. This is in accordance with the virulence survey, in which the pathotype distributions did not show any structuring within locations or years. Populations from individual locations were highly diverse with Simpson indices around 0.9. The five most frequently occurring pathotypes were found in every year, their frequency, however, never exceeded 10 % indicating low selection pressure by the host.

Diversity and complexity in leaf-rust populations of rye drastically deviate from other cereal rust populations like wheat leaf rust or wheat stripe rust where usually a few pathotypes dominate the population (Park & Felsenstein 1998, Flath & Bartels 2002). This may be caused by the outcrossing nature of rye that confronts the pathogen with a large host diversity and by the fact that race-specific resistances have not been widely used in present rye breeding. Thus the pathogen population could maintain a broad spectrum of virulences that might have already arisen during the long historical phase of rye growing in Germany. This is likely to change if race-specific resistances will become more common. According to the present results, a vast number of virulences exists in the pathogen population and therefore even gene deployment or pyramiding strategies will not provide sufficiently durable resistance. The authors therefore recommend to select for race non-specific quantitative resistances and to employ effective race-specific genes in a transition phase only.

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Genetic diversity of hull-less barley (*Hordeum vulgare* L.) landraces in the highlands of central Nepal as revealed by SSRs

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ABSTRACT: A set of 107 naked barley genotypes from mountains and highlands of Annapurna, Manaslu and Lamtang Himalaya range in central Nepal and 8 selected German and Canadian cultivars were analysed for genetic similarity using 40 SSRs covering the barley genome. Based on this, 258 alleles were detected with an average of 6.45 alleles per SSR. Taking into account only Nepalese barley on average 4.8 alleles were found. The overall diversity index was estimated at 0.53 and within Nepalese landraces at 0.52. The UPGMA clustering based on Dice similarity coefficient clearly separates Nepalese landraces from European and Canadian cultivars. The Nepalese collection is grouped into two distinct clusters and several sub-clusters, which in most cases are in accordance with the collection sites. The SSR markers revealed considerable genetic diversity within the Nepalese naked barley landraces.

Key words: Conservation – genetic resources – Himalaya barleys – microsatellites

Introduction

The Himalayan region is considered rich in barley diversity. In Nepal, barley is cultivated in hills and mountains in the north from the east to the west along the Himalayas and naked barley is common predominantly above 2800 m sea level. Hull-less barley is used as staple food, animal feed, for alcohol preparation and considered as a high-value crop in the Himalayan highlands. Because of a high level of variation in agro-ecology and primitive farming practices, a large number of hull-less barley landraces has been preserved in farmer's fields. Previous studies have shown considerable variation in morphology, maturity and disease reaction among the landraces (Sharma et al. 1994). However, detailed information on genetic diversity is sketchy. Such information is vital for crop improvement and conservation of genetic resources for future use. In this study, a large number of naked barley accessions originally collected from the highlands of central Nepal was analysed using SSRs.

Materials and methods

A total of 115 barley genotypes were analysed using 40 SSR markers (Ramsay et al. 2000) covering each of the barley chromosomes (Table 1). The plant material consisted of 107 six-rowed Nepalese hull-less barley genotypes originally collected from mountains and highlands of Annapurna, Manaslu and Lamtang Himalaya-range in central Nepal (Catalogue of Barley Germplasm, Okayama Univ., 1983), five Canadian hull-less cultivars and three German hulled cultivars. SSR analysis on genomic DNA was carried out according to Ramsay et al. (2000) with some modifications. Respective SSR profiles were detected on an automatic DNA-sequencer (LiCor 4200-S2) and scored using the software RFLP-scan 2.1. The resulting 1/0 data matrix was used to compute the Dice similarity coefficient. Based on these data UPGMA-clustering using the SAHN option of the software NTSYS-pc was carried out. Polymorphic information content was calculated: $PIC = 1 - \Sigma (p_i)^2$, where p_i is the frequency of the i^{th} allele of the SSR locus across the 115 genotypes studied. The mean diversity index (DI) was estimated as follows:

$$DI = n_a(1/n_l \sum_j (1 - \sum_i x_{ij}^2))/(n_a - 1)$$

where x_{ij} is the frequency of the ith allele of locus j, n_l is the number of genetic loci, and n_a is the number of accessions. The response of Nepalese hull-less barley landraces to BaMMV was tested by creating artificial infection in greenhouse and presence of virus particles was assessed by DAS-ELISA using the BaMMV-specific antiserum.

Table 1. SSRs, repeat motifs, chromosomal location, number of alleles and PIC values for Nepalese genotypes and on all of the 115 genotypes analysed

SSR	Repeat	Chromosome	No. alleles		PIC	
			a[1]	b	a	b
Bmac0399	(AC)21	1H	9	7	0.72	0.69
Bmag0032	(AC)7T(CA)15(AT)9	1H	17	15	0.85	0.83
Bmag0211	(CT)16	1H	9	8	0.69	0.65
HvHVA1	(ACC)5	1H	3	1	0.03	0.00
WMC1E8	(AC)24	1H	3	2	0.10	0.07
Bmac0093	(AC)24	2H	5	5	0.67	0.66
Bmac0134	(AC)28	2H	5	3	0.58	0.54
Bmag0378	(AG)14	2H	6	3	0.50	0.46
EBmac0415	(AC)17	2H	3	3	0.66	0.65
HVM36	(GA)13	2H	8	4	0.56	0.51
HVM54	(GA)14	2H	4	4	0.65	0.60
Bmac0067	(AC)18	3H	9	7	0.77	0.76
Bmac0209	(AC)13	3H	8	4	0.50	0.43
Bmag0013	(CT)21	3H	11	8	0.36	0.26
Bmag0136	(AG)6-(AG)10-(AG)6	3H	3	3	0.16	0.04
Bmag0225	(AG)26	3H	6	4	0.72	0.69
HVM62	(GA)11	3H	8	6	0.68	0.51
Bmag0353	(AG)21	4H	7	6	0.68	0.66
Bmag0384	(AG)18	4H	6	4	0.74	0.73
EBmac0701	(AC)23	4H	9	4	0.56	0.49
HVM40	(GA)6(GT)4(GA)7	4H	6	4	0.55	0.49
HVM67	(GA)11	4H	7	5	0.43	0.34
HvMLO3	(CTT)6	4H	2	2	0.43	0.42
Bmac0113	(AT)7(AC)18	5H	12	7	0.85	0.83
Bmag0222	((AC)9(AG)17	5H	5	3	0.65	0.63
Bmag0223	(AG)16	5H	12	11	0.86	0.85
EBmac0684	(TA)7(TG)11-(TG)11(TTTG)5	5H	6	6	0.63	0.60
EBmac0970	(AC)8	5H	2	2	0.11	0.07
HvLOX	(AG)9	5H	2	1	0.03	0.00
HVLEU	(ATTT)4	5H	2	1	0.07	0.00
Bmac0018	(AC)11	6H	3	3	0.56	0.51
Bmac0040	(AC)20	6H	5	2	0.13	0.02
Bmac0316	(AC)19	6H	3	2	0.07	0.02
Bmag0009	(AG)13	6H	6	5	0.60	0.54
Bmag0218	(AG)6(AG)6	6H	3	2	0.35	0.25
EBmac0806	(AC)4(GA)(CA)8-(CA)5	6H	7	4	0.48	0.40
Bmac0273	(AC)20(AG)20	7H	11	11	0.89	0.88
Bmag0007	(AG)16(AC)16	7H	15	14	0.88	0.87
Bmag0120	(AG)15	7H	7	4	0.59	0.53
HVCMA	(AT)9	7H	3	2	0.43	0.35
Mean			6.45	4.8		

a: all genotypes analysed (n=115), b: Nepalese genotypes

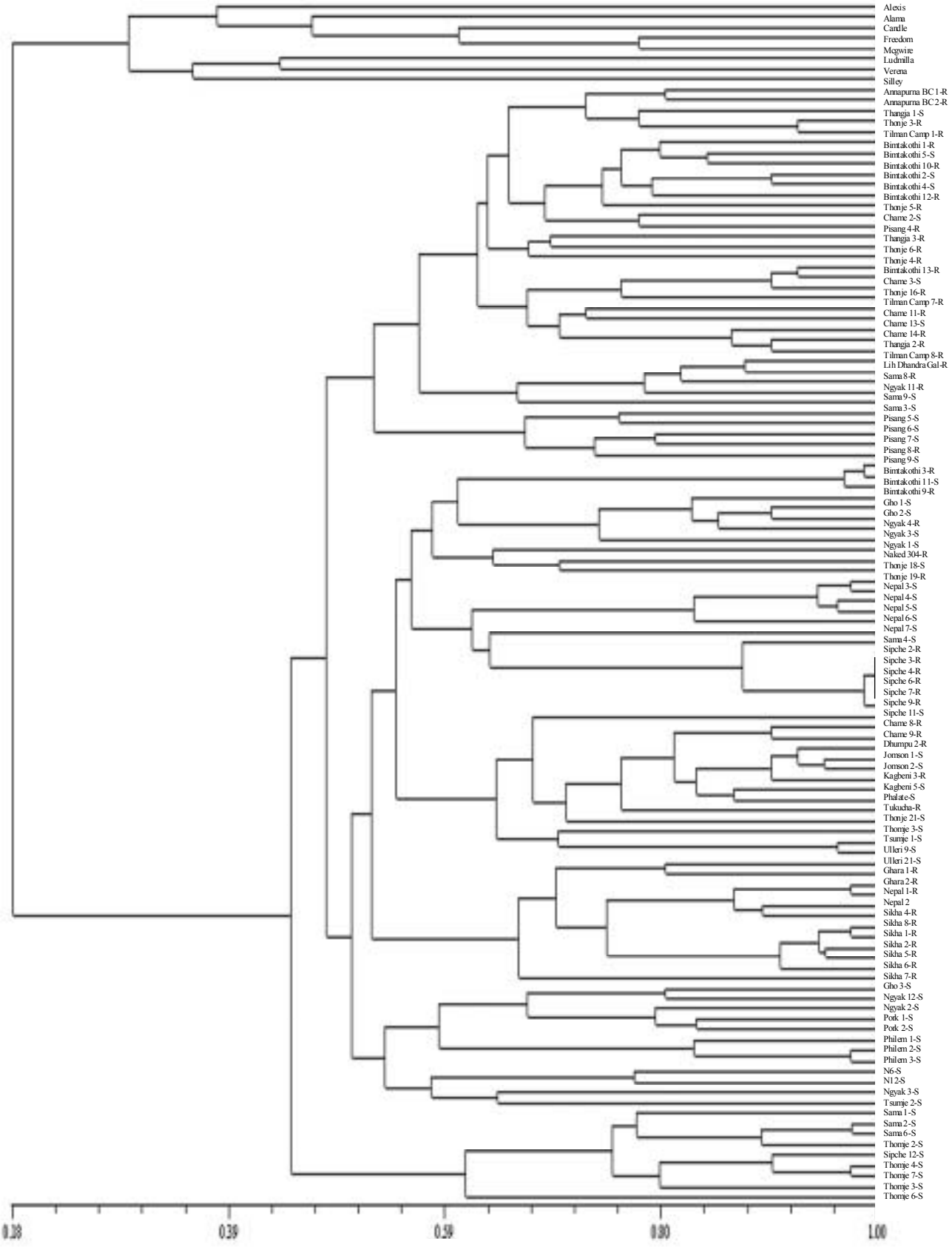

Figure 1. UPGMA-cluster of 115 barley genotypes (German cvs: Alexis, Ludmilla, Verena; Canadian cvs: Alama, Candle, Freedom, McGwire, Silley; Nepalese landraces) and reaction of landraces to BaMMV (-R, resistant; -S, succeptible)

Results and discussion

All 40 SSRs applied were polymorphic and a total of 258 alleles were detected across the 115 genotypes. The number of alleles per locus ranged from 2 to 17 with an average of 6.45 alleles per SSR (Table1). The PIC values varied largely among the 40 SSRs; minimum estimated for HvHVA1, HvLOX (0.03) and maximum for Bmac0273 (0.89). The total genetic diversity (DI) was estimated at 0.53 for all the 115 genotypes analysed. The Dice similarity coefficient varied from 0.08 to 1.00; minimum between 'Ludmilla' vs. Nepalese landraces: *Pork 1*, *Thangja 2*, *Chame 14*, between *Silley* vs. *Gho 1* and maximum between landraces: *Sipche 3*, *Sipche* 4, *Sipche* 6, *Sipche 7* and *Siphce 9*. The UPGMA clustering of 115 genotypes resulted in two well distinct groups clearly differentiating German and Canadian cultivars from Nepalese landraces (Figure 1). The Mantel test as a measure of goodness of fit for the clusters was estimated at r = 0.897 revealing a good fit. The result of BaMMV test is presented at the end of each accession name on Figure 1. Many genotypes from Bimtakothi, Sipche and Sikha sites have shown resistance reaction.

Genetic diversity within Nepalese germplasm

Out of 40 SSRs applied to 107 Nepalese genotypes, except HVLUE, HvHVA1 and HvLOX other 37 were polymorphic and in total 192 alleles were scored resulting in 4.8 alleles per SSR (Table1). The maximum number of alleles (15) scored for Bmag00032 and minimum (1) for HVLUE, HvLOX and HvHVA1. The DI was 0.52 based on the 37 polymorphic SSRs. The DICE similarity coefficient ranged from 0.27 to 1.00 of which minimum value was found between *Pisang 7* vs. *Ghara 1*, *Pisang 6* vs. *Sama 1* and maximum between the landraces: *Sipche 3*, *Sipche 4*, *Sipche 6*, *Sipche 7* and *Sipche 9*. The genotypes *Sipche 3*, *Sipche 4*, *Sipche 6*, *Sipche 7* and *Sipche 9* are not separated by the 40 SSRs. These accessions do not differ among them for reaction to BaMMV. The clustering of landraces in two broad groups do not show a defined geographic differentiation, rather several sub-clusters are formed in accordance with the collection sites. For example, many genotypes from Bimtakothi, Nepal, Pisang, Ngyak, Philem, Sikha and Thomje locality formed distinct sub-groups and/or placed within the same broader group (Figure 1). In contrast to this, *Bimtakothi 3*, *Bimtakothi 11*, *Bimtakothi 9*, *Sama 4*, *Chame 8*, *Chame 9*, *Sama 4* and *Thomje 6* belong to different clusters. Like in other studies, e.g. based on morphological traits (Witcombe & Murphy 1986) or isozymes (Konishi & Matsuura 1991) results of the present study indicate considerable genetic variability in Nepalese hull-less barley landraces.

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Development and use of genomic tools for cereal introgression breeding

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ABSTRACT: There is an increasing amount of public model sequence information in model plants available with potential use in agriculture. In cereals, the complete sequence of the rice genome is the basic resource. Additionally, there is an increasing amount of sequence information for the main cultivated species, such as wheat and barley. Wild relatives with introgression potential can also benefit from such resources through a comparative genomic approach. One of such species is *Hordeum chilense*. It exhibits great levels of polymorphism and high crossability with different cereal genera. In addition, interesting biotic and abiotic stress resistance genes, and important quality traits like carotene content and seed storage protein variability shown in the species are also expressed on wheat backgrounds and are the basis of a breeding program. In the past years, the search for the most suitable DNA marker system for tagging *H. chilense* genomic regions in a wheat background has lead to the development of RAPD and SCAR markers for this species. RAPDs represent an easy way of quickly generating suitable introgression markers. SCARs are more specific assays, suitable for automation or multiplexing. Direct sequencing of RAPD products is a cost-effective approach that reduces labour and costs for SCAR development. Transfer of SSTs from wheat and barley have also been useful approaches. More recently, SNP development is being accomplished for the species. A practical application of the different marker approaches has been the generation of derived introgression products.

Key words: *Hordeum chilense* – marker assisted selection – molecular breeding – wheat

Introduction

The wild South American barley species *Hordeum chilense* Rome. et Schult. has an indirect but interesting potential in agriculture through introgression breeding (Martin et al. 1998). It belongs to a heterogeneous group of South American *Hordeum* species (Sec. Anisolepsis Nevski). It is very polymorphic and has been hybridised with species from the genera *Aegilops*, *Agropyron*, *Dasypyrum*, *Hordeum*, *Secale*, and *Triticum* (Fedak 1992). It contains interesting genes for biotic and abiotic stress resistance as well as important quality traits such as carotene content and seed storage proteins, many of which are expressed in a wheat background (Martin et al. 1998). *Tritordeum*, the barley-wheat amphiploid, is the basic genetic material for using *H. chilense* genetic variability in wheat breeding (Martin et al. 1996). The use of this wild species to increase wheat genetic resources will be greatly facilitated by marker-assisted introgression. To do this, molecular markers that enable tracking of *H. chilense* chromatin in a wheat background are needed.

With the breeding aims outlined above, a series of marker development attempts have been undertaken in the past decade. The overall rationale was, firstly, to assess if marker-assisted selection in our system was possible, and later, to simplify and set up methods to be useful in the routine of a cereal breeding program.

Adapting protocols for routine applications

The practical application of molecular markers in a breeding program requires simple and economic methods, due to the high number of individuals that need to be characterised in every generation. The advent of PCR-based molecular markers has made molecular tools accessible for breeders use. Shortening and simplification of methods is always desirable. For example, a RAPD profile run usually takes five to six hours. Starting from the standard

RAPD PCR cycling method (Hernandez et al. 1996), the temperature profile was optimised in order to obtain a high level of polymorphism, reducing as much as possible the time of the run. In order to maintain the RAPD profiles, a slow ramp between annealing and polymerization temperature (1°C per second) was needed, but it was possible to reduce denaturation, annealing and polymerization times without affecting the degree of polymorphism obtained. Using such thermal profile and thin-walled tubes, we reduced in two hours the time for each run. Amplification was performed in a System 9600 Cycler (Applied Biosystems, Foster City, CA, USA). The cycling was performed as follows: 94°C / 3 min, followed by 40 cycles of amplification (94°C / 20 sec, 35°C / 20 sec, then increase to 72°C / 1 min with a ramp of 1°C per second) and by a 7 min final extension. This cycling profile (Hernandez 1998) has been successfully used later for RAPD amplification in olive (Beluga et al. 2001) and miscanthus (Attunes et al. 2002).

A further development on the search of faster and cost-effective systems for the use of molecular markers in breeding, is a method to transform RAPDs in SCAR markers by direct sequencing of the RAPD products, thus avoiding the costs on labour and consumables derived from cloning (Hernandez et al. 1999). Additionally, PCR primer designs are optimized when possible for shorter 2-step-PCR amplification runs in a search for high specificity to avoid electrophoresis (Hernandez et al. 2002). This has been the starting point to the development of markers suitable for automation using real-time PCR detection systems.

Generation of novel plant material

Traditionally, marker-assisted selection of basic breeding material has been accomplished using morphological or biochemical markers. The advent of DNA molecular markers widens the possibilities of application. Marker-assisted selection of basic breeding material has been carried out, in a first approach, with the development of chromosome-specific markers for *H. chilense*, detectable in a wheat background. These markers have been used to obtain new addition forms of *H. chilense* in wheat (Hernandez 1998) that have been useful so far to identify carotene genes expressed on wheat background (Alvarez et al. 1998).

Future trends

Microsatellite markers have become the marker of choice for cereal molecular breeders (see Korzun et al. 1997, Pestsova et al. 2000, 2001, Hernandez et al. 2002), for example on cereal introgression applications. Unfortunately, methods for microsatellite analysis rely on electrophoretic separation of the amplified products. Semi-automated systems, based on capillary gel electrophoresis using fluorescently labelled primers, combine a resolute power of ca. 2 bp with semi-automation, thus avoiding the set up of sequencing gels and manual sample loading. This is the preferred system for microsatellite analysis. Nevertheless, requirements for a high throughput genotyping system amenable for MAS include an increased level of automation both for set up and scoring. Markers based on allele-specific PCR and real-time detection systems or allele-specific oligonucleotides also amenable for microarrays meet these automation requirements and deserve the attention of breeders.

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Genetic divergence of maize inbred lines based on molecular markers

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ABSTRACT: As comprehensive characterization and genetic analysis is useful in choosing appropriate parental lines, the present study attempted to analyze ten maize inbred lines of different origin by protein and RAPD markers. Both types of markers can be used for characterization of maize inbred lines, because each inbred lines had a specific banding pattern. Genetic distance for 45 crosses among 10 inbred lines based on protein markers ranged from 0.094 between lines ZPPL 200 and ZPPL 204 and up to 0.359 between ZPPL 148 and ZPPL 15 as well as ZPPL 148 and ZPPL 52. The higher level of polymorphism in maize has been obtained with RAPD markers. Genetic distance calculated from RAPD marker data ranged from 0.124 in the combination ZPPL15 and ZPPL 149 to 0.674 in the lines ZPPL 148 and ZPPL 15. Analysis of genetic relationship using both types of markers grouped the ten inbred lines into two main clusters generally consistent with expectations based on known pedigrees. Results from this study corroborate the usefulness of protein and RAPD markers for characterization of inbred lines and for assigning inbreds into heterotic groups.

Key words: Heterotic group – polymorphism – protein – RAPD – *Zea mays*

Introduction

Knowledge of genetic relationship among inbred lines or populations is important for planning breeding programmes, hybrid development and germplasm conservation. Three methods are mostly used in assessing genetic diversity among maize germplasm: pedigree records, field testing methods and molecular markers. The analysis of genetic diversity and relationships based on pedigree data is not easy to carry out because in many cases pedigree data are unreliable or unavailable. Molecular markers allow a direct comparison of the similarity of genotypes at the molecular level. Several analyses of the maize genetic variability have been performed using molecular markers to obtain genotype characterization (Gethi et al. 2002, Amorim et al. 2003), to assigning lines to heterotic groups (Melchinger et al. 1992, Pinto et al. 2003), or to estimate the heterosis among inbred lines (Drinic et al. 2000). The RAPD technique has been useful in studying polymorphism, identifying genes of interest and characterizing genetic resources. RAPD markers are used for characterization of maize inbred lines (Hahn et al. 1995, Heun & Helentjaris 1993) and hybrids (Stojsin et al. 1996, Sun et al. 2001). The objective of the present study were to assess the genetic diversity based on protein and RAPD markers among ten maize inbred lines and examine usefulness of molecular markers for assigning inbred lines to heterotic groups.

Material and methods

A ten maize inbred lines, ZPPL 149, ZPPL 225, ZPPL 15 from Iowa Stiff Stalk Synthetic (BSSS); ZPPL 151, ZPPL 200, ZPPL 204, ZPPL 80 from Lancester Sure Crop heterotic group; ZPPL 52 and B97 not related to BSSS and Lancester heterotic groups and ZPPL 148 from Wf9, were analysed. The proteins were isolated from hybrid germs according to Wang et al. (1994) and separated by polyacrylamide gel electrophoresis according to Leammli (1970). The genomic DNA was isolated from inbred germs following the protocol of Saghai-Maroof et al. (1984) and RAPD was performed using modified protocol of Williams et al. (1990). The amplified bands were scored based on 1/0 (presence/absence) system. Genetic distances among all possible pairs of inbred lines were estimated from protein and RAPD data

according Nei and Li (1979). Cluster analysis were carried out on the matrix of genetic distances using the unweighted pair group method with arithmetic averages (UPGMA) clustering algorithm. The dendogram were constructed with NTSYS-pc software (Rohlf 2000).

Results and discussion

The analysis of embryo salt soluble proteins showed that all genotypes studied have a specific protein pattern. Totally, 42 protein fractions of different molecular weight were observed, from which 76 % of protein fractions were polymorphic. Quantitative differences in concentration of protein fractions were not analysed further.

Genetic distances ranged from 0.094 for inbred lines ZPPL 204 and ZPPL 200 to 0.359 for ZPPL 148 and ZPPL 15, as well as ZPPL 148 and ZPPL52. The average genetic distance was 0.234. Obtained results are in good agreement with the pedigrees of the lines. The inbred line ZZPL 148 is derived from Wf9, ZPPL 15 is obtained by recurrent selection from Iowa Stiff Stalk Synthetic and ZPPL 52 is an European dent line. The cluster analysis based on genetic distance computed from protein marker data classified each of 10 inbred lines into one of two principal heterotic groups (Figure 1).

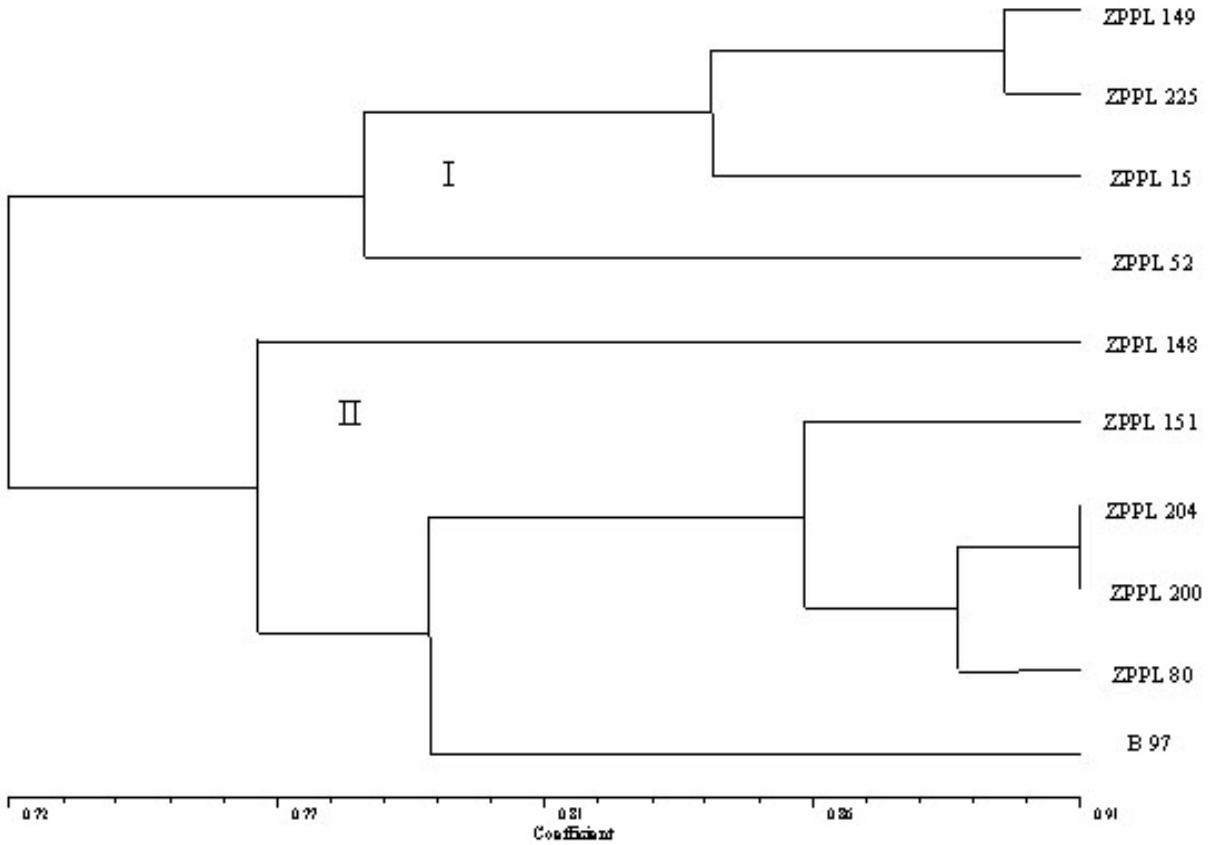

Figure 1. Dendogram of 10 maize inbred lines obtained using protein markers

The first group encompasses inbred lines developed or related to BSSS germplasm and inbred ZPPL 52 loosely linked to this subcluster. The second group consists of inbred lines belonging to the Lancester Sure Crop germplasm. The inbred ZPPL 204 have one common parent with inbred ZPPL 200, and second parent is inbred ZPPL 151. Two inbreds B97 and ZPPL 148 are loosely linked to that heterotic group.

Results of previous investigations of molecular marker applications in genetic studies showed that RAPD markers reveal a high level of polymorphism in the maize genome (Heun & Helentjaris 1993). Based on results of a previous screening of RAPD primers for polymorphism with maize inbred lines (data not shown), only primers that gave highly reproducible RAPD patterns were used for present study. The reproducibility of RAPD fragments was tested in two rounds of amplification with all inbreds. Ten random 10-mer primers from Genosys Biotechnologies were used to amplify fragments from the DNA templates of 10 inbred lines. A total of 68 RAPD fragments of different molecular weight were scored. Of the 68 fragments 81 % were polymorphic and gave from 3 to 11 fragments per primer. The genetic distance calculated from 45 combination of 10 parental inbreds ranged from 0.124 in the combination ZPPL15 and ZPPL 149 to 0.674 in the inbreds ZPPL 148 and ZPPL 15. Cluster analysis based on RAPD markers showed clear grouping of inbred lines into two main heterotic groups (clusters not shown). In cluster I there are two subclusters. The first formed three inbred lines: ZPPL 52 (European germplasm), B97 (BSSS and Lancester not related) and ZPPL 148 (Wf9) and second subcluster formed inbred lines from BSSS germplasm. Cluster II comprises inbreds ZPPL 200, ZPPL 204, ZPPL 80 and ZPPL 151 from Lancester germplasm.

The results of our study agree with previous reports (Senior et al. 1998, Sun et al. 2001) that the level of polymorphism for different types of molecular markers is high in maize. The polymorphism of proteins markers can be used for characterization of maize inbreds, because each inbred had a specific banding pattern. They can be used as preliminary method for characterisation of inbred lines which is in agreement with result of several authors (Wang et al. 1996). In general, the RAPD markers detected larger genetic variability than protein markers in the ten assessed genotypes. It could be concluded from the present study and previous studies done by other researchers that grouping of inbred lines generally agrees with the pedigrees of these lines and clusters are representative of heterotic groups but with few discrepancies.

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Genetic analysis of drought tolerance in tropical maize

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ABSTRACT: A population of recombinant inbred lines derived from a cross between a drought tolerant and a drought susceptible maize line was used to construct a genetic linkage map and was phenotyped in field experiments under different water regimes. The anthesis-silking interval as well as the ear and silk dry weight at pollen shedding seem to be good secondary traits for drought tolerance, as indicated by the phenotypic correlations and the results of the QTL analysis. Moreover, the population segregated well for leaf chlorophyll content which could be related to stay green characteristics. These first results will be complemented by more evaluations in the field in Mexico and Zimbabwe as well as under controlled high temperature conditions.

Key words: Drought stress – QTL – recombinant inbred lines – *Zea mays*

Introduction

Drought stress is evenly distributed across the world's major maize production regions and is a particularly severe problem for slightly more than one-fifth of the tropical and subtropical maize planted in developing countries (Pingali & Pandey 2000). Maize is unusually susceptible to drought around the flowering period. Drought tolerance is closely associated with a short anthesis-silking interval (ASI), reduced kernel barrenness (Chapman & Edmeades 1999), increased growth rate of ovaries (Betrán et al. 2003) and the stay green characteristics (Sanchez et al. 2002).

Materials and methods

'CML444' is a drought tolerant maize line developed at CIMMYT. 'SC-Malawi' is a local line used in Africa and is very susceptible to water limited conditions. By crossing these two lines, a segregating population of recombinant inbred lines (RILs) has been developed. Individual DNA samples of the F5S6 plants have been used to construct a genetic linkage map, which consists of the allelic information of 236 RILs at 160 marker loci.

The 236 RILs were used for the phenotypic evaluation in the field. To date, three field experiments have been performed in Tlaltizapán, Morelos, Mexico. All of them were repeated twice and designed as an alpha (0, 1) lattice (Patterson & Williams 1976) with 10 plots per block and 12 plants per plot. Standard traits like male flowering, plant height, ear height, leaf chlorophyll content, leaf senescence and root conductance were measured in all the experiments. Two experiments were performed under drought stress conditions (DS), one to record female flowering (which allows calculating ASI) and grain yield related parameters, and one to measure dry weight of the ears and silks at pollen shedding and seven days after pollen shedding (It is not possible to collect data on female flowering and ear and silk growth in together in one trial, since the last measurement is destructive.). The third field experiment was performed under well-watered conditions (WW) and was used as a control for female flowering, ASI and grain yield.

Phenotypic data in combination with genetic marker data allowed to calculate quantitative trait loci (QTLs) to identify genomic regions involved in the expression of the traits. QTLs were considered significant if the corresponding LOD-score was higher than 2.5.

Results and discussion

The RIL population segregated well for plant height and leaf chlorophyll content. The segregation for ASI was less pronounced. However, there was a negative correlation between ASI and grain yield, both under well-watered and drought stress conditions. The QTL "A" (cf. Figure 1 and Table 1) on chromosome 1 has been detected for ASI, ear number and grain yield in the well-watered trial, indicating that ASI is closely related to kernel set and grain yield. The QTL "F" was involved in the expression of ASI under stress and influenced ear and silk weight measured seven days after pollen shedding. These two parameters reflect how fast the ears and the silks grow during the flowering period. They were under a strong genetic control (Carcova et al. 2003) and good secondary traits for drought tolerance (Edmeades et al. 2000). Unsurprisingly, ASI was negatively correlated with ear and silk growth during stress. The objective of the QTL study was not to present entire series of all the QTLs identified for individual traits, but we rather focused on target regions where multiple QTLs were detected. There was a QTL at the end of chromosome 5 ("G") for grain yield and kernel number under stress conditions, which could reflect a genetic response to drought, since this QTL was not detected under control conditions.

Plant height and ear height were highly correlated in all environments. Ear position, however, did not depend on plant height. There was a small positive correlation between plant height and grain yield under well-watered conditions. Under drought stress, however, these two traits were not correlated, and there was also no correlation between plant height and ASI. QTLs for plant and ear height ("B", "E", "H", "I", "J") showed high LOD values and were consistently detected across environments.

Chlorophyll content of the youngest leaf and the ear leaf were negatively correlated with ASI and positively correlated with grain yield both under stress and non-stress growing conditions. Sanchez et al. (2002) and Xu et al. (2000) reported that leaf chlorophyll content in sorghum was significantly correlated to the stay-green trait, which improves resistance to premature senescence under soil moisture stress during grain filling. Three main QTLs involved in the expression of the leaf chlorophyll content have been identified: QTL "C" on chromosome 2 under drought stress and QTL "D" under well-watered conditions, reflecting a shift in gene expression under drought stress. The third QTL for leaf chlorophyll content was present on chromosome 10 ("J") and was also involved in the expression of other traits like plant height, ear height, progress of senescence and lodging, which is in accordance to Sanchez et al. (2002).

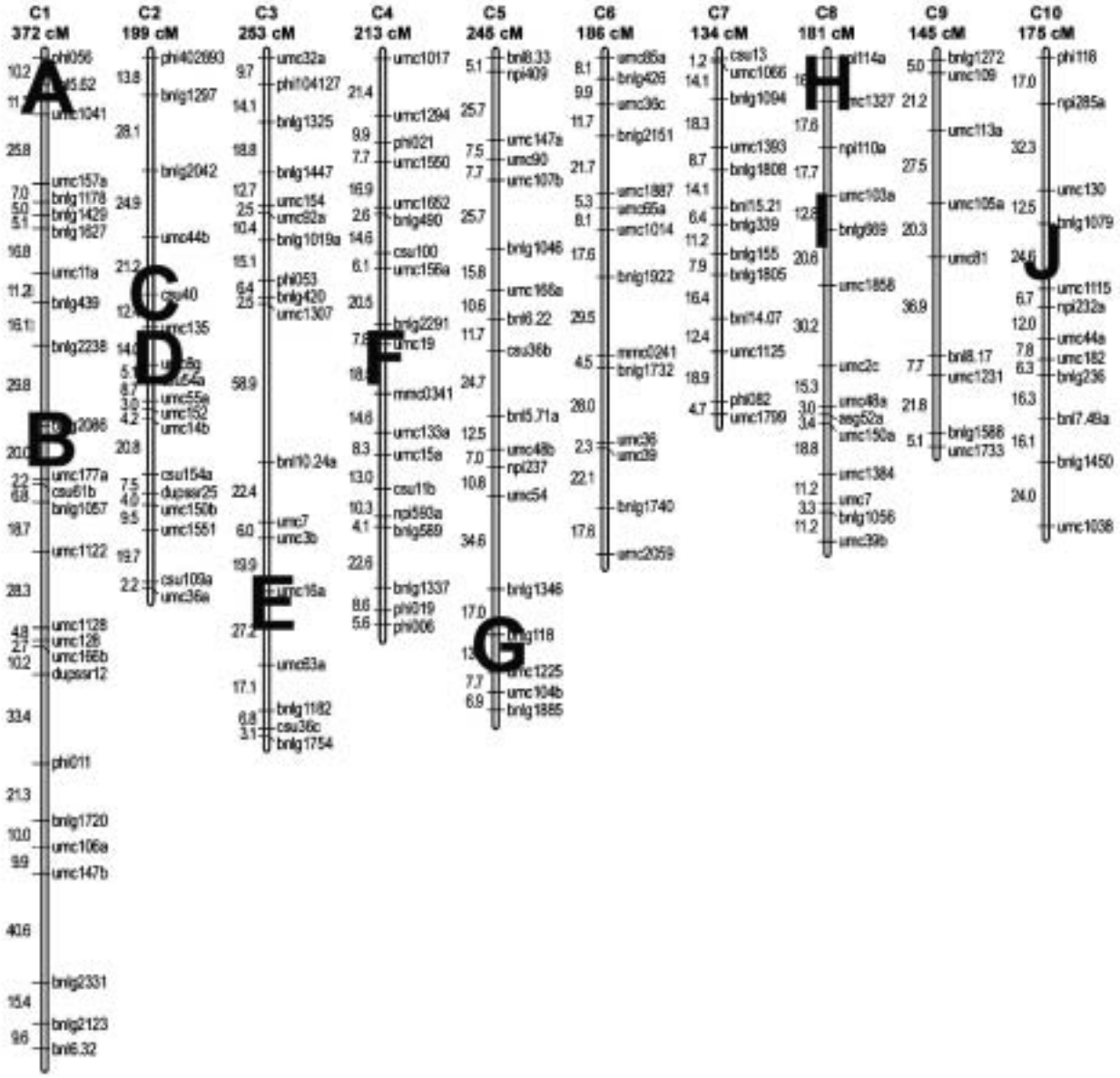

Figure 1. Genetic linkage map based on the allelic information of 236 recombinant inbred lines of the cross 'CML444' x 'SC-Malawi' at 160 marker loci. Genetic distances in cM are on the left of each chromosome and marker names on the right. Capital letters refer to regions where QTLs have been identified.

Table 1. QTL results based on phenotypic data of two drought stress and one well-watered field experiment. Capital letters in the column 'Map', indicate the location of the QTL on the genetic map in Figure 1

Map	Cond[1]	Trait[2]	Chr	cM	Marker	LOD	add	%
A	WW	ENO	1	5	2	5.2	0.5	13.2
A	WW	GY	1	6	2	3.6	24.0	6.0
A	WW	ASI	1	7	2	2.8	-0.4	6.9
B	WW	PHT	1	131	11	12.4	-8.0	18.7
B	DS	PHT	1	137	11	9.3	-5.8	20.4
B	DS	PHT	1	138	11	12.4	-6.6	22.2
B	WW	EHT	1	142	11	8.3	-5.2	19.2
B	DS	EHT	1	150	12	11.1	-4.9	19.7
B	DS	EHT	1	152	12	8.1	-4.9	15.5
C	DS	CHYL	2	83	5	7.3	0.9	13.7
C	DS	CHEL	2	83	5	6.6	0.8	12.9
D	WW	CHYL	2	113	7	7.2	1.3	12.6
D	WW	CHEL	2	115	7	2.9	0.7	5.7
E	DS	EHT	3	199	14	4.2	3.0	4.0
E	DS	EHT	3	200	14	3.3	3.1	4.9
E	WW	EHT	3	200	14	3.7	3.3	3.5
F	DS	EW7D	4	105	10	3.1	0.1	4.9
F	DS	SW7D	4	105	10	2.6	0.0	3.3
F	DS	ASI	4	108	10	3	-0.6	5.3
G	DS	KNO	5	225	16	4.2	-21.1	7.8
G	DS	GY	5	235	17	3.8	-3.7	6.8
H	WW	EHT	8	0	1	3.2	-2.7	6.7
H	DS	EHT	8	9	2	5.4	-4.4	14.5
H	DS	PHT	8	17	2	5.6	-4.5	13.4
H	DS	EHT	8	19	2	5.4	-3.6	12.4
H	DS	PHT	8	21	2	4	-3.9	10.5
I	DS	EHT	8	64	5	5.2	-3.2	7.0
I	DS	EHT	8	67	5	5	-4.1	9.9
I	WW	EHT	8	73	5	4.4	-3.9	6.4
J	DS	CHYL	10	60	4	3.3	0.6	5.4
J	DS	EHT	10	69	4	3.7	-3.6	6.0
J	DS	CHEL	10	74	5	8.4	0.8	11.8
J	WW	EHT	10	74	5	2.3	-2.9	1.4
J	DS	PHT	10	75	5	4	-4.2	4.3
J	WW	PHT	10	76	5	4.7	-5.2	5.0
J	WW	LODG	10	77	5	9.7	-0.7	16.2
J	DS	CHYL	10	78	5	7.1	0.5	16.1

[1] DS, drought stress; WW, well watered

[2] ASI, anthesis-silking interval; CHEL, chlorophyll content ear leaf; CHYL, chlorophyll content youngest leaf; EHT, ear height; ENO, ear number; EW7D, ear weight 7 days after pollen shedding; GY, grain yield; KNO, kernel number; LOD, base 10 logarithm of the ratio between likelihoods under the null and alternative hypothesis; LODG, lodging score; PHT, plant height; SW7D, silk weight 7 days after pollen shedding

Outlook

There will be more field evaluations under well-watered conditions and drought stress in Mexico as well as in Zimbabwe in order to improve the physiological understanding of drought tolerance and to enhance the stability of the QTL results. Additionally, seedlings of the RILs are currently being analyzed under high temperature conditions in the growth chamber, since drought and heat often occur together. The gene expression analysis of the two

parental lines, which will be done in a second phase of the project, will hopefully allow to better understand the genetic responses to drought, particularly in this tropical maize population.

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DNA recovery and AFLP analysis of common millet (*Panicum miliaceum* L.) from the 4th and 15th centuries compared to the current variety 'Topaz'

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ABSTRACT: Ancient DNA was extracted from seed remains of common millet preserved in the Hun's age settlement of the 4th century AD (Mongolian) and from a 15th century well settlement in the King's Palace of Budapest (Hungary). Sediment samples were processed by floatation and sub-samples of seed remains were subsequently sorted and determined in the laboratory. Identical seeds of common millet (*Panicum miliaceum* L., 2n = 4x = 36) were surface sterilized thoroughly and kept in aseptic tissue culture for a long period of three months to exclude bacterial or fungal infected seeds. Genetic material of DNA from the non infected seeds was extracted successfully and analyzed by fAFLP (fluorescent labeled amplified fragment length polymorphism). Agarose gel electrophoretic analysis suggested that extensive DNA degradation had occurred in the 4th century sample. This was confirmed in the AFLP analysis, in which only 2 fragments (92.8 %) were observed. In contrast, the DNA from the 15th century sample (middle ages millet, *mam*) was much less degraded (40.0 %) with high molecular weight DNA still present. Consequently, 158 AFLP bands could be detected. However, this was still substantially less than the 265 AFLP bands that we detected in the contemporary millet variety 'Topaz'. Our results begin to develop an AFLP-based DNA database of 500 years old common millet (*P. miliaceum*).

Key words: AFLP – archeogenetics – common millet – Hungary – Mongolia

Introduction

Ancient/aged DNA (aDNA) has been successfully isolated from a number of plants, human and animal tissues either from extinct or fossilized samples, or amber-preserved insects (Austin et al. 1997). The recovered DNA was found to be extensively amplifiable with PCR (polymerase chain reaction) resulting in comparative fragment patterns for evolutionary, migration or domestication studies (Paabo 1989, Parducci & Petit 2004). In the study presented here DNA samples of a 1600 years old common millet from the 4th century (Mongolia) and a 500 years old one from the 15th century (Hungary) were analyzed and compared to a current variety 'Topaz' by AFLP.

Materials and methods

Archeological samples were taken from the excavation layers of the 4th (Mongolian) and 15th (King's Palace of Budapest, Hungary) centuries. Sediment samples were processed by floatation and sub-samples of seeds were subsequently sorted and identified in the laboratory. A registered common millet variety 'Topaz' was used as a control. Seed samples were surface sterilized by commercial bleaching of NaOCl (20 %) followed by a three month incubation period in aseptic tissue culture media F6 (Gyulai et al. 2003). Total DNA samples of 0.1 g seeds in each case were extracted by the CTAB-method followed by an RNase-A (Sigma) treatment. The undiluted genomic DNA samples were subjected to fAFLP analysis. The AFLP method (amplified DNA-fragment length polymorphism) described by Vos et al. (1995) was used with modifications (Cresswell et al. 2001, Skot et al. 2002).

Results and discussion

Archeological sediments from excavation layers of the 4th (Mongolian) and 15th (King's Palace of Budapest, Hungary) centuries were processed by floatation followed by taxonomic characterization (Figure 1a, b). The excavations from the 15th century layer at the King's Palace of Budapest (Hungary) revealed a number of plant remains including about 300.000 seeds of 88 plant species (Nyekhelyi 2003). Remains of common millet seeds appeared extremely well preserved (Figure 1b) due to the anaerobic conditions in the slime of a deep medieval well covered by water. Some seeds of *mam* (middle age millet) looked so intact without any carbonized or burnt damages that we tried to germinate them as was successfully done with 172 year old seeds of barley (*Hordeum vulgare*) and oat (*Avena sativa*) recovered in Nürnberg (Aufhammer & Fischbeck 1964), and with 127 year old hexaploid Hungarian wheat (Szekesfehervari – Stuhlweissenburger) (*Triticum aestivum*) recovered from 1877 in Vienna (Ruckenbauer 1971). We also tried to induce callus proliferation from single putative surviving seed cells on aseptic medium F6 (Gyulai et al. 2003) but had no success with either approach.

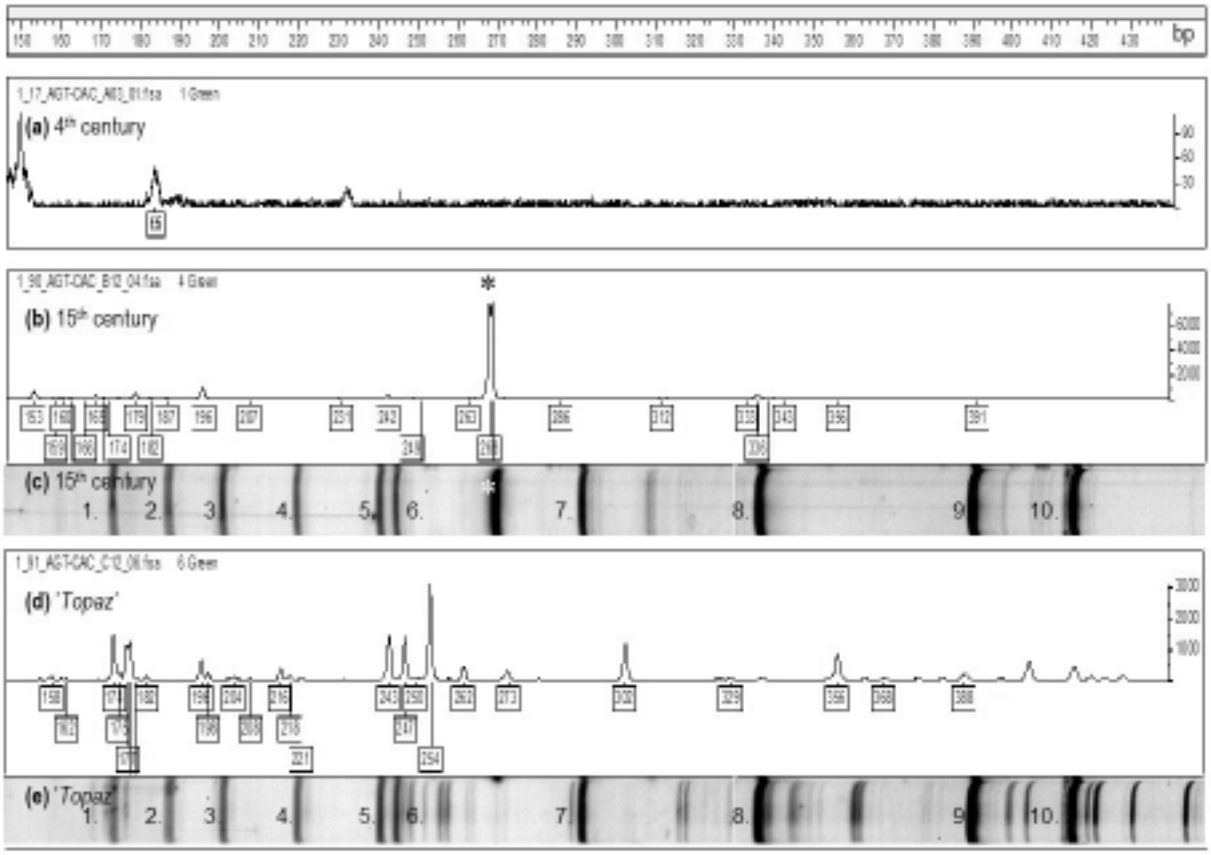

Figure 1. Samples of the fAFLP (*Eco-AGT – Mse-CAC) analysis of the aDNA samples extracted from seed remains of common millet (*Panicum miliaceum*) recovered from the 4th century (a), and from the 15th century (*mam*) (b), compared to 'Topaz' (d). Fragment sizes are indicated by boxed numbers. Samples of PAGE (c, e) show the quantity of AFLP fragments with size standards of [1]160, [2]180, [3]201, [4]212, [5]238, [6]242, [7]307, [8]404, [9]527, [10]622 in bp. * indicates a characteristic fragment of 272 bp in the 15th century sample.

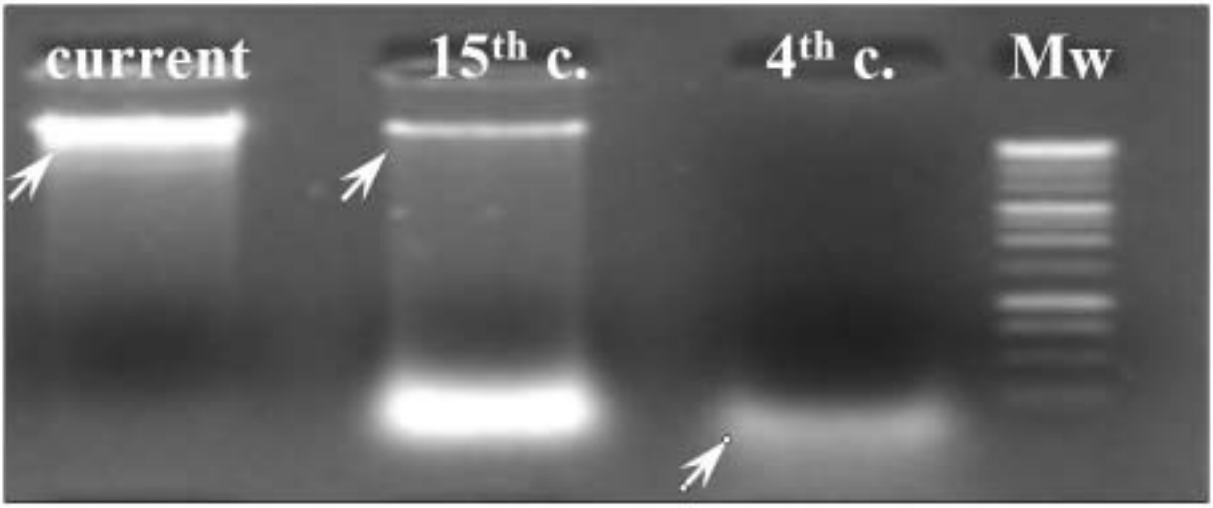

Figure 2. Total DNA (arrows) of archeobotanical samples of common millet compared to variety 'Topaz' (current). The electropherogram shows different levels of degradation (Mw, DNA ladder 3 kb)

Common millet remains have been excavated from various different ages such as the 1st century at Pompeii or the 13th - 14th centuries at Prague (Robinson 2002), but no DNA recovery has been reported. For safe DNA analysis the most important step is to eliminate both the exogenously and endogenously infected seeds because bacterial or fungal DNA-remains can contaminate the plant DNA being studied. So, we surface sterilized the individual seeds thoroughly and kept them in aseptic tissue culture *in vitro* for a three month period. DNA was extracted only from the non-infected seeds. For comparative analysis the modern common millet variety 'Topaz' was included (Figure 2).

Agarose gel electrophoresis suggested that extensive DNA degradation had occurred in the 4th century sample (Figure 2), and it was so serious that even no RAPD amplification succeeded in the preliminary experiments (not detailed) using sixty primers of Operon A, J, and AB sets. This was an unexpectedly negative result compared to the successful RAPD fragment amplification of a 1200 year old rice sample (Nakamura & Sato 1991) and even with 13010 and 17310 year old carbonized/burnt quasi-rice samples recovered from the famous excavation in South Korea (Suh et al. 2000). The samples from the 15th century and the modern variety 'Topaz' showed regular RAPD polymorphisms but with less band numbers in *mam* (not shown).

The AFLP analysis of the 4th century millet revealed a fragment of 85 bp length, and a longer one at about 150 bp length (Figure 1a), but both had such low intensity that further fragment purification did not succeed. In contrast, the DNA from the 15th century sample was much less degraded (40.0 %) with high molecular weight DNA still present (Figure 1, Table 1). Consequently, 158 AFLP bands could be detected (Figure 1). However, this AFLP fragment number was still substantially less than the 265 AFLP bands (100.0 %) that we detected in the contemporary millet variety 'Topaz' (Table 1). All the AFLP samples were loaded onto PAGE gel. Twenty-one PAGE fragments (Figures 1b, 1c) were recovered from the gel and were cloned for further sequence analysis. Further AFLP analysis with more primer combinations and subsequent fragment analysis is in progress.

Table 1. Total numbers, % and degradation (%) of the fAFLP-ABI fragments (relative intensity over 100 units, 150-600 bp) of common millet recovered from the 4th, and 15th centuries compared to 'Topaz'. The selective AFLP primer combinations are: Mse-CAC- combined with -[a]Eco-AAT*, -[b]Eco-ACC*, and -[c]Eco-AGT*; and Eco-AGT* combined with -[d]Mse-CAA, -[e]Mse-CAG, -[f]Mse-CAT, -[g]Mse-CCC, -[h]Mse-CCT, -[i]Mse-CGA, -[j]Mse-CGC, and -[k]Mse-CTA

	fAFLP fragments / AFLP primer pairs (a to k)											Total		
	a	b	c	d	e	f	g	h	i	j	k	n	%	degr. %
4th c.			2									2	0.8	99.2
15th c.	10	18	24	34	29	12	16	5	5	3	2	158	60.0	40.0
'Topaz'	32	23	38	42	34	33	18	17	7	4	16	265	100.0	0.00

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Molecular and agro-morphological variation in sorghum (*Sorghum bicolor* (L.) Moench) germplasm from Sudan and ICRISAT under drought stress condition

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ABSTRACT: Low and erratic rainfall constitutes a major constraint to sorghum cultivation and impedes sorghum yield improvement in semi-arid climate zones. For estimating genetic and agro-morphological variability of sorghum under drought stress conditions, 40 sorghum genotypes were analysed with 16 simple sequence repeats (SSRs) and evaluated under natural drought condition across three environments. In total 98 polymorphic bands were detected with a mean of 6.1 alleles per SSR locus. By this approach each accession is uniquely fingerprinted. Genetic similarity estimates ranged from 0 to 0.88 with a mean of 0.32. The polymorphic information content (PIC) for SSRs ranged from 0.33 (SB34) to 0.86 (SB10). Diversity index for all accessions was 0.67. Within sub-groups, DI ranged from 0.47 (Mugud group) to 0.72 (Milo group). Mantel statistics revealed a good fit of the UPGMA cluster to the original genetic similarity data (r = 0.88). UPGMA clustering produced two main clusters comprising genebank accessions cluster and cultivars, landraces and synthetic cluster. Grouping of accessions by UPGMA cluster analysis matched with the pedigree information and morphological characters (Feterita, Mugud, and Milo types), indicating the strong differentiation among the sorghum materials. Significant differences were detected among the genotypes for all traits measured under stress condition, which included: days to flowering, plant height, grain yield/plant, growth rate, biomass, 1000-grain weight and harvest index. Based on the yield superiority under stress condition, relative yield, and days to 50 % flowering some genotypes could be selected to be grown under stress condition, and included as parents in breeding programs.

Key words: Drought tolerance – genetic diversity – molecular marker – SSRs

Introduction

Sorghum (*Sorghum bicolor* (L.) Moench) is a very important crop in the Sudan serving as a primary source of food, beverage and total livelihood for millions of people in the country (Grenier et al. 2004). Nearly 80 % of the total grain production in the country is obtained from sorghum (FAO 2001). However, the average yield per unit area in Sudan is relatively low (583 kg/ha) in comparison to the average world yield level (1370 kg/ha) demonstrating the vital need for improving the efficiency of sorghum breeding to increase the productivity. Moreover, Sudan is within the geographical range where sorghum is believed to be domesticated for the first time (Mann et al. 1983) and where the largest genetic variation in sorghum is found (Doggett 1988).

Materials and Methods

A total of 40 sorghum genotypes of five different groups (16 *Feterita*, 8 *Milo*, 10 *Synthetic*, 3 *Mugud* and 3 *Hegiri* types) were analysed with 16 SSRs markers (Abu Assar et al. 2004).

Based on these SSR fingerprints genetic similarity (GS) was estimated according to the formula of Nei & Li (1979):

$$GS = \frac{2a}{2a + b + c}$$

where *a* refers to alleles shared between two accessions, and *b* and *c* refer to alleles present in either one of the accessions regarding a pair-wise comparison of the genotypes: UPGMA-cluster analysis was carried out by using NTSys-pc. The goodness of fit of the UPGMA-clustering in comparison to the computed similarity indices was estimated by Mantel statistics. Polymorphic information content (PIC) for each of the SSRs was estimated by determining the frequency of alleles per locus and further on genetic diversity (DI) across all loci was estimated according to Nei (1973):

$$DI = n_a(1/n_l \sum_j (1 - \sum_i x_{ij}^2))/(n_a - 1)$$

where x_{ij} is the frequency of the *i*th allele of locus *j*, n_l is the number of genetic loci, and n_a is the number of accessions.

The same 40 genotypes were evaluated in field trials in Sudan at two locations (Medani and El-Rahad) in two seasons (2002 and 2003) and under two water regimes namely: normal (fully irrigated) and stress (partially irrigated) by using split-plot design with three replicates, water treatments assigned in main plots and genotypes randomised in sub-plots. The agro-morphological assessment was carried out on 40 genotypes and includes the following traits: days to 50 % flowering (DFF), plant height (PHT) in cm, yield/plant (YS) in g, plant growth rate (GR) in cm day^{-1}, biomass (BM) in g 1000-grain weight (TGW) in g and harvest index (HI) in %. Statistical analysis was done using SPSS 11.5 software. The traits performance under stress and normal conditions were used to estimate drought parameter index (relative performance, P_{rel}) according to the formula $P_{rel} = \frac{P_{stress} \times 100}{P_{normal}}$ where, P_{stress} and P_{normal} stand for the performance under stress and normal conditon, respectively.

Results and discussion

Molecular characterization

The 40 accessions of 5 different morphological groups and some genebank accessions included in this study encompass a relatively broad array of germplasm diversity (Sudan and ICRISAT, India). The 16 SSR primers cover all of the 10 linkage groups (A to J) and were able to uniquely fingerprint each of the 40 sorghum germplasms. All SSR markers generated polymorphic patterns confirming their usefulness for genetic analysis of sorghum germplasm. In total 98 alleles were detected with an average of 6.1 alleles per locus, varying between 3 to 11 alleles per locus. The estimated PIC values ranged from 0.33 (SB34) to 0.86 (SB10). The genetic similarity of the investigated set ranges from 0 to 0.88 resulting in a low mean value of GS = 0.32. The dendrogram generated from the UPGMA cluster analysis shows two main clusters differentiated in nine significant sub-clusters related to morphological characters and/or pedigree (Figure 1). Mantel statistics revealed a good fit of the cophenetic values to the original similarity data set (r = 0.88). Accordingly, these results suggest that the dendrogram based on the estimated genetic similarity reflects pedigree and varietal relationships, according to previous reports on sorghum-inbred lines (Ahnert et al. 1996). The overall mean genetic diversity (DI) in this study was 0.67. However, the mean DI found within the *Feterita* group was 0.65, *Milo* group 0.73, *Synthetic* group 0.48, *Mugud* group 0.47 and *Hegiri* group 0.57. The highest genetic diversity was found within *Milo* group (DI = 0.73), but this is not surprising since Sudan is a part of the centre of origin of sorghum (Doggett 1988), where high natural variability is expected.

Agro-morphological assessment

Significant differences were detected among the genotypes for all measured traits (Table 1). The yield per plant under stress condition varied from 20 to 57 g with an average of 39 g plant^{-1}, while the relative yield ranged from 52 to 85 % with an average of 75. Days to 50 % flowering, which is an indicator for escaping drought, varied from 61 to 94 days with an average of 75 days and stress caused 5 days delay in flowering. Plant height also showed significant variation among the genotypes, it ranged from 119 cm to 265 cm with an average of 183 cm, and the relative plant height ranged from 81.1 to 91.9 % with an average of 86 %. The growth rate under stress varied from 1.6 to 3.4 cm day^{-1} with an average of 2.5 cm day^{-1}, while the relative growth rate ranged from 77.1 to 87.7 % with an average of 81.0 %. 1000-grain weight ranged from 16.1 to 28.9 g with an average of 23 g. Stress on average caused 13 % reduction in relative 1000-seed weight. Biomass varied from 82 to 223.1 g plant^{-1} with an average of 142.4 g. The relative biomass was reduced by 25.5 %. On the other hand, harvest index under stress ranged from 22 to 42 % with an average of 31 % and the relative HI ranged from 73.7 - 132.8 % with an average of 102.1 %. Under stress condition the harvest index increased by 2.1 %, which could result from the reduction in the biomass. Based on yield superiority under stress conditions, the best genotypes were: 'Wad Ahmed' (57 g), 'ICSR 92003' (54 g), 'Feterita Eriana' (53 g), 'El Najada' (53 g) and 'ICSR 91030' (52 g). Upon relative performance, the best genotypes were: 'Arfa Gadamak' (85%), 'Wad Ahmed' (84 %), 'El Nnajada' (84 %), 'Koracola' (83 %), 'ICSR 92003' (83 %), and 'Sham Sham' (83 %). However, based on days to 50 % flowering, the earliest genotypes were *PI 569695* (60 days), *PI 570446* (60 days), *PI 569953* (60 days), 'Dwarf White Milo' (61 days) and *PI 569951* (61 days). All high yielding genotypes were more or less clustered together in the first main cluster across two sub-groups, while the early genotypes were clustered together in the second main cluster with exception of 'Dwarf White Milo' (Figure 1).

Table 1. Environmental means, ranges, standard errors, and significances for grain yield, days to 50% flowering, plant height, growth rate, 1000-grain weight, biomass plant^{-1} and harvest index under stress condition and as relative value of the control across 3 environments

Traits	Unit	Mean	Min.	Max.	SE±
Yield/plant (YS)	g	39	20	57	0.58**
Relative YS	%	75	52	85	0.67**
Days to 50% (DF)	days	75	61	94	0.59**
Relative (DF)	%	107.2	102.9	111	0.34**
Plant height (PHT)	cm	183	119	265	2.67**
Relative PHT	%	86.2	81.1	91.9	0.55**
Growth rate (GR)	cm/day	2.5	1.6	3.4	0.04**
Relative (GR)	%	81	77.1	87.7	0.53**
1000-grain weight (TGW)	g	23	16.1	28.9	0.29**
Relative TSW	%	86.4	77.7	94.9	0.71**
Biomass/plant (BM)	g	142.4	82	223.1	2.9**
Relative BM	%	74.5	61.6	87.8	0.76**
Harvest index (HI)	%	0.31	0.22	0.42	0.01**
Relative HI	%	102.1	73.7	132.8	0.01**

** significant at 0.01 probability level

From this study the SSR data proved to be useful in identifying genetic relationships among a diverse collection of accessions, with the majority of the accessions clustering in concordance with pedigree relationships and morphological information. Based on the yield superiority under stress conditions, the superior genotypes can be grown in moderately dry areas, and on

the basis of relative performance and days to 50 % flowering, the genotypes can be used as parents in further breeding programs. Based on both the molecular information and morphological traits it is expected to enhance the process of incorporation of many desirable genes into well-adapted varieties.

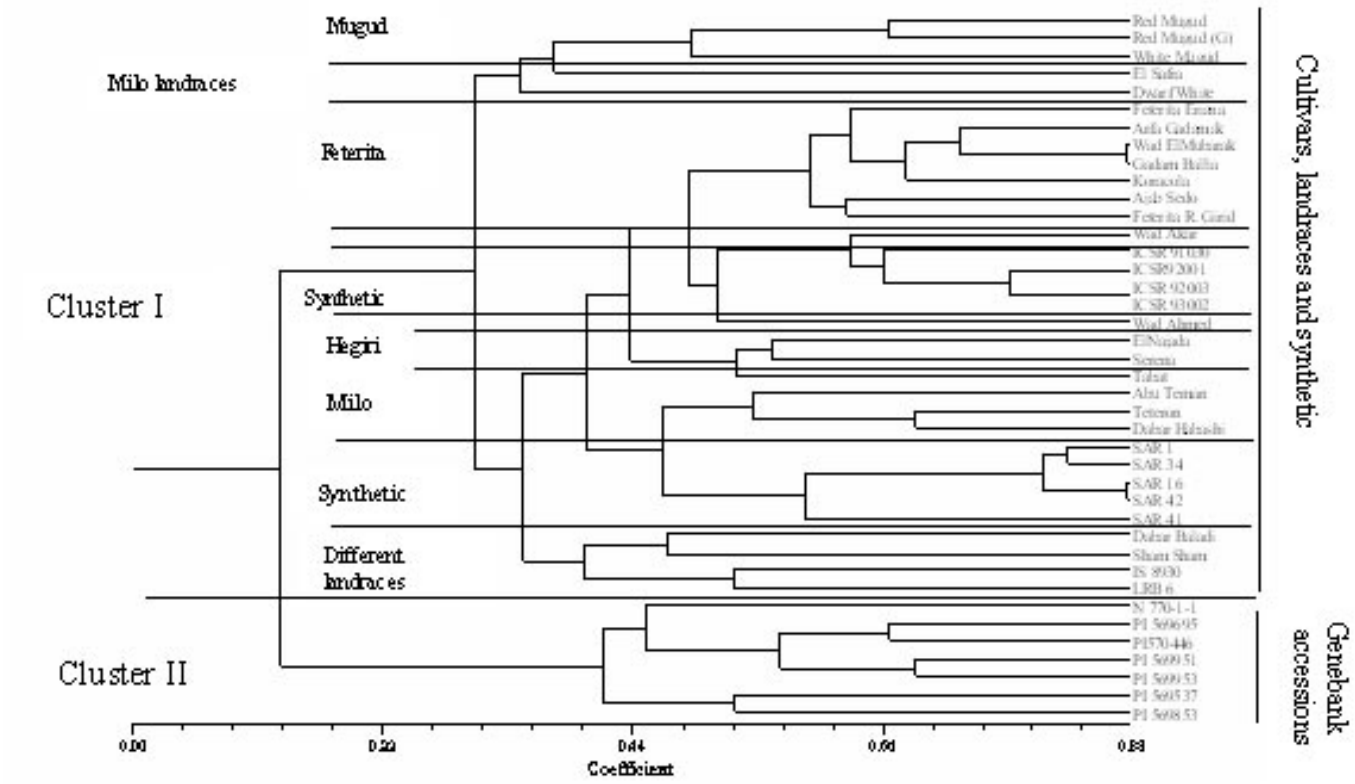

Figure 1. Dendrogram generated by UPGMA cluster analysis showing the relationships among 40 sorghum accessions based on Nei & Li similarity.

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A mutation altering auxin sensitivity and root morphology in rice (*Oryza sativa* L.)

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ABSTRACT: Formation of lateral root is the key way by which a plant increases its absorptive area. In an effort to understand the mechanism of lateral root formation in rice, we isolated a monogenic recessive mutant, *lrm1* (lateral root mutant) which has been isolated in rice. The mutant exhibited significant reduction both in the number and length of lateral roots. The mutant produced lateral root primordia normally as WT, but most of the primordia failed to emerge through the epidermis of the seminal root. The emerged lateral roots in the mutant also showed defects in elongation and as a result the length of lateral roots was significantly reduced as compared to WT. The mutant was also defective in seminal root elongation. The mutant was more resistant to exogenous IAA but did not show resistance to 2,4-D. To exogenously applied NAA, the mutant showed resistance response only at lower concentration. Exogenous application of NAA significantly increased the number of lateral roots in the mutant, but did not promote lateral root elongation in the mutant. In contrast, IAA failed to increase the number and length of lateral roots in the mutant. Phosphate availability was found to restore the growth of lateral roots and sensitivity to exogenous IAA inhibition of root elongation in the mutant.

Key words: Auxin – lateral root – mutant – *Oryza sativa* – phosphate – rice

Introduction

Lateral roots contribute to water-use efficiency and facilitate the extraction of micro- and macronutrients from the soil. Lateral root is derived from the pericycle of parent root. The mature cells in pericycle, once stimulated, dedifferentiate and proliferate to form a lateral root primordium (LRP) and then lead to LR (lateral root) emergence. After emergence, LRP undergoes an activation step to form a fully functional meristem and a mature lateral root can develop through the activity of this LR meristem (Malamy & Benfey 1997). Auxins have been shown to regulate the formation of lateral roots. The *axr1* and *axr2* mutants of *Arabidopsis* are auxin resistant and produce fewer lateral roots than WT (Estelle & Somerville 1987). Lateral root development is influenced strongly by environmental factors. The molecular basis of the adaptive responses of plants root growth to different environmental stimuli remained poorly understood in rice. In this paper, we describe a novel mutant in rice that initiates LRP normally but shows defects in LRP emergence and elongation. Exogenous phosphate was able to restore the growth of lateral roots in the mutant.

Materials and methods

Mutant screening

Screening was performed using R_1 generation of 768 lines generated by means of retrotransposon *Tos17*-mediated mutagenesis system from a japonica rice cultivar, Nipponbare (Hirochika 1999). Twenty R_1 seeds from each line together with WT were germinated and grown in water culture for two weeks. We selected one line that was specifically affected in lateral root formation. R_2 seeds from this line were harvested separately and the altered root phenotype was verified in R_2 generation. Southern analysis with *Tos17*-specific probes confirmed that the mutation is not tagged by the retrotransposon *Tos17*.

Morphological characterization
The mutant and WT seedlings were grown in water culture at 25°C in an incubator and data on different morphological characters were taken. For lateral root primordia observation, the whole roots were fixed in ethanol: glacial acetic acid mixture (3:1) and cleared by sodium hypochlorite solution and then stained with methylene blue and observed under the microscope.

Growth regulator assays
To control the sensitivity of the mutant roots to exogenous auxins, the germinated seeds of WT and the mutant were grown in different concentration of IAA, NAA, 2,4-D or distilled water as the control. After seven days of growth, seminal root length was measured.

Response to phosphate availability
The germinated seeds of WT and *lrm1* mutant were grown either on the nutrient solution containing 0.18 mM $(NH_4)_2SO_4$, 0.27 mM $MgSO_4.7H_2O$, 0.09 mM KNO_3, 0.1 mM KH_2PO_4, 0.05 mM K_2SO_4, 0.18 mM $Ca(NO_3)_2.4H_2O$, 0.04 mM NaEDTA-$Fe.3H_2O$ and 0.08 mM Na_2SiO_3 (+P) or on phosphate depleted solution containing all the nutrients mentioned above except for 0.1 mM KCl instead of 0.1 mM KH_2PO_4 (–P). After 12 days of growth data on lateral root number and length were recorded. To see the sensitivity of root growth to exogenous auxin in presence of phosphate, the seeds of WT and mutant were grown in +P solution with different concentration of IAA.

Results and discussion
In F_2 population of the cross between the mutant and WT, the segregation of the WT and mutant phenotype statistically fitted to the 3:1 ratio, indicating that the mutation was caused by a single recessive gene. Morphological characterization revealed a significant reduction both in the number and length of lateral roots in *lrm1* mutant as compared to WT. Observation of lateral root primordia formation revealed that the mutant can produce LR primordia normally, but showed defects in subsequent emergence and elongation (Table 1, Fig. 1). The mutant also showed shorter seminal root than WT but showed similar plant height as WT.

Table 1. Morphology of WT and *lrm1* mutant at the seedling stage

Character	WT	*lrm1*
Plant height (cm)	11.1 ± 0.3	10.6 ± 0.4
Seminal Root length (cm)	11.8 ± 0.2	7.6 ± 0.2
Number of LR/cm	10.3 ± 1.2	2.1 ± 0.6
Number of (LR+LRP)/cm	14.3 ± 1.5	15.0 ± 2.7
% LRP not emerged	27.3 ± 10.0	86.5 ± 4.5
Lateral root length (mm)	6.25 ± 0.7	1.3 ± 0.2

Rice seedlings were grown in water culture for 10 days. Number of lateral root (LR) and lateral root primordia (LRP) were counted from each seminal root. For lateral root length, the length of the 10 longest lateral roots from each seminal root were measured. Data are averages of 10 seedlings (± S.D.).

As auxins are known to play important roles in LR development, we checked the sensitivity of the mutant to inhibition of root elongation by exogenous auxins. Results showed that the mutant has stronger resistance response to IAA (Fig. 2) but did not show any resistance response to 2,4-D. To NAA, the mutant showed more resistance than WT only at lower concentration (data not shown). These results suggest that the defective lateral root formation

in *lrm1* mutant might be due to the reduced sensitivity to auxin. In *Arabidopsis*, auxin resistant mutants were reported to produce fewer LRs (Estelle & Somerville 1987).

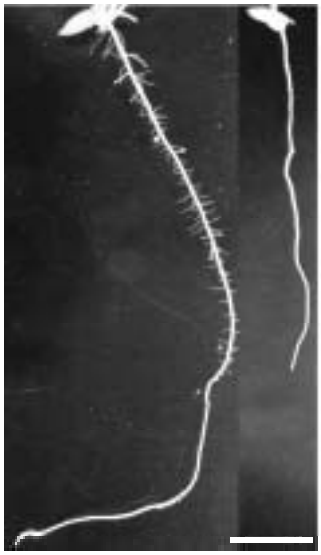

Figure 1. Lateral roots on seminal root of WT (left) and *lrm1* mutant (right). Bar = 10 mm.

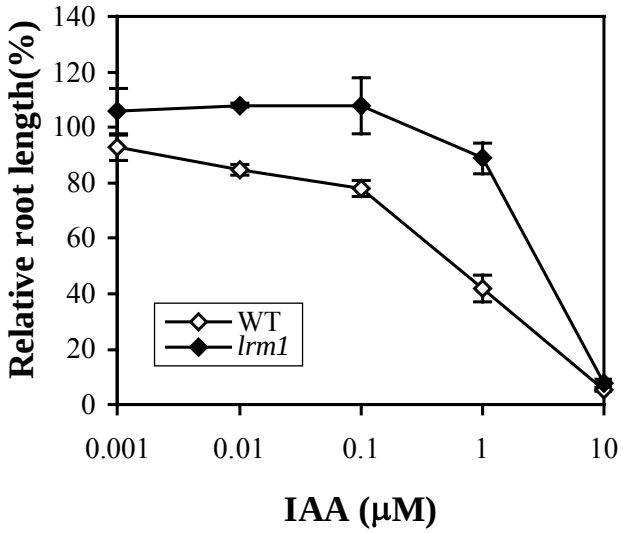

Figure 2. Effect of exogenous IAA on root growth of WT and *lrm1* mutant seedlings. Values represent the mean of 10 seedlings and bars indicate S.D.

We checked the effect of exogenous IAA and NAA on LR formation in WT and *lrm1* mutant. The results showed that NAA at a concentration of 0.1 μM significantly increased the number of LR in mutants but failed to stimulate LR elongation in *lrm1* mutant. In contrast, IAA failed to promote either LR number or LR elongation in the mutant (data not shown).

Lateral root development is greatly influenced by the environmental stimuli and the adaptive response to environmental stimuli sometimes regulated by changing hormone sensitivity (Lopez-Bucio et al. 2002). As phosphate influences LR development in *Arabidopsis*, and the sensitivity of root growth was also reported to be influenced by phosphate availability, we checked the effect of phosphate on LR formation in WT and *lrm1* mutant. Our results showed a dramatic increase of LR density by phosphate in *lrm1* mutant (Table 2). The length of LRs also increased significantly in the mutant while grown in +P solution, that is comparable to WT of untreated control (–P solution).

Table 2. Effect of phosphate on lateral root formation in WT and *lrm1* mutant

Treatment	LR density		LR length (mm)	
	WT	*lrm1*	WT	*lrm1*
–P	9.5 ± 0.9	3.2 ± 0.9	8.6 ± 0.9	1.5 ± 0.3
+P	10.4 ± 0.7	32.4 ± 2.9	8.7 ± 0.8	6.9 ± 1.1

Seedlings were grown in –P and +P solution for 12 days, and then the number of LR per centimetre of seminal root (LR density) were calculated. For LR length, the length of 10 longest LR from each seminal root was measured. The nutrient solutions were renewed every 3 days. Data are averages (± S.D.) of 10 seedlings.

As the *lrm1* mutant is resistant to IAA and phosphate application restored the LR growth in the mutant, we checked the sensitivity of the mutants root growth to IAA in the presence of phosphate (Figures 3 and 4). Our results showed that the *lrm1* mutant showed normal sensitivity to IAA in the presence of phosphate. These results suggest that the defects in LR

formation in *lrm1* mutant might be due to reduced auxin sensitivity of the roots and that the sensitivity of auxins is being regulated by phosphate availability.

Figure 3. Lateral root formation by phosphate availability. a) Roots of the seedlings grown in –P solution. WT (left) and *lrm1* (right). b) Roots of the seedlings that were grown in +P solution. WT (left) and *lrm1* (right). Bar = 10 mm.

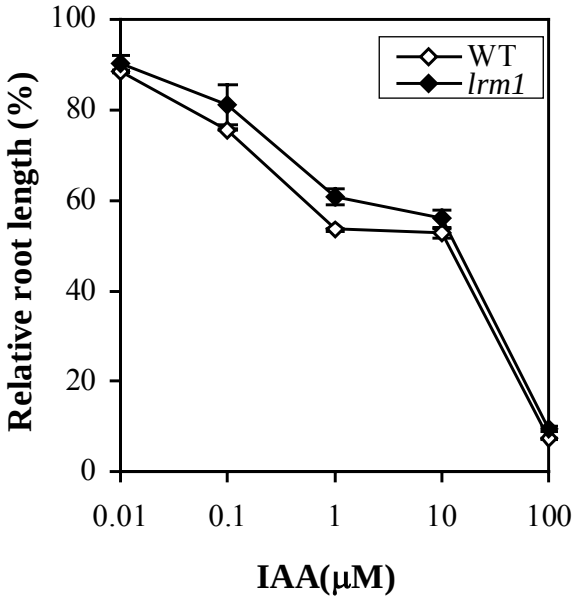

Figure 4. Sensitivity of root growth to IAA in the presence of phosphate (+P). Seedlings were grown in indicated concentrations of IAA in +P solution for 7 days and seminal root length was measured.

Acknowledgements

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Assessment of genetic erosion in Slovenian common bean germplasm

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ABSTRACT: Common bean cultivation has a long history in Slovenia. Numerous landraces have been developed that are still used in bean production. More than 1000 accessions that differ in various morphological, biochemical and molecular characteristics are stored in the germplasm collection at the Agricultural Institute of Slovenia, including a set of 55 accessions more than 50 years old. Genetic similarity between newly acquired and the old set of 'Češnjevec' landrace accessions collected in the 1950ies was analyzed with RAPD and microsatellite markers in order to determine the level of genetic erosion. The results of molecular analysis did not reveal any complete allele loss in the new Slovenian 'Češnjevec' landrace collection, but introgression of new alleles and allele frequency shifts were detected.

Key words: Common bean – genetic erosion – genetic variability – *Phaseolus vulgaris*

Introduction

Common bean (*Phaseolus vulgaris* L.) is one of the most important food legumes for human consumption in the world. At first, two major gene pools, Mesoamerican and Andean were recognized (Gepts 1988). Later, the third gene pool was described in Northern Andes, which contains unique genetic diversity and which could be ancestral in the evolution of common bean (Debouck et al. 1993, Debouck 1999).

In Slovenia, common bean has been cultivated already for centuries, which resulted in development of numerous landraces; many of them are cultivated still nowadays. In 1990's, accessions of common bean were collected from various parts of Slovenia, and today a fairly large *ex-situ* collection of almost 1000 accessions exists at the Agricultural Institute of Slovenia (AIS). Newly acquired accessions were classified using biochemical and molecular data into two distinct groups in which the majority of accessions clustered near the Andean group (Meglič et al. 1999). 15 Slovenian accessions did not contain any Andean gene pool check lines. This suggests that these genotypes are significantly different from any *P. vulgaris* genotypes previously characterized with RAPDs, representing a unique set of germplasm, which is valuable to be preserved and to be used in the breeding programme.

The continuing adaptive change and development of agriculture has always been associated with genetic erosion, this being the loss of formerly favoured crop varieties, genes or alleles (Engels & Wood 1999). The collection at the AIS holds also a set of 55 bean accessions collected in 1950's. In this study, an attempt was made to analyze genetic similarity between newly acquired and the old set of 'Češnjevec' landrace accessions based on molecular marker analysis.

Materials and methods

Plant material

20 newly acquired 'Češnjevec' and five old 'Češnjevec' accessions were selected on the basis of seed morphology and passport data. 15 morphologically different accessions from the collection at AIS were included as outgroups.

DNA isolation and genetic analysis

DNA was isolated from single seeds with GenElute Plant Genomic DNA Miniprep Kit (Sigma). Genetic variability was studied using RAPD and microsatellite markers. Seven RAPD markers from Operon series were tested on 21 samples. Data were scored for presence and absence of amplification fragments. The Jaccard (J) coefficient of similarity was used to construct a UPGMA dendrogram. All calculations were performed using the NTSYS computer package (Rohlf 1993). 46 bean samples were analysed for ten microsatellite loci (ATA2, ATA3, ATA4, ATA5, ATA6, ATA7, ATA9, ATA13, ATA16, ATA20; Metails et al. 2002). Population genetics statistics were computed using the GENETIX software (Belkhir et al. 1998).

Results and discussion

An UPGMA dendrogram, representing relationship among new and old 'Češnjevec' landraces, based on RAPD analysis is presented in Figure 1. S17, S30, S36, S47 and S48 are old 'Češnjevec' landraces, whereas accessions 316, 358 and 'Emergo' were chosen as outgroups. Genetic erosion of common bean was evaluated by comparing microsatellite allelic diversity of 5 old and 20 new local populations of landrace 'Češnjevec'. Microsatellite markers are highly polymorphic; their length polymorphism results from a variable number of tandem repeats. Thus, they are the markers of choice for genetic studies (Metais et al. 2002). 10 microsatellite primer pairs used in this study detected polymorphism with 4-14 alleles per locus (Figure 2). However, the results did not reveal any complete allele loss in the new Slovenian 'Češnjevec' landrace collection. Moreover, introgression of new alleles and allele frequency shifts were detected which could be considered as a genetic pollution of the original 'Češnjevec' landrace genotype, possibly due to population mixture, recombination or mutation during the past five decades. The results of RAPD and microsatellite analysis were very congruent; they both did not reveal any complete allele loss in the new Slovenian 'Češnjevec' landrace collection.

The research with microsatellite markers has recently been extended, with higher number of markers being applied. More Slovenian accessions as well as control accessions for the Andean, Mesoamerican and Northern Andean genepools were included in the study. A more comprehensive study of genetic variability of common bean has also been launched recently in which 120 accessions are being examined by AFLP-marker system. First results of the analysis confirm the existence of unique Slovenian germplasm previously determined by RAPD marker system (Meglič et al. 1999).

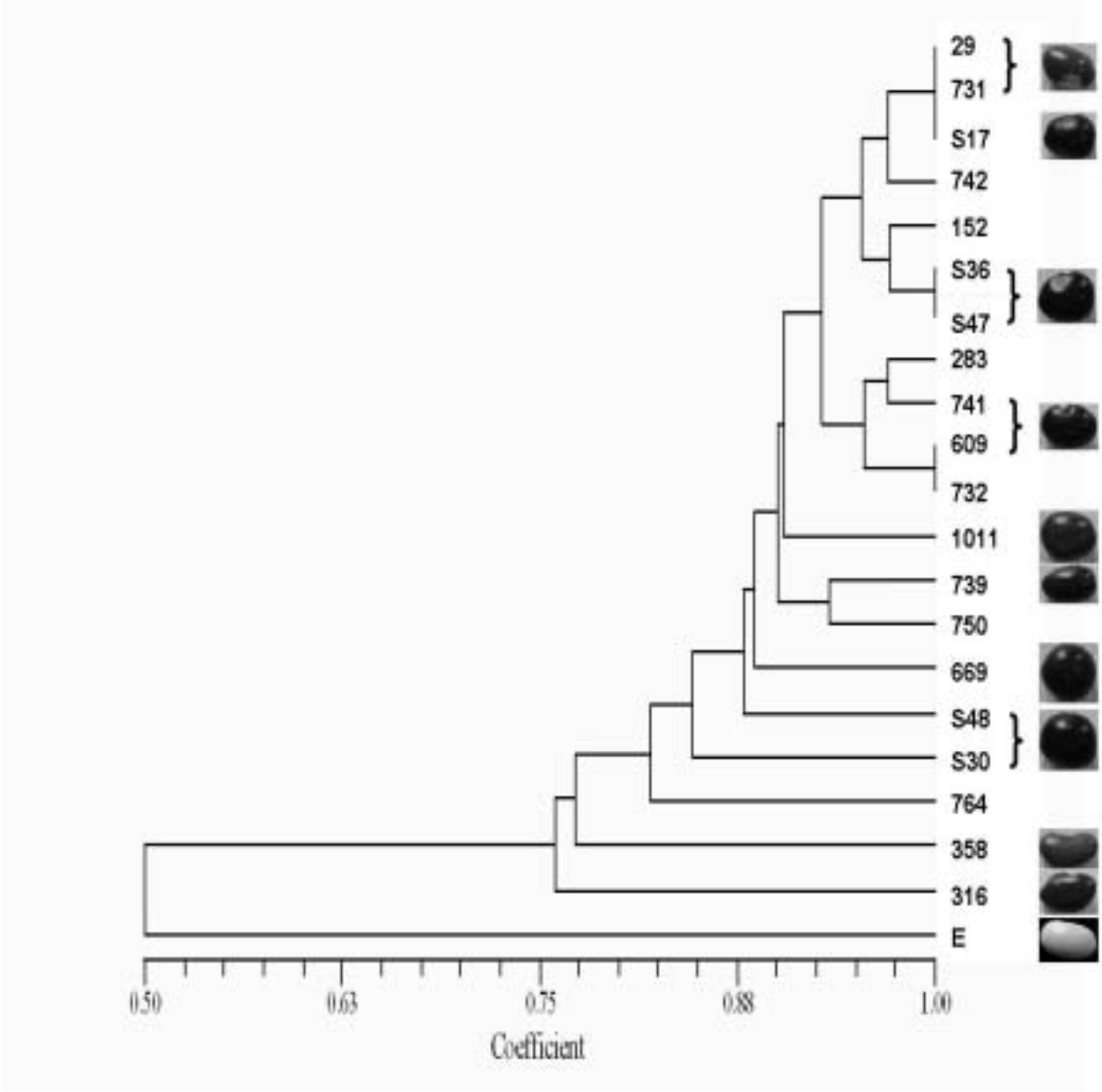

Figure 1. UPGMA dendrogram representing the relationship among the present and the old 'Češnjevec' landraces (S17, S30, S36, S47, S48) based on RAPD analysis

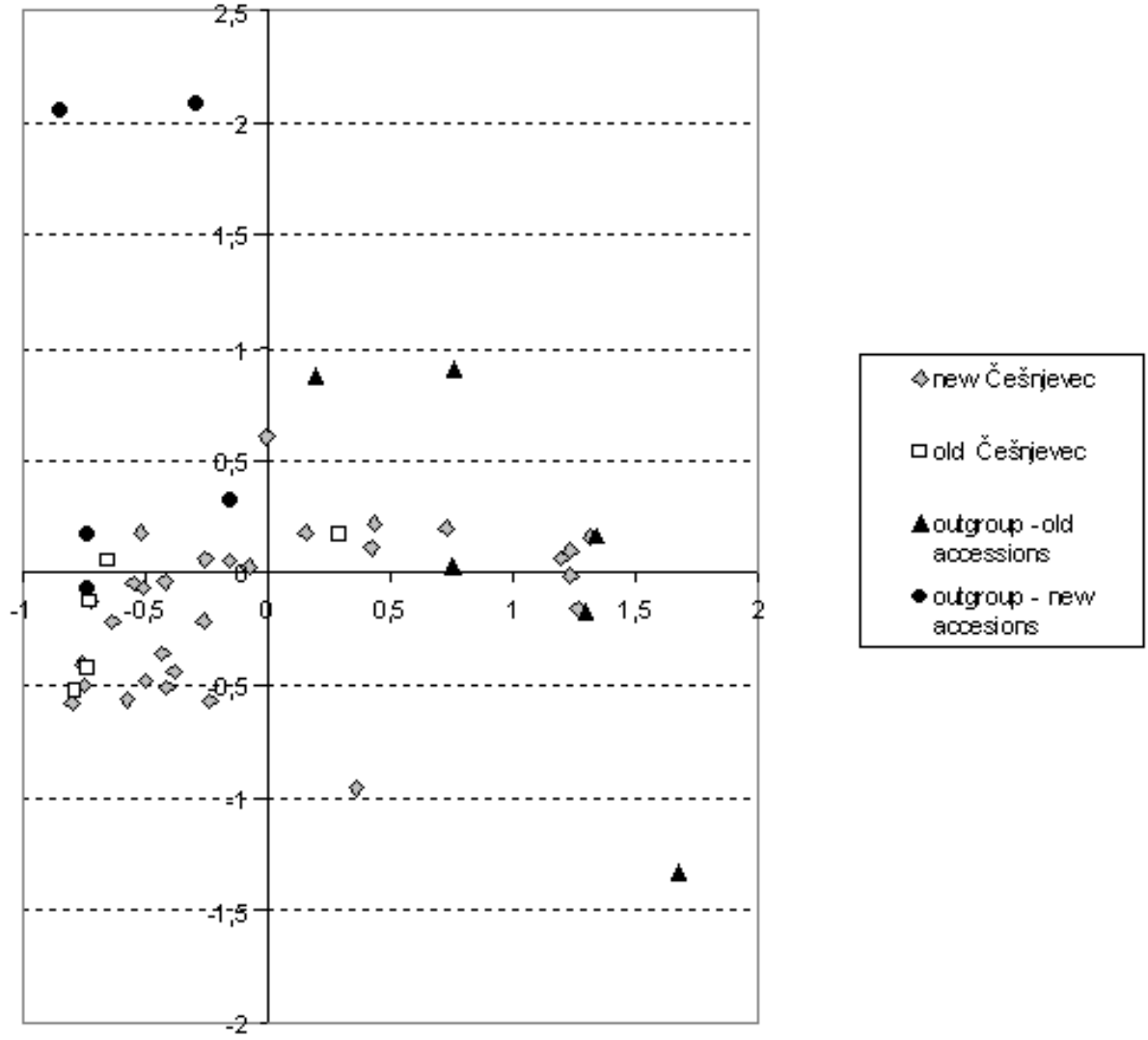

Figure 2. Distribution of 'Češnjevec' and control accessions (out-group) according to correspondence analysis based on 10 microsatellite loci

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Ecogeographical distribution and biodiversity of winter vetch (*Vicia villosa* Roth) in Lithuania

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ABSTRACT: Winter vetch (*Vicia villosa* Roth.) belongs to the element of European flora. It is known as a fodder plant and agrophytocenoses component. In the "Genefund" program the vetch has been investigated at the Lithuanian University of Agriculture since 1998. A collection of 48 accessions of different cenopopulations has been accumulated. Ecogeographical investigation demonstrated that the plant is mostly found in sandy eastern, south-eastern and south-western regions. *Ex situ* investigation revealed great phenological and biomorphological diversity of cenopopulations. The most varying parameters were as follows: the number of stem branchings, inflorescences of one plant, number of pods, stem height at the beginning of flowering, stalk length. The least varying were pod parameters: length, width. Correlations were established between stem height and the number of branchings (r = 0.34), stem height and the number of pods (r = 0.51), stem height and raceme stalk height (r = 0.52). According to morphometric parameters three groups of winter vetch were identified. The biggest seeds (weight of 1000 seeds - 23.3 g) were found in the group of lowermost vetch. In the flowering stage the amount of raw protein was up to 25 percent in the aboveground part. Differences in protein amount within cenopopulations were lower than differences between parameters. Under the same growing conditions (*ex situ*), phenological, morphometric and immunological differences were established in separate (geographically remote) cenopopulations of *Vicia villosa*. Seed number in a pod and weight of 1000 seeds were the most genetically determined parameters. Winter vetch is a polymorphic plant. Investigations revealed high diversity both of intracenopopulations and cenopopulations (individuals).

Key words: Adaptation – diversity – phenotypic plasticity – *Vicia villosa*

Introduction

Plant species are vanishing and species biodiversity is shrinking due to global warming and other processes on the earth. Since each individual is genetically determined, extinction of one plant impoverishes species' genetic resources. High species biodiversity provides better chances for survival. The main aim of preservation of genetic resources is to preserve genetic diversity formed in the adaptation process, i.e. to preserve such level of genetic diversity which would provide sufficient reserve for adaptation of populations in changing conditions and their evolution. In the world there are around 50 000 species of plants that animals are feeding, but only a small part of these species is cultivated. One of such plants is winter vetch (*Vicia villosa* Roth.).

V. villosa Roth. is found in Europe, the Mediterranean, Central Asia, Caucasus (Vulf & Maleeva 1969) mainly in winter crops, especially rye (Grigas 1971). It's mainly the plant of light, poor and acidulous soils. In many countries it is used for fodder as a productive, well-eaten annual plant (Lazauskas & Dapkus 1992). As it is rich in green mass with a more powerful root system (Ziolek et al. 1987) than the common vetch (*V. sativa* L.), it is usually sown in mixtures with cereals in Lithuania. In various countries winter vetch is grown not only for fodder but as green mulch for vegetables (Hanano et al. 1987), in weedy soils or as a means against soil erosion (Fujii & Araki 2000).

In Lithuania winter vetch, like the wild one, is a rather rare plant (Vilkonis 2001), mostly occurring in the southern and eastern parts of the country and hardly found in the northern districts. In Lithuania, four forms of *V. villosa* (Grigas 1971) were found and

identified. Its breeding was carried out from 1934 till 1952. Over 30 accessions of local winter vetch were accumulated from which several winter hardy breeding numbers and variety 'Pūkiai' were released. Meanwhile, after the breeding had been stopped, seed viability greatly declined due to inadequate seed storage, aberration of chromosomes increased and the variety was lost (Sliesaravičius 1999). Variety 'Pūkiai' was preserved only at the Russian Plant-Growing Institute (Sankt Petersburg), wherefrom in 2001 a small sample of seeds was obtained and at present we try to produce seed for long-term storage and research. The aim of this work was to investigate and preserve the diversity of *V. villosa*,as well as to select perspective forms for breeding.

Materials and methods

The research material (seeds) of *V. villosa* was accumulated in all the territory of Lithuania in the period 1998 - 2002. Specific composition of phytocenoses, granulometric composition and acidity of soil were determined.

The accumulated seeds were sown and investigated in the collection of LUA Experimental Station (central Lithuania, medium loam, pH 7) in 1998 - 2003. Winter vetch was sown at the end of August. Phenological observations of vegetation stages were performed. Morphometric parameters of 20 plants from each cenopopulation were assessed. They were: plant height, number of branches, inflorescences of a plant, stalk length, pod number, pod parameters, the number of seeds in a pod, weight of 1000 seeds, raw material of one plant, qualitative traits, shoot hairiness, leaf colour and shape, flower colour. Protein amount was established in air dry matter at the beginning of flowering. The summer (May-June) hydrothermal coefficient, i.e. the ratio of precipitation and the sum of above zero temperatures over the investigated period was calculated for evaluation of climatic conditions. The morphometric research of plants *ex situ* was carried out in the laboratory of Crop Science Department, the Lithuanian University of Agriculture. Soil analysis and raw protein analysis of the study material were performed at the Agrochemical Research Centre, the Lithuanian Institute of Agriculture.

Results and discussion

In 1998 - 2002, 48 vetch accessions were collected from different places of the country, and ecogeographical occurrence of the species was assessed. Winter vetch was found primary in the south-eastern and eastern part of Lithuania (74 %) and less frequently in central Lithuania (18 %), south-western (16 %) and north-western Lithuania (2 %). Winter vetch was not found in the northern part of Lithuania. 82 % of sites were characterised by sandy and sandy loam soils, 18 % by light loam, pH 5.4 - 6.9. In the main regions of rye vetch occurrence, south-eastern and eastern Lithuania, acid sandy and sandy loam soils prevail.

Winter vetch grew in winter crop, especially rye, agrophytocenoses, waste fields, where rye had been grown. However, it also occurred in summer crops like oats, barley-oats mixtures and potatoes. In agrophytocenoses other vetch species grew simultaneously, *V. angustifolia* and *V. hirsuta.*

Under the same agrotechnical conditions (*ex situ)* plants of different cenopopulations highly varied in all parameters. Most distinctively (Table 1), winter vetch differed in stem branching, number of inflorescences and pods, and stem height at the beginning of flowering. The least varying parameters were pod length and width, and stalk length. According to stem height winter vetch cenopopulations were subdivided into three groups (Table 1). Vetch of group I was behind the rest of cenopopulations by morphological parameters. Side shoots grew from buttons formed in anlobe, while side shoots in the remaining two groups grew from the main stem, but were distinctive for the large size of seeds. Vetch of group II flowered later, pods had the largest amount of seeds, but the weight of which was lowest. The

highest (group III) vetch was most productive, branched most and matured the highest amount of seeds.

Table 1. Morphometric characteristics of winter vetch

Character	Group		
	I	II	III
Stem height (cm)	75 - 119	120 - 159	160 - 230
Beginning of flowering	June 10 - 20	June 15 - 25	June 10 - 16
Height of plant:			
Beginning of flowering	28.5	32.1	35.4
End of vegetation	99.9	135.8	183.0
Number of branches:			
From anlobe	2.4	1.0	1.0
From main stem	1.2	1.9	3.1
Inflorescences	5.5	10.5	11.4
Pods	14.6	29.7	33.8
Seeds in the pod	4.1	4.7	4.2
Pod length (mm)	28.5	29.8	29.6
Pod width (mm)	8.6	8.9	8.9
Raceme stalk length (mm)	96.0	104.0	116.8
1000-seeds weight (g)	23.3	21.6	22.0
Raw yield (g plant^{-1})	18.5	33.2	42.6
Protein content (%)	19.9	20.1	20.0

The amount of raw protein in air dry matter of vetch of all three groups (in the flowering stage) was similar, around 20 percent, though in separate cenopopulations protein content reached up to 25 percent. Negative correlation was established between summer hydrothermal coefficient and protein content in the aboveground mass of vetch ($r = -0.44$). Positive correlations were established between plant height and number of branches ($r = 0.34$), height and pod number ($r = 0.51$), height and raceme stalk length ($r = 0.52$), branches and inflorescences number ($r = 0.34$), and branches and pod width ($r = 0.41$).

Intracenopopulation differences were established for qualitative parameters: stem colour and hairiness, colour intensity of flowers. Cenopopulations with abundantly hairy shoots make up 35 %, thinly hairy 25 %. Shots of the remaining (40 %) cenopopulations were medium hairy. The intensity of flower colour varied from dusty blue to dark violate. In separate cenopopulations individuals occurred with dark red or russet flowers. Winter vetch is a xenogamic, polymorphic plant. Some cenopopulations were distinguished for their very high variation of individuals in morphometric parameters, shape of shoots and leaves, and colour (Figure 1).

Disease resistance in separate cenopopulations of winter vetch varied. Cenopopulations undamaged by *Erysiphe communis* Grev.f. *vicea* Jacz. made up 9 %, severely damaged 47.5 %. The rest of the cenopopulations were slightly or medium damaged. Irrespective of ecological and geographical conditions of growth sites, winter vetch *ex situ* was well adapted in the collection. The Lithuanian population of winter vetch is polymorphic, phenotypically varying both in intracenopopulations and cenopopulations. High phenotypic plasticity stipulates ability of cenopopulations to adapt in changing ecological conditions.

Figure 1. Intracenopopulation diversity of winter vetch

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Assessment of genetic diversity among Persian clover cultivars as revealed by RAPD markers

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ABSTRACT: Yield crop landraces are valuable sources of genetic variations that the knowledge and implication of these variations are critical in the plant breeding programs. Legume forage clover due to high forage yield, quality, nitrogen fixation and improvement of soil textures is cultivated worldwide. Persian clover (*Trifolium resupinatum* L.) is grown worldwide. Molecular markers are used efficiently for assessment of genetic diversity in crop plants. In this study 20 Persian clover cultivars collected from different areas were used. DNA extractions were carried out using minipreparation method with equal amount of leaves from 30 plants of each cultivar. DNA samples from 20 cultivars of clover was evaluated using RAPD markers. Eight primers out of 30 used primers produced repeatable bands. Cluster analysis was conducted using NTSYS software and UPGMA method based on Jaccard's similarity matrix. Primers totally produce 83 bands, of which 66 bands (%80) were polymorphic among clover genotypes. The greatest and least amplification fragments belonged to OPH_{12} and OPG_{06} primers, respectively and average band number of primers was estimated as 10.4 bands. According to cluster analysis and cutting dendrogram in 0.7 similarity coefficient, clover populations divided into six groups in which 'Kazerun' and 'Haftun-Isfahan' individually formed the separate clusters. According to similarity matrix, the least similarity (%36) belonged to 'Haftchin-Brujerd' and 'Kazerun' and the highest similarity belonged to 'Chegeni' and 'Haftchin-Hamedan'. Clustering based on RAPD method almost substantiated the grouping based on geographical origin. Considering the results, it is concluded that RAPD technique can be used for genetic diversity study of Persian clover as well as discrimination of its cultivars.

Key words: Diversity – Persian clover – RAPD marker – *Trifolium resupinatum*

Introduction

Assessment of genetic diversity in cultivated crops have important implications for breeding programs and for the conservation of genetic resources. Some 250 species of *Trifolium* are recognized throughout the world. They contribute nitrogen fixation and thus promote the growth of associated grass. Persian clover adapted to heavy, moist soils but is not tolerant to low winter temperatures. Under cultivation it is seeded in the fall and grows rapidly during late winter and early spring. Seed pods are inflated and light in weight, subject to distribution by wind or floating on water. Natural reseeding occurs generally. Although used mainly for grazing, Persian clover is also excellent for silage and hay. Persian clover as a self-pollinated crop, is a prolific seed producer which yields up to 675 kg/ha (Cope & Taylor, 1985). Evaluation of genetic diversity and similarity between Persian clover cultivars is a first step toward its germplasm utilization for a breeding program.

Materials and methods

Plant materials

Twenty cultivars of Persian clovers collected from different areas of Iran were used in this study: (1) 'Kermanshahi-1' and (2) 'Kermanshahi-2' from Kermanshah, (3) 'Haftun', (4) 'P513', (6) 'Sechin' and (7) 'Haftchin' from Isfahan, (5) 'Nehavandi', (8) 'Haftchin-Hamadani', (9) 'Chegini', (10) 'Doroud', (11) 'Harati', (12) 'Dehpir', (13) 'Silakhor', (14) 'Alashtar' and (15) 'Haftchin-Boroujerd' from Lorestan, (16) 'Kasaroun' from Fars, (17) 'Bazneh', (18) 'Elvijan', (19) 'Reihan' and (20) 'Tajra' from Markazi.

Random amplified polymorphic DNA markers

Genomic DNA was extracted from young leaf tissue using Dellaporta et al. (1983) procedure with modifications. Briefly, 100 mg of leaf tissue was powdered by grinding in liquid nitrogen and incubated in extraction buffer (100mM Tris-HCl pH 8.0, 50 mM EDTA pH 8.0, 100 mM NaCl and 1.25 % (w/v) SDS) containing 200 µl 5 M potassium acetate at 65 °C for 30 min. The slurry was extracted with 0.8 volume of chloroform – isoamyl alcohol (24:1 v/v), and the emulsion was centrifuged at 5000 rpm at 4 °C for 15 min. Extracted DNA was precipitated from supernatant with 950 g/L alcohol and washed with 700 g/L alcohol three times. After drying, DNA was dissolved in Tris-EDTA pH 8.0 (10 mM Tris, 1 mM EDTA) containing 100 µg RNAase. DNA concentration was quantified by comparing its intensity with those of DNA standard (known concentrations) on an ethidium bromide stained 1 % agarose gel.

Thirty decamer random primers obtained from Primm Co., Italy were used in PCR analysis. The PCR reaction was performed in a 25-µl volume using a Techgen thermocycler (Techgen, UK). Polymerase chain reactions based on genomic DNA performed according to Gustine & Huff (1999). PCR amplified products were separated by electrophoresis in 1.5 % agarose gel. Gels were stained with ethidium bromide (0.5 µg/ml). DNA banding patterns were visualized using Vilber Lourmat gel documentation (Model IP-008-SD, France).

For each genotype, the presence of a band (1) or its absence (0) was scored. Cluster analysis was conducted using NTSYS software and UPGMA method based on Jaccard's similarity matrix.

Results and discussion

Of 30 primers used for initial screening of polymorphism using Persian clover cultivars, 8 primers gave amplified polymorphic products (Table 1). Amplification of 20 genotypes with these primers yielded a total of 83 scorable bands, of which 17 were polymorphic (Table1). An average of 10 bands per primer and 4 bands per genotype were obtained. The highest number of bands (was obtained with primers OPH_{12} and OPH_{10} while the lowest number was obtained with primer OPG_{06}.

Figure 1 shows a representative amplification pattern obtained using primer OPH_{10}. A dendrogram showing genetic relationships among the 20 clover genotypes were presented in Figure 2. According to cluster analysis and cutting dendrogram in 0.7 similarity coefficient, clover populations divided into six groups in which ‘Kazerun’ and ‘Haftun Isfahan’ individually formed the separate clusters. According to similarity matrix, the least similarity (36%) belonged to ‘Haftchin Brujerd’ and ‘Kazerun’ and the highest similarity belonged to ‘Chegeni’ and ‘Haftchin Hamedan’. Clustering based on RAPD method almost substantiated the grouping based on geographical origin. Considering the results, it is concluded that RAPD technique can be used for genetic diversity study of Persian clover as well as discrimination of its cultivars.

Table 1. Percent polymorphism revealed by random primers in Persian clover

Primer	Nuclotide sequence 5' → 3'	Total number of bands	Polymorphic bands	Percentage of polymorphism
OPH_{04}	GGAAGTCGCC	9	8	89
OPA_{08}	GTGACGTAGG	8	7	88
OPH_{12}	ACGCGCATGT	14	11	79
OPH_{10}	CCTACGTCAG	14	11	79
OPB_{14}	TCCGCTCTGG	13	10	71
OPG_{13}	CTCTCCGCCA	13	10	71
OPH_{07}	CTGCATCGTG	9	7	78
OPG_{06}	GTGCCTAACC	3	2	66

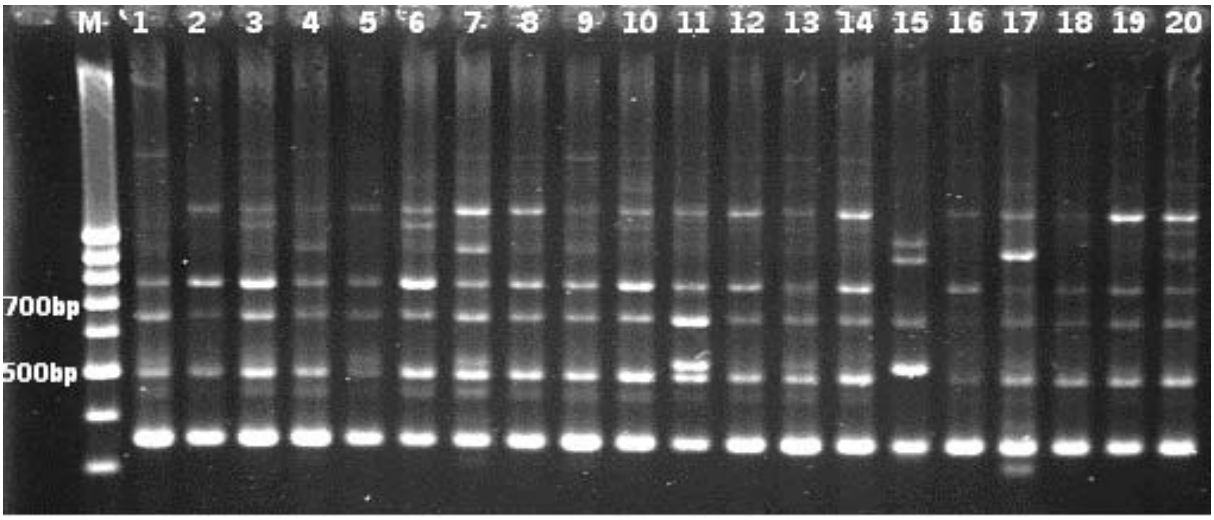

Figure 1. RAPD profile of 20 Persian clover cultivars using the primer OPH_{10} (M = 100bp ladder, for cultivar number see Materials and methods, p. 85)

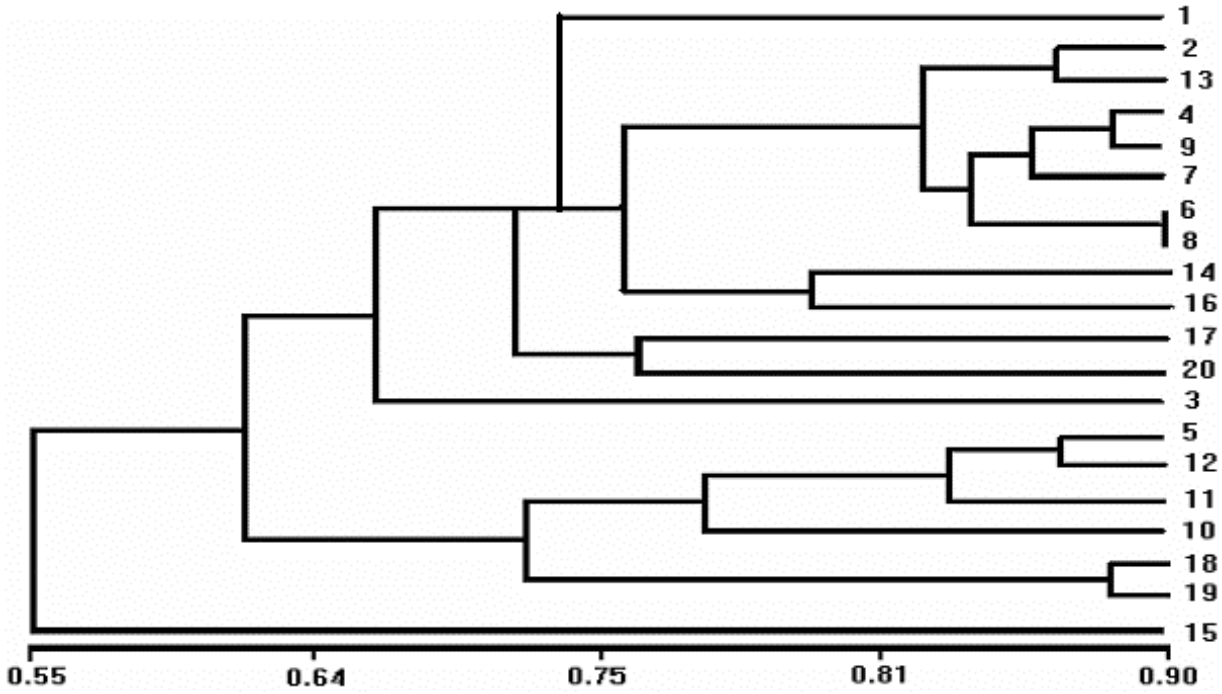

Figure 2. Dendrogram showing interrelationships of 20 Persian clover genotypes

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National collection of *Vitis* as a source of valuable genotypes for new breeding goals

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ABSTRACT: The effective utilization of gene resources for new breeding programmes depends on a well chosen set of descriptors specific for agronomically important traits as well as on the appropriate mathematic model for evaluation of plant variability. The objective of this contribution is to show one way how to select genotypes bearing desirable traits from the whole collection of quite diverse varieties.

Key words: Cluster analysis – genetic resources – variability – *Vitis vinifera*

Introduction

The description of *Vitis* genetic resources is conducted using the internationally accepted descriptor list, consisting of 72 descriptors. Most of them are morphological characteristics with high taxonomical value, but some of them are traits of principal concern in plant breeding programmes, which can be thought of as inherited agronomic qualities. Breeding programmes nowadays should reflect changing of natural conditions and changing in the demands of consumers as well as modern trends in healthy nutrition. There are reasons, why breeders started to look for specific traits of old or local varieties or wild ancestors of commercially growing crops. We put to the centre of our interest traits important for Czech vintners, as they are growing vine in regions where grape plants are endangered by hard frost in winter and by late frost in spring, and the quality of grapes is often negatively influenced by cold, rainy and early coming autumn.

Material and methods

According to the objective of our investigation we selected the following traits with great importance to earliness of grape and good quality of must: number of days from bud bursting to early mature state (days of vegetative period), the amount of sugar in must, pH of must and must yield. These traits were measured in three years (2001–2003) in 34 accessions of the *Vitis* collection maintained at the Research Station of Viticulture in Karlštejn. All accessions are planted in the same vineyard, 218 m above see level. The results were evaluated by variance analysis, and also cluster analysis with Ward´s method was used to see whether the cultivars fell into groups or clusters, having similar phenotype in evaluated traits. The dissimilarity matrix was calculated using squares of Euclidean differences. All analyses were carried out using the STATGRAPHIC or SPSS packages.

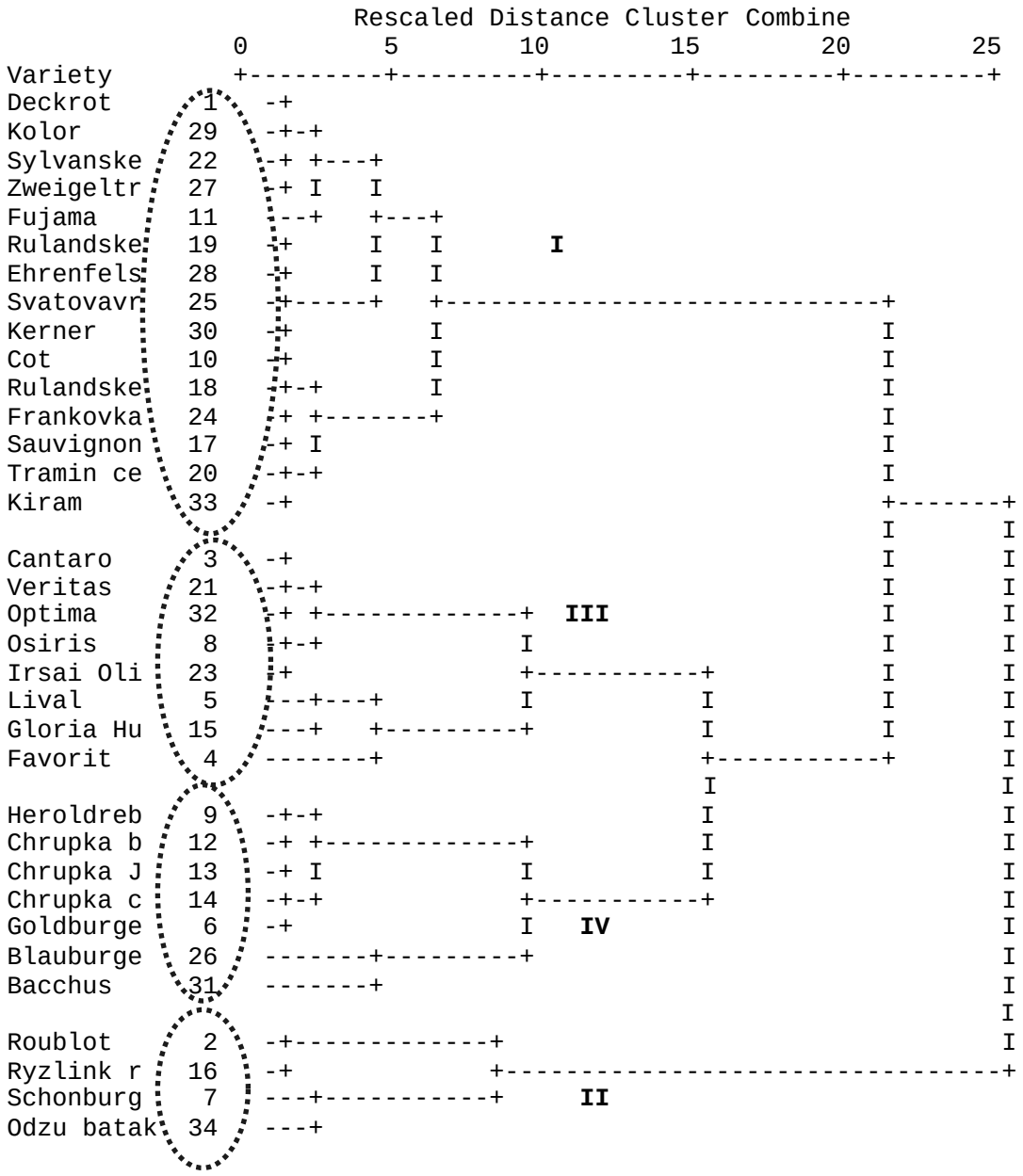

Figure 1. Dendrogram from a hierarchical cluster analysis using Ward's Method

Results and discussion

The application of cluster analysis (Hebák 1987) using Ward's method of cluster formation led to separation of 34 genotypes into four groups, as shown in Figure 1. The main characteristics of the above mentioned clusters: The cultivars with shortest vegetative period are grouped in cluster III. It consists of three table cultivars ('Lival', 'Gloria Hungaria' and 'Favorit') and five wine varieties ('Cantaro', 'Optima Veritas', 'Osiris' and 'Irsai Oliver'). The other characteristics, sugar content, pH and yield suggest that these varieties are well adapted to the natural conditions of the Czech vine growing region. Cluster IV consists of well known and taxonomically closely related table varieties 'Chrupka' (white, red and 'Jalabert'), and there are also German wine varieties 'Bacchus', 'Heroldrebe' and Austrian 'Blauburger' and 'Goldburger' grouped into that cluster. In spite of longer vegetative period, these varieties did not reach the average of sugar content of cluster III. , but their yields were higher. Cluster I contains 15 varieties, and we can find there a lot of varieties commercially gown in Czech Republic. The sugar content was higher in this group but the yield hardly

reached the mean value of the most early group. The latest maturity group II represents Bulgarian table variety 'Odžu Batak' and three wine varieties ('Ryzlink rýnský', 'Shonburg' and 'Roublot'), which are not well adapted to the natural conditions of the Czech vine region. Table 1 shows statistical parameters of all four clusters for the evaluated traits.

Table 1. Mean values and SD of individual traits in four clusters (The indexes [123] indicate significant differences among clusters.)

Cluster	I	II	III	IV
Varieties (n)	15	4	8	7
VEGPER[1] (d)	119.0 ±.03[3]	121.08±1.66[1]	108.17 ±1.19[124]	116.54±1.97[3]
SUGAR	18.01 ±0.25[24]	14.65±1.85[1]	16.33 ±0.66	14.94±0.3[1]
pH	3.07 ±0.02[2]	2.06±0.05[134]	3.05 ±0.11[2]	2.96±0.16[2]
Yield	0.59 ±0.04[4]	0.55±0.05[4]	0.61 ± ±0.08[4]	0.99±0.09[123]

[1] VEGPER, vegetation period (d); SUGAR, sugar content in the must

Finally we can conclude, that cluster analysis represents a very useful method to compare variability of distant genotypes, but as most of agronomically important traits are strongly influenced by environment, we have to test all genotypes under the same growing conditions.

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Assessment of genetic diversity among Portuguese melon landraces by molecular markers

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ABSTRACT: The melon, *Cucumis melo* L., is one of the agronomically most interesting and diversified species of the genus *Cucumis*. This species has one of its secondary centres of genetic diversity the Iberian Peninsula (Portugal and Spain). In Portugal, several landraces of melon (belonging to the *Cantalupensis* and *Inodorus* groups) have been traditionally cultivated throughout the different regions of its geography. Nevertheless during the last decades these well adapted landraces are being replaced by modern commercial cultivars with the subsequent genetic erosion of this traditional horticultural crop. Variation in random amplified polymorphic DNA (RAPD) loci in twenty-six melon landraces representative of the Portuguese germplasm were used to determine the utility of these markers for assessing genetic variation among them. A standard molecular marker array consisting of 463 random amplified polymorphic DNA (RAPD) marker bands (30 primers) was considered. Results show that high genetic variability is still conserved in these landraces because the variability estimated as heterozygosity from Hardy-Weinberg proportions is nearly 40 %. The estimated proportion between the inter- and intra-accession variability shows that the total variability estimated is mainly maintained at inter-population level as could be expected for a crop grown in small market gardens without seed interchanges between farmers. Genetic distances between samples show some important differences between pairs of accessions, higher than previously described, but not directly associated with their adscription to the *Cantalupensis* and *Inodorus* groups or with their geographical origin.

Key words: *Cucumis melo* – genetic distance – RAPD – variability

Introduction

The cultivated species of *Cucurbitaceae* include plants from the Old and the New World. One of them is the cultivated melon, *Cucumis melo* L., that was brought into cultivation in south-west Asia or Egypt. Moreover, other secondary centres of diversity have been described in China, Korea and the Iberian Peninsula (Portugal and Spain) (Esquinas-Alcazar & Gulick 1983). *C. melo* has been described as the most diversified species of the genus *Cucumis*, including netted muskmelon, salmon-flesh cantaloupe, smooth-skinned and green-fleshed Honey Dew, wrinkle-skinned casaba, long shelf-life Hami melon, small and thin-pericarp makuwa, and several non-sweet pickling and cooking oriental melons (Liu et al. 2004). Several years ago Whitaker and Davis (1962) subdivided *C. melo* subsp. *melo* into seven horticultural groups that were later reviewed by Munger and Robinson (1991). Only two of them (*Cantalupensis* and *Inodorus*) are of commercial interest in Europe, Asian countries and the USA.

In Portugal, the melons, all of them belonging to the *Cantalupensis* and *Inodorus* groups, is cultivated from north to south, and from inland to littoral regions. A high amount of variability can be found for different morphological parameters, such as fruit shape, fruit skin texture, writing, netting or flesh colour. However, in the last decades modern cultivars have replaced the Portuguese landraces and this source of variability and genetic resource is being lost.

The aim of the present study was to evaluate by RAPD markers the molecular diversity among Portuguese germplasm of melon and to establish genetic relationships among the landraces belonging to two different groups of *C. melo*.

Materials and methods

Twenty-six melon landraces representative of the Portuguese germplasm were analysed. Sixteen of the landraces belonged to the *Cantalupensis* group and the other ten to the *Inodorus* group. Furthermore, another four genotypes were used as controls: line 'PAK312' from Pakistan, line 'PMR45' from the USA, cultivar 'Doublon' from France, and cultivar 'Kirkagaç' from Turkey.

Thirty individual plants per accession were analysed for RAPD markers. Genomic DNA from young leaves was extracted following the procedure described by Martins et al. (unpublished). Forty five random decamer oligonucleotides from Operon Tecnologies were used as single primers for the amplification reactions with the genomic DNA of each one of the individuals. The optimized amplification reaction contained 700 ng DNA, 25 ng primer, 2.5 mM dNTPs, 25 mM $MgCl_2$, 2.5 µl of 10x Taq DNA polymerase buffer and 2.5 U of Taq polymerase MBI Fermentas in a 25µl final volume. After 5 min of heating at 94°C, amplifications were performed using the following regime: 9 cycles of 15 sec at 94°C, 45 sec at 33°C, and 75 sec at 72°C; followed by 35 cycles of 15 sec at 94°C, 45 sec at 37°C, 75 sec at 72°C, and a final extension reaction of 7 min at 72°C. PCR products were separated by 1.5 % agarose gel electrophoresis, and stained with ethidium bromide. Each polymorphic band was scored as present (1) or absent (0) for all genotypes, resulting in a binary data matrix. Genetic heterozygosity index (H_S) according to Nei's method (Nei 1973) and genetic variability (K_X) of Nei-Kumar's method (Nei & Kumar 2000) were estimated. In order to describe genetic relationships among Portuguese landraces and the controls samples, genetic distances (GD) were estimated. Finally, cluster analysis (UPGMA) from genetic distance data was carried out to obtain a dendrogram showing the genetic similarity between accessions used. All statistical procedures were carried out by TULKAS software program developed by Sáenz de Miera (unpublished).

Results and discussion

Thirty of the forty five primers were selected since they revealed a higher level of polymorphism and reproducible patterns. These 30 primers generated 463 bands, of which 124 were polymorphic (26.8 % of bands detected). The mean number of bands per primer was 15.4 (ranging from 3 to 22), the mean number of polymorphic bands per primer was 4.3, (ranging from 0 to 11) and the mean polymorphism level was 24.2 % (ranging from 0 to 55 %), among the 30 primers. A relatively high level of polymorphism was revealed by 11 primers (five showed a polymorphism level higher than 40 % and six showed a polymorphism level ranging between 30 and 40 %). The percentage of monomorphic bands within the accession was 39.4 % (ranging from 21.8 to 53.2 %), and the counterpart of polymorphic bands was 22.1 % (ranging from 21.8 to 53.2 %). The average of the estimated number of alleles was 1.18 (from 1.04 to 1.36). Total heterozygosity (H_T) was 0.40, and total genetic variability (K_XT) was 0.50, with a mean level per accession of 0.079. ST/S proportion calculated for the total number of accessions was 5.329 suggesting that inter-accession variability is higher than the intra-accession variability. In this way the ratio ST/T show that the inter-accession variability explains the 80 % of the variability found in this study. Genetic drift acting over these historically adapted landraces could explain this high value of the inter-accession variability.

The dendrogram generated by cluster analysis (UPGMA) allowed the detection of four groups. Group 1 consists of six landraces, five of which come from an inland part of the

country and one from a littoral region (Algarve). Three landraces belong to the *Cantalupensis* group and the other three to the *Inodorus* group. Group 2 contains eight landraces and the control 'PMR45'. All accessions belong to *Cantalupensis* group except one that belongs to *Inodorus* group. All of the six landraces from the north littoral region (Minho) are in this cluster. Group 3 includes five landraces, originating from north, south, inland and littoral parts of the country. Of these landraces, three are from *Inodorus* group and two from *Cantalupensis* group. Group four consists of seven landraces, the Pakistan line PAK312, the French cultivar Doublon and the Turkish cultivar Kirkagaç. This cluster can be divided in two subgroups, one with the two cultivars and three landraces, all from inland, and a second subgroup with four landraces and the line PAK312. All the landraces of this group belong to the *Cantalupensis* group except one. The average genetic distance between any two pairs of landraces/controls was 0.48±0.10, higher than that described by López-Sesé et al. (2003) for Spanish melon accessions. For all accessions the minimum genetic distance (GD) (most related accessions) was observed between two landraces, originating from the same area (GD = 0.007). The maximum genetic distance (most distantly related accessions) (GD = 0.73), was registered between two landraces originating from north littoral and central inland Portugal, respectively. Genetic distance in the *Cantalupensis* group, ranged between 0.14 ('PAK312 and a landrace from south Portugal) and 0.76 ('PMR45' and a Portuguese landrace from the inland of Portugal). For the *Inodorus* group the minimum genetic distance among accessions was 0.09 and was registered between two landraces from south inland Portugal and the maximum 0.74 between two landraces both from north inland Portugal.

The RAPD analysis revealed that a great variability among Portuguese landraces of melon exists, which was also detected with morphological parameters. The six landraces from the north littoral region showed morphological characteristics that do not allow their inclusion in any market class previously defined (Gómez Guillamon et al. 1985, McCreight et al. 1993, Nuez et al. 1996, Gómez Guillamon et al. 1998, Staub et al. 2000). This specificity is confirmed by cluster analysis with RAPD markers, once all landraces from this region have been grouped separately. No association was found between the geographical origin of the Portuguese landrace or taxonomic groups with molecular analysis. Similar results were obtained by López-Sesé et al. (2003) for Spanish melon accessions.

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aDNA analysis of cantaloupe (*Cucumis melo* L.) from the Middle Ages compared to modern varieties

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ABSTRACT: Archaeological excavations from a 15th century sediment layer at the King's Palace of Budapest, Hungary, revealed a number of plant remains (Gyulai et al. 2004). Seed remains of cantaloupe (*Cucumis melo* L., 2n = 4x = 24) appeared extremely well preserved due to anaerobic conditions in the slime at the bottom of a deep medieval well covered by water. Identical seeds were surface sterilized individually and kept in aseptic tissue culture for a three month period to exclude bacterial or fungal infected seeds. DNA was extracted from non-infected seeds and from a number of modern cantaloupe varieties and analysed using PCR-based techniques. Some seeds showed fungal ITS sequence-remains which indicated endogenous contamination and these were excluded from further experiments. DNA samples from '*mac*' (middle ages cantaloupe) were analyzed using RAPD and SSR primers. Twelve of the 48 RAPD primers (25 %) amplified polymorphic bands (44 out of a total 105 bands) in '*mac*' and also in modern varieties. Ten of the twenty SSR primers amplified microsatellite fragments in identical fragment ranges. Sequence analysis (using an ABI-310) of the CmCTT144 fragment showed different fragment lengths depending on changes in the number of trinucleotide core sequence repeats $(cct)_n$ in the imperfect type microsatellite sequence $(cct)_{10}ctac(ctt)_4$. Cluster analysis, based on the presence vs. absence of PCR fragments, indicates that the '*mac*' sample is most closely similar to the modern muskmelon variety 'Hogolyo' at a 0.25 similarity level.

Key words: *Cucumis* – molecular marker – microevolution – muskmelon – RAPD – SSR

Introduction

Analysis of ancient plant DNA provides valuable data concerning crop domestication events that have occurred during previous centuries (Ruckenbauer 1971, Lagler et al. 2004). In this study, PCR based analysis (ITS, RAPD and SSR) was carried out and DNA fragments were sequenced in samples extracted from cantaloupe seed remains dating from the 15th century (found in Budapest, Hungary) in comparison to ten modern cantaloupe varieties.

Materials and methods

Seed samples

Seed samples were excavated from a 15th century sediment layer in a well at the King's Palace of Budapest, Hungary. Samples were processed by floatation and sub-samples of seeds were subsequently sorted and identified in the laboratory. The registered modern cantaloupe varieties 'Ezust-Ananas', 'Fortuna', 'Hales Best', 'Hogolyo', 'Javitott-Zentai', 'Magyar-Kincs', 'Muskotaly', 'Sweet-Ananas', 'Tetenyi-Cseresheju' and 'Top' were used for comparisons. Seed samples were surface sterilized using commercial bleach followed by a three month incubation period in aseptic tissue culture medium to exclude fungally or bacterially contaminated seeds (Lagler et al. 2004).

DNA extraction and PCR
Total DNA from 0.1 g of seed in each sample was extracted by the CTAB-method followed by an RNase-A (Sigma) treatment. DNA analysis was carried out using RAPD, SSR (Danin-Poleg et al. 2001) and ITS (Jobst et al. 1998) techniques. A minimum of three independent DNA preparations of each sample was used. Each successful reaction with scoreable bands was repeated at least twice (Figure 1).

Fragment analysis
PCR fragments were scored for the presence or absence of band profiles. A negative control which contained all the necessary PCR components except template DNA was included in the PCR runs.

Sequencing
Fragments isolated from an agarose gel (spin column, Sigma 5-6501) were analysed in an automated fluorescent DNA sequencer (ABI PRISM 3100 Genetic Analyzer).

ALF (Automated Laser Fluorometre) analysis
SSR fragments were analysed by ALF ExpressII (Pharmacia) using cy-5 labelled primers and markers (Amersham, AP Hungary) following the method of Röder et al. (1998).

Primers
For ITS analysis, primers (f 5'- tcg taa caa ggt ttc cgt agg tg-3', and r:5'-tcc tcc gct tat tga tat gc-3') complementary to the evolutionary conserved spacer regions of the nuclear ribosomal (rDNA) genes (ITS1 and ITS2 internal transcribed spacer) were used. For RAPD analysis, 48 primers were used (Operon Technology). Microsatellite DNA regions were amplified using twenty SSR primers (Katzir et al. 1996, Danin-Poleg et al. 2001). Cluster analysis was carried out using the SPSS-11 programme and a Jaccard index.

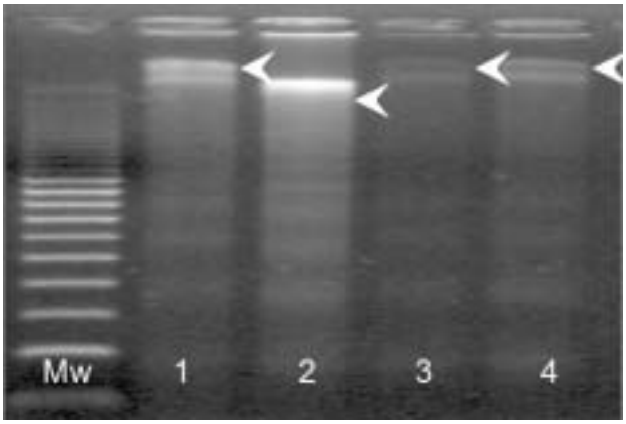

Figure 1. Total ancient DNA (arrows) isolated from single cantaloupe seeds (1-4); Mw - 100 bp ladder

Results and discussion
Molecular analysis
RAPD analysis was used to test the amplification ability of the DNA samples. Twelve of the 48 RAPD primers (25 %) amplified polymorphic bands (44 out of a total of 105 bands) in '*mac*' and modern varieties. ITS analysis was used for monitoring the microevolution of the '*mac*' samples in comparison to modern varieties (Jobst et al. 1998).

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                .|....| ....|....|  ....|....| ....|....|  ....|....| ....|....|  ....|....| ....|....| ....|....| ....|....| ....|
varieties/bp    85        95         405        415         465        475         605        615        625        635
Z48805          CATGCTC TTTGCCTGTC  CCCACCACAC AACACTCCCC  CCGTACGCAT CGTCGTGCGG  CCGCCCCTTA AAAGGACGAC GCTCTCGACG CGACC
Ezust Ananasz   ....... ...AG...C.  .......... ...T......  .........Y ..........  .........G .......... .......... .....
Fortuna         ....... ...RS...Y.  .......... ...T......  .........Y ..........  .........R .......... .......... .....
Hógolyó         ....... ...RC...Y.  .......... ...T......  .......... ..........  .......... .......... .......... .....
Hales Best      ....... ...RC...T.  .......... ...T......  .......... ..........  .......... .......... .......... .....
Javitott ZentaI ....... ...AG...C.  .......... ...T......  .........C ..........  .........G .......... .......... .....
Magyar Kincs    ....... ...AG...C.  .......... ...T......  .........C ..........  .........G .......... .......... .....
Muskotaly       ....... ...RC...T.  .......... ...T......  .......... ..........  .......... .......... .......... .....
Sweet Ananas    ....... ...AG...C.  .......... ...T......  .........Y ..........  .........G .......... .......... .....
Tetenyi Cseresheju ..... ...RS...Y.  .......... ...T......  .........Y ..........  .........R .......... .......... .....
Top             ....... ...AG...C.  .......... ...T......  .........C ..........  .........G .......... .......... ..G..
'mac'           ....... ...RC...C.  .......... ...T......  .......... ..........  .......... .......... .......... .....
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Figure 2. Sequence alignment of the ITS1 and ITS2 regions of rDNA sequences amplified by primer pairs ^{f}5'-tcg taa caa ggt ttc cgt agg tg-3' and ^{r}5'-tcc tcc gct tat tga tat gc-3' (Jobst et al. 1998) in middle age cantaloupe ('*mac*') samples compared to ten modern current varieties

The amplified fragments included the full ITS1-5.8S-ITS2 sequence at about 690 - 700 bp length. Obviously the highly conserved 5.8 S regions did not show any polymorphism in the sequences, nevertheless sequences of ITS1 (218-219 bp) and ITS2 (234 bp) showed several nucleotide changes in dinucleotides at 94-95 bp (ITS1) and SNPs (single nucleotide polymorphism) at 414 bp, 470 bp, 610 bp, and 633 bp (ITS2) in the '*mac*' samples and modern varieties, respectively (Figure 2).

In the microsatellite analysis, ten (CmTC13, CmCT44, CmAG59, CmGA104, CmGA128, CmTA134a, CmCT134b, CmCTT144, CmTC168 and CmCT170b) of the twenty primers amplified identical SSR fragments in the middle age cantaloupe ('*mac*') samples compared to ten current varieties 'Ezust-Ananasz', 'Fortuna', 'Hales Best', 'Hogolyo', 'Javitott-Zentai', 'Magyar-Kincs', 'Muskotaly', 'Sweet-Ananas', 'Tetenyi-Csereshejú' and 'Top' (Figure 3). Sequence analysis of the CmCTT144 fragments from '*mac*' samples and modern varieties showed different fragment lengths according to changes in the number of trinucleotide core sequence repeats $(cct)_n$ in the imperfect type of SSR sequence $(cct)_{10}ctac(ctt)_4$ (Figure 4).

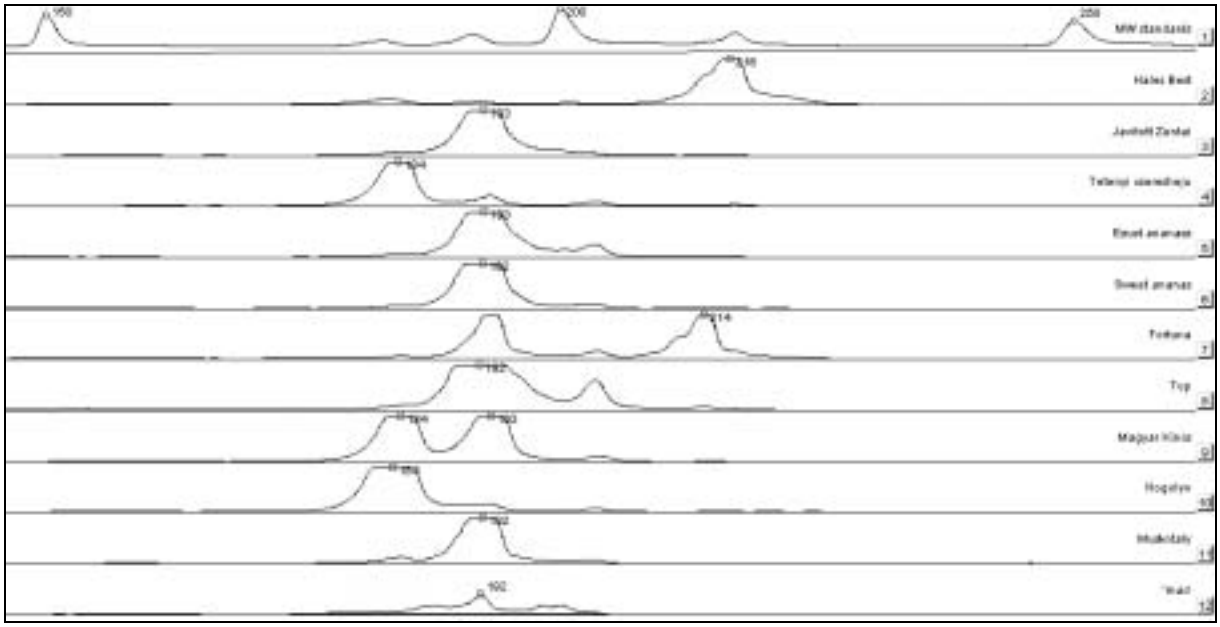

Figure 3. ALF analysis of the microsatellite marker CmCTT144 in the middle age cantaloupe ('*mac*') sample compared to ten current varieties amplified by primers (Danin-Poleg et al. 2001) of cy-5 labelled ^{f}caa aag gtt tcg att ggt ggg, and ^{r}aaa tgg tgg ggg ttg aat agg

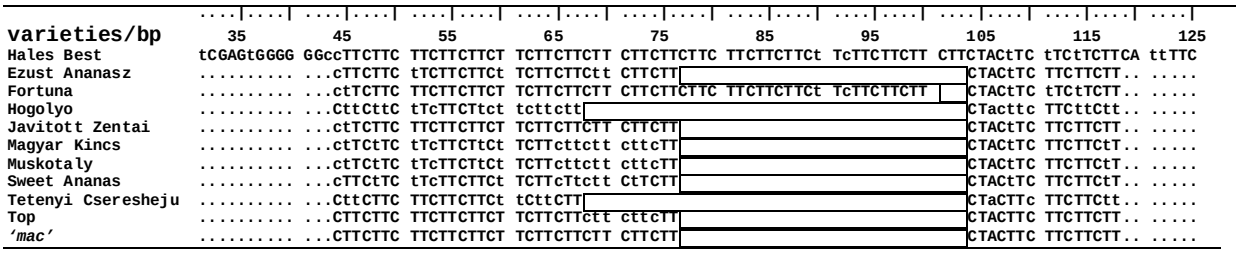

Figure 4. Sequence alignment of microsatellite marker $CmCTT_{144}$ (184-216 bp) amplified by primers ^{f}caa aag gtt tcg att ggt ggg and ^{r}aaa tgg tgg ggg ttg aat agg (Danin-Poleg et al. 2001) in middle age cantaloupe ('*mac*') samples compared to ten modern varieties.). □ – sequence deletions

Cluster analysis based on the presence versus absence of amplified fragments from ITS SSR was carried out in order to assess genetic distances among '*mac*' samples and modern varieties. On the dendrogram '*mac*' showed the closest similarity to the current muskmelon variety 'Hogolyo' but at a low similarity level (0.25) (Figure 5). 'Hogolyo' is the oldest registered variety in Hungary still kept in cultivation and may provide further information on the genetic reconstruction of the '*mac*' seed.

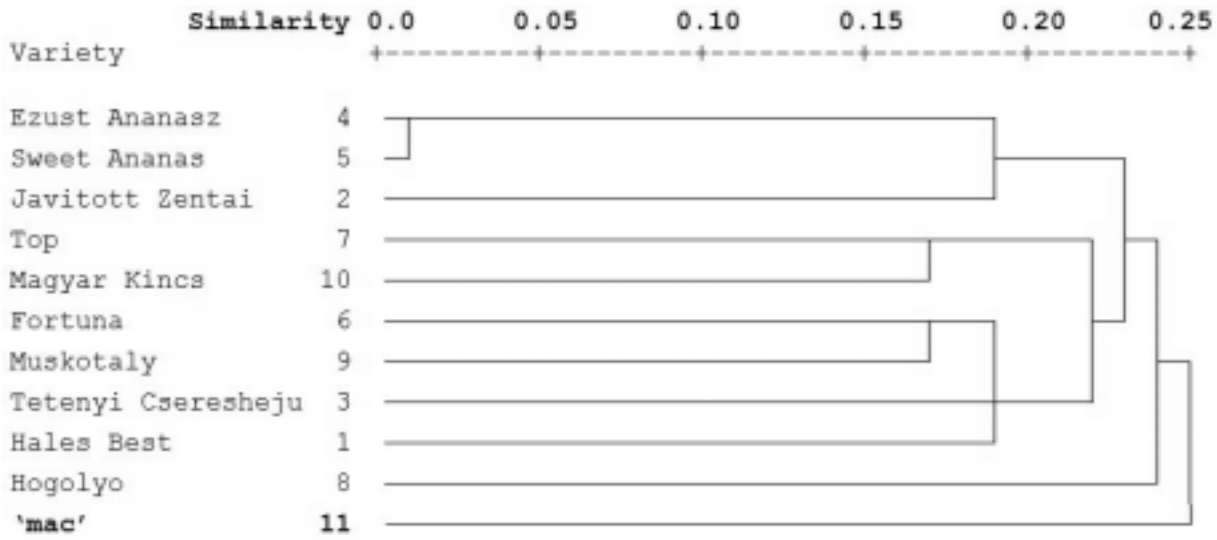

Figure 5. Genetic similarity based on the presence versus absence of amplified DNA fragments (ITS, SSR) of '*mac*' (middle ages cantaloupe) samples compared to ten modern varieties

Acknowledgements
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Isozyme variation in European *Lactuca serriola* germplasm

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ABSTRACT: *Lactuca serriola* L. is one of the most common species of the genus *Lactuca* and has two different forms of rosette leaves. They are described as *L. serriola* f. *serriola* and *L. serriola* f. *integrifolia*. Altogether 56 samples (accessions) of both forms of *L. serriola* originating from twelve European countries were analyzed for isozyme polymorphism. Eleven enzymatic systems were used for characterisation of variation and nine of them showed polymorphism. From 66 bands (isoforms) observed, 42 displayed polymorphism. According to isozyme polymorphism the studied set was divided into two groups. The first group comprised mostly samples from Austria, Czech Republic, Slovenia and Sweden; the second group contained mostly samples from France, Germany, Great Britain, Italy, Netherlands and Switzerland. A a good relationship was recorded between isozyme polymorphism and taxonomic status of both *L. serriola* forms.

Key words: Geographic distribution – prickly lettuce

Introduction

The genus *Lactuca* L. is represented by approx. 100 species distributed in different biogeographical areas and ecological conditions (Lebeda et al. 2004) and is characterised by a very broad variation of different characters (Doležalová et al. 2003, Lebeda & Astley 1999). *Lactuca serriola* is a cosmopolitan and common weedy annual species found in disturbed and ruderal sites (Lebeda et al. 2001a, 2001b). For the geographical distribution of *L. serriola*, it was found that it reaches the western limit of Europe approximately to 5 °W. The northern limit can be drawn between 50 and 55 °N. Some records from Eastern Europe at 65 °N are known (Feráková 1977). Two forms of *L. serriola* are recognized according to leaf morphology: *L. serriola* f. *integrifolia* with unlobed leaves and *L. serriola* f. *serriola* with pinnatified to pinnatisect leaves (Prince & Carter 1977). The existence of intermediate forms has not been confirmed exactly. Lobbing of the leaves is under genetic control (Lindqvist 1958).

Until now only a few articles focusing on *Lactuca* spp. isozyme variation have been published (Dziechciarková et al. 2004). Isozyme analysis has been used to study inter- and intraspecific diversity during the last thirty years (Vallejos 1983). The results showed a lower level of intra- than interspecific diversity. Isozyme markers for *Lactuca* spp. displayed a high level of polymorphism and can be useful for characterization of genetic variability, phylogenetic and geographical relationships (Kesseli & Michelmore 1986; Dziechciarková et al. 2004). Isozyme variability was used as a criterion for taxonomic characterization and detection of variation among European populations of *Lactuca serriola*.

Material and methods

Plant material

A set of 56 samples of *L. serriola* and one accession of *L. sativa* cv. 'Norden' (CGN 13390) as a control were analyzed. The plant material originated from twelve European countries (Austria, Czech Republic, France, Germany, Great Britain, Greece, Italy, Netherlands, Slovakia, Slovenia, Sweden and Switzerland), different geographical regions, altitudes and

habitats (Doležalová et al. 2001, Lebeda et al. 2001b). Ten accessions were determined as *L. serriola* f. *integrifolia*, thirty-nine as *L. serriola* f. *serriola*, plants of three accessions possessed both leaves forms, three displayed an unknown type of lobing and one was determined as a primitive form of *L. sativa*.

Isozyme analysis

The plants were cultivated in plastic pots in a greenhouse under controlled conditions (day/night temperatures 18 - 30 / 12 - 16°C). 4-5 weeks after transplanting, 4 young basal rosette leaves were harvested from 4 plants per accession and a bulk sample was used for analysis. Samples were homogenised by grinding one volume of fresh young leaf material (0.5g) in three volumes of extraction buffer (0.1M Tris-HCl, pH 8.0; 78 mM 2-mercaptoethanol; 26 mM sodium disulphite; 11 mM sodium salt ascorbic acid, 4 % PVP-40 (polyvinylpyrrolidone)) (Vallejos 1983) with a little addition of sea sand and sucrose. Crude extract was centrifuged at 14000 rpm for 10 min (-4 °C). Supernatant was split to the tube and stored in the freezer at -80°C. Samples were thawed and loaded on polyacrylamide gel (8.16 % separation ge1, 13.5 x 10 cm, 4 % concentration gel). Electrophoresis was run at 35 mA, 390 V for 2 h on adjustable height dual gel electrophoresis units (Sigma) and ESP 601 power supply (Amersham Pharmacia Biotech).

Polyacrylamide gels were specifically stained for 11 enzymatic systems: diaphorase (EC 1.6.99.1, DIA), esterase (EC 3.1.1.1, EST), glutamate-oxaloacetate transaminase (EC 2.6.1.1, GOT), phosphoglucomutase (EC 5.4.2.2, PGM), glucose-6-phosphate isomerase (EC 5.3.1.9, GPI), leucine aminopeptidase (EC 3.4.11.1, LAP), malate dehydrogenase (EC 1.1.1.37, MDH), malic enzyme (EC 1.1.1.40, ME), NADH dehydrogenase (EC 1.6.5.3, NADH DH), shikimate dehydrogenase (EC 1.1.1.25, SHDH) and 6-phosphogluconate dehydrogenase (EC 1.1.1.44, 6-PGDH) following the methods of Vallejos (1983). The dendrogram was constructed using UPGMA (average linkage) cluster analysis. The clustering method and similarity coefficient were tested using the procedure NCSS 97 (Statistical Solutions Ltd, Cork, Ireland).

Results and discussion

Isozyme variability

Fifty-six *L. serriola* and one *L. sativa* accessions were tested for 11 enzyme-staining systems. Two systems (MDH, SHDH) were homogeneous, the remaining ones (DIA, EST, GOT, PGM, GPI, LAP, ME, NADH DH, 6-PGDH) expressed polymorphism. The tested enzyme systems showed 23 zones of activity. Among all staining systems there were observed 66 bands (isoforms) from which 42 showed polymorphism.

Cluster analysis

The dendrogram was constructed from the set of 42 polymorphic markers. The dendrogram of 56 studied *L. serriola* and one *L. sativa* (CGN 13390) accessions is divided into the three major clusters (groups). In the group 1 there is only *L. sativa* cv. 'Norden' (CGN 13390). The group 2 is formed by two accessions, one of *L. serriola* f. *integrifolia* (*09H5801445*, France), and *L. serriola* mixture (*09H5801441*, Italy). For above mentioned accessions the following phenological and morphological features were characteristic: (1) they do not form full developed rosettes, and (2) cauline leaves were entire and spathulate in outline. The group 3 is formed by two sub-groups. Sub-group 3.1 comprises one accession (*09H5801524*) from Sweden determined as *L. serriola* f. *serriola*. Sub-group 3.2 consists of one accession (*09H5801261*) from Slovakia determined as a primitive form of *L. sativa*. Sub-group 3.3 contains *L. serriola* accessions of both leaves forms. First cluster (3.3.1) represents *L. serriola* f. *serriola* (22 samples), second cluster (3.3.2) of this sub-group is formed by 31 accessions of

L. serriola f. *integrifolia* and *L. serriola* f. *serriola.* Cluster 3.3.2 is composed by accessions from Great Britain (5), France (5), Switzerland (4), Germany (3), Italy (3), Slovenia (2), Slovakia (2) and from Austria (1). Their eastern limit is from 0 to 21 °E, western limit from 0 to 5 °W and northern limit between 39 and 52 °N. Accessions in this cluster originate from low (0 - 300 m) to high (600 - 1600 m) elevations, however with some exceptions. On the basis of these data we suggest determination of some accessions (*CGN 17433*, *CGN 16251*, *09H5801468*, and *09H5801334*) as *L. serriola* f. *integrifolia* (Table 1). Cluster 3.3.1 is formed by accessions from Czech Republic (6), Austria (3), Sweden (3), Slovenia (3), Germany (2), Slovakia (2), Italy (1), Netherlands (1) and Switzerland (1). Their eastern limit is between 7 and 18 °E and northern limit from 46 and 59 °N. Accessions in this cluster originate mostly from medium altitudes (ca. 200 - 700 m). Accession CGN 16194 (Netherlands) was determined as *L. serriola* f. *serriola* (Table 1).

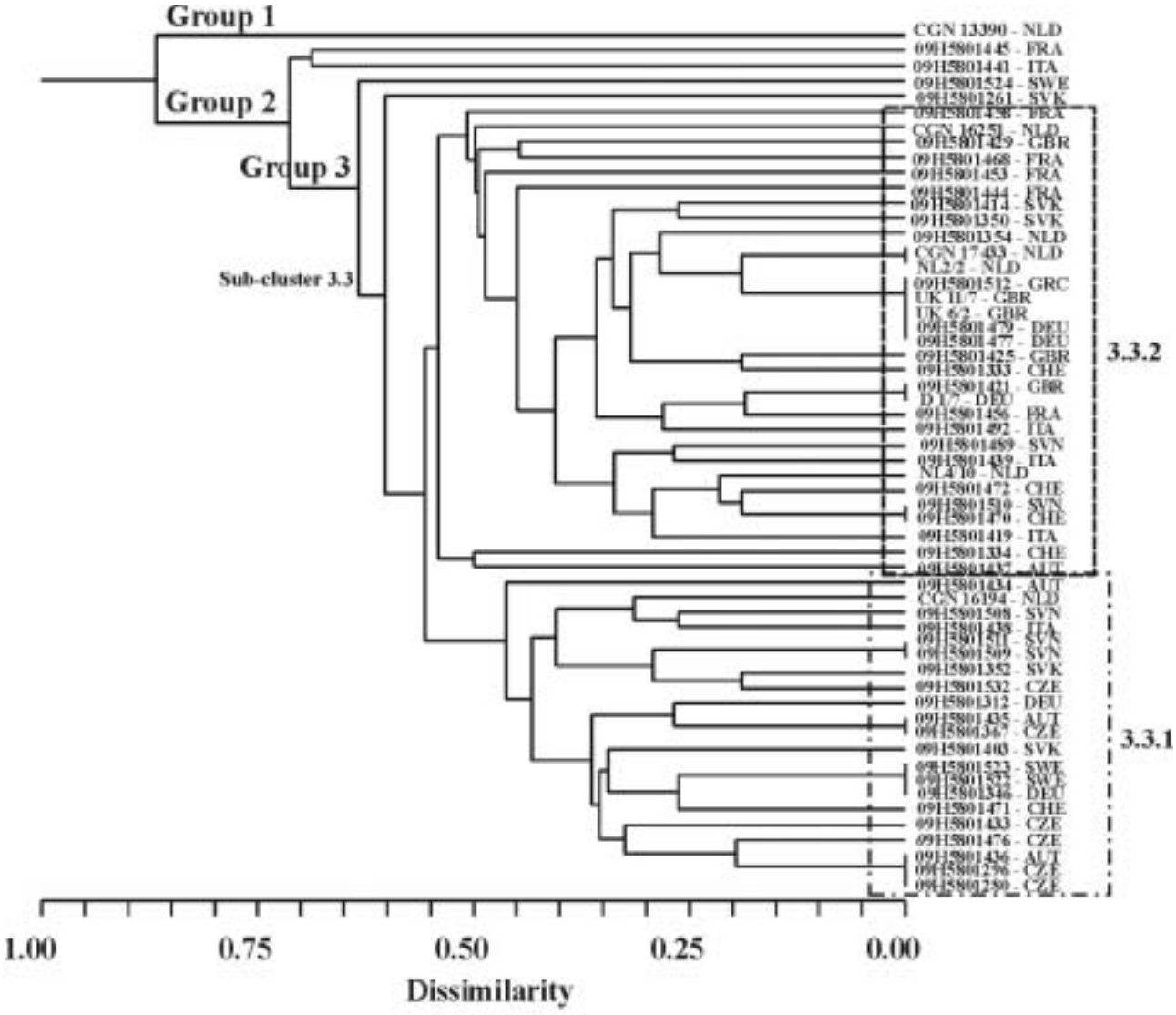

Figure 1. Dendrogram of 56 accessions of *L. serriola* and 1 accession of *L. sativa* cv. 'Norden', based on data of 9 isozyme staining systems

Table 1. Taxonomic determination of *L. serriola* accessions based on isozyme variation

Original description	Accession number	Determination
L. serriola L.	*CGN 16251*, *CGN 17433*	*L. serriola* f. *integrifolia*
L. serriola f. *serriola*	*09H5801468*, *09H5801334*	*L. serriola* f. *integrifolia*
L. serriola L.	*CGN 16194*	*L. serriola* f. *serriola*

The studied *L. serriola* material was represented by an eastern limit of distribution in Europe of approx. 0 to 21 °E and a western limit from 0 to 5 °W. The northern limit of the 12 countries is from 46 to 59 °N. Two basic clusters also follow the geographical distribution of *L. serriola*. The first one mostly comprises accessions from Austria, Czech Republic, Slovenia and Sweden. Their eastern limit is between 7 to 18 °E and northern limit from 46 to 59 °N. The second cluster contains mostly accessions from France, Germany, Great Britain, Italy, Netherlands and Switzerland with an eastern limit from 0 to 21 °E, a western limit from 0 to 5 °W and a northern limit between 39 and 52 °N. It is evident that *L. serriola* f. *serriola* is distributed mostly in Mediterranean areas at an elevation from 200 to 600 m. *L. serriola* f. *integrifolia* was found frequently in low elevations (0 - 100 m) in Germany, Great Britain and Netherlands or in highlands in altitudes from 890 m up to 1000 m (accessions from France and Italy) (Lebeda et al. 2001b). These observations support the hypothesis that the lobed leaf-form could be more successful in warmer, more arid habitats (Werk & Ehleringer 1984).

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Systematics, geography and biodiversity of wild *Lactuca* spp. germplasm

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ABSTRACT: At least 98 wild *Lactuca* spp. have been described in world literature. There are enormous differences in the continental distribution and biodiversity between *Lactuca* taxa. A total of 27 wild *Lactuca* spp. has been reported in germplasm collections summarized in the International *Lactuca* database (ILDB); but due to incorrect taxonomical determination the real number is lower. Only 20 % of the known *Lactuca* spp. mostly belonging to the European group, is available in gene banks. About 90 - 95 % of all available wild *Lactuca* accessions represent three species *L. serriola*, *L. saligna* and *L. virosa*.

Key words: Collecting strategy – *ex-situ* conservation – genetic resources – taxonomy

Introduction

The genus *Lactuca* includes a relatively large number of species (98) distributed in most of the world's important biogeographic and phytogeographic regions. However, there are enormous differences in the continental distribution and biodiversity, which is why the generic concept of the genus *Lactuca* is still in question. This information is not only important for plant taxonomists, but it is also a useful tool for the development of strategies for the collection, regeneration, *ex-situ* conservation and genebank management (van Soest & Boukema 1997). Moreover, wild relatives and progenitors of cultivated plants are of a great importance for resistance research and practical breeding exploitation owing to their broad genetic variability (Guarino et al. 1995).

Materials and methods

An overview of the wild *Lactuca* spp. distribution has been developed from local floras of European and other wider geographical areas, articles on ecology and biogeography and our own ecogeographical observations made during collecting expeditions of *Lactuca* spp. germplasm in northern, central and southern Europe. These data were compared with the representation of wild *Lactuca* spp. in world genebank collections (Lebeda el al. 2004), summarized in the recent International *Lactuca* database (ILDB; web-address: http://www.plant.wageningen-ur.nl/cgn/ildb) (Stavělíková et al. 2002).

Results and discussion

Survey of systematics

The taxonomic treatment follows the wide generic concept of *Lactuca* and the subgeneric division suggested by Lebeda (1998) and Lebeda and Astley (1999), whereby seven sections and two heterogeneous geographical groups (the African and North American one) are recognized (Table 1). New data on biochemical and molecular variability within *Lactuca* (Dziechciarková et al. 2004a, Kesseli & Michelmore 1996) did not significantly influence the generic and infrageneric divisions based on fundamental cytological, morphological, ecological and biogeographic differences.

Table 1. Sections (groups) and subsections of the genus *Lactuca* L. (modified according to Doležalová et al. 2002, Lebeda 1998, Lebeda & Astley 1999, Lebeda et al. 2004)

Groups	Sections/ subsections	Spec. (n)	Chrom. (n)
Europe[1]		17	
	Lactuca L.		
	subsection *Lactuca* L.		9, ?[2]
	subsection *Cyanicae* DC.		8
	Phaenixopus (Cass.) Bentham		8, 9,?
	Mulgedium (Cass.) C.B. Clarke		9
	Lactucopsis (Schultz Bip. ex Vis. et Pančić) Rony		8, 9,?
Asia		51	
	Tuberosae Boiss.		8, 9, ?
	Micranthae Boiss.		8, ?
	Sororiae Franchet		9, ?
North America		12	17, ?
Africa		43	8, ?

[1] Splitting the European and Asian species from the geographical viewpoint is somewhat complicated, because some species occur in both continents, and moreover new data on crossability show the presence of west Asian species in the section *Lactuca* where the majority of European species is found.
[2] For the majority of species the number of chromosomes has not been reported.

Geography

From the 98 wild *Lactuca* spp. reported in the literature, 17 are distributed in Europe, 51 in Asia, 43 in Africa and 12 in America (Table 1). The European *Lactuca* group includes 16 wild species occurring mostly in temperate and warm regions, and the cultivated lettuce (*L. sativa*). The highest diversity of European *Lactuca* spp. was found in the Mediterranean region. Altogether there are 51 *Lactuca* spp. recorded from Asia, representing about 52 % of the known species. The greatest species richness was recognized in Iran, India and Pakistan with 15, 18 and 23 species, respectively. There are only a few species found in Mongolia, Israel, Lebanon and Syria (3 to 7). From the 43 *Lactuca* spp. known on the African continent about 75 % can be considered as autochthonous. The highest species richness is evident in central and south Africa (23 species), with 18 and 10 taxa, respectively, from east and west tropical Africa, while only 8 species are reported in north Africa. A total of 12 *Lactuca* spp. were recorded on the American continent, of which seven *Lactuca* spp. can be considered as autochthonous.

Biodiversity and its representation in genebanks

Analysis of the *Lactuca* spp. genebank collections showed that the structure of the collections is skewed with regard to biogeography and distribution (Lebeda et al. 2004) The largest part of collections (90 - 95 %) is represented mainly by *L. serriola* (70 %), *L. virosa* (12 %) and *L. saligna* (10 %) (Lebeda et al. 2002) (Figure 1). A summary of the continental distribution of wild *Lactuca* spp. in genebank collections (Figure 2) showed that the majority of accessions originate from Europe (59 %) and Asia (37 %) with very low numbers of accessions coming from Africa and America. Nevertheless, these two later continents, especially Africa, are characterized by a very broad spectrum of *Lactuca* spp. totalling 59, which are mostly autochthonous or endemic. The only material available from Australia is for one accession of *L. serriola.* Information relating to the *Lactuca* spp. accessions is rather poor and sometimes misleading because the primary country of origin description does not correspond with natural distribution areas. The data for secondary sources of origin are

generally donor sources (Institutes and Botanical Gardens). The best represented species originate from and were collected in Europe, 9 species representing 55 % of the European species. Of the 23 European countries, *Lactuca* genetic resources have been collected intensively only in the Czech Republic, Germany, Greece, France, Hungary and Italy. *Lactuca serriola* is the most common species as far as geographic variation, the spectrum of different habitats and genetic variation are concerned (Lebeda et al. 2002, Dziechciarková et al. 2004b, Lebeda & Petrželová 2004).

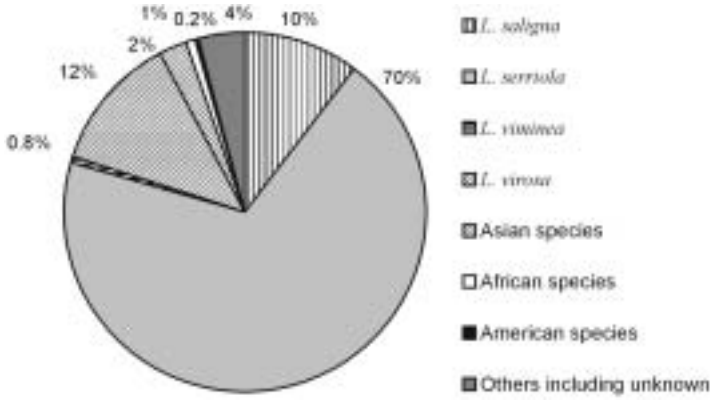

Figure 1. Representation of the common species and geographic groups of wild *Lactuca* in *ex-situ* collections (Lebeda et al. 2004)

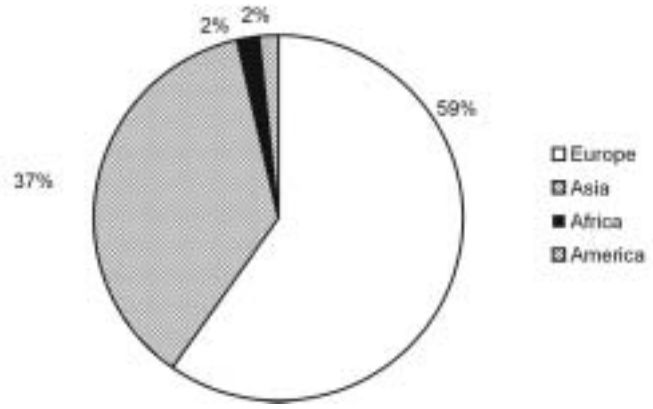

Figure 2. Representation of the continental origins of wild *Lactuca* spp. in *ex-situ* collections (Lebeda et al. 2004)

Conclusions

The collection and maintenance of more localised and autochthonous (area-specific) species, which are absent from the genebank collections, is considered essential for future progress in research of the genus *Lactuca*. A future collecting strategy must be concentrated in the hotspots of *Lactuca* spp. biodiversity (Asia, central and south Africa, north America). The study of *Lactuca* spp. in natural habitats is valuable providing underpinning basic knowledge on taxonomy, ecobiology, phylogeny and evolutionary relationships. The correct determination of wild *Lactuca* spp. accessions is the first step required to enable efficient germplasm management, utilization and the identification of true duplicates (Doležalová et al. 2003). The development of high quality validated collections with the associated international

database will serve as a base for global activities in biodiversity conservation (Programme of 'The Global Biodiversity Information Facility').

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Occurrence of race-specific resistance to *Bremia lactucae* in *Lactuca serriola* germplasm originating from four European countries

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ABSTRACT: To date there are no data available on variation and distribution of race-specific resistance to *Bremia lactucae* in populations of *Lactuca serriola* (prickly lettuce). Present research was focused on variation of resistance to *B. lactucae* within and between 50 populations of *L. serriola* in four European countries (Czech Republic, Germany, Netherlands and United Kingdom). *L. serriola* samples were investigated for their resistance against 10 isolates (races) of *B. lactucae*. Populations of *L. serriola* were found as extremely variable from the viewpoint of their resistance to *B. lactucae* and there were also clear differences in recognized resistance phenotypes and their diversity among individual distribution areas investigated. However, race-specific resistance was the most common in studied populations of *L. serriola*. Preliminary genetic interpretation of recorded resistance data has been done but for more precise characterization of the studied *L. serriola* samples further resistance studies are needed.

Key words: lettuce downy mildew – prickly lettuce

Introduction

Bremia lactucae (lettuce downy mildew) is a world-wide distributed pathogen of cultivated lettuce (*L. sativa*) and many other species from the family *Asteraceae* (Lebeda 1998, Lebeda et al. 2001). Among them, *L. serriola* (prickly lettuce) is the most common wild host species of this pathogen (Lebeda et al. 2001, Lebeda 2002, Lebeda & Petrželová 2003). Many recent papers, e.g. Lebeda and Zinkernagel (2003a) have shown that the majority of recently used resistance genes do not provide sufficient protection against lettuce downy mildew because of extremely high frequencies of complementary virulence factors recorded in recent populations of *B. lactucae*. During the last two decades much progress has been made in utilization of new resistance derived from wild *Lactuca* species (Lebeda et al. 2002). Thus, research of variation in the wild *Lactuca* spp. – *B. lactucae* pathosystem proves to be the main precondition for successful resistance breeding of lettuce against lettuce downy mildew.

Numerous papers have been published on the variation in populations of *B. lactucae* and the distribution of virulence phenotypes of this pathogen, both in crop and wild pathosystems, e.g. Crute 1987, Lebeda 1984, 2002, Lebeda and Petrželová 2004, Lebeda and Zinkernagel 2003b. However, until now there is no comparable data available concerning variation of resistance and its distribution in populations of the host *Lactuca* spp. The main objective this study was to determinate variation for resistance characters against *B. lactucae* within and between wild populations of *L. serriola* from four European countries.

Materials and methods

Samples of 50 populations of *L. serriola* were collected in four European countries (Czech Republic, Germany, Netherlands and United Kingdom) in 2001 within the frame of EU project Gene-Mine. Seeds originating from individual plants were regenerated at the Department of Botany, Laboratory of Plant Pathology, Faculty of Science, Palacký University in Olomouc. In total 752 *L. serriola* samples were included in our tests (Table 1). Samples were screened for their resistance against 10 isolates (races) of *B. lactucae* (NL1, NL5, NL12, NL14, NL15, NL16, BL17, BL18, BL21 and BL24) with known virulence patterns (van

Ettekoven & van der Arend 1999). Pathogen isolates were maintained and multiplied on seedlings of *L. sativa* cv. 'Cobham Green' which served also as a susceptible control.

Table 1. Tested *L. serriola* seed samples

Origin of seed samples	Populations (n)	Seed samples (n)
Czech Republic (CZ)	16	250
Germany (D)	16	241
The Netherlands (NL)	8	120
United Kingdom (UK)	10	141
Total	50	752

Tests were carried out according to methods described by Lebeda (2002). Circa 25 seeds of each *L. serriola* sample were sown. At the stage of fully expanded cotyledon leaves (mostly 7 days after sowing) the seedlings were inoculated with a conidial suspension of a particular *B. lactucae* isolate. Inoculated plants were incubated at 10 - 15°C, first at 12 - 24 hours after inoculation in dark and then under 12 h photoperiod. The degree of infection was assessed quantitatively 14 days after inoculation using a 0 - 3 scale (Lebeda 2002). Tests giving unambiguous results were repeated.

Results and discussion

Broad variation in resistance to *B. lactucae* was recorded within and between investigated European populations of *L. serriola*. In general, populations of *L. serriola* were highly susceptible to the used isolates of *B. lactucae* (Table 2) but great differences in recorded resistance patterns were found among individual samples. Samples resistant to all ten isolates represented only 7.8 % of all tested samples of *L. serriola* and were found in CZ and D populations only. On the contrary, about 21 % of all tested samples displayed high susceptibility to all ten isolates of *B. lactucae* (Table 3). In addition, there was a large diversity of other *L. serriola* samples characterized by race-specificity.

Table 2. Variation of resistance to *B. lactucae* in investigated European populations of *L. serriola*

Populations	*L. serriola* populations (n)				
	Resistant	Susceptible	Race-specific response	Intra-population variation in race-specifity	Total
CZ	4	9	0	3	16
D	0	6	1	9	16
NL	0	2	6	0	8
UK	0	0	10	0	10
Total	4	17	17	12	50

Table 3. Assessment of resistance of *L. serriola* samples to ten isolates of *B. lactucae*

Populations	*L. serriola* populations (n)			
	Completely resistant	Completely susceptible	Race-specific response	Total
CZ	24 (9.6)	76 (30.4)	150 (60.0)	250
D	35 (14.5)	66 (27.4)	140(58.1)	241
NL	0 (0)	15 (12.5)	105 (87.5)	120
UK	0 (0)	0 (0)	141 (100)	141
Total	59 (7.8)	157 (20.9)	536 (71.3)	752

The highest diversity of various resistance phenotypes (R-phenotypes) was recognized in CZ and D populations of *L. serriola* (Table 4). The lowest variation at the level of resistance phenotypes was recorded in UK populations (totally 10 resistant phenotypes with only 2 prevailing among 84.4 % of all UK samples). Our previous studies aimed on variation of virulence in natural populations of *B. lactucae* showed high diversity of virulence phenotypes in Czech populations of this pathogen and clear genetic affinity of the studied isolates to *L. serriola* accessions (Lebeda 1984, 2002, Lebeda & Petrželová 2003, 2004). High diversity of reaction patterns recorded in populations of *L. serriola* and *B. lactucae* indicates there is strong co-evolutionary relationship between host and pathogen populations. However, there are no data on virulence variation of *B. lactucae* in natural populations of *L. serriola* for other European countries to compare.

Table 4. Diversity of resistant phenotypes in European populations of *L. serriola*

Populations	R-phenotypes (n)	Most frequent R-phenotypes (%)
CZ	45	8 (79.2)
D	34	7 (78.0)
NL	27	5 (64.2)
UK	10	2 (84.4)

Recorded resistance data indicate that race-specific resistance is the most common type of resistance in European populations of *L. serriola*. Within some CZ and D populations high intra-population variation in race-specificity to the used isolates of *B. lactucae* was recorded, while NL and UK populations were more homogeneous (Table 2). Reaction patterns of some *L. serriola* samples (ca. 28 % of all screened samples) can be interpreted by the presence of the known R-factors/genes. In many other samples some additional known R-factors/genes could be probably identified, however presence of numerous unknown ones could be expected, too. Clarification of the presence of race specific R-factors/genes would have been based on further screening with the use of some additional differential isolates with distinct virulences (v-factors) combination. Especially isolates originating from wild *L. serriola* may have a special importance for revelation of these R-factors/genes (Bonnier et al. 1994). Recent data on the level of populations strongly support previously reported results that in *L. serriola* race-specific resistance is a common phenomenon (Lebeda et al. 2002).

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Genetic diversity among north Portugal landraces of *Brassica oleracea* subsp. *capitata* analysed with RAPD markers

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ABSTRACT: *Brassica oleracea* subsp. *capitata* L. is one of the most interesting vegetable cabbages. In Portugal, the Tronchuda cabbage comprises a large number of landraces, which are spread all over the Portuguese geography, and show a high diversification for morphological characters. In order to improve the utilization of these traditional germplasm a study to estimate the genetic diversity at inter- and intra-population level, for RAPD loci, affecting to a collection of eight landraces was carried out. The data indicate that inter- and intra-population genetic variability contributed in a similar form to the genetic diversity in this set of populations. The Portuguese landraces show a higher genetic variability than Spanish and French populations analyzed previously. A considerable differentiation between the Portuguese landraces was found. This preliminary approach suggests that a genetic resource conservation program would be advisable.

Key words: Cabbage – genetic variability – molecular markers – RAPD

Introduction

The *Cruciferae* family is a worldwide plant group with a high agronomic and economic importance. *Brassica oleracea* subsp. *capitata* L. is one of the most interesting vegetable cabbages. During antique Greek and Roman times cabbage was already established as a garden vegetable and it was probably in general use as early as 2000 or 2500 B.C. (Chiang et al. 1993). The wild cabbage is native to the coasts of northwestern Europe and the Mediterranean. Cabbage can be used cooked, as a green vegetable, raw or preserved as in sauerkraut or pickle. In the Portuguese diet the cabbage variety *Tronchuda* is frequently consumed. It can only be found in Portugal, in Galicia (northwest Spain) or in regions with strong Galician or Portuguese influence all over the world (Larkcom 1977, Msikita & Skirvin 1989). *Tronchuda* comprises a large number of landraces which are spread throughout Portugal. One of the regions that have these landraces is Trás-os-Montes, situated in the north inland of Portugal. These landraces are very diverse and differ in many morphological characters. In order to improve the utilization of germplasm resources in a crop breeding program, the estimation of genetic diversity at inter- and intra-population level of landraces is a primary goal for future projects.

Traditionally, phenotypic markers have been selected by farmers while molecular markers are free of conscious selection when the morphological selection is carried out by farmers. In this way the variability estimated using these last markers will be more representative of the genetic diversity conserved in populations. Molecular marker techniques based on polymerase chain reaction (PCR), such as random amplified polymorphic DNA (RAPD) have been used to characterize and differentiate *Brassica* species or cultivars (Kresovich et al. 1992, dos Santos et al. 1994, Margalé et al. 1995, Foisset et al. 1996, Lannér-Herrera et al. 1996, Divaret & Thomas 1997, Lázaro & Aguinagalde 1998, Divaret et al. 1999, Crockett et al. 2000, Geraci et al. 2001). In this paper, we present preliminary results

from the evaluation of the genetic diversity in north Portuguese populations of *Tronchuda* cabbage and their genetic relationships.

Materials and methods

In order to assess inter- and intra-accession variability, cabbage accessions from seven different localities (Vidago, Mirandela, Sampaio, Santo Estevão, Vila Verde, Póvoa, Macedo de Cavaleiros) were evaluated by RAPD markers. The variation at RAPD loci in these accessions was compared with the Portuguese commercial cultivar 'Penca de Chaves'. Genomic DNA from young leaves was extracted following the procedure described by Martins et al. (unpublished). 20 individuals per accession were analyzed using 11 arbitrary primers from Operon Technologies (set S and C). PCR was conducted in 25 µl of a mixture containing 15 ng of DNA, 2.5 mM of each dNTP, 15 ng of a single decamer primer, 2.5 µl of 10x *Taq* polymerase buffer, 25 mM $MgCl_2$ and 1.5 units of *Taq* DNA polymerase. A thermocycler Biometra UNO II was programmed as follows: an initial denaturing step of 3 min at 94°C, 45 cycles each consisting of 1min at 94°C, 1 min at 36°C and 2 min at 72°C, with a final step of 10 min at 72°C. The reaction products were electrophoresed on 1.5 % agarose gels containing 0.2 µg/µl of ethidium bromide, during 2 hrs at 90 V. Gels were then photographed under UV light Polaroid 667 film. Each polymorphic band was scored as present (1) or absent (0) for all genotypes, resulting in a binary data matrix. Genetic heterozygosity index (H_S) according to Nei's method (Nei 1973) and genetic variability by K_X parameter of Nei-Kumar's method (Nei & Kumar 2000) were estimated. In order to describe genetic relationships between Portuguese landraces and the control samples, genetic distances (GD) were estimated. Finally, cluster (UPGMA) analysis from genetic distance data was carried out to obtain a dendrogram. All statistical procedures were carried out by TULKAS software program developed by Sáenz de Miera (unpublished).

Results and discussion

The 11 primers used generated 228 reproducible bands to analyze, and ranged from 200 to 2750 bp. Of these amplified fragments 129 were polymorphic (56.6 % of loci detected) across the analyzed populations. The mean number per primer was 20.7. The number of polymorphic fragments varied, per primer, from two to seven. On average, 13.2 % of the markers were present in every individual in a population within a population, and ranged between 4.4 for 'Penca de Chaves' and 22.8 % in Mirandela population. Except for the commercial control (4.4 %), Macedo de Cavaleiros (4.8 %) and Vila Verde (6.1 %) populations, all the remaining populations registered a number of monomorphic markers higher than the average (Table 1). From all the markers examined, 57.9 % in average, did not amplified RAPD bands in every individual in any population. The number of polymorphic loci within any population was on average 66. The percentage of polymorphic RAPD loci was very high for populations originating from Vila Verde (52.6 %), Macedo de Cavaleiros (49.6 %) and for 'Penca de Chaves' (44.3 %). The lowest value (11 %) was registered for the Mirandela population.

A fingerprinting study with the 228 markers analyzed showed that all populations, with one exception, the Vidago population, can be identified with the amplification of only a single, fixed and specific marker. Elsewhere other markers were specific of a concrete population but showed some polymorphism at intra-populational level. Total heterozygosity (H_T) was 0.205 and total genetic variability (K_XT) was 0.525, with mean levels per population of 0.097 and 0.246, respectively. Heterozygosity ranged between 0.036 (Mirandela) and 0.176 (Vila Verde). For the K_X parameter, the minimum value was observed for the Mirandela population (0.071) and the maximum value (0.462) was registered for the population originating from Macedo de Cavaleiros (Table 1).

Table 1. Parameters of genetic variability

Pop.[1]	AB[2]	% AB	FB(1)	% FB(1)	FB(0)	% FB(0)	PoB	% PoB	H_S	K_X
VID	85	37.3	41	18.0	144	63.2	44	19.3	0.061	0.131
MIR	77	33.8	52	22.8	152	66.7	25	11.0	0.036	0.071
EST	76	33.3	41	18.0	151	66.2	35	15.4	0.060	0.128
SAM	80	35.1	34	14.9	148	64.9	46	20.2	0.068	0.161
POV	78	34.2	37	16.2	145	63.6	45	19.7	0.062	0.142
VVE	134	58.8	14	6.1	95	41.7	120	52.6	0.176	0.442
MCA	124	54.4	11	4.8	103	45.2	113	49.6	0.150	0.462
PEN	111	48.7	10	4.4	118	51.8	101	44.3	0.161	0.427
Mean	95.6	42.0	30	13.2	132	57.9	66	29.0	0.097	0.246

[1] Populations: VID, Vidago; MIR, Mirandela; EST, Santo Estevão, SAM, Sampaio; POV, Póvoa; VVE, Vila Verde; MCA, Macedo de Cavaleiros; PEN, 'Penca de Chaves';
[2] AB, amplified bands (n); % AB, amplified bands (%); FB(1), fixed bands (1) (n); % FB(1), fixed bands (1) (%); FB(0), fixed bands (0) (n); % FB(0), fixed bands (0) (%); PoB, polymorphic bands (n); % PoB, polymorphic bands (%); H_S, genetic heterozygosity; K_X, genetic variability

The calculated proportion between the inter- and intra-population heterozygosity (D_{ST}/H_S) was (1.113) and the counterpart ratio between the inter and total ($G_{ST} = D_{ST}/H_T$) was 0.527. These values suggest that inter- and the intra-population genetic variability contributed in a similar form to the genetic diversity in this set of populations. This G_{ST} value was higher than the one estimated by Lannér-Herrera et al. (1996) for Spanish and French populations, and similar for British ones. The average genetic distance between any two pairs of populations estimated by RAPD variation was 0.456±0.180. Distances ranged between 0.093 (most related populations Santo Estevão and Sampaio) and 0.667 (distantly related populations Póvoa and 'Penca Chaves'). The most distant population from others was the one originated from Macedo de Cavaleiros, with an average genetic distance of 0.594±0.073.

Cluster analysis (UPGMA) divided the eight populations into two clusters. One cluster contains the populations originated from Macedo de Cavaleiros, Vila Verde and the commercial control. In this cluster, populations were associated by GD ranging between 0.419 and 0.533. The second cluster includes five populations and can be divided into two subclusters. One subcluster contains Vidago and Mirandela populations (GD = 0.170), and the second subcluster contains the remaining populations (Sampaio, Santo Estevão, Póvoa), that were associated by GD ranging between 0.093 and 0.224. These results suggest that the populations included in subcluster two, possessed more genetic affinities than any other cluster considered. In the same way, the populations originating from Macedo de Cavaleiros and Vila Verde, along with the commercial control originating from Chaves, must be considered the most diverse of all populations included in this study.

Comparing with other studies (Divaret & Thomas 1997, Lázaro & Aguinagalde 1998, Geraci et al. 2001), in the present study RAPDs revealed that they are able to detect high levels of polymorphism. RAPD markers are able to distinguish among geographically close populations (Lázaro & Aguinagalde 1998) such as Vidago and Vila Verde or Vidago and the commercial control originating from Chaves, which had a GD value, in both cases, higher than 0.5. The high genetic variability found in this set of landraces of cabbage and its inter-population differentiation suggests that it is useful for future breeding programs. In this way a genetic resources conservation program would be advisable.

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Assessment of genetic diversity among Iranian mints using RAPD markers

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ABSTRACT: *Mentha* is a genus of aromatic perennial herbs belonging to the family *Lamiaceae*. The taxonomy of mints is complex because of their high morphological polymorphism, their ability to hybridize and their domestication. There are many landraces of mints in Iran, that their genetic diversity has not been characterized. In the present study, the genetic diversity and taxonomic relationships between 17 accessions of four *Mentha* species were examined by random amplified polymorphic DNA (RAPD) markers. Out of 70 primers, 31 provided reproducible results. A total of 608 reliable and polymorphic RAPD band were detected which were used to estimate genetic similarity among pair-wise combinations of accessions. The percentage of polymorphic markers ranged from 409 to 3446 across all the accessions studied. Jaccard similarity value ranged from 13.24 to 68.8 %, with mean of 0.25, which reflects a rather high genetic variability among the accessions evaluated. Based on the RAPD markers two major clusters were at 80 genetic distances. The result indicated that RAPD as molecular marker system was useful for detecting the genetic variation among the accessions. The maximum and minimum similarity observed between *M. spicata* 10 and *M. longifollia* 11 (68.8%), and *M. piperita* 13 with *M. spicata* 2 (13.24 %), respectively. In the view of taxonomists and studies on the evolution of the mints, *M. piperita* is believed to be a hybrid between *M. spicata* and *M. aquatica*. According to our analysis, *M. piperita* 14 is about 45.19 % similar to *M. spicata* 10 and clustered in the same group. The clustering between these two accessions is in agreement with the earlier observation.

Key words: Genetic diversity – *Mentha* – RAPD molecular marker

Introduction

Mints are herbaceous plants and perennial aromatic herbs that are cultivated for their essential oils used both for medicinal and culinary purposes. These plants belong to the genus *Mentha* L. (*Lamiaceae*), which produces secondary metabolites such as alkaloids, flavonoids, phenols, gummy polysaccharides, terpens and quinines that are used in food, pharmaceutical, cosmetics and pesticide industries (Khanuja et al. 2000). According to a high polymorphism in morphology and a great diversity in essential oil composition, the number of species of the genus *Mentha* L. has been a matter of speculation for many years. Harley and Brighton (1977) recognized five sections (*Audibertia, Eriodontes, Pulegium, Preslia, Mentha*) on the basis of basic chromosome numbers and morphological features. There is no problem of identification for the first four sections because no example of natural interspecific hybridization exists. The fifth section, *Mentha*, includes five species: *M. suaveolens, M. longifolia, M. spicata, M. arvensis* and *M. aquatica*. Natural interspecific hybridization occurs with high frequency in section *Mentha*, this leads to a large diversity of chromosome numbers (24-120), and much of the taxonomy of section *Mentha* has been complicated by hybridization, by high morphological polymorphism, as well as polyploidy and vegetative propagation (Gobert et al. 2003).

The assessment of genetic diversity at the DNA level for these accessions has been considered as the desirable step in the process of developing taggable markers to aid genetic improvement in the variety development programme. The accurate identification of plant material is essential for effective germplasm characterization; without such information breeders have no means of selecting appropriate material for entry into breeding programmes.

In recent years, molecular markers have been applied in a wide number of genetic and breeding studies, including fingerprinting individuals and the positional cloning of important genes. One of the most extensively used molecular markers are RAPDs (Williams et al. 1990), which have been applied to address genetic diversity issues in plants (Vilanova et al. 2001, Ray Choudhury et al. 2001, Campos-de-Quiroz et al. 2001, Gichuki et al. 2003). They are especially suited to species with little molecular information such as mint due to the following attributes: 1) No previous knowledge of the genome is required; 2) Rapid results can be obtained when compared with alternatives such as RFLPs; and 3) A universal set of primers, which can be used for genomic analysis in any species, is commercially available.

There is no information available on genetic diversity of Iranian aromatic mints. Hence, in the present study, the extent of genetic diversity and taxonomic relationships between 17 accessions belonging to *Mentha spicata, M. piperita, M. suaveolens*, and *M. longifolia* were assessed by RAPD fingerprinting.

Materials and methods

Plant material

Four taxa of mints, namely *Mentha spicata* L., *M. piperita* L., *M. suaveolens* and *M. longifolia* (L.) Hudson were selected for RAPD analysis. These included nine accessions of *Mentha spicata* (1, 2, 3, 4, 7, 8, 9, 10, 12), four accessions of *Mentha piperita* (13, 14, 15, 16), three accessions of *Mentha longifolia* (5, 6, 11) and one accession of *Mentha suaveolens*. The plant materials were obtained from Agriculture Research station of Isfahan University of Technology, University Jehad Research Farm (sponsored by Shahid-Beheshti University) and Research Farm of Mayor in Isfahan. The plant materials were identified by Randy Olson, W.P. Fraser Herbarium, Canada. The plants analyzed in this study were maintained in the Shahrekord University glass house and leaf materials were taken for DNA isolation.

Total DNA isolation, RAPD fingerprinting and data analysis

Total cellular DNA was isolated from freshly germinated young leaves by following a modification of the CTAB method of Murray and Thampson (1980): about 3 g of fresh leaf tissue was crushed to powder in liquid nitrogen and transferred to pre-warmed (60 °C) 2 × CTAB buffer containing 1 % PVP (w/v) (polyvinyl pirrolidone).

Thirty one arbitrary 10-mer primers (Genset, France) were used for PCR amplification of the total genomic DNAs. Polymerase chain reaction was performed based on the protocol of Williams et al. (1990) with minor modifications. Amplifications were carried out in 25 μL of reaction mixture containing 2.5 μL of PCR buffer, 200 μM each of dATP, dCTP, dGTP, dTTP (Amersham Biosciences), 15 ng of the primer, 0.7 unit of Smar*Taq* DNA polymerase (Cinnagen) and 25 ng of DNA template. DNA amplification was performed in a Eppendorf Mastercycler Gradiant programmed as follows: pre-denaturation 3 min at 94°C followed by 45 cycles, each of 1 min at 94 °C, 1 min at 35 °C, 2 min at 72 °C, followed by one final extension cycle of 10 min at 72 °C. The amplification products were size-separated by gel electrophoresis in 1.2 % agarose gels with 0.5 × TBE and stained with ethidium bromide. *Eco*R1/*Hin*dIII digested lambda DNA served as molecular size marker. PCR reactions were repeated at least twice to establish reproducibility of results. After AGE, the gel was photographed in UV light.

The molecular size of each fragment was calculated by using UVI bandmap software 10.02. All the accessions were scored for the presence of band size (1) or its absence (0). The data were analyzed to generate Jaccard's similarity coefficients. These similarity coefficients were used to construct dendrograms using the unweighted pair group method with arithmetic averages (UPGMA) employing the NTSYS-pc software, version 2.02.

Results and discussion

The amplification profiles produced by 31 primers gave a total of 617 bands, out of which only nine were monomorphic while the rest 608 were polymorphic (98.7 %). The maximum number of bands was produced by the primer RAPD68 (32), the minimum number by primer RAPD70 (1) (Figure 1). Each primer generated an average 19.9 bands per individual, ranging in size from 409 to 3446 base pairs (bp). Further analysis of these RAPD profiles for band similarity indices could clearly differentiate all the taxa of *Mentha* viz. *M. spicata, M. longifolia, M.* × *piperita and M. suaveolens* from one another.

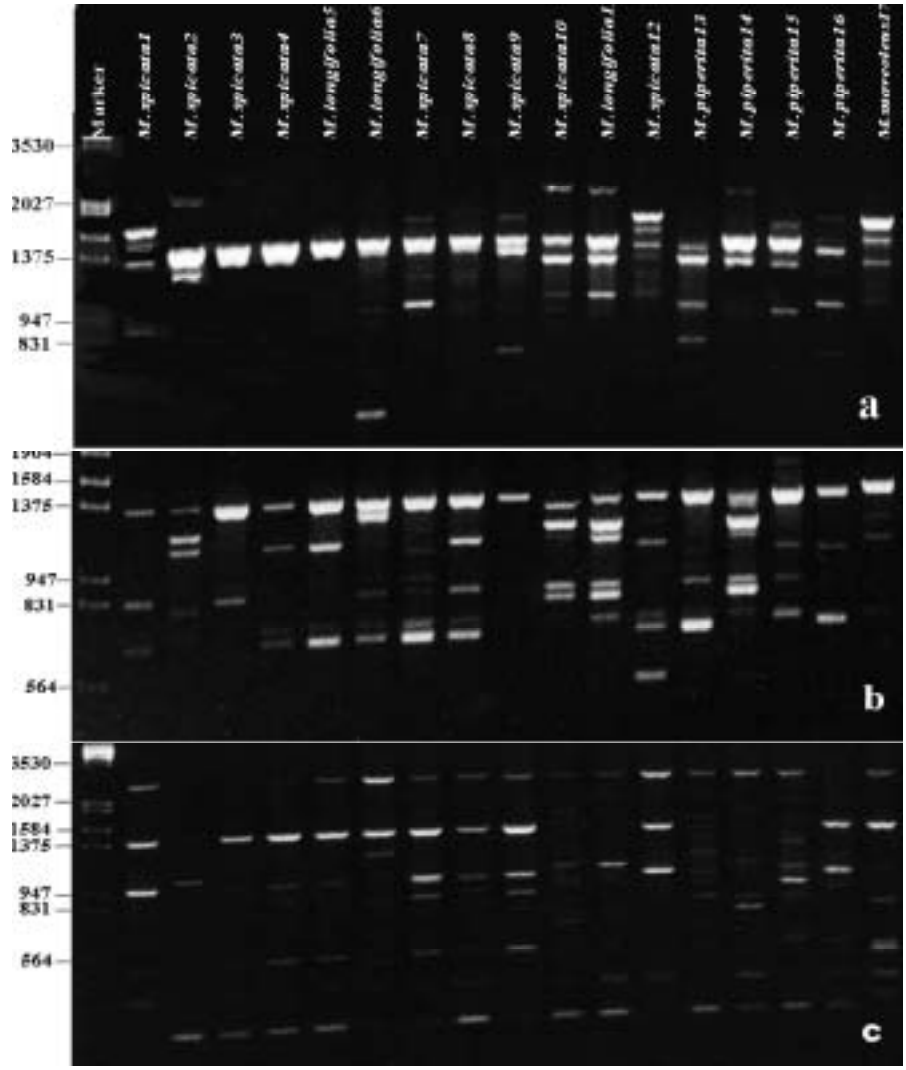

Figure 1. PCR products amplified from *Mentha* accessions DNA using primers RAPD4 (a), RAPD10 (b) and RAPD67 (c)

One of the main objectives of the study was to analyse the genetic similarity/distance between these species of mints. The similarity coefficients were obtained using Jaccard coefficient. Similarity coefficients ranged from 13.24 to 68.85 across all 17 accessions. The range of similarity coefficients values within *M. longifolia*, *M.* × *piperita* and *M. spicata* were 0.22 - 0.28, 0.18 - 0.34 and 0.18 - 0.45, respectively. These similarity coefficients were used to generate a tree for cluster analysis using UPGMA method (Figure 2). Based on the RAPD markers, 2 major clusters were formed at 80 % genetic distance: (I) *M. piperita* 13 along with *M. piperita* 15 were grouped into one, and had 19.6 % similarity with the rest of accessions;

and (II) the rest of accessions. The most well known hybrid, *M.* × *piperita* (peppermint) showed a range of similarity coefficients, from 0.18 to 0.34, and was separated by UPGMA into two clusters.

There were two distinct sub-clusters in cluster II: (1) *M. spicata* 2, *M. spicata* 10, *M. longifolia* 11, *M. piperita* 14 and *M. suaveolens* 17, and (2) *M. longifolia* 5, *M. longifolia* 6, *M. piperita* 16 and the rest of accessions of *M. spicata*. Within sub-cluster 2, two subgroups are clearly defined: (1) *M. spicata* 1 and *M. spicata* 12, and (2) *M. spicata* 3, 4, 7, 8, 9, *M. longifolia* 5, 6 and *M. piperita* 16. In the sub-cluster I, maximum similarity was observed between *M. spicata* 10 and *M. longifolia* 11 (68.8%). *Mentha spicata* is a hybrid between *M. longifolia* and *M. suaveolens* (Harly and Brighton 1977). These results confirm the progenitor of *M. spicata*. Interestingly, *M. suaveolens* 17, *M. piperita* 14, *M. spicata* 10, *M. spicata* 2 and *M. longifolia* 11 formed a subgroup in the cluster II with about 23% similarity. Our results, based on molecular markers showed that *M. suaveolens*, *M. longifolia*, *M. spicata* and *M.* × *piperita* are closely related. In the view of taxonomists and studies on evolution of the mints, *M.* × *piperita* is believed to be a hybrid between *M. spicata* and *M. aquatica* (Murray at al. 1972). *M. aquatica* is octoploid, while *M. spicata* is a triploid or tetraploid. Therefore, two-thirds of the *M.* × *piperita* genetic pool is composed of the *M. aquatica* genome. According to our molecular analysis, *M. piperita* 13 and *M. piperita* 15 showed the highest similarity with one of its octoploid progenitors, rather than with *M. spicata*. The primers provided enough RAPD polymorphisms to resolve genetic diversity between and within species. The similarity between these accessions was in agreement with their earlier observations. The degree of polymorphism displayed with RAPDs in *Mentha* was expected from a cross-pollinated nature. The analysis of molecular markers detected a larger fraction of genetic variation within species. This is in accordance with what would be expected from a cross-pollinated species. This genetic variability could in future be exploited through molecular approaches for gene introgression in breeding programs to produce desired genotypes.

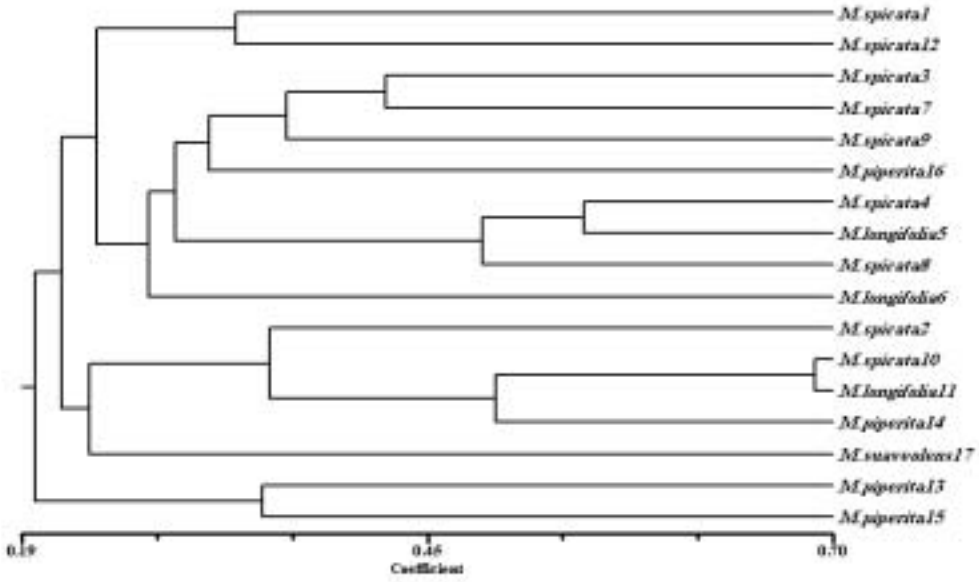

Figure 2. Dendrogram generated using Jaccard coefficient of *Mentha* accessions based on RAPD data

Acknowledgements
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Variation in relative DNA content in maca and yacon germplasm

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ABSTRACT: A set of 12 maca (*Lepidium meyenii* Walp.) genotypes and 24 genotypes of yacon (*Smallanthus sonchifolius* (Poepp. & Endl.) H. Robins.) were analyzed for relative DNA amount variation. Nuclear DNA content, estimated by using flow cytometry (DAPI staining), showed that 2C DNA amount ranged from 1.63 to 1.69 in maca. A mean 2C DNA content ranging from 5.81 to 6.12 was found for yacon accessions. Statistical analysis of data based on relative DNA contents does not correspond to either morphological features or to isozyme polymorphism of both crops studied.

Key words: Andean crops – nuclear DNA – genetic resources – *Lepidium meyenii* – *Smallanthus sonchifolius*

Introduction

Several approaches have been used to characterize plant material stored in world genebanks. Classical morphological features of descriptive databases have been completed with biochemical and molecular characters which become to be a part of evaluation databases (Ayad et al. 1995). From the viewpoint of the conservation of genetic diversity, the molecular techniques can be used for screening of material maintained in genebanks in order to precise determination of individual accessions (van de Wiel & Vosman 1998).

The American Indians in Peru have been used maca (*Lepidium meyenii* Walp.) and yacon (*Smallanthus sonchifolius* (Poepp. & Endl.) H. Robins.) as food and medicinal plants for centuries. However, to Europe they have been introduced as vegetables having health optimizing effects in last twenty years (Valentová et al. 2001). Recently, morphological and yield parameters as well as antidiabetic, nutritioal and fertility-enhancing properties of both crops have been studied in detail (Lebeda et al. 2003a). A recent paper is focused on maca and yacon relative DNA amount and contributes to the knowledge on a complex variability of both Andean crops (Lebeda et al. 2003b, 2003c), since this basic information has been still missing.

Materials and methods

Plant material

The plant material represented 12 genotypes of maca and 24 genotypes of yacon maintained as genetic resources for new crop development in Potato Research Institute Havlíčkův Brod, Ltd., Czech Republic (Frček 2001, Lebeda et al. 2003a, 2003b). Genotypes of maca originated from Peru and a set of yacon genotypes was imported from New Zealand (primary centre of origin having been Ecuador).

Relative DNA content assessment

Relative nuclear DNA content estimation was conducted by using a PAS flow cytometer (Partec GmbH, Germany). Approximately 20 mg fresh leaf tissue was chopped in 500 µl OTTO I buffer (Otto 1990). After addition of 1000 µl OTTO II buffer (Otto 1990) containing DAPI stock solution, the suspension of isolated nuclei was filtered through a nylon mesh (40 µm pore size) and examined. The DNA amounts of maca plants were determined relative to the radish (*Raphanus sativus*) cv. 'Saxa' (2C DNA 1.11 pg, PI) as an internal standard. Pea

(*Pisum sativum*) cv. 'Ctirad' (2C DNA 9.09 pg, PI) was used as an internal standard for analysis of the yacon germplasm set. The data of sample analysis were processed through the Partec computer. Statistical analysis was performed using Statistica statistical software. Analysis of variance, Scheffe's and LSD tests were employed to analyse the variation in mean relative DNA content.

Results and discussion

Flow cytometric analysis showed that means of 2C DNA relative amount ranged from 1.63 to 1.69 in maca (Table 1). A flow histogram of measurements of maca nuclear DNA content (peak no. 1) in comparison with radish (peak no. 2) is shown in Figure 1. Significant differences (Scheffe's test) in the relative DNA amount were found among three groups of maca genotypes (Table 1). Group A comprises genotypes 151, 265 and 310 with the lowest DNA amount. Most of genotypes (280, 314, 145, Unalm amarilla, 153, 168, 146 and 136) belong to group B with medium relative DNA content. Only genotype 290 falls to a group C with the largest value of nuclear DNA amount.

Table 1. Means of relative nuclear DNA content and differences in maca genotypes

Genotype	2C nDNA content		Variation	Group/Significance of differences[1]
	Mean	SD		
151	1.634	0.029	1.630 - 1.640	A
265	1.641	0.014	1.630 - 1.650	A
310	1.642	0.019	1.623 - 1.657	A
280	1.649	0.016	1.640 - 1.660	B
314	1.651	0.009	1.650 - 1.653	B
145	1.651	0.030	1.640 - 1.667	B
Unalm amarilla	1.653	0.011	1.657 - 1.650	B
153	1.654	0.025	1.640 - 1.663	B
168	1.657	0.029	1.650 - 1.660	B
146	1.657	0.016	1.663 - 1.667	B
136	1.662	0.012	1.653 - 1.670	B
290	1.691	0.016	1.680 - 1.697	C

[1] Genotypes with the same letter are not significantly different ($P \leq 0.05$)

From the viewpoint of morphological and biochemical variation the studied accessions represent different groups of morphotypes and show various isozymes spectra (Lebeda et al. 2003b, 2003c). The genotypes belonging to groups A and C (according to relative DNA amount) form the most often observed morphotype of maca hypocotyl called 'Raku chupa' (Lebeda et al. 2003a). For the isozyme variation, all accessions representing groups A and C exhibit the same EST banding pattern (Lebeda et al. 2003b, 2003c). Thus, it is evident from the obtained data, that variability on relative DNA content in maca genotypes does not correspond directly to either morphological variation or isozyme polymorphism.

The variability in nuclear DNA content was found among 24 yacon genotypes as well. In yacon the means of relative 2C DNA amount ranged 5.81 to 6.12 (Table 2). Statistical analysis (LSD test) based on relative DNA data showed five significantly differring groups of yacon genotypes (Table 2). The majority of yacon genotypes (57, 17, 5, 64, 47, 1237, 6, 31, 88, 85, 74, 25, 60, 84 and 75) belong to the group A with low relative DNA content within the set studied. The groups B (genotypes 68, 48 and 22) and C (51, 90, 28 and 18) were characterized with medium 2C DNA content. The largest nuclear DNA amount was observed in groups D (genotype 83) and E (genotype 92). The variability observed on the level of nuclear DNA amount resp. isozymes (EST and ACP) is very low in contrast to a large

variation in morphological characters and yield parameters (Lebeda et al. 2003a). The statistical differences in relative DNA amounts among yacon genotypes were verified, however, a direct relationship between 2C DNA content and morphological resp. isozyme variation was not found.

Table 2. Means of relative nuclear DNA content and differences in yacon genotypes

Genotype	2C nDNA content		Variation	Group/Significance of differences[1]
	Mean	SD		
57	5.816	0.231	5.757 - 5.907	A
17	5.820	0.305	5.720 - 5.910	A
5	5.859	0.239	5.830 - 5.883	A
64	5.883	0.242	5.810 - 5.973	A
47	5.939	0.211	5.867 - 6.050	A
1237	5.941	0.144	5.857 - 6.037	A
6	5.961	0.121	5.943 - 5.977	A
31	5.968	0.161	5.953 - 6.040	A
88	5.972	0.258	5.863 - 6.060	A
85	5.976	0.259	5.817 - 6.063	A
74	5.980	0.220	5.840 - 6.067	A
25	5.982	0.257	5.887 - 6.030	A
60	5.987	0.201	5.920 - 6.047	A
84	5.988	0.086	5.947 - 6.030	A
75	5.993	0.121	5.930 - 6.047	A
68	6.012	0.360	5.750 - 6.293	B
48	6.012	0.190	5.873 - 6.120	B
22	6.013	0.212	5.987 - 6.113	B
51	6.019	0.114	5.948 - 6.067	C
90	6.027	0.126	5.973 - 6.060	C
28	6.031	0.176	5.977 - 6.080	C
18	6.039	0.092	5.980 - 6.093	C
83	6.058	0.090	6.020 - 6.123	D
92	6.118	0.290	6.050 - 6.167	E

[1] Genotypes with the same letter are not significantly different ($P \leq 0.05$)

The results obtained from the analysis on relative DNA amount in maca and yacon show that variability detected on this level exists. Unfortunately, a clear relationship among morphological characters, isozyme polymorphism and variation in relative DNA content in both crops was not found. Nevertheless, for the very first time such a high number of original Andeans genotypes has been analyzed and characterized by using this approach.

Acknowledgements
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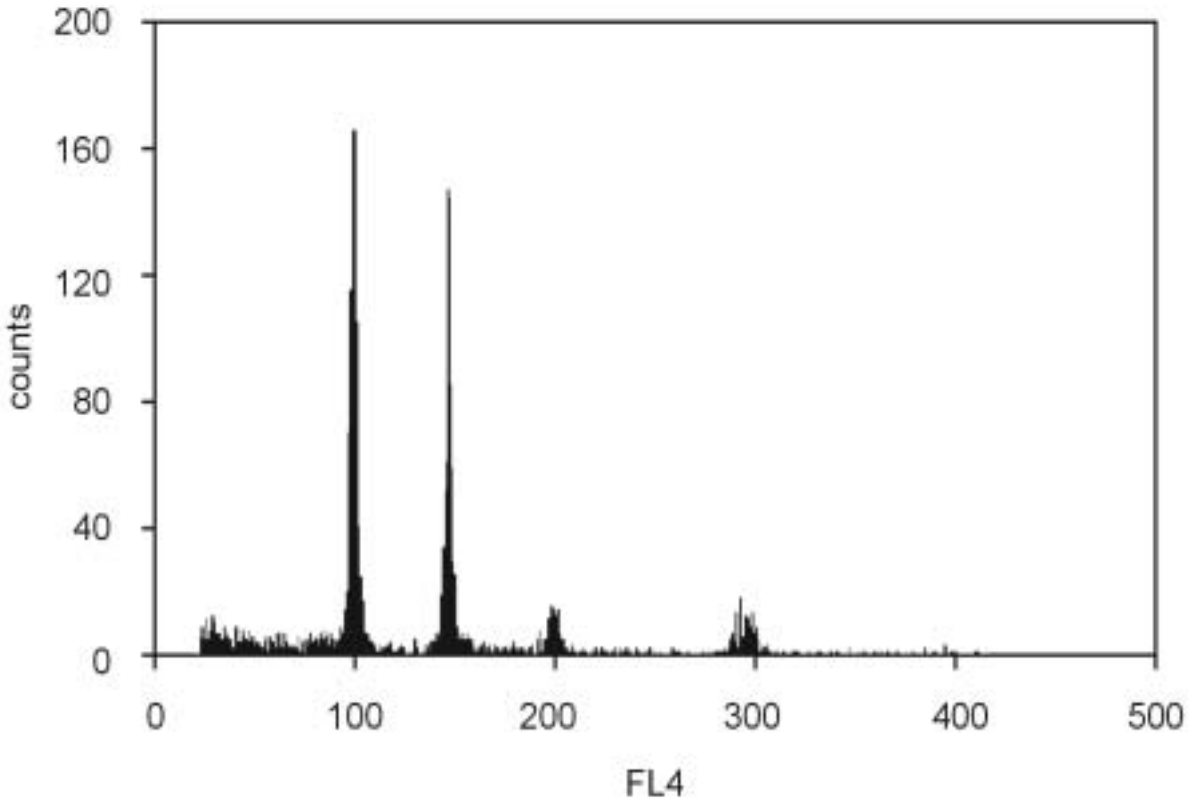

Figure 1. Histogram showing relative nuclear DNA content of maca in comparison to an internal standard *Raphanus sativus* cv. 'Saxa' (2C DNA 1.11 pg, PI). Peak no. 1 – maca (relative 2C DNA = 1.63); peak no. 2 – radish.

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The landraces – An inexhaustible source of variability

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Abstract
In less than 10 years (from 1956 to 1964) the corn landraces cultivated on 3,000,000 ha have been replaced by new hybrids. A huge germplasm made in a long process of evolution, from 1611, was in danger. Nobody knows how many and what kind of genotypes have been collected and how many disappeared forever. In contrast with Jean Rostand's sceptic affirmation "The nature arrived to their fulfilment" we look for new genotypes with their own evolution in local ecological niches.

The Banat and Maramure counties as well as other areas of Romania represent a valuable genepool for many cultivated and spontaneous plants. The collecting and evaluating strategy depends on the economical interest due to the introduction of new cultivars with more profitable parameters. In the beginning cultivars of major agricultural importance had been collected. The horticultural species were in the second stage. Our first search was extended to collect and to study corn landraces from southern Banat (1955 - 1965). Here, 173 corn landraces were collected and maintained in *ex situ* conditions through the SIB method. The best of them, *Giulvaz*, *Gottlob* and *Recas* were used in the corn breeding program. From the *Recas* landrace an inbreed line was selected which was used as parent of the Romanian simple 'Hybrid 400'.

After 1980, the interest in landraces dropped and was only sporadic. In 1984 the Department of Agriculture supported the national program for landraces collection. From this time up to 2003, we collected 1383 landraces with unequal effort. In the years of financial support (2001 - 2003) the amount of collected genotypes was high (646), but we continued to collect them even now, without financial support anymore, because in isolated villages of mountainous valleys there exist still valuable landraces.

859 landraces of major importance for agriculture were collected. The majority of them were *Zea mays* L. (241, 28.05 %) and *Phaseolus vulgaris* L. (360, 41.9 %). A total of 318 major horticultural species were also collected. *Allium cepa* (82, 25.8 %) and *Allium sativum* L. (63, 19.8 %) were collected and studied *in situ* and *ex situ*. Among the other horticultural species collected (99) were *Lactuca sativa* L. (37, 37.4 %) *Satureja hortensis* L. (20, 20.2 %), and Papaver *somniferum* L. (18, 18.2 %). The most interesting tomato landrace is *Porodice*. It grows spontaneously in the unherbicided corn fields of the Alma area.

Up to now the medicinal plants were not in our attention even if their chemical composition is well appreciated. The main reasons consist in the incapacity to determine the active compounds. Nevertheless, we collected four *Ocymum basilicum* L. genotypes, a plant appreciated for its antiseptic effect. In our attention were also forage landraces (88), in particular flowers (8), endemics (3) and honey ones (5). Some of them were sent to the Research Institute for Cereals and Industrial Crops in Fundulea (1984 - 1989) and to the genebank in Suceava (1986 - 2001). Some landraces are retained in our collection (corn: 90; bean: 53; others: >200).

It can be summarized that the southern and northwestern parts of Romania are a valuable genepool area, not yet enough explored. In the spontaneous flora there are *Linum usitatissimum* L. with white flowers, different *Melilotus officinalis* genotypes, white clover and species adapted to salinity or acid soils. All of them bear the local breeder's imprint, the phenotype being in concordance with his conception, taste and proud. Landraces exchange, especially bean, corn and *Allium* sp. are rarely practised by the 'local breeders'. The Vest University Vasile Goldis from Arad in co-operation with our University started to gather up the landraces in a genebank at Socodor, a small town near Arad.

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Remarks on the safeguarding actions for autochthonous crop genetic resources in Mediterranean countries

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Abstract

A study has been carried out on crop safeguarding actions in the twenty countries directly facing the Mediterranean Sea. Per each country a short overview is given on the most important measures related to the local plant genetic resources (PGR) situation, and some proposals for safeguarding autochthonous plant germplasm threatened by genetic erosion are suggested. In general, almost everywhere, an integrated approach *ex situ*/*in situ* conservation represents the best solution to protect PGR. In all the proposed cases, the role of the genebanks can be seen as a catalytic one, combining the efforts of farmers and nature protection institutions, but also as critical observers not only of the process of genetic erosion but also of the ongoing evolution. Several examples are also given of the practical utility of landraces as gene reservoir for classical and modern breeding. In conclusion, nowadays, some countries are still particularly rich in PGR (e.g. Turkey), others need urgent actions to stop the local crop genetic erosion and/or extinction, some others have not been well studied yet, while very few countries already have a satisfying situation and a fine scientific and technical organisation.

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Effective germplasm collection management by evaluation of plant genetic resources status with molecular markers

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Abstract

To serve as an effective improvement source for modern varieties, plant genetic resources (collections) should have rational organization and management. Each accession should be identified and registered. Preservation of genetic integrity of accessions and identification of duplicate accessions are also included in the category of basic problems. An important goal of genebank activity is to provide understanding of genetic structure of biodiversity (relationships inside a gene pool). Molecular markers are successfully used on all steps of work with plant collections (in VIR, protein markers are mainly used). Protein markers are as a rule inherited codominantly; analysis of a genotype is possible by the protein phenotype. They are multiple, the most polymorphic and located in morphogenetically homogeneous tissues – in the endosperm and cotyledons of mature seed. It is possible to use for analysis only part of the seed, using another one for planting. Seed storage proteins (SSP) are widely used as marker systems for variety verification. ISTA confirmed the advantage of protein markers as standard methods for variety and species identification. In VIR, the system of identification and documentation based on SSP was elaborated. This system is successfully used (1970 - 2004) for management of many crop collections (Konarev et al. 2002).

The system of registration includes: nomenclature of electrophoretic components, standard (etalon) patterns and methods of recording components in the form of protein formula, and the structure of information databases founded on protein formulae. Varieties and biotypes of many crops have been written down in the form of protein formulae, e.g. catalogues and databases of such formulae were released. For wide practical application of these approaches in genebanks it is necessary to improve and develop all procedures and adapt protocols for a certain gene bank (key problems, dominant crops, other circumstances).

Identification simplifies documentation of a gene pool of cultivated plants and their wild relatives with the aim of its registration, preservation and effective utilization in breeding. It is important to be able to reveal and isolate desirable genotypes from complex natural varietal and hybrid populations. Identification of varieties and lines is especially topical for intensification of breeding and seed production, which demand high accuracy and efficiency of seed control. The first stage of the use of novel germplasm in breeding projects involves its identification. In order to develop a flexible and reliable nomenclature and system of pattern recording, practically all intraspecific (or intrageneric) variability of a given protein marker should be investigated (Konarev et al. 2002). The pattern of protein bands produced is related to genetic constitution and can be considered as a "fingerprint" of biotype, variety or accession. SSP were taken as basic marker proteins for practically all major crops.

By this comprehensive investigation of biodiversity, almost all possible locations of protein (or DNA) components in electrophoretic patterns can be identified. Cultivars, wild populations and landraces from the global collections have to be analyzed. It was implemented in VIR and this principle was laid in the base of nomenclatures and systems of recording electrophoretic components for many crops. This approach was first developed for

wheat and then spread on all *Triticeae* Dum. and other crops**.** The structure of a computerized catalog of the VIR collection based on protein formulae with electrophoretic pattern was developed. The software for management of this database is being designed.

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Activities of the Botanical Garden of Vilnius University at the Lithuanian National Plant Genetic Resources Programme – description and use of genetic diversity

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Abstract

At present the main work on genetic resources is carried out in ten research and educational institutions of Lithuania. All of them are actively involved in the fulfilment of the National Genetic Resources Programme that was launched in 1998 by Lithuanian Ministry of Education and Science. The programme encompasses the conservation and research of the genetic resources of agricultural and horticultural crops, medicinal and ornamental plants and forest trees. Since 2002, the Ornamental Plant's Genetic Resources Research and Protection Coordination Centre is working at the Botanical Garden of Vilnius University. Since 2002 the Central European data registration base for *Ribes* L. and *Rubus* L. genera has been created under authorisation of IPGRI (Italy). There are about 10000 taxa plants of 190 families and 866 genera in the collections of the Botanical Garden of Vilnius University. The collection of woody plants is the biggest in Lithuania (more than 2500 species and cultivars). The work contains two parts: investigation of woody plants in old manor parks, forests and other green areas of Lithuania, research work in the department's collections. In Lithuania the breeding of flowers is concentrated in amateur breeder's hands. Nurseries are established for the conservation of the genetic resources of these flowers at the Botanical Garden of Vilnius University (about 900 number of flower varieties). The collection of induced and natural mutants of cereal crops is maintained and investigated only at Vilnius University. There are natural mutants (about 80 species), but the most important is the original collection of induced barley mutants and revertants – about 300 collection numbers. It includes several genetically unstable loci. This collection can be used as donor for chromosomal gene-markers and genes for resistance to fungal infections. The *Rubus idaeus* L. collection from different Lithuanian administrative regions is stored too. The oldest and most abundant collection of horticultural plants is the currants and gooseberries collection (over 400 species and cultivars). The other collections include 86 Lithuanian A. Gailiūnas grape clones, 37 Lithuanian honeysuckle clones and 40 quince collection numbers. The collection of Lithuanian A. Gailiūnas grape clones, induced barley mutants and revertants, 123 *Rubus idaeus* L. accessions from different locations of Lithuania are investigated by methods of estimation DNA polymorphism too.

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Genetic erosion in crop plants? A case study

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Abstract
Human activities like urbanization, replacement of traditional agriculture systems by modern industrial methods or the introduction of modern high-yielding varieties may pose a danger to the biological diversity. In a case study, samples of cultivated wheat (*Triticum aestivum* L.) collected in intervals of 40 to 50 years in four comparable geographical regions of Europe and Asia were analysed using microsatellite markers. The material was originated from Albania (1941; 1994), Austria (1922-1932/1982), Nepal (1937; 1971) and North India (1937; 1971). For the total number of year specific alleles detected, there was no clear tendency. Whereas this number was slightly higher for the early missions in the material collected in Albania and Nepal the opposite case was detected for Austria and India. At the single locus level, however, contrasting tendencies were observed. Analogous results were obtained for the PIC values. Applying the *U*-test, no significant differences were detected both in the number of alleles per locus and in the mean PIC values, comparing the material of the repeated collection missions in all four regions analyzed. About two third of the alleles were in common for both collection periods. One third, however, represented collection mission specific alleles. These findings demonstrate that an allele flow took place during the adaptation of traditional agriculture to modern systems, whereas the level of genetic diversity was not influenced significantly. In other words the genetic diversity has been maintained within hexaploid wheat since genebank activities started in the first half of the last century. However, there was clear evidence for qualitative changes in the observed diversity. The data clearly demonstrate that in a certain period of cultivation a certain amount of unique alleles is present. This may have consequences for the conservation of plant genetic resources. The exploitation of the whole range of allelic variation makes it necessary both to maintain the already existing *ex situ* collections but also to collect new material. One can only preserve the allelic composition of a present situation.

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Influence of wild donor D-genome chromosome substitutions on wheat architecture and flowering time

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Abstract

Plant architecture and flowering time are key traits influencing cereal yield and adaptation. Morphological and agronomic characterisation of a set of wheat ('Chinese Spring'/Synthetics) D-genome substitution lines has been carried out under Mediterranean conditions during a two-year field trial. The substitution of *Ae. tauschii* (D-genome) chromosomes influenced both morphological traits (like flag leaf length, flag leaf width, plant height, peduncle length or tiller diameter) and flowering time traits. The substitution of chromosome 1D increased flag leaf size, whereas chromosome 5D had the opposite effect. Chromosomes 2D, 4D and 6D promoted early ear emergence and flowering while chromosomes 5D and 7D delayed ear emergence and flowering under short photoperiod Mediterranean conditions in both years tested. Spike shape was altered by the introgressions of chromosomes 2 (speltoid) and 7D (shorter and squared spike). Disease pressure was observed during one year only, but provided preliminary data on the chromosomal location of wheat leaf rust susceptibility genes. Mapping of selected traits will be carried out in the future by the agronomic characterisation of the introgression lines available for the involved chromosomes.

Acknowledgements

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Assessment of genetic diversity in bread wheat based on RAPD and seed storage protein markers

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Abstract
Assessment of genetic diversity in a crop species is prerequisite to its improvement. The use of germplasm with distinct DNA profiles will help to generate genetically diversified breeding populations. The aims of this investigation were to study of genetic diversity based on RAPD data and HMW glutenin subunits among twenty-eight bread wheat genotypes. Combined data of both mentioned marker systems helps to identify parents with adequate genetic distance and high quality of allele composition. Eight of fifty primers of a 10-mer showed scorable polymorphic bands. The genetic similarity based on RAPD analysis by simple matching coefficients ranged from 41 % to 91 % with an average of 64 %. The highest similarity was for Azadi and Khazar 1 cultivars, where as the highest genetic distance was for Line7107 and Karaj3 cultivar. The study of HMW glutenin subunits diversity by SDS-PAGE detected 13 alleles and 15 allele compositions. The most frequencies were related to N/7+8/2+12 and 2*/7+8/2+12 allele compositions. 'Navid 8' showed the highest quality score (8) and 'Alamoot 1' and 'Alamoot 2' had lowest quality score (5). The genotypes with acceptable quality alleles occupied the same groups base on RAPD analysis. Also this study demonstrated that combined data of different marker systems will provide much breeding information for crop improvement, especially for the selection of parents for hybridization breeding programs.

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Salt tolerance within genetic tester stock collections of the Gatersleben Genebank

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Abstract

Bread wheat *Triticum aestivum* L. possesses a genetic variation for the ability to survive and reproduce under salt stress conditions. Obviously genetic factors of the D Genome are responsible for a higher salt tolerance. A set of *T. aestivum* L. - *Aegilops tauschii* Coss. introgression lines, former developed at the IPK Gatersleben, were tested to find introgressed segments in respect of tolerance against salt stress. Germination tests were carried out on filter paper in plastic boxes by using three different sodium solutions (1%, 1.5% and 2%) and aqua dest. as control. After ten days in a climatic chamber with a light and dark photoperiod of 12 hours and a constant temperature of 20°C, the lines were scored according the scheme of Mano et al. (1996). Positive and negative factors against salt stress were detected on chromosome 3D, 4D and 7D.

However, survival in a saline environment requires multiple adaptations of the plant and salt stress tolerance is a character with quantitative inheritance. Molecular marker could facilitate the breeding for quantitative traits. Therefore, the 'Oregon Wolfe Barley' mapping population well saturated with different DNA marker has been used to find marker connected with salt tolerance at the germination stage. The tests were arranged in the same manner described above. The concentrations of the sodium solutions were 1.5%, 2% and 2.5%, because barley is more tolerant than wheat. QTLs were detected on chromosomes 5H and 7H.

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Changes in genetic diversity of wheat varieties of Odessa's breeding center

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Abstract

Plant Breeding and Genetics Institute (PBGI, Odessa) is one of the main breeding centers in the Ukraine. About 35 % of bread wheat varieties that have been registered in the official list of varieties of Ukraine in 2001 were developed in this center. The history of this center began at the end of XIXth century, and the breeding station had been created in 1912. It's aim was to develop new wheat varieties with good adaptation for ecologic-climatic conditions of south Ukrainian steppe. The famous geneticist A.A. Sapegin had organized this activity. Increase in effectiveness of breeding resulted from theoretical and experimental work in genetics. First bread wheat varieties involved in the breeding process by A.A. Sapegin were local populations of *Banatka* and *Krymka*. Ninety years have passed from the start of scientific based breeding program in the south of Ukraine, and since then more than hundred wheat varieties have been developed in this center. For our work, samples of seeds of *Banatka*, *Krymka* and *Kooperatorka*, originated from the Genebank of IPK Gatersleben and from the PBGI collection were examined together with a set of main wheat varieties developed in PBGI during 90 years of breeding. The aim of the study was to analyze the changes in genetic diversity of wheat varieties of Odessa's breeding center. For investigation, the microsatellite analysis with twenty markers were used. Microsatellite analysis was performed in the laboratory of Gene and Genome Mapping of IPK (Getersleben, Germany). Microsatellite analysis showed 84 alleles in the pool of seed samples of *Banatka*, *Krymka* and *Kooperatorka*, while in the pool of tested PBGI wheat varieties 121 alleles were discovered. Among them, about 60 new alleles were discovered which had not been found previously. This fact illustrates that, during 90 years of breeding, genetic diversity has been enriched. However, 23 alleles tested in old wheat varieties were not revealed in genetic pool of PBGI varieties. These alleles could be lost by the selection process. About 50 % of alleles detected in PBGI wheat varieties were found in old varieties like *Banatka*, *Krymka* and *Kooperatorka*. Allelic characteristics of seed samples of old wheat varieties grown in Chersoneses province have shown that the main part of their alleles are still present in the genetic pool of PBGI bread wheat varieties. During the scientific breeding programs, the allelic compositions were enriched with new alleles, while a number of original alleles previously discovered in pool of seed samples of *Banatka*, *Krymka* and *Kooperatorka* were lost.

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Genetic diversity assessment among Bulgarian and western European wheat germplasm collections using microsatellite markers

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Abstract

Wheat (*Triticum aestivum* L.) is one of the most economically important cereal crops for Bulgarian agriculture. Bulgaria has a rich collection of local varieties, land races, and exotic germplasm. In the last few decades the collection was exponentially enriched with introduced modern cultivars from Europe and over the world. The gene bank collections (DZI, G. Toshevo and IGR, Sadovo) contain more than 500 accessions which are characterized by valuable agronomic traits. For rational use of Bulgarian wheat genetic resources, selection of parental genotypes for hybridizations and creation of a modern molecular database, a program for characterization and assessment of the genetic diversity in wheat germplasm from DZI, G. Toshevo gene bank and western Europe (CRA, Gembloux, Belgium) was recently initiated. The study is also aimed at determination of the level of heterogeneity and heterozygosity in varieties. A set of 23 microsatellite markers representing at least one marker/chromosome was used to characterize and evaluate the genetic diversity of 40 modern wheat varieties originating from south-eastern and western Europe. On average, SSR markers detected 7.03 different allelic variants per locus, with mean diversity index (DI)≈0.60, thus revealing a diversity content comparable to those in other small-grain cereal gene pools. A high variation in allele number was detected among SSR loci, suggesting different suitability in genotyping and estimating the genetic diversity in wheat. The study showed that B and D genomes are characterized with an overall polymorphism significantly higher than A genome. The lowest gene diversity among all 7 homeologous groups was observed in the group 4 and the highest in the group 7, followed by the groups 5 and 6. Comparative analysis of microsatellite diversity among both geographical regions revealed that south-eastern germplasm exhibited more genetic diversity than those from western Europe. It was shown that SSRs are a fast and powerful tool in genotyping and discriminating of closely related varieties such as sister lines.

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Genetic variability and phenotype values for the harvesting index in some wheat genotypes

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Abstract
The main purpose of this three year research was to determine the genetic variability of some wheat genotypes for their production capacity of biomass and grain per plant and ear through phenotype values. These phenotype values create the wheat yield and are determined in the moment of harvesting. Differences of phenotype values between biomass/plant and grain/plant represent the harvest index of wheat. Researches include the variability from sixteen wheat genotypes with origin from nine different countries (institutions) compared with the international check 'Bezostaja-1' and with the Local check 'Evropa'. Experiments were are performed in agro-ecological conditions of Prishtina during the seasons 1999/00, 2000/01 and 2001/02. Phenotypic performance of genotypes and genotype by year interactions were highly significant. Average values were 2.837 g/fertile tiller for biomass and 1.311 g/fertile tiller (ear) for grain. The variability of harvest index was in the range from 0.43 to 0.50 (total mean 0.46). Phenotypic values of the production of biomass and grain have a strong correlative connection (r = 0.849).

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Genetic stability of wheat varieties containing rye 1R chromatin

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Abstract

Nine-teen varieties of soft winter wheat have been studied with the use of storage proteins (such as gliadin, glutenin, secalin and high molecular secalin) as markers of short and long arms of soft wheat chromosomes of first homeologous group and 1R chromosome of rye. These varieties contain the whole 1R rye chromosome or its short arm introduced by 1R (1B) replacement or T1BL.1RS translocation. A comparative analysis of protein electrophoretic spectra of individual seeds of originals and reproductions of varieties from the collection of the State Scientific Center of All-Russian Research Institute of Plant Growing named after N.I. Vavilov has shown that the varieties are characterized by a different level of genetic stability as related to the preservation of 1R rye chromatin.

All varieties originals were monotypical on gliadin banding patterns for exception of 'Winnetou' and 'Lovrin 13'. The markers of 1RS and 1RL were presented simultaneously in 'Mironovskaja 10', 'Soladin', 'Burgas 2', 'Orlandi', 'Neuzucht 14/14', and *WRH 48/49*, that specifies about substitution of 1B for 1R. The remaining varieties carried the translocation T1BL.1RS. After reproduction the varieties 'Hamlet', 'Linos', and 'Mironovskaja 10' were completely identical to the originals. The high stability is marked for 'Feldkrone', 'Perseus', 'Aurora', 'Caucasus', 'Lovrin 10', and 'Burgas 2'. However, some varieties have undergone considerable changes: 'Winnetou' lost a likeness with the original; 'Orlandi' corresponded to the original on 18 %; and 'Benno' 58 %. In one reproduction of 'Lovrin 13' the relation of the biotypes has varied; in another reproduction of the same cultivar, the impurity of that wheat variety in spectra of which the gliadin markers of 1R are absent, has appeared.

Detected spectrum modifications are evidence of the existence of the following probable genotypic reconstructions: (1) elimination of 1R chromosome or its individual arms, (2) heterozygosity in foreign chromosome or in translocation, (3) recombination of genes included into *Sec1* and *Gli-B1*- loci, which control rye and wheat protein markers, and (4) spontaneous translocation of T1DL.1RS types. These genetic changes were represented in different degree in the studied varieties. The obtained results testify the necessity of a strict control for saving of originality and integrity of varieties and show perspectives of usage of grain storage proteins as markers for these purposes.

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Genetic structure of the Portuguese wheat landrace 'Barbela' by SSR's

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Abstract
The bread wheat landrace 'Barbela' is mentioned in literature more than a hundred and fifty years ago as cultivated in the region of Trás-os-Montes. This wheat landrace includes some genotypes with rye chromatin introgression on chromosome 2DL (Ribeiro-Carvalho et al. 2001). We used 27 simple sequence repeats (SSR's) that enabled the identification of 34 loci located in 18 chromosomes distributed in the A (7), B (6) and D genome (5), and present in at least 22 different chromosomes arms. The 34 loci allowed identification of 162 alleles in the 59 analysed lines, with an average number of alleles per locus of 4.8 and an average value of the polymorphism information content (PIC) of 0.52. The number of alleles per locus ranged between 2 and 11, while the average value of the PIC was situated between 0.06 and 0.86.

The genetic distance (GD) calculated among all the 1711 possible pairs of the 'Barbela' wheat genotypes was situated between 0.23 and 2.43, while the average genetic distance observed was 0.94. The dendrograms obtained with the genetic dissimilitude, using UPGMA discriminated all the genotypes of the 'Barbela' wheat lines and showed two clusters: one with the 'Barbela' wheat lines and the other with the 'Chinese Spring' cultivar. As six SSR's used belong to the 2DL chromosome, it was also possible to distinguish 35 'Barbela' wheat lines, with similarity coefficient above 0.15. The other lines formed groups with 2 or 3 'Barbela' wheat lines with similarity coefficient above 0.83 confirming the high polymorphism in this landrace, even when using a small number of SSR's.

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Molecular characterization of durum wheat germplasm with NBS markers as compared to AFLPs and SSRs

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Abstract

Resistance to fungal diseases is one of the major goals of wheat breeding. In wheat, up to ca. 50 genes conferring resistance to leaf rust (*Puccinia recondita* f. sp. *tritici*) and ca. 20 for powdery mildew (*Blumeria graminis* f. sp. *tritici*) have been identified and used in breeding programs. Most of the R-genes cloned up till now are members of families coding for NBS-LRR proteins. Genetic variation in resistance genes (R-gene) and R-gene analogous (RGA) can be analyzed using a PCR-based approach based on NBS primers designed to match the conserved sequences of the nucleotide binding site (NBS) domain of R-genes. We performed an NBS profiling to measure genetic diversity in a collection of 58 durum wheat accessions representative of the elite Italian germplasm. Two frequent-cutter restriction enzymes (MseI, AluI) and four NBS primers were utilized. In total, 190 polymorphic bands were scored. A dendrogram based on NBS genetic similarity identified a number of sub-clusters each composed of genetically related cultivars. The NBS results were compared with SSRs (70 loci) and AFLP (234 loci) already profiled in the same set of genotypes. The NBS dendrogram well resembles those obtained with the AFLP and SSR data. A significant correlation was found between NBS and SSR ($r = 0.76$) / AFLP ($r = 0.73$) markers. The high r values herein obtained can be due to the high level of linkage disequilibrium of durum wheat, to the random distribution of R-gene clusters on all linkage groups and to the restriction enzyme (MseI) having an AATT recognition site, known to be rare within coding sequences. Our study indicates that the NBS marker system can be used with confidence to assess genetic diversity in durum wheat.

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Genotype by environment interaction in spring barley varieties of Baltic and Nordic origin bred during 120 years

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Abstract

The genotype by environment interaction of agronomical traits was evaluated in Nordic and Baltic spring barleys from different breeding periods. The material includes landraces, cultivars released during the 20th century and breeding lines from Latvia, Estonia, Lithuania, Sweden, Norway, Denmark and Finland. The grain yield and its relationships with the length of vegetation period, plant height, lodging resistance, harvest index, hectolitre weight and 1000 kernel weight (TKW) were analysed in 196 accessions. Trials were carried out in Priekuli Plant Breeding Station (Latvia) during the years 2002 and 2003. The meteorological conditions were dissimilar: in 2002 it was dry and warm, but in 2003 rainy and cooler (precipitation during vegetation 211 and 294 mm, respectively). In the year 2003 heavy lodging and sprouting were observed, whereas in 2002 plants were shorter, therefore lodging was of minor importance. In 2002 the vegetation period was on average 3 days shorter than in 2003. In 2002 hectolitre weight, TKW and harvest index were higher than in 2003. The analysis of variance showed, that the effect of genotype, year and also genotype by year interaction on grain yield was significant ($P<0.0001$). In both years grain yield correlated significantly positively with hectolitre weight, TKW, length of periods from sowing to flowering and maturity, and lodging resistance ($P<0.01$). The positive correlation of yield with harvest index and negative correlation with plant height was significant only in 2003 ($P<0.01$). In 2003, 29 genotypes significantly surpassed mean yield (4.6 t ha^{-1}, $LSD_{0.05} = 1.37$). Almost all of those were varieties and breeding lines released after 1990 (e.g. 'Anni', 'Fager', 'Alsa' and lines SW 2102, SW 2517, LIA 6186). A different situation was observed under the dry conditions of 2002: no tested genotype had significantly higher yield than the mean (4.9 t ha^{-1}, $LSD_{0.05} = 1.73$) and only one landrace had significantly lower yield. Therefore, modern varieties had similar yield as old varieties, which are probably more resistant to the low moisture conditions. Comparison of the individual genotype yield in 2002 and 2003 showed that 12 % of them were significantly better yielding in the growing conditions of 2002, all those (with exception of one) are older varieties released before 1974. Only 7 genotypes (3.6 %) had significantly higher yield in 2003 than in 2002, all of them were bred after 1981 including 4 new breeding lines. Two-row genotypes were on average significantly higher yielding than 6-row genotypes in both harvest years ($P<0.0001$). In general, results showed that particularities of genotype by environment interaction are depending from the breeding period and region of the origin of varieties. More detailed analysis of behaviour of yield components of genotypes bred in different countries in different time periods as well as 2 and 6-row varieties and breeding lines will be presented.

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Diversity of SSRs and agronomic traits of barley accessions from the Nordic and Baltic regions

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Abstract
Spring barley is one of the most important crops in the Nordic and Baltic regions. Intensive plant breeding carried out in this area during more than one hundred years has certainly had an impact on the gene pool of the crop. The aim of this study was to evaluate phenotypic and genotypic changes of spring barley during the last century due to commercial plant breeding. We therefore looked at agronomic performance, change of molecular diversity through SSRs, and the relationship between these variables in 196 spring barley varieties. Agronomic traits studied included days to heading and maturity, harvest index, plant height, volumetric and thousand-kernel weight. The field trials were carried out in Latvia, Norway and in Sweden during the years 2002 and 2003. For 22 polymorphic SSR loci a mean number of 8.6 alleles per locus was found. We found significant differences in the studied material showing that diversity changes over time were related to both the geographical origin and the ancestry. The present investigation is part of a larger study with the general objective to visualise changes in genetic diversity and putative genetic erosion over time in Nordic and Baltic spring barley material.

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Characterization of Bulgarian and Cypriot barley germplasm collections by microsatellite markers

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Abstract

Barley (*Hordeum vulgare* L.) is one of the most important cereal crops after wheat and occupies about 1/3 of the cereals cultivation area in Bulgaria and Cyprus. Bulgarian germplasm collection is represented mainly of winter type brewing and feeding barley varieties in comparison to the Cypriot germplasm which is consisting of spring type feeding barley. Bulgarian germplasm collection was increasingly enlarged in the last few years by introducing new European and other wide-spread varieties. The renewal and re-shaping of the national cereal breeding program taking place at present requires precise selection new cultivars for high yield, tolerance to multiple abiotic and biotic stresses and adaptation to the extremely changeable Bulgarian climatic conditions. The genetic structure of the barley germplasm cultivated in Cyprus encompasses varieties, directly obtained from local materials, more recent and successful ICARDA-derived cultivars and cultivars from countries with similar climatic conditions characterized by yield potential and adaptability to dryland climate. The assessment of morphological and agronomic traits of Bulgarian and Cypriot germplasm showed that it is diverse and has sources for resistance/tolerance to biotic and abiotic stress factors. Responding to the demands for improvement of cereals in both countries a bilateral program for characterization of the native and new valuable barley germplasm was initiated by molecular markers. Totally 14 microsatellite markers encompassing all 7 barley chromosomes were selected for this study. 60 accessions covering a wide spectrum of genetic diversity of the cultivated barley gene pool were characterized at 7 genomic and 3 EST-derived SSR loci until now. The mean genetic diversity index (DI) observed in Cypriot barley germplasm is slightly higher than those in Bulgarian (European) barley. The observed PIC (polymorphic information content) of the genomic SSRs is higher in comparison to EST-derived SSRs. Alleles not observed in European barley were detected in some cultivars and selections from Cypriot collection in the studied EST-derived SSR loci.

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Genetic variation of fertile forms in progeny of *Hordeum marinum* (2x) × *Triticum aestivum* (6x) amphidiploid by seed storage proteins

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Abstract

Interspecific hybridization is a source of genetic variability extension of cultivated plants. Genetic diversity of fertile forms in progeny of *Hordeum marinum* (2x) x *Triticum aestivum* (6x) amphidiploid were studied using seed storage proteins as molecular markers and changes of wheat gliadin and barley hordein gene expression in the process of amphidiploid stabilization were investigated. We analysed storage proteins from the seeds of parental forms, BC2, BC3 plants and self-pollinated generations by one-dimensional electrophoresis in 6.5 % PAGE.

Seeds of backrossed forms mainly had 'Chinese Spring' type of protein electrophoretic pattern and only 15 % of grains had some changes: presence of *Hordeum* proteins without alteration in wheat patterns or, in some cases, presence of barley proteins accompanied by disappearance of a few wheat's bands. Self-pollinated progenies of such forms were studied too. Two types of changes in seed proteins were found: elimination or decrease of the intensity of individual proteins. Elimination of the barley proteins can be related with the loss of corresponding *Hordeum* chromosomes in meiosis, because chromosome number in their parental plants was not stable on hexaploid level yet. Decreasing of the intensity can be connected with decreasing of corresponding gene dose. As for wheat proteins, gliadins, the changes of the expression of gene blocks on chromosomes 1B and 1D were observed. Range of protein variability was in the following way: from parental 'Chinese Spring' type till the total disappearance of gliadin block. The intermediate forms with storage protein composition without a few protein bands from corresponding gliadin blocks in electrophoretic pattern or forms with electrophoretic bands of low intensity were found too. According to the changes in storage protein composition we can suggest the following types of corresponding gene expression: normal expression, disturbance of one, two or three genes, disturbance of the whole gene cluster. Thus, in process of fertility restoration by backcrossing and self-pollination of BC plants the alterations of gliadin and hordein genes were detected. Some gliadin components were detected in self-pollinated progeny of seeds without those components. It is possible that the loss of individual components or whole protein clusters determined by genes of 1B chromosome is a result of gene silencing. Besides, it is impossible to explain the loss of some components of the same cluster by recombination only because the gliadin blocks are very stabile and recombination inside a cluster is scarcely probable (1:1000). Such alteration of gene expression may be explained by interaction of genes determined the analogous functions: synthesis of seed storage proteins ensured the growth and development of the embryo in the process of germination.

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Evaluation of genetic diversity among Korean, Chinese and Japanese barley landraces based on SSR markers

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Abstract

Barley (*Hordeum vulgare* L.) is one of the most important crop species in the world and has been subjected to considerable genetic study. Knowledge of germplasm diversity has a significant impact on the crop breeding and conservation of genetic resources. The objectives of this study were (1) to reveal the diversity of SSRs in landrace barley, *H. vulgare*, from populations in Korea, China, and Japan, (2) to compare SSR diversity in these countries, (3) to evaluate the discrimination ability of SSR markers distributed uniformly throughout the barley genome.

Eleven SSR primers were used to screen a set of 650 diverse barley landraces from Korea, China, and Japan. For the polymorphic SSR loci, 1-12 alleles were detected. The PIC ranged from 0 for the primers HVM68 and HVBKASI to 0.796 for the primer HVM60. The average number of alleles was highest for Chinese landrace (5.16) and those of Korean and Japanese landraces were 4.00 and 2.66, respectively. The allele size ranges were varying from 79 - 113 to 180 - 222 bps. Based on the UPGMA dendrogram, Korean landraces were divided into two groups while Japanese landraces were clustered together. Chinese landraces were scattered throughout the dendrogram indicating their wide geographic distribution. A further study should be conducted to explain the relationships between barley landraces among the three countries.

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Genetic diversity among a world collection of spring oat (*Avena sativa* L.)

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Abstract

We have evaluated 116 oat lines and cultivars for genetic diversity using the AFLP fingerprinting method. The oat lines originated from breeding programs in Europe (75), North America (30), South America (6), Asia and Oceania (5). Leaf tissue was harvested and genomic DNA was extracted. The DNA was digested with *Mse*I and *Sse8387*I. Two step PCR amplification was applied and eight primer combinations with two selective nucleotides each were used to generate the genetic fingerprints. For fragment detection we used a LI-COR 4200 DNA analyser. We could visually score 87 clear polymorphisms and collected these using a binary code in a spreadsheet. Genetic similarity was calculated according to the Jaccard index. The similarity matrix was further analysed by applying UPGMA cluster analysis and multi-dimensional scaling.

Based on the cluster tree 12 groups were detected, primarily reflecting geographic origin of the lines. Many of the European lines grouped closely together in two main cluster-branches. As expected often lines from the same breeding program and lines related by descent were grouped together, with a few exceptions. Multi-dimensional scaling showed close clustering of most European lines around the center of the scatterplot. Lines from overseas origin were scattered more widely. This indicates that within the European material the genetic diversity is smaller compared to the diversity in overseas germplasm.

We expect that European oat breeding could benefit from introducing adapted overseas lines in the crossing program because this may increase genetic variation in the breeding populations and thus enhance the potential for selection of improved lines.

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Genetic diversity of cowpea landraces from Korea determined by simple sequence repeats

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Abstract

Cowpea, belonging to the family *Leguminosae*, genus *Vigna,* is an economically important crop in Africa. Although cowpea has been cultivated in Korea for several hundred years, it is not a stable food crop in Korea. A rapid decrease of its recent cultivation has caused the loss of Korean cowpea landraces. Estimation of its genetic diversity and the establishment of a core set will provide the formulation of appropriate strategies for conservation and germplasm management and maximize use of available germplasm. The objectives of this study, therefore, were to (1) estimate genetic diversity in the landraces collected from different geographical regions in South Korea and (2) establish a core collection. Genetic diversity was estimated using six SSR markers in 492 accessions of *Vigna unguiculata* subsp. *unguiculata* collected from South Korea. Six SSR markers generated 42 polymorphic bands ranging from 141 bp to 282 bp. Genetic relationships among the genotypes were evaluated by generating a similarity matrix based on the Dice coefficient. One hundred landraces (ca. 20 % of the amount of the collection) were selected by random sampling after stratification of the entire landrace collection based on a phenetic dendrogram in order to form a core collection representing genetic diversity of cowpea in South Korea.

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Genetic variability among and within dry bean local landraces: field and molecular data

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Abstract

Local bean landraces are expected to be promising genetic material for utilization in modern breeding programs. A preliminary evaluation of the genetic variability among and within local common bean landraces based on field performance and molecular analysis data was the objective of this study.

Nine local bean landraces from the species *Phaseolus vulgaris* and one from *Phaseolus coccineus*, were used. Genetic similarity estimates and relationships among accessions was studied according to morphological characters (plant type, leaf and flower type, size and scheme of seeds), agronomic performance (earliness, adaptation to low inputs), yield components and molecular marker analysis by RAPDs. Field evaluation during the 2003 growing season for eight *P. vulgaris* populations was based on 32 individually spaced plants under low input growing conditions. Furthermore, the evaluation of PR-1 population (*P. vulgaris)* in the same growing season was based on 14 pure lines selected for dry bean yield and earliness following the preliminary evaluation during the 2002 growing season. The corresponding evaluation of the PG-1 population (*P. coccineus*) was based on 14 HS families selected in the same manner as for population PR-1.

Molecular analysis of random amplified polymorphic DNA fragments (RAPD) was conducted using 20 primers. Genomic DNA was extracted from young leaves of ten random plants in each population and bulked to form representative samples for the eight local bean populations. In the same manner one genomic DNA sample for each line of PR-1 population and for HS families of PG-1 population were used. For genetic distance analysis, Jaccard similarity coefficients were estimated and clustering using UPGMA analysis was calculated.

Field data showed differences among and within the eight landraces studied for phenotypic characters. Based on phenotypic data landraces were classified in four groups and this classification was confirmed from molecular data. In the same manner PR-1 lines and PG-1 HS families were differentiated based on their field performance and two promising lines along with two HS families were selected accordingly. The selected material was clearly distinguished based on the molecular data from RAPD analysis.

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Variability analysis between different Portuguese olive (*Olea europaea* L.) cultivars by RAPD and ISSR

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Abstract
Olea europaea L. was among the first fruit trees domesticated in the Near East. Cultivars of this area and the Mediterranean hold a great range of genetic variation as shown by DNA markers like random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), and microsatellites (SSR). In Portugal olive represents 10 % of the agricultural production with a high economical impact. Genetic variability was studied in eleven Portuguese cultivars of *O. europaea* using RAPD and intermicrosatellites (ISSR). DNA amplification fragments were separated by agarose gel electrophoresis. A set of primers was defined, 20 from 104 primers tested in RAPD and 17 from 48 primers tested in ISSR, based on their ability to describe the genetic relationships between cultivars. The data analysis was based on the genetic similarity (SM, UPGMA) using NTSYS-PC software. Comparison between the two different markers and their ability to discriminate the olive cultivars will be discussed. Our data seems to point out that RAPDs showed a higher genetic distance between olive tree cultivars than ISSRs. Also, Portuguese olive cultivar clusters obtained with each molecular marker (RAPD and ISSR) analysis and with both of them, are discussed in comparison with cultivars from different Mediterranean countries.

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SNP discovery in olive tree

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Abstract

The olive tree (*Olea europaea* L.) is an important oil crop that has been traditionally cultivated throughout the Mediterranean basin. Its cultivation has spread to North and South America and to Australia. The current consideration of olive oil as a healthy source of fats has led to its incorporation into the diet in many countries, besides the ones on the Mediterranean region. Olive oil varietal composition is an important quality parameter. With the aim of developing variety-specific PCR-based molecular markers in olive tree amenable to high-throughput detection systems, we have explored the levels of single nucleotide polymorphisms (SNPs) present within sequence characterized amplified regions (SCAR) markers. So far, eight SCAR loci in eight olive tree varieties and three wild olive trees have been sequenced, yielding an average of 5.5 SNPs per loci (2.75 SNPs per loci among cultivated varieties). These numbers correspond to one SNP every 82.5 bp (one SNP per 165 bp for cultivated varieties). All polymorphic loci with the exception of a retrotransposon-like sequence contained SNPs among varieties. The retrotransposon-like sequence contained nine SNPs differentiating the cultivated varieties from the wild olive trees, confirming the phylogenetic value of such sequence and its use as internal amplification control. In total, 17 C → T transitions, 14 A → G transitions, one G → T transition, 5 A → C transversions, 6 A → T transversions and one G → C transversion were found. SCAR markers have therefore proven useful targets for SNP development in olive tree.

Acknowledgements

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Morphological variation in Turkish sesame (*Sesamum indicum* L.) landraces

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Abstract

In this study, diversity for agro-morphological traits in 52 landraces of sesame (*Sesamum indicum* L.) originating from Turkey was estimated through multivariate analysis. The populations were evaluated for time to flowering, branching, capsule number per axil, carpel number per capsule, seed coat colour, capsule pubescence, capsule order, plant height to first capsule, plant height, number of seeds per capsule, number of capsules on main stem, total number of capsules per plant, and 100 seed weight. This dataset was reduced to 6 significant principle components (PCs) that cumulatively explained 79% of the variance. The 6 retained PC scores were subjected to a hierarchical cluster analysis (Ward's minimum variance). The populations were clustered in 4 different major groups according to their similarity levels. Most of the populations of the southern, southeastern, and western regions tended to cluster as outliers outside their region of adaptation. The distribution of the populations from the northwestern region, however, was according to their geographic origin. This study can help breeders better understand the genetic structure of Turkish sesame populations which can be used for parental selection.

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Analysis of allelic diversity in genes involved in lipid biosynthesis in rapeseed

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Key words: Association mapping – molecular markers – oil content – SNPs

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Assessment of the genetic diversity of local cassava varieties in Uganda using simple sequence repeat markers

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Abstract

The genetic diversity and differentiation of 245 cassava landrace accessions from Uganda was assessed in this study. In addition, 20 cassava accessions from a previous diversity study in Tanzania, 20 from another study in Ghana, 20 from Guatemala and 18 from holdings at CIAT and IITA representing the core collection from Latin America were included. All together 9 groups based on country of origin were created to study genetic diversity and differentiation among countries. Also the effect of various turbulences in Uganda since the introduction of cassava in the late nineteenth century, notably cassava mosaic virus disease (CMD) epidemics and government intervention programs, to cassava genetic diversity in six agro-ecologies in Uganda was further elucidated upon. Using simple sequence repeat (SSR) markers, variation in allele frequency at 35 unlinked loci, parameters of genetic diversity and differentiation, and the strengths of various forces shaping them were estimated. Results affirm a genetic divergence between African and Latin American accessions and more alleles in Latin America. Some of the alleles that occurred in Uganda, 5 of the common and 18 of the 40 rare, were not represented in the Latin American accessions but most occurred in the other African countries. This could be partly explained by the introgression of *Manihot glaziovii* genes in African breeding programs. No clear distinction between East and West Africa was observed, possibly as an effect of the regional breeding programs. The data from this study suggest that cassava has a considerable genetic diversity in Uganda despite its short history in the country and that the repeated multiplication schemes have had a limited effect on this diversity. However, the occurrence of rare alleles was much higher in low than in high impact agro-ecologies of CMD and government programs, indicating that this turbulence has had an effect on the genetic composition.

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Evaluating genetic resources of reed canary grass at northern conditions

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Abstract

Reed canary grass, *Phalaris arundinacea* L., is a native species to northern temperate regions. It has a long history as a feed crop, but it is also a potential crop for bioenergy and paper pulp production under northern conditions. Furthermore, it has environmental value for filtration and evaporation of runoff water and in erosion control. At the present time reed canary grass is cultivated on 2700 hectares in Finland, but the aim is to manifold the cultivation area for bioenergy up to 2010.

This study described variation in agronomic traits in reed canary grass germplasm. Geographic variation in traits was also identified among wild populations. The potential of current germplasm was evaluated for non-food and seed production and for breeding. Fifty-three wild populations of Finnish origin (60 - 66 ºN) were included to the experiment together with eight cultivars and fourteen breeding lines mainly from central Europe and North America. In total, 23 agronomic traits were evaluated between 1994 and 1998 in field trials at Jokioinen in Finland. The ideal plant type for non-food use was described. It should have many tall, strong and unbranched stems and only few and small leaves. It should also be winter hardy, have low mineral content and be resistant to pests and diseases.

This study revealed that wild populations and elite material were characterized by considerable variation for each trait. Wild populations also exhibited geographic distribution, which should be exploited in locating particular traits for breeding and targeting new germplasm collections. Forage cultivars and breeding lines had better biomass and seed yield traits than wild populations. Wild populations had better winter-hardiness, higher straw or leaf proportion and unbranched stems. Some wild populations from southern coast were especially promising for non-food uses having high biomass yield combined with high straw proportion. Those sites could be potential collection areas.

The material of this study provides a realistic coverage of reed canary grass genetic resources in Finland. Thus, it forms the basis of a breeding programme for improved cultivars. Promising local populations identified in this study serve as well-described candidates for breeding. On the basis of this work, the first domestic non-food cultivar should be released around 2010.

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Genetic variation in natural populations of perennial ryegrass in relation to flowering time and soluble carbohydrates

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Abstract

An association mapping approach in natural populations of perennial ryegrass (*Lolium perenne* L.) has been used to identify molecular markers associated with heading date, an important trait affecting seasonal production, tillering, digestibility and grassland management regimes. Twenty-three natural populations originating from throughout Europe, with heading date phenotypes ranging from very early to very late, as well as three synthetic populations (varieties) were used for molecular marker genotyping using AFLP. In total, 589 polymorphic markers were identified. Hierarchical clustering, Principal Coordinate and other statistical analyses identified four outlying populations forming a clearly distinct sub-group. Removal of those four populations from the subsequent analysis reduced population sub-structure two-fold. However, this made relatively little difference to the result of the association analysis. Linear regression identified three markers whose frequency of occurrence correlated with the heading date phenotype. Moreover, these markers were shown to be closely linked to each other within a major QTL on Chromosome 7, explaining 70% of the total variation in heading date. Pair-wise linkage disequilibrium among them was also significant. These results suggest that association mapping approaches may be feasible in *L. perenne*, and that the use of natural populations could provide a useful source of genetic variation in traits of importance in crop improvement.

To further exploit the genetic variation in these populations, they were used in a candidate gene approach to identify single nucleotide polymorphisms (SNP) in an alkaline invertase gene, the mRNA sequence of which has previously been determined. Alkaline invertase is a key enzyme in carbohydrate metabolism, particularly in species such as ryegrass, where the dominant storage carbohydrate in vegetative tissue is fructan. The concentration of this soluble carbohydrate in the leaves and stems of ryegrass is a very important heritable quality trait. The alkaline invertase gene described here has been mapped to a region of Chromosome 6 associated with soluble carbohydrate levels. It therefore seems reasonable to assume that this gene controls some of the variation of this trait. Variation between genotypes in their flowering time is often paralleled with variation in the concentration of water soluble carbohydrates, so the populations described above should provide genetic variation with regard to this trait as well. The 4.7 kb genomic sequence of the gene was determined, and SNPs identified. Preliminary results show that in three sections covering a total of 2.1 kb of the sequence, more than 60 SNPs were identified. Four of those were non-synonymous (amino acid changing). The remainder were either synonymous or occurred in introns or non-translated regions. An analysis of intragenic linkage disequilibrium and haplotype structure is being carried out.

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Molecular genetic diversity within and among Irish ecotypes of perennial ryegrass and white clover collected from old pastures

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Abstract
Presently white clover (*Trifolium repens* L.) and perennial ryegrass (*Lolium perenne* L.) are the most important forage species in Ireland. They are growing together with other grasses and herbs in a plant community. In Ireland more than 90 % of the agricultural output is produced from grassland. Because of its high economic importance, and the foreseeable need for improvement and adaptation of these crops to changing agricultural needs, charting the existing genetic diversity is an important long-term investment. In recognition of this, a collection has already been made from Teagasc researchers at old pastures sites all over Ireland in the beginning of the 1980's, comprising more than 400 perennial ryegrass ecotypes and 100 white clover ecotypes. Characterisation of the existing material is necessary to be able to utilise the available resources, to pinpoint possible gaps in the collection, and to inform future plant breeding programmes based on these accessions. Eleven white clover and eight perennial ryegrass accessions with 48 individual plants each have been included in this initial study. From these accessions eight white clover populations were collected at the same field site as the eight perennial ryegrass accessions. Furthermore for comparison reasons three white clover accessions from outside of Ireland were included. The plant materials were raised from single seeds in the greenhouse, transplanted to larger pots, and subsequently planted to the field in 2003. For the outlined purposes, microsatellite DNA markers have particular advantages over other molecular marker systems. For white clover seven microsatellite markers and for perennial ryegrass eight microsatellite markers were applied. Absolute allele frequencies and relative allele frequencies were summarised to create an inventory of alleles. Relative allele frequency data were used as input data matrix to compute genetic distance coefficients for the genetic data. Nei's distance measure was chosen for the calculations. Calculations were performed with the help of the NTSYS statistics package for genetic data. Generally, Irish white clover populations displayed a large number of different alleles. At some marker loci genetic drift towards a bottleneck in the populations was observed. Similar bottleneck effects were also found in the non- Irish populations. Irish populations contained several unique alleles and are therefore as valuable as sources of rare alleles as the 'exotic' gene bank accessions. However, all white clover accessions were rather similar using the Nei's genetic distance coefficient (genetic distances between 0.09 and 0.15). The exotic 'Tajikistan' and 'Iran' populations were rather distant related to all other accessions investigated. Closely related to the Irish white clover populations was 'Dutch White Clover'. According to the dendrogram within the Irish populations little grouping according to the geographical origin was found. Irish perennial ryegrass accessions displayed a rather large number of alleles. All perennial ryegrass accessions were rather distinct using the Nei's genetic distance coefficient (genetic distances between 0.06 and 0.36). For perennial ryegrass further accessions will be investigated to group the Irish accessions into a European context.

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Description and use of genetic diversity in perennial ryegrass

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Abstract
Perennial ryegrass (*Lolium perenne* L.) is the most important grass species, both for agricultural use, but also for non-agricultural purposes (lawn, turf). Perennial ryegrass is spread all over Europe and is well adapted to various types of habitats. Grass breeders took profit from the large amount of variation available in natural and cultivated grasslands. For many decades, ecotypes were collected, and, after one or several cycles of recurrent selection, new cultivars could be released. With the establishment of genebanks a more scientific approach towards the collection of ecotypes was introduced. However, because of the large number of accessions evaluation is lacking and only very limited information is available so far.

From a collection of ecotypes from three geographic regions of Germany morphological and molecular data are available. Among the morphological traits, over 80 % of the variation accounts for the differences in time of flowering (40 days). Molecular marker data revealed genetic distance between ecotypes from the north vs. the south of Germany. In a comparison with marker data from European cultivars, it could be shown that most of the molecular variation of ecotypes has already been exhausted by cultivar breeding. Thus, ecotypes and cultivars of west and central Europe build one single genepool. This is due to the fact that breeders take advantage from breeding progress by intercrossing new cultivars with their own material. According to a survey made among European grass breeders, the use of ecotypes in breeding programmes has decreased tremendously throughout the last two decades. Although breeding progress in the forages is rather slow, experimental data showed that ecotypes had a yield performance of only 95 % of standard cultivars.

To enlarge the west European genepool, east European ecotypes come into focus. A pre-breeding strategy applied to ecotypes from Poland will be described on the basis of experimental data. Molecular marker data suggest the presence of a different gene pool. Future use of gene bank accessions will largely depend on the availability of their description in terms of specific agronomic traits and related marker data.

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Identification of interspecific barriers in the genus *Trifolium*

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Abstract

The study of pre- and post-fertilisation barriers of crossability after an interspecific hybridisation of *Trifolium pratense* and wild species *T. alpestre*, *T. medium* and *T. sarosiense* is presented in this work. Growth of pollen tubes was arrested after interspecific crosses to a relatively small extent. The decisive meaning for hybrid embryo viability had post-fertilisation barriers traced by two clearing treatments of immature seeds by means of chloral hydrate and a mixture of benzyl benzoate and dibutyl phthalate. In interspecific combinations *T. pratense* (4x) x *T. alpestre*, *T. sarosiense*, enlargement of immature seeds occurred, but no hybrid embryo was traced. Of wild species used as a male parent for crosses, *T. medium* was the only exception from the point of view of fertilisation. Globular, heart and early torpedo stages of hybrid embryos were observed 7 days after pollination (DAP) only when *T. pratense* was on the tetraploid level. When *T. pratense* (2x, 4x) was used as a male parent for interspecific crosses with *T. alpestre*, *T. medium* and *T. sarosiense*, strong defects in various stages of embryogenesis were observed, in particular wrinkled and narrowing embryo sacs caused by expansion of endothelial cells. This seemed to have the decisive meaning for the lack of hybrid embryo viability. Wild species used as a female parent for crosses with *T. pratense* were entirely unsuccessful from the aspect of hybrid embryo development. We could conclude the following findings: to make crosses only in one direction with *T. pratense* as a female parent and *T. medium* as a male, to keep up the tetraploid status of *T. pratense* and to carry out hybrid embryo excision at early torpedo stage at about 7 DAP.

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Linkage disequilibrium in cultivated grapevine (*Vitis vinifera* L. *sativa*)

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Abstract
We report here the first estimation of linkage disequilibrium (LD) in grapevine, *Vitis vinifera* L., an outcrossing, heterozygote, perennial species. Our goal in this study was to assess the pattern of LD at the cM scale between 38 SSR loci located on 5 linkage groups. LD presence was evaluated through association tests, and its extent through the estimation of parameters *D*' and Δ (Weir), using both GDA and Arlequin softwares. We used two different samples: (1) a "genotypic" sample of 214 cultivars representing the diversity of the cultivated compartment (including a core collection of 142 cultivars); (2) a "gametic" sample of 30 haplotypes from 15 cultivars with known parentage. Significant genotypic LD was found only within linkage groups, extended up to 14.5 cM, and declined as a function of distance. It appeared to be little influenced by structure, which was weak. Slight differences in LD pattern were observed between table and wine grapes. Genotypic LD seemed to be mainly of gametic origin. Its slow decline with distance suggests that the genetic basis of the cultivated compartment was initially narrow and that the impact of recombination has been low. The consequences of these first results for marker assisted selection and fine mapping of QTLs will be discussed.

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Detection of intravarietal variability in Portuguese grapevine cultivars by RAPDs

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Abstract

Vitis vinifera includes between 5000 and 15000 cultivars grown all over the world. Grapevine, a perennial crop, reveals a great genetic diversity between and whithin cultivars. The National List of Grapevine Synonyms of Portugal refers about 450 varieties, most of which are specific to Portugal. Clonal selection in Portugal began 25 years ago and in recent years more than 15000 ha of modern vineyards with the selected material were planted. The certification of the several clones of a cultivar, showing variability for yield components and productivity, is an important goal for the Portuguese viticulture. Some clones of the 'Aragonez' cultivar are already available. The characterization of the grapevine cultivars, using different molecular markers (RAPDs, SSRs, AFLPs, etc.), has been carried out by several authors with good results. However, the search of intravarietal (clonal) diversity has not been so successful. In fact, there are few cases reported where molecular markers allowed to differentiate grape clones within a cultivar, although the first results with RAPDs and AFLPs are nowadays being obtained. The aim of our study was to search the genetic variability between 50 clones in two cultivars, 'Viosinho' (VS) and 'Aragonez', (RZ) by RAPD markers, in order to discriminate the clones within the cultivars. A total of 65 primers (60 from Kits A, E and O - Operon Technologies and 5 from the Kit BC - British Columbia) were tested for both cultivars. One hundred and seventy nine and one hundred and fifty nine clear and unambiguous RAPD markers were amplified in 'Viosinho' and 'Aragonez', respectively. The number of bands obtained ranged from four to eleven in all the primers tested and its size varied between 150 bp and 2995 bp. In 'Viosinho' it was possible to detect polymorphism for the 1267 bp marker with the primer OPO 12 being absent in the most part of the clones (47) and present in three of them (VS 0331, VS 0601 e VS 1328). In 'Aragonez', polymorphism was detected with the primer OPE 01 for the 600 bp marker on the RZ 6407 clone and with the primer OPO 20 for the 563 bp marker for the RZ 0707 clone. Our data pointed out that the use of RAPDs allowed the identification of some clones, although in a small number (4 to 6 % of the total studied) for the set of primers tested.

Acknowledgements

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Retrotransposon (TRIM) based markers reveal genetic diversity in *Malus* and other *Rosaceae*

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Abstract

Retrotransposon based markers have been found to be powerful tools for investigating genetic diversity in plants. Some direct comparisons have indicated for example that they are 25 - 50 % more polymorphic than AFLP markers. Therefore retrotransposons could be expected to reveal some additional variation in apple compared to other markers, and detect even very small differences caused by mutations. Full size TRIM (terminal-repeat retrotransposon in miniature) - group retrotransposon elements were cloned from apple (*Malus domestica*) cultivar 'Antonovka'. Sizes of these elements range from 510 to 659 bp. The elements have long terminal repeats about 290 bp that flank an internal domain part about 70 bp containing a PBS (located 2 - 3 bases downstream of the 5'LTR and complementary to the methionine tRNA), and a PPT (located immediately upstream of the 3'LTR). TRIM element is flanked by 5-bp direct repeats, which were generated after TRIM insertion. Primers were designed using the FastPCR - programme to match the LTRs of the apple TRIM - sequences. Even single primers could produce multiple bands from genomic apple DNA, which suggests that the copy number of TRIM - elements is relatively high. DNA fingerprints produced with either only the TRIM - primers (IRAP method) or in combination with selected anchored microsatellite primers (REMAP) resulted in polymorphic patterns, which are able to distinguish even closely related cultivars. In addition, apple TRIM - primers turned out to be useful also in fingerprinting other species in the family *Rosaceae*. PCR amplification of genomic DNA samples of *Chaenomeles japonica*, *Rubus idaeus*, *Rosa x hybrida* and *Prunus domestica* revealed multiple polymorphic bands.

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Utilization of plant genetic resources for the improvement of vegetable crops

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Abstract

Plant genetic resources (PGR) represent the main source of variation for developing modern varieties of vegetable crops for either old (yield, uniformity, resistance to diseases, extended shelf-life) or new goals (organoleptic and nutritional quality, adaptation to organic production, diversification of types, etc.). Conventional techniques and other tools of biotechnology have contributed to significant advances in the use of PGR in vegetable crops breeding. New developments, especially in the field of genomics, are opening the way to a qualitative step forward in the use of PGR by improving the efficiency in the utilization and by extending the range of PGR that can be effectively used in plant breeding. Here we present some examples of the past and present achievements as well as future prospects in the use of PGR in the genetic improvement of several vegetable crops.

Tomato (*Lycopersicon esculentum*; *Solanaceae*) is one of the vegetable crops to which more effort has been devoted to using PGR for the development of new cultivars. All wild species of *Lycopersicon* have been used to some extent in tomato breeding and have resulted in the transfer to the tomato of genes conferring resistance to diseases and improved quality, as well as genes that modify the plant architecture. Genes of interest from *L. peruvianum* and *L. chilense*, which present a strong sexual incompatibility with *L. esculentum*, have been introgressed into the crop by using embryo rescue. The recent advancements in the molecular genetics of this crop have allowed the detection of favourable genes and QTLs for many traits of interest in the wild materials, as well as the development of molecular markers and ultra-dense genetic maps that facilitate the use of PGR. The high degree of conservation of the synteny with other related species and crops of this family promotes the use of PGR not only in tomato, but also in other *Solanaceae* vegetable crops, like pepper (*Capsicum annuum*) or eggplant (*Solanum melongena*).

Wild species have not had an important role in the genetic improvement of melon (*Cucumis melo*; *Cucurbitaceae*), mainly due to strong prezygotic incompatibility barriers and to the sterility of somatic hybrids. However, an increase in the efficiency of the utilization of the PGR is being fostered by the use of molecular markers and the development of genomic tools. At this respect, landraces of Asian melons have represented a source of variation, not fully exploited, for the introduction of resistance to diseases in this crop.

Breeding of cabbages, cauliflowers, broccoli, and other types of *Brassica oleracea* (*Brassicaceae*) has benefited from the extensive use of the different gene pools. Somatic hybridization techniques have allowed the development of interspecific and intergeneric hybrids. Genes introgressed into cultivated *Brassica* vegetable crops include genes of resistance to diseases and abiotic stresses and genes of cytoplasmic androsterility, which have greatly contributed to the improvement of this crop. The possibility of using the PGR in the improvement of *Brassica* crops is greatly benefited by the fact that *Arabidopsis thaliana*, the model plant for the study of the plant genome, is a member of *Brassicaceae*. Therefore, the

comprehensive data on molecular markers, location and function of genes of *Arabidopsis* are of immediate application to the use of PGR in *Brassica* breeding.

In definitive, advancements in new biotechnologies and in genomics are allowing a greater efficiency in the exploitation of PGR and will make an important contribution to vegetable crops breeding.

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Arbutin polymorphism in the genus *Origanum*

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Abstract

Arbutin ((4-hydroxyphenyl)-β-D-glucopyranoside) is a hydroquinone derivative present in marjoram (*Origanum majorana*), pears (*Pyrus communis*, *Rosaceae*) and species of the *Ericaceae* family. The leaves of *Arctostaphylos uva-ursi* (*Ericaceae*) are internally used due to their content of arbutin as a mild urinary antiseptic for moderate inflammatory conditions of the urinary tract and bladder. Extracts of the leaves have been widely used in cosmetic preparations to lighten the skin, with the active principle hydroquinone which derives from arbutin.

Arbutin, however, exhibits some adverse effects. Hydroquinones are hepatotoxic and nephrotoxic, mutagenic and carcinogenic in animal studies (Nowak et al. 1995, Peters et al. 1997). That is the reason why the use of *Arctostaphylos uva-ursi* is always restricted to a short period. Due to the low amounts consumed the daily intake of an aromatic plant may not seen as critical. Arbutin, however, can be regarded as a substance non-desired in the human diet.
Arbutin is present in marjoram while it can not be found in oregano. Within the genus *Origanum*, hybridisation occurs quite often, a fact that was used to establish a species hybrid between marjoram (*Origanum majorana*) and oregano (*Origanum vulgare*).

Arbutin was analysed by quantitative thin-layer chromatography (TLC). In the segregating F_2-generation, the presence of arbutin followed a clear Mendelian segregation of 3:1 indicating that only one gene was different between both parents leading to the formation of arbutin.

Polymorphism of the presence of arbutin in the genus *Origanum* enables the elimination of undesired arbutin from marjoram by classical breeding methods.

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Molecular survey of the genetic diversity from commercial *Pelargonium* X *hortorum* cultivars compared to botanical accessions

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Abstract
The genus *Pelargonium* contains more than 250 species. Most of them are found in southern Africa and were imported to Europe mainly during the 17th and 18th centuries. From the collected accessions several cultivated groups have been created by hybridisation resulting in more than 4000 varieties. The so-called geraniums (*P*. x *hortorum* Bailey) are the most economically important. Nowadays commercial cultivars look like quite similar in shape and colour. This similarity may be due to a narrow genetic basis according to the limited number of specimens at the origin of the European germplasm. In order to test this hypothesis, we compared the level of variability available in a set of 16 tetraploid and 4 diploid commercial cultivars belonging to 3 breeding companies with the level of variability evidenced in 16 botanical accessions. These accessions were mainly chosen in the *Ciconium* section from which modern *P*. x *hortorum* cultivars are considered to originate. We used 10 microsatellites primer pairs developed during a previous work (EU Project FAIR3 CT96-1796, Becher et al. 2000) in order to characterise the diversity. We scored 74 different fragments which were used to build an UPGMA phenogram based on Jaccard's dissimilarity indices. Botanical accession were clearly separated from cultivars. Nevertheless a specimen of *P. inquinans* and another *Ciconium* named 'Robert Fish' clustered with the commercial cultivars. This could reflect their genetic relatedness to the modern germplasm. Two accessions pairs were not resolved. This was not a surprise according to their phenotypical similarity. This result addresses the problem of specimen identification and emphasises the use of molecular markers for genetic resources management in the genus *Pelargonium*. The level of homozygosity seems to be high in the cultivated group and the average allelic diversity is lower than in the accessions group. Therefore it seems that genetic diversity can be recovered from accessions specimen which could represent a source of disease resistance genes and other potentially interesting traits.

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Genetic structure and temporal allozyme variation of *Cotylelobium melanoxylon* Pierre in Thung Khai genetic resource area, Trang, southern Thailand

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Abstract

The level and structure of genetic variation, as determined by allozyme genetic markers in a genetic resource area (GRA) of *Cotylelobium melanoxylon* Pierre were assessed and compared to that of three natural populations in Thailand. The GRA harbored a higher level of genetic variation than that observed in the other three natural populations (mean number of alleles per locus 2.5 vs. 2.4, percentage of polymorphic loci 83.33 vs. 75.00, and observed heterozygosity 0.20 vs. 0.09). Genetic differentiation among the three natural populations was 9.9, this value increased to 21.1 after the GRA was included in the analysis. Genetic distance analysis indicated that the GRA was genetically different from the other populations. The mean genetic distance among the three natural populations was 0.044, this value increased to 0.199 after the inclusion of the GRA in the analysis (i.e. greater differentiation).

Increase in genetic diversity among cohorts in the GRA, from seedling cohort (H_e = 0.1554) to mature cohort (H_e = 0.2655) indicated temporal genetic variation in GRA which might be caused by loosely connection of temporal breeding web that ensures genetic flow throughout a population, which is common to a large population.

In summary, the present study has indicated that the establishment of GRA is an effective conservation method. It should be emphasized that this study is restricted to the species southern range. A general and more effective conservation strategy should, however, consider the natural distribution of the entire species.

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Genetic diversity and conservation of forest trees in Thailand

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Abstract

In Thailand many economically important species are under threat due to human pressure and overexploitation. Therefore, understanding of the status of genetic resources of forest trees is imperative before any forest gene conservation can be designed. One of the fundamental requirements for conservation and use of forest tree genetic resources is understanding the biological dynamics of genetic variation within and between species. Considerable variation exists among tree species with respect to the extent of genetic diversity and the way such diversity is organised within and among populations. The extent and the pattern of this diversity are strongly dependent on the amount of genetic polymorphism, pattern of gene flow and mating system.

To estimate the status of genetic resources, genetic diversity of keystone forest tree species in Thailand such as teak (*Tectona grandis*), pine (*Pinus merkusii* and *P. kesiya*), mangrove trees (*Rhizophora mucornata* and *R. apiculata*), dipterocarp and bamboo species have been investigated by using isoenzyme genes, RAPDs (randomly amplified polymorphic DNA), AFLPs (amplified polymorphic DNA), micosatellite markers and DNA sequences. According to the information provided from the mentioned study, the development of appropriate conservation strategies is discussed and suggested.

STRATEGIES FOR EXPLOITATION OF GENETIC DIVERSITY

Part 2

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DNA sequence polymorphisms and their application in bread wheat

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ABSTRACT: Single-nucleotide polymorphisms (SNPs) constitute an abundant source of DNA polymorphisms, which have been successfully used to identify loci that are associated with a particular phenotype. Information on such markers in plants is still scarce. For bread wheat, SNP data are restricted to few genes. This can be explained by the hexaploidy of *Triticum aestivum* making difficult SNP discovery. We developed a novel method for SNP discovering in bread wheat. This strategy was based on the development of highly specific PCR-primers used to sequence 27 lines. SNPs were discovered from sequence alignment. Some of them were genotyped by mass spectrometry in a collection of ~155 lines, which were both evaluated for agronomic traits and genotyped at 42 neutral microsatellites. Traits estimated were protein content, the quantity of high molecular weight glutenins and that of the *Glu-B1*x subunit. Neutral markers were used to infer population structure, further included in linear models for association studies. The results showed 89 SNPs in approximately 20 kpb, i.e. one SNP every 223 bp on average. Among SNPs genotyped, 3 SNPs were located along the *Glu-B1-1* gene, two of them in the promoter and one in the coding sequence, while 3 nonsynonymous SNPs were located along the gene coding for transcriptional factor *SPA* (Storage Protein activator). In both years, SNPs from *Glu-B1-1* had a significant effect on the studied variables, whereas those of *SPA* had no effect. Such results might indicate that some haplotypes for *Glu-B1-1* were linked with higher protein content through an increased amount of high molecular weight glutenins, especially the subunit GluB1x.

Key words: Association study – polyploidy – single-nucleotid polymorphism (SNP) – *Triticum aestivum*

Introduction

Single nucleotide polymorphism (SNPs) and small insertions/deletions are the most abundant form of DNA sequence variations in the genome of many organisms. Therefore, they are useful markers for genetic mapping, population genetics or genotype/phenotype association studies. Moreover, they can be adapted to high-throughput automated methods. Information on SNPs in plant genomes is still limited, with the exception of *Arabidopsis*, maize and soybean. Maize is considered as highly polymorphic with an average of one SNP every 104 base pairs (bp) (Tenaillon et al. 2001), while in soybean, a self-fertilized species, Zhu et al. (2003) found about one SNP every 273 bp. Data concerning linkage disequilibrium (LD) are also available for these species. In *Arabidopsis*, LD declines in about 250 kbp, i.e. 1 cM (Nordborg et al, 2002). Results on LD are more pronounced in maize, where very variable rates of declines among genes were reported (Tenaillon et al. 2001, Remington et al. 2001). A relatively long range of LD was observed in soybean (Zhu et al. 2003).

In other plant species, SNP studies were generally confined to single genes or DNA fragments. In bread wheat (*Triticum aestivum*), only a few genes such as *puroindoline b* (Giroux & Morris 1997), *SPA* (Guillaumie et al. 2004) have been studied for sequence variations. SNP discovered in these studies were used for genotype-phenotype associations or for genetic mapping. The lack of intensive studies of SNPs in wheat can be due to its large

genome size and its polyploidy which makes SNP identification difficult, since allelic sequence variations can be confused with homeologous (differences found between gene copies on A, B and D genomes) and paralogous variations (differences in duplicate copies which may exist within a given genome).

In this study, we present a novel strategy to identify SNPs in hexaploid wheat. Approximately 20 kb were sequenced from a panel of 27 diverse hexaploid wheat genotypes to discover sequence variations. The frequency and the nature of SNPs in wheat are presented as well as an example of association between SNP and agronomic traits.

Materials and methods

Plant material

A panel of 27 hexaploid accessions was selected from a large collection on the basis of their geographical origin, their growth habit (winter or spring type), their registration date (old and recent cultivars) and their neutral polymorphism at 42 SSR loci. They were obtained from the Centre of Biological Resources on Cereal Crop of Clermont-Ferrand (France). Leaves were harvested from a pool of five to seven 3-weeks old seedlings (from bagged spikes to prevent cross-pollination) and bulk genomic DNA was extracted using the Sigma GenElute Plant Genomic DNA Kit (G2N-350).

SNP discovery

In hexaploid wheat, SNP discovery is hindered by the presence of several gene copies, usually at least one on each genome. Direct sequencing of genes from PCR-products requires the design of locus-specific PCR primers to avoid co-amplification of the different copies. Designing such primers requires several steps: (1) deducing the structure of studied gene from wheat ESTs aligned with wheat (when available) or rice genomic sequences, (2) designing PCR-primers pairs in conserved regions of putative exons, (3) testing the primers with DNA of each diploid ancestor, *T. urartu* (AA), *Aegilops speltoïdes* (BB) and *T. tauschii* (DD), and that of two hexaploid wheat cultivars ('Chinese Spring' and 'Renan'), (4) pairs giving an expected signal, one single band per genotype, were then used to screen the BAC (Bacterial Artificial Chromosome) library from 'Renan', developed by B. Chalhoub, (5) sequencing positive pools, and (6) designing specific locus-primers based on sequence differences between each identified copy. PCR-products obtained from the panel of 27 lines with these specific primer pairs were sequenced. SNP were deducted from sequence alignment.

SNP genotyping

Among all discovered SNPs, a few of them were genotyped by mass spectrometric analysis technique at CNG (Centre National de Génotypage, France) according to the method reported by Sauer et al. (2000). For illustration, SNPs located in two candidate genes related to storage protein synthesis, the structural gene *Glu-B1-1* and the gene coding for a transcriptional factor likely to interact with its promoter, were used for association studies.

Field trial

135 and 155 cultivars were sown in nursery design trials at Clermont-Ferrand in 2001 and 2002, respectively. For each genotype, protein content was predicted by NIRS (near infra-red spectroscopy) and protein fractions were quantified using capillary electrophoresis.

Association genetics

In order to declare true associations between polymorphism at a given candidate gene and a phenotypic trait, it is necessary to take into account the genetic structure of the studied population. Indeed population structure may cause linkage disequilibrium between unlinked

genes, thus leading to spurious associations. Genetic structure among the lines of each trial was inferred from marker data at 42 SSR loci, one per chromosome arm (Roussel et al. 2004) using the admixture option in STRUCTURE software (Pritchard et al. 2000). Five independent replicates of 300,000 Markov chain iterations were used for each parameter set tested. The effect of polymorphism was then tested by linear modelling, with ancestor group as covariates and polymorphism as tested factor. Two kinds of analyses were carried out: first by considering each individual SNP as a factor (with one degree of freedom), the second one by combining every SNP in haplotypes for each gene.

Results and discussion

Polymorphism

A total of 89 SNPs (87 single base changes and 2 indels) and 3 insertion-deletion events longer than one base were identified in 18644 bp of sequence (Table 1). Among 69 SNPs located in the coding sequence, 45 were nonsynonymous. These data showed that wheat has an average of one SNP every 223 bases. Thus, wheat appears as being less polymorphic than maize (Tenaillon et al. 2001), but as polymorphic as soybean (Zhu et al. 2003). Our results are probably overestimated due to the sequenced panel which includes a synthetic hexaploid wheat. As polymorphism of *PinB* was studied, this line revealed many SNPs due to the *T. tauschii* parent, which showed much higher polymorphism than the corresponding region in wheat D genome. Surprisingly, our results indicate that coding sequences are more polymorphic than non coding regions. This can also be partly explained by the high level of polymorphism of *Glu-B1-1*. This gene which has no intron encodes for storage protein, which probably has not been submitted to any selection pressure until very recently.

Table 1. Nucleotide diversity in wheat

	Total	Non coding regions			Coding regions
		Promotor	UTR regions	Intron	
Nb of bases sequenced	18644	2131	380	7218	8915
Nb of SNPs:					
single base changes	87	13	0	6	68
indels (1 base)	2	0	0	1	1
Nb of nonsynonymous SNPs	45				45
Nb of indel > to 1 base	3	1	0	0	2

Association study

Three SNPs located in the *Glu-B1-1* were genotyped. Two of them were in putative *cis*-element. The last SNP, in the coding sequence, induces an arginine/histidine. Three non-synonymous SNPs of the B homeologue of *SPA* were also genotyped. The first polymorphism leads to a stop codon (Guillaumie et al. 2004), the second one to a leucine/proline change and the last one to a glutamic acid/asparagin change.

STRUCTURE software shows that the population studied in 2002 and 2003 had 6 and 5 ancestor genomes, respectively. Moreover the data obtained from these trials are highly correlated. The analysis indicates a strong effect of each SNP and haplotypes located along *Glu-B1-1* (see an example in Table 2) on each variable (protein content, quantity of HMWG and *GluB*x) in 2002 and in 2003. SNPs in *SPA* have never significant effects. This result is quite surprising especially for the SNP leading to a stop.

Table 2. F-values and probabilities (in parenthesis) for the haplotype effect; the model took into consideration the structure of the population

Effect	Year	% Protein	Quantity of HMWG	Quantity of B1x
Haplotype for *Glu-B1-1*	2001	10.38 (0.00)	9.37 (0.00)	62.42 (0.00)
Haplotype for *Glu-B1-1*	2002	3.87 (0.00)	5.06 (0.00)	31.69 (0.00)
Haplotype for *SPA*	2001	1.71 (0.20)	1.45 (0.09)	2.86 (0.06)
Haplotype for *SPA*	2002	0.46 (0.67)	0.10 (0.90)	0.45 (0.64)

To conclude, we can discard an influence of *SPA* on HMWG, contrarily to its effect on LMWG synthesis reported by Albani et al. (1997). As the protein content and the quantity of HMWG is highly influenced by the SNPs in *Glu-B1-1*, these markers can be useful in breeding programmes aimed at enhancing grain protein content and composition.

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New results in wheat breeding and their use in a global scale

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ABSTRACT: During the last 25 years several high quality wheat cultivars were developed, accepted and registered in Bulgaria on the one side, and highly productive, ecologically adaptable and economically effective cultivars, on the other. The most representative of them are cvs. 'Milena', 'Preslav', 'Albena', 'Yantar', 'Priaspa', and 'Todora'. They possess high milling and bread making qualities and proper agronomic traits and yield potential. The complex of these different types of cultivars and their combination may ensure stable wheat production with high grain quality. Besides classical breeding the basis of hybrid wheat breeding was developed, too. This includes the new cms (k) and (m) systems. By these, several new hybrids were produced in experimental stage.

Key words: cms – hybrid wheat – quality – yield

Introduction

As a part of the world breeding processes, Bulgarian wheat breeding follows the same peculiarities for quality and grain yield. After 1962 three breeding programmes were formulated (Panayotov 1992) in which the main purposes are yield potential, quality and developing new material to improve these characteristics. After creating and using 'Bezostaya 1' several good quality wheat cultivars were developed in Bulgaria, e.g. 'Ludogorka', 'Slavianka' and others. Almost all cultivars possessed the 'Bezostaya 1' HMW glutenin subunits 2*/7+9/5+10 (Todorov et al. 1998). All high quality cultivars used in the country are hard red winter (HRW) types. The ecologically productive type of cultivars are with semi hard and semi red grain and good and acceptable quality. The tendency is the red grain color to be changed to white (Pike & Mac Ritchie 2004).

The increasing of yield potential is a main task of breeding. One of the methods for this purpose is development of hybrids by using cms or other genetic systems. After 1970, in Bulgaria several new cytoplasms of male sterility were discovered. On this basis some experimental hybrids were developed by using alloplasmy for male sterility and fertility restoration of the hybrids. The aim of this paper is to present the main results of the classical wheat breeding and the development of the new genetic basis for hybrid wheat.

Material and methods

All common wheat cultivars are created by the pedigree breeding method. For this investigation they were tested in randomised complete block design trials with six replicates. Plot size was 15 m². For milling, baking and electrophoretical analysis, the common methods were used.

The cms alloplasmic lines were created by using original donor species. The analogues were produced by backcrossing. The plants, A, B lines and F_1, were grown in the greenhouse and the field as single plants. The sterility/fertility was determined after selfing and seed set.

Results and discussion

According to the last breeding program, development of high quality cultivars is the main aim and the second one is the group of cultivars with high productivity, good ecological adaptability, stress tolerance and acceptable economical efficiency (Panayotov 2001).

High quality cultivars

Table 1 presents the results of yield potential and grain characteristics of high quality cultivars. All cultivars are with hard red grain, vitreous, and with good hectolitre weight. Flour yield is 68 - 72 % and water absorption is larger than 62 %. The yield potential and the agronomic characteristics are very good, too.

Table 1. Grain characteristics of high quality cultivars and yield potential (2002 harvest)

Cultivar	TGW[1]	HW	VITR	KH	YLD	Test years
Preslav	42.5	80.4	79	74	7730	1993 - 2002
Milena	39.7	81.3	93	78	7630	1999 - 2002
Dobrudjanka	37.6	81.7	76	47	6740	1993 - 2002
Albena	48.7	81.0	75	61	7660	1993 - 2002
Progress	50.3	79.1	72	75	7360	1994 - 2002
Zlatina	38.4	81.2	95	78	7340	1999 - 2002
Demetra	44.3	80.3	76	75	7780	1999 - 2002
Bezostaya 1	44.9	81.0	80	78	6200	1999 - 2002

[1] TGW, 1000-grain weight (g); HW, hectolitre weight (kg); VITR, vitreousness; KH, kernel hardness; YLD, grain yield (kg/ha)

Rheological and bread making qualities are presented in Table 2. Wet gluten content ranged from 24 to 32 %, which, for the year 2002, is a good index. The gluten quality is excellent because the dough resistance and degree of softening are after 19 min and to 5 farinograph units, respectively. Additional evidence for gluten strength is the valorimeter value, which reached 92 units. This reveals a high quality of the gluten, regardless of the environment. Loaf volumes reached 890 cm^3 without any additives. Bread crumbs were white, fine, with a uniform texture.

Table 2. Dough and bread making qualities of high quality wheat cultivars

Cultivar	WG[1]	R	SOFT	VAL	VOL	H:D
Preslav	26.6	16	28	92	645	0.48
Milena	28.4	8	35	72	835	0.50
Dobrudjanka	32.0	19	5	90	782	0.54
Albena	27.0	7	55	67	705	0.46
Progress	29.5	13	25	87	717	0.50
Zlatina	26.7	9	30	75	890	0.48
Demetra	28.2	17	10	84	707	0.55
Bezostaya 1	28.7	6	55	67	800	0.45

[1] WG, wet gluten (%); R, dough resistance; SOFT, dough softening (FU); VAL, valorimeter value; VOL, loaf volume (cm^3/100 g flour); H:D, loaf relation height:diameter

Electrophoretic analysis of HMW and LMW glutenin subunits shows the allele components of 2*/7+9/5+10 (Payne score 9) and 2*/7+8/5+10 (Score 10) of 'Zlatina'. 'Albena' possessed score 8 because of null allele, but bread making is very good. It can be seen that most of the cultivars possessed the allele composition of 'Bezostaya 1'.

Highly productive and economically effective cultivars

The data of these cultivars are presented in Table 3. The average results of five years show stem height ranging from 81 to 98 cm, so these are single or double dwarfs, probably with *Rht1* and *Rht2*. It can be assumed that *Rht8* is involved, too. The spike traits are well

performed for the continental climate, and seed/spike reached 71 grains. Yield capacity is up to 7.8 t/ha for 'Todora' and 'Priaspa'. These cultivars are very adaptable and tolerant to biotic and abiotic stresses, including diseases and drought. In different international trials, including WWEERYT, organized by CIMMYT, 'Priaspa' and 'Todora' are on the top almost every year. Both varieties also possessed very good combining ability for yield capacity, but less for grain quality.

Table 3. Agronomic traits of productive wheat cultivars (1998-2002)

Cultivar	HGHT[1]	EL	SPE	KPE	YLD
Yantar	91.0	9.5	20.5	56.1	7357
Priaspa	89.6	10.0	20.0	57.3	7739
Todora	92.3	9.2	20.3	71.3	7823
Laska	89.5	7.9	15.6	43.4	7571
Lilia	98.8	9.6	20.3	62.0	7627
Prostor	80.8	7.3	15.4	40.4	7262
Bezostaya 1	98.4	8.2	16.8	42.3	6150
LSD 5%	3.9	0.9	1.1	5.8	25.0

[1] HGHT, stem height (cm); EL, ear length (cm); SPE, number of spikelets per ear; KPE, number of kernels per ear; YLD, yield (kg/ha)

The grain and bread making qualities are quite high. The 1000 grain weight is up to 54 g, and hectolitre weight (HW) up to 80 kg. The wet gluten is acceptable. Gluten strength is quite sufficient and valorimeter values reached 80 - 100 units. Loaf volume and bread quality are also acceptable. These results reveal that basic wheat cultivars, grown in the country, are with very good productivity and grain quality. The cultivars spectrum of the first and second group may provide raw material with excellent quality for the bread industry.

Hybrid cultivars

During the last 35 years more than 25 cms sources were discovered in Bulgaria. As a result of this effort two new systems for ms-Rf were established, with *Aegilops kotshyi* (k) and *Ae. mutica* (m) cytoplasms: (1) (k)-system: the new element of this system is male sterility with normal 1B chromosome, without 1B/1R translocation. It can be presumed this is caused by one recessive allele of the *Rg3* gene. By this system, several experimental hybrids were produced with relatively good heterosis, 15 - 18 % more than MP. Probably the fertility restoration is determined not only by *Rf3*, but some modifyers play a role too. The segregation in F_2 is 357:216 (fertile:sterile plants), which is deviates from a 3:1 segregation. By avoiding the 1B/1R translocation the negatives of the ms analogues are not observed (Table 4). There is almost no differences among A and B lines, the fertility restoration and seed set is complete, the kernels are with normal shape and viability. This system can be used immediately on a large scale. (2) (m)-system: this system is universal for male sterility, because no *Rf* genes are discovered among *Triticum* species and varieties until now. The *Rf* gene(s) can be transferred from *Ae. mutica* only (Panayotov & Tsujimoto 1997). The process of transferring *Rf* gene(s) is already done, and the first hybrids may be created during the next two years. The morphology of A and B lines is similar, but two peculiarities are noticed, as follows: (1) (m) Cytoplasm forced the heading of late maturing cultivars, e.g. 'Mercia', 'Soissons' and 'Pernel', and delayed the heading time of early cultivars, like 'Pliska' and 'Fundulea 4' (Table 5). (2) (m)-System recognized the cultivars with 1B/1R translocation, because such varieties into this cytoplasm produce shrivelled kernels. The cultivars without 1B/1R translocation produce normally plump seeds. All other characteristics of morphology and plant growth of A and B lines are similar. The genetics of fertility restoration is not known for the time being.

Table 4. Male sterile cultivars and lines without 1B/1R translocation with (k) type cytoplasm

Lines	BC	Deviation from A analogue		
		HEAD[1]	HGHT	EL
326/04	7	0	+3	+0,5
330/04	3	0	+5	-1,0
334/04	3	0	-2	+1,0
336/04	3	+1	0	-1,0
348/04	3	0	+5	0

[1]HEAD, heading (d); other abbreviations see Table 3

Table 5. Male sterile cultivars and lines (B lines) with *Ae. mutica*-(m)-cytoplasm

Genotype	BC	Deviation from A analogues		
		HEAD[1]	HGHT	EL
Pliska	10	+5	+6	+3,5
Soissons	9	-3	+5	0
KS-HB 60332	3	-1	+9	+1,0
Fundulea 4-31	9	+1	0	+1,0
Zlatina	4	0	+9	0
Pernel	9	-3	+11	0
Mersia	9	-3	-1	+3,0

[1] For abbreviations see Table 4

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Studies on the resistance of wheat genotypes to two different races of *Pyrenophora tritici-repentis* (Died.) Drechsler

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ABSTRACT: Young plants of wheat varieties bred in Martonvásár and others with known genetic backgrounds were inoculated under greenhouse conditions with two isolates of *Drechslera tritici-repentis* (Pti2 → race 1, DW5 → race 5). The area under the disease progress curve (AUDPC) was calculated from the values of lesion types at various dates. Statistically significant differences in susceptibility were revealed by analysis of variance on the varieties studied. On the basis of two years of data, a degree of infection similar to that observed for susceptible 'Glenlea' was recorded for the durum wheat varieties 'Martondur 3', 'Mv Makaróni' and 'Mv Maxi', and for the bread wheat varieties 'Bezostaya 1', 'TAM 107' and 'Martonvásári 4'. For 'Atlas 66' and 'Disponent', which are regarded as resistance sources, the degree of infection was negligible. Several varieties bred in Martonvásár, 'Mv Mezőföld', 'Mv Magvas', 'Mv Emma' and 'Mv Magdaléna', exhibited resistance similar to that of the resistance sources. The resistant control genotype 'M-3' exhibited the lowest AUDPC value. All the other varieties tested had a significantly higher degree of infection.

Key words: Artificial inoculation - resistance – tan spot – wheat

Introduction

The pathogen causing tan spot in the *Gramineae* is a fungus belonging to the *Ascomycota*, the sexual form of which is *Pyrenophora tritici-repentis* (Died.) Drechsler and the asexual form *Drechslera tritici-repentis* (Died.) Shoemaker. It is found mainly on wheat (*Triticum aestivum* L.) and durum wheat (*Triticum durum* L.). Rye (*Secale cereale* L.) and triticale are also susceptible to the pathogen. Symptoms have been recorded on 26 other species from the *Gramineae*. The pathogen was first isolated from various grass species and from *Agropyron repens* in the 1850s. Its presence on wheat was demonstrated in the 1930s, but until the 1970s it did not cause any significant losses of yield (De Wolf et al. 1998). It has been recorded in Europe, Asia, America and Africa, and is listed as one of the major pathogens world-wide (Farkas 2000). It was first reported that the fungus exhibited only a limited amount of physiological specialisation and no races could be distinguished within the pathogen (Obst 2000). Later studies, however, revealed a number of races. For the identification, test collections consisting of six (Ali & Francl 2001) or eight (Strelkov et al. 2002) wheat genotypes were compiled. In 1965 in Hungary the pathogen was still thought causing damage only to *Agropyron repens* (Vörös & Husz 1965). Aponyiné et al. (1988) were the first to report symptoms on wheat and hybrid rye.

Since this first attack it has been regularly isolated from cereal fields to different extents each year. Investigations carried out by Csőszné et al. (2003) indicated that it was dominant among the diseases causing leaf spots. If wheat is grown in a monoculture, yield losses may be as much as 30 %, due to a reduction in the number of grains per spike and the thousand grain mass. The spread of the pathogen and the development of epidemics are promoted by the large number of possible hosts, monoculture, the large area sown to cereals, shallow soil cultivation without ploughing, the susceptibility of the cultivated varieties (Farkas 2000) and favourable weather conditions (Rátainé & Pecze 1997). Differences in susceptibility are known to occur between wheat varieties, but the level of resistance of the Hungarian varieties

currently grown is unknown. A large quantity of inoculum and an exact method of evaluation will be required if this is to be determined.

The aim of the present experiments was to determine the resistance of young plants of various wheat genotypes after artificial inoculation with *Drechslera tritici-repentis*.

Material and methods

The experiments were set up in the greenhouses of the Agricultural Research Institute of the Hungarian Academy of Sciences. Twenty grains of each Martonvásár bred wheat variety and line, and of each genotype from the test collection published by Ali and Francl (2001) were sown in 15 cm Ø pots filled with a 2:1 mixture of earth and sand in three replications. The seedlings were grown at a day/night temperature of 22 / 18°C with a 16 h photoperiod.

Inoculation was carried out with two different races of *Drechslera tritici-repentis* (Pti2 → race 1, DW5 → race 5). Fungus multiplication and conidium development took place in Petri dishes containing PDA and V8 medium (Raymond et al. 1985). The number of conidia required for inoculation (5000 conidia/ml) was adjusted under a light microscope by means of a Bürker chamber count. The inoculum was sprayed onto the leaf surface when the plants were in the 2-leaf stage. In order to promote infection, the plants were covered with polythene for 48 hours, after which the 80 - 90 % relative humidity required for pathogen development was ensured using a humidifier. The genotypes were evaluated from the 7th day after inoculation, scoring the lesion types on a 1 to 5 scale (Lamari & Bernier 1989). The area under the disease progress curve (AUDPC) was calculated from the lesion type values recorded at various dates (Shaner & Finney 1977).

Results and discussion

Based on the results of analysis of variance, a significant difference was observed in the 2003 and 2004 series of experiments between the mean AUDPC values of varieties infected with the two isolates and between the resistance levels of the individual varieties. The year effect was not significant. Based on the data of two years, isolate Pti2 was significantly more aggressive than DW5, since the former exhibited an AUDPC value of 33.4, compared with only 20.0 for the latter. The difference in aggressiveness between the two isolates is also clear from the percentage distribution of varieties suffering different degrees of infection. For Pti2, 70 % of the varieties had AUDPC values greater than 30, while only 10 % of the genotypes fall into this category for DW5. AUDPC values greater than 40 were recorded for none of the genotypes with this latter isolate, but for 30 % of the genotypes with Pti2 (Figure 1).

In greenhouse tests carried out in 2003 and 2004, the varieties were found to differ in their susceptibility to the two isolates (Figure 2). In 2003, the significantly most severe infection with Pti2 was observed for the susceptible variety ‘Glenlea’. Varieties ‘Atlas 66’ and ‘Disponent’, reported by Rees and Platz (1990) as resistance sources, had significantly smaller AUDPC values than the average, though the values recorded for ‘Kavkaz’ and for the Martonvásár varieties ‘Mv Magvas’, ‘Mv Mezőföld’ and ‘Mv Magdaléna’, did not differ significantly from these. The durum wheat varieties, ‘Martondur 3’, ‘Mv Makaróni’ and ‘Mv Maxi’, exhibited the greatest degree of infection with DW5 isolate. In regard to the latter isolate ‘Mv Magvas’, ‘Mv Mezőföld’, ‘Mv Emma’ and ‘Mv Magdaléna’ exhibited similar resistance to that of the resistance sources. The variety ‘M-3’, included in the experiments as resistant control, was completely free of symptoms when inoculated with either pathogen.

For both isolates the infection figures in 2004 were similar to those of the previous year. In the case of Pti2, the most severely infected genotypes were the durum wheat varieties and, from among the bread wheats, ‘TAM 107’ and susceptible ‘Glenlea’. Resistant ‘M-3’ had significantly smaller AUDPC values. ‘Glenlea’ and the durum wheats also had the greatest AUDPC values after inoculation with DW5, while in this year ‘Bezostaya 1’ also exhibited a

higher rate of infection. Resistant ‘M-3’ was completely free of symptoms. The Martonvásár varieties listed above exhibited good levels of resistance similar to those of the resistance sources to both isolates.

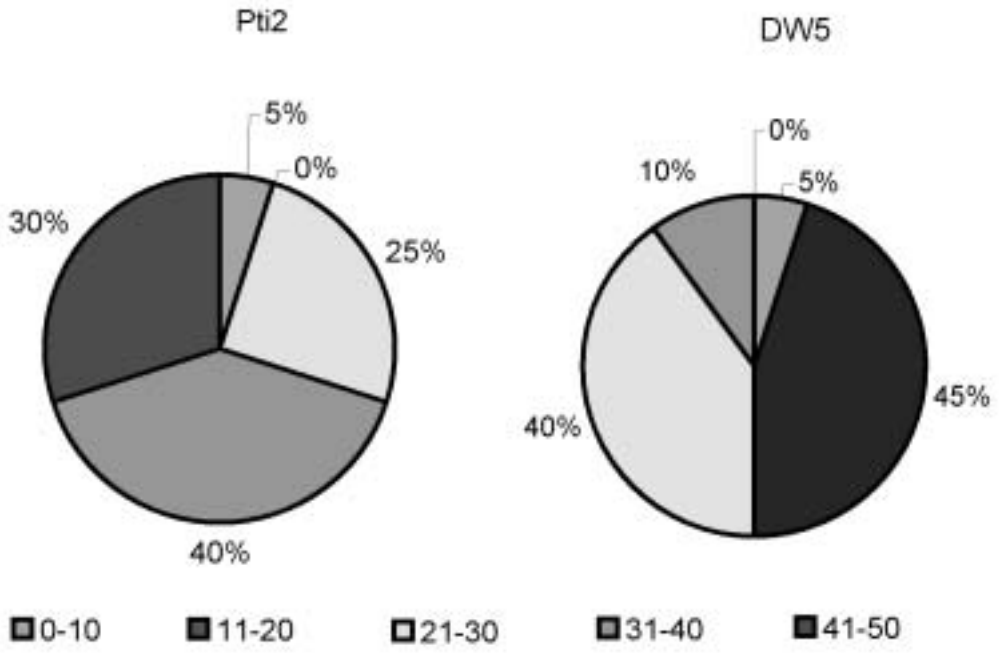

Figure 1. Distribution of AUDPC values for the tested genotypes

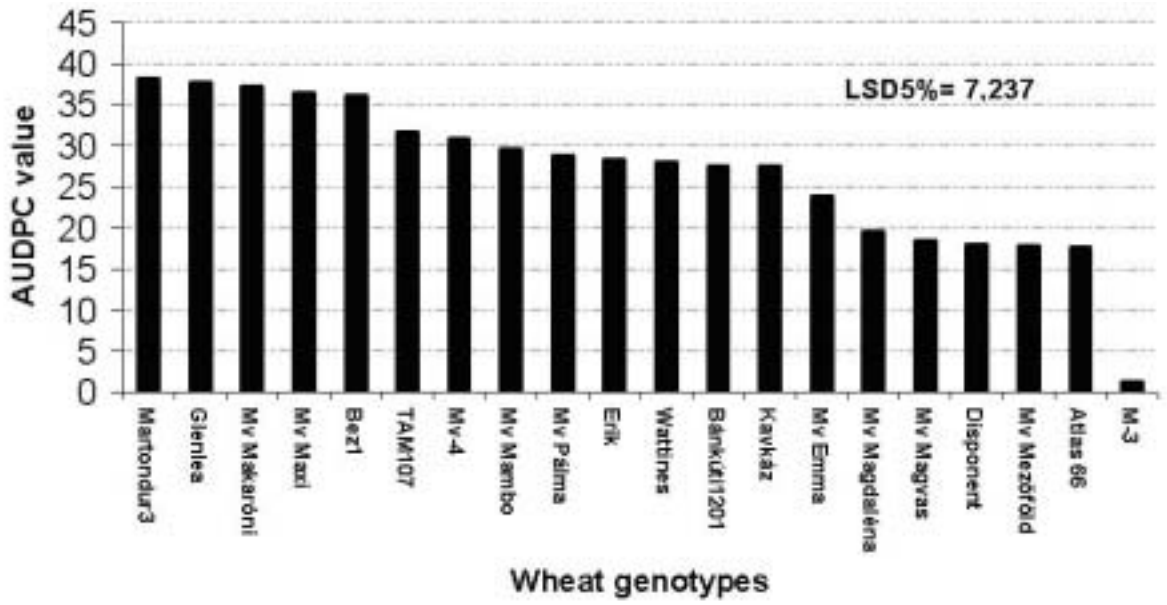

Figure 2. Resistance of wheat genotypes infected with *Pyrenophora tritici-repentis* as revealed by the area under the disease progress curve (AUDPC)

In summary, on the basis of two years’ experiments, infection levels similar to that of susceptible ‘Glenlea’ were observed for the durum wheats, ‘Martondur’, ‘Mv Makaróni’ and ‘Mv Maxi’, and for the bread wheats ‘Bezostaya 1’, ‘TAM 107’ and ‘Martonvásári 4’. The resistance sources ‘Atlas 66’ and ‘Disponent’ exhibited a negligible level of infection. Several varieties bred in Martonvásár (‘Mv Mezőföld’, ‘Mv Magvas’, ‘Mv Emma’ and ‘Mv Magdaléna’) showed resistance similar to that of the resistance sources. Genotype ‘M-3’,

used in the experiments as the resistant control, had significantly the lowest AUDPC value, exhibiting significantly less infection than any of the other varieties included in the experiments (Figure 2).

The greenhouse test currently employed appears to be suitable for the relatively simple and reproducible determination of resistance in young wheat plants. It is hoped that the testing of a larger number of advanced lines will contribute to further improvements in the complex disease resistance of the next series of Martonvásár wheat varieties and in the efficiency of selection.

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The isolates required for artificial inoculation and the varieties in the test collection were put at our disposal by Ali Shaukat (North Dakota State University).

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Analysis of Fusarium head blight resistance QTLs in the 'Ning 8331' x 'Martonvásári 17' population

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ABSTRACT: A population of 228 recombinant inbred lines (RILs) from the wheat cross 'Ning 8331' / 'Martonvásári 17' was used in the examinations. The testing of Type II resistance to Fusarium head blight was carried out in the greenhouse. In field trials the surface of wheat ears was completely inoculated by spraying. In the laboratory the lines were screened with 35 microsatellite markers to reveal the inheritance of chromosome segments. Correlation and simple regression analysis were used to search for associations between the phenotypic and molecular data. Significant correlations were found between the Fusarium head blight resistance and 7 SSR markers. The head blight evaluations of the lines in the Type II resistance trial were associated with the Xgwm539 and Xgwm608 markers on the 2D chromosome, explaining 16.3 and 10.6 % of phenotypic variance, respectively. Five markers proved to be linked with the seed infection levels of the lines in the field resistance trial. Four of these were located on chromosome 3B and one (Xgwm169) on 6A. For the Xgwm389, Xgwm493, Xgwm108, Xgwm533 and Xgwm169 primers the coefficients of determination (R^2) were 11.0, 8.4, 7.2, 7.0 and 7.0 %, respectively.

Key words: Microsatellite markers – QTL – scab – *Triticum aestivum* – wheat

Introduction

Among the fungal diseases attacking wheat in Hungary, Fusarium head blight (FHB) may cause serious infection, resulting in considerable yield and quality losses. The incidence of FHB can be traced from the beginning of the 20th century (Husz 1925), but really severe epidemics have only been experienced since the 70s, when intensive production technologies were introduced (Kükedi 1988). Up to now numerous *Fusarium* species have been identified from diseased heads (Szunics & Szunics 1981), but *F. graminearum* and *F. culmorum* can be found most frequently in epidemic years (Békési & Hinfner 1971).

Wheat plants employ various defence mechanisms to protect themselves against FHB (Mesterházy et al. 1999). The two main types of resistance, to initial infection (Type I) and to the spread of infection within the wheat head (Type II), were described by Schroeder & Christensen (1963).

Studies dealing with the genetic background of FHB resistance are focussed on the highly resistant variety 'Sumai 3' and its derivatives. The results show considerable differences depending on the selected population, infection method and molecular marker type. Linkage between the Type II resistance QTL and the markers mapped on the chromosome 3BS were detected in almost all cases, while the 6BS is also frequently implicated (Waldron et al. 1999). Further chromosomes were also found to be correlated with Fusarium head blight resistance in populations of various 'Sumai 3'-derived lines, such as 6AS and 3AL from *ND2603 (*Anderson et al. 2001), 2BL and 2AS from 'Ning 7840' (Zhou et al. 2002), 5A and 1B from *CM82036* (Buerstmayr et al. 2002), and 7A from 'Ning 894037' (Ren et al. 2003). Since Type I resistance is hard to evaluate separately, only very few examinations have aimed at revealing its genetic background. According to Buerstmayr et al. (2003), it is possible that chromosome 5A may play an important role in resistance against

fungal penetration into the wheat heads. Xu et al. (2001) found an additional marker connected to the Type I resistance QTL on 5BS.

Materials and methods

Plant material

A population of 228 F_5-derived recombinant inbred lines (RILs) developed by single seed descent from the wheat cross 'Ning 8331' (moderately resistant) / 'Martonvásári 17' (moderately susceptible) was used in the examinations.

Inoculum production

Macroconidia of 'IFA 104' *F. culmorum* and 'IFA 65' *F. graminearum* isolates were prepared as described by Buerstmayr et al. (2000, 2002).

Field resistance trial

The parents of the population and the RILs were sown in 2-metre long rows in the middle of October, 2002, in Martonvásár. In consequence of the extremely hard winter, a large proportion of the plants were frost-killed, so the yield-decreasing effect of FHB was not recorded in this study. At the beginning of flowering in each line, the heads were sprayed with *F. culmorum* spore suspension (conc. 5×10^4 /ml) using a back-pack sprayer. The inoculation was repeated 2 and 4 days later. To ensure high humidity for the penetration of the fungus, a mist irrigation system was operated during the trial. Significant head blight symptoms could not be evaluated on the spikelets until ripening due to the extended infection type of the moderately susceptible 'Martonvásári 17' ('Mv17'). In July ten ears per row were harvested and threshed, and the degree of seed infection was determined.

Type II resistance trial

The resistance of RILs against the spread of *Fusarium* was studied in the greenhouse in 4 - 6 replications in Martonvásár. The inoculation was carried out when the main ear of each plant flowered. Using a Nichiryo 8100 repetitive syringe dispenser, 2×5 µl *F. graminearum* inoculum (containing about 1000 macroconidia) was injected into the two basal florets of the 4^{th} or 5^{th} spikelet from the tip of the heads. The inoculated plants were placed in a high humidity chamber for 3 days. On the 21^{st} day after inoculation the discoloration of the rachis and the ratio of infected spikelets were determined. The plants were classed in five groups following the evaluation scheme of Xu & Fan (cit. Bai & Shaner, 1994).

Microsatellite analysis

DNA extraction, PCR amplification and fragment analysis were carried out in Tulln as described by Buerstmayr et al. (2002). More than one hundred SSR (simple sequence repeat) primer pairs (Röder et al. 1998) were tested for polymorphism between the parental DNAs. Apart from chromosomes 4A and 7D, at least one Xgwm primer pair was chosen for each chromosome and more for the 3B, thought to be linked to the head blight QTL. Up to now 35 SSRs have been used to screen the population.

Statistical analysis

Correlation analysis and simple regression analysis were used to calculate correlation coefficients between pairs of experiments and the coefficients of determination (R^2) for the markers. Both were performed using Microsoft Excel.

Results and discussion

Correlation analysis on the phenotypic results of the spray and point inoculation methods and the pattern of inherited chromosomal segments in the recombinant inbred lines revealed significant correlations between Fusarium head blight resistance and 7 SSR markers. These primer pairs were subjected to further analysis by simple regression (Table 1). In this study the markers on chromosome 2D, Xgwm539 and Xgwm608, were the most tightly associated with Type II resistance against FHB (R^2 values of 16.3 and 10.6 %, respectively).

Table 1. Coefficients of determination (R^2) and P values for SSR markers associated with FHB resistance in the 'Ning 8331' / 'Mv17' population using the point and spray inoculation methods

Infection type	Microsatellite	Chromosome	R^2 (%)	P
Point inoculation	Xgwm539	2D	16.3	1.69×10^{-8}
	Xgwm608		10.6	8.25×10^{-6}
Spray inoculation	Xgwm108	3B	7.2	0.000495
	Xgwm389		11.0	1.48×10^{-5}
	Xgwm493		8.4	0.000148
	Xgwm533		7.0	0.000424
	Xgwm169	6A	7.0	0.00041

When the whole surface of the wheat heads was sprayed, five microsatellite markers proved to be significantly linked to resistance. According to Röder et al. (1998), four of these are located on chromosome 3B and one on 6A. The Xgwm389 marker on 3B explained 11.0 % of phenotypic variance, while the coefficients of determination for the other 3 markers (Xgwm493, Xgwm108 and Xgwm533) on the same chromosome ranged from 8.4 to 7.0 %. The histogram in Figure 1 shows the distribution of RILs with different rates of seed infection after spray inoculation with *F. graminearum*.

Comparing these results with those of previous studies, two of the chromosomes associated with FHB resistance can be found in other 'Sumai 3'-derived lines, but are usually connected to resistance against the spread of *Fusarium* within wheat heads. In the experiments of Anderson et al. (2001), the marker associated with Type II FHB resistance explained 1.0 % of phenotypic variance, while this value was 7.0 % for Xgwm169 in the 'Ning 8331' / 'Mv17' population. Among the markers on chromosome 2D, Shen et al. (2003) found two SSR primer pairs in the 'Ning 894037' / 'Alondra' population, for which R^2 ranged from 0.05 to 0.121, but the resistance QTL originated from 'Alondra'.

In order to confirm these results, further resistance trials and molecular markers will be necessary for screening recombinant inbred lines of 'Ning 8331' / 'Mv17'.

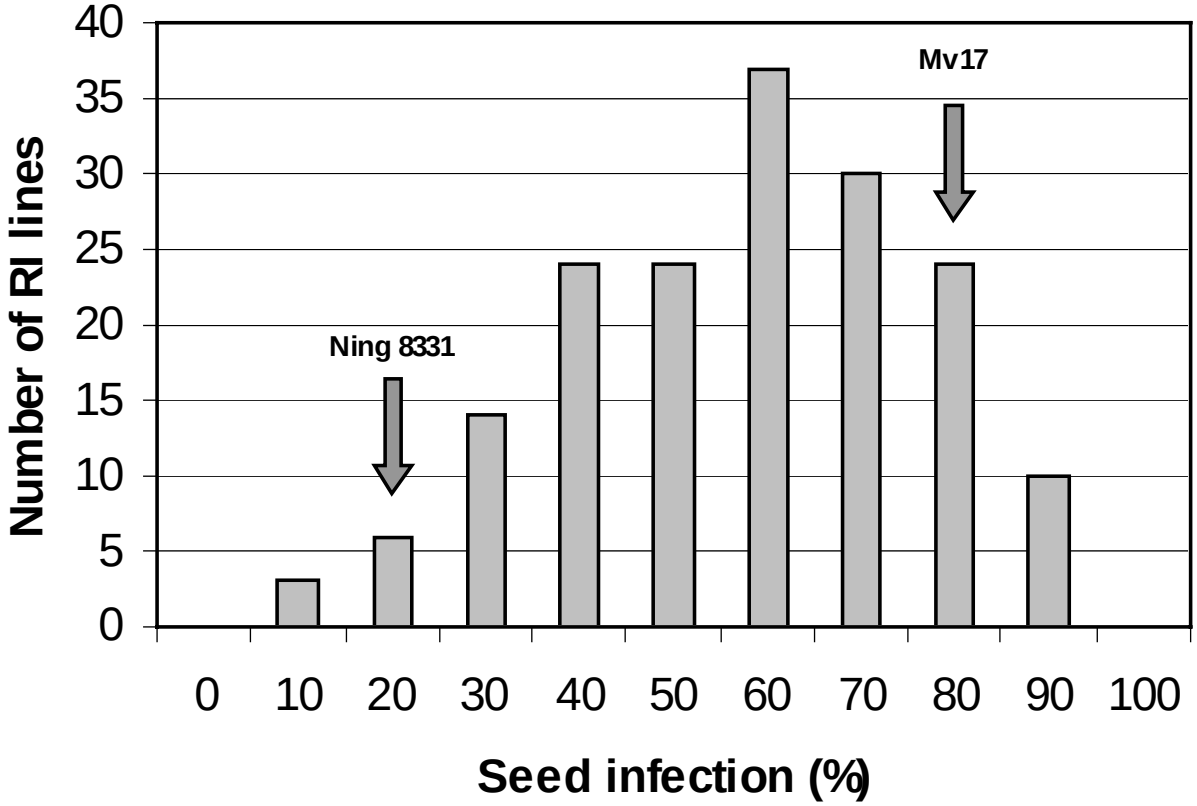

Figure 1. Distribution of seed infection level after spray inoculation in recombinant inbred lines derived from the 'Ning 8331' / 'Mv17' population

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QTL mapping of vegetative characters in wheat (*Triticum aestivum* L.)

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ABSTRACT: A set of 114 recombinant inbred lines (RILs) of the ITMI mapping population was grown under greenhouse conditions in Gatersleben. The lines were evaluated for the vegetative characters early growth habit, basal culm colour, leaf arrangement, length and width of different leaves, shape of the ligule, presence and absence of an auricle, plant height and waxiness. For the character basal culm colour a major QTL could be detected on chromosome 7AS. This corresponds with a known QTL for coleoptile anthocyanin colour but not with the QTLs for the colour of the upper part of the culm. A major QTL could be confirmed for early growth habit on chromosome 2DS. For the characters leaf arrangement, length and width of different leaves, ligule, and auricle no distinct major QTLs could be found. However, for plant height and waxiness major QTLs were determined on chromosomes 3BS, 5AL and on chromosome 2DS, respectively.

Key words: QTL mapping – *Triticum aestivum* – vegetative characters

Introduction

Vegetative characters are important economic traits for wheat (*Triticum aestivum* L.). Analyses of genes controlling vegetative characters are of practical importance because of the effects on plant adaptation and crop yield (Kato et al. 1999). For the discovery of the QTLs of vegetative characters in hexaploid wheat high density linkage maps are necessary. In wheat such a map was established by the "International Triticeae Mapping Initiative" (ITMI) using the ITMI mapping population, which was created by crossing the hexaploid spring variety "Opata 85" (male parent) with the synthetic hexaploid wheat "W7984" (female parent; "Altar 84", a tetraploid durum-wheat was crossed with the diploid *Aegilops tauschii* "CIGM86.940"). World-wide about 800 RFLP and 1000 microsatellite markers are mapped. With the development of high-density linkage maps the discovery of QTLs became possible in many species (Börner et al. 2002).

Materials and methods

A set of 114 recombinant inbred lines (RILs) of the ITMI mapping population was grown under greenhouse conditions in Gatersleben in 2004. The lines were evaluated for the vegetative characters early growth habit, basal culm colour, upper culm colour, leaf arrangement of the third and fourth leaf, length and width of different leaves (basal and third leaf), shape of the ligule (coneshaped or collarshaped), presence or absence of an auricle, waxiness, and plant height. The RFLP map used was created by J. C. Nelson, Cornell University, Ithaca, USA. QTL analysis was performed using the programme QGENE (Nelson 1997).

Results and discussion

Table 1 summarizes the positions of each QTL discovered on the chromosomes. For some characters QTLs with a LOD-score >3.0 could be detected. Some other characters could not be localised definitely. The known QTL from the ITMI population for the character early growth habit (Kulwal et al. 2003) could again be found on the short arm of chromosome 2D.

Table 1. Detected QTLs on chromosomes

Character	LOD > 3.0	LOD > 2.0 < 3.0	LOD > 1.5 < 2.0
Early Growth Habit	2DS		2AS; 3DL
Basal Culm Colour	7AS	5AL	1AL; 3DL; 4AL
Culm Colour	7DL	7DS	4DL; 4DS; 6AS
Leaf Arrangement (3rd Leaf)	2DS; 6AL	2DL; 6BL; 7DL	4DL; 6AS
Length Basal Leaf		3DL; 6DS	2DL; 4AL; 7DS
Length 3rd Leaf			1BL; 2DL; 4DL; 5BL
Width Basal Leaf	7DS	1DL; 6DS	2AS; 2DS
Width 3rd Leaf	6DL	1DL; 2BL	3BS; 5AL; 6AL; 6BL; 7AS; 7DS
Ligule			2BL; 2BS; 6BS
Auricle		2BL; 5DL	4DL
Waxiness	2DS		2DL; 3AS; 7AS
Plant Height	3BS; 5AL	3DS; 4AL	6AL; 6BL

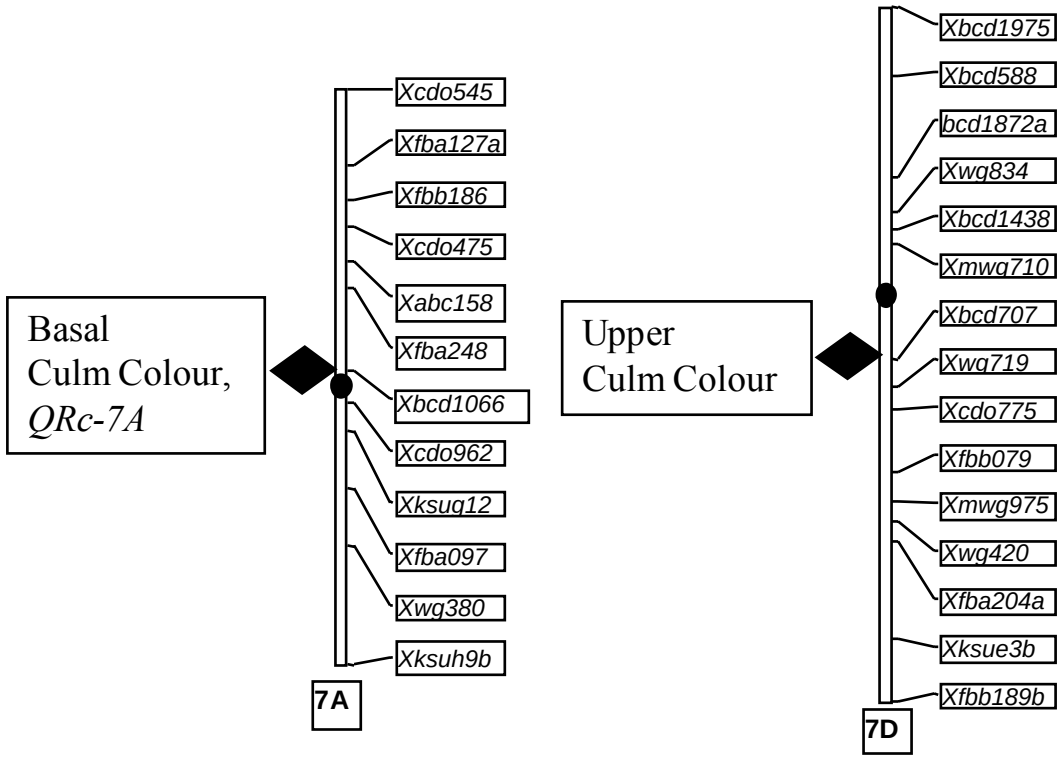

Figure 1. QTLs for basal and upper culm colour, and for one *Rc*-gene (Khlestkina et al. 2002) (◆ contributed by W7984; LOD-score > 3.0)

The QTL for basal culm colour could be detected on the short arm of chromosome 7A. This corresponds with the gene for anthocyanin colour of the coleoptiles (Khlestkina et al. 2002) but not with the QTLs for the colour of the upper part of the culm which were discovered on chromosome 7DL (Lohwasser et al. 2004) (Fig. 1).

New QTLs were found for the character leaf arrangement of the third leaf but the calculated QTLs could not be confirmed for the other leaves. It is necessary to replicate the evaluation of leaf arrangement to control the determined QTLs. For leaf length, however, no major QTLs could be found. Leaf length is probably dependent on environmental factors. Loci for leaf width were detected on two different chromosomes. The QTL for the character width of the basal leaf was different from the QTL for the width of the third leaf (Fig. 2). Possibly there are differences here between an early growth habit and a later growth habit. For the shape of the ligule (coneshaped or collarshaped) and for the presence or absence of an auricle no major QTLs could be found. Perhaps the segregation of these two characters is not pronounced enough. In the ITMI population the QTLs for waxiness and plant height on chromosome 2DS and on chromosome 3BS and 5AL, respectively, could be confirmed from previous field results and from earlier published results (Börner et al. 2002).

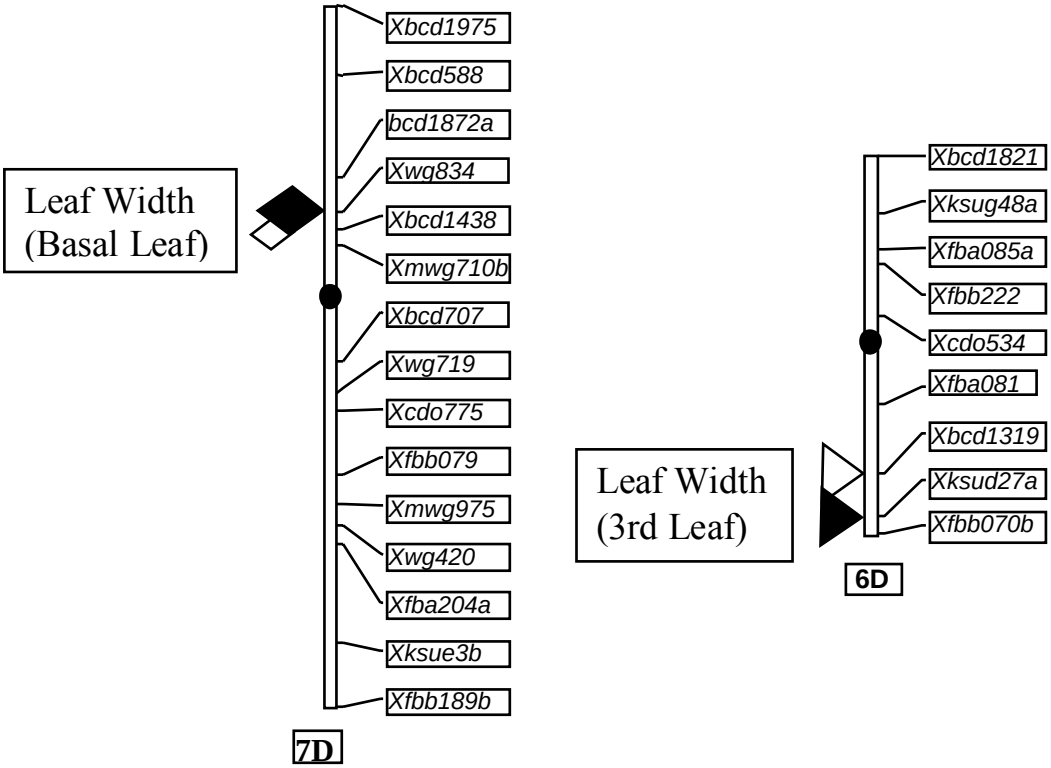

Figure 2. QTLs for leaf width (basal leaf, 3rd leaf)
(◆ contributed by W7984; LOD-score > 3.0, ◇ LOD-score >1.5 < 2.0;
▶ contributed by Opata; LOD-score > 3.0, ▷ LOD-score > 2.0 < 3.0)

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A comparison of genetic stability in haploid plants derived from wheat × maize crossing and anther culture using molecular markers

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ABSTRACT: The utility haploid-culture methods depends on the amount of genetic stability and the aim of the study. In this study, RAPD and AFLP techniques were used to evaluate the genetic stability of produced haploids via wheat x maize crossing and anther culture methods. None of the six random primers used in PCR reactions showed detectable polymorphic bands in haploid plants derived from two techniques. Most of the paired primers used in AFLP technique performed polymorphisms among the haploids. Analysis of the results indicated that the frequency of polymorphic bands varied from 2.6 % to 7.8 % among anther-regenerated plants whereas this frequency was 2.6 % to 3.3 % among haploids derived from wheat × maize crossing. These results indicated that RAPD analysis is not sufficiently sensitive to detect genetic changes that occur during haploid production from two methods. The results also showed that utilization of wheat × maize crosses might be more efficient than anther culture for producing stable haploid plants.

Key words: AFLP – Anther culture – RAPD – wheat × maize crosses

Introduction

Haploids are useful in basic studies and breeding programs. In wheat, haploid plants can be produced through anther (or isolated microspore) culture and wheat × maize crossing. Tissue culture methods can induce variation in regenerated plants. These variations may be due to several factors such as *in vitro* conditions, explant, genotype, media composition and time of culture (Silvarolla 1992). The utility from different methods for haploid production depends on the amount of the genetic stability and the purpose of the study.

In last years, morphological, cytological and molecular techniques have been described for identifying genetic polymorphism, but molecular markers are widely used to detect tissue culture induced variations at the DNA level (Muller et al. 1990, Sabir et al. 1992, Shenoy & Vasil 1992). The objective of this study was to evaluate of genetic stability of haploids regenerated plants via anther culture and wheat × maize crossing using molecular markers.

Materials and methods

Wheat genotype 'Grebe' was used for haploid production using wheat × maize crossing and anther culture. Wheat florets were pollinated with fresh maize pollen. Then 2,4-D (100 ppm) was injected into the uppermost internodes of pollinated spikes. Embryo rescue was made 14 to 16 days after pollination using 1/2 MS medium (Murashige & Skoog 1962). Anthers containing uni-nucleate microspores were cultured on CHB medium (Chu et al. 1990) modified by using 2 mg/l 2,4-D, 1 mg/l Kinetin, 90 g/l maltose and 2.8 g agarose (type 1A, Sigma) and incubated in the dark at 27^{o}C. After 4 to 6 weeks, calli were sub-cultured on regeneration medium (MS + 1 mg/l BA and IAA).

To determine the evaluation of genetic stability, ten haploid regenerated plants from each method and 5 mother plants ('Grebe') were selected and their DNA was extracted using the Dellaporta et al. (1983) method. Six RAPD primers (OPA-19, OPB-05, OPB-09, OPB-10, OPB-17 and OPC-05) were tested on the selected plants. The PCR and electrophoresis techniques were done as described by Williams et al. (1990). The AFLP was analyzed using

PstI and Tru9I as restriction enzymes and followed as described by Soleimani et al. (2002). PCR were carried out using three selective primer combinations (P+ACC-M+CGT, P+ACC-MCTG and P+ACC-M+CCC). Electrophoresis of PCR products was carried out using 6 % denaturing polyacrylamide gels and silver staining.

Results and discussion

All of the haploid plants regenerated from wheat × maize crossing were similar to the field-grown source plants, however, regenerated plants via anther culture varied morphologically. These variations consisted of chlorophyll defects, dwarfness and curly leaf.

The sizes of amplified fragments from RAPD technique ranged from 200 bp to 2 kb. The RAPD patterns of regenerated haploid from both techniques were similar to the patterns of mother plants and no polymorphism bands were detected. It appears that RAPD analysis is not sufficiently sensitive to detect genetic changes in haploid-derived plants. Therefore, we used the AFLP approach because of its capacity to reveal several bands in a single amplification and generating a large number of highly reproducible markers in a short period of time.

Analysis of the AFLP results showed polymorphism bands on haploid plants derived from both techniques. In anther culture, 16.9 % of amplified bands were polymorphic whereas 3.5 % of them showed polymorphism on haploid-regenerated plants from wheat × maize crossing. The frequency of polymorphism varied among individuals from 2.6 % to 7.8 % among anther-regenerated plants and 2.6 % to 3.3 % among haploids derived from wheat × maize crossing.

Genetic instability revealed in anther culture method can be the result of one or a combination of several possible causes including: insertion, deletion, duplication, translocation and inversion within DNA, alteration in DNA methylation pattern, activation of transposable elements, changes in the copy number of repeated sequences, point mutations and epigenetic changes. These phenomena may be due to *in vitro* stress, elongation time of culture, components of medium, concentration and the kind of regularity hormones, genotype and type of explants. For example, 2,4-D causes a general increase in DNA methylation.

Explanation of genetic instability for haploid-regenerated plants via wheat × maize crossing can be related to transposable elements within maize genome and injection of 2,4-D after pollination. Zucchi et al. (2002) reported genetic instability of directly regenerated plants from meristem culture in sugarcane. Similarly, Duvaux et al. (1993) found increase and decrease of methylation in doubled-haploids of barley.

In conclusion, the results obtained from this study indicated that the utilization of wheat × maize crossing may be more efficient than anther culture for the production of stable haploid wheat plants.

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Accurate whole-plant phenotyping: An important component for successful marker assisted selection (MAS)

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ABSTRACT: Accurate whole-plant phenotyping that involves the genome as a whole, constitutes a very essential component for successful marker assisted selection. The honeycomb field designs that use individual plants as evaluation units, enable accurate whole-plant phenotyping principally by three innovative properties: The first is that every plant occupies the center of a complete circular replicate, the plants of which may serve as a common multi-plant check for single-plant yield evaluation. The second is that the progenies of each plant are laid in the field in the corners of a triangular lattice pattern that samples environmental diversity more efficiently than random allocation. The third is that the progenies are evaluated on the basis of the three genetic components of the crop yield potential, i.e., yield potential per plant by the progeny mean $(\bar{x})$, stability of performance by the plant's progeny standardized mean $(\bar{x}/s)$, and responsiveness to inputs by the plant's progeny standardized selection differential $[(\bar{x}_{sel} - \bar{x})/s]$ at a chosen selection pressure.

Key words: Crop yield – genetic components – honeycomb breeding

Introduction

Since the appearance of the thought-provoking paper on a cautiously optimistic vision for marker-assisted breeding (Young 1999), additional work has drawn more attention to the importance of conventional phenotypic selection and the fact that, despite the current genomics revolution, plant breeding is presently and will continue to be driven primarily by selection in breeders plots (Bernardo 2001, Koebner & Summers 2003).

By their very nature, the molecular and genomics approaches have the capacity to focus on the individual plant genome. Thus, for exploiting their full capacity for MAS schemes, it is necessary to combine them with a robust phenotyping technology that will also focus on the individual plant genome, both in order to discover new markers and to validate already known markers in different environments.

This is particularly important for the so-called quantitative traits, which represent the majority of traits with breeding significance. In their case, the practical difficulties encountered are such that it is still considered a great challenge to identify the relationships between genetic variation in gene sequences and phenotypic variation in traits, in order to enable the full exploitation of the genomics revolution (Morgante & Salamini 2003).

The aim of this paper is twofold: First, to draw attention to the feasibility and the importance of accurate whole-plant phenotyping for successful marker-assisted-selection programs with emphasis on quantitative traits, including yield potential per plant, stability of performance, and responsiveness to inputs, and second, to present the details of an innovative field methodology that can greatly assist this goal.

Materials and methods

Selection within a well-known local barley cultivar 'Athinaida' was carried out for two growing seasons (2001 - 2002 and 2002 - 2003) in a series of honeycomb trials, according to the general principles of honeycomb breeding (Fasoula & Fasoula 2000). The honeycomb

breeding is a methodology that was conceived for evaluation and selection of individual plants, with the aid of a series of innovative field designs, known as the honeycomb selection designs (Fasoulas & Fasoula 1995). All honeycomb field designs have common properties, five of which are depicted in Fig. 1. The first is the easy establishment. The second is their capacity to allocate genotypes in the field in a regular triangular pattern that samples effectively environmental diversity and allows reliable selection among progenies. The third is that each plant occupies the center of a complete circular replicate, the plants of which may serve as a common multi-plant check for single-plant yield evaluation. The fourth and fifth are their capacities to handle large numbers of progeny lines and replicates.

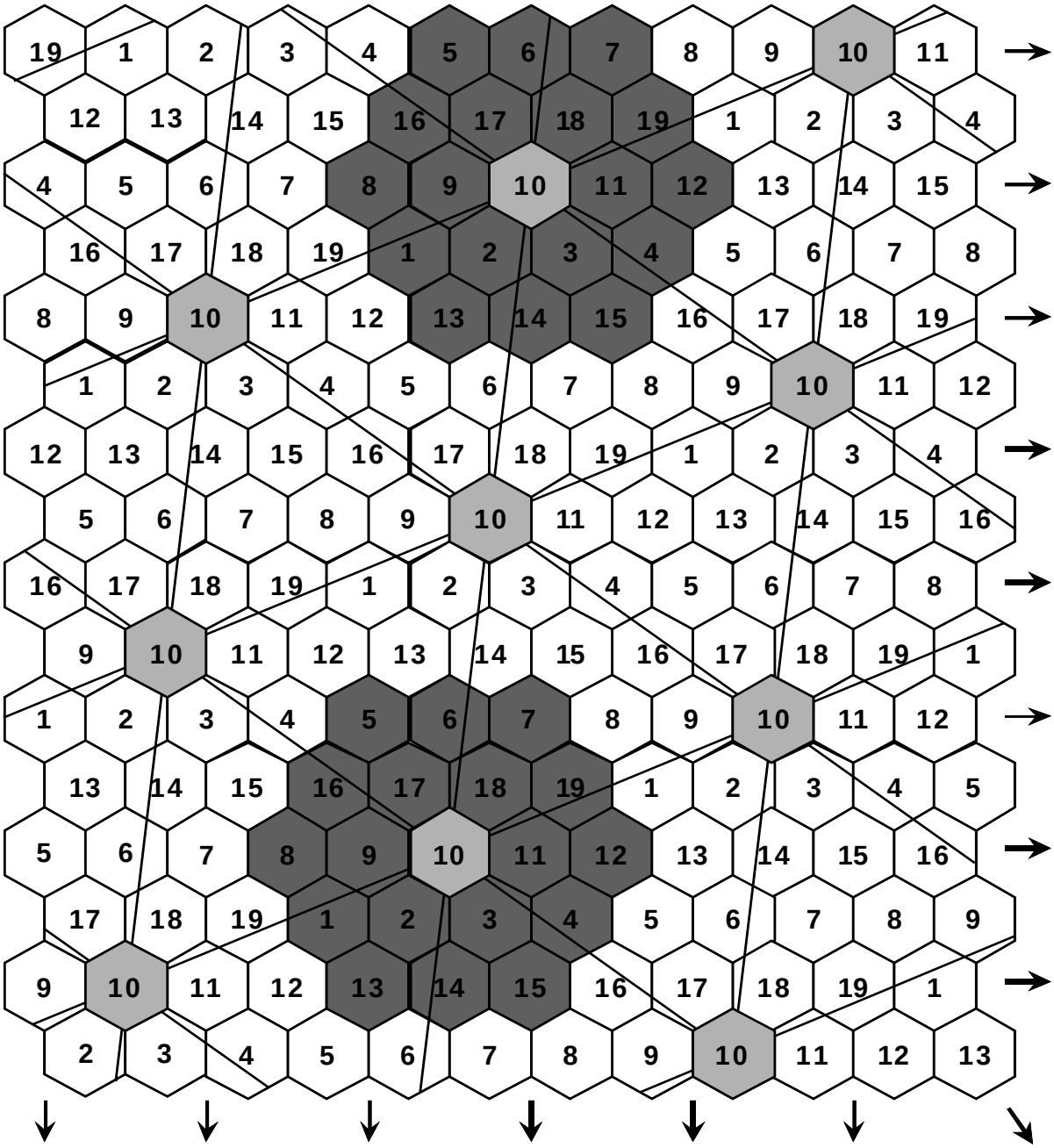

Figure 1. The replicated-19 honeycomb design (left) is capable to compare 19 progeny lines (1 to 19) in such a way that each plant is surrounded by plants belonging to all other lines forming complete circular replicates.

The capabilities of the honeycomb field designs were recently enhanced by the partitioning of crop yield potential into three genetic components (Fasoula & Fasoula 2002, 2003). The first component estimates the yield potential per plant by the progeny mean $(\bar{x})$, the second component reflects the stability of performance and is estimated by the plant's progeny standardized mean $(\bar{x}/s)$, and the third component estimates the responsiveness to inputs by the plant's progeny standardized selection differential $[(\bar{x}_{sel} - \bar{x})/s]$ at a chosen selection pressure. The use of the three components ascertains accurate whole-plant phenotypic evaluation.

Results and discussion

Up to now, the principles of the honeycomb breeding have been successfully applied in various crop species for applied breeding purposes, but not for the purpose of phenotyping in order to accompany a MAS program. In Table 1, it is presented the evaluation of 18 progeny plus the check 'Athinaida' for the three genetic components of crop yield.

Table 1. Evaluation of 18 progeny lines selected for high yield per plant within the barley cv. 'Athinaida'. Lines were evaluated at ultra low densities (90 cm) for the three components of crop yield potential. Mean separation is at 5% level (*t*-test for independent samples and different standard deviations), with 12.5% selection pressure within lines

Progeny lines	YLD[1] (g)		STAB		RESPONSE		GM
	$(\bar{x})$	(%)***	$(\bar{x}/s)$	(%)***	$[(\bar{x}_{sel} - \bar{x})/s]$	(%)***	(%)
6	125.23a	100	2.98	100	1.58	66	89
7	115.93abc	93	2.17	73	2.05	86	84
2	114.87	92	2.46	83	2.18	91	89
12	111.05	89	2.61	88	1.68	70	82
17	110.05	88	2.46	83	1.79	75	82
16	109.21	87	2.28	77	1.48	62	75
15	107.65a	86	2.57	86	2.16	90	87
Check	107.89	86	2.43	82	1.92	80	82
3	105.86	85	2.40	81	1.69	71	79
8	105.79	84	2.16	72	2.14	90	82
1	105.75	84	2.40	81	1.74	73	79
9	105.31	84	2.48	83	2.13	89	85
13	105.15	84	2.73	92	2.18	91	89
5	104.18	83	2.26	76	2.39	100	86
4	103.39	83	2.73	92	1.87	78	84
14	103.26	82	2.51	84	1.97	82	83
18	101.44	81	2.09	70	1.86	78	76
11	100.85b	81	2.00	67	1.88	78	75
10	97.57c	78	2.23	75	1.97	82	78

[1] YLD, mean yield per plant; STAB; stability performance; RESPONSE, responsiveness to inputs; GM, grand mean

Highly significant yield differences among selected lines were obtained and the correlation coefficients between single plant yields and height ranged from r = 0.33 to 0.53 ($p<0.001$). Molecular breeding needs accurate phenotypic evaluations if the molecular data are to be reliable and accurate. The enhanced resolution at the phenotypic level, afforded by the principles of honeycomb breeding, can successfully complement the enhanced resolution at the DNA level, afforded by the methods of molecular breeding. In both molecular and

honeycomb breeding, the unit of evaluation is the single plant. Honeycomb breeding practices multi-site single-plant progeny testing in every generation (starting at the F_1 and the F_2) and selects effectively for high and stable yield, both among and within single-plant progeny lines. The exploitation of the capabilities of honeycomb breeding by molecular breeding programs can be very beneficial, because of the possibility of honeycomb phenotyping to assess the merit and assist the task of genotyping. This approach has the potential to identify multiple loci with cumulative minor effects in the expression of quantitative traits and provide markers of value in MAS breeding schemes. Alterations at such loci, which are very hard to identify with conventional approaches, including QTL mapping, determine whether a new cultivar yields 102 % or 106 % of a standard (Thomas 2003). In addition, the orderly arrangement of honeycomb trials renders them amenable to full mechanization and computerization of the experiments, which is an important precision component.

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Detection of QTL for agronomic traits in an advanced backcross population with introgressions from wild barley (*Hordeum vulgare* ssp. *spontaneum*)

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ABSTRACT: The objective of this study was to map QTL for yield, and yield determining traits in a BC2DH population derived from a cross between the spring barley cultivar 'Thuringia' and the wild barley accession ISR42-8 (*H. v.* ssp. *spontaneum*). Phenotypic data for the traits: time to heading, plant height, ears per m^2, thousand kernel weight and yield were recorded at four locations in Germany. The BC2DH population was genotyped with 69 SSR markers. We detected QTL for all analysed traits except for thousand kernel weight. QTL clustered at certain genomic regions indicating that these may be domestication related loci with strong effects. Favourable exotic alleles were detected for all traits including yield. We could thus show that QTL mapping in advanced backcross populations can uncover "cryptic" beneficial exotic alleles. These QTL are the basis for unraveling the genetic basis of yield and may eventually help breeding new cultivars.

Key words: Domestication-related loci–*Hordeum vulgare* ssp. *spontaneum* – QTL – yield

Introduction

The first important step in plant breeding is the generation of genetic variation. Genetic and morphological studies, however, have shown a general trend of declining genetic diversity in cultivated barley as a response to domestication and selection. Wild barley has often been considered a valuable resource for increasing variability in modern breeds. Exotic germplasm has already been successfully applied in order to (1) introduce new beneficial alleles into modern cultivars and (2) to increase genetic variation and thereby enable the study of gene function. Wild barley, however, has primarily been used as a source for the improvement of simply inherited traits, e.g. for disease and stress resistance. Only few studies attempted to use wild barley for the improvment of quantitative characters such as yield, because these show complex patterns of inheritance, and because wild barley has an overall inferior phenotype with respect to agronomic traits (Pillen et al. 2003, Matus et al. 2003). The study of yield determinants such as those influencing plant architecture, development and different yield components may provide insight into the causes of yield differences. Next to dissecting yield into its different components it has been proposed to partition individual quantitative trait loci, by developing advanced backcross populations. This allows isolating individual QTL, thereby providing insight into gene function and allowing a rapid transfer of exotic alleles into the elite varieties.

Here we report preliminary results of a QTL analysis of yield and and yield determining characters in an advanced backcross population (BC2DH) derived from a cross of the spring barley cultivar 'Thuringia' and the wild barley accession *ISR42-8* from Israel.

Materials and methods

Plant material and genotyping

For the generation of the BC2DH populations an exotic accession of *H. vulgare* ssp. *spontaneum* from Israel (*ISR42-8*) was crossed with the spring barley cultivar 'Thuringia' (*Hordeum vulgare* ssp. v*ulgare*). After two cycles of backcrossing with the recurrent parent 'Thuringia', the BC2DH population with 84 DH lines was developed by anther culture in the

lab of the Saaten-Union Resistenzlabor (Leopoldshöhe, Germany). The BC2DH population was genotyped with 69 SSR markers from different published sources and these were integrated in a consensus map (von Korff et al., submitted).

Evaluation of agronomic traits

Phenotypic evaluation of the BC2DH plants was done in field trials in 2003, carried out at the experimental stations of the University of Bonn (western Germany), Nordsaat Saatzucht (Gudow, northern Germany), Saatzucht Josef Breun GdbR (Morgenrot, eastern Germany) and Dr. J. Ackermann (Irlbach, southern Germany). The experiments were designed in randomised plots without replications. As a control, the recurrent parent was tested with 10 replications per block. Plot size (6 m² - 10 m²), seedling rate (300-330 kernels/m²), and field management were in accordance with the local practice. Investigated traits were: time to heading, plant height, 1000 kernel weight, ears per m^2, and yield.

Table 1. Presentation of QTL for time to heading, plant height, ears per m^2 and yield detected in the BC2DH population ('Thuringia' x *ISR42-8*)

Trait	Marker	*Chr*	*Bin*	Pos[1] (cM)	*RP[exotic]*
Time to heading	HVALAAT	1H	7	63-70	- 3.0
	HVM36	2H	2	17-67	- 8.41
	MGB334	2H	14	159	- 2.1
	HVLTPPB	3H	3	25-30	+ 0.9
	Bmac29	3H	16	175-190	+ 2.7
	HVM40	4H	2	14-25	+ 1.4
	EBmac0701	4H	10	130-150	+ 3.8
Plant height	GMS21	1H	2	14	- 2.3
	GMS3	2H	8	86	- 11.5
	MGB334	2H	14	159	+ 4.6
	HV13GEIII	3H	13	152-175	+ 7.3
	MGB338	5H	8	85	+ 7.0
	Bmag613	6H	9	120	- 8.1
Ears per m^2	HVM36	2H	2	17	- 12.2
	HVSS1	7H	5	62	+ 5.5
	MGB317	7H	10	155	- 5.5
Yield	HVALAAT	1H	7	63-85	- 6.8
	Bmag125	2H	10	122	- 4.2
	MGB358	3H	15	175	- 8.4
	HVJASIP	4H	12	180	+ 3.8
	Bmag0613	6H	9	120-135	- 8.1
	MGB317	7H	10	155	- 7.3

The chromosome assignments and positions in cM are given for the most significant marker of each group of linked markers. The cM position of the QTL are indicated from the position of the first significant to the last significant marker in the linkage group.

[1] Marker positions from von Korff et al. (submitted). Bin classification follows Kleinhofs and Graner (2001). RP[exotic] = relative performance (aa-AA)*100/AA).

QTL analysis

The QTL detection was conducted using the procedure GLM (SAS Institute 1999). The GLM model included the marker (M) as fixed effect, and the environment (E) and the MxE interactions as random effects (mixed model). Here we report on significant marker main effects (M) based on a 0.01 probability threshold. Only the most significant single locus from each group of linked loci is recorded. The relative performance (RP [exotic]) of the homozygous exotic genotype was calculated as follows:

$$\text{RP [exotic]} = \frac{aa - AA}{AA} * 100$$

For each trait aa and AA are the least square means of the homozygous exotic and the homozygous elite genotypes, respectively, calculated across all environments.

Results and discussion

Time to heading

Seven QTL controlling time to heading were detected by QTL analysis. QTL with exotic alleles reducing time to heading were detected on chromosomes 1H and 2H (Figure 1, Table 1). Exotic alleles postponing flowering time were localised on chromosomes 3H and 4H. The strongest effect of the exotic allele was measured on the short arm of chromosome 2H where Laurie et al. (1994) mapped the photoperiod response gene *Ppd-H1*.

Height

For height six QTL were localised on chromosomes 1H, 2H, 3H, 5H, and 6H (Figure 1, Table 1). The exotic allele reduced height on 1H, 2H and 6H, while it increased height on 2H, 3H and 5H. The strongest effect was detected on the long arm of chromosome 3H where the dwarfing gene *denso* is located. Here the exotic allele increased height by 7.3 %.

Ears per m^2

Three QTL were identified for ears per m^2 on chromosomes 2H and 7H (Figure 1, Table 1). The exotic allele at $HVSS1_{7H}$ was associated with an increase in the number of ears. At the loci $HVM36_{2H}$ and $MGB317_{7H}$ the exotic allele reduced the number of ears per m^2 by 12.2 and 5.5 %.

Yield

For yield six QTL were detected (Figure 1, Table 1). The exotic allele improved yield by 3.8 % at the locus $HVJASIP_{4H}$. For the remaining QTL the elite parent contributed the high-yielding alleles. The strongest yield reducing effects of the exotic alleles were measured at $MGB358_{3H}$ and $Bmag613_{6H}$. At both loci yield was decreased by about 8 %.

Yield, being the most important trait for breeders, is also the most difficult to manipulate, because of its complex nature. In this preliminary study, we analysed yield and yield determinants in order to gain insight into the causes of yield differences. We examined time to heading demonstrating developmental differences, plant height indicative of plant architecture and ears per m^2 as a yield component. It is interesting to note, that we detected 'QTL hotspots', chromosome regions, which have an effect on several traits. For example, markers at the telomere of 2HL had significant effects on time to heading, height and yield. Clustering of QTL may be caused by physiological constraints due to trait correlation. The exotic allele increased, for example, height at $HV13GEIII_{3H}$ by 7.3 % and decreased yield by 8 %. Genetic correlations among traits may also be the reason for QTL clusters. Clusters of agronomically important traits may have been selected for in the process of domestication. Peng et al. (2003) found clustering and strong effects of some QTL in *T. dicoccoides* and association of domestication related QTL with gene-rich regions. Matus et al. (2003) mapped

QTL for seed and awn retention, which is characteristic for wild barley. These were located on chromosome 1H, 2H and 6H close to the loci with a yield decreasing effect of the exotic allele detected in this study. Favourable exotic alleles were detected for all investigated traits including yield. We could thus show that QTL mapping in advanced backcross populations can uncover 'cryptic' beneficial exotic alleles. These QTL are the basis for unravelling the genetic basis of yield and/or domestication factors and may eventually help breeding new cultivars.

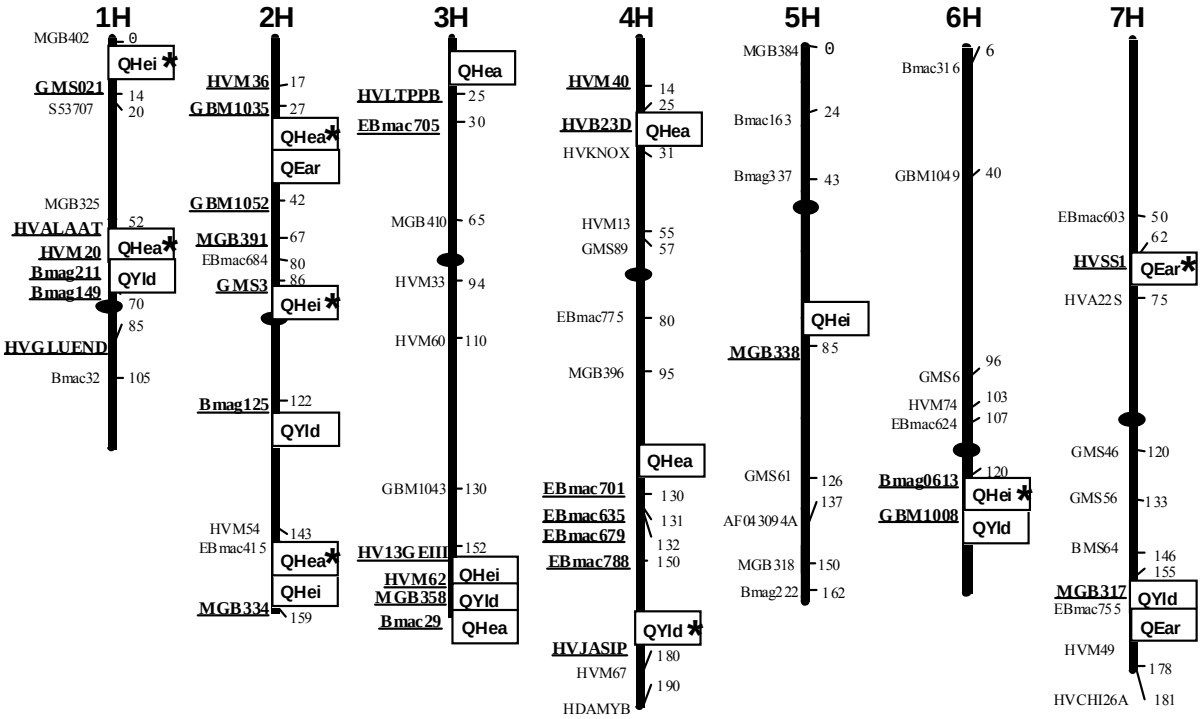

Figure 1. Locations of QTL for time to heading (QHea), plant height (QHei), ears per m² (QEar) and yield (QYld) on a consensus map with 69 SSR markers. The QTL are indicated to the right of the significant markers. These are typed in bold and underlined. The asterisks next to the QTL indicate that the wild barley contributes the favourable allele.

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Selection of a core set of informative gene-derived SSR and SNP markers for assaying the genetic variation in germplasm collections of barley for abiotic stress tolerance

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ABSTRACT: A core set of informative gene (EST)-derived SSR (simple sequence repeat or microsatellite) and SNP (single nucleotide polymorphism) markers was selected for the analysis of the genetic background and to make inferences about population structure of a set of >220 barley lines comprising landraces, accessions, cultivars, etc. In this direction, a total of 50 SSR and 50 SNP markers from the transcript map of barley were used with a set of 6 highly diverse barley accessions by employing fluorescence-based fragment analysis (for SSR) and allele-specific sequencing (for SNP) techniques. Finally, a core set of 28 SSR and 28 SNP markers was identified on the basis of the following criteria: (i) randomly distributed markers (2 markers per chromosome arms), (ii) high PIC (polymorphic information content) value, and (iii) producing good quality peaks. Details on different features like PIC, number of alleles or SNPs detected per marker, the haplotypes, and the calculated nucleotide diversity index (π value) for selected SSR and SNP markers are discussed. With the help of SNP2CAPS tool (http://pgrc.ipk-gatersleben.de/snp2caps/), putative restriction enzymes were identified for 20 of 28 targeted SNPs; therefore, they can be assayed on agarose gel by using CAPS marker assay. Furthermore, comparison of SSR and SNP marker data for cladistic analysis suggest that both marker types yield similar groupings.

Key word: Abiotic stress – diversity – EST-SSRs – EST-SNPs – functional markers – *Hordeum vulgare*

Introduction

Abiotic stresses are major barriers for sustainable crop production in temperate and tropical environments because the yield potential of any particular cereal variety is rarely maximized in any one season or area due to limitations imposed by abiotic stresses (Quarrie et al. 1999). Therefore it is important to identify the sources of genetic variation for tolerance to important abiotic stresses especially from unadapted germplasm and then to develop strategies for their deployment so that eventually plant breeders can develop new cultivars with greater yield potential and yield stability over seasons and ecogeographic locations (Ellis et al. 2000).

The majority of the markers developed and used in the past, for detection of genetic variation for abiotic stress, belonged to genomic DNA, and therefore could belong to either the transcribed region or the non-transcribed region of the genome without any information available on their functions. Molecular markers developed from known sequences like ESTs or fully characterized genes in recent times represent the class of functional markers and have been considered useful in this direction (Kota et al. 2001, Eujayl et al. 2001).

To meet the future challenges, a barley collection held at ICARDA will be explored for assaying the genetic diversity and functional diversity at DNA and RNA level by using functional/gene (EST)-derived SSR (simple sequence repeats or microsatellite), SNP (single nucleotide polymorphism) and cDNA macroarrays. In the present study a core set of informative genetic markers has been selected for the analysis of the genetic background and to make inferences about population structure of a set of >220 barley genotypes.

Material and methods

Plant material

In the present study, a set of six diverse barley (*Hordeum vulgare* L.) genotypes was used as a representative subset of the complete collection of >220 barley lines. The accession numbers of these genotypes at ICARDA are 123722 (IG128088), 123793 (IG128159), 123804 (IG128170), 123807 (IG128173), 123834 (IG128200) and 123838 (IG128204). DNA was isolated from these genotypes as given in Thiel et al. (2003).

SSR analysis

Amplification of microsatellite loci using fluorescent-dye labeled primer pairs was carried out as given in Thiel et al. (2003). Amplification products were separated on an ABI377 fragment analyzer and evaluated using GenoTyper 3.7 (Applied Biosystems). The PIC (polymorphic information content) value of SSR markers was calculated as given in Thiel et al. (2003).

SNP analysis

Allele-specific sequencing by using the primer pairs for ESTs was done with the set of genotypes examined as given in Kota et al. (2001). The PIC value, Pi (π), haplotypes, etc. were calculated as described in Kota et al. (2003).

Cladistic analysis

The 0/1 matrix obtained by selected SSR and SNP markers with the set of six genotypes was used for the calculation (NTSYSpc 2.1) of genetic dissimilarity according to Nei, SAHN clustering and the construction of UPGMA (unweighted pair group method arithmetic average) phenograms.

Results and discussion

Marker analysis

A total of 100 gene/EST-derived markers including 50 EST-SSR and 50 EST-SNP markers, distributed more or less uniformly throughout all the linkage groups of the transcript map of barley were used with the set of six diverse genotypes. 47 SSR and 46 SNP markers showed polymorphism among the genotypes used (Table 1). Since the remaining seven markers were polymorphic between parents of at least one mapping populations ('Igri' x 'Franka', 'Steptoe' x 'Morex', OWBRec x OWBDom) and were mapped, it suggests that the examined genotypes are less diverse than the parents of above mapping populations.

Core set of informative functional markers

SSR markers. Out of 47 SSR markers that were polymorphic in the examined genotypes, 28 SSR markers were selected as a core set of informative genic-SSR markers. These markers are randomly distributed (2 markers per chromosome arms) and produce good quality peaks without any stuttering. Selected markers exhibit 2 - 4 (average 2.93) alleles with the PIC value of 0.23 to 0.52 (average 0.36) in the analysed set of six genotypes (Table 1).

SNP markers. 28 out of 46 SNP markers were selected as a core set of informative genic-SNP markers. Like SSR markers, these markers also represent both the chromosome arms of all the linkage groups of barley. After screening the 217 - 798 bp sequence data (average 409 bp) of the six genotypes, the selected set of SNP markers yields 1 to 29 SNPs (average 7.6) with 2 to 6 haplotypes (average 4.1) per marker (Table 1). The PIC values of identified SNPs and haplotypes are in the range of 0.14 - 0.28 (average 0.23) and 0.44 - 0.83 (average 0.67), respectively. The calculated nucleotide diversity index (π value) for each of the selected SNP markers is in the range of 0.16×10^{-2} to 2.79×10^{-2} with a mean π value of 1.07×10^{-2}.

Assaying the single nucleotide polymorphism in the whole set of >220 barley lines with the selected set of SNP markers by using the allele-specific sequencing or any other SNP genotyping platform would be very expensive. To make the SNP assay cost-effective in a large germplasm collection, targeted SNPs (with the higher PIC value) were investigated for presence of the restriction site with the help of SNP2CAPS tool (Thiel et al. 2004; http://pgrc.ipk-gatersleben.de/snp2caps/). As a result, it is possible to assay 20 out of 28 'targeted' SNPs on agarose gel following CAPS assay with commonly used restriction enzymes.

Comparison of SSR and SNP markers

In order to compare the results of SSR and SNP markers for cladistic analysis, a total of 120 and 520 data points obtained by using 47 SSR and 46 SNP markers, respectively, were used to prepare the phenograms. Both phenograms classify the examined genotypes more or less in similar way. In both phenograms, three genotypes (123722, 123807 and 123834) are in one cluster, two genotypes (123804 and 123838) in another cluster while one genotype (123793) is very distant to the above clusters (Figure 1). These results are in accordance with our earlier results (Kota et al. 2001), where it was concluded that different types of markers derived from genes are more suitable for fingerprinting studies as they yield similar groupings of unrelated germplasms.

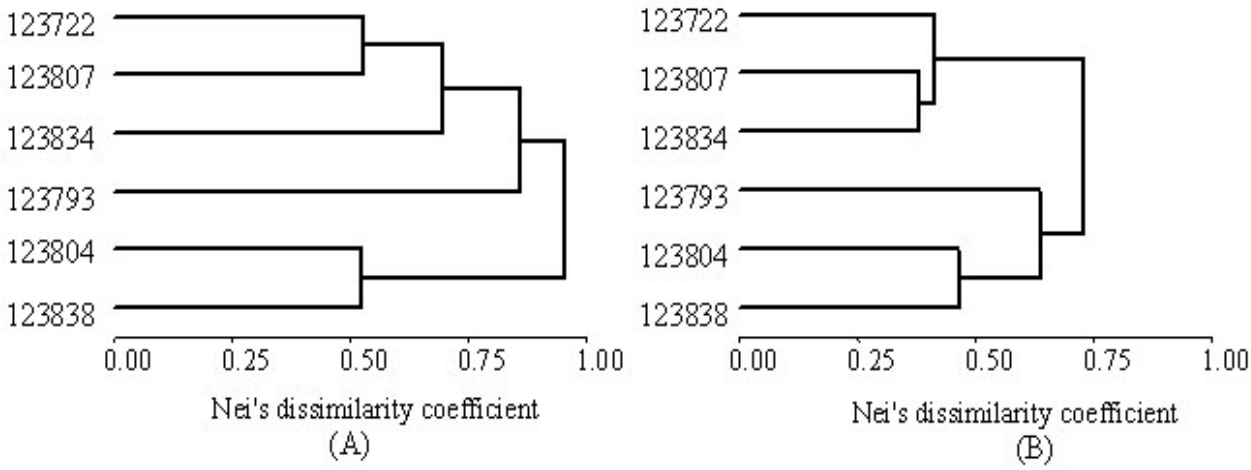

Figure 1. UPGMA phenograms showing the genetic diversity among the six barley genotypes using (A) SSR and (B) SNP data, respectively

Table 1. Details on the identified core set of SSR and SNP markers

Chromosome	1H	2H	3H	4H	5H	6H	7H	Sum / *Means*
SSR markers								
Used	7	7	8	7	7	7	7	50
Polymorphic	7	6	8	7	7	6	6	47
Selected	4	4	4	4	4	4	4	28
Alleles (n)	2-4	2-4	3-4	3-4	2-3	2-4	2-4	2.93
PIC (min-max)	0.29-0.52	0.29-0.42	0.37-0.47	0.37-0.47	0.23-0.42	0.25-0.42	0.25-0.48	0.36
SNP markers								
Used	7	7	8	7	6	7	8	50
Polymporphic	6	7	7	7	6	7	6	46
Selected [1]	4 (2)	4 (3)	4 (3)	4 (3)	4 (4)	4 (4)	4 (1)	28 (20)
SNPs (n)	7-14	2-29	5-11	2-7	1-11	1-9	4-15	7.25
PIC (min-max)	0.25-0.29	0.25-0.29	0.25-0.29	0.25-0.29	0.25-0.29	0.18-0.25	0.25-0.28	0.26
SEQL (bp)	307-507	293-526	217-798	237-445	261-653	326-430	311-542	409
HAPLO (n)	3-6	2-6	3-6	2-5	2-5	4-5	4- 6	4.10
PIC_{haplo}(min-max)	0.61-0.83	0.44-0.83	0.50-0.83	0.44-0.80	0.44-0.78	0.66-0.80	0.48-0.63	0.68
Average Pi	0.0106-0.0126	0.0027-0.0279	0.0050-0.0170	0.0050-0.0074	0.0016-0.0225	0.0066-0.0102	0.0089-0.0139	0.0107

[1] Number in parenthesis for selected SNP markers stand for those markers that can be assayed by CAPS assay; SEQL, sequence length examined; HAPLO, haplotypes obtained; PIC_{haplo}, PIC values of haplotypes

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Reversions from genetically unstable mutants as a means of expanding the genetic diversity of barley

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ABSTRACT: A collection of lines based on reversions from the homeotic pleiotropic genetically unstable barley mutants *tweaky spike* (*tw*) was created and investigated for productivity, resistance to plant diseases, protein content and other traits. Results of multi-year investigations show that reversions *tw* → *Tw* do not exactly return to wild type, and a high diversity of lines in all the test characters has been observed. Several revertants are of economical value.

Key words: Diversity of revertants – genetic resources – *Hordeum vulgare* – unstable mutants

Introduction

The true reversions from mutant allele to *WT* (wild type) gene are very rare, and in the broad sense reversions are all incidents in which a gene returns gene from 'loss-of-function' (null, knock-out) to its active state or from 'gain-of-function' to normal expression. Reversions from nonsense, frameshift mutations restore activity of gene, but give mutant proteins. The same is true for the suppressible alleles in the tRNR anticodon sequences. Duplications of several nucleotide pairs remain in revertants (Rvs) also after a ME (mobile element) passes the target place by the 'cut-and-paste' mechanism. Insertion mutagenesis and mutations in UTR (untranslated regions) and introns, ectopic expression of mutations in the developmental genes added the new types of Rvs among whose diversity and absence of the exact return to *WT* have been also observed (Bradley et al. 1993; Girard & Freeling 2000). Two states of ME, active and inactive, cause two phenomena – somatic instability of mutants (Bradley et al. 1993) and Rvs of epigenetic nature (Girard & Freeling 2000). Transcriptional gene silencing or gene silencing via RNA interference give the new incidents of reversions to *WT* with varying their expression (Moffatt et al. 2002).

The first barley mutant *tweaky spike* (*tw*) induced by treatment of cv. 'Auksiniai II' with aziridine was intriguing because of its genetic instability and high protein content. Eleven new allelic *tw* mutants were induced, but only two of them were genetically unstable. The *tw* mutants are recessive homeotic and pleiotropic, characterized in the first order by a characteristic spike form and lodicules converted to stamens and/or carpels. The genetic instability of mutants *tw*, tw_1 and tw_2 allowed us to compile a collection of Rvs and to examine their pecularities (Rančelis et al. 2001; Balčiūnienė et al. 2002). Splitting of the complex of pleiotropic characters has been proposed, and we could expect that several characters should be preserved in Rvs to *WT*, easily determined by return to the normal spike structure. These characters and the economical value of Rvs were investigated in the current work.

Materials and methods

Only spontaneous Rvs from the three unstable pleiotropic allelic *tw* type mutants were used in the present work. All Rvs arose from mutants induced in the same initial barley cv. 'Auksiniai II'. Its seed material was obtained from the Lithuanian Institute of Agriculture and was used also as wild type (*WT*). Two series of Rv investigations were realised. They differed by the source of Rvs and by the traits studied. In the first part Rvs from *tw* mutant were mainly used, and in the second part Rvs from tw_1 and tw_2 were investigated. All determinations were made in many years (3 - 4) of investigations. The stability of Rvs was examined by genealogical and hybridological analysis. Protein content was determined by the standard micro Kjeldahl method and starch content by hydrochloric acid dissolution. The size of plots for productivity determination was 2 m^2 in 3 replications in both Botanical Garden of Vilnius University and Lithuanian Institute of Agriculture (Dotnuva). The susceptibility of Rvs to the pathogens *Drechlera teres* (Sacc.) Shoem. (syn. *Helminthosphorium teres* Sacc.), *Puccinia hordei* G. H. Otth, *Erysiphe graminis* DC. ex Merat (syn. *Blumeria graminis*), *Claviceps purpurea* (Fr.: Fr.) Tul. was evaluated in their natural field conditions. For *Ustilago nuda* (Jens.) Rostr. in the first part of investigations artificial infection was also used. The distribution of internal micromycetes in germinating seeds was studied by isolation of a pure culture using malt extract agar (MEA) medium with streptomycin (250 mg l^{-1}) added. Micromycetes were identified on the basis of their morphological and cultural characteristics (Arx 1981).

Results and discussion

In the first series of investigations our attention was focused on the traits that are exclusive for *tw* type mutants. The peculiarity of the first part of investigations was that mutant *tw* was divided into sublines, progenies of the one individual plant, and Rv was compared with the initial *tw* subline. The results were partially published (Rančelis 1993, Rančelis & Vaitkūnienė 1998). In the second part of investigations our attention was directed to the characteristics having an economic value, such as productivity and quality of seed production, resistance to diseases and lodging. Diversity of Rvs in a wide range of traits was observed in both parts of investigations and independently of the source of Rvs – from *tw,* tw_1 or tw_2 (Tables 1 and 2). It confirmed the observations of other authors (Bradley et al. 1993, Girard & Freeling 2000, Moffatt et al. 2002) about the diversity of Rvs, but exploitation of Rvs for breeding purposes is unknown to us. All data are results of three and more years of investigations and expressed in coefficients (coef) to *WT,* and coef above 1.00 means a higher expression of a trait in Rvs than in *WT*, or vice versa. So, coefs show that protein content is higher in all 12 Rvs $tw \rightarrow Tw$, presented in Table 1, while not all Rvs from tw_2 have a higher protein content than *WT*. However, well known is the opposite correlation between grain yield and protein content. Therefore, these two traits were expressed in the common coef. Only those Rvs are of interest for which the common coef is higher than 1.00. Among $tw \rightarrow Tw$ there are six such Rvs, among $tw_1 \rightarrow Tw$ two and among $tw_2 \rightarrow Tw$ also two. From barley mutants tw_1 and tw_2 arose not only *WT* with normal spike, but also plants with a longer spike or, more contrasting, with compacted spike. Compactoids in Table 2 are presented separately. All of them are resistant to lodging. In our previous work (Balčiūnienė et al. 2002) we have concluded that several Rvs are very resistant to *Erysiphe graminis*. Comparison of resistance to *E. graminis* with resistance to *Puccinia hordei* gave quite an unexpected effect of interference. In the main part, Rvs that were resistant to *E. graminis* were very susceptible to *P. hordei*, i.e. *P. hordei* blocked the development of *E. graminis*.

Table 1. Summarized data of the first series of investigations, *tw* → *Tw*. Results are expressed in coefficients to *WT* (1.00).

Character/	Mutant			Revertant		
Property	no	Mean	Variation	no	Mean	Variation
Productivity	12	0.4±0.12	0.29–0.68	12	0.9±0.11	0.72–1.07
Protein in grain	12	1.3±0.06	1.06–1.42	12	1.1±0.06	1.07–1.21
Productivity + protein	12	0.9±0.09	0.68–1.00	12	1.0±0.14	0.95–1.09
Sensitivity to						
– *Ustilago* (artificial)	5	1.0±0.07	0.53–1.60	5	0.7±0.07	0.32–1.16
– grains to micromycetes	5	1.7±0.06	1.33–2.31	5	0.7±0.10	0.47–0.87
Resistance to aziridine	5	2.3±0.23	2.13–2.57	5	1.3±0.20	1.05–1.62
Free radicals in grains	5	1.1±0.02	0.89–1.36	5	0.9±0.02	0.74–1.05
– seed-coats	5	1.2±0.02	0.93–1.65	5	0.9±0.04	0.72–0.96
Chromosome aberrations						
– in mitosis anaphase	4	1.7±0.09	1.41–1.90	5	1.6±0.06	1.54–1.74
– in the first meiosis division metaphase	4	2.8±0.20	2.57–3.30	4	1.4±0.27	1.19–2.30
SCE	4	1.1±0.03	1.06–1.22	4	1.1±0.05	0.86–1.24

Table 2. Summarized data of the second series of investigations, $tw_1 \rightarrow Tw$, $tw_2 \rightarrow Tw$. Results are expressed in coefficients to *WT* (1.00)

Character/		Revertant			Compactoid		
Property	Mutant	n	Mean	Variation	n	Mean	Variation
Productivity	Tw_1: 0.56±0.04	8	0.9±0.07	0.79–0.95	1	1.0±0.09	–
	Tw_2: 0.56±0.04	7	0.9±0.08	0.78–1.04	4	0.9±0.06	0.82–0.94
Protein in grain	Tw_1: 1.26±0.06	8	1.1±0.02	0.99–1.11	1	1.0±0.05	–
	Tw_2: 1.28±0.03	7	1.0±0.02	0.96–1.10	4	1.1±0.03	0.92–1.10
Productivity + protein	Tw_1: 0.91±0.05	8	1.0±0.05	0.92–1.08	1	1.0±0.07	–
	Tw_2: 0.92±0.04	7	1.0±0.05	0.95–1.07	4	1.0±0.04	0.90±1.02
1000 grain mass	Tw_1: 1.02±0.03	8	1.0±0.07	0.97–1.09	1	1.1±0.03	–
	Tw_2: 1.05±0.02	7	1.1±0.06	1.03–1.13	4	1.0±0.05	0.96–1.05
Resistance to lodging	Tw_1: 1.44±0.16	8	0.8±0.21	0.70–1.06	1	1.1±0.31	–
	Tw_2: 1.42±0,18	7	0.7±0.23	0.56–1.20	4	1.2±0.32	1.04–1.31
Sensitivity to diseases[1]	Tw_1: –	3	4.0	2.78–6.29	1	2.3	–
	Tw_2: 1.60	4	3.6	1.00–6.97	2	8.7	8.16–9.14

[1] Mean coefficients for *Drechlera, Puccinia, Erysiphe*

Ten Rvs, previously characterised by a higher protein content, were for three years tested at Lithuanian Institute of Agriculture (Dotnuva), where conditions significantly differ from those of Vilnius. Rvs were compared with the standard cv. 'Ula'. Among the lines tested, three had longer spikes and three were compactoids. Like under Vilnus conditions, these traits have been inherited. The grain yield of Rvs was lower. The best line was compactoid K1. Its yield was about the same as of cv. 'Ula' (coef. 0.99). Protein content in all Rvs tested was higher than cv. 'Ula'. Two compactoids (K7 and K8) alongside a higher protein content accumulated the highest starch content (57.3 - 57.5 %), but did not significantly surpass cv.

'Ula'(55.6 %). Almost all Rvs matured earlier than cv. 'Ula' (84 days), but one compactoid (K7) was significantly superior (79 days) to cv. 'Ula'. In Vilnius there was fixed one very early Rv (N 32) which was not examined in Dotnuva. In Dotnuva, after a three-year evaluation four Rvs were selected for breeding purposes. These Rvs were characterised by short straw, good lodging resistance, shorter maturity time and high grain number per spike.

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A retrotransposon sequence is related to DNA instability in barley microspore culture

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ABSTRACT: In order to assay gametoclonal variation in barley, a set of doubled haploid (DH) plants (cv. 'Igri') regenerated from isolated microspore culture by direct embryogenesis, was especially developed. AFLP analysis was performed with eleven enzyme-primer combinations, that represented the study of 719 band polymorphisms. Only one change in the banding pattern was observed, indicating a high degree of genetic stability. An extra band was present in DH lines regenerated from microspores, treated or not with sodium azide, representing a 'hot spot' of instability. The sequence of this band was related to barley 'sukkula' retrotransposon element. Our preliminary results suggest the activation of retrotransposon by barley isolated microspore culture.

Key words: Doubled haploid – genetic stability – *Hordeum vulgare* – retrotransposon

Introduction

Doubled haploid (DH) production is a valuable tool for plant breeders because it provides a rapid way to produce a large number of homozygous plants at any stage in a breeding program (Thomas et al. 2003). Furthermore, DHs have been very useful for mutation, gene mapping and genomic studies. Androgenesis, which involves the regeneration of plants through a tissue culture phase, is the most efficient method for barley DH line production.

It is well documented that some tissue culture methods generate genetic modifications such as numerical and structure changes of chromosomes, gene mutation, activation of transposable elements and also changes in DNA methylation patterns (Lee & Phillips 1988). Although early studies described genetic modifications in barley plants obtained by androgenesis, this instability was attributed to a callus regeneration phase. However, in systems where plants were regenerated by direct embryogenesis, there was little evidence of induced genetic changes, suggesting that point mutations and larger DNA rearrangements had not occurred but differences in levels of methylation (Devaux et al. 1993, Logue 1996)

In the present work, a molecular analysis of barley DH lines produced from isolated microspores was performed in order to broaden the few reports related to gametoclonal variation obtained by direct embryogenesis.

Materials and methods

Plant material and isolated microspore culture

The model cultivar 'Igri' was chosen for this study due to its good androgenic response. Dissected anthers were pre-treated with 0.7 M mannitol (Cistué et al. 1994). Microspore isolation, culture and plant regeneration were performed as described by Castillo et al. (2000). Treatment with 10^{-5} M sodium azide was applied for 1 h according to Castillo et al. (2001).

DNA extraction and AFLP analysis

Leaf-DNA was extracted using minor modifications of the CTAB procedure according to Saghai-Maroof et al. (1984). AFLP analysis was performed according to AFLP Analysis System I (Invitrogen) specifications. Eleven primer - enzyme combinations were used for

AFLP analysis (E33M61, E35M47, E36M50, E36M59, E40M49, E40M50, E41M49, E35M60, E37M61, E41M60, E35M48). Acrylamide gels were silver stained. AFLP-band was cloned using the pGEM-TEasy vector system I (Promega) and sequenced.

Results and discussion

Of the different methods used to detect DNA changes, AFLP analysis was chosen due to its high level of polymorphism. Other methods such as RFLP and RAPD have been used before in the same cultivar 'Igri' without success (Devaux et al. 1993).

In order to rule out any other possible variability source such as pre-existing variation in the starting material or additional stress caused by mutagenic treatment, a complete set of phenotypically normal DH lines was developed. From a single 'Igri' DH plant (I-DH), six daughter plants (I-DH1 to I-DH6) were grown. The plant I-DH2 was used as the donor plant for microspore isolation and self-pollinated seeds production (I-DH2a1 to I-DH2a5). DH plants were regenerated from microspores treated (I-DH2sat1 to I-DH2sat15) or untreated (I-DH2mc1 to I-DH2mc15) with sodium azide after isolation. 'Igri' plants developed from commercial seeds (Is) were also included in the analysis.

The study of eleven primer - enzyme combinations, that represented the analysis of 719 band polymorphisms, revealed only one change in the banding patterns, with the presence of an extra band of 320 pb in the E36M50 combination, indicating a high degree of genetic stability (Figure 1).

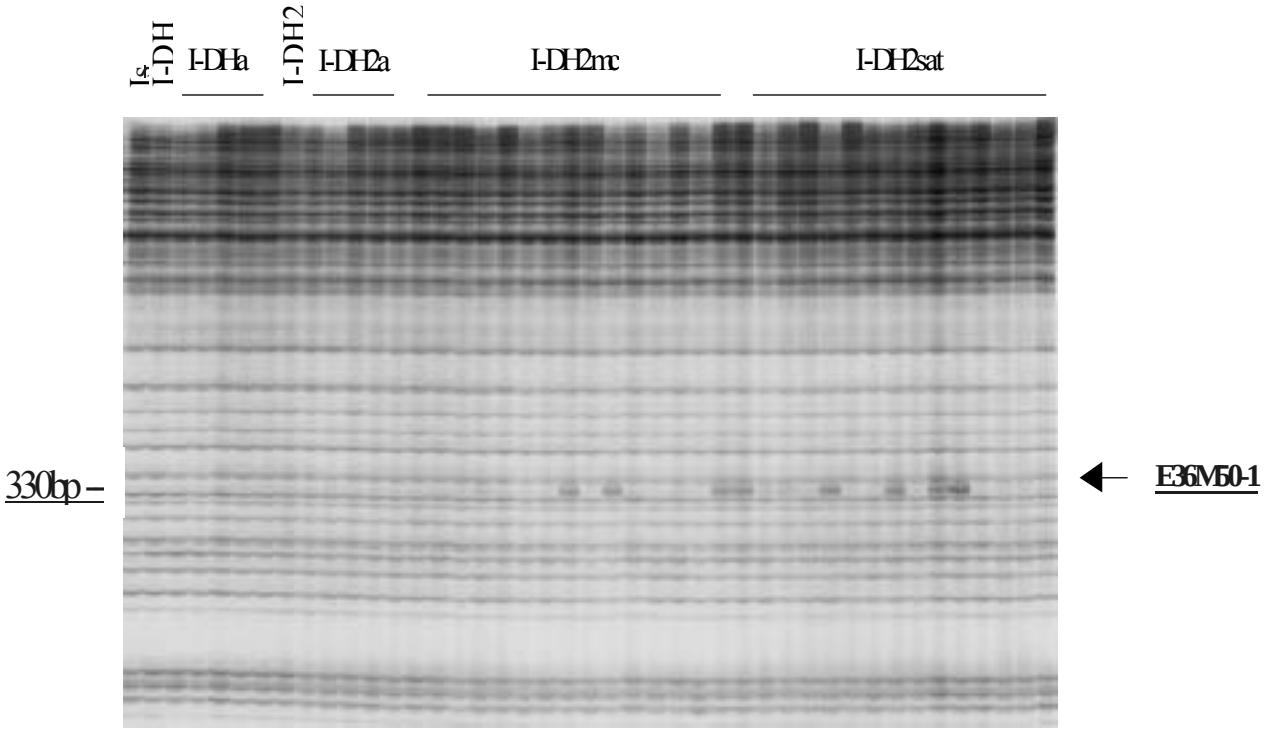

Figure 1. Identification of AFLP-band polymorphism in barley DH plants obtained by isolated microspore culture

The amplified band was clearly present in three I-DH2mc and five I-DH2sat lines out of fifteen. The presence of the band in independent DH lines indicates that this band represents a hypervariable 'hot spot' of DNA instability (Linacero et al. 2000). The higher number of lines with additional band in the sodium azide treated material could indicate that the mechanism of

this instability induction is related to stress pressure, increasing the rate of induction with severe stress conditions.

In order to know the sequence involved in the new AFLP polymorphism the band was excised, cloned and sequenced. The cloned band corresponded to a 294 bp fragment that showed homology to different barley retrotransposon 'sukkula' elements. The 'sukkula' family is unusual, having terminal sequences similar to LTR terminal regions of rice gypsy-like retrotransposons (RIRE), but lacking a protein-coding domain (Shirasu et al. 2000).

Substantial reports have shown that retroelements are activated under different stress conditions and specifically during cell and tissue culture (Grandbastien 1998). However, as far as we know, there is only one report on transposon activation in DH lines derived from anther culture, where rice plants were regenerated through a callus phase (Kikuchi et al. 2003).

These preliminary results suggest the activation of a retrotransposon by barley isolated microspore culture. Further analysis should be performed in order to clarify the mechanism generating this genetic variability.

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Attempts to establish an embryogenic callus culture in two Portuguese maize (*Zea mays*) landraces using leaves and mature embryos

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ABSTRACT: A total of five different experiments were conducted in order to obtain an embryogenic callus culture from two Portuguese maize landraces of northern Portugal. Leaves and mature zygotic embryos were used as explants and several hormonal conditions were tested for callus induction using several plant growth regulators such as N^6-benzyladenine (BA), 2,4-dichlorophenoxyacetic acid (2,4-D), zeatin or 1-naphtaleneacetic acid (NAA). No embryogenic callus culture was formed in any of the five experiments. The use of 2.25 μM 2,4-D and 4.5 μM BA in the induction medium led to the formation of several green shoots that did not develop after excision from the calli.

Key words: *In vitro* culture – regeneration – somatic embryogenesis

Introduction

Food security is one of the most important issues in the 21st century. In fact, in a context of water scarcity and soil degradation for agricultural purposes, agricultural production of several crops is expected to increase in order to answer the demands of a growing world population, especially in developing nations. Genetically modified crops are hence expected to play a major role in the answer to such problems (Herrera-Estrella 2000). Maize is the third most important crop in the world being an important staple food in several countries of Africa and South America and the key feature of poultry, pork and dairy sectors in North America and Europe.

In order to be able to genetically modify one crop, adequate regeneration systems must be developed. Generally, monocots and especially maize are very difficult plants to regenerate *in vitro* limiting regeneration and hence transformation systems to only a few varieties lacking of agronomical interest or adaptation to local conditions (soil, climate and marketing) (Komari et al. 1998). The establishment of regeneration systems of agronomically important varieties, landraces included, seems therefore to be of capital interest. Various *in vitro* culture conditions are suggested to obtain somatic embryos, callus and multiple shoot clumps, using several explants sources such as immature embryos (Ishida et al. 1996), embryos (Shen et al. 1999), leaf segments (Conger et al. 1987), immature tassels (Zhong et al. 1992a) and seedling shoot tips (Zhong et al. 1992b). In this work we report several attempts to establish reproducible protocols for induction of somatic embryogenesis using two types of explants: leaves and seedling shoot tips in two Portuguese maize landraces.

Materials and methods

Plant material

Two Portuguese maize landraces *PB64* and *PB369*, kindly supplied by the Centro de Germoplasma Vegetal, Braga, Portugal, were used.

Seed disinfections protocol
Overnight immersion in Benlate solution (1g l^{-1}), wash in running tap water for 20 min, 70 % ethanol for 5 min, commercial bleach for 20 min and final wash in double distilled water for 15 min. Disinfected seeds were germinated on MS medium (Murashige & Skoog 1962).

Experiments
Experiment 1: Five days old germinated shoot tips (1.5 to 3 cm long) were excised and placed in Petri dishes with MS medium supplemented with 500 mg l^{-1} casein hydrolysate and 20 g l^{-1} sucrose with several plant growth regulator combinations: 2.25, 4.5, 9.0 or 18 μM BA (N^6-benzyladenine); 2.25 μM 2,4-D (2,4-dichlorophenoxyacetic acid) plus 4.5 or 9.0 or 18 μM BA and finally 9 μM BA plus 4.5 or 9.0 or 18 μM 2,4-D. Shoot tips were cultured in the dark at 27ºC for 35 days. Experiment 2: After germination, seedlings were placed in culture tubes to allow plantlets to develop. At day 20, segments of the youngest leaf were excised and placed in Petri dishes with the medium and growth regulator combinations previously described in Exp. 1. Leaf segments were cultured in the dark at 27ºC for 35 days. Experiment 3: Five days old germinated shoot tips (1.5 to 3 cm long) were excised and placed in Petri dishes with MS medium supplemented with 500 mg l^{-1} casein hydrolysate, 1 mg l^{-1} DTT (dithiothreitol) and 20 g l^{-1} sucrose with the following growth regulator combinations: 2.25 μM 2,4-D plus 4.5 or 9.0 or 18 μM zeatin and 0.45 μM 2,4D plus 0.9 μM zeatin. Shoot tips were placed in such medium for 21 days (at 27ºC in dark conditions) and were later transferred to plant growth regulator free MS medium and placed in light conditions in a growth chamber (phytotron EDPA 700, ARALAB), 16 hours photoperiod and a day/night temperature of 22ºC to 24ºC. Experiment 4: Same conditions of Exp. 3, the only exception was that no casein hydrolysate was added to the medium. Experiment 5: After desinfection, seeds were hydrated in double distilled sterile water for two hours. Zygotic embryos were immediately excised and placed in N6 (Chu et al. 1978) initiation medium (N6 salts and vitamins, 20 g l^{-1} sucrose, 1 mg l^{-1} 2.4D, 25 mM L-Proline, 100 mg l^{-1} casein hydrolysate, 10 mg l^{-1} silver nitrate). After 21 days in the dark at 27ºC, calli were transferred to regeneration medium 1 (N6 salts and vitamins, 6 % sucrose, 1 mg l^{-1} 1-naphtaleneacetic acid - NAA) for another 21 days period and placed in the light conditions previously described. At day 42 forming calli were transferred to regeneration medium 2 (MS salts and vitamins, 20 g l^{-1} sucrose, 2 g l^{-1} myo-inositol) maintaining light conditions. In all experiments the pH of the media was adjusted to 5.8 before autoclaving (121ºC, 20 min) and solidified with 0.4 % gelrite (Duchefa, The Netherlands).

Results and discussion
Results obtained in Experiments 1 and 2 are depicted in Figure 1. Calli such as those shown in Figures 1c and 1d were the result of Experiment 2. None of the BA concentrations used led to the formation of an embryogenic calli but instead a phenolysed calli that subsequently died was formed. Regarding Experiment 2, we can therefore conclude that our media conditions and the use of leaves as explants are not adequate to the development of an embryogenic callus culture for the two landraces used in this study.

Experiment 1 produced similar results to those previously described, since most of the calli formed were similar to those shown in Figure 1a and 1b. Initially such calli had a white or light yellow color but as the experiment progressed, phenolisation was heavily increased and material gained a characteristic brown color and eventually died.

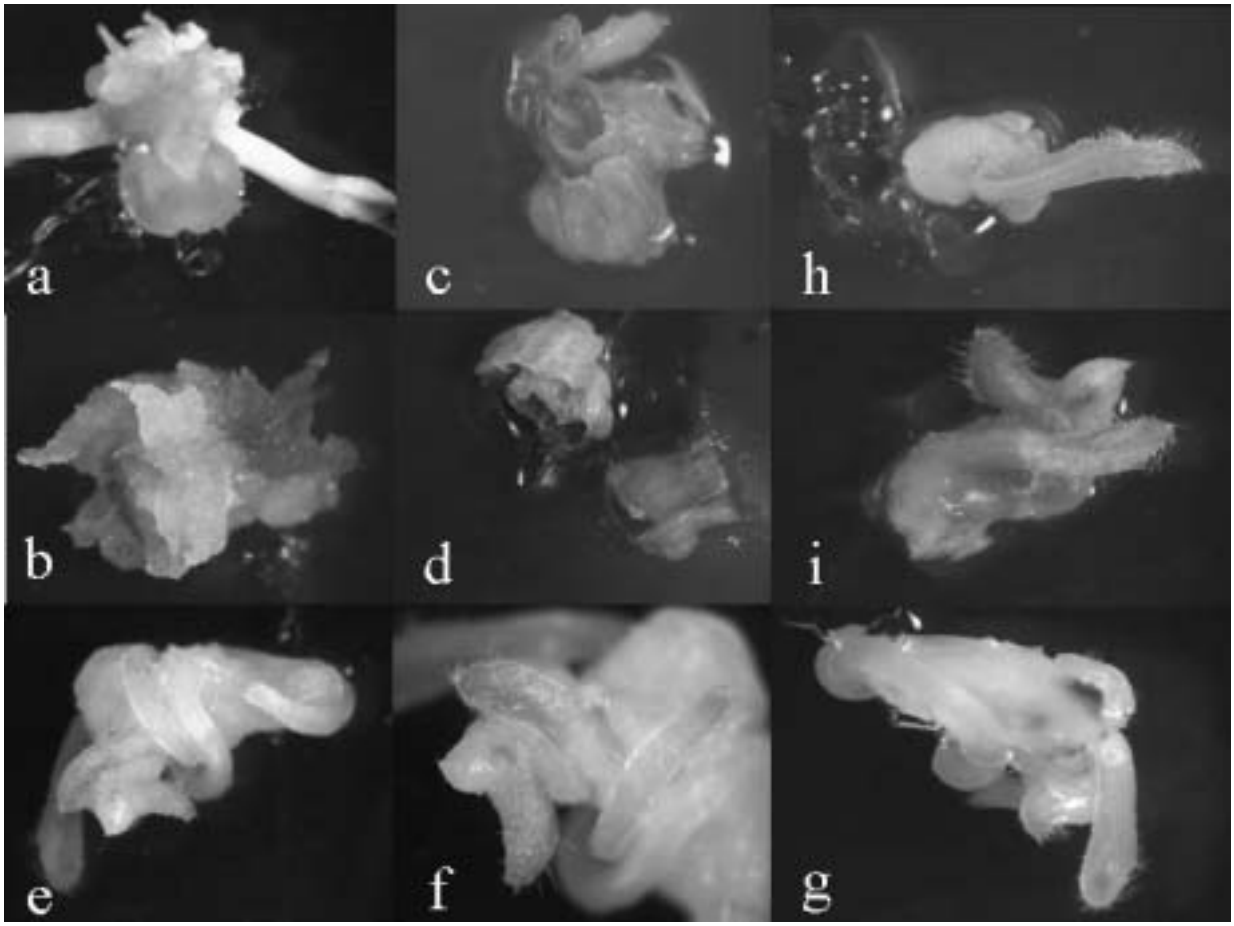

Figure 1. Calli obtained from experiments 1 and 2: (a) and (b): phenolysed calli obtained in experiment 1; (c) and (d): calli obtained in experiment 2; (e), (f) and (g): green morphogenic structures developing from the calli obtained in experiment 1; (h) and (i): shoots after isolation

Our observations indicate that calli derived from *PB369* landrace showed phenolisation at an earlier stage than *PB64* calli. Some of the calli derived from *PB64* landrace and cultivated in medium with 2.25 μM 2.4-D and 4.5 μM BA were in fact able to remain with the initial white or light yellow color throughout all the tissue culture period. Two to five days after being placed in the light, we were able to detect green morphogenic structures in the calli such as those described in Figure 1e and 1f. Such structures developed in size and at a later stage (15 days), we were able to excise and culture them separately from the calli. A total of six shoots such as those presented in Figure 1h and 1i were excised. After separation from the calli, shoots failed to root and they eventually died. Zhong et al. (1992a) tested twenty different genotypes under the media and hormonal combinations described for Experiment 1 and, although they were able to obtain multiple shoot clumps in all varieties tested, their results demonstrated to be strongly genotype dependent. Our results suggested that the two Portuguese varieties used in this study also showed different responses to the culture conditions tested and one of them was able to produce structures similar to shoots. Besides this fact, callus induction and plant regeneration from mature zygotic embryos is an inefficient approach (Wang 1987) and therefore it would be interesting to extend the number of embryos excised and possibly repeat Experiment 1 in some of the hormonal concentrations used.

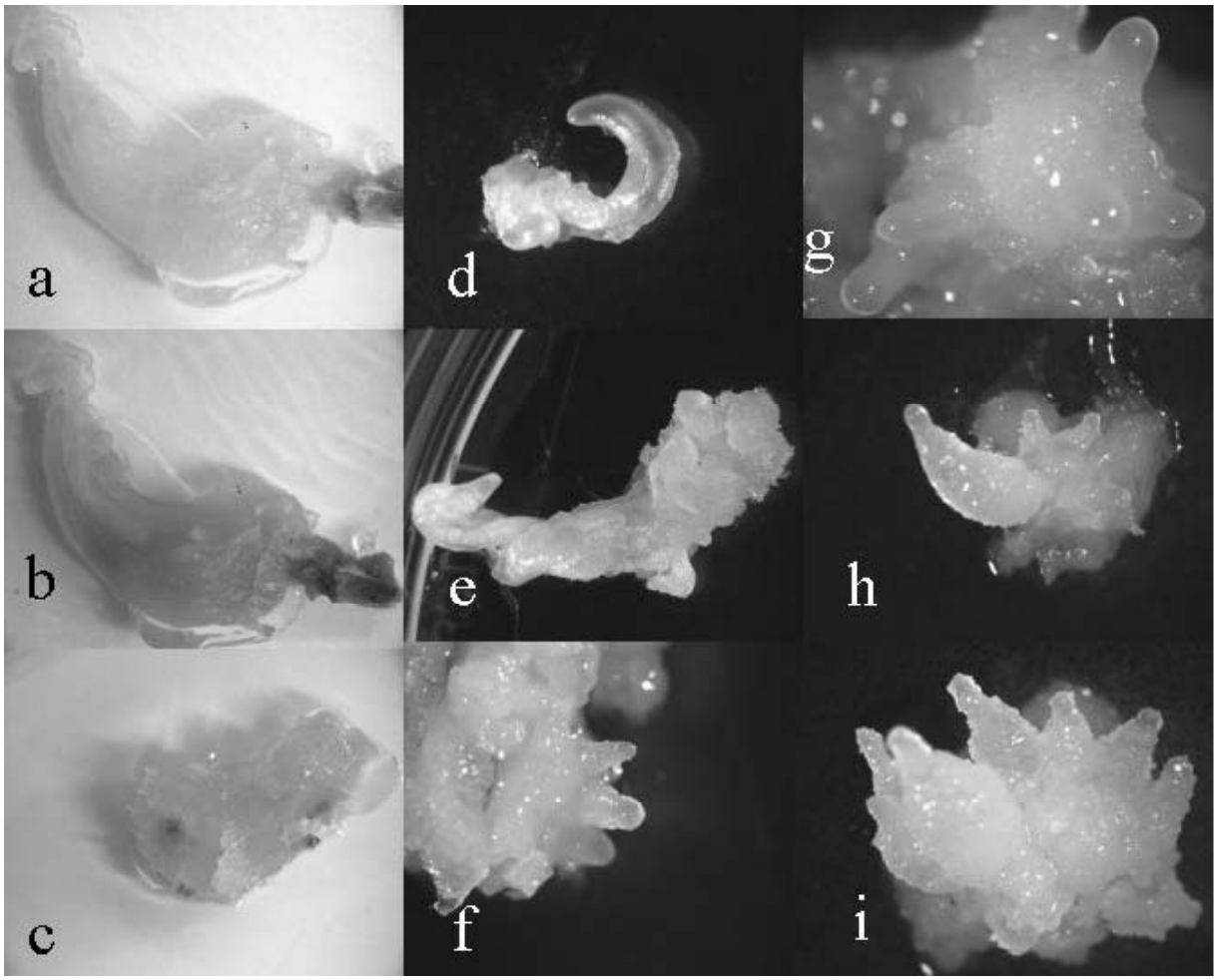

Figure 2. Calli obtained in experiments 3 to 5: (a), (b) and (c): examples of calli produced in experiments 3 and 4. Note growing phenolisation in the same callus seen in Figures 1a and 1b (respectively at transfer date and 30 days after transfer); (d) and (e): callus obtained from experiment 5 at day 5 (d) and 11 (e) after initiation phase; (f), (g), (h) and (i): different aspects of rhyzogenic calli obtained at day 7 of the regeneration phase of experiment 5

Examples of calli produced on Experiments 3 and 4 are depicted in Figures 2a, 2b and 2c. Results were very similar for both experiments since no embryogenic callus was obtained in any case. Similarly to the general aspect of Experiment 1, obtained calli displayed a characteristic whitish color at the end of the 21 days (see Figure 2a), but a week later the oxidation levels were severely increased and calli had changed to a distinct brown color (see Figure 2b). Equally, *PB369* genotype presented higher levels of oxidation than those showed by *PB64*. In the case of this experiment, replacing BA by zeatin did not lead to an improvement in the callus development and no somatic embryo was obtained. The inclusion of casein hydrolysate under the hormonal conditions of the experiment seems to have little effect on callus development. Under the conditions of Experiment 5, we were able to obtain calli earlier than any of the previously described experiments. Five days after the initial induction phase a callus had already been formed (see Figure 2d) and at day eleven, the calli had grown in size (see example in Figure 2e). In both varieties, calli showed the characteristic white color throughout the experiment, an effect possibly due to the inclusion of silver nitrate in the media. At day 21, calli were transferred to regeneration medium and placed in a culture chamber under light conditions. Fourteen days later, after the transfer (day 35) several structures had emerged from the callus (see Figures 2f, 2g) that retained the characteristic white color showed since the beginning of the experiment. Such structures were roots as

illustrated in Figure 2h and 2i and rhyzogenic calli had formed as a result of the Experiment 5. Our results seem to indicate that the cultivars under study are highly averse to somatic embryogenesis induction using mature zygotic embryos as explants since no somatic embryos and hence regenerated plants were obtained with any of the experiments. Nevertheless, some of the results from Experiment 1 are relatively encouraging since green structures or shoots were developed. Besides the already mentioned amplification of Experiment 1 in order to test a higher quantity of explants under the effects of 2.25 μM 2,4-D and 4.5 μM BA, another possible approach to obtain embryogenic calli for these two varieties could be the use of immature embryos as explants, since they are more efficient than any other tissue in somatic embryogenesis (Wang 1987).

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Broadening genetic variation in rapeseed (*Brassica napus*) aided by molecular methods

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ABSTRACT: Resynthesized (RS) rapeseed generated via interspecific hybridisation between suitable forms of *Brassica rapa* L. (syn. *campestris*; genome AA, 2n = 20) and *B. oleracea* L. (CC, 2n = 18) represents a potentially important resource to expand genetic diversity in the narrow gene pool of oilseed rape (*B. napus* L., AACC, 2n = 38). In this study 165 RS rapeseed lines originating from crosses between an Indian yellow sarson (*B. rapa* ssp. *trilocularis*) and five different cauliflower (*B. oleracea* convar. *botrytis*) cultivars were studied using amplified fragment length polymorphism (AFLP) markers, and their genetic diversity was compared in relationship to a collection of 40 diverse spring oilseed and fodder rape varieties. Using three AFLP primer combinations, a total of 467 polymorphic bands were scored. Cluster analysis allowed differentiation among the different RS lines, which, as expected, were genetically highly divergent from the cultivars. The genetic diversity of the material is discussed in relation to its morphological variability with a view to the implementation of RS lines in oilseed rape breeding.

Key words: AFLP – *Brassica oleracea* – *B. rapa* – genetic diversity – resynthetic rapeseed

Introduction

Oilseed rape (*Brassica napus* L.) is one of the most important oilseed crops worldwide. *B. napus* (2n = 38, genome AACC) is a relatively young species that was derived only a few hundred years ago from spontaneous hybridisation between turnip rape (*Brassica rapa* L., syn. *campestris*; 2n = 20, AA) and cabbage (*Brassica oleracea* L.; 2n = 18, CC). However, the limited geographic range of rapeseed and intensive breeding has led to a comparatively narrow genetic basis in current breeding material. As a consequence, genetic variability in this important oilseed crop is restricted with regard to valuable characters. 'Resynthetic' (RS) rapeseed genotypes developed via interspecific hybridisation between the diploid parents and assisted by embryo rescue techniques have the potential to significantly increase the available gene pool and provide important basic germplasm for further improvements of agronomical traits. Both progenitor species exhibit a broad genetic and phenotypic diversity that gives the potential for a huge variety of different resynthesised rapeseed forms (e.g., Chen & Heneen 1989, Song et al. 1993, Becker et al. 1995, Lühs et al. 2002, cit. in Seyis et al. 2003a).

Furthermore, RS material is potentially of interest for hybrid breeding, since heterotic effects are higher in crosses of genetically more distant materials. On the other hand, RS genotypes are generally unsuitable to use them directly for variety development, because they usually display inferior seed quality traits such as high erucic acid, high seed glucosinolates (GSL), low oil content, and other undesirable oil quality traits from the progenitor parents. As crop plant gene pools narrow through continued selection for yield traits, it becomes more and more important for breeding to have tools that allow to effectively distinguish among breeding materials. Over the last years an array of genetic marker systems based on DNA polymorphisms has been developed that potentially allows increased efficiency in breeding by enabling a more accurate definition and exploitation of genetic variation. Many studies have also demonstrated the use of molecular marker techniques for analysis of genetic variation in eventually all crop species. In rapeseed, Becker et al. (1995) compared cultivars and resynthesised lines using allozyme and restriction fragment length polymorphism (RFLP) markers and concluded that resynthesised forms are a suitable resource for broadening the

genetic base of rapeseed (for more details and references cf. Seyis et al. 2003a). In particular, amplified fragment length polymorphism (AFLP) markers provide a large amount of information in relatively short time at low cost and therefore represent an extremely useful tool for genetic diversity studies and related aims (cf. Snowdon & Friedt 2004).

In the present study 165 RS rapeseed lines originating from crosses between an Indian Yellow Sarson (*B. rapa* ssp. *trilocularis*) and 5 cauliflower (*B. oleracea L.* convar. *botrytis*) cultivars were studied using AFLP markers, and their genetic diversity was compared to a collection of diverse spring oilseed and forage rapes from Germany, Canada, France, Sweden, Denmark, Australia and New Zealand by unweighted pair-group method algorithm (UPGMA) clustering and principal coordinate (PCO) analysis. Because the same *B. rapa* parent was used for all crosses, the RS material was closely related. However, phenologic data from a field evaluation of morphologic and agronomic traits including plant height, leaf morphology, flowering time and period, time of maturity, vegetation period and seed yield components showed a clear differentiation between the RS families, which in turn could be clearly differentiated from natural spring rapeseed cultivars, fodder types and Canadian oil types, respectively. The main intention of the present study was to characterise and quantify the molecular genetic variability present in the RS lines to describe their variation in relation to existing spring rapeseed breeding material.

Materials and methods

Plant material

RS rapeseed lines were generated from crosses between different cauliflower cvs (*B. oleracea L.* convar. *botrytis*) and an Indian yellow sarson (*B. rapa* ssp. *trilocularis*) accession. 165 RS lines were tested in field trials at Rauischholzhausen (Germany) in 1999 together with 40 spring oilseed and fodder rape cvs from European gene banks. All RS lines exhibit high erucic acid and GSL contents, self-fertility, a lack of or moderate vernalisation requirement and deficiency of winter hardiness.

AFLP based analysis of genetic diversity

DNA was isolated from young leaves from plants in the field using a standard CTAB extraction protocol. AFLP amplification products were generated, separated and analyzed as described earlier (Seyis et al. 2003a).Genetic similarity among all samples based on AFLP markers was calculated using the similarity index of Dice. A dendrogram was generated with the unweighted pair-group method algorithm (UPGMA). Furthermore, a principal coordinate (PCO) analysis was performed (for more details see Seyis et al. 2003a).

Results and discussion

With three selected primer combinations a total of 467 polymorphic AFLP bands were scored in the complete set of RS lines and varieties. This extremely high number of polymorphic bands underlines the high capacity for the AFLP technique to detect polymorphism and reflects the distinct genetic origin of the RS lines from novel combinations of *B. oleracea* and *B. rapa* genotypes. As expected this high degree of polymorphism was reflected in a clear differentiation between the RS material - closely related due to its common *B. rapa* parent - and the spring rapeseed cultivars, both in an UPGMA dendrogram and in a PCO analysis. Many of the polymorphic loci identified in the present study can be attributed to the high genetic distance of the RS lines from the variety material, however the number of loci polymorphic within the varieties alone was far greater than the number of polymorphic loci in previous studies. The 40 rapeseed cultivars in our study formed a distinct cluster in both the UPGMA and PCO analyses, whereby the dendrogram contained smaller sub-clusters that also segregated with relatively high genetic distances. Most genetically distant from the bulk of the

material were the old German landrace 'Janetzki', the Canola varieties 'Regent', 'Tristar' and 'Excel', and the two German 00 cvs 'Callypso' and 'Evita'. Also genetically distinct were the Canadian oilseed variety 'Nugget' and the fodder type 'Moana' from New Zealand along with the Australian cvs 'Wesbrook' and 'Marnoo' (both 00). These 9 varieties also segregated distinctly in the PCO analysis, whereas all other cultivars were relatively closely grouped. In the dendrogram, however, the French 00 cv 'Drakkar' was also genetically distinct from the other cultivars, as were the Danish and Polish oilseed types 'Industry' and 'Bronowski'. The German hybrid variety 'Profitol' clustered separately together with the Danish variety 'Star', whereas the Canola cv 'Tower' was also genetically distinct. The remainder of the varieties, comprising oilseed forms from Sweden, Germany and Canada along with the German fodder rapes 'Petranova', 'Jumbo' and 'Tiger', formed a large cluster in the dendrogram with no obvious differentiation related to origin or type. It should be remembered, however, that most fodder rapes originate from the same narrow genetic pool that also gave rise to low GSL oilseed rape forms, and their morphology and phenology is not necessarily different from that of oilseed forms (cf. Seyis et al. 2003a).

The inability of our AFLP data to clearly distinguish genotypes from different geographical origin is not surprising, bearing in mind that plant breeders often implement material from international sources in order to maximise genetic diversity. In some cases genetic distances separating cultivars deriving from the same origin was as high or even higher than the genetic distance among cultivars of different origin. Nonetheless, material from more exotic sources tended to be more genetically distinct in comparison to the majority of cultivars from Canada, Germany and Sweden. Without detailed pedigree information it is difficult to interpret all sub-clusters among the cultivars, however it appears, as expected, that relatedness of pedigrees plays a more important role than the country of origin of a given variety.

The observation of RS lines in the present study with quite distinct AFLP genotypes to sibling lines from the same cross, or to clones of the same line, may be a consequence of relatively frequent intergenomic recombination between A and C genome chromosomes in early generations of RS rape (cf. Song et al. 1995, Lydiate et al. 1995, cit. in Seyis et al. 2003a). In some cases the genetic differences were reflected in a distinct or unusual morphology of the line in question, demonstrating the potential of such genetic variation to manifest itself in novel phenotypic variation. The large genetic distance between the RS lines and existing rapeseed cultivars is of particular interest in terms of increasing heterotic potential for hybrid development. In order to investigate heterosis for yield components, selected RS lines from the material described were used to develop experimental hybrids with male-sterile breeding lines (Seyis et al. 2003b). In field trials at three locations the experimental hybrids based on the RS lines demonstrated the potential of resynthesised rapeseed material in hybrid breeding. The average seed yield of the 25 genotypes over all locations was 33.8 dt ha^{-1} (check cv. 'Senator' = 32.6 dt ha^{-1}). The experimental hybrids TH1 (37.2 dt ha^{-1}) and TH2 (36.8 dt ha^{-1}) developed from the RS line 578d (BK2256 x Y.S) with the best GCA as pollinator showed the highest average yields. Contrary, the old spring rapeseed cultivars, 'Janetzkis Sommerraps', 'Bronowski' and 'Svaloefs Gulle', showed negative GCA effects (Figure 1).

The enormous potential of resynthesis in rapeseed breeding will make efforts dealing with germplasm conservation and well-directed use of the diploid parents more important in future. As the yield potential of RS rapeseed is low the use of such forms and the new genetic variability thus created must be directed, particularly with regard to quality and yield, to facilitate its integration into high-yielding breeding material. Specifically this can be achieved by producing semi-synthetic rapeseed forms or by developing hybrids. Obviously, the establishment of a new gene pool based on artificial *B. napus* is limited by its inferior agro-

nomic performance and seed quality (erucic and GSL content), hence this approach must be considered under more long-term perspectives.

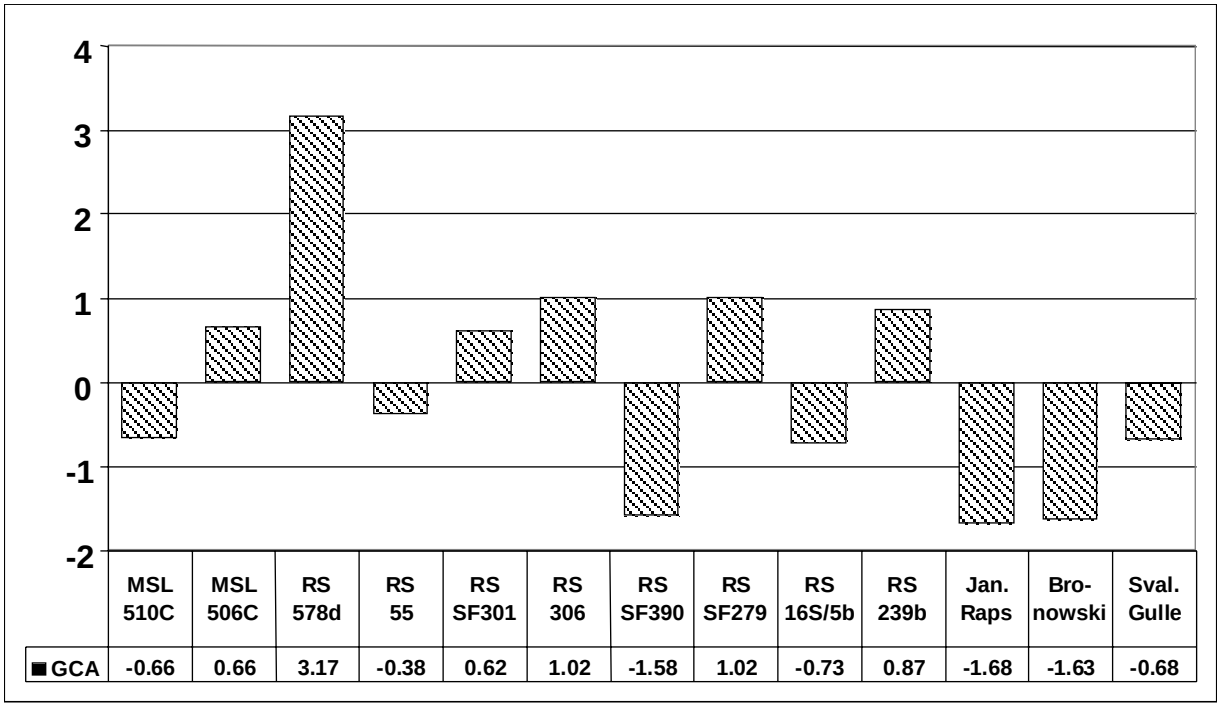

Figure 1. General combining ability (GCA) regarding seed yield of nine selected RS lines, three old spring cultivars, i.e. 'Janetzkis Sommerraps' (Ja. Raps), 'Bronowski', 'Svaloefs Gulle', and two male sterile MSL-lines ('MSL-506C' and 'MSL-510C'). Data from field trials in 2001 at three locations (Seyis et al. 2003b).

The discovery of low-erucic acid mutants among *B. oleracea* accessions and the development of synthetic rapeseed forms via interspecific crosses with interesting 0 or 00 quality *B. rapa* genotypes will open the possibility to use RS rapeseed material as a genetic resource for quality and yield improvement in oilseed rape (Lühs et al. 2002). As demonstrated in the present paper, the use of AFLP markers will assist in the evaluation of RS lines in terms of describing their genetic distance from existing breeding material in order to enrich the available gene pool for breeding of this important oil crop species.

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Full modification of the coding sequence for enhancing potato expression of insect control protein *cry3a* gene

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ABSTRACT: *Bacillus thuringiensis* genes (*cry* genes) contain some sequences that reduce their expression in plants. These are splicing and polyadenylation sites, the ATTTA sequences, mRNA degradation signals and transcription termination sites as well as the codons that are rare in plants. The truncated *cry3a* gene was obtained from the *Bt* genome (subspecies *tenebrionis*) by means of PCR. Our preliminary results on expression of the truncated gene in eukaryotic cells (yeasts) showed that the expression level was low. To enhance the expression in potato cells for control of CPB, we modified the *cry3a* gene. A new strategy was used for the chemical synthesis of the modified gene. The native (truncated) and synthetic genes with the reporter gene *licB* were cloned into bacterial and yeast expression vectors. A comparative analysis of expression in *E. coli* cells has shown that the synthetic gene was 1.5 times less active than the native gene, which may reflect the fact that the codon composition of the synthetic gene was optimal for eukaryotes. Quantitative and qualitative assays of lichenase in the hybrid proteins and of gene expression has shown that the synthetic gene was expressed in yeast cells approximately 10-fold stronger than the truncated gene.

Key words: *Bacillus thuringiensis* (*Bt*) – *Leptinotarsa decemlineata* (*CPB*) –*Saccharomyces cerevisiae*

Introduction

Current efforts to develop crop genetic pools through biotechnology are based primarily on intra- and interspecific hybridizations, or transforming plants with a single gene encoding a new protein (insecticidal, herbicidal, fungicidal enzyme or toxin). The most widely used in this approach are the δ-endotoxins gene of *Bacillus thuringiensis* (*Bt*), a spore-forming, and gram-positive bacterium. The insecticidal activity of *Bt* is due to its ability to produce large amounts of one or more insecticidal crystal proteins (ICPs) during sporulation. After ingestion by a susceptible insect, crystalline δ-endotoxin is solubilized and proteolytically cleaved from an inactive protoxin, to an active toxin form within the insect midgut. The activated toxin binds to receptors in the midgut and forms ion channels (Crickmore et al. 1998). The δ-endotoxins from *Bt* comprises a group of over 100 related proteins, which were previously categorized by insecticidal activity but currently by amino acid similarity.

The insecticidal protein from the *Bt* var. *tenebrionis* (Cry3a) is a specific toxin for coleopteran insects (*Tenebrio molitor*, *Leptinotarsa decemlineata* and *Chrysomela scripta*) yet exhibits no toxicity toward humans, other vertebrates or beneficial insects (Secar et al. 1987). The three-dimensional structure of the Cry3a toxin consists of three functional domains: (I) a cluster of seven α-helices predicted to be involved in membrane interaction; (II) three antiparallel β-sheets involved in receptor binding; and (III) a β-sandwich implicated in receptor binding and ion channel activity (Perlak & Stone 1993). To enhance the expression in potato for control of the Colorado potato beetle (CPB), we modified the *cry3a* gene. The native and synthetic genes were then fused with the reporter gene *licB*, which codes for a *Clostridium thermocellum* thermostable lichenase (Piruzian et al. 1998, 2000, 2002), and cloned into bacterial and yeast expression vectors. Using quantitative and qualitative assays of lichen in the hybrid proteins, expression of *cry3a* genes was compared in eukaryotic cells.

Material and methods

Strains and plasmids

The *Bt* var. *tenebrionis*, the *E. coli* strain XL1-Blue ('Stratagene') and BL21(DE3) ('Novagene', USA), the *Saccharomyces cerevisiae* strain YPH 857 were used as the host organisms, and plasmids pTZ57 R/T, pUC18, pET32b(+), pGEM-easy, pGAL were used as the cloning vehicles. Bacterial strains used, cloning vectors and growth conditions for them were the same as previously described (Sambrook et al. 1989).

Chemical synthesis and cloning of the full-modified cry3a gene

A new strategy was used for the chemical synthesis of the modified gene. First the gene sequence was divided into four fragments about 500 bp long. Then, the coding (first) strand of each of the fragments was subdivided into 50-55 bp fragments, and the corresponding oligonucleotides were synthesized by means of phosphoramide chemistry. This was followed by synthesizing a set of supporting complementary oligonucleotides 30-35 bases long which were complementary to the junctions between first chain oligonucleotides, so that 15-17 5'-end bases of the supporting oligonucleotides were complementary to the corresponding 3'-end of one oligonucleotide of the first strand and the remaining 15-17 3'-end bases of the same supporting oligonucleotide were complementary to the corresponding bases of the next first strand oligonucleotide. The combined mixture was exposed to DNA polymerase I and the dNTPs to complete synthesis of the second strand, and then T4 DNA ligase was added to join the adjacent oligonucleotides. The assembled fragments were then subjected to PCR, and cloned to T-vectors. To obtain the complete gene sequence, all the four fragments in the correct reading frame were cloned into a pGEM vector.

Molecular cloning

The 1.8 kb *cry3a* gene was isolated from the genomic DNA of *Bt* var. *tenebrionis* by PCR using primers: 5'-ggatccatgactgcagataataatacgg-3' and 5'-gagctcattcactggaataaattcaattt-3'. The amplified fragment then was isolated and cloned to pTZ57 R/T (T-vector), and then into pET-32a(+) and yeast expression vector pGAL. The pET-*synthetic* plasmid was constructed by cloning a *BamHI-XhoI* fragment of pGEM-*synthetic* into the *BamHI-XhoI* sites of pET-32a. The native and synthetic genes were then fused with the reporter gene *licB* which codes for a *Clostridium thermocellum* thermostable lichenase (licB), and cloned into bacterial and yeast expression vectors (fig 1). pGAL-*cry3a-licB*, pGAL-*synthetic-licB* were introduced into YPH 857 yeast cells using the lithium-acetate protocol (Soni et al. *1993*).

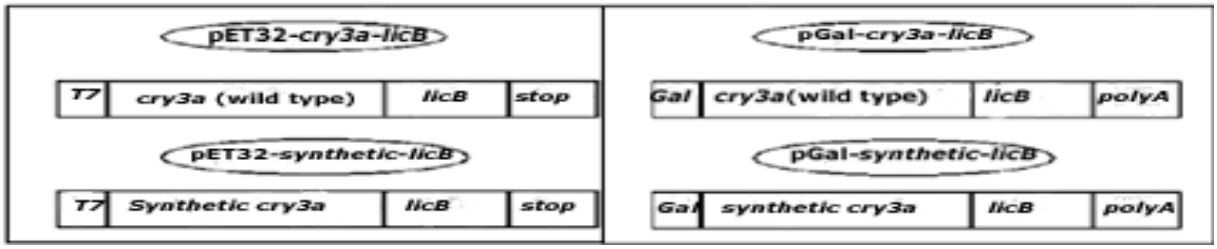

Figure 1. Bacterial vectors: pET32-*cry3a-licB* and pET32-*synthetic-licB* (left), yeast vectors pGAL-*cry3a* - *licB* and pGAL - *synthetic-licB* (right). *T7*- promoter, *Gal* – inducible galactose promoter, *polyA* – polyadenylation signal, *licB* – gene encoding lichenase enzyme

Protein production, determination and enzyme assay

The bacterial and yeast protein extracts were extracted according to Piruzian (1998, 2002). Lichenase activity was measured by a plate test according to Teather and Wood (1982). Protein was measured according to Bradford (1976) using BSA as standard. Lichenase

activity in the lysates was determined at 65°C using lichenan as substrate. Reducing sugars released from the substrate were determined with the dinitrosalicyl reagent according to Wood and Bhat (1988). The reaction mixture contained 200 µl of 0.5 % lichenan and 100 µl of the protein sample. It was incubated for 10 min, then 1.2 ml of the dinitrosalicyl reagent was added, and heated at 100°C for 15 min. The concentration of the colored product was determined with an spectrometer.

Results and discussion

The *cry3a* gene has an open reading frame equivalent to a 73 kDa protein (Secar et al. 1987). The protein is accumulated during *Bt* sporulation, then processed to a 67 kDa polypeptide at high level during late sporulation, and the crystal within the spore is formed. Since it was determined that the 67 KDa protein starting at met-48 was toxic (McPherson 1988), we cloned the gene (1794 bp) at this position. Our sequencing results showed that the obtained fragment is *cry3a* gene and showed 100 % homology with gene cloned by Secar and others. We transformed *E. coli* and yeasts for determination of expressing level of *cry3a* gene. Our preliminary results on expression of the gene in eukaryotic cells (yeasts) showed that the expression level was low. In order to overcome this trouble, we redesigned and modified the *cry3a* gene. The purpose of our work was to create a synthetic gene that would be highly expressed in plant cells and thus confer resistance to CPB.

The modified *cry3a* coding region has a codon usage pattern altered to resemble that of the average dicot gene. The dinucleotide frequencies used at the second and third positions in codons of dicot and monocot genes were considered in the *cry3a* gene design. The CG and TA dinucleotides are strongly avoided in plant genes possibly due to regulation involving methylation. In parallel with codon usage analysis of the native *cry3a* gene revealed that the coding region is 64 % A+T. This level of A+T is 10 % higher than that found in a typical plant gene coding region. The A+T content of synthetic *cry3a* gene was decreased from to 51 %. All known DNA sequences that might contribute to RNA instability in plants were eliminated from the synthetic gene. Notable among such sequences are the plant polyadenylation signals, such as AATAAA and its variants. The eukaryotic mRNA degradation signals, poly-ATTTA, were also eliminated. Finally a single modification, to introduce guanine in lieu of adenine at the forth nucleotide position in the *cry3a* coding sequence was made to form a consensus plant initiation sequence. The redesigned gene differs from the native gene in 21 % of its nucleotides (Table 1).

Table 1. Comparison of wild and synthetic *cry3a* genes

	Wild type gene	Synthetic gene
Bases different from wild type		350 / 1812 (20%)
Codons different from wild type		320 / 597 (54%)
G+C content (%)	37	49
Potential polyadenylation sites	24	0
ATTTA sequences	12	2
A+T rich regions (>6 consecutive A and/or T)	37	0

To analyse wild type and synthetic *cry3a* gene expression, protein production was induced in *E. coli* and yeast cells. For this purpose, were used many quantitative (measuring of lichenase activity and protein concentration), and qualitative (zymogram, electropherogram, Western Bloting, Petri dish test, Congo Red test and DNSO test) assays (Figures 2 and 3). The qualitative detection of lichenase activity by the zymogram and electropherogram methods

allowed one to determine directly the molecular masses of lichenase and fused Cry3a proteins. The molecular masses of lichenase and fusion Cry3a proteins measured in this work is in a good agreement with theoretically predicted masses (100 kD) (Figures 2 and 3). A comparative analysis of expression in *E. coli* cells has shown that the synthetic gene was l.5 times less active than the native gene, which may reflect the fact that the codon composition of the synthetic gene was optimal for eukaryotes. Quantitative and qualitative assays of lichenase in the hybrid proteins and of gene expression in yeast cells has shown that the synthetic gene was expressed approximately 10-fold stronger than the native gene. These results suggest that a high level of expression of the synthetic *cry3a* gene can be expected in plant cells.

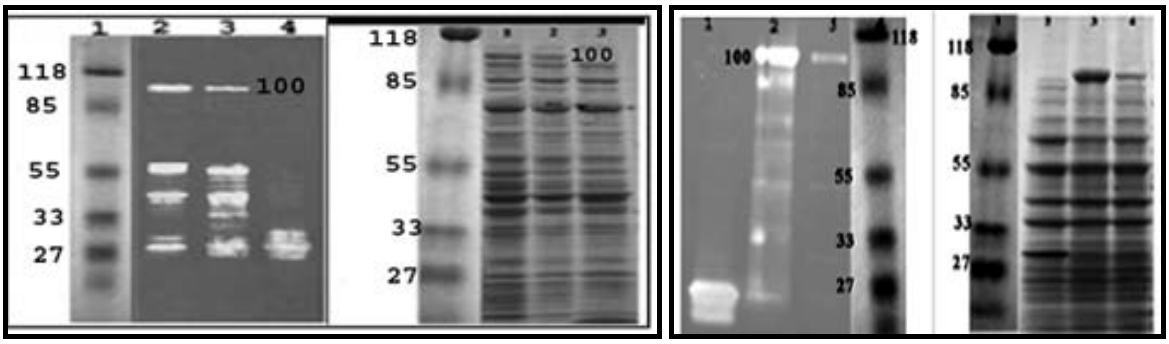

Figure 2. (A) Zymogram of bacterial protein extracts at the presence of 0.1 % lichenin as a substrate. 1- markers, 2- *cry-licB*, 3- *synthetic-licB*, 4- *licB*; (B) Electrophoresis of bacterial protein extracts, 1- *cry-licB*, 2- *synthetic-licB*, 3- BL21 extracts; (C) Zymogram of yeast protein extracts at the presence of 0.1 % lichenan as a substrate, 1- pGAL-*licB*, 2- pGAL-*synthetic-licB*, 3- pGAL-*cry-licB*, 4- marker; (D) Electrophoresis of yeast protein extracts: 1- marker, 2- pGAL-*licB*, 3- pGAL-*synthetic-licB*, 4- pGAL-*cry-licB*

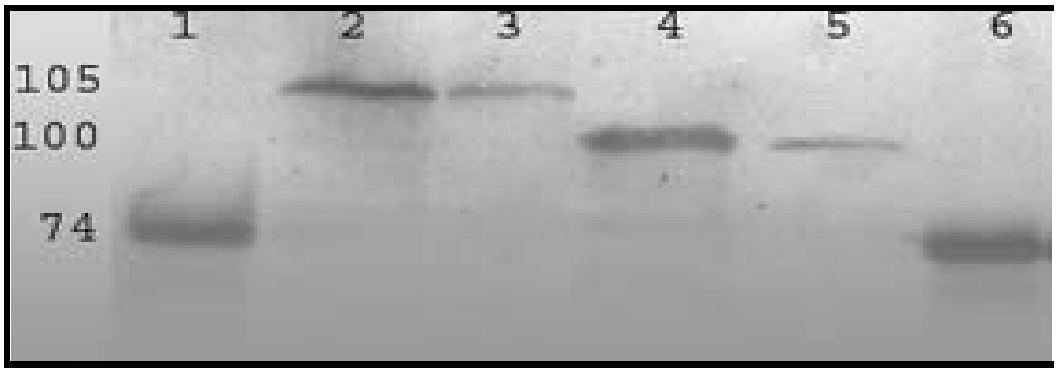

Figure 3. Protein gel-blot analysis of relative expression levels of the synthetic and wild-type *Bt* toxin genes in *E. coli* and yeast cells. 1- *cry3a* (in *E. coli*), 2- *leader-cry3a-licB* (*E. coli*), 3- *leader-synthetic-licB* (*E. coli*), 4- *synthetic-licB* (yeast), 5- *cry3a-licB* (yeast), 6- crystalline protein, extracted and purified from *B.t.* var. *tenebrionis*

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Identification of RAPD molecular polymorphism and cloning of polymorphic bands in potato late blight (*Phytophthora infestans*)

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ABSTRACT: Seven accessions of the potato late blight (PLB) fungus *Phytophthora infestans*, derived from different potato varieties and different Romanian locations, were isolated and multiplied *in vitro* on selective *Zea* medium with Vancocine (200 ppm) and Nystatine (100 ppm) antibiotics. The extracted DNA was amplified with several decamer primers. The amplification products were separated by electrophoresis in 1.4 % agarose gel and revealed in UV. Relying on molecular polymorphism characterization, genetic distances and phenetic relationship among the seven potato late blight accessions were established. It seems that the seven accessions belong to three major genotypes, with higher or lower levels of differentiation within them, according to location and potato variety. Thus, there are the A genotype, with two identical accessions isolated from one variety of potato and two different locations; the B and C genotypes, with three and two relatively different accessions isolated from different potato varieties but from relatively close locations. This differentiation could be a sign of an evolution process. DNA from polymorphic bands was purified using Gene Elute Agarose Spin Columns (Sigma 5-6500) and cloned by pGEM T Easy Vector Sistem II (Promega A 1380). The genetic transformation of host bacteria (JM 109 *E. coli*) with polymorphic bands integrated in pGEM T vector was confirmed by blue/white reaction. Thus, there were nine cloned polymorphic bands obtained from six PLB accessions with one primer; two polymorphic bands were obtained from two PLB accessions with other two primers and one polymorphic band from one PLB accession with another primer. Some of the cloned polymorphic bands were specific for a certain genotype and are to be sequenced in order to convert the RAPD markers into more stable SCAR markers.

Key words: Cloning – molecular marker – potato – RAPD – *Solanum tuberosum*

Introduction

One of the most important potato diseases in Romania is the late blight caused by *Phytophthora infestans*. At the moment it is difficult to perform an accurate identification of potato late blight pathotypes based on morphological characteristics. This is limited to phenotypic markers like virulence, fungicide resistance, mating type etc. (Maufrand et al. 1995). RAPD molecular markers could be beneficial for revealing the genetic variability of different pathotypes (Botez et al. 2003). In spite of its simplicity, the repeatability of this technique could be a problem. This type of markers could be converted into more stable SCAR markers. The first step in this procedure should be the cloning of DNA sequences specific for different potato late blight pathotypes. Actually, this is exactly what we have done and the results are presented in this paper.

Materials and methods

We have isolated in our experiment seven accession of potato late blight (*Phytophthora infestans*) derived from different potato varieties and different Romanian locations (Table 1). The isolation was done from infected leaves on selection *Zea* medium with Nystatin (100 ppm) and Vancocin (200 ppm). On this medium there could be seen (microscopically) a lot of pure *Phytophthora* spores (Figure 1).

DNA extraction was made by CTAB protocol (Rogers & Benedich 1994) modified by using a DNA precipitation with isopropanol. DNA amplification was performed with five decamer primers using 100 picomoles for each decamer primer (Table 2). The amplification

program consisted of denaturation at 94°C for 30 sec. followed by 3 long cycles with a denaturation step at 94°C for 1 min., an annealing step at 35°C for 1 min. and an extension step at 72°C for 2 min. and 32 shorter cycles with a denaturation step at 94°C for 15 sec., an annealing step at 35°C for 30 sec. and an extension step at 72°C for 1 min. The cycling program was terminated by a final extension step at 72°C for 4 min. (Maufrand et al. 1995).

Table 1. Different accessions of potato late blight (*Phytophthora infestans*)

Accession	Source (potato cultivar)	Site of collection
K	Kondor	Harghita
10	Desiree	Cluj (Gilau)
24	Desiree	Miercurea Ciuc
25	Ostara	Miercurea Ciuc
26	Rozana	Miercurea Ciuc
27	Superior	Miercurea Ciuc
28	Sante	Miercurea Ciuc

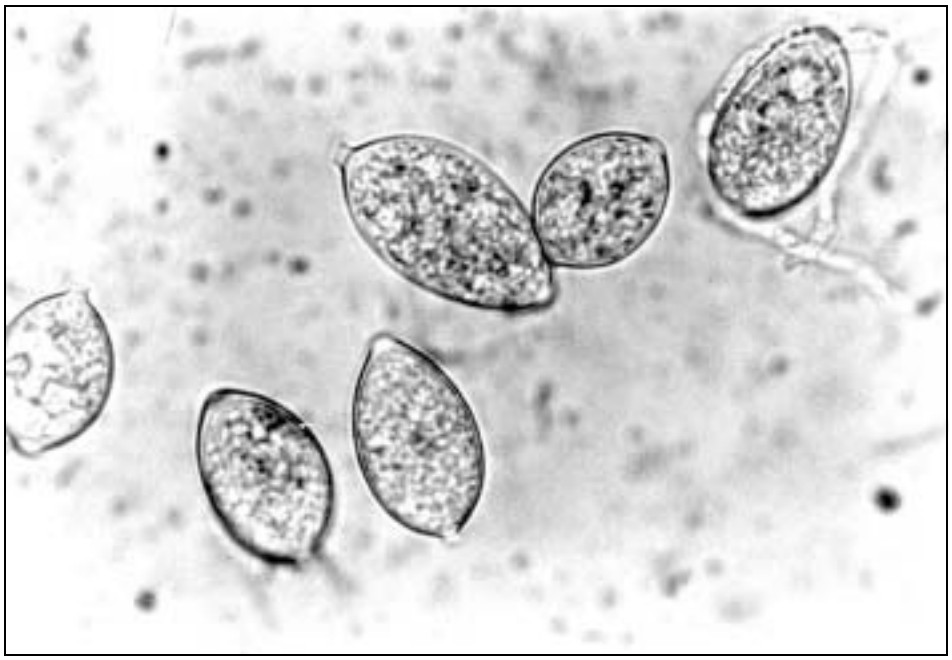

Figure 1. Isolated spores of *Phytophthora infestans* on *Zea* selection medium

Table 2. Decamer primers used for RAPD amplification

Primer	Primer specification
P_{07}	5`-TGTCTGGGTG-3`
P_{09}	5`-TGTCATCCCC-3`
P_{12}	5`-CACACTCCAG-3`
P_{13}	5`-TTCCCCCCAG-3`
P_{14}	5`-TGAGTGGGTG-3`

The amplification products were separated by electrophoresis in 1.4 % agarose gel and revealed in UV light. The polymorphic bands were purified using Gene Elute Agarose Spin Columns (Sigma 5-6500) and cloned using pGEM T Easy Vector Sistem II (Promega A 1380). Based on the obtained molecular polymorphism, the similarity coefficient of different accessions was computed and the dendrogram was built using RAPDistance v. 1.04 Program.

Results and discussion

The amplification product separated in agarose gel shows a significant variability concerning the degree of polymorphism in relation with the primer used and the compared accessions. Thus, with primer P_{12} the seven accessions were almost monomorphic, while for primer P_{13} we can see that a significant molecular polymorphism was obtained (Figure 2).

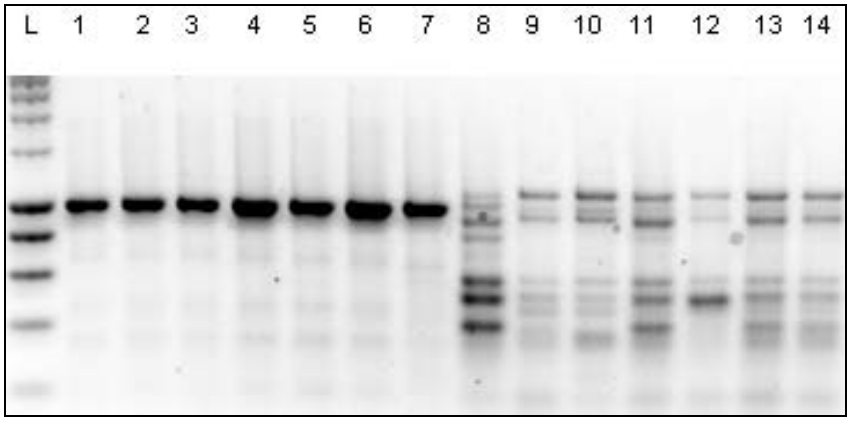

Figure 2. Amplification products obtained from seven accessions of potato late blight with primer P_{12} (lines 1 to 7) and primer P_{13} (lines 8 to 14); L: Smart ladder

Relying on molecular polymorphism, genetic distances and phenetic relationship among the seven potato late blight accessions were established (Figure 3). It seems, from the dendrogram, that the seven accessions belong to three major genotypes with higher or lower levels of differentiation within them according to location and potato variety. Thus there are the A genotype with two identical accessions (10 and 24) isolated from one variety of potato ('Desiree') and two different locations (Cluj and Miercurea Ciuc); the B and C genotypes, with three (27, 28 and 26) and two (K and 25) relatively different accessions isolated from different potato varieties, but from relatively close locations. This differentiation could be a sign of an evolution process. It is inferred that the three genotypes could be in fact pathotypes. We noticed in two relatively different accessions (26 and 27) the same biological process, meaning spore conjugation (Figure 4).

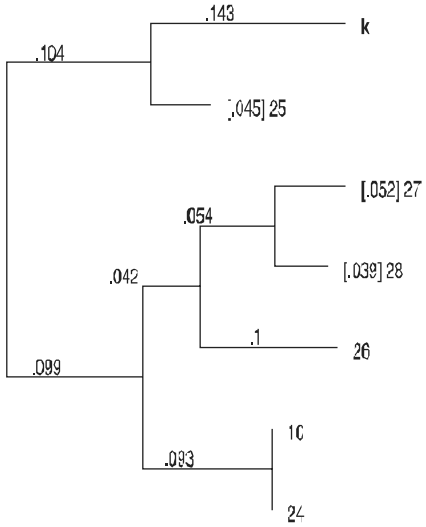

Figure 3. Dendrogram for the seven accessions of potato late blight resulting from the five primers

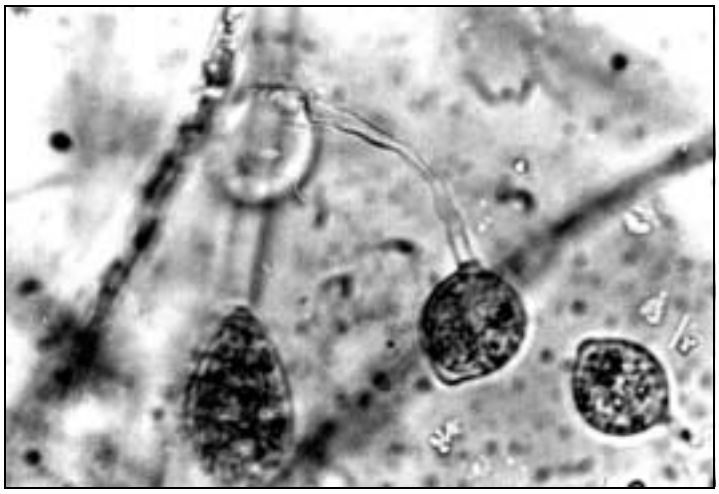

Figure 4. Potato late blight spores conjugation

The polymorphic bands were cloned (Table 3) and some of the more specific bands will be sequenced in order to try conversion of RAPD markers into more stable SCAR markers.

Table 3. Polymorphic bands cloned from amplified DNA with different primers in the seven analysed accessions of potato blight

Primer	Accession	Polymorphic bands cloned		
		All bands	Molecular weight (bp)	Specific bands
P_{13}	K	13.K.B	1077	-
		13.K.D.	800	13.K.D.
		13.K.G.	400	-
	10	13.10.K.	458	13.10.K.
	24	13.24.L.	449	-
		13.24.M.	360	-
	25	13.25.G.	449	-
	27	13.27.M.	337	-
	28	13.28.M.	360	-
P_{07}	27	7.27.F.	275	7.27.F
P_{09}	28	9.28.C.	311	9.28.C.
P_{14}	25	14.25.E.	456	14.25.E.

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Mapping genes of *Solanum caripense* involved in resistance to *Phytophthora infestans*, the causal agent of potato late blight

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ABSTRACT: Reciprocal, bi-parental cross progenies of *Solanum caripense*, a diploid (2n = 2x = 24), self-incompatible, non-tuberizing, wild herb occurring throughout the Andes from Bolivia to Costa Rica, were used for the construction of parental framework linkage maps. Unexpectedly, low levels of polymorphism were encountered. The map of crp-1 comprises in total 287 cM and contains 89 markers in 10 linkage groups, whereas the map of crp-4 spans a genetic distance of 310 cM distributed over 66 markers in 12 linkage groups. Gene-specific markers detecting R gene analogs, a kinase similar to the tomato *Pto* gene, and a homolog of *SGT1* were also mapped. Two QTLs associated with resistance were located on the parental linkage maps by composite interval analysis of marker-trait associations. Within the cytoplasmic background of parent crp-4, a portion of the susceptible progenies carried the marker alleles for resistance at the position of the QTLs. This suggested that resistance may have been abrogated by an additional, independent nuclear factor that interacts with the cytoplasm of parent crp-4 to cause a susceptible phenotype. By single-marker analyses of marker-trait association, three additional marker loci independent of the QTLs were detected that significantly contributed to the resistance phenotype. *S. caripense*, a wild plant that has not been subjected to domestication and breeding should be a valuable source of resistance to late blight.

Key words: Genetic map – late blight – potato – QTL – resistance genes – *Solanum caripense*

Introduction

Late blight, the economically most important disease of potato, caused by the oomycete *Phytophthora infestans*, occurs almost everywhere where potatoes are grown. At around 1850, potato breeders started to introduce resistance from related *Solanum* species. Until present, 11 *R* genes from *S. demissum* have been included into potato breeding stocks. But most of the *R* genes used have been overcome by the pathogen throughout a large geographic area (Müller 1936). New resistance genes, such as *RB* from *S. bulbocastanum* (Song et al. 2003, van der Vossen et al. 2003) have been found in the gene pool of wild *Solanum* species, which are valuable sources for breeding. We detected late blight resistance in the wild *S. caripense*. Although this resistance segregates in the fashion of single dominant factors it proved intact against a wide array of *P. infestans* isolates representing most likely all strains of this pathogen occurring outside of South America (Trognitz 1998a). We report about the development of genetic maps and the localization on these maps of loci involved in the resistance. This work will facilitate eventual positional cloning of the blight resistance gene(s) of *S. caripense*.

Material and methods

Phenotyping of resistance to the late blight

S. caripense seedlings were grown and tested for resistance to late blight as described in Trognitz (1998b). Two parental genotypes, crp-1 and crp-4, were intercrossed reciprocally to produce two unselected cross progenies of 60 individuals each, designated crp-1xcrp-4 for the forward cross and crp-4xcrp-1 for the reverse cross. For the resistance tests using two highly virulent *P. infestans* isolates, either entire pot plants were exposed or lateral leaflets were detached from plants prior to flowering. The criteria percent leaflet area affected (A) and

sporulation intensity (S) were assessed. Typically, resistant plants developed none or very small lesions covering up to 10 % of the total leaflet area and no sporulation, whereas in susceptible interactions, a single lesion covered 60 - 90 % of the leaflet and there was abundant sporulation of the pathogen. Numbers of resistant and susceptible individuals within each cross progeny were compared to ratios of segregation expected under several plausible models of inheritance using a χ^2 test for goodness-of-fit.

DNA extraction and molecular marker techniques
DNA extraction and RFLP analysis were carried out as described in Trognitz et al. (2002). The AFLP procedure was carried out according to Vos et al. (1995), the digestion and ligation was done in one step overnight. For the sequence specific amplification polymorphism (S-SAP) procedure the protocol of Waugh et al. (1997) was followed using primers specific for conserved domains of R genes and a primer for the *Eco*RI adapter. Parental maps were constructed and marker-trait regression calculated in Joinmap v 3.0 (van Oijen & Voorrips 2001). QTL were detected by interval mapping in QTL cartographer (Wang et al. 2001-2003).

Results and discussion

Segregation of the resistance phenotype
We detected binomial distribution of resistant and susceptible cross progenies that indicated inheritance of blight resistance by few dominant genes. In the crp-1xcrp-4 cross the figures could be explained by segregation of two genes. However, the frequency of resistant and susceptible plants in the reverse cross could be explained by this model only under the assumption of an additional, interfering cytoplasmic factor.

Map construction
Of 140 EcoRI/MseI and 28 EcoRI/PstI-specific primer combinations tested, 48 primer combinations were used for construction due to their sufficient levels of polymorphism. For these, on average not more than 4.6 polymorphic bands per primer combination were obtained. Of 26 RFLP probes of known position on the potato map tested in addition, only two were polymorphic in the *S. caripense* crosses and one of these could be placed on the map. To map resistance gene analogs (RGAs), conserved motifs were used for amplification of RGAs from genomic DNA. Fragments amplified with specific primers for the *R1* gene from *S. demissum* were used as a probe and two fragments were polymorphic for parent crp-1. Based on sequence information of known R genes specific primers were designed and used in an S-SAP approach. We detected polymorphism for several *R1* like genes, for a kinase based on the tomato *Pto* gene (Jia et al. 1997), for a TIR-NBS resistance gene based on the "motif 2" sequence (Hammond-Kosack & Jones 1997), and a homolog of the *SGT1* gene involved in resistance signaling. Of several potato SSR markers tested, only one (STM0025-1/EcoR00) was polymorphic and could be mapped. Additional molecular marker techniques (SSR and CAPS-cleaved amplified polymorphic sequence- from potato) failed to detect polymorphic fragments in *S. caripense*. The map of parent crp-1 (Figure 1) consists of 89 markers distributed throughout ten linkage groups, of a total of 128 segregating markers obtained for this parent. This map spans a genetic distance of 287 cM, its markers are spaced on average at 3.2 cM. Several clusters of markers spaced at small genetic distance are apparent, whereas other, frequently distal regions on individual linkage groups present large spaces between adjacent markers. For the crp-4 parent, a total of 94 markers were obtained, 66 of which were sufficiently informative to construct a map of 12 linkage groups covering a total genetic distance of 310 cM (average spacing of markers at 4.6 cM). The total number of 12 linkage groups corresponds to the number of chromosomes in the haploid genome of *S. caripense*. Because of the reduced levels of polymorphism encountered within the mapping populations,

no assignment of the linkage groups to the consensus potato/tomato chromosomes can be made at present. Nonetheless, the maps have been useful for detection of genomic regions associated with late blight resistance.

Detection of marker-trait associations

Single factor analyses of variance were carried out for 89 markers from crp-1 and 66 markers from crp-4 as included on the genetic maps and phenotype values of four resistance criteria (A and S following inoculation with two *P. infestans* isolates), in separate for the crp-1xcrp-4 and crp-4xcrp-1 populations. Within crp-1xcrp-4, two clustered markers from crp-1, E+AAA/M+ACG-319 and E+AAC/M+AAG-188, localized on linkage group crp-1-10 were significantly associated with the resistance phenotype and this position coincided with a QTL, as detected by interval mapping (Figure 2). On the map of crp-4, 11 clustered markers significantly associated with resistance were detected. AFLP marker E+ACT/M+CTG-67 had the largest LOD score in the QTL analysis. When the data for both reciprocal cross populations were combined, the marker-trait associations and QTLs tended to disappear. Close inspection of the data revealed the unexpected presence of marker alleles associated with resistance in twenty individuals of the crp-4xcrp-1 population. When these twenty individuals were omitted from the analysis, the QTLs re-appeared with large LOD scores (as indicated in Figure 2). The most likely hypothesis to account for this phenomenon at present is to assume an additional, independent nuclear genetic factor that would interact with an unknown factor present within the cytoplasm of parent crp-4. Additional crosses and investigations to test this hypothesis are underway.

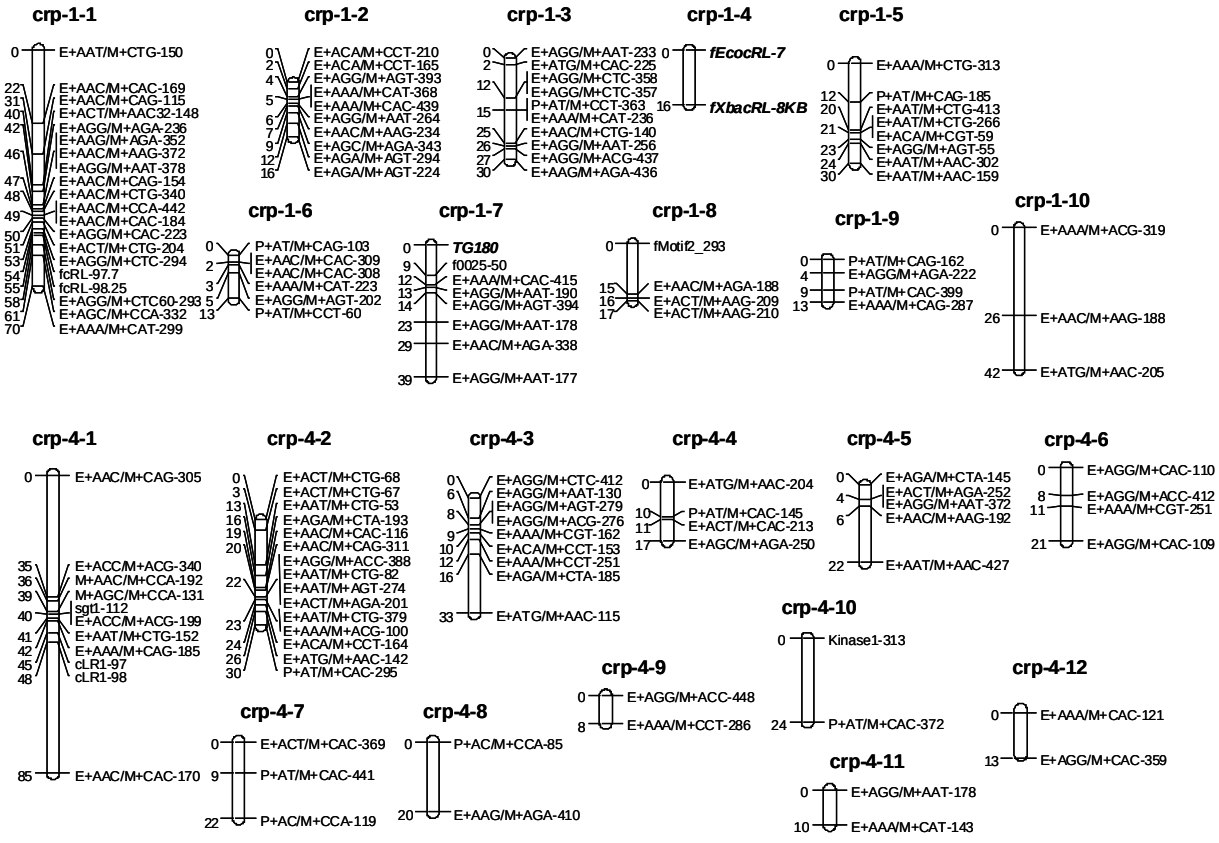

Figure 1. Genetic linkage maps of two *S. caripense* parental genotypes, crp-1 (top) and crp-4 (bottom).

AFLP markers are indicated to the right of a linkage group and genetic distance (cM) to the left. LOD scores are plotted along the genetic distance. Dotted line; LOD score of original data for population crp-1xcrp-4, dashed line; data combined for reciprocal crosses (excluding 20 individuals from population crp-4xcrp-1 with unexpected marker alleles; see text), solid line; population crp-4xcrp-1 (excluding 20 individuals with unexpected marker alleles).

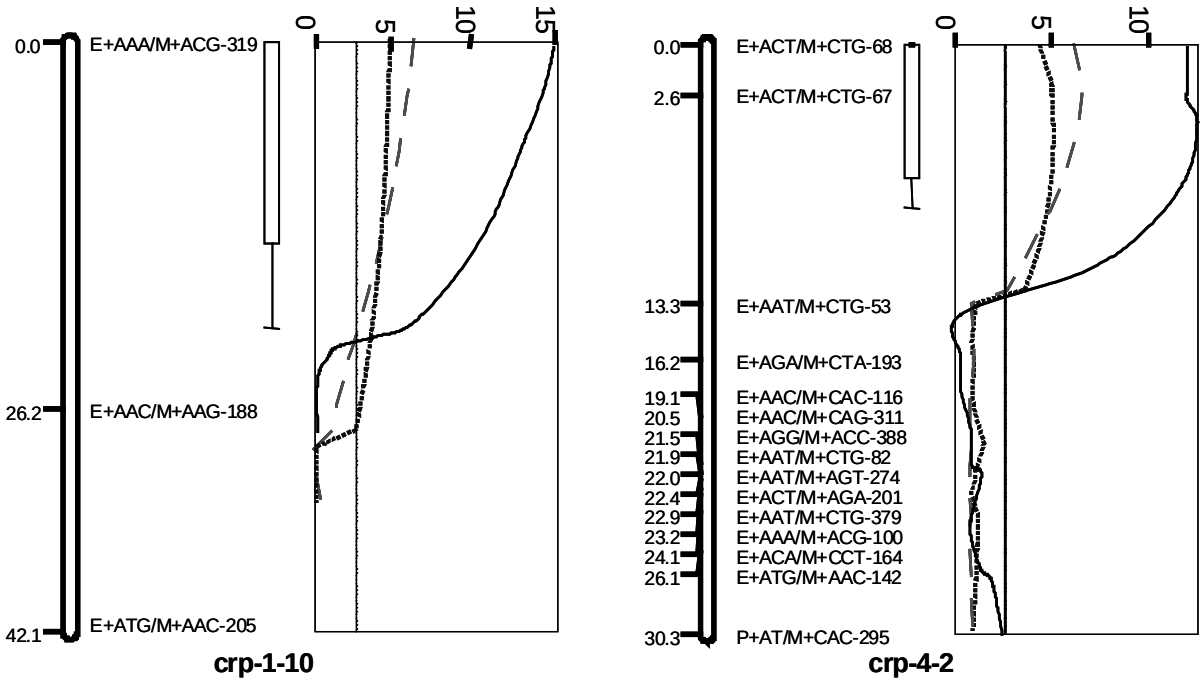

Figure 2. QTL positions as detected by composite interval analysis on the maps of parental genotypes crp-1 and crp-4.

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Fine mapping of the bolting gene of sugar beet using BAC-derived SNP markers

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ABSTRACT: The gene *B* is responsible for early bolting in sugar beet. Plants carrying the dominant allele make shoot elongation followed by flowering without prior exposure to cold temperatures. Among 54 BACs selected from the *B* region on chromosome 2 of sugar beet, seventeen BAC ends and five ORFs were used to identify SNPs (single nucleotide polymorphisms). Nine SNP markers were developed from *single-* or *low-copy* BAC end sequences, and 5 SNPs from ORF sequences were identified. Genotyping by DNA sequencing is costly and time consuming. One alternative is the ARMS (amplification refractory mutation system) technique, which relies on PCR amplification of two allele specific fragments using two primer pairs in one PCR reaction. Here, we report on the routine detection of SNPs by PCR-based ARMS. To minimize genotyping efforts, only the class of homozygous recessive plants (*bb*) were selected from the entire population and were used for fine mapping. The genotypes of these 64 plants had been verified by phenotyping their F_3 progenies. No bolting plants had been identified within 1344 F_3 plants analyzed. Two-point linkage analysis between each marker locus and the *B* locus revealed recombination values ranging between 0.00 and 0.058. Two markers did not show any recombination with the *B* locus. This analysis helps to determine the orientation of the BACs in the physical map, to assemble overlapping BACs and to identify candidate BACs carrying the bolting gene. Moreover, the BAC-end derived sequences can be used directly as diagnostic markers to detect bolting plants.

Key words: ARMS – *B* gene – BAC derived markers – two-point linkage analysis

Introduction

Sugar beet is a biennial root crop that grows vegetatively in the first season. It initiates stem elongation (bolting) after exposure to a period of low temperature (vernalization) followed by cultivation under long-day conditions. The *B* locus controlling early bolting (annuality) was first described in a commercial sugar beet cultivar by Munerati (1931). It had been mapped with molecular markers to chromosome 2 of sugar beet using RFLP markers (Boudry et al. 1994). A high-density map of the region around the B locus has been published (El-Mezawy et al. 2002). Two AFLP markers were mapped to positions 0.14 and 0.23 cM from the *B* gene, respectively.

There has been recent interest in the development of high-density linkage maps based on biallelic markers that can be assayed by PCR. Although most SNPs reside within non-coding genomic regions, an important subset of SNPs correspond to mutations within genes. The increasing production of genomic sequence data in combination with improved methods for SNP analysis are leading to the systematic generation of genetic maps based on SNPs. Mapping of SNPs can be facilitated by rapid, simple, low cost and high-throughput methods for SNP genotyping. Tetra-primer ARMS-PCR is one of such methods, which employs two primer pairs to amplify, respectively, two different alleles that differ by one SNP in a single PCR reaction (Ye et al. 2001).

Materials and methods

Identification of single nucleotide polymorphisms (SNPs)

Due to the development of CAPS (Cleaved Amplified Polymorphic Sequences) from BAC ends was inefficient, the strategy was to discover SNPs by sequencing BAC ends and BAC

ORFs. Briefly, the purified PCR products amplified with the primers from BAC end sequences and BAC ORFs (exon and intron primers) were sequenced on a MegaBACE 500. Sequence comparisons of amplicons from bolting and non-bolting genotypes were performed with the SeqMan program (DNASTAR) potential SNPs identified.

Analysis of clones from a sugar beet BAC library
BACs were isolated from a representative library of sugar beet (Hohmann et al. 2003). Three BACs, B1, B70 and B131 from the central gene *B* region were selected for subcloning and *shotgun* sequencing. Potential open reading frames were detected by DNASTAR software. The longest 8 open reading frames (ORFs) were choosen for marker development.

Mapping of SNPs
The population was an F_2 derived from a cross of 930190 (*BB*) and A906001 (*bb*) and consisted of 1359 plants. Of those, 64 F_2 plants homozygous for the non-bolting alleles (*bb*) were selected on the basis of analysis with markers closely linked to the B locus and on phenotyping the F_3 progenies. None of the 1344 F_3 plants was bolting. SNPs were converted into tetra-primer ARMS-PCR. Allele specific primers were designed to contain one base pair mismatch at position 2 from 3`-terminus. Two primer pairs (two inner allele specific primers and two outer primers) were used in one PCR reaction to amplify two allele specific fragments, which were separated on 2% agarose gels. Nucleotide diversity across different BAC sequence fragments was calculated according to Nei and Li (1979).

Linkage analysis
According to Hühn (1995), two-point linkage analysis can be done based only on the recessive class of the mapping population using the following equation (1). Here, $\hat{R}$ is the maximum likelihood estimate of the recombination fraction *R*, and Z_1, Z_2, Z_3 are the observed absolute frequencies of different classes of recessive non-bolting individuals. Using the following equation (2), two point analysis has been done. The recombination between each two markers were calculated by determining the recombination between each marker and the B locus.

$$(1) \qquad \hat{R} = \frac{2Z_1 + Z_2}{2(Z_1 + Z_2 + Z_3)} \qquad (2) \qquad \hat{R}M1M2 = \frac{\hat{R}M1B - \hat{R}M2B}{1 - 4(\hat{R}M1B * \hat{R}M2B)}$$

Here, $\hat{R}M1M2$ is the recombination value between marker 1 (*M*1) and marker 2 (*M*2), $\hat{R}M1B$ and $\hat{R}M2B$ are the recombination values between the marker 1, marker 2 and *B* locus respectively (Liu 1998).

Results and discussion

SNPs developed from BAC sequences
Seventeen BAC ends were used for discovering SNPs by sequencing the amplicons from bolting (930190, *BB*) and non-bolting (A906001, *bb*) genotypes. Nine (52.9 %) BAC end sequences showed polymorphisms (SNPs) (Table 1). The SNP nucleotide diversity between bolting and non-bolting genotypes varied between 2.2 to 30.4. In total, 46 SNPs were detected among 3,892 bp analyzed and this corresponds to 1 SNP/84.6 bp. In addition, four indels were detected. Among partial sequences from 3 BACs, 61 ORFs were found using the DNASTAR software. Eight BAC ORF sequences were used to identify SNPs. Among these, five BAC ORFs showed polymorphisms (Table 2). The SNP nucleotide diversity based on potential ORFs ranged from 0.0 to 7.1 which was lower as compared to sequences from BAC ends. In total 17 SNPs were detected among 5,757 bp which means 1 SNP /338.6 bp. This result is in agreement with Schneider *et al.* (2001), who used EST-derived primers to determine SNPs in

sugar beet. They found 1 SNP/283 bp, in the potential coding regions, on average using fragments of 37 genes amplified and sequenced in two different inbred lines of sugar beet.

Table 1. SNP nucleotide diversity and indel frequency in the F_2-population as determined from comparative sequencing of amplicons from BAC ends

BAC end	PCR product length	No. of SNPs	Nucleotide diversity (π x 10^{-3})	No. of indels
B10s	316 bp	2	6.3	-
B15s	428 bp	13	30.4	3 (one bp)
B18s	415 bp	8	19.3	-
B18t	564 bp	5	8.9	-
B21s	509 bp	4	7.9	1 (one bp)
B44s	467 bp	6	12.8	-
B29s	461 bp	1	2.2	-
B70s	360 bp	3	8.3	-
B73t	372 bp	4	10.8	-
Total	3892 bp	46	11.8	4

Table 2. SNP nucleotide diversity and indel frequency in the F_2-population as determined from comparative sequencing of amplicons from BAC ORFs

BAC end	PCR product length	No. of SNPs	Nucleotide diversity (π x 10^{-3})	No. of indels
B01co15F2^{+}	797 bp	0	0	-
B01co15F3^{+}	630 bp	4	6.3	-
B01co36F4^{+}	692 bp	1	1.4	-
B70co3F4^{+*}	808 bp	4	4.9	-
B70co9F1^{-}	494 bp	0	0	-
B70co9F3^{+}	874 bp	0	0	-
B131co15F2^{+}	761 bp	3	3.9	-
B131co15F6^{+}	701 bp	5	7.1	1
Total	5757 bp	17	2.9	1

+ Exon region was amplified by using intron primers. - Intron region was amplified by using exon primers.

Mapping of SNPs

The SNPs identified from BAC sequences (BAC end or ORF sequences) were converted into tetra-primers PCR-ARMS. To accelarate the mapping procedure, only the non-bolting recessive (*bb*) plants from F_2 population were used. The recombination values between the *B* locus and each marker were calculated (Table 3). Then the R-values were converted into map distances (cM) using Kosambi's equation. One BAC end sequence (B18t) and another BAC ORF sequence (B01co36F4) showed no recombination with the *B* locus. Three-point linkage analysis was done in order to find the most likely order of markers in relation to *B* locus. Linked significance test of markers to *B* locus was done. Based on this LOD SCORE test, all markers showed significant linkage to the *B* locus. The markers described here, are useful for identification of seeds carrying the early bolting allele and for map based cloning of the bolting locus from its position on chromosome 2. This study also demonstrates the usefulness of BACs as sources for SNP markers.

Table 3. Two-point linkage analysis with SNP markers developed from BAC ends and ORF sequences

Marker	Recombination value (R)	cM	LOD score
B01co36F4	0.0000	0.0000	∞
B18t	0.0000	0.0000	∞
B10s	0.0078	0.0039	35.99*
B44s	0.0078	0.0039	35.99*
B70co3F4	0.0078	0.0039	35.99*
B131co15F6	0.0078	0.0039	35.99*

*: Marker is significantly linked to B locus

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Cloning and physical mapping of a wild beet (*Beta procumbens*) translocation in sugar beet

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ABSTRACT: Resistance against the beet cyst nematode *Heterodera schachtii* was transferred from wild beet into sugar beet. The *Hs1pro-1* resistance locus is located on a translocation (1.5 Mb) from chromosome I of *Beta procumbens*, which is attached to chromosome IX of sugar beet. To clone the whole translocation aiming at identification of a second nematode resistance gene Hs1-1pro-1, a BAC-library was made. The library consists of 61,056 clones with an average insert size of 100 kb, resulting in a 7x coverage of the haploid sugar beet genome. The first screening of the BAC library using *B. procumbens* specific probes led to the identification of 54 clones, which were assembled into four contigs. In addition, 112 BAC-clones were identified, which carry sequences with homology to cloned disease resistance genes. Among these, translocation specific clones were detected by hybridisation with genomic DNA of *B. procumbens*.

Key words: BAC-library – *Beta vulgaris* – *Heterodera schachtii* – nematode resistance genes

Introduction

The beet cyst nematode, *Heterodera schachtii*, is a major parasite of the sugar beet. No resistant sugar beet cultivars are known. Nematodes can be controlled by crop rotation, by fumigation with nematicides or by growing resistant crops. But, breeding of resistant varieties offers the most promising control alternative. Complete resistance against *H. schachtii* was found in the three species of the *Procumbentes* section, *Beta procumbens*, *B. webbiana*, and *B. patellaris*. At least three different resistance genes were located on different chromosomes of wild beets: *Hs1* on the homoelogous chromosomes I of each species, *Hs2* on the homoelogous chromosomes VII of *B. procumbens* and *B. webbiana* and *Hs3* on chromosome VIII of *B. webbiana* (see review by Jung et al. 1998). Crosses between wild species and sugar beet resulted in various nematode resistant beet lines. They are monosomic (2n = 19) and fragment addition lines (2n = 19) as well as translocation lines (2n = 18) carrying a translocation from the wild beet chromosome I encompassing the *Hs1pro-1* locus. Translocation lines show a stable inheritance of nematode resistance and were therefore intensively used as introduction lines for breeding programs as well as for molecular cloning of the resistance genes. The first nematode resistance gene *Hs1pro-1* was cloned from the translocation line A906001 (Cai et al. 1997). The translocation was genetically mapped to the end of chromosome IX (Heller et al. 1996). A YAC-library of line A906001 was constructed and positive clones from the translocation were identified, however, due to the low genome coverage of the library no complete physical map could be build. Due to the fact that another translocation line PRO4, which also carries a translocation from chromosome I of *B. procumbens* is lacking the *Hs1pro-1* gene gave rise to the hypothesis that there is a second resistance locus containing a resistance gene analog sequence (RGA). This gene is referred to as *Hs1-1pro-1*. In this work physical mapping of the translocation and identification of RGA containing BACs is described.

Materials and methods

Plant material

For construction of the BAC-library sugar beet line KI-000520 was used, which carries the *B. procumbens* translocation and the gene *B* for early bolting.

High-molecular-weight (HMW) DNA isolation

Nuclei were isolated from leaves according to Jacobs (2001) with a few modifications. 15 g of young leaves were ground in liquid nitrogen and incubated with 1 x extraction buffer with 2 % PVP at 4°C for 1 hour. The leaf homogenate was filtered through nylon filters (250, 134 and 55 µm, respectively). Centrifugation of the filtrate was performed at 4°C for 10 min at 1200 x g. Nuclei were re-suspended in 200 µl of extraction buffer without Triton-X-100, β–mercaptoethanol and 2 % PVP and embedded in 1.4 % InCertTM-Agarose plugs. Agarose plugs were incubated for 48 hours at 50°C in lysis buffer and stored in 0.5 M EDTA solution at 4°C. The HMW-DNA was controlled by pulsed-field gel electrophoresis (PFGE) using a CHEF II apparatus (Biorad, UK) under the conditions: 6 V/cm, 60 sec constant pulse, 10°C, for 16 h, 1 % agarose gel in 0.5x TBE-buffer.

Construction and screening of BAC library

Agarose-plugs were cut into 4 pieces. Eight agarose-plugs were undergone a pre-electrophoresis and then incubated with 1x TE-buffer, 1x restriction-buffer and 1x restriction-buffer with 7.5 Units HindIII for one hour at 4°C, respectively. Restriction was performed for 1 hour at 37°C and was stopped with 0.5 M EDTA. Restricted plugs were separated on a 1 %-SeakemTM-Agarose-PFGE for 18 h, 6 V/cm, 60 to 90 sec pulse, and 14°C. Seven regions between 50 kb and 745 kb were cut from the gel and undergo a second size selection on a 1 %-SeakemTM-Agarose-PFGE under the same conditions. The seven fragment regions were cut from the gel again. The isolated DNA of each block was dialysed for 1 h with 70 V in an electrophoresis chamber against 1x TAE-buffer. Ligation was performed in the vector pCC1-BAC cloning vector (Epicentre, USA) with 100 ng DNA and 10 ng of vector-DNA at 55°C for 10 min and cooled down for 15 min at RT. One-times reaction-buffer, 1 µl 100 mM ATP and 4 units of Fast-LinkTM DNA ligase were added and incubated at 16°C for 4 h. The ligation reaction was dialysed against 1x TE-buffer with 100x polyamids for 1.5 h. Transformation was performed in ElektroMAXTM DH10BTM cells. The transformation efficiency was determined by the white/blue colony ratio. Insert-DNA-size was estimated by PFGE analysis with randomly chosen colonies with the restriction enzyme NotI. The ligation reactions were chosen for further application, which gave an average DNA insert of 100 kb in size, a white colony content over 90 % and a high transformation efficiency (over 300 colonies in 100 µl transformation reaction). White colonies were picked with the Q-Pix-Roboter (Genetics) into 384-microtiter-plates containing LB-freezing-medium. All plates were gridded on Hybond-N+ nylon high-density-filters (Amersham) with a 3x3 spotting pattern. 50 ng of probe DNA were radioactively labelled and used for hybridisation on the BAC-filters at 60°C overnight. Filters were washed with 1x SSC and 0.1% SDS for 20 min and 0.5x SSC and 0.1% SDS for 15 min. The filters were overlaid with hyper-X-ray film overnight.

Probes for screening the library

For screening the library, probes were used which are specific for the *B. procumbens* genome. There are three different cDNA single-copy probes available *Hs1pro-1*, 23a and 14b. Three YAC-end-sequences, 128R, 104R and 58R derived from YAC-clones from the translocation gave single- and low-copy signals. The *B. procumbens* specific repetitive element X2.1 has three copies on the translocation. All probes were tested on Southern-blots with HindIII-

restricted gDNA of *B. procumbens*, A906001 and sugar beet to confirm specifity and copy-number. For screening of NBS-LRR-sequences a probe derived from cDNA cZR-7, which is an RGA cloned from *B. procumbens* (Tian 2003), was used.

BAC fingerprinting and BAC contig construction
1.75 µg of BAC-DNA were digested with 10 units of HindIII and EcoRI at 37°C for 4.5 h, respectively. The digestion was controlled by loading 250 ng DNA on a 1 % agarose gel. For fingerprinting, 500 ng restricted BAC-DNA was loaded on a 1 % SeakemTM-agarose gel running at 70 Volt, 4°C and for 16 h. The DNA was stained with ethidiumbromide for 1 h and visualized with gel documentation provided by Biorad. The DNA was blotted on a Hybond-N+ filter for further hybridisation. Detection of restriction fragments was performed interactively using the Image 3.9 program (Sanger Centre). The contig assembly was conducted by the program FPC (C. Soderlund).

Results

Construction and screening of BAC-library
The BAC library consists of 61056 clones. 6.63 % are without DNA-insert, 0.87 % carry mitochondrial DNA and 1.98 % carry plastid DNA as revealed by Southern-blot analysis. Thus, the BAC library represents approx. 7 copies of haploid sugar beet genome (758 Mb). The average insert size was about 100 kb. The clones were distributed in 159 microtiter-plates and stored at -70°C. For hybridisation, BAC clones of each plate were spotted on 7 Hybond N+ high-density filters in a 3x3 spotting pattern. Eight *B. procumbens* specific probes were employed for the screening the BAC library resulting in identification of 54 candidate BAC clones. Tight physical linkage of probe pairs X2.1 and 23a and 104R and 128R was revealed by hybridisation with one and the same BAC.

To obtain the BAC clones containing RGAs, a sugar beet NBS-LRR consensus sequence was used as a probe for colony-hybridisation. In total, 112 candidate BACs were identified. To identify translocation-specific RGA-containing BACs in a fast and efficient manner, all candidate BACs were subsequently subjected to a further hybridisation using whole genomic DNA of *B. procumbens* as a probe. The hybridisation revealed several BACs giving strong hybridisation signals with the *B. procumbens* DNA, but not with the sugar beet DNA. Further characterization of these BACs and their integration into the existing contigs are in progress.

BAC-contig-construction
To produce the image suitable for BAC-fingerprinting with Image 3.9 software, 500 ng of HindIII-restricted BAC-DNA were used for each sample. On the average, about 20 fragments per clone were detected, which were in a range from 500 bp to 12,000 bp. So far, 32 BAC-clones out of 54 could be assembled into 4 contigs across the translocation. In addition, 34 T7- and 32 Sp6-BAC-ends were cloned and sequenced. Southern analysis revealed that approx. 90 % of the cloned BAC ends gave hybridisation signals with DNA of *B. procumbens*, the translocation line A906001 and sugar beet 93161p as well. Only 5 BAC-ends, corresponding to 8 % of the cloned BAC-ends, are *B. procumbens* specific. They gave single-, low- or high-copy signals with genomic DNA from *B. procumbens* and the translocation line A906001, but not on the sugar beet DNA.

Discussion
The BAC-technology provides a powerful tool for cloning of high-molecular-weight-DNA (HMW). Several factors have significant impact on the cloning efficiency. In this study, we used the pCC1-BAC-cloning-vector that had been restricted and dephosphorylated for cloning

of the restricted sugar beet HMW-DNA. The isolation of HMW-DNA from sugar beet is well established in our institute and contamination of plastid-DNA was less than 2 %. To get BAC clones with large DNA-inserts, we performed two times size-selection to get rid off smaller DNA-fragments co-separated. The ratio between vector and insert for ligation reaction is also important for the ligation efficiency. In our experiments a ratio of 1:10 gave the highest efficiency. For screening of the BAC-library 8 translocation specific probes were used. Because some of these probes cross hybridised with sugar beet, stringent conditions for filter washing were used for elimination of sugar beet background signals. In total, 54 translocation-specific BAC-clones could be identified. Four BAC contigs could be constructed on the basis of the FPC analysis. But the gaps between the contigs could not be closed even after intensive screening of the BAC library. Thus, several BAC ends were employed for development of the probes for chromosome walking. Unexpectedly, 92 % of the BAC-ends obtained showed high homology with sugar beet DNA and could therefore not be used for screening the library by hybridisation. Only 8 % of the cloned BAC ends are wild beet- and translocation-specific with various hybridisation patterns. Thus, these probes will be intensively used for further screening of the BAC library to close the gaps between the existing contigs. To obtain the BAC clones containing the *B. procumbens* RGAs, we used a novel strategy. A conserved sugar beet NBS-LRR sequence was first used as a probe for the colony-hybridisation, and selected candidate BACs were subjected to a second round of hybridisation with the *B. procumbens* genomic DNA. In this way, several candidate clones have been identified. Further characterization of such NBS-LRR containing BACs and the integration of these BACs into the existing contigs are in progress.

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Changes of sucrose, glucose and fructose content in illuminated leaves of transgenic carnation (*Dianthus caryophyllus* L.) containing decreased fructose 2,6-bisphosphate levels

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ABSTRACT: The physiological role of fructose 2,6-bisphosphate (Fru-2,6-P_2), an important regulator of photosynthetic carbon metabolism was examined in carnation. The amount of Fru-2,6-P_2 in leaves of carnation (*Dianthus caryophyllus* L. cv. 'Improved White Sim') was reduced by introduction of a modified mammalian gene, encoding a functional fructose 2,6-bisphosphatase. Sucrose, glucose and fructose content were influenced by modification of the Fru-2,6-P_2 metabolite concentration. Differences could be observed in the morphology and the ontogeny of plants. The development of sucrose overproducing plants was faster than the wild type ones. Since the energy and carbon transport occurs in form of sucrose, rise in the amount of sucrose results in the increase of energy in tissues causing accelerated growth.

Key words: *Agrobacterium tumefaciens* – fructose 2,6-bisphosphate – transformation

Introduction

Carnation is one of the most important floricultural crops in the world. In addition to its economic importance it can be regarded as a model plant for petal senescence and pigmentation processes. Both are important characteristics of species utilized mainly as cut flower. Understanding the biochemical pathways in carnation could help not only in improving the aesthetic traits but is of scientific significance. Photosynthesis and carbohydrate metabolism are fundamental physiological processes of plants, providing energy for normal growth and development.

Fructose 2,6-bisphosphate (Fru-2,6-P_2), as a signal metabolite is an important regulator of the carbohydrate metabolism in plants (Stitt 1990). During photosynthesis this molecule co-ordinates the rates of CO_2 assimilation and sucrose synthesis and partitions the carbon between sucrose and starch (Stitt 1997). These are based on the following: Fru-2,6-P_2 is a strong inhibitor of cytosolic fructose 1,6-bisphosphatase enzyme, which has key regulatory role in sucrose synthesis (Stitt et al. 1985) and a potent activator of pyrophosphate:fructose 6-phosphate phosphotranspherase, promoting the glycolysis. The concentration of Fru-2,6-P_2 in plants is determined by a bifunctional enzyme (Larondelle et al. 1986): the relative activities of 6-phosphofructo-2-kinase and fructose 2,6-bisphosphatase (6-PF-2-K/Fru-2,6-P_2ase).

To clarify the physiological role of Fru-2,6-P_2 in carnation in our experiments a transgenic approach was applied. Carnation was transformed by a modified rat liver bifunctional enzyme (6-phosphofructo-2-kinase/fructose 2,6-bisphosphatase) cDNA encoding a polypeptide with the capacity to decrease the Fru-2,6-P_2 concentration. This system has already been described in tobacco (Scott et al. 2000).

Materials and methods

Plant material

Carnation (*Dianthus caryophyllus* L.) variety 'Improved White Sim' (IWS) was obtained from the Óbuda Horticultural Laboratory Budapest, Hungary. Sterile shoot cultures were maintained under 16 h light / 8 h dark regime, 200 W/m^2 light intensity at 25°C on Murashige and Skoog basal medium (Murashige & Skoog 1962) containing 3 % sucrose and 0.8 % agar (Oxoid). After selection of the regenerated transgenic shoots, they were transferred into soil and grown in a greenhouse.

Gene construct

The modified coding region of the rat liver 6-phosphofructo-2-kinase/fructose 2,6-bisphosphatase contains a point mutation: arginine-195 was replaced for alanine (Li et al. 1992). This sequence was inserted *via* subcloning steps into pBIN 19 under the control of CaMV 35S promoter and introduced into *Agrobacterium tumefaciens* strain LBA 4404. Binary vector pBIN 19 carries neomycin phosphotranspherase (*nptII*) as a selectable marker gene.

Plant transformation

Carnation leaves were transformed by *Agrobacterium* as described by van Altvorst et al. (1995) and selected on 50 mg/l kanamycin containing medium. Transgene integration was proved by PCR and Southern hybridisation. The regenerated kanamycin resistant and positive plants were potted into soil and grown in greenhouse.

Measurement of metabolites

Prior to carbohydrate analyses plants were kept under controlled environment in a climatic chamber. Extraction and measurement of sucrose, glucose and fructose was performed with Boehringer Mannheim test-combination kits according to the manufacturer's instructions in three replications. To monitor the effect of decreased Fru-2,6-P_2 on carbohydrate metabolism in carnation leaves, sucrose, glucose and fructose content were compared to that of the wild type plants. The amounts of these compounds were measured before and after the start of illumination at different time intervals over a 9 h light period (Figures 2A, B, C).

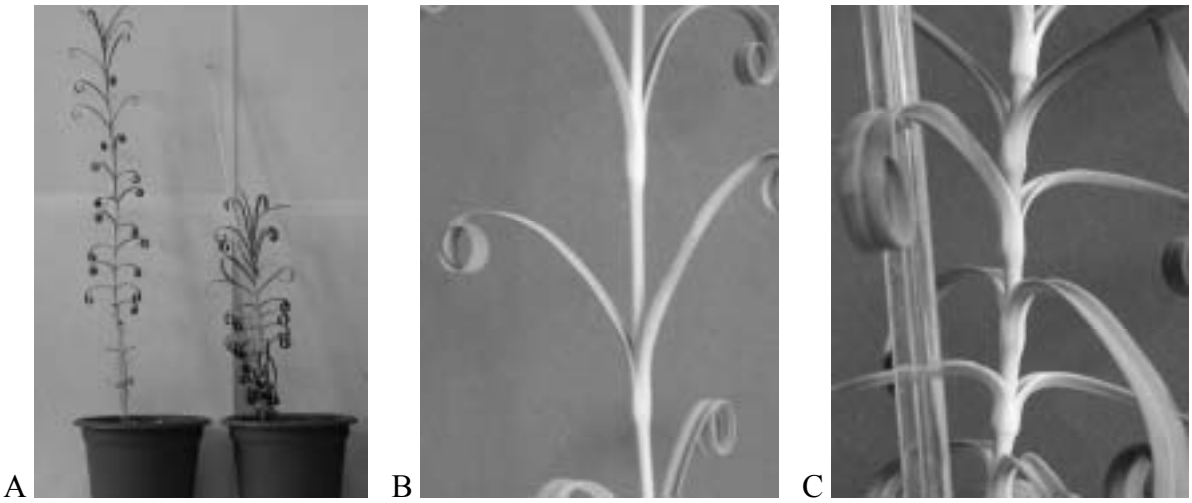

Figure 1. Differences in growth shape of plants of the same age. From left to right: (A): transgenic (left) and wild type plant (right) 6 months after potting; internodal length differences between transgenic (B) and wild type plants (C)

Results and discussion

When the phenotypic effect of the decreased Fru-2,6-P_2 amounts was investigated, a difference could be observed in growth and development of transgenic and wild type plants (Figure 1A). The more intensive development of the transgenic lines can be due to the elevated sucrose content in the tissues, as it is shown in Figure 2A, since the energy and carbon transport takes places in form of sucrose. In previous studies the modification of sucrose export capacity caused phenotypical and morphological alterations (Riesmeier et al. 1994, Bürkle et al. 1998).

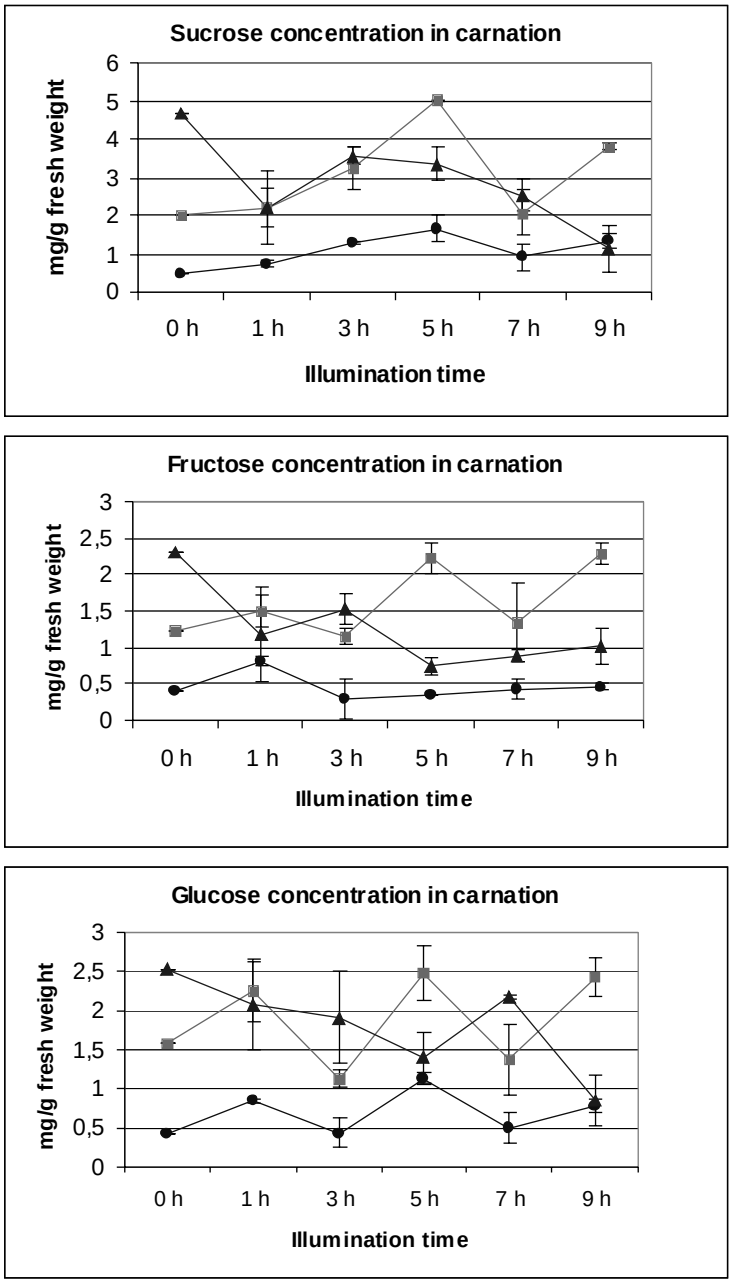

Figure 2. Sucrose, fructose and glucose levels over the 9 h light period in wild type (circles) and transgenic plants [squares (fb406), triangles (fb407)]. Each value is the mean ± SE of three replicate measurements from leaves of individual plants

There were differences in the length of internodes, which can be a characteristic trait for carnation, internodes of Fru-2,6-P_2 transgenic plants were 4 cm long compared to the 2 cm length of the wild types (Figures 1B, 1C). The sucrose accumulation was greater in both transgenic lines than wild type over the entire light period (Figure 2). The glucose and the fructose content were similar to sucrose. Tendency of the curves in fb406 transgenic lines and wild type is similar, but the actual concentration values belonging to fb406 line are higher than the control. Further analysis is necessary to explain the differences obtained between curves of fb407 and 406 transgenic lines.

Sucrose overproducing plants grew faster and bloomed by 3 - 4 weeks earlier, their tillering capacity and flower production per plant was higher than controls. These changes can be important for growers, meaning higher annual flower yield. Comparing our results to previous studies (Scott et al. 1995, 2000) indicates that modification of cytosolic Fru-2,6-P_2 concentration can significantly alter carbohydrate metabolism in photosynthetically active tissues.

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Effect of an apple derived antisense ACC-synthase cDNA on the ethylene production and the vase life of carnation (*Dianthus caryophyllus* L.)

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ABSTRACT: Transgenic carnation regenerants were potted in the glasshouse in order to make observations on the phenotypic effect of the heterologous antisense ACS transformation on the intact plants, how the antisense inhibition influences the ethylene production itself and how the decreased ethylene production affects, if at all, the flowering time, the senescence and vase-life of the flowers. All the transformed plants showed normal growth and were true-to-type. Ethylene production – measured at full opening stage – was decreased by 30 - 60 %; no plant with 100 % decrease was identified.

The vase-life of the cut carnation flowers has been observed for 4 years, but the season and the weather influences the onset of flowering to a great extent, therefore a relative value was calculated for comparison from the data of transgenic and non-transgenic plants. As for the prolongation of vase life, 38 % of the transformed carnations showed relative values between 2 to 6 days compared to the controls. Three plants were found exhibiting the most relevant alterations in each tested trait. In these plants ethylene production decreased by 37 - 67 %, they had longer vase life (4 - 6 days). The control and the precise mechanism of the transgene integration is not well understood yet, but it is very important, that in our experiments fragrance carnations were produced with prolonged vase life as a result of down-regulated ethylene production.

Key words: *Agrobacterium tumefaciens* – flower prolongation - transgenic

Introduction

Carnation is one of the leading varieties in the global flower trade; therefore genetic improvement of carnation in respect of several ethylene dependant traits such as flowering time, petal senescence characteristics or vase life can have beneficial economic effect in addition to scientific significance. Carnation is a typical ethylene-sensitive flower plant (Woltering & van Doorn 1988).

Our aim was to decrease the ethylene production in flowers to prolong the vase life of the flowers. Several methods are available to modify the vase-life of cut flowers: treatment with chemicals, application of inhibitors of ethylene biosynthesis such as aminooxyacetic acid (Fujino et al. 1980), 1-methylcyclopropene (Hassan & Gerzson, 2002, Ichimura et al. 2002) and α-aminoisobutyric acid (Onazaki et al. 1998), or with inhibitors of ethylene action, such as silver thiosulfate (Veen 1979). There are several ways of genetic improvement, for example crossing and selection (Onazaki et al. 2001) or genetic modification of carnation with antisense ACC oxidase (Savin et al. 1995) and synthase genes (Florigene Pty Ltd.). In our experiments, an apple-derived cDNA clone was used in antisense orientation (Kiss et al. 1995, Rosenfield et al. 1996) for down-regulating ethylene biosynthesis (Kiss et al. 2000, Veres et al. 2001) in order to study, how the reduced ethylene production influences flowering characteristics of carnation varieties. The carnation ACC synthases are homologues to the apple ACC synthases. The known carnation ACC synthase sequences are very similar (*Dc*ACS3 66 %, *Dc*ACS2 68 %, *Dc*ACS 65 %; NCBI database) to the apple *Md*ACS 2 cDNA clone.

It is interesting that there is a negative correlation between long vase life and the fragrance characteristics of flowers (Priel 1999); at the same time, trade demands flowers of excellent fragrance and long vase life. For transformation, varieties having good fragrance quality were chosen so that they produce flowers with long vase life.

Materials and methods

Plant material

The marketable fragrance varieties 'Bíbor'/'Purple' and 'Improved White Sim'/'IWS' (Óbuda Horticultural Laboratory, Budapest, Hungary) were transformed by *Agrobacterium tumefaciens* LBA 4404, harbouring an antisense apple derived ACC synthase gene construct (Kiss et al. 1995, 2000) named CCA (Veres et al. 2001). Carnations were transformed at the same time with *Agrobacterium tumefaciens* carrying the binary vector pBI 121 with GUS (β-D-glucoronidase) gene, these are referred as J4, and the strain without the binary vector is called J2, too.

In vivo growth conditions

Wild-type and transgenic carnation plants (*Dianthus caryophyllus* L. 'IWS' and 'Bíbor') were potted in glasshouse and grown under the same normal greenhouse conditions, as the market varieties.

Measurement of ethylene production

Transgenic and non-transgenic flowers at the full-opening stage were cut to 10 cm and put into special glass with a rubber septum. Following a 24 h incubation period with 1 % exogenous ethylene, they were kept in fresh air for 24 h and put back into the glass. Ethylene production was measured at full flowering stage and was compared to that of non-transformed plants. Ethylene was measured with a gas chromatograph GC 6000 equipped with an activated alumina column and a flame-ionization detector.

Measurement of flowering time

Transgenic and non-transgenic flowers at the full-opening stage (their outermost petals were at right angles to the stem of the flowers) were cut to 50 cm (day 0). The flowers were put into 2 l test-tube containing 1 l distilled water. Flowers were kept at a controlled air temperature of 25°C, 70 % RH. The vase life longevity was estimated by the number of days, when the petals showed in-rolling since June of 1999. Data were statistically evaluated by the *t*-test.

Results and discussion

All transformed plants showed normal growth and were true-to-type. Transformed plants flowered 2 weeks earlier than non-transformed ones. Ethylene production of the non-transformed carnation was 100 ± 13 %, the production of CCA plants was lowered by 28 - 85 ± 15 %, no plant with 0 % decrease was identified (Figure 1). There is a negative correlation between ethylene production and vase life (Figure 2).

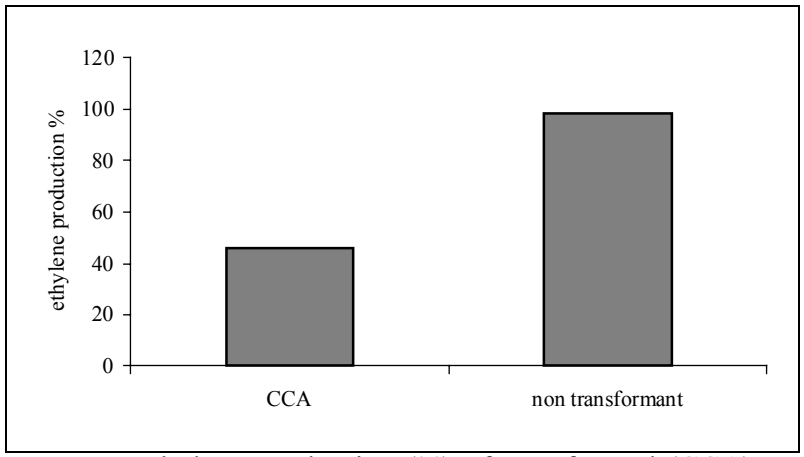


Figure 1. Ethylene production (%) of transformed (CCA) and control (non-transformed) carnations

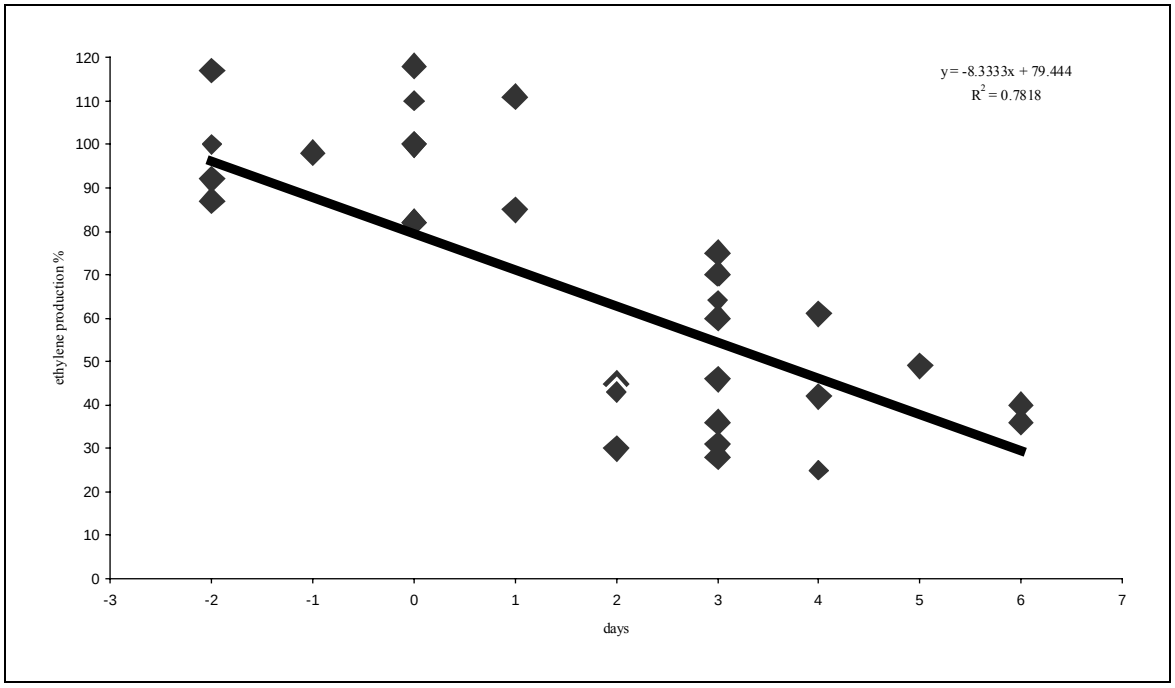
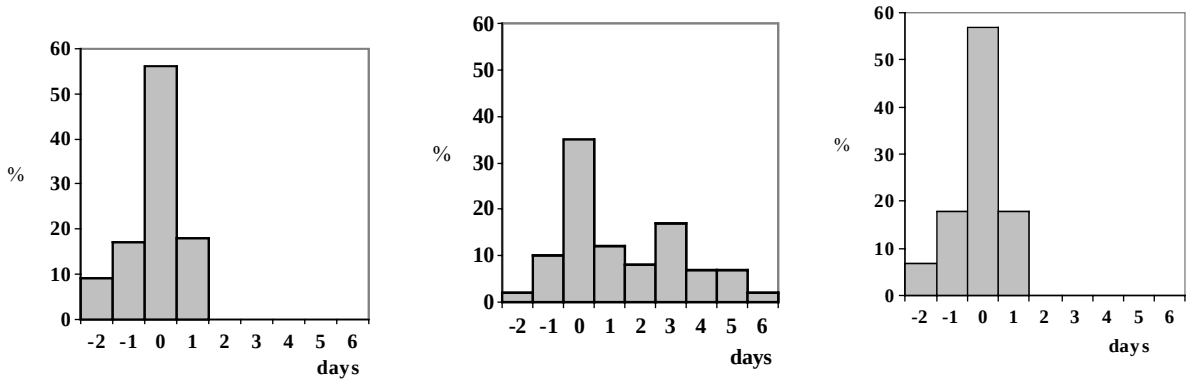


Figure 2. Correlation between the ethylene production (%) and the vase life (days) of carnation

The vase-life of the flowers has been observed for 4 years, the season and the weather influence the length of flowering to a great extent, therefore a relative value was calculated for comparison from the data of transgenic and non-transgenic plants. The relative value of non-transformed carnations never exceeded more than two days Figure 3a, the situation is the same for J2 and J4 carnations, used as transformed control (neutral for ethylene synthesis, (Figure 3c), but 38 % of the transformed carnations has a relative value higher than 2 days (Figure $3b_1$, $3b_2$).

The data of vase-life values (Figure 3) showed that the transformants can be divided into two groups. The first group (62 % of the plants: b_1) does not show any change in vase life, while the other group (the 38 % of the plants b_2) exhibits longer-life phenotype. These transgenic plants are used as stock material for further propagation. There were plants showing 3 - 6 day longer vase life compared to the control.



a	b 1	b 2	c
n = 87	n=98	n=60	n = 89
mean=-0.17	mean=-0.03	mean=3.38	mean=-0.13
s=0.84	s=0.69	s=1.01	s=0.77

Figure 3. Vase life (days) of flowers: non-transformed (a), CCA (b) and J2, J4 (c)

Three plants were found exhibiting the most marked alterations in each tested trait. In these plants, ethylene production decreased by 37 - 67 %, they have longer vase life (by 4 - 6 days). Consequently, we could produce fragrance carnations with down-regulated ethylene production, resulting in longer vase life than the control plants (Figure 4).

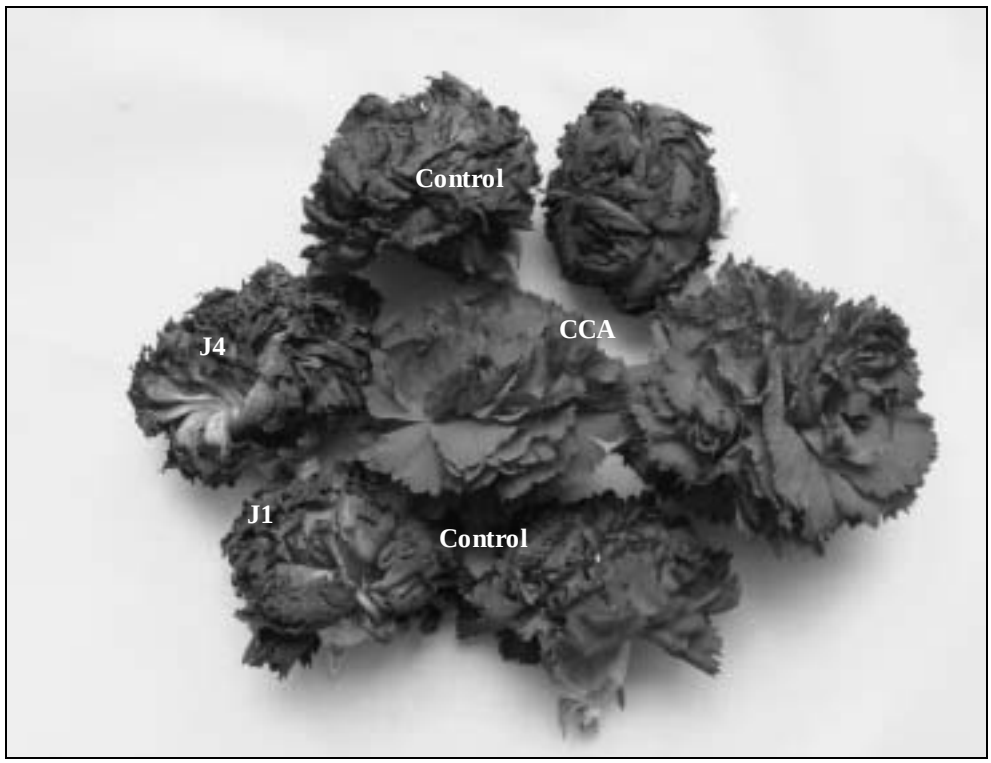


Figure 4. Carnation flowers 'Bíbor' 10 days after harvest. Control: non-transformed; J1, J4 ethylene-neutral transformants; CCA: antisense ACC-synthase transformants

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Stress capacity and RT-PCR analysis of transgenic *gshI* poplar clones (*Populus canescens*) in response to paraquat exposure

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ABSTRACT: Stress response capacity of non-transformed hybrid poplar *Populus canescens* (*P. termula* x *P. alba*) and two transgenic lines overexpressing γ-ECS either in the cytosol (cyt-ECS) or in the chloroplast (chl-ECS) were studied in response to the herbicide paraquat. Discs (8 mm) from leaves of micropropagated clones were cut and placed onto low sucrose (1 %) woody plant media (WPM) supplemented with a concentration series (4.0×10^{-9} to 4.0×10^{-6} M) of paraquat and incubated for 21 days. For analysis of phytotoxicity chlorophyll fluorescence ratios of Fv/Fm [(Fm-Fo)/Fm] and F690/F735 at F_{max} were calculated using a two-wavelength fluorometer (CFM-636973). Semiquantitative RT-PCR was used for gene expression analysis of chloroplast encoded *rbcS*-gene (RuBPCase SSU). For expression analysis of the stress responsive *gst* gene (GST, glutathione-S-transferase) specific primers were used. Expression of the constitutive mitochondrial *26S rRNA* gene was probed as a control for all RT-PCR reactions.

Key words: Paraquat – PCR – stress tolerance – transgene

Introduction

Hybrid poplar (*Populus canescens*) has significant phytoremediation capacity which may be increased by cell manipulation and genetic transformation (Bittsanszky et al. 2004). Recently *P. canescens* was transformed to overexpress the bacterial (*Escherichia coli*) gene *gsh1* encoding γ-glutamylcysteine synthetase (γ-ECS, EC 3.2.3.3). γ-ECS is the rate-limiting regulatory enzyme in the biosynthesis of the ubiquitous tripeptide (γ-L-glutamyl-L-cysteinyl-glycine) thiol compound GSH (glutathione) (Arisi et al. 1997, Noctor et al. 1998). GSH plays a central role in the plant detoxification processes supplying the initial molecule for sulfur-rich peptides of metallothioneins (MTs), metal transporter proteins (MTPs) and phytochelatins (PCs). In the present study stress response capacity of the wild-type hybrid poplar *P. canescens* (*P. termula* x *P. alba*) and two transgenic lines overexpressing γ-ECS in the cytosol (*ggs11*, cyt-ECS) and in the chloroplasts (*lgl6*, chl-ECS) was studied in response to paraquat (4×10^{-9} to 4×10^{-6} M) *in vitro*.

Materials and methods

Plant material

Clones of the non-transformed INRA 717-1-B4 hybrid poplar and two genetically transformed lines *ggs11* (also designated as cyt-ECS; Arisi et al. 1997) and *lgl6* (also designated as *Lggs6* and chl-ECS; Noctor et al. 1998) were micropropagated *in vitro* (Koprivova et al. 2002).

Paraquat treatment
Leaf discs (8 mm) were cut and placed onto the surface of aseptic tissue culture medium WPM (Lloyd & McCown 1980) with low sucrose content (1 %) and containing a paraquat concentration series (4.0 x 10^{-9} to 4.0 x 10^{-6} M). The discs were incubated for 21 days in a 16/8 h dark/light (1000 lux) photoperiod.

Fluorometry
Photosynthetic activity was determined using a laser induced (635 nm), two-wavelength chlorophyll fluorometer (CFM-636973) detecting fluorescence at 690 nm and 730 nm (Barocsi et al. 2000). Chlorophyll fluorescence ratios of Fv/Fm [(Fm-Fo)/Fm] at 690 nm, and F690/F735 at F_{max} were calculated according to Lichtenthaler & Rinderle (1988). Ten leaf discs were measured in each treatment.

RT-PCR analysis
Total RNA was extracted from 0.1 g leaf disc tissues using TRI-reagent (SigmaT9424) following the manufacture's protocol. First strand cDNA was synthesized on the mRNA templates by RT (reverse transcriptase) using an oligo$(dT)_{18-23}$ primer following the protocol of Fermentas (K1622). For gene expression analysis, cDNA (2.5 ul) samples were probed by gene specific primers. Degenerative primers for *gst* were designed using the Primer3 computer program and blasting the *gst27* (*Zea mays*) gene on sequences of *P. tremula* x *tremuloides* (Populus Database, http://poppel.fysbot.umu.se), *gst*1-5 (5'- gca caa gaa aga gcc (a/g)tt cc -3' and 5'-(a/t)gc tcc ca(c/g) (a/t)(g/t)(t/c) ag(c/t) ttt ga-3' (Fig. 3/5-6), and *gst*3-4 (5'-(c/g)t(c/a) tca ac(a/c) (g/a)c(c/t) ac(g/a) ca(a/g) cg-3' and 5'-(t/g)(g/t)(a/g) a(g/a)(a/g) (t/c)a(t/g) c(a/g)(c/g) (c/t)(t/c)c (c/g)c(a/g) ag -3') (Fig. 3/7-8).

For the chloroplast encoded *rbcS*-gene (RuBPCase SSU) the expression analysis primers used were *f:* 5'-agc ttg taa gag atg gct tcc tc –3' and *r:* 5'-cca cat agt cca gta gcg tcc at –3'; and for the mitochondrial *26S rRNA* gene the expression analysis primers used were *f:* 5'-ttc cat ggt tcg atc ctt cc-3', and *r:* 5'-gca ggg cga tcg tgt ttt tc -3' (Gray-Mitsume et al. 2004).

Results and discussion
Paraquat herbicide (1,1'-dimethyl-4.4'-bipyridilium) primarily affects the electron transport chains located in the chloroplasts and mitochondria as electron acceptors (Will et al. 2001). A reduced sucrose concentration (1 %) was used in the leaf disc culture medium to stimulate activity of the photosynthetic electron transport chain (PETC) which switches off at a higher concentration (2 to 3 %).

A concentration of 4 x 10^{-6} M paraquat led to bleaching of leaf discs, but with some growth activity retained in all the poplar clones. A concentration of 4.0 x 10^{-7} M paraquat caused chloroplast sublethality with a mixture of bleached and green spots on the leaf discs. No toxic effects were observed at lower paraquat concentrations in either of the clones (Figure 1).

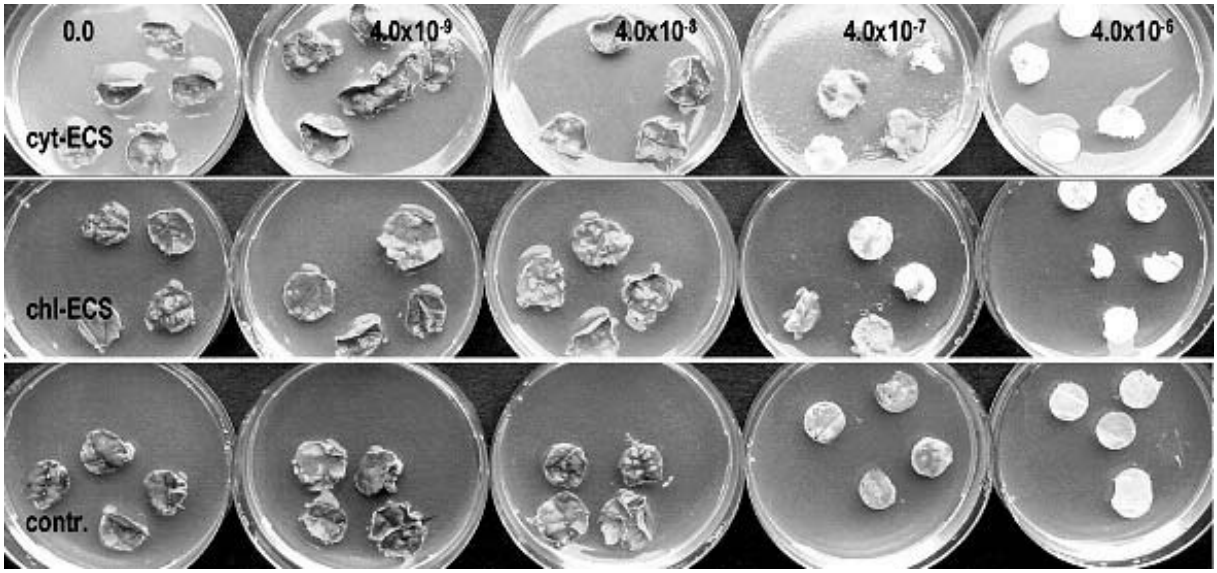

Figure 1. The effect of paraquat concentration on leaf discs of hybrid poplar clones incubated on 1 % sucrose WPM media. *cyt-ECS,* ^{gshI}transgenic clone overexpressing γ-ECS in the cytosol; *chl-ECS,* ^{gshI}transgenic clone overexpressing γ-ECS in the chloroplasts; *contr*., untransformed wild type clone

The phytotoxic effect of paraquat was determined by measuring chlorophyll fluorescence of leaf discs and calculating the ratio of P690/P730 at F_{max} (Lichtenthaler & Rinderle 1988, Barocsi et al. 2000). The results showed no significant differences at concentrations of 4.0 x 10^{-9} to 4.0 x 10^{-8} M compared to untreated samples (Figure 2a). Significant differences at sublethal (4.0 x 10^{-7} M) and bleaching (4.0 x 10^{-6} M) concentrations of paraquat were observed with about a two-fold and eight-fold (total) level of damage, respectively (Figure 2a). The phytotoxic effect of paraquat was also characterized by measuring photosynthetic activity (Fv/Fm = (Fm-Fo)/Fm) at 690 nm (Figure 2b) and by the inversely correlated chlorophyll fluorescence ratio F690/F730 at F_{max} (Figure 2a).

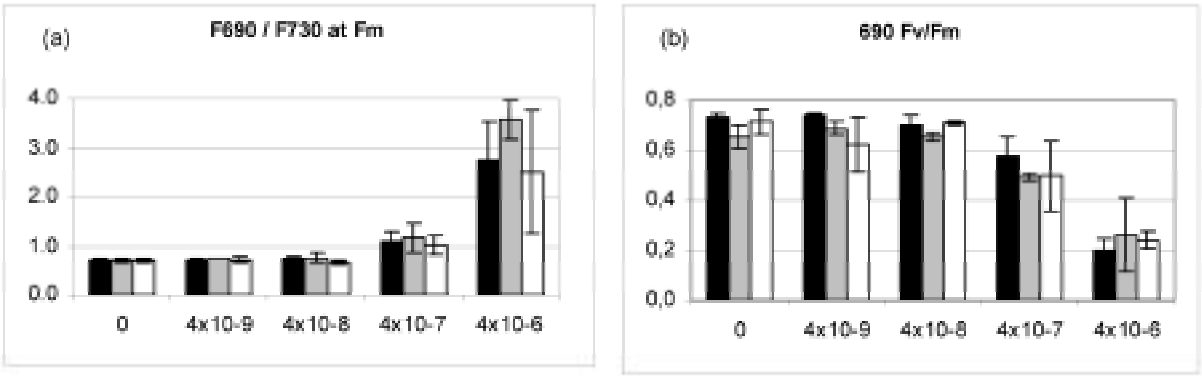

Figure 2. The effect of paraquat concentrations on the chlorophyll fluorescence ratio F690/F730 at F_{max} (a), and photosynthetic activity [Fv/Fm = (Fm-Fo)/Fm] at 690nm (b) of leaf discs from hybrid poplar clones: *cyt–ECS* (■), ^{gshI}transgenic clone overexpressing γ-ECS in the cytosol; *chl–ECS* (■), ^{gshI}transgenic clone overexpressing γ-ECS in the chloroplasts; *control* (□), untransformed wild type clone. Error bars shows standard deviation. Means of 10 leaf discs are presented

Gene expression analysis was carried out with RT-PCR using cDNA samples from leaf discs after paraquat treatment at a sublethal concentration of 4.0 x 10^{-7} M compared to untreated samples (Figure 3).

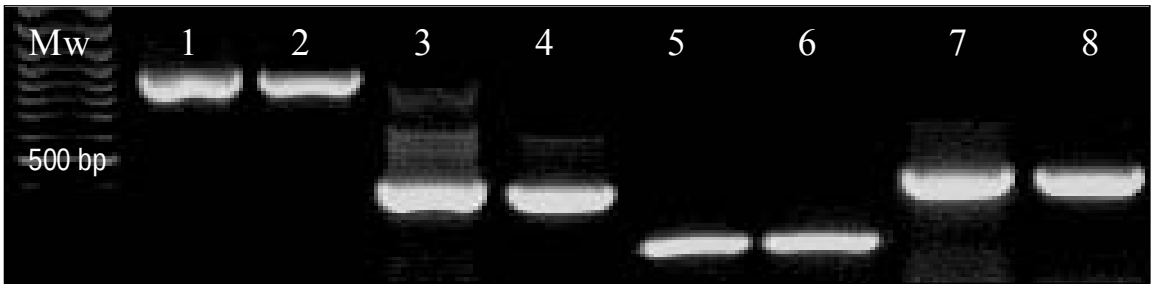

Figure 3. RT-PCR analysis of gene expression of the mitochondrial gene *26SrRNA* (1-2), the chloroplast encoded *rbcS* (3-4), and the stress responsive *gst* with two primer combinations (5-6 and 7–8) in paraquat treated (4.0 x 10^{-7} M) leaf disc cDNA samples (2, 4, 6 and 8) and untreated samples (1, 3, 5 and 7) from ^{gshI}transgenic poplar

The mitochondrial gene *26SrRNA*, used as standard marker for studying general transcriptional activities (Gray-Mitsume et al. 2004), showed equal gene expression in the untreated and treated samples (Figure 3, lanes 1 and 2). As a control, the *rbcS* gene - the SSU (small subunit) of RuBPCase (ribulose-1,5-bisphosphate carboxylase) - was probed because its transcriptional activity was found to depend on the presence and vitality of chloroplasts in the cell (Gray-Mitsume et al. 2004) (lanes 3-4). The stress response expression of *gst* in paraquat treated (4.0 x 10^{-7} M) samples (lanes 6 and 8) did not show higher activity compared to untreated samples (lanes 5 and 7).

In general, gene expression is controlled by regulatory processes of transcription (at a transcriptional level), pre-mRNA processing and mRNA turnover (at a post-transcriptional level), and translation (at translational and post-translational levels). The total of these regulatory processes accounts for gene expression. Because of post-transcriptional events affecting mRNA stability and translation, expression levels of genes do not directly correlate with steady-state levels of mRNAs (Gygi et al. 1999). In our results stress response expression of *gst* in paraquat treated samples did not show higher activity compared to untreated samples which indicates that the increased phytoextraction capacity of the ^{gshI}transgenic poplars (Gyulai et al. 2004) is due to post-transcriptional regulation.

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Clonal propagation and improved phytoextraction activity of *gshI* poplar clones (*Populus canescens*) *in vitro*

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ABSTRACT: *In vitro* phytoextraction ability of vegetative clones of *Populus canescens* including two transgenic clones (*ggs11* and *lgl6*) was studied in leaf disc cultures. Clone stability was determined by fAFLP (fluorescent labeled amplified fragment length polymorphism). In total, 679 AFLP fragments were identified. Only one of them was different (99.85 % genetic similarity). Presence of the *gsh1* transgene in the transformed clones (*ggs11* and *lgl6*) was detected in PCR reactions using *gsh1* specific primers. For the study of phytoextraction activity leaf discs (8 mm) were exposed to a concentration series of $ZnSO_4$ (10^{-1} to 10^{-5} M) incubated on tissue culture media WPM for 21 days. Zn^{2+} showed phytotoxicity only at high concentrations (10^{-1} to 10^{-2} M). Transgenic poplars showed elevated heavy metal (Zn and Cu) uptake as compared to the non-transformed clones. These results suggest that transgenic poplars may be suitable for phytoremediation of soils contaminated with Zn^{2+}.

Key words: AFLP – detoxification – heavy metals – molecular marker – soil pollution – transgene

Introduction

Poplars are known to take up and detoxify several pollutants from the soil, such as atrazine and chloroacetanilide herbicides, organic pollutants such as trinitrotoluene and trichloroethylene, and the heavy metals mercury and selenium. This remediative capacity of poplars may be significantly increased by cell and genetic manipulations. Recently *Populus canescens* was transformed to overexpress the bacterial gene encoding γ-glutamylcysteine synthetase (γ-ECS, EC 3.2.3.3) which is the rate-limiting regulatory enzyme in the biosynthesis of the ubiquitous tripeptide thiol compound glutathione (GSH, γ-L-glutamyl-L-cysteinyl-glycine) and plays a central role in plant detoxification processes (Arisi et al. 1997, Noctor et al. 1998). In the present study phytoextraction activity was investigated in the wild type poplar hybrid *P. canescens* (*P. termula* x *P. alba*) and two transgenic lines overexpressing γ-ECS in the cytosol (*ggs11*, cyt-ECS, and *lgl6*, chl-ECS) following 21 days exposure to $ZnSO_4$ (10^{-1} to 10^{-5} M) *in vitro*.

Materials and methods

Plant material

Clones of the non-transformed INRA 717-1-B4 hybrid poplar *P. canescens* and two genetically transformed lines overexpressing the *gshI* (*Escherichia coli*) gene product of glutathione (GSH) either in the cytosol (line *ggs11* of Arisi et al. 1997) or in the chloroplasts (line *lgl6* also designated as *Lggs6*; Noctor et al. 1998) were used following *in vitro* micropropagation. Leaf discs (0.8 mm) were cut and placed onto the surface of tissue culture

media WPM (Lloyd & McCown 1980) with supplementation of a concentration series of $ZnSO_4$ (10^{-1} to 10^{-5} M) for 21 days.

AFLP analysis

DNA samples were extracted from 0.1 g leaf tissues by the CTAB-method followed by a RNase-A (Sigma) treatment. Undiluted DNA samples of 10 individuals of each clone were pooled and subjected to fAFLP analysis following the basic method of Vos et al. (1995). For the digestion-ligation reaction the *Eco*RI – *Mse*I pair of restriction endonucleases were used (Table 1). The sequences of the preselective primers were as follows: *Eco-A*: GAC TGC GTA CCA ATT C-A, and *Mse*-C: GAT GAG TCC TGA GTA A-C. For selective amplification 24 primer combinations were used with JOE (green) fluorescent labelled **Eco*-primers. In the primer combinations from 1 to 12 the primer *Mse*-CAC was combined with labelled primers of **Eco*-AAA, -AAC, -AAG, -AAT, -ACA, -ACC, -ACG, -ACT, -AGA, -AGC, -AGG and **Eco*-AGT. In the primer combinations from 13 to 24, the labelled primer **Eco*-AGT was combined with primers of *Mse*-CAA, -CAG, -CAT, -CCA, -CCC, -CCG, -CCT, -CGA, -CGC, -CGG, -CGT, -CTA (Table 1). In all PCR reactions the AmpliTaq Gold DNA polymerase was used.

Fragment analysis

PCR-amplified samples were analysed using an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) with a G5 filter set in two repetitions. The fragment patterns were analyzed by the Genotyper 3.7 NT software.

Table 1. Sequence data of the restriction enzymes (rare cutter *Eco*RI, and frequent cutter *Mse*I), adapters, non-selective primers, and the effective selective primers (1 to 11) used for AFLP analysis

	*Eco*RI	*Mse*I
Restriction sites		
	5'-…….G**AATT**C……-3'	5`-……T**TA**A….-3'
	3'-…….C**TTAA**G……-5'	3'-….. A**AT**T….-5'
Adapter sequences		
	5`-**CTC**GTAGACTGCGTACC	5`-**GACG**ATGAGTCCTGAG
	CATCTGACGCATGG**TTAA**-5`	TACTCAGGACTC**AT**-5`
Non-selective primers		
	EcoA:	MseC:
	5'-GACTGCGTACCAATTC-**A**	5'-GAT GAG TCC TGA GTA A-**C**
Selective primers		
1.	5'-GACTGCGTACCAATTC-***AAT***	
2.	5'-GACTGCGTACCAATTC-***ACC***	5'-GAT GAG TCC TGA GTA A-**C*AC***
3.	5'-GACTGCGTACCAATTC-***AGT***	
4.		5'-GAT GAG TCC TGA GTA A-**C*AA***
5.		5'-GAT GAG TCC TGA GTA A-**C*AG***
6.		5'-GAT GAG TCC TGA GTA A-**C*AT***
7.		5'-GAT GAG TCC TGA GTA A-**C*CC***
8.	5'-GACTGCGTACCAATTC-***AGT***	5'-GAT GAG TCC TGA GTA A-**C*CT***
9.		5'-GAT GAG TCC TGA GTA A-**C*GA***
10.		5'-GAT GAG TCC TGA GTA A-**C*GC***
11.		5'-GAT GAG TCC TGA GTA A-**C*TA***

PCR

The *gshI*-transgene (*E.coli* No. X03954) in the transformed poplar clones was amplified by the *gshI* specific primers ^{f}ATCCCGGACGTATCACAGG (position bp. 341 - 359 in *gshI*) and reversed ^{r}GATGCACCAAACAGATAAGG (position bp 939 - 920 in *gshI*). Amplification reactions were run at a volume of 25 µl containing 20 ng DNA by a PE-9700 thermocycler according to the manufacturer's instructions (WestTeam, Pecs, Hungary).

Phytoextraction

Leaf discs (8 mm) from leaves of micropropagated poplar clones were cut and placed on to woody plant media (WPM) supplemented with a concentration series of $ZnSO_4$ (10^{-5} to 10^{-1} M). Zn-free WPM basal medium contained 63.5 µg (1.0 µM) Cu.

ICP analysis

After 21 days exposure to $ZnSO_4$ heavy metal contents (mean values of three independent measurements) of discs (Zn and Cu) were determined by inductively coupled plasma emission spectrometry (Zarcinas et al. 1987). At least three independent parallel experiments were carried out in each case. Differences between mean values were evaluated by Student's *t*-test and were considered to be significant at $P \leq 0.05$.

Results and discussion

AFLP analysis

Hybrid poplar was transformed to overexpress the bacterial gene encoding γ-glutamylcysteine synthetase (γ-ECS, EC 3.2.3.3) which is the rate-limiting regulatory enzyme in the biosynthesis of the ubiquitous glutathione tripeptide thiol compound (GSH, γ-L-glutamyl-L-cysteinyl-glycine) (Noctor et al. 1998). Increased production of GSH contributes to the antioxidative protection of plant cells against oxidative stress caused by various environmental factors. Clones were micropropagated in aseptic *in vitro* shoot culture by nodal segments for over ten years which prompted a study of the genetic stability of cut clones using fAFLP (fluorescent labeled amplified fragment length polymorphism) analysis as described by Vos et al. (1995) with modifications. Eleven of the 24 selective primer combinations applied were effective in producing AFLP banding patterns.

Table 2. The numbers of fAFLP fragments (rel. int. over 100 units, 150 - 600 bp) of the *gshI*-transgenic poplar clones of *ggs11*, *lgl6* compared to the non-transformed (contr.) clone. The selective AFLP primer combinations are: Mse-CAC- combined with -^{a}Eco-AAT*, -^{b}Eco-AAC*, and -^{c}Eco-AGT*; and Eco-AGT* combined with -^{d}Mse-CAA, -^{e}Mse-CAG, -^{f}Mse-CAT, -^{g}Mse-CCC, -^{h}Mse-CCT, -^{i}Mse-CGA, -^{j}Mse-CGC, -^{k}Mse-CTA, and -^{l}Mse-CGC

Clone	fAFLP fragments / selective primer pairs (a - l)												
	a	b	c	d	e	f	g	h	i	j	k	l	total
ggs11	25	6	17	30	25	35	16	14	11	9	17	21	226
lgl6	25	6	17	30	25	35	16	14	11	9	17	21	226
Control	25	6	17	30	25	35	17	14	11	9	17	21	227

A total of 679 common fragments were detected (Table 2). Only one fragment was different in the control clone (99.85 % genetic similarity) which indicated an unexpectedly low level of bud mutation in *P. canescens* clones and thus genetically uniform plant material for phytoextraction experiments. The AFLP fragment numbers were higher than in an analysis of black poplar (*P. nigra*) which gave only 104 AFLP fragments (Smulders et al. 2002).

Transgene detection

When the poplar clones were analyzed for transgene presence by PCR amplification with gene-specific primers, no elimination of the *gshI*-gene was observed in the transgenic clones (*ggs11*, *lgl6*) (Figure 1).

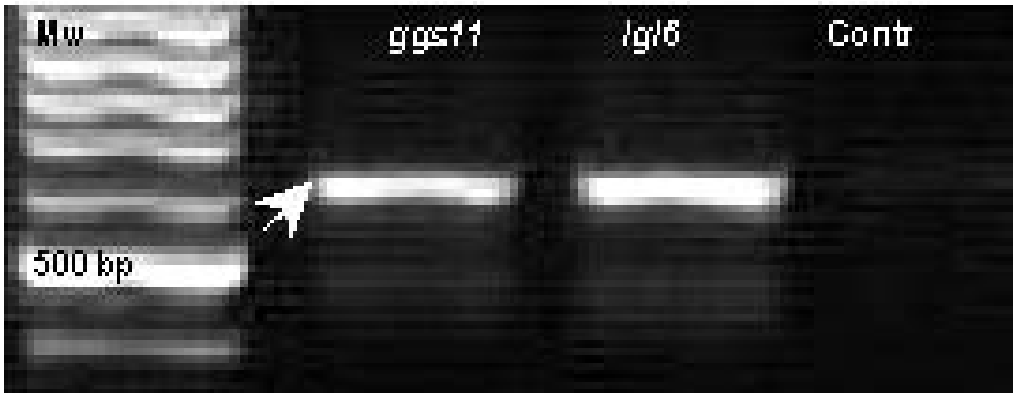

Figure 1. PCR detection of a part (561 bp) of the *gshI*-gene (No. X03954) in the transformed poplar clones (*ggs11, lgl6*). Amplification was carried out by primer pair ^{f}atc ccg gac gta tca cag g (position bp 341-359 in *gshI*) and reverse ^{r}gat gca cca aac aga taa gg (position bp 939 - 920 in *gshI*). Arrow indicates the transgene (561 bp).

Phytoremediation in vitro

The heavy metal contents of leaf discs analyzed in the poplar clones after Zn stress showed a complex pattern (Table 3). The Zn uptake showed a linear increase with the exogenously applied concentrations of $ZnSO_4$ (10^{-1} to 10^{-5} M) in all clones. None of the *gshI* transgenic (*ggs11* and *lgl6*) clones showed elevated Zn uptake capacity.

Table 3. Mean values (n = 3) of heavy metal (Zn and Cu) contents (µg /g dry matter) in leaf discs of non-transformed poplar clones *P. canescens* (contr.), and two transgenic lines *ggs11* and *lgl6* after 21 days exposure to concentration series of $ZnSO_4$ (10^{-1} to 10^{-5} M) using *in vitro* leaf disc cultures on Zn-free WPM basal medium containing 0.0635 µg / g (1.0 µM) Cu

	Zn		Cu	
	µg /g DM	%	µg /g DM	%
Contr.:10^{-1}	53,643.37	100.0	32.47	100.0
10^{-2}	26,822.07	49.9	30.59	94.2
10^{-3}	8,434.80	15.7	13.96	43.0
10^{-4}	907.48	1.7	10.95	33.7
10^{-5}	171.97	0.3	9.31	28.7
ggs11:10^{-1}	51,729.04	96.4	96.32	296.6
10^{-2}	32,124.33	59.9	107.76	331.9
10^{-3}	5,561.96	10.4	32.78	100.9
10^{-4}	1,213.45	2.1	21.16	59.7
10^{-5}	258.73	0.5	16.51	50.8
lgl6:10^{-1}	50,751.02	94.9	37.17	114.5
10^{-2}	25,973.51	48.4	39.87	122.7
10^{-3}	8,601.29	16.0	31.77	97.8
10^{-4}	1,013.98	1.8	24.91	76.7
10^{-5}	218.90	0.4	15.56	47.9

In the case of Cu uptake, an unexpected Zn-stimulated Cu uptake was observed in the transgenic *ggs11* clone with a peak at 10^{-2} $ZnSO_4$ concentration (331.6 %). Enhanced heavy metal uptake in the *ggs11* poplar clone was also found in *ex vitro* experiments. Our results confirm that under *in vitro* conditions the accumulation of Zn and Cu in the transgenic *ggs11* clone is regulated by the *gshI*-transgene and is independent of other physiological or ecological factors such as mycorrhizal symbiosis.

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Transformation of tobacco with an *Arabidopsis thaliana* gene involved in trehalose biosynthesis as a model to increase drought resistance in crop plants

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ABSTRACT: Trehalose plays an important role in desiccation stress protection and its accumulation is an effective way of increasing drought tolerance in both model and important crops using genes of bacterial origin. In this work we aimed to improve desiccation tolerance in tobacco by increasing trehalose accumulation through transformation with the *Arabidopsis thaliana* trehalose phosphate synthase gene (*AtTPS1*) that is involved in trehalose biosynthesis. A cassette harboring the *AtTPS1* gene under the control of the CaMV35S promoter and the bialaphos resistance gene *Bar* as a selective agent was inserted in the plasmid vector pGreen0229 and used for *Agrobacterium*-mediated transformation of tobacco. Several T_0 plants were obtained and analyzed by PCR for the presence of the *AtTPS1* gene. Thirty lines were positive and their seeds were germinated in media with 6 mg l^{-1} PPT to obtain T_1 plants that were grown in the greenhouse to obtain T_2 seeds. T_2 seeds were germinated in selective media (6 mg l^{-1} PPT) and those lines which seeds showed 100 % survival rate were considered homozygous transgenic T_1 lines. Three lines were selected and T_2 seeds were germinated on media with different concentrations of mannitol to score their resistance to osmotic stress. Transgenic plants showed higher germination rates at higher levels of mannitol than did wild type plants.

Key words: *Agrobacterium tumefaciens* – desiccation tolerance – model plant – transformation

Introduction

Three important factors are believed to be determinant for world agricultural production in the 21st century: increase in world population, especially in developing nations, continuous scarcity of fresh water available for irrigation and a continuous deterioration of arable land. These three factors combined suggest the urgent need to dedicate considerable effort to the development of means to improve abiotic stress resistance of crop plants (Siedow 2001).

Trehalose is a non-reducing disaccharide of glucose that has a capacity to stabilize proteins and membranes under stress conditions (Wingler 2002). Engineering trehalose accumulation in important crop plants is considered an important way of increasing their drought and salinity tolerance (Romero et al. 1997). Synthesis of trehalose-6-phosphate synthase (TPS) is considered to be the key factor in the regulation of trehalose biosynthesis, as demonstrated by Romero et al. (1997). Trehalose accumulation in both model and crop plants by means of genetic engineering has been described. The objective of our work is to generate transgenic tobacco (*Nicotiana tabacum*) plants with increased levels of trehalose, via *Agrobacterium*-mediated transformation with a trehalose-6-phosphate-synthase gene of plant origin and to validate the hypothesis that trehalose accumulation improves drought resistance and can be used for transformation of important crop plants. In this paper, we describe the DNA construct and the process of transformation and of obtaining transgenic plants. Some preliminary results regarding stress tolerance to osmotic stress by mannitol are presented as well as considerations and future prospects.

Materials and methods

Plant material and bacterial strains

Tobacco (*Nicotiana tabacum*) cultivar 'Petit Havana SR1' was used. *Escherichia coli* strain DH5α was the bacterial host of every plasmid used for cloning the *AtTPS1* gene. *Agrobacterium tumefaciens* strain EHA105 was used for tobacco transformation. Bacteria were cultured on Luria Broth medium (LB 10 g l^{-1} triptone, 5 g l^{-1} NaCl, 5 g l^{-1} yeast extract).

Construction of vector and transformation

For construction of vector pGreen0229/35S-AtTPS1 to be used for tobacco transformation, the cDNA of *TPS1* gene from *Arabidopsis thaliana* was used. Plasmids used were pJIT60 and pGreen0229 (Hellens et al. 2000). Plasmid pGreen0229 contains a cassette with the bialaphos resistance gene, plasmid pJIT60 contains the CaMV35S promoter with double enhancer regions. Standard cloning and plasmid manipulation procedures were used (Sambrook et al. 1989). Transformation via *A. tumefaciens* was used for tobacco transformation as described by Horsch et al. (1985). After rooting, plants were transferred to the greenhouse in order to obtain T_1 seeds. Plants were screened by PCR for the presence of the *AtTPS1* gene. T_1 seeds of plants positive to PCR were germinated on MS media with 6 mg l^{-1} PPT and surviving plantlets were rooted and transferred to the greenhouse in order to obtain T_2 seeds. T_2 seeds were germinated in Petri dishes with MS media with 6 mg l^{-1} PPT. Those with 100 % germination rate and a total absence of T_2 plantlets sensitive to PPT were considered to have an origin in a homozygous T_1 line. Three transgenic homozygous T_1 lines were selected for stress tolerance assays.

Genomic DNA extraction and PCR analysis

DNA was extracted according to the method described by Dellaporta el al. (1983). Polymerase chain reaction (PCR) was followed on a UNO II (Biometra, Germany) thermal cycler with Taq polymerase (Invitrogen, USA). The following parameters were used: 94ºC for 5 min, followed by 30 cycles of 94ºC for 1 min, 58ºC for 1min, 72ºC for 1 min. Final extension time of 10 min at 72ºC was used. For identification of TPS1 transgenic plants 2 primers were used AtTPS L (5'–GAA TTT GAG GCC AGA TGG ATA G–3') and TPS 2 (5'–TAT CTC AGA CGA AGG GAA TGG T–3'). Primers were designed in order to obtain a 400 bp amplification of the AtTPS1 sequence. PCR products were separated by a 2 % agarose gel electrophoresis.

Stress tolerance assay

T_2 seeds of each of the three homozygous T_1 lines were germinated on MS medium with different mannitol concentrations (0 M, 0.25 M, 0.5 M, 0.75 M). Wild type tobacco seeds were used as control. Seeds were disinfected in commercial bleach for 10 min followed by rinsing in sterile double-distilled water. One hundred seeds were sown per Petri dish and three dishes for each variety were used. Each dish was divided in four sections and germination rates were calculated for each section by dividing the number of germinated seeds by the total number of seeds in each section. Results were compared by multifactorial variance analysis.

Results and discussion

Cloning of the cassette 35S-AtTPS1 into vector pGreen 0229 to be used for tobacco transformation purposes was successful and confirmed through enzymatic restriction (data not shown). Figures 1A and 1B depict regeneration of tobacco plants under selective media. Figure 1A shows the last stage of the regeneration phase. A considerable amount of shoots, resistant to PPT are visible in the right dish while in the left dish non-transformed control

explants present severely damaged necrotic tissue, lacking any sort of shoot formation. In Figure 1B, selected T_0 shoots are growing in jars prior to being transferred to the greenhouse.

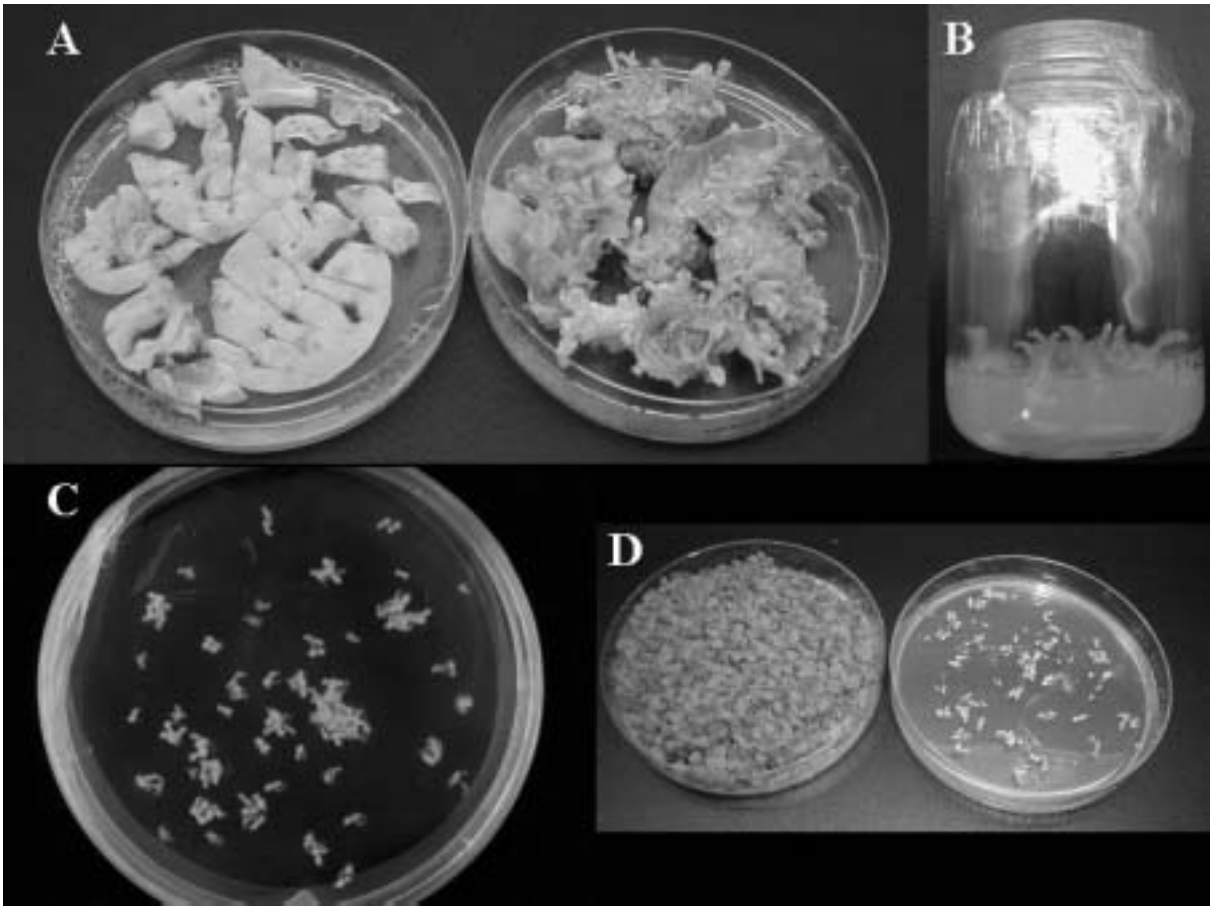

Figure 1. Regeneration and selection of transgenic tobacco plants: (A) Regeneration in selective media (right) of transformed plants as opposed to non-transformed plants (left); (B) Regenerated plants after isolation; (C) Germination of T_1 seeds on MS medium with 6 mg l^{-1} PPT; (D) Germination of T_2 seeds (left) and control (right) on MS medium with 6 mg l^{-1} PPT

A total of 30 T_0 plants were successfully transferred to the greenhouse and tested for the presence of the *AtTPS1* gene by PCR analysis. Wild type SR1 plants were used as control. An example of PCR analysis is presented in Figure 2. As expected, no amplification was registered in wild type plants while several T_0 plants were found to be positive for the *AtTPS1* gene, as indicated by a PCR amplification band of 400 bp. Stunted growth and lancet shaped leaves are frequently reported in tobacco transformed with genes involved in trehalose synthesis (Romero et al. 1997). Abnormal phenotypes were not reported in this experiment although transformed plants were smaller than wild type plants. Seeds from T0 plants (T_1 generation) were germinated on MS medium with 6 mg l^{-1} PPT (Figure 1C), showing both PPT sensitive and resistant T_1 plantlets. Several surviving T_1 plantlets were transferred to the greenhouse in order to obtain seeds (T_2 generation). Germination of such T_2 seeds on media with 6 mg l^{-1} PPT is presented in Figure 1D. Those lines where no PPT sensitive T_2 plantlets were recorded (Figure 1D, left Petri dish) were considered homozygous.

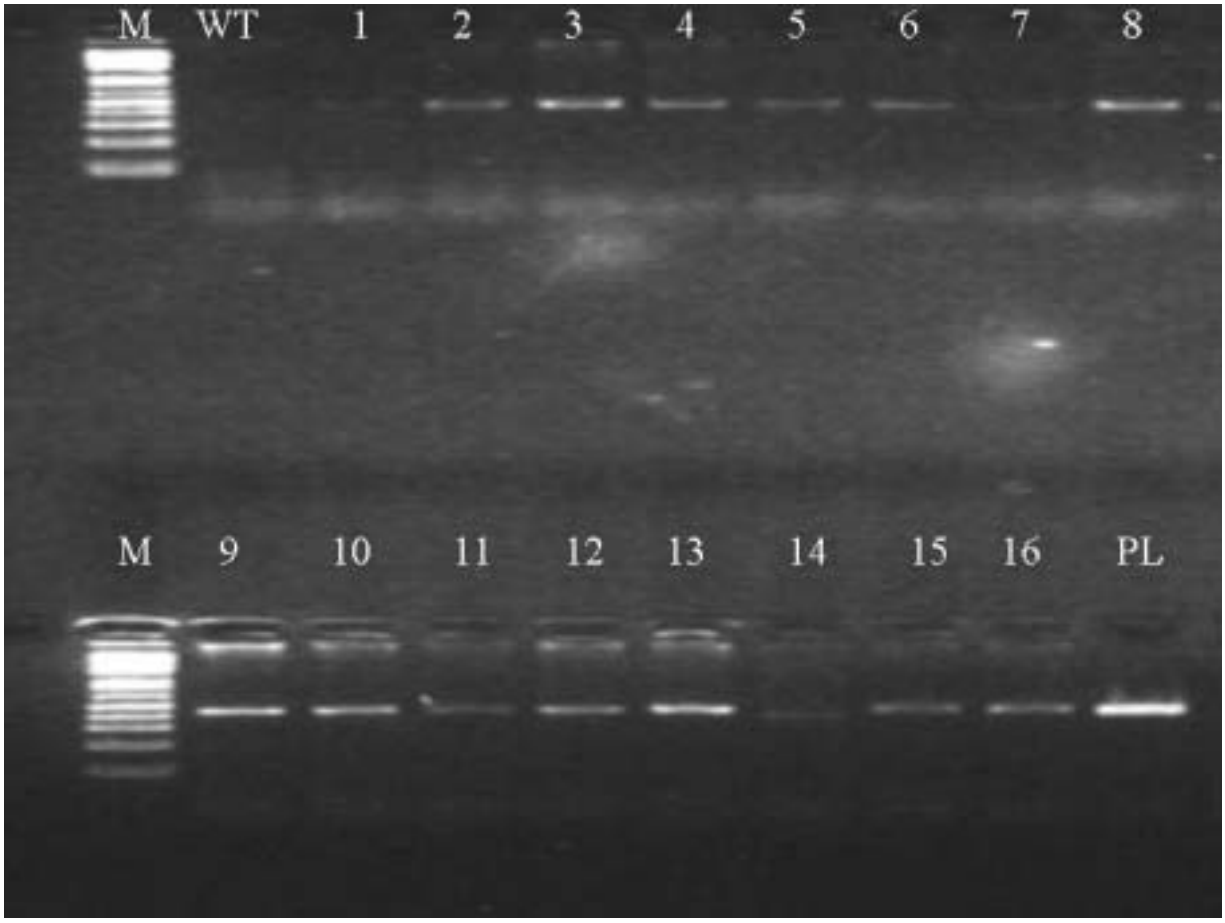

Figure 2. PCR analysis of putative transgenic T_0 tobacco plants using TPS1 and TPS2 primers. M: Molecular weight DNA ladder 1kb+ (Invitrogen); WT: Control wild type plants; Lanes 1-16: putative transgenic tobacco lines. PL – Plasmid construct pGreen0229/35S-AtTPS1 (positive control)

Several homozygous lines were obtained and three were selected for osmotic stress tolerance analysis, using mannitol to induce the osmotic stress. Seed germination of the three transgenic lines and wild type under several mannitol concentrations is presented in Table 1. At a concentration of 0 M mannitol, both transformants and wild type had similar germination rates of 99 to 100 %. With 0.25 M mannitol, germination of wild type seeds was severely affected since only 55 % germinated. Germination of transgenic lines did not seem to be affected by the stress imposed by 0.25 M mannitol as the results obtained were statistically similar to those registered for the same varieties at 0 M Mannitol (94 to 97.5 %). With 0.5 M of mannitol, a germination rate of 8 % for the wild type seeds is statistically inferior to the results obtained at the same concentration for transgenic varieties (33 to 58 %). The stress imposed by the concentration of 0.5 M mannitol affected the germination of transgenic lines as germination rates were lower than those observed at lower concentrations of this inducer of osmotic stress. A mannitol concentration of 0.75 M was lethal to both wild type and transformants, as no seed germination was recorded.

Drought resistance has been reported as a feature of transgenic plants transformed with trehalose biosynthesis genes of bacterial origin (Romero et al. 1997). Our preliminary results indicate that transgenic tobacco plants have higher germination rates under an osmotic stress situation imposed by mannitol than those observed for wild type plants under the same stress conditions. Assuming that our plants may have the ability to withstand drought, we plan to expand the assays of stress tolerance to other osmotic stress conditions (salt, PEG, high

temperatures). To our knowledge, this is the first time that a *TPS1* gene of plant origin (*A. thaliana*) is used in plant transformation and, as a consequence, phenotypes tolerant to osmotic stress are observed. Such results indicate that the construct designed can be used for the transformation of other plants, namely important crop plants such as maize, rice or wheat in order to increase drought tolerance of such plants.

Table 1. Germination rates (%) of three transgenic lines at three mannitol concentrations

Line	Mannitol concentration			
	0 M	0.25 M	0.5 M	0.75 M
WT	99.0^{aA} (1.0)	55.0^{aB} (2.0)	8.0^{aC} (1.0)	0.0^{aD} (0.0)
B5A	99.0^{aA} (1.5)	94.5^{bA} (1.0)	58.0^{bC} (3.0)	0.0^{aD} (0.0)
B5H	99.0^{aA} (2.0)	96.0^{bA} (1.0)	33.0^{cB} (1.0)	0.0^{aD} (0.0)
B1F	100.0^{aA} (2.0)	97.5^{bA} (1.0)	57.0^{bB} (1.0)	0.0^{aD} (0.0)

[A] Lines with different superscripts indicate statistical significance ($p<0.05$); [a] columns with different superscripts indicate statistical significance ($p<0.05$); standard deviations are in parenthesis; WT, wild type plants; B5A, B5H, B1F, transgenic lines

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Generation Challenge Programme: the global effort to apply genomic sciences for the benefit of the resource poor

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Introduction

Farmers in the developing world face agricultural challenges far different from their counterparts in industrialized countries. Malnourishment is a major global problem. Agricultural research and development can and should play a role in the attempts to alleviate poverty and hunger. Major players in applying agricultural research to the resource poor are the so-called 'Future Harvest Centers' of the Consultative Group on International Agricultural Research (CGIAR), including amongst others the International Rice Research Institute (IRRI) in the Philippines and the International Center for Research in Wheat and Maize (CIMMYT) in Mexico. These institutes have been and are conducting and stimulating research and adoption of new crop varieties and practices in countries where the production of staple foods is limited and where agriculture is often the primary livelihood.

While the advances made by the CGIAR institutes and other players averted starvation among millions of people, many experts – including the poor farmers themselves - believe that the pace of improvement is too slow. The causes for concern are many:

- The rate of increase in potential and realized productivity of key crops under favourable conditions is leveling off.
- Rural and urban populations continue to grow.
- Chronic environmental stresses continue to limit productivity.
- Catastrophic events, such as war, floods, sustained drought, and fire, cause nearly total losses in crops, which in most countries are not buffered by food reserves.

Creating the varieties that can better cope with the stresses in the given settings (in terms of society, farming system, etc.) appears to be a major problem. More effective methods of identifying useful genetic resources, and of using these resources are urgently needed. The genomic sciences coupled with the developments in the information and communication technology might offer a solution.

The developments in the genomic science, often referred to as the genomics revolution, have yielded unprecedented quantities of information about biological systems and a new understanding of the functioning of plants. Parallel to this 'revolution' another revolution has taken place, that in the field of information and communication technology. This 'revolution' resulted in unprecedented abilities to store, exchange, access, and process data. Together they offer the ability to uncover new biological phenomena at the gene level. Certainly it appears possible to use knowledge generated on one species on widely different species, since the processes at the level of genes appear to be strongly conserved. This genetic homology (synteny) among widely different species and across species, genera, families, orders, and kingdoms unlocks genetic diversity in completely new ways enabling new diversity to be used in crop improvement. Many believe that this new approach, properly harnessed and vigorously applied, will take crop performance to new levels, just as hybrid vigor, crop

morphology, mechanization of field research, computers, and crop management interventions have done over the past eight decades.

Obviously this new approach to crop improvement is highly relevant to developing countries in their fight against hunger and malnutrition. However, the developments in these technologies have been primarily based on private initiative, and as a result ownership issues play an important role in the application. Furthermore, many of the applications of the new technology require vast investments both in equipment and knowledge. These factors make the promising results of the two technological revolutions difficult to use for those who need it most.

A new initiative

The Generation Challenge Program (GCP, initially called "Unlocking Genetic Diversity in Crops for the Resource-Poor") hopes to develop and use the new approach to crop improvement in a way that will contribute to the solution of the problems described above. It unites a wide range of actors in its effort to alleviate poverty by applying advances in the biological and computer sciences to the agricultural constraints of the developing world. It will produce a new, unique public platform for accessing and developing new genetic resources using advanced molecular technologies and traditional means. The GCP will also ensure that the technologies developed will remain in the public domain, as far as possible.

The consortium

The GCG involves three groups of partners, who have committed themselves to work on unlocking genetic diversity with molecular tools and use it to improve the productivity and sustainability of farming systems throughout the world:

- The CGIAR Centers, who, in addition to their molecular research, hold in trust for the world vast amounts of plant diversity, the basic resource for crop improvement.
- The national agricultural research systems (NARS) of developing countries are the primary experts on assessing and breeding plants under their own conditions, in consultation with the farmers for and with whom the work is undertaken.
- The advanced research institutes (ARIs), both public and private, of the developed world are developing the novel molecular techniques and strategies to decode diversity, such as that held by the CGIAR Centers and the NARS.

By capturing the synergies that result from this type of broad-based collaboration, the GCP will contribute to increasing the rate of potential and realized productivity for keystone crops in marginal environments.

The approach

The research of the GCP is focused in three areas, separate but interconnected:

(1) The genetic resource collections will provide the raw materials for crop improvement. However, tools and techniques need to be developed to identify useful genetic variation among these germplasm collections (held by the CGIAR centers and elsewhere), and these tools need to be applied to identify and characterize this genetic diversity.

(2) Genomic science provides the means to identify and better understand the mechanisms that cause the desired traits. For this purpose, comparative and functional genomics will be used to identify genes and pathways to use in crop improvement programs, and identify marker systems to speed up selection for these.

(3) Crop improvement applies traditional and modern methods of gene/allele transfer into functional crop varieties, utilizing knowledge of gene function and location to improve efficiency and scope of breeding programs for formally intractable traits.

To support these research areas, a vital area can be added to this list:

(4) Bioinformatics in the wide sense, using the advances in information and communication technology to allow an optimal use of information and analysis tools, irrespective of their location on the globe: develop integrated crop genetic resources, improvement, and bioinformatics systems to facilitate and optimize implementation of the discoveries.

And finally, to make sure the knowledge is properly managed:

(5) Capacity building, a major goal of this Challenge Programme. The platform that will be developed in the GCP will provide the materials and technology for application to research and applied plant breeding. For NARS scientists to utilize the materials and technology in their own research and plant breeding programmes, considerable capacity-building will be organized.

The GCP will operate over two phases of approximately five years. In the first the GCP will acquire and/or develop the tools and techniques needed to identify useful genetic variation among the collections held by the CGIAR Centers and elsewhere. It will identify genes and pathways to use in crop improvement programs, identify marker systems to speed selection for these, and develop integrated crop genetic resources, improvement, and bioinformatics systems to facilitate and optimize implementation of the results. The feasibility of this integrated, collaborative approach will be demonstrated by the case study on drought tolerance. In the second phase, optimal alleles and novel genes identified in the first phase will be incorporated into elite breeding materials and locally adapted landraces in the most efficient way and in partnership with NARS. The new lines will ultimately be passed to farmers for comprehensive assessment in concert with NARS. New systems for producing and disseminating seed will also be tested and promoted. Improvements gained in some crops will be transferred to other crops, which are vital for better nutrition and for the economic health of farming systems. In both phases, information and materials developed will be freely available to the resource poor.

Fair access and benefit sharing will be in harmony with the Convention on Biological Diversity and the International Treaty on Plant Genetic Resources for Food and Agriculture. It is important to emphasize that the applications of the Challenge Program are generic. They can and will be used for any crop, any gene, and any trait, although, as noted, the Challenge Program proof-of-concept will focus initially on studying the tolerance of the major food crops to drought-stressed environments. The GCP works across four crop groups: cereals, root and tuber crops, legumes and *Musa* and forage species.

Demonstrating the concept: drought tolerance

To demonstrate the application of the GCP's output, a problem of global importance has been chosen: drought tolerance. At the basis of this choice that is to be used as a proof-of-concept, several factors have been considered. It had to be a problem that was important in many places and for many crops, to allow the involvement of a broad spectrum of scientists and institutions within and outside the CGIAR. Other element in the choice were the progress achieved through more conventional plant breeding efforts, the possibilities for future gains, the level of past and current investment in research, and the likelihood that a comparative genomic approach would result in a positive outcome.

Drought is a problem faced by farmers all around the world. It is one of the oldest and most pervasive threats posed to agriculture by the environment. And the threat of drought is likely to increase with the increasing lack of water due to the rising demand for non-agricultural uses, but also as a result of climatic changes.

Breeding for drought tolerance has been only moderately successful. It is a complex trait and tolerance is a trait that involves quite different processes in different situations. Our understanding of these processes is still in its infancy, although it is advancing rapidly in several species, especially the cereals and *Arabidopsi*s. Most studies focus on one species. The GCP will apply genomic tools to better understand drought tolerance mechanisms across a subset of the crop species.

In conclusion

The Generation Challenge Programme for Cultivating Plant Diversity for the Resource-Poor will use plant genetic resources to improve livelihoods and increase food security in developing countries. It will do so by enhancing the use of genetic resources in particular in breeding programmes through innovative initiatives to generate, manage, and apply genomic information derived from comparative studies. It will enhance the public domain as the best means to ensure fair access and benefit sharing for resource-poor farmers.

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Breeding by Design

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Abstract

The development of PCR-based DNA marker technologies together with appropriate robotics and software tools, has enabled the cost-effective production of large marker data-sets and the broad implementation of markers in breeding. Moreover, large-scale application of DNA markers for a wide variety of agronomic traits has increased our understanding of ongoing genetic processes during the execution of breeding programs. Application of markers in breeding enables the reduction of cost, space and time in a breeding program. More interestingly, the knowledge on the genetic basis of complex traits in concert with the unlimited access to DNA markers enables the breeder to create new varieties that would not have been obtained without the utilization of markers. This process of marker assisted breeding can now be extended to controlling all relevant genes in the genome.

Breeding by Design is a concept which aims at controlling all allelic variation for all genes of agronomical importance. This concept can be achieved through a combination of large-scale genetic mapping, high-resolution chromosome haplotyping and extensive phenotyping. Thanks to marker technology, software tools and know-how available today, this goal can be achieved now. Depending on the crop specific generation time, controlled marker assisted selection strategies can lead to the production of superior varieties within five to ten years.

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Development of near-isogenic lines for validation of QTL for Fusarium head blight resistance

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Abstract

Fusarium head blight (FHB) is one of the most serious fungal diseases which threat wheat (*Triticum aestivum* L.) producers and consumers world-wide. Marker assisted-selection (MAS) seems one of the promising possibilities to obtain regionally adapted and FHB resistant wheat cultivars. Two major QTL were previously found on chromosomes 3B and 5A in 'CM-82036' and these regions are well covered with SSR markers.

The objectives in this work are quantifying the effectiveness of MAS by using linked SSR markers, evaluating the effects of two QTL: *Qfhs.ndsu-3BS* and *Qfhs.ifa-5A* in near isogenic lines in different genetic backgrounds (highly susceptible to moderately resistant), and developing adapted winter wheat lines with all possible combinations of two QTL for FHB resistance derived from 'CM-82036'. To achieve these goals different populations of wheat were created by crossing 'CM-82036' as donor plant with 15 lines and cultivars of winter wheat (recurrent parents) differing in their genetic background to produce F_1. F_1 plants were back-crossed with their recurrent parent. Approximately 30 BC_1F_1 seeds were planted and genotyped with flanking SSR markers (*Gwm389*, G*wm493* and G*wm533* were used as flanking markers for the 3B QTL, and G*wm156*, G*wm293* for the 5A QTL). The BC_2F_2 plants were screened with the same SSR markers, and only plants homozygous for resistance and susceptible alleles with different combinations for each cross (3B3B5A5A, 3B3B5a5a, 3b3b5A5A, and 3b3b5a5a) were selected and selfed to produce near-isogenic lines. These seeds ($BC_2F_{2:3}$) will be sown in the field and inoculated artificially with *Fusarium graminearum* for FHB resistance testing in 2004/05.

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QTL mapping and validation of Fusarium head blight resistance in the spring wheat cultivar 'Frontana'

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Abstract

Fusarium head blight (FHB) is a devastating disease of wheat in many areas of the world. The main threat associated with the disease is the contamination of cereals with mycotoxins. Complex inheritance of resistance and confounding environmental effects have hampered progress in FHB resistance breeding. Identifying resistance genes and understanding the complex genetic structure of FHB resistance will greatly enhance breeding for FHB resistance.

The Brazilian spring wheat cultivar 'Frontana' is a widely used FHB resistance source. Molecular mapping of a 'Frontana/Remus' doubled haploid population led to the identification of several FHB resistance QTL. Effects on chromosomes 3A and 5A showed consistent association with FHB severity and accounted for 16 % and 9 % of the phenotypic variation, respectively. The study indicated that FHB resistance of 'Frontana' primarily inhibits fungal penetration, but has minor effect on fungal spread after infection.

To validate the two major FHB resistance QTL of 'Frontana' on chromosomes 3A and 5A, a population of 110 F_7 recombinant inbred lines from a cross of 'Frontana' and 'Inia 66' (FHB susceptible) was evaluated for FHB resistance after spray inoculation in the field. The severity and incidence of the disease were assessed by visual scoring. The population was genotyped with 320 DNA markers. The aim is to verify molecular markers linked to the major FHB resistance QTL of 'Frontana' on chromosomes 3A and 5A for marker assisted selection, e.g. for combining FHB resistance QTL of 'Frontana' and Asian wheats.

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Molecular mapping of Fusarium head blight resistance in two winter wheat populations using AFLP and SSR markers

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Abstract

Fusarium head blight (FHB), mainly caused by *Fusarium graminearum* and *F. culmorum*, can significantly reduce wheat grain yield and quality. In this study quantitative trait loci (QTL) associated with FHB resistance were identified in two winter wheat recombinant inbred line (RIL) populations. These two mapping populations were developed by crossing (1) resistant line G16-92 with susceptible cv. 'Hussar', and (2) resistant cv. 'Dream' with susceptible cv. 'Lynx', respectively. Both populations were evaluated in the field to their response to spray inoculation of a *F. culmorum* suspension. The field trials were performed in 2002 in four environments. For the construction of genetic maps the RILs were genotyped using about 600 AFLP and 40 SSR markers for each population. For the detection of resistance, QTLs composite interval mapping was applied for the mean value across the environments of the recorded FHB symptoms.

In the G16-92/Hussar population three QTLs associated with FHB resistance were located on the chromosomes 1AS, 1BS and 2BL explaining 13.5 %, 7.7 % and 17.3 % of the phenotypic variation, respectively. Four FHB resistance QTLs were identified in the population Dream/Lynx on the chromosomes 6AL (16.7 %), 1BS (14.9 %), 2BL (10.3 %) and 7BS (17.4 %). The resistance QTL on chromosome 1B was common in both populations and associated with the rye arm of a 1RS.1BL translocation. The resistance QTL on 2BL is located in a similar chromosomal region in both populations.

The resistance QTLs on 6AL and 7BS identified in the Dream/Lynx population and the QTL on 2BL in the G16-92/Husssar population were linked with SSR markers. In order to validate the association of these three SSR markers with FHB resistance, a population with an independent genetic background was developed by a four-fold crossing approach. Phenotypically resistant lines of both mapping populations were crossed with two highly susceptible winter wheat cultivars. The F_1-progeny of about 600 lines was classified with the three SSR markers to determine which lines possess the different possible combinations of resistance QTLs in the heterozygous state. The 600 lines were evaluated in 2002 after spray inoculation with *F. culmorum* in four environments. Examining the marker classes and the phenotypic data revealed a shift towards resistance of lines carrying all three markers linked to the corresponding resistance QTLs compared to lines without these markers.

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Spontaneous 7BS.7RL translocation in F_1 triticale x tritordeum hybrids

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Abstract

Many authors reported the production of different wheat-rye translocation (e.g. 1BL.1RS, 1AL.1RS, 2BS.2RL, 6BS.6RL) and substitution lines. The interest in these lines for plant breeders is the possible transference of useful agronomic characteristics from rye to wheat. Another breeding strategy consists in the production of multigeneric hybrids by interspecific sexual hybridisation. In our work, we detected a spontaneous wheat-rye translocation in all mitotic cells of one triticale 'Douro' x tritordeum HT9 ($AABBRH^{ch}$, 2n = 42) F_1 hybrid plant, after fluorescent *in situ* hybridisation using total genomic DNA from *Hordeum chilense* and rye as probes. We aimed to identify the rye and wheat chromosomes involved in the translocation. For that purpose, we reprobed the chromosome spreads with the repetitive DNA sequences pTa71 and pSc119.2 which allowed the identification of nine rDNA loci and the discrimination of all rye chromosomes, respectively. The pSc119.2 also weakly hybridised on some wheat chromosomes. The wheat-rye translocation was identified as being 7BS.7RL after comparison of the *in situ* hybridisation patterns obtained with those previously published. As far as we know this translocation was never referred in scientific literature. Although it occurred spontaneously, this translocation could incorporate useful rye genes in the wheat background, such as the leaf-rust resistance, located on chromosome 7RL. The multigeneric F_1 triticale x tritordeum hybrids could be an interesting starting material for the production of new translocation lines. The fluorescent *in situ* hybridisation technique proved to be useful both for parental genome discrimination and for intergenomic translocation identification.

Acknowledgements

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Molecular dissection of transpiration efficiency, early vigour and reduced tillering in wheat (*Triticum aestivum*)

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Abstract

Using water more efficiently (greater transpiration efficiency), reducing water loss from the soil surface (early vigour) and partitioning more assimilates into fertile shoots (reduced tillering) are important goals for crops in temperate Australia. We have identified important variation in wheat for the above traits and have developed specific segregating families so as to develop linked markers and to investigate their molecular basis. We are using the level of carbon isotope discrimination as an estimate for transpiration efficiency and identified several putative chromosomal regions in wheat that are associated with high C_{13}/C_{12} ratios in the leaf. To improve early vigour we are replacing widely used dwarfing genes (*Rht-B1b* and *Rht-D1b*) with alternative GA responsive dwarfing genes that have no known negative effects on early growth. We have developed 'perfect markers' to select against *Rht-B1b* and *Rht-D1b* genes and identified chromosomal locations and linked markers for a number of new dwarfing genes (*Rht4*, *Rht12* and *Rht13*) that are currently being evaluated as replacements. To further enhance early crop growth, we are selecting for genes that produce greater leaf area and longer coleoptiles. We identified one region on chromosome 6 that is associated with greater leaf width – the most heritable component of early vigour. The same chromosomal region may also be associated with longer coleoptiles. Robust markers are currently being developed to validate this association in different genetic backgrounds. All Australian wheat varieties produce more tillers than they can sustain to grain maturity representing a waste of water and nutrients which could otherwise be invested into fertile spikes. We identified a single gene that reduces tillering (known as tiller inhibition gene, *tin*) and increases kernel size. Appropriate genetic material was developed through backcrossing that allowed the mapping of the tiller inhibition gene to chromosome 1AS, tightly linked to microsatellite marker gwm136. This marker has been validated across a wide range of germplasm and is being used to transfer *tin* in the Graingene crop improvement program.

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A significant improvement of wheat transformation through optimization of *in vitro* culture conditions

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Abstract
Poor plant regeneration is often the limiting step for the efficient genetic transformation of wheat, especially of elite genotypes. Through optimizing three critical factors of *in vitro* culture, carbohydrate source, hormones for callus induction, and hormones for shoot regeneration, we have developed an efficient biolistic transformation protocol for both, winter wheat 'Certo' and spring wheat 'Bobwhite'. Immature embryos of both cultivars were used as source material. They were harvested and pretreated according to the protocol published by the Montana State University (MSU). Plasmid DNA containing the *bar* gene under the control of *ubi* promoter was delivered to the embryos via particle bombardment. The bombarded embryos were cultured on MS based medium with 1 mg/l 2,4-D for induction of embryogenic callus, then on medium with 4 mg/l thidiazuron for shoot regeneration. The carbohydrate source in the media during these two phases of culture was 6 % sucrose, instead of 3 % maltose used in the MSU protocol. By using the improved protocol, more plants with normal phenotype and tolerance to L-phosphinothricin have been obtained from both cultivars. PCR analysis of regenerants for the presence of the *bar* gene showed a transformation frequency of 3.9 % for 'Certo' which is almost 7 times higher than what could be obtained through using the MSU protocol. For spring wheat, 'Bobwhite', PCR analysis is ongoing, yet the number of PPT tolerant plants showed a 70 % increase over the previous protocol.

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Isolated microspore culture in wheat (*Triticum aestivum* L.)

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Abstract

Green haploid plants were generated via wheat micropore culture, and the effect of stress factors on the induction and course of androgenesis was studied (Pauk et al. 2003). The experiments were carried out on the spring wheat genotype CY-45. The effect of cold pre-treatment on the induction of androgenesis was studied and a two-week cold pre-treatment on the donor heads was found to have a positive effect.

Modified AA medium, which had given good results in previous experiments on somatic cell culture, was used to co-culture with ovaries (Poulimatka et al. 1996). The AA medium was supplemented with two different hormone components. Embryoid production was significantly higher with the 2,4-D (6.8 mmol/L) and kinetin (2.3 mmol/L) hormone combination than the medium supplemented with 2,4-D (4.5 mmol/L) alone.

Albino and green plantlets were regenerated from induced embryoids. A significant proportion of the regenerants of all the embryoids (234) obtained in microspore culture only three regenerated green plantlets. After these were grown in the greenhouse, tests proved that they were haploids. Further examinations will be focused on raising the ratio of green/albino plantlets.

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Genetic diversity for resistance to SBWMV in durum wheat: a phenotypic and molecular analysis

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Abstract

Soilborne wheat mosaic virus (SBWMV), a furovirus transmitted by *Polymyxa graminis* Led., is the causal agent of an important wheat disease in North America, Europe and Asia. In the presence of high infection levels and favourable conditions for its development, SBWMV may reduce grain yield by as much as 80 %. Various studies suggest that in bread wheat (*Triticum aestivum* L.) the genetic control of resistance is mono- and/or oligogenic. However, durum wheat (*T. durum* Desf.), one of the most important crops in Italy, undoubtedly expresses a wide range of responses to SBWMV and relatively few cultivars are either highly susceptible, e.g. 'Vesuvio' and 'Simeto', or highly resistant, e.g. 'Ionio' and 'Neodur'. Previous investigations on the genetic diversity and linkage disequilibrium (LD) in the durum wheat cultivated germplasm showed the presence of detectable long-range LD levels, extending on a cM-wide scale, thus rendering these materials suitable for gene/QTL discovery and/or validation through association mapping analyses by whole genome scan with molecular markers. Presently, we are characterizing a collection of 114 durum wheat cultivars, representing the world's major cultivated gene pools**,** both at the phenotypic level, i.e. in terms of SBWMV response, as well as at the molecular level**,** using mapped dinucleotide SSRs selected for their reliability, level of polymorphism and genome coverage. The collection is being characterized in a two year experiment distributed according to a randomized complete block design (two replicates) in a field, highly and uniformly infected of SBWMV, located in the Po Valley, near Bologna. Each cultivar is being evaluated for symptom severity and virus concentration on 2 - 3 dates per season, during the late tillering stage; cv. 'Grazia', highly susceptible to SBWMV, is used as the susceptible control. The SSR data will be used to investigate the population structure present within the collection, in order to account for it in the association analysis and thus reducing the occurrence of false positive associations. The LD analysis should allow the identification of the markers linked to the chromosome regions with major effects on SBWMV resistance. This will provide the molecular tools necessary for marker-assisted selection (MAS) actions directed toward improving resistance to SBWMV.

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Introgression of a grain protein content QTL from *Triticum turgidum* subsp. *dicoccoides* in durum wheat

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Abstract
Grain protein content (GPC) and gluten quality are the most important factors affecting pasta-making technology characteristics and resistance to overcooking. The relationship of protein content to cooking quality is complex and is influenced by other factors, but generally as protein content increases, pasta becomes firmer and less sticky. Breeding efforts to increase grain protein content have been marginally successful and the production of high protein grain is usually obtained through high rates of nitrogen fertilizers. The lack of sufficient genetic variation within the cultivated wheat has limited the ability of plant breeders to improve grain quality. A good source of genetic variation has been found in wild wheat and related species. Particularly, *Triticum turgidum* subsp. *dicoccoides,* a wild relative of cultivated durum wheat, contains a large reservoir of high GPC genotypes. Quantitative trait loci (QTLs) influencing protein concentration in cultivars and wild wheats have been found located on all chromosomes. Seven QTLs for GPC were detected in a recombinant inbred line population derived from the cross 'Messapia' x *T. turgidum* subsp. *dicoccoides*. This study was initiated to introgress alleles for high grain protein content (GPC) from *dicoccoides* into more adapted and agronomically acceptable durum wheat germplasm and to identify PCR-based markers to be efficiently used in marker-assisted selection. A high GPC recombinant inbred line was backcrossed twice to 'Messapia' and the BC_2F_3 progenies were evaluated in three replicted field trials for GPC and some grain yield components. The analysis of variance revealed highly significant differences ($P<0.01$) among BC_2F_3 progenies for grain protein content. The pattern of variability was typical of a quantitative trait, suggesting a GPC control by more than one segregating genetic factor. Ten high and ten low GPC progenies with similar grain yield per spike were selected for making two DNA bulks of extreme phenotypes to be screened according to the bulked segregant analysis. Fifty-five primer pairs of microsatellite markers localized on chromosome regions known to be involved in the control of GPC were used to amplify bulked and parental DNA simultaneously. Three polymorphic markers between the parents were also found to be polymorphic in the two bulks and then screened on the complete population of BC_2F_3 progenies. The putative linkage with protein content QTLs was tested by simple regression analysis of each marker locus on the F_3 population. The marker analysis indicated a short region on chromosome 4B having a significantly effect on GPC. Selection for the markers associated to the GPC would be effective in introgressing the QTL(s) into wheat breeding programs.

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Genetic Variation for Plant Breeding, p. 305 (2004)

Individuation of powdery mildew resistance genes from *Triticum turgidum* subsp. *dicoccum*

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Abstract
Powdery mildew is a common disease induced by the biotrophic fungal pathogen *Blumeria graminis* f. sp. *tritici*, which causes considerable damage to wheat cultivations, particularly in areas with temperate climates. The most environmentally safe way to control powdery mildew is the cultivation of resistant varieties. However, due to the pathotypes continuous evolution, the resistance against a specific strain of the pathogen usually becomes ineffective within a very short period and new sources of resistance are requested. Up to now, more than 30 loci conferring resistance to powdery mildew were individuated in the wheat gene pool and some of these loci have been associated to molecular markers. Molecular markers (SSR AFLP, RAPD, RFLP etc.) are very useful to study segregant populations for monogenic and polygenic traits.

Triticum turgidum subsp. *dicoccum* is a good source of powdery mildew resistance. Previous germplasm screening individuated accession MG5323 very resistant to powdery mildew both in greenhouse to different isolates and in field conditions to the natural population. The accession MG5323 was crossed to *Triticum turgidum* subsp. *durum* cv. 'Latino', susceptible to powdery mildew. A set of 120 recombinant inbred lines (RILs) was produced by single seed descent method. RILs were tested for resistance on field conditions using a randomised complete block design of 120 plots with 3 replications. Adult plant resistance assays were performed at the end of stem extension using a modified 0 - 4 scale, where the level of infection reflects the percentage of plant surface infected. The distribution frequency suggested the presence of more than one locus involved in the control of powdery mildew resistance.

Eight resistant and eight susceptible RILs were used in bulked segregant analysis. Two hundred microsatellites were assayed between the two parents, showing a polymorphism of 50 %. Polymorphic markers were analysed between the two bulks. The SSR WMC25, polymorphic between resistant and susceptible bulk, was screened on the complete set of RILs. The regression analysis showed the linkage of WMC25 with powdery mildew resistance. The present study indicated the presence of a major locus influencing the powdery mildew resistance, identified by the molecular marker WMC25 located on the short arm of chromosome 2B.

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Construction of a linkage map using a rye doubled haploid population

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Abstract

A preliminary linkage map of rye (*Secale cereale* L.) was constructed using a doubled haploid (DH) population from a cross between doubled haploid parents cv. 'Voima' and cv. 'Amilo'. The population contained 90 DH individuals. The DHs were produced by an anther culture method mainly as described in Immonen & Tenhola-Roininen (2003). Different marker types were used when building the map: microsatellites, RAPDs (Random Amplified Polymorphic DNA), IRAPs (Inter-Retrotransposon Amplified Polymorphism) and REMAPs (Retro-transposon-Microsatellite Amplified Polymorphism). All seven linkage groups of rye were identified. The DHs were crossed to cv. 'Riihi' to get seed for α-amylase activity measurements. α-Amylase activity is negatively correlated with pre-harvest sprouting. Three QTL (quantitative trait loci) affecting pre-harvest sprouting were found.

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Detection and mapping of SSRs in rye ESTs related with aluminium tolerance

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Abstract
Aluminium toxicity is considered to be a major problem for crop production on acid soils. Plants species differ in their Al tolerance, some are inherently more tolerant than others. Rye (*Secale cereale* L.) has one of the most efficient groups of genes for aluminium (Al) tolerance among cultivated species of *Triticeae*. This tolerance is controlled by at least three independent and dominant loci (*Alt1*, *Alt2* and *Alt3*) located on chromosome arms 6RS, 3RS and 4RL, respectively. In order to obtain simple sequence repeat (SSR) markers related with Al tolerance more than 1189 publicly accessible rye cDNA sequences from Al-stressed roots were exploited as a resource for SSR marker development. A total of 12 new *S. cereale* microsatellite (SCM) loci were located using wheat-rye addition lines or mapped using an F_2 segregating for Al tolerance. Several interesting EST-derived SCM loci related with aluminium tolerance were located on chromosomes 1R, 2R, 3R, 4R and 5R. Moreover, five of these SCM loci could be associated with proteins of known or unknown function. Finally, a new Al tolerance gene (*Alt4*) was detected on rye chromosome 7R and a map of this chromosome with the *Alt4* gene, sixteen SCIM and RAPD markers and two SCM markers was obtained.

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Use of SCAR markers for cytoplasm identification in rye (*Secale cereale* L.)

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Abstract
In cultivated rye three genetically different cytoplasms are known - the normal N-cytoplasm and two sterility-inducing ones: Pampa (*cms*-P) and Vavilovii (*cms*-V). Cytoplasm identification in rye is routinely performed using a laborious and time-consuming plasmotype/genotype interaction test. The purpose of our study was to determine if recently discovered SCAR markers (Szklarczyk et al. – in preparation) could serve as an alternative method of cytoplasm genotyping in rye.

The study included 16 inbred lines with known type of cytoplasm and 172 randomly chosen single plants originating from 4 Turkish ('Candar', 'Ancora', 'Turkey 75', 'Harlan I.R. 6982') and 4 South American ('Don Enrique', 'Pasteoro Massaux', 'Pico Gentario', 'Pico Mag') open-pollinated cultivars. The cytoplasm type (normal vs. sterilizing) was determined using both the conventional plasmotype/genotype test as well as the SCAR markers. Additionally, 31 plants known to carry the sterilizing cytoplasm were analyzed by conventional testing in order to specify the source of CMS (Pampa vs. Vavilovii).

For all tested inbred lines the profile of SCAR markers confirmed the known plasmotypes. Similarly, full agreement between conventional testing and PCR assay was found for plants originating from OP cultivars. The normal N-cytoplasm was found only in 63 out of 172 tested plants. All N-cytoplasmic plants represented Turkish cultivars. Sterility-inducing cytoplasms were present in 7 tested populations. Only cultivar Ancora appeared to be free of plants carrying sterilizing cytoplasm.

In 29 cases discrimination between P- and V-cytoplasm yielded consistent results for both methods of testing. Among those, the majority of South American materials represented Pampa cytoplasm, while Turkish CMS sources were assigned to *vavilovii* type. However, for another two plants (one from 'Pasteoro Massaux', the other from 'Harlan I.R.6982') inconsistent results were obtained with these methods. Use of conventional testing assigned both plants to Pampa type, whereas the SCAR markers indicated the presence of Vavilovii cytoplasm. Explanation of this discrepancy will be addressed in future research. Despite these two cases, general agreement between genetic and molecular data shows that our SCAR markers can be very useful for cytoplasm identification in cultivated rye.

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Doubled haploids and genetic mapping in barley, rye and oat

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Abstract

Production of doubled haploid plants through anther culture offers possibilities for both efficient genetic mapping and breeding. At MTT Agrifood Research Finland we have developed anther culture methods for barley, rye and oat (Manninen 1997, Immonen & Tenhola-Roininen 2003, Kiviharju et al. 2000). Although genotypic differences in anther-culture response still exist between genotypes, these methods work on a wide array of genotypes. In barley, anther culture has been used to produce several doubled haploid progenies for genetic mapping of agronomic and resistance traits (Manninen 2000a, Manninen et al. 2000). Doubled haploids have been produced from F_1, F_2 and BC_3 progenies. Segregation distortion has been detected in many cases and at least some of the distorted genomic regions have been shown to control anther-culture response (Manninen 2000b). Boreal Plant Breeding Ltd. currently uses this anther culture method in their barley breeding programmes. In rye, a doubled haploid progeny has been produced for mapping purposes and loci affecting pre-harvest sprouting have been located on the preliminary linkage map. At Boreal Plant Breeding, selected doubled haploids homozygous for a dominant dwarfing gene have been used in pair crosses in rye breeding. In order to locate genes affecting quality traits in oat, a doubled haploid progeny has been produced by anther culture. In addition, genetic markers associated with anther-culture ability have been recognized in a cross between oat and wild red oat (Kiviharju et al. 2004).

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Development of a TILLING resource in barley

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Abstract
TILLING (Targeting Induced Local Lesions IN Genomes), first described by McCallum et al. (2000) is a reverse genetics technique that can identify a series of alleles for a target gene. TILLING can be adapted for use in a relatively high-throughput facility and can be applied to virtually any organism for the detection of either natural or induced variation. We are in the process of developing a TILLING facility for identifying mutations in barley genes. We choose to produce a reverse-genetic resource in barley because this species is both of significant economic importance and a good model for any *Triticeae* species. A mutagenized barley population (variety 'Morex') has been obtained by sodium azide (NaN_3) seed treatment. Three concentrations of NaN_3 (1, 5 and 10 mM) have been tested on a total number of ca. 60000 treated seeds which were then sown in the field. The effects of the different mutagen concentrations were evaluated on coleoptile elongation rate and frequency of chimeric sectors on M_1 plants, fertility of M_1 spikes and germinability of M_2 seeds. In all cases, the 10 mM NaN_3 concentration was the most effective. The TILLING resource is currently being tested using a sub-set of ca. 1600 M_2 plants in order to verify the effectiveness of the mutagenized population obtained. A screening system based on fluorescently-labeled PCR primers, digestion with CELI nuclease and detection with a Licor automatic sequencer is being implemented. Within the mutagenized population a large number of morphological mutants have also been identified and are being characterized.

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Genotyping of the intron III – exon IV region polymorphisms of the β-amylase gene *Bmy1* in north European barley varieties

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Abstract

Allelic diversity and inheritance of polymorphic sites of the intron III - exon IV region of the seed specific β-amylase gene *Bmy1* were studied in a set of 55 barley accessions composed mainly of old Latvian and Scandinavian commercial varieties and three *Hordeum spontaneum* lines from Israel. A CAPS-marker was used for genotyping the $C^{698}\rightarrow T$ polymorphism encoding alleles of β-amylase with different thermostability. The genotype C^{698} which is diagnostic for a more thermostable isoform of the β-amylase was detected in 13 of the investigated accessions. In most cases the origin of the C^{698} genotype could be traced back to the old Danish variety 'Binder' in the pedigree. However, this genotype was lost in later varieties originating from 'Binder'. A 6+1 bp deletion event in intron III of the β-amylase gene was in all cases linked to the presence of the C^{698} mutation, while the repeat number of a microsatellite in intron III had no correlation to the presence of the C^{698} mutation. Sequencing of the microsatellite $(TG)n(G)_n$ of intron III revealed several new motifs additionally to the previously reported. Sequence analysis of a number of haplotypes within exon IV did not result in amino acid changes due to the degenerated genetic code.

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New barley mutants in phenological traits

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Abstract

In many members of the *Triticeae*, there is genetic variation for growth habit, which can broadly be divided as winter and spring types. Winter types differ from spring types in their requirement for a long period of low temperature, known as vernalisation, to become competent to flower. To obtain mutants that show different phenological response, pure seed of the 2-row winter malting barley 'Angora' was treated with N_3Na, 10^{-3} M. The M_2 generation was sown in late April. Most of the 300.000 plants and all of the untreated control plants remained vegetative during summer. Only 54 plants reached the flowering stage and were selected. Plants that segregated in the next generation or showed other mutations were discarded and thus 23 mutants were selected to be evaluated in glasshouse experiments together with 8 F_1-generations from the cross of the most promising mutants with the wild type. Four treatments were applied combining a vernalised and unvernalised period with long and short photoperiod. Several of the mutants showed typical spring response, and the F_1 from these showed either spring or winter type, indicating dominant or recessive mutations. The F_1 was backcrossed again to clean other spurious mutations and F_2BC_2 is being re-selected. These lines will be studied at the transcriptional level and will be used in physiological field experiments.

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Microspore transformation in barley

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Abstract

Our investigation was focused towards improving the method for gene transfer into androgenic tissue in barley, which has a number of technical and scientific advantages over existing systems for gene transfer in this important crop species. The experimental approach involved use of microspores as a source tissue for gene transfer. Five barley varieties were used: 'Pearl' (winter), 'Siberia' (winter, six row), 'Golden Promise', 'Chime' and 'Derkado' (spring genotypes). Anther culture and culture of mechanically isolated microspores were involved in our experiments. We tested different microspore isolation and cultivation methods. The suitable methods were optimised with the aim to isolate embryogenic competent microspores among the population of all microspores.

Gene transfer was conducted by the biolistic method into both isolated microspores and intact anthers using a *Gus* gene driven by a ubiquitin promoter. Histochemical analysis was performed on regenerated material to assess initial transformation efficiencies.

Efficiencies of gene transfer into microspores were very low due to technical limitations of the used methods. Bombardment of intact anthers resulted in 0.2 % of transformed microspores which continued into androgenic development. Presented results indicate, that using the system based on bombardment of anthers is likely to be more technically efficient than the use of a microspore isolation, transformation and regeneration system. Bombardment of anthers can be considered as an alternative method to existing methods for genetic tansformation in barley.

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Molecular assessment of genetic diversity in barley and its use in breeding

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Abstract

During the last decades extensive progress has been achieved in winter barley breeding with respect to grain yield and disease resistance. This progress is mainly due to the efficient use of genetic diversity present within high yielding adapted cultivars and - with respect to resistance - to the extensive evaluation of genetic resources followed by genetic analyses and transfer ('introgression') of respective genes by sexual recombination. Detailed knowledge on genetic diversity present on the molecular level with respect to specific traits as well as on the whole genome level is expected to efficiently enhance future barley breeding. In winter barley breeding, resistance to viral diseases, i.e. the Barley yellow mosaic virus complex (BaMMV, BaYMV, BaYMV-2) and Barley yellow dwarf virus (BYDV), has gained evident importance. By extensive screening programmes and segregation analyses different recessive resistance genes efficient against the soil-borne mosaic inducing viruses have been identified and mapped. For most of these resistance genes, i.e. *rym4, rym5, rym9, rym11, rym13, rym15* and for the BaYMV/BaYMV-2 resistance of ´Chikurin Ibaraki 1´ which is assumed to be allelic to *rym3* closely linked PCR based markers (RAPDs, AFLPs, SSRs STSs and SNPs) have been developed facilitating an efficient incorporation of these resistance genes in the frame of marker assisted selection (MAS) and back-crossing procedures as well as pyramiding of resistance genes. In contrast to barley yellow mosaic virus disease no complete resistance to BYDV is known in barley. However, genotypic differences in the reaction to BYDV-PAV have been observed and two stable QTL on chromosomes 3H and 2H could be detected in independent crosses and trials. These QTL can be efficiently used in barley breeding as they explain about 50 % of the phenotypic variance for this trait. Besides marker development for specific traits, genome covering SSRs and SNPs available in barley provide the basis for efficient genotyping and estimation of genome wide genetic diversity. Knowledge on genetic variability in combination with data on yield and additional agronomic traits may facilitate the detection of marker-trait associations and a more efficient selection of parental genotypes. In order to get detailed information about changes regarding genome composition and allele frequencies during barley breeding in Germany, 64 six-rowed and 49 two-rowed winter barley cultivars, having been the most important at their time of release (1959 - 2003) and representing important ancestors of modern cultivars, are presently genotyped using SSRs. In parallel these cultivars are tested for agronomic traits in multi-site field experiments and are analysed for pathogen resistance under controlled conditions. First results have shown a clear separation between six and two-rowed cultivars by PcoA, and a non-homogenous allele distribution has been found between six-rowed and two-rowed cultivars for most SSRs tested.

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Development of SNP markers for the oat dwarfing gene *Dw6*

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Abstract
Short straw is one of the principal aims in oat cultivar breeding. Its selection efficiency could be improved using markers especially because the dwarfing gene is dominant. Finnish spring oat cultivar 'Aslak' (Boreal Plant Breeding Ltd.) was crossed with the Dutch cultivar 'Kontant' (bred by Zelder BV. and represented by Wiersum BV.) containing the dwarfing gene *Dw6*. One F_1 seed produced a progeny of 111 plants. Bulked segregant analysis was used to find markers linked to the dwarfing gene. Five hundred RAPD (randomly amplified polymorphic DNA), 42 IRAP (inter-retrotransposon amplified polymorphism), 24 ISSR (inter simple sequence repeat) primers, and 325 REMAP (retrotransposon-microsatellite amplified polymorphism) primer combinations were first tested in the parents. Primers that produced polymorphic markers in the parents were used to screen the short and the tall bulks, which were constructed from pooled DNAs of the nine shortest and nine tallest F_2 plants. If primers revealed polymorphisms between the bulks, individual plants from the bulks were further analysed. Two markers (one RAPD and one REMAP) were associated with height and were tested in the whole F_2 progeny. For more efficient and codominant scoring in marker-assisted selection, the markers were planned to be converted into SNP (single nucleotide polymorphism) markers. The RAPD marker has already been converted, and the development of the SNP from the REMAP marker is underway. The SNP markers can be used to facilitate selection for homozygous short individuals in those breeding programmes that contain lines carrying the *Dw6* gene.

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Development and application of functional markers in maize

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Abstract

Functional markers (FMs) are derived from polymorphic sites within genes causally involved in phenotypic trait variation. FM development requires allele sequences of functionally characterized genes from which polymorphic, functional motifs affecting plant phenotype can be identified. In maize and other species with low levels of linkage disequilibrium, association studies have the potential to identify sequence motifs, such as a few nucleotides or insertions/deletions, affecting trait expression. In one of the pioneering studies, nine sequence motifs were shown to be associated with genetic variation for flowering time in *dwarf8*. Proof of sequence motif function can be obtained by comparing isogenic genotypes differing in single sequence motifs. At current, the most appropriate approach for this purpose in crops is targeting induced local lesions in genomes (TILLING). In central Europe, maize is mainly grown as forage crop, with forage quality as major trait, which can be determined as proportion of digestible neutral detergent fiber (DNDF). *Brown midrib* gene knock out mutations have been shown to be beneficial for forage quality but disadvantageous for overall agronomic performance. Two *brown midrib* genes (*bm1* and *bm3*) have been shown to be involved in monolignol biosynthesis. These two and additional lignin biosynthesis genes have been isolated based on sequence homology. Additional candidate genes putatively affecting forage quality have been obtained by expression profiling using, e.g., isogenic *bm* lines. Results from association studies between selected genes and DNDF in a collection of European elite inbred lines will be presented.

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Variability among and within local maize populations for agronomic performance, early growth traits and RAPD molecular markers: field and growth room data

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Abstract

A preliminary evaluation of the genetic variation among and within local maize populations based on field performance, early growth traits under controlled room conditions and RAPD molecular marker analysis were the objectives of this study.

Five local maize populations maintained in the Cereal Institute were used. One hundred spaced individual plants from each population were field evaluated in a grid mass selection arrangement during 2002 growth season. Plant height, flowering and anthesis, leaf greenness in SPAD units, prolificacy and grain yield data were recorded. In the same manner 100 plants from each population were evaluated under controlled room conditions, 25°C and 16 - 8 h day-night regime. Data on emergence, leaf greenness in SPAD units, fresh biomass and root mass measured with the 810 A capacimeter were recorded. A molecular analysis using 20 RAPD markers was also conducted. Each population was represented by five genomic DNA samples extracted from five random plants. Jaccard similarity coefficients were estimated and UPGMA cluster analysis was conducted.

Controlled room growth data indicated that variation among and within the five populations exists for all traits studied and traits like leaf greenness and root mass are worth of further study to determine their value to be utilized in breeding programs. Field data showed that although the average yielding ability of all populations studied was low as compared to modern hybrids, variation among and within populations exists for grain yield mainly associated with variability in leaf greenness and prolificacy. Data further indicated that leaf greenness measured either in the vegetative or in reproductive stage is worth using as indirect selection criterion for yield. Molecular analysis data indicated that variation within and among population is existing confirming the phenotypic data discussed.

Summarizing the evidence presented it seems that the populations studied are worth of further evaluation to determine their usefulness as germplasm sources along with the need of more data on the value of leaf greenness and the root mass traits as potential selection criteria.

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Identification of genes differentially expressed in association with SCMV resistance in maize by combining SSH and macroarray techniques

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Abstract

The molecular mechanisms underlying the development and progression of SCMV infection are poorly understood. The identification of differentially expressed genes has been used to recognize candidate genes involved in plant infection processes. In this study, we combined suppression subtractive hybridization (SSH) and macroarray hybridization to identify genes whose expression is differently expressed between resistant and susceptible cultivars. Five SSH libraries were constructed using lines F_7 and 10940 (BC_5 derived SCMV resistant isogenic line from susceptible parent F_7 using FAP1360A as donor of resistance genes). 400 cDNA clones from each library were arrayed onto nylon filter membranes.

In order to control the quality of cDNA array hybridizations, the sensitivity, linearity and reproducibility of array hybridizations were evaluated. Using TIGR MIDAS and TIGRMEV (http://www.tigr.org/software/tm4/index.html), genes significantly increased or repressed were identified by statistical analysis. After sequencing, 283 genes differentially expressed have been revealed, which account for 10.5 % of cDNA clones deposited in the macroarray.

Similarity search (http://mips.gsf.de/proj/thal/db/tables/tables_func_frame.html) shows classified genes mostly involve in four functional categories (cell rescue, defense, death and ageing; metabolism; signal transduction and transcription). Promising clones from macroarray hybridization experiments are currently studied in more detail by EST mapping.

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Androgenic responsivity in anther and microspore culture of maize hybrids

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Abstract

The application of anther culture techniques in maize breeding is strongly dependent on the production of large numbers of microspore derived plants and on the frequency of spontaneous or induced chromosome doubling. Although commercial hybrids were already produced using the anther culture technology, maize remained a recalcitrant species in regard to *in vitro* androgenesis when compared to other cereals (e.g. barley, wheat and triticale). Genotypes most responsive to anther culture have been found in non-commercial maize germplasm. It was shown before, that androgenic responsivity can be incorporated into commercial recalcitrant genotypes by hybridisation with responsive ones, since androgenic responsivity is a heritable trait. We focused our experiments to study androgenic responsivity of different hybrids of interesting commercial maize breeding lines in anther and microspore culture and to test their suitability for practical maize breeding.

In our experiments we used the hybrid material *A 632 x F 7*, *Mo 17 x F 7*, *B 73 x F 7*, *B 84 x F 7*, *Tva 45-9 x F 7*, *Tva 36-9b x F 7*, and *F 7 x A 632*. Our results proved high recalcitrance of maize for *in vitro* androgenesis. Androgenic response of the hybrids was low, even when optimised protocols were used. The results of androgenic embryo production using microspore cultures of hybrid combinations supports the assumption that the androgenic responsivity is a heritable trait that can be incorporated into elite lines by hybridisation with highly responsive exotic lines, e.g. *F 7 x Seneca 60* and *Tva 55-9 x F7*. The higher number of androgenic embryos obtained in microspore cultures compared to anther response in anther culture can be due to elimination of the interaction of the microspores with the anther wall. The results presented here and our earlier experiments demonstrated that lines with high responsivity can be a good source for this trait and via crossing this valuable trait can be incorporated into elite lines. Till now it is not clear to what extent this trait from exotic maize lines can be incorporated into commercial maize lines and how this trait will influence other agronomic characteristics of the elite lines.

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Altered organ-specific expression of Adh1 gene in autotetraploid maize (*Zea mays* L.)

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Abstract

The mechanisms underlying the tissue- and organ-specificity of maize *alcohol dehydrogenase-1* (*Adh1*) expression are unknown. *Adh1* is expressed aerobically in younger developing endosperm, aleurone, older scutellum, root cap, certain cells of the vascular system and pollen. There are over 100 *Adh1* mutants that have been studied. None of these *Adh1* mutants have been shown to have altered organ-specific expression or altered anaerobic induction. A new organ-specific *Adh1* mutant was isolated from autotetraploid S_{25} Wf9 maize line by germinating kernels under water for two days before extraction. Enzyme activity in anaerobic scutellum was found to be normal or slightly reduced. No activity or approximately 33 - 75 % of normal levels of ADH1 in pollen grains were observed. The organ-specific phenotype of the *Adh1-mF* could be maintained for at least six subsequent generations of male transmission. The mutant line was crossed to a standard inbred line, *Adh1-SSSS* at both direction to study the transmission of mutant allele. The *Adh1-mF/Adh1-S* vs. *Adh1-S/Adh1-mF* F_1 hybrids were then twice self-pollinated to recover any recessive mutations which had occurred in the parental or F_1 generations. Quantification of *Adh1-mF* expression in matured scutellum was accomplished by measuring ADH1 allozyme-activity ratios in native PAGE. Fifty two mutant heterozygous F_1 seeds were analyzed to determine the activity of the mutant F allele. In all cases two-banded patterns could be detected on the gel slabs, indicating that F · F homodimers were missing. But the abnormal ADH1 subunit was able to be complemented by a wild type ADH1 subunit. This was indicated by the presence of the S · F heterodimers. So the contribution of the *Adh1-mF* to the ADH activity was about 10 %. To be sure that transmission and expression of *Adh1-mF* was normal in pollen grains, ADH1 activity was visualized by direct enzyme staining of whole pollen grains. ADH1-normal cells stain an opaque dark blue, ADH1-low cells stain pink blue, and ADH1-negative cells remain light yellow. Every pollen grain deriving from *Adh1-SSSS* homozygotes and both types of heterozygotes, *Adh1-mF/Adh1-S* and *Adh1-S/Adh1-mF,* stained to the same homogeneous dark blue at the same rate. PCR analysis of *Adh1-mF* families and F_1 plants with *Mu* and promoter specific primers are carried out to reveal insertion in the promoter region.

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Heterotic grouping of Sudanese sorghum landraces

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Abstract

Sorghum (*Sorghum bicolor*) is the most important grain crop in the Sudanese economy and diet. The Sudan and the adjacent areas in Eritrea and Ethiopia are considered as centers of diversity of sorghum. In Sudan sorghum ranks first in terms of cultivated area (6.4 mill. ha) and production (5 mill. metric tons). However, average yield per unit area is very low (540 kg/ha) in comparison to the world average (1300 kg/ha). The only released sorghum hybrid variety is sensitive to drought and the parasitic weed *Striga hermonthica*. The aim of this study therefore is to characterize the pattern of genetic diversity in a representative samples of Sudanese sorghum landraces and to determine genetically distinct pools which shall serve as base materials for hybrid breeding. Seed samples of 52 landraces from a broad range of the sorghum growing area in Sudan were provided by ARC. Most of the landraces belong to the races *durra* and *caudatum*. For comparison, a world-wide collection of 25 inbred lines and 2 wild sorghums *(S. arundinaceum)* were included.

A total of 31 simple sequence repeat (SSR) markers were employed to establish clusters of potentially heterotic groups. An UPGMA dendrogram was generated from distance-matrix data using modified Roger's distance. The results show that Sudanese sorghum landraces are highly variable providing abundant diversity for the development of hybrids and open-pollinated varieties. SSR clustering revealed distinct sorghum landrace groups which are considered as promising base materials for building up heterotic gene pools for the development of high-yielding hybrid varieties. Presently, all landraces and inbred lines will be test-crossed with two cytoplasmatic male sterile lines derived from different gene pools. Individuals of differential clusters will be manually crossed in a diallel manner. The landraces, inbred lines, test crosses and diallel crosses will be evaluated in regular yield trials at three sites in Sudan in 2005 and 2007. Final grouping will be based on molecular markers as well as field data.

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Occurrence of diploid and polyploid gametes in *Sorghum bicolor* as the result of cytomixis

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Abstract

Sorghum bicolor (L.) Moench is a crop species of *Poaceae* that can be grown in harsh environments where other crops such as rice and corn grow or yield poorly. This crop is an important food and feed crop in the world. Also this crop is still the principal source of energy, protein, vitamins and minerals for millions of the poorest people in India, Africa and other regions of world.

The phenomenon of cytomixis consists in the migration of chromosomes between meiocytes through cytoplasmic connections. Since cytomixis creates variation in the chromosome number of the gametes, it could be considered a mechanism of evolutionary significance. Until now cytomixis has been investigated in numerous species, including some grass species, but never in *Sorghum bicolor*. The results obtained from meiotic studies showed the 10 bivalents in most of pollen mother cells at diakinesis and first metaphase. In some cells, occurrence of cytomixis and chromosome migratiom were observed. Analysis of 230 pollen mother cells at first metaphase stage showed 73.91 % haploid (n = 10), 8.64 % diploid (n = 20), 8.69 % triploid (n = 30), 4.34 % tetraploid and 4.34 % pentaploid (n = 50), respectively. Pollen diameters showed that the cytomictic cells differed from the normal cells, since it has been demonstrated that the size of pollen grain is related to the ploidy level. These results indicate that cytomixis can really be an effective mechanism for the production of polyploid gametes and plant breeding.

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Molecular mapping of AB-QTLs affecting salt tolerance in rice (*Oryza sativa* L.)

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Abstract

Rice is moderately sensitive to salinity. Salinity affects virtually all aspects of rice growth in varying degree at all stages starting from germination through maturation. It is now recognized that tolerance to salinity is genetically and physiologically complex and also inherited quantitatively. Molecular marker aided selection techniques for salinity tolerance would accelerate breeding progress by increasing selection efficiency.

In order to map the genes / QTLs for salinity tolerance in rice, 63 advanced backcross lines (BC_2F_5) derived from the cross between 'IR64' as recurrent parent and 'Tarom molaei' as donor parent were investigated at the International Rice Research Institute (IRRI). The map length was 1692.6 cM with an average interval size of 16.3 cM. The phenotypic traits under study included: Sodium (Na) and potassium (K) concentration in root and shoot, dry and wet weight of root and shoot, salinity tolerance at 15 and 22 days after salt treatment in phytotron, and Na-K ratio in root and shoot. 235 SSR markers with uniform coverage on all 12 linkage groups were analyzed for parental survey by agarose and polyacrylamide gels, through that 114 markers showed polymorphism and were assigned for genotyping. Transgressive segregation was observed in all traits. We found QTLs with additive effects for K in shoot, dry weight of root and shoot and Na-K ratio in root and shoot. On all chromosomes except number 9, at least one QTL mapped for Na-K ratio in root. All QTLs under discussion in this paper have significant threshold (LOD > 4) and are also approved by both IM and CIM methods of analysis.

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Testing the 'Suweon 472' non-transgenic mutant lines for improving agronomical traits of *japonica* rice

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Abstract

For creating new favourable allele types to improve agronomic traits, we adopted the sodium azide (SA) as the mutagen for a high yielding rice cultivar, the 'Suweon 472'. Dose effect of SA was negatively correlated with germination rate, seedling height, and fertility at M_1 generation, and the ratio of chlorophyll mutants, at M_3 generation, reached up to 13.5 % at SA concentration of 4 mM. Various kinds of mutations for viable and agronomic characters were also induced. Interestingly, the ratio of waxy endosperm was much higher than other observed mutant types. To increase the practical usefulness of the induced favorable allele types, we selected 2000 lines at M_5 generation depend on two selection criteria, favorable agronomic traits and similar morphological plant types as the wild type, 'Suweon 472'. During preliminary screening procedures, we identified several conditional mutants line with significant durable resistance under biotic stresses (blast and bacterial blight) as well as desirable endosperm mutant types such as waxy, dull, floury, and high lysine. Along with more sophisticated and systematic evaluation of agronomic phenotypes and molecular analysis, the 'Suweon 472' mutant lines could offer not only useful alleles for important breeding goals of *japonica* rice cultivars with less impacts of deleterious gene modifications but also valuable materials for functional genomics.

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Induction of transposition events by a thermal stress reactivates the *P* gene in common bean line 'Fin de Bagnols'

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Abstract
In common bean (*Phaseolus vulgaris* L.), colour is controlled by a group of well described genes. Colour expression in either the seed coat or flower is completely dependent on the multiple alleles at the *P* locus. The dominant allele at *P* potentiates colour in the seed coat and flower. Retrotransposons are present in all plant genomes and can constitute a very large part of them. A common feature of most retrotransposons is that they are activated by stress and environmental factors. We grew 'Fin de Bagnols' plants in a green-house until the development of the first flowering buds. We then performed a controlled thermal stress by cultivating the plants under glass at 30°C continuously for 15 days during flowering and then under standard conditions until the seed maturation. Three independent experiments were annually performed on ten mother plants.

'Fin de Bagnols' plants have brown-red seed coat with beige variegation and an orange hilum ring. Leaves were large with three light green leaflets. The flower buds and the flowers are light pink to nearly white. After the thermal stress, the seeds harvested on the parental plant had a normal phenotype as the seed colour patterns are maternal traits. When the G1 seeds were sowed, seedlings showed several phenotypic alterations. First, the first leaf appearing after the cotyledonary leaves had a modified number of leaflets 2, 4 or 5. The flower buds and the flowers were dark pink. There was no alteration of the pods phenotype, but the seeds harvested from these plants were nearly black differing from the control. An IRAP (inter retrotransposon amplified polymorphism) profile was generated using primers designed to match the 5'LTR of the bean Tpv2 retrotransposon sequence. 'Fin de Bagnols' displayed only a 4500 bp band. The pattern of the plants grown from G1 seeds harvested after the thermal stress showed one more band of 2500 bp. The same profile was observed from all G1 plants coming from the different experiments. Each band was cloned and sequenced. The 4500 bp fragment corresponds to the Tpv2 sequence located between both LTR. The 2500 bp fragment presents a Tpv2 sequence lacking from 2000 bp, the deleted part includes integrase genes. The alteration of IRAP profile relies to transposition events in response to thermal stress leading to incomplete retrotransposon integration.

High temperature during bean flowering induced transposition. If such events occurred in field this could lead to gene alteration resulting in genotype or phenotype changes and could affect seed batch quality during the multiplication process. We interpret the modification of seed and flower colour as the result of the reactivation of the *P* allele by the way of excision of a retrotransposon. This could represent a possibility of characterising the sequence of the *P* gene. Nevertheless there are at least two retrotransposon classes in bean (gypsy like, copia like) and it remains to establish which one is involved in *P* gene inactivation.

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Utility of high copy number 'Ogre' / 'Cyclop' retrotransposons for molecular genotyping of pea (*Pisum sativum* L.) germplasm

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Abstract
Large genome size of most crop plants is mainly attributed to the presence of repetitive sequences and mobile elements. Retrotransposons as representatives of the later, are present in high copy number in many if not all plant genomes and become increasingly used as molecular markers due to their considerable degree of sequence heterogeneity and high insertional polymorphism. In pea (*Pisum sativum* L.), numerous retrotransposons have been identified and described, with predominant use of relatively low abundant *Ty1/copia-like* PDR-1, for pea diversity and evolution studies.

We have used two highly abundant retrotransposons, representative of one of the major group of long terminal repeats (LTR) - containing retrotransposons of *Ty3/gypsy-like* class, 'Ogre' and 'Cyclop', for development of molecular markers for pea cultivar genotyping.
The aim of this study was to access retrotransposon based markers to evaluate the genetic diversity of pea. To test the utility and reliability of the method we have chosen 20 commercially used pea cultivars with well known pedigree. High copy number of both elements (estimated 1 - 3 x 10^4 copies per haploid genome) has allowed to use and compare IRAP (inter-retrotransposon amplified polymorphism) and REMAP (retrotransposon-microsatellite amplified polymorphism) with other techniques such as ISSR (inter-simple sequence repeats) and RAPD. The presence and absence of generated DNA fragments was scored and converted into binary matrix using Cross Checker 2.9 and Bio 1D++ programmes. Data obtained were used to generate relative genetic-similarity (GS) matrices using Nei and Li measurements. Finally dendrograms were generated based on GS values using the unweighted pair-group method arithmetic average (UPGMA).

Both retrotransposon procedures are more simple than commonly used highly polymorphic but technically challenging AFLP technology. The advantage over other currently used DNA genotyping techniques is ability to track individual insertion history resulting in better pedigree and genetic diversity estimation. Data will be presented demonstrating that simple IRAP approach can be effectively applied in the fingerprinting of pea cultivars and for genetic similarity analysis. Due to the sequence knowledge, IRAP markers are better scorable and result in more accurate genetic distance estimations over the REMAP, ISSR and RAPD techniques. With the use of just two outward-facing primers annealing to LTR 'Ogre' and 'Cyclop' target sequences 56 and 43 polymorphic bands per cultivar and reaction were produced which clearly distinguished and separated 20 investigated cultivars into groups related to known pedigree. Since numerous plant retrotransposons have been shown to be activated for transposition by several forms of stress to the host plants, we currently investigate this together with marker stability during ontogenesis and mainly over generations for the purpose of reliable and reproducible cultivar identification and description. The method will be further tested and tuned over the genotyping of entire Agritec Ltd. pea germplasm collection comprising about 2000 accessions with the final aim of core collection development while preserving highest possible genetic diversity.

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Molecular tools to facilitate breeding of false flax (*Camelina sativa* Crtz.) 'low-input' genotypes

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Abstract

The re-discovered crop species *Camelina sativa* (false flax, Gold of Pleasure, camelina) is basically suitable for low-input production systems because of its adaptability to adverse environmental conditions and its comparatively short vegetation time. This annual oilseed crop belongs to the mustard familiy (*Brassicaceae*) and its seed oil is rich in poly-unsaturated fatty acids. At present, the oil is mainly used in non-food applications as drying oil and also in pilot projects as bio-fuel. The revival of interest in camelina oil for food purposes is due to its comparatively high α-linolenic acid content (35 - 40 %), an ω-3 fatty acid which is generally found in substantial quantities only in linseed and fish oils. Camelina offers an opportunity to diverse crop production and supply the growing demand for edible oils rich in ω-3 fatty acids.

Very little breeding work has been carried out on camelina, yet. As a result very few named varieties and breeders selection lines are available. However, on the basis of the achieved selection progress towards productivity further improvements of the major agronomic and quality characteristics should be possible. Previous work concentrated in particular on analyses of inbred lines derived from various crosses for grain yield, seed quality and their molecular genetic relationships. These investigations aimed at the identification of superior genotypes and breeding lines regarding adaptability to marginal conditions (locations, N-fertilization) as basic material for subsequent breeding programmes. From a cross between two phenotypically different varieties, 'Lindo' and 'Licalla', inbred lines were created using the single-seed decent (SSD) method. In addition this mapping population of 186 SSD-lines is used for field performance trials and accompanying molecular analyses using AFLP (amplified fragment length polymorphisms) and SSR (simple sequence repeats) markers. The analysis of genetic maps and phenotypic data from replicated trials in several years at different locations will allow the identification of quantitative trait loci (QTL) related to genetically complex agronomic characters, i.e. oil content, seed and oil yield, and others.

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Development of a divergent winter oilseed rape gene pool for hybrid breeding and QTL mapping of agronomic characters

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Abstract

Exploitation of genetic potential with regard to seed and oil yield is a primary breeding objective in order to further increase the economic value of winter rapeseed (*Brassica napus* L.) as an oilseed crop. With the advent of hybrid breeding, seed yield has undergone considerable enhancement in recent years. In addition to individual yield performance the availability of useful genetic diversity between the potential crossing partners - as a prerequisite for a superior combining ability - is necessary. In the course of a winter oilseed rape breeding programme emphasis is placed on the development of a separate gene pool for hybrid breeding, based on high-erucic acid and high-glucosinolate rapeseed (HEAR). In the first step, this material was used as pollinator in order to determine its combining ability with male sterile double-low rapeseed lines. Following test crosses, suitable HEAR lines were selected which formed the basic material for all project work. Regarding seed yield the general combining ability (GCA) was estimated for the parental HEAR lines selected due to their yield performance. The yield performance of the inter-pool hybrids (double-low quality x HEAR) based on a three-location field trial of 20 selected HEAR lines (V1 to V22). Concomitantly, promising HEAR parental lines are improved towards double-low seed characters through a quality conversion procedure. Based on the results of GCA testing, a cross 'good combiner' (double-low quality pool) x 'poor combiner' (HEAR pool) was selected to develop a new mapping population of doubled-haploid (DH) lines for analysis of quantitative trait loci (QTL). In order to identify gene loci contributing to 'combining ability' with regard to relevant quantitative traits, such as seed and oil yield as well as oil content, a segregating mapping population of about 220 DH lines was developed by microspore culture, which is now used for subsequent genetic mapping by AFLP and SSR markers.

For the production of test hybrids a male sterile female line ('MSL Falcon') was sown in the field, and artificially vernalised clones of 110 DH individuals were planted in the experimental field (Rauischholzhausen, Germany) in spring 2003 in order to produce seed material of test hybrids of the mapping individuals. These experimental hybrids are going to be tested in subsequent field trials for the assessment of the GCA effects of individual DH lines and finally generating phenotypic data for QTL analysis.

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Analysis of the genetic basis of heterosis in oilseed rape (*Brassica napus*) via comparative QTL mapping

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Abstract

Today, oilseed rape (*Brassica napus* L.) is one of the most important oilseed crops world-wide The overall aim of this project is a comparative analysis of the genetic control of heterosis in oilseed rape by QTL mapping of heterosis-relevant loci in different *B. napus* mapping populations. A doubled-haploid (DH) mapping population that has been generated in preliminary work from a cross between two lines with high and low general combining ability, respectively, will be used for construction of a genome map containing a set of consensus SSR markers that also show polymorphisms in another population segregating for heterotic effects, to be constructed by the University of Göttingen in a closely-integrated parallel project. Field trials of test hybrids from crosses between the individual DH lines of the respective populations with common male-sterile tester lines will enable the identification and dissection of QTL that correspond to the expression of heterosis. By using common markers it will be possible to align the genetic maps from the two populations, meaning that the positions, effects and interactions of the respective heterosis-relevant QTL identified in the two populations can be aligned and compared. Based on the results of the respective mapping experiments, the genetic control of heterosis in the two populations will be studied and compared.

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The use of microspore culture for genetic improvement of winter oilseed rape (*Brassica napus* L.)

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Abstract

The development of methods of efficient production of haploids is stimulated mainly by the potential of using homozygous lines in breeding programmes and basic research. The efficiency of production of haploids and doubled haploids depends mainly on the efficiency of microspore embryogenesis and plant development from microspore-derived embryos. It is also important to introduce doubled haploid lines properly into the breeding cycle or to use them adequately in basic research. This study presents an efficient method of production of doubled haploids of winter oilseed rape (*Brassica napus* L.) from cultures of isolated microspores. The paper presents also results of research on stimulation of organogenesis in microspore-derived embryos and various methods of doubling the number of chromosomes in haploids. On the basis of the results, a system has been developed for assessment of doubled haploids derived from one donor plant of oilseed rape and for selection of doubled haploid lines meeting the widely applied criteria conditioning their practical value in breeding. The usefulness of research on DH lines of oilseed rape has been confirmed. Many examples of application of haploids and doubled haploids of this species in breeding programmes are given. These include the breeding of high-yielding lines with favourable qualitative traits, of yellow-seeded lines, and of lines with a very high erucic acid content and very low level of glucosinolates. The usefulness of application of homozygous lines in hybrid breeding based on CMS *ogura* is indicated. Finally, the recently developed methods for application of oilseed rape haploids in mutation breeding and transformations are presented.

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Improving *in vitro* culture of canola in Iran

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Abstract

Canola is a type of rapeseed that has been developed to contain less than 2 % erucic acid in the oil and less than 30 µmol/g glucosinolates in the meal. Preliminary investigations indicate that canola can be successfully grown *in vitro*. Tissue culture is a useful tool which allows the rapid production of plantlets with using relatively small amounts of space, supplies and time. Since gene transformation into plant cells is one of the important problems in genetic engineering and its base is on tissue culture, so in this research, we attempted to optimize the regeneration percentage of *Brassica napus* cultivars. We tested several factors for optimizing regeneration like explants (hypocotyle - cotyledone), hormons of shoot inducing medium and root inducing medium, and seedling age. Canola cultivars most important in Iranian cultivation (SLM, NSA, 'Maluka', 'Hyola 401', 'Hyola 308', 'Global') were used. Objective of this experiment was to find out conditions for the best regeneration of complete plants from single cells. Statistical analyses revealed that the optimum shoot inducing medium was with BAP (4.5 mg/l) of different hormons tested [Kinetin (3.5, 10 mg l^{-1}), GA (0.75, 1.5 10 mg l^{-1}), BAP (2, 4.5 10 mg l^{-1})] and for root inducing medium IBA (2 mg l^{-1}) of other hormons [(NAA (0.1, 0.3, 0.5 mg l^{-1}), IBA (2.4 mg l^{-1})]. The best explant was cotyledone, the best seedling stage was 3 days age, and the best cultivar for tissue culture was 'Global'. In this project we obtained a regeneration rate of 73 % complete plants.

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Resynthesised *Brassica napus* as a genetic resource for improvement of Verticillium wilt resistance in oilseed rape

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Abstract

Oilseed rape (*Brassica napus* L.) is today the most important oil crop in Europe, with a cultivation area in Germany of 1.3 million ha in 2003. Because of the relatively high crop rotation rate and the increasing area under rapeseed cultivation, disease problems are a major issue in current breeding efforts. Verticillium wilt of *B. napus*, caused by the host-adapted pathogen *Verticillium longisporum*, can cause grave yield losses in affected areas, e.g. of Sweden, Denmark, Great Britain and the north of Germany. Chemical plant protection is not possible, because accredited fungicides against *V. longisporum* are not available. *V. longisporum* is a soil-borne pathogen. The fungus persists by forming microsclerotia on plant tissues. These microsclerotia overlay in the soil for time periods of more than a decade and represent the inoculum source for later grown oilseed rape. For both winter and spring rapeseed, breeding for resistance against *V. longisporum* is severely hampered by the absence of sufficient resistance in available breeding material. The aim of this study is to identify and combine quantitative resistance sources from cabbage (*Brassica oleracea* ssp.) and turnip rape (*Brassica rapa*) in novel resynthesised *B. napus* forms with an enduring resistance against *V. longisporum*. Potential resistance donors have been identified in an ongoing resistance screening of diverse turnip rape and cabbage accessions, and resistant donor genotypes are being combined in resynthesised *B. napus* forms by interspecific hybridization assisted by embryo rescue (ovule culture). The resynthesised rapeseed lines will be tested to verify resistance behaviour before introduction to breeding programs. Although resynthesised rapeseed forms represent only pre-breeding material that generally exhibits unsuitable seed quality characters and poor yield, the genotypes developed in this study should provide a long-term source of genetically diverse *B. napus* for breeding of resistance against an increasingly important oilseed rape pathogen. Future work will concentrate on genetic mapping and marker development for accelerated use of the material in advanced backcross breeding.

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Nematode resistance in a disomic rapeseed-radish chromosome addition line

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Abstract

In genus *Raphanus* genetic resistance against the beet cyst nematode (*Heterodera schachtii*) is present. From the breeders point of view, the transfer of nematode resistance to rapeseed is desirable to convert this tolerant host plant into a resistant crop. Nematode-resistant winter rapeseed would be of interest as an agronomically important trap crop usable over a complete cultivation period. As a first step towards this goal, a complete series of nine rapeseed-radish disomic chromosome addition lines (DAL), *a* to *i*, were developed. Counting and identification of the added alien chromosomes was carried out by means of FISH and molecular marker techniques. Each DAL with $2n=4x+2=40$ chromosomes has a single pair of a radish chromosome added to the genetic background of winter rapeseed variety Madora. To determine, if resistance will be expressed and which radish chromosome is contributing to it, the DAL's, together with the susceptible rapeseed (chromosome recipient) and resistant radish (chromosome donor), were inoculated with L2 juveniles of *Heterodera schachtii*, cultivated under controlled conditions and checked for root cyst number after 42 days. The results of this experiment show that nematode resistance was transmitted by one radish chromosome. The resistant DAL for radish chromosome *d* had the same resistance level as the radish donor variety, whereas the other eight DAL's were as susceptible as the rapeseed. In a second experiment with the F_2 generation of an intraspecific cross between the susceptible fodder radish variety 'Siletta Nova' and the nematode-resistant variety 'Pegletta', a gene with major effect explaining more than 60 % of phenotypic variance was localized on a single linkage group by means of molecular marker mapping and QTL analysis. Markers assigned to the nine radish chromosomes in the DAL's were also enclosed for mapping. Markers of a single chromosome mapped in the same linkage group. The radish chromosome *d* conferring resistance to rapeseed was consistent with the linkage group carrying the major QTL. Some radish chromosome *d* markers were closely linked to the QTL for nematode resistance, allowing marker-assisted selection for intergenomic recombination. Analyses in progenies of DAL having radish chromosome *d* with molecular and cytogenetic chromosome-specific markers revealed a nearly unaffected transmission of the radish chromosome *d* through meiosis. The resulting high phenotypic stability of the resistant rapeseed-radish DAL of chromosome *d* allows its direct use in agronomical evaluation.

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Detection of allelic diversity in resistance gene candidate sequences for association studies with blackleg disease in oilseed rape (*Brassica napus*)

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Abstract

Blackleg disease caused by the fungal pathogen *Leptoshaeria maculans* (anamorph *Phoma lingam*) is one of the most important oilseed rape (*Brassica napus*) diseases and can cause heavy yield loss of up to 50 % in epidemic years. A broad variation for *L. maculans* resistance is present in the *Brassica napus* gene pool, however due to the quantitative nature of the resistance it is difficult to develop effective selection markers. This project aims at detection and exploitation of genetic diversity of resistance gene candidates associated with blackleg resistance in *B. napus* via association studies between resistance data and single nucleotide polymorphism (SNP) haplotypes in a core set of 50 diverse oilseed rape genotypes. Resistance gene analogue (RGA) sequences have been amplified in resistant and non-resistant *B. napus* genotypes using degenerate primers for resistance gene motifs (TIR, NBS, LRR), and the resulting RGAs were cloned and sequenced. 48 unique RGAs were detected that showed the expected RGA sequence motifs. In addition to these anonymous RGA sequences, a further set of potential resistance gene candidates (RGCs) is being developed from 277 publicly available cDNAs derived from the *B. napus* defense reaction to inoculation with *L. maculans*. Sequence-specific primers were used to amplify and sequence the unique RGCs in eight genotypes showing a broad range of blackleg disease responses. Altogether we detected 82 SNPs and 16 InDels in RGAs and RGCs via sequencing, demonstrating the effectiveness of SNP markers for detection of allelic diversity. For all available sequences, sequence-independent SNP-detection is being performed with locus-specific primers in the entire core set, in order to construct haplotypes for association studies with *L. maculans* resistance/susceptibility data from field and greenhouse tests. Optional high-throughput procedures for SNP-detection are BessT/G (base excision sequence scanning) or EcoTILLING (targeting induced local lesions in genomes). SNP markers for haplotypes associated with quantitative resistance loci will be useful not only for marker-assisted selection, but also for identification of new resistance sources in the broader *B. napus* gene pool.

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Genetic map for pumpkin (*Cucurbita pepo* L.) using random amplified polymorphic DNA markers

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Abstract
A genetic map for *Cucurbita pepo* L. (2n = 40) will be a useful tool for immediate application in plant breeding and comparative genetic analysis. Using random amplified polymorphic DNA (RAPDs), simple sequence repeats (SSRs) and morphological traits, a molecular map for pumpkin was constructed. The map was developed using an F_2 population obtained from a cross between an Austrian oil-pumpkin inbred line (SZG1) and a zucchini genotype resistant against the zucchini yellow mosaic virus (ZYMV). From a total of 290 loci scored, 254 RAPD markers, 24 of which are co-dominant, 3 SSRs and one qualitative trait could be mapped at a LOD score of 3 and maximum distance of 35; 22 markers are for the time being unlinked. The map covers 1425 cM and contains 36 linkage groups with 17 of them including less than 4 markers. The average distance between markers is 5.5 cM. However, a number of gaps (>20 cM) are still to be filled. One-way ANOVA revealed a significant association of RAPD markers to fruit length, they explain more than 40 % of variation of this trait.

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Development of SSR markers for *Cucurbita pepo* L.

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Abstract
Microsatellites are tandemly repeated di-, tri-, tetra- or penta-nucleotide sequences, dispersed throughout eukaryotic genomes. The nonradioactive establishment of a genomic library of C*ucurbita pepo* from *MseI* restricted genomic DNA enriched by SSR-containing fragments is described. Enrichment was done using a biotinylated probe bound to streptavidin-coated paramagnetic beads. Fragments were cloned into Novagen AccepTor Vector. Out of 1704 insert containing colonies, 858 SSR- positive clones were found (GT/AC repeats). Sequence data of presumed SSR-positive clones are discussed. Genetic relationship of *Cucurbita* species and varieties, as revealed by using the new SSR-primers, is shown.

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Development and selection of partial interspecific lines in cotton

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Abstract

Doubled haploids could be a useful tool in the hands of cotton breeders for the production of homozygous lines. An efficient protocol for the production of dihaploid plants originating from *Gossypium barbadense* x *Gossypium hirsutum* could lead, upon chromosome doubling, to homozygous partial interspecific hybrids effectively combining high yielding ability and earliness of *Gossypium hirsutum* (2n=4x=52) with superior quality and disease resistance of *Gossypium barbadense* (2n=4x=52). An approach leading to this goal could be the pollination of F_1 interspecific cotton hybrids with alien pollen. For that reason, flower buds of field grown F_1 *G. barbadense* x *G. hirsutum* plants (B_{403} x Acala Sindos, B_{403} x Coker 310, Carnak x 4S, Carnak x Acala Sindos) were pollinated with pollen from *Hibiscus cannabinus*. Finally, 4 parthenogenetically developed plants (Pg_0) were produced. These ones exhibited morphological traits from both cotton species and they were fertile. Chromosome counts revealed that the chromosome number of the Pg_0 plants ranged from 27 to 42. This number however was increased progressively from generation to generation up to 52 after four years of self-pollination and visual phenotypic selection of the plants. In addition, 31 Pg_4 partial interspecific cotton lines were evaluated for the yielding ability and quality by performing one cycle of honeycomb selection (R-31) in two locations. Moving grid selection for seed cotton was carried out by desk computer ('Honey' software). In each grid were included 19 plants and the selection pressure applied was 5.3 %. Following this procedure 56 plants were selected for high seed cotton yield in each location. In each of the selected plants, three lint quality traits (fineness, length and strength) were measured in HVI**.** Finally 15 plants from each location that combined high seed cotton yield and lint quality traits were selected for a new selection cycle.

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Molecular analysis of *Phytophthora infestans* induced gene expression in two potato cultivars with different levels of resistance

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Abstract

Late blight, caused by the oomycete *Phytophthora infestans*, is the most devastating disease in potato production. From an economical and ecological point of view potato cultivars incorporating durable forms of genetic resistance are needed. R gene mediated resistance is essentially short-lived, as virulent races of *P. infestans* rapidly overcome the resistance provided by R genes. Therefore, most breeding effort is at present devoted to increasing the level of quantitative resistance which is more durable than the qualitative resistance since quantitative resistance is controlled by many genes. To gain deeper information about the host pathogen interaction in the system potato – *P. infestans* subtractive hybridization in combination with cDNA array hybridization was used. Leaflets of a moderately resistant and a susceptible potato cultivar were inoculated with *P. infestans*. Using infected and control tissue, two cDNA libraries highly enriched for *P. infestans* induced genes were prepared. Within 531 clones randomly picked and sequenced from the libraries 285 unigenes were found, from which 182 clones were selected for further analysis by cDNA array hybridization. 72 h post inoculation induction of gene expression was clearly detectable. In both cultivars, 143 genes were induced moderately (≥ 2-fold), 35 of the selected genes appeared to be strongly induced (≥ 7-fold). Among these clones were mainly genes associated with stress and/or defense mechanisms. The strongest gene induction was found in four-week-old susceptible plants. In the moderately resistant cultivar, transcripts of a number of genes accumulate with plant age; as a result, induction of gene expression upon infection was less pronounced. Down regulation of three genes was observed in both cultivars upon infection. Transcript levels of these three genes increased in uninfected plants within four weeks of growth.

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Microarray based identification of genes involved in the drought stress response of sweet potato

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Abstract

Abiotic stresses and adaptation strategies in plants are numerous and interlinked. Limited tolerance to extremes in temperatures have traditionally been restricted the cropping alternatives available to farmers and threatened the sustainability of agriculture industry. Drought is known as the most significant limiting factor for plant agriculture world-wide. Due to salinity and water deficit of soil, world-wide reduction of the cultivated area by 30 % is predicted in the next 25 years. Plants have to exploit their immediate environment to maximum effect. Their inability to move means that the best way of dealing with many stresses is to adapt physiologically or morphologically. This can be followed either at the protein or gene expression level.

In our study cDNA microarrays were used to identify genes involved in immediate drought-stress response as well as in acclimatisation to drought conditions of sweet potato. Sweet potato is one of the most economically important basic food crops in Asia and Africa. There are drought-tolerant sweet potato varieties available, however, this tolerance is mostly not accompanied by other good agronomic qualities. The results of this work will deepen our understanding of drought response of sweet potato and will also provide data for marker-assisted breeding to achieve better quality phenotypes by through inducing higher tolerance for environmental stressor.

cDNA clones representing both the root and the leaf mRNA populations were obtained either from the North Caroline State University (Bryon Sosinski) or were isolated in our laboratory. Altogether the PCR products representing 3072 cDNAs were spotted on Corning slides. Plant material was collected at 1, 3, 7 and 14 days after drought-stress induction (desert climate).Total RNA was isolated, reversely transcribed, fluorescently labelled using Cy3 and Cy5 dye and hybridized to microarrays. Analyses were performed using GenePix and GeneSpring software packages. Results were verified with the use of quantitative real-time PCR. Evaluation of the results was done comparing the gene expression of the stressed plants to that of the non-stressed ones. Setting signal ratio threshold higher than 3 yielded 478 up-regulated genes and 487 down-regulated genes. At the threshold of fifteen, 25 genes were up-regulated and 121 down-regulated. Up-regulated genes included, for example, metallothioneins, lipid-transfer proteins, mannose-binding lectin, down-regulated ones involved proline-rich proteins, carbonic anhydrase and chlorophyll a/b related proteins. Genes selected by signal ratios showing important functions as well as expression profile were validated by quantitative RT-PCR.

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Use of single nucleotide polymorphisms (SNPs) of expressed genes as a marker system to merge existing linkage groups in hexaploid sweetpotato

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Abstract

The most popular markers (AFLP, RFLP, RAPD, etc.) used in developing linkage maps can not identify homologue chromosomes in hexaploid organisms like sweetpotato (*Ipomoea batatas* (L.) Lam. 2n = 6x = 90). In order to overcome this drawback single nucleotide polymorphisms (SNP) were identified in selected genes, in order to use them as markers for merging and anchoring existing linkage groups in the 'Tanzania' x 'Beauregard' mapping family; and to elucidate the type of inheritance of sweetpotato. Three genes were selected for the study: The 'cold induced gene', lycopene β-cyclase and the farnesyl-diphosphate synthase. SNP sites were validated by using the SnaPshot Multiplex Kit, and direct sequencing reactions. All SNPs were tested for goodness of fit simplex (1:1), duplex (3:1, 4:1, 19:1), and double simplex segregation ratio (presence : absence) according to the three alternative cytological theories of sweetpotato using a χ^2 test. The type of ploidy was analysed based on homologous simplex markers by comparing the observed progeny genotypic distribution with the expected distribution for autohexaploid (hexasomic), tetradiploid (tetra-disomic) and allohexaploid (disomic) inheritance. Segregation analysis in the 192 individuals 'Tanzania' x 'Beauregard' mapping population detected 7 simplex and 6 triplex-markers for the cold induced gene. These 7 simplex-markers could be separated into three segregation groups, representing three different homologous alleles. For FPP gene 10 simplex markers were detected. All the 10 presented exactly the same segregation pattern. Three simplex-markers and 4 double-simplex markers were found in the lycopene β-cyclase gene. All 3 simplex markers presented the same segregation pattern; however the double simplex markers could be separated into two different segregation groups, showing no association with the simplex markers. The type of ploidy (autopolyploidy or allopolyploidy) is uncertain in sweetpotato and was examined in this study using the simplex markers obtained from the cold induced gene. The study supported an autopolyploid type of inheritance.

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Marker assisted backcross of resistance genes in sugar beet (*Beta vulgaris* L.): Some practical cases

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Abstract
Selection for disease resistance is one of the major activities in sugar beet breeding to create improved varieties. For economically important diseases like leaf spot disease and crown root rot, caused by the fungal pathogens *Cercospora beticola* and *Rhizoctonia solani*, respectively, resistance is available and its inheritance has been identified as oligogenic. Disease screenings in greenhouse are rather labourious and can lack good correlation to field results, whereas field observations are season-depending and often vary due to uncontrolled climatic conditions. Selection based on molecular markers in the process of backcrossing disease resistance QTLs has enabled to speed up the backcross process as well as to visualize the efficiency of the backcross breeding. In the case of leaf spot disease, 2 major resistance QTLs have been successfully backcrossed into an elite pollinator. Three resistance QTLs to crown root rot have been backcrossed into 2 elite monogerm lines and their male sterile equivalents. Practical results and some implications related to marker assisted breeding will be discussed.

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Genetic Variation for Plant Breeding, p. 342 (2004)

Development of sugar beet germplasm of gynogenetic origin and its evaluation with AFLP fingerprinting

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Abstract

Diploid and tetraploid sugar beet germplasm of mainly Belarusian origin has been involved in experiments aimed at gaining gametoclonal variants. Each gametoclonal line originated from a single unfertilized ovule of a separate beet donor plant representing a definite line or a cultivar. In this way more than 70 sugar beet haploid, doubled haploid and dihaploid lines have been induced through *in vitro* gynogenesis. For polyploidization purposes in 8 gynogenetic lines besides colchicine, three antimicrotubular agents – amiprophos-methyl, trifluralin and pronamide in concentrations 10, 100, 300, 1000 µM were used. Preliminary flow cytometry (FCM) results of ploidy level analysis in somatic tissues of cultured beet shoots after treatment have shown both doubling and toxicity effects. FCM measurements in more than 60 non-treated originally haploid lines have demonstrated spontaneous chromosome doubling (from x to 2x) while subculturing *in vitro* shoots on MS medium containing cytokinin BA. Recultivation of mixoploid (x+2x) beet gynogenetic plants with high frequency of diploid cells (up to 60 %), using apical meristems of generative shoots allowed to overcome mixoploidy. Arising of heterozygous diploid regenerants from unfertilized ovules of diploid donors (5 of 64) as well as of tetraploid donors was also observed (Svirshchevskaya & Dolezel 2000). Experiments on AFLP-fingerprinting were applied to 31 sugar beet accessions, representing parental plants and their gynogenetic progeny. The technique was based on a selective amplification of a limited number (50 - 100) DNA restriction fragments. Band patterns of 50 - 300 bp fragments out of total genomic EcoR1/Mse1 DNA digests were generated. In total, 9 primer combinations were applied. The possibility of differentiation between lines originated from different ovules of the same donor plant was shown. The genetic relationships between 6 groups of sugar beet accessions derived from different cultivars were revealed. Identification of three groups out of six was proved to be achieved by one (E + ACC/M + CTA) primer combination (Svirshchevskaya 2002). AFLP fingerprints were also utilized for assessment of heterozygosity in 3 parental forms from 'Belorusskaya 69' and 'Ganusovskaya 55' diploid cultivars-standards. The rate of heterozygosity in mentioned genotypes varied (55.3 % at maximum) and was influenced by the primer combinations chosen. The study is in progress.

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Development of molecular marker diversity in polycross breeding populations of *Lolium perenne*

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Abstract

Genetic diversity substantially influences the success of plant breeding programs through various mechanisms such as heterosis, general combining ability, inbreeding depression and self incompatibility. This may be particularly important in outbreeding forage species where cultivars consist of populations obtained by polycrossing several parental individuals. We used amplified fragment length polymorphism (AFLP) markers to select suitable parental combinations in a perennial ryegrass breeding program in order to study the effect of genetic diversity of parental plants on genetic diversity and performance of synthetic cultivars.

The genetic diversity observed among 108 putative parental plants from three groups (early flowering, intermediate and late flowering) was almost entirely due to variation among plants within groups while no significant variation was observed among groups. Based on AFLP marker diversity, two polycrosses of six parental plants with contrasting levels of diversity were composed for each of the three groups. The genetic diversity, expressed as average Euclidean squared distance, among "low diversity" parents was on average 36 % lower when compared to "high diversity" parents. While cluster analysis revealed a distinct cluster containing the six parents of the low diversity polycross of the early flowering group, the remaining 30 parents did not form distinct clusters.

Parental plants of the respective group and diversity level were grown in six isolated polycrosses in the field. Seed was harvested for each mother plant separately and F_1 progenies were analysed using the same AFLP markers used for the 36 parental plants. In contrast to the initial 108 putative parental plants, analysis of molecular variance of the 212 F_1 individuals revealed significant variation (6.6 %) among the three groups, while 12.0 % of the variation was observed among populations within groups and 81.4 % of the variation was due to variation among individuals within populations. Genetic diversity within F_1 populations was proportional to the diversity within the respective parental populations but differences were less pronounced. Diversity within F_1 populations of low diversity polycrosses was on average 16 % lower when compared to populations of high diversity polycrosses. In general, diversity within F_1 populations of high diversity polycrosses was clearly lower when compared to the respective parental populations while these differences were only small between parents and progeny of low diversity polycrosses. Multivariate analyses allowed a clear separation of the six F_1 populations based on AFLP markers and demonstrated the genetic distinctness of these populations.

Morphological characterisation based on five traits used for variety testing according to UPOV guidelines showed no systematic increase in variability within F_1 populations derived from high diversity polycrosses when compared to populations from low diversity polycrosses. Thus, selection for parents with high AFLP marker diversity does not increase the risk of reduced uniformity in synthetic cultivars.

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QTL mapping of vernalization response in perennial ryegrass (*Lolium perenne* L.) reveals cosegregation with an orthologue of wheat *VRN1*

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Abstract

The objective of this study was to map quantitative trait loci (QTL) for vernalization response in perennial ryegrass (*Lolium perenne* L.). The mapping population consisted of 184 F_2-genotypes produced from a cross between one genotype from the synthetic perennial ryegrass variety 'Veyo' and one genotype from the perennial ryegrass ecotype 'Falster'. 'Veyo' and 'Falster' were chosen among four different varieties because of contrasting vernalization requirements. In total, five QTL for vernalization response measured as days to heading were identified and mapped to LG2, LG4, LG6 and LG7. Individually, QTL explained between 5.4 and 28.0% of the total phenotypic variation. The overall contribution of these five QTL was approximately seventy-five percent of the total phenotypic variation. A putative orthologue of *Triticum monococcum Vrn1* (*TmVrn1*) was identified in perennial ryegrass. DNA fragments from both parents of the mapping population revealed a 95 % DNA sequence identity to *TmVrn1*. Several polymorphisms were identified between 'Veyo' and 'Falster' in the promoter as well as in the 5' end of this putative *TmVrn1* orthologue. A CAPS marker, vrn-1, was developed and found to cosegregate with a major QTL on LG4 for vernalization response. This indicates, that the CAPS marker vrn-1 is located in an orthologues gene of the wheat *TmVrn1*.

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Transfer of genes governing freezing tolerance from *Festuca* spp. into *Lolium multiflorum* genome

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Abstract

Enhanced resistance to abiotic stress (drought resistance and winter hardiness) is an important breeding objective for forage and turf grasses. Species within the *Lolium-Festuca* complex offer many valuable and complementary traits, including the high productivity and forage quality of *Lolium*, and the persistency and resistance to abiotic stress of *Festuca*. *Festuca arundinacea* and especially *F. pratensis* have better adaptations to cold than *Lolium multiflorum*, so both species can be used as sources of genes for winter hardiness. *Lolium* and *Festuca* species hybridise and their chromosomes pair and recombine in hybrids; it is possible to transfer stress resistance genes from *Festuca* into *Lolium* by conventional introgression procedures. The identification of *Festuca* introgressed chromosome segments in *Lolium* cultivars by genomic *in situ* hybridisation (GISH) and the co-segregation over generations of *Festuca* DNA and genes for improved winter hardiness allows us to locate the genes of the stress resistance and to assign them to specific chromosome arms.

In our experiments, triploid hybrids *L. multiflorum* × *F. pratensis* (2n = 3x = 21) and pentaploid hybrids *F. arundinacea* × *L. multiflorum* (2n = 5x = 35) were backcrossed three times onto diploid *L. multiflorum* cultivars. BC3 plants from both hybrid combinations were selected in simulated conditions for freezing tolerance. The stress resistant genotypes were then screened by GISH. Most of the selected freezing tolerant plants had a single *Festuca* chromosome segment. FISH experiments revealed that *F. pratensis* and *F. arundinacea* chromosome segments with stress resistance genes were located on the short arm of chromosome 2 *Lolium*; in some cases, *F. pratensis* chromatin occurred in centromere and both pericentromeric regions of chromosome 4. Amplified fragment length polymorphism (AFLP) analyses were performed on BC3 populations and their parent genotypes to identify markers that co-segregate with introgressed *Festuca* segment and to tag alleles that provide improved stress resistance. The selected plant materials carrying freezing tolerance genes can be a good starting point for the development of stress resistant cultivars of *L. multiflorum*.

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Genetic mapping in *Actinidia* species (kiwifruit), and the effects of dioecy and polyploidy

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Abstract

The genus *Actinidia* (kiwifruit) has over 60 species which form a polyploid series from diploid to octaploid. It is also probable that ancient polyploidy is involved as n = 29 and the diploid species show signs of multiple loci. All species are dioecious, so dioecy preceded speciation in the evolution of the genus. As kiwifruit are a major horticultural crop in New Zealand, breeding and genomes programmes are underway to develop new cultivars and advance knowledge of fruit and vine characters.

Microsatellites obtained from enriched genomes and cDNA libraries and from ESTs from a genomes programme are being used to create a framework map and map genes in the diploid species *Actinidia chinensis*. Segregating microsatellite alleles are recorded in 240 progeny of a controlled intraspecific cross, and linkage analysed with the map-making software Joinmap3. Almost 500 segregating microsatellite markers have now been scored in progeny of the test family. Three linkage maps have been created, a female-informative map, using the female and fully informative alleles, a male-informative map using male and fully informative alleles, and a consensus map constructed using fully and partially informative markers to unify female and male markers. Data on the occurrence of loci with multiple alleles, sex linkage and information about the possible function of the ESTs from which the microsatellites have been obtained, has been recorded. A parallel project to score the mapping family for phenotypic characters for which QTL information is required is also underway.

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Genetic Variation for Plant Breeding, p. 347 (2004)

Identification of self-(in)compatibility alleles in apricot (*Prunus armeniaca* L.) by PCR and sequence analysis

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Abstract

Molecular typing of *S*-alleles has been already achieved in several *Prunus* species. However, this approach had not yet been applied in apricot (*Prunus armeniaca* L.). In this work, the eight self-(in)compatibility alleles (S_1 to S_7 and *S*c), present in a collection of apricot accessions of known *S*-genotype, have been identified by PCR. A primer pair developed from conserved regions of *Prunus armeniaca S*-gDNA sequences was used to amplify the first *S*-RNase intron. Fragments containing the second intron were obtained by using two sets of primers designed from previously published *Prunus avium S*-cDNA sequences. These two primer pairs amplified two alleles in most of the cultivars except in those homozygous for the self-compatibility allele (*S*c). The identity of the amplified *S*-alleles was verified by sequencing the first intron and 135 bp of the second exon. The deduced amino acidic sequences of these fragments showed the presence of the C1 and C2 *Prunus S*-RNase conserved regions in all *S*-alleles analyzed. These results allowed confirmation of genotypes previously established by other methods and to establish five self-(in)compatibility groups in apricot. The application of this methodology will permit to easily determine *S*-genotypes in apricot cultivars and in seedlings from breeding programs as well as the identification of new *S*-alleles.

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Genetic Variation for Plant Breeding, p. 348 (2004)

Linkage disequilibrium in lettuce and maize

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Abstract
With the aim of investigating the extent of linkage disequilibrium (LD) within the lettuce and maize genomes, methods for measuring LD in relation to genetic distance were evaluated, using single marker scores as well as marker haplotypes.

High density genetic molecular marker maps consisting of mainly AFLP markers were used in this study to position the marker scores of fingerprinted lines in genetic map order. For lettuce, 93 *Lactuca sativa* and *L. serriola* lines selected out of a lettuce genebank collection were fingerprinted. For maize a marker dataset consisting of 200 inbred lines divided over the 'flint' and 'dent' heterotic groups was produced.

LD was examined by calculating the R^2 values for all marker-pairs as well as haplotype-pairs. Using marker haplotypes, a stronger relation between LD and genetic distance was observed, as compared to using single marker scores. We argue therefore that marker haplotypes have superior power for LD-mapping studies.

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Three different onion male-sterile cytoplasms share a mutated mitochondrial locus – a study of sequence occurrence, organization and expression

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Abstract

Hybrid seed production of onions is based on cytoplasmic male sterility (CMS). In addition to normal male-fertile N-cytoplasm, three different male-sterile plasmatypes are used in onion breeding: S, C and T. We applied vectorette PCR to screen for mitochondrial polymorphisms among different cytoplasmic genotypes. Using this approach, a region of the mitochondrial genome was identified which appeared to be specific for the male-sterile cytoplasms. Sequence analysis revealed the chimeric character of this region – it contains segments of standard mitochondrial genes as well as a stretch of unknown origin. We also studied the genomic surroundings of the chimeric locus. The upstream sequences differentiate S- and C-plasmatypes from T-cytoplasm. The sequences located directly downstream of the chimeric locus are the same in all three CMS sources. On the basis of sequence data a SCAR marker was designed which can be used for convenient genotyping of onion cytoplasms.

The chimeric sequence is represented on RNA level – the respective transcripts were detected using both RT-PCR and Northern hybridization. Differential processing of this mRNA was observed for male-sterile vs. restored plants with T-cytoplasm. These data indicate that the chimeric mitochondrial sequence may be involved in expression of CMS in onions.

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Genetic Variation for Plant Breeding, p. 350 (2004)

Linkage analysis and genetic constitution of 2 populations of F_6 lines derived from *Lycopersicon pimpinellifolium* and *L. cheesmanii*

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Abstract

A population of recombinant inbred lines presents several advantages in comparison to its F_2 population counterpart for quantitative trait loci (QTL) and genomic studies. It facilitates more accurate estimates of the location of the QTL, with less variance, and each genotype can be replicated allowing its evaluation several times, or in several environments, different years, locations and for as many traits or molecules as needed. The objective of the present work is the comparative characterisation by SSR and SCAR markers of two populations of F_6 lines derived from *L. pimpinellifolium* (P population, consisting of 142 lines) and *L. chesmannii* (C population, consisting of 115 lines) and sharing the female parent, *L. esculentum* var. cerasiforme (E 9 line).

The same percentage of polymorphic markers was found for each population although involving a different set of markers. The proportion of SSR primer pairs (93 total) that resulted in polymorphism was larger (79.5 %) than for SCAR ones (54 % from a total of 42). Twenty nine and fifty two percent of markers deviated significantly from the F_6 expected ratio in P and C populations, respectively. They also differed in the distribution of heterozygosity per marker, being the most frequent class 5 - 7.5 % in P and 10 - 12.5 % in C population. The distribution of the percentage of *esculentum* alleles was much more narrow for P than for C population. The modes of these distributions were different too, 30 and 50 % of *esculentum* alleles in P and C populations, respectively.

A linkage map for each population was obtained using JoinMap 3.0 and average distances between consecutive markers were 3.8 or 3.4 cM depending on the population. Loci with skewed allelic ratio were scattered throughout the genome. In most genomic regions the degree of deviation was similar for linked markers except for 4 markers in chromosomes 3, 6 and 10. Their sequence analysis revealed the presence of duplicate marker loci at three of them. The linear order of markers was the same between maps except for 3 cases. Just 1 cM discrepancy in the location of two of them in chromosomes 3 and 12 was found while the distance involved in the third case (also in chromosome 3) was 4 cM.

This marker characterisation of both populations will continue and will permit the comparative QTL and candidate analysis of complex traits towards a more efficient utilisation of genetic resources and breeding strategies.

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Genetic Variation for Plant Breeding, p. 351 (2004)

Investigation of adaptive traits in forestry to differentiate between two closely related species by gene expression and allelic variation (single nucleotide polymorphisms)

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Abstract

The interspecific genetic exchange within *Quercus* is widespread. Though the species are genetically compatibel, their ecological preferences are distinct. *Q. robur* and *Q. petraea* have evolved the adaptability to wet and dry conditions, respectively. Their preference to populate different microclimatic niches was used as a basis for population differentiation. Plantlets of both species were grown in 4x concentrated P24 medium to induce osmotic stress. Leaf material was harvested at 4 different time points. The expression of seven genes out of 25 previously isolated from induced oak cell culture was monitored by real-time PCR. Single nucleotide polymorphisms (SNPs) were identified in 12 osmotic stress related genes and their inheritance was followed in the progeny of two crosses (one interspecific and one intraspecific *Q. robur* cross). In 8 polymorphic genes Gst based on genetic diversity was estimated on allele frequency distributions of 23 SNP markers in 10 mixed European oak populations. Six genes displayed significantly different expression under moderate stress conditions regarding *Q. robur* and *Q. petraea*. Early gene induction as well as delayed response could be observed. Twelve genes were located on 9 different linkage groups of oak. Nearly 50 % of the investigated SNP markers showed significant species differentiation.

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Detection of microsatellite instability during somatic embryogenesis of oak (*Quercus robur* L.)

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Abstract

Plants regenerated from organ cultures, calli, protoplasts and via somatic embryogenesis often show phenotypic and DNA variation, a phenomenon which has been termed somaclonal variation. Various methods are available for characterizing mutations induced by tissue cultures, including phenotypic identification, cytological methods and DNA analysis techniques. Many reports on tree species have dealt with the assessment of somaclonal variation using various molecular markers. Both dominant and co-dominant marker systems have been successful in detecting genetic instability. Among them, RAPDs (random amplified polymorphic DNA) and AFLP (amplified fragment length polymorphism) have been employed and have resulted both in the detection of variation induced by tissue culture as well as in cases in which no variation was found. In contrast to RAPDs and AFLPs, simple sequence repeats (SSRs) or microsatellites are co-dominant markers and show a high rate of mutability in all the species studied. Due to their relatively high degree of variability, microsatellite markers have been widely used for assessing genetic diversity. In order to evaluate the effects of *in vitro* culture on the genetic integrity of oak SEs, we applied SSR analysis. For several years we have been establishing a system of somatic embryogenesis for pedunculate oak (*Quercus robur* L.) with the aim of demonstrating the potential use of this vegetative propagation technique for tree improvement programs as well as for studying tree physiological aspects. In the course of the study reported here, we evaluated the applicability of SSRs for assessing genetic variability in SEs and derived plantlets of pedunculate oak (*Quercus robur* L.).

Five microsatellite loci (QpZAG1/5, QpZAG9, QpZAG36, MSQ4, MSQ13) were used to test for genetic stability of five somatic embryogenic culture lines of *Quercus robur* L. and plantlets derived from them. DNA variation was detected among somatic embryos within all embryogenic lines, thus indicating a genotypic effect, whereas no genetic instability was found among the regenerated plants. All microsatellite loci revealed variations and a locus-dependent instability could be observed. The most frequent polymorphic and useful microsatellite locus for detecting genetic variation was OpZAG9, with 34.6 % of the investigated loci being variable.

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OLD AND NEW GOALS IN PLANT BREEDING

Part 3

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Genetic Variation for Plant Breeding, pp. 355-357 (2004)

Application of breeding value prediction (BLUP) in crop plants

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ABSTRACT: The goal of this project is to establish the BLUP breeding value prediction (best linear unbiased prediction) under the conditions of plant breeding. The BLUP breeding value prediction is so far only used in animal breeding. On a long-term basis the number of test crosses which need to be performed to develop a new cultivar is supposed to be reduced by a more efficient selection. This procedure is to estimate the genetic disposition of different lines with consideration of the relation between the lines and different environmental conditions. Thus the average performance of the descendants concerned can already be judged before the execution of a cross. This method is particularly favourable for traits with low heritability, e.g. yield. As yet the plant breeder is forced, in order to be able to judge the genotypic caused achievement of his lines, to examine these at several locations and in several years. In the end only a very small portion of the accomplished crosses and tested lines is later developed to a new cultivar. The associated time and costs are to be clearly reduced by the employment of the breeding value prediction.

Key words: BLUP – breeding value prediction – plant breeding – selection

Introduction

The goal of breeding is to change individuals genetically in such a way that they are better adapted to the needs of humans (Becker 1993). Thereby those individuals from a population are selected whose descendants are closer in their achievements to the breeding goal than the parent's generation (Eßl 1996). Breeding goals in plant breeding can be e.g. the enhancement of yield, the improvement of the quality or the increase of disease resistance.

In order to reach these breeding goals there are different procedures. On the one hand a selection can be accomplished only on the basis of the phenotype. This method has however the disadvantage that it is difficult to select on traits with low heritability because these traits are characterized by a high environmental influence. Another possibility is the marker-assisted selection where in addition to the phenotypic selection marker data of these traits are incorporated. A marker is a short DNA sequence which is easy to recognize and is coupled with interesting genes (Becker 1993). The third method deals with the breeding value prediction. This method is characterized by the fact that the value of an individual for breeding will be estimated on the phenotypic data of the individual and on the performance data of the relatives. In addition different environmental conditions are considered. So the genetic disposition of an individual is to be recognized as exactly as possible and consequently the efficiency of the selection is increased (Comberg 1980).

So far in plant breeding the descendants from a cross are judged predominantly on the phenotypic data. A purely phenotypic selection is however based particularly on traits with low heritability and from this resulting in high environmental influence on a reduced statement about the genotype and thus the breeding value of an individual or a line. In this case it is difficult to compare achievements, which were generated under different environmental conditions (e.g. at different locations) and in different years. A further problem is present if there are unbalanced data records, e.g., that all examined descendants were not cultivated at all locations (Bernardo 2002). These designated problems frequently arise in plant breeding. Thus a correct selection decision can be made only if the genotype is known

as good as possible. A method with which one can judge the genetic disposition of an individual is the breeding value prediction.

Therefore, it shall be examined in this project whether the breeding value prediction can be accomplished also under the conditions of plant breeding. For this the BLUP method (best linear unbiased prediction) which is applied by default in animal breeding for the available populations is intended to be used. It is to be analyzed whether additional information for the selection decisions can be consulted with the help of this procedure. A special attention is given here to the genetic variances and covariances. In this work it is to be tried to establish the BLUP breeding value prediction under the conditions of the plant breeding. In addition there are simulated situations as they occur with self- and cross-pollination. Then the practical application of the BLUP breeding value prediction in plant breeding is to be tested by data from field trials. Furthermore the integration of marker data into the breeding value prediction is examined. On a long-term basis the number of test crosses which need to be performed to develop a new cultivar shall be reduced and at the same time the quality of the crosses is increased. Here, the meaning of the term quality is that the portion of very promising crosses is higher with than without BLUP because one is now able to select all "unfavourable" crosses directly. Additionally the breeding costs of a new cultivar are lowered.

Literature overview

The routine application of the BLUP breeding value prediction was so far exclusively limited to the animal breeding. In plant breeding and forestry this procedure was not used (White & Hodge 1989).

Cross-pollination

Hill and Rosenberger (1985) used alfalfa (*Medicago sativa* L.) showing that the BLUP breeding value prediction was superior to all other selection methods. Similar results were achieved by Bridges (1989) with cucumber (*Cucumis sativus* L.), Purba et al. (2001) with oil palm and White and Hodge (1988) with forest trees. Bernardo (1994, 1995, 1996a, 1996b) illustrated that with the help of the BLUP procedure the performance of maize crosses (*Zea mays* L.) could be measured by means of phenotype data before realization of field trials. Also based on the performance of hybrids already tested in field trials the achievement of still untested hybrids can be estimated (Bernardo, 1995). Duel et al. (1998) applied REML estimations in apple breeding. Here in contrast to the other attempts all relationship information was included in the estimation. The obtained breeding values were more informative for future breeding strategies than all values determined with standard methods.

Self-pollination

Panter and Allen (1995) accomplished investigations for the employment of the BLUP procedure in the breeding of soybean (G*lycine max*). He stated that with the aid of BLUP more favourable crosses could be detected before execution of field trials than with other selection methods. Apart from Duel et al. (1998) however in all studies mentioned no relationship information was included in the predictions. In order to reach an even more efficient prediction of the breeding values and to establish the breeding value prediction into plant breeding, the data of the relatives have also to be taken into account.

Materials and methods

This project intends to examine the application of the BLUP procedure on the data of field trials. For this a model for the breeding value prediction adapted to the conditions of plant breeding must be developed in which the relatedness of the lines among each other is considered as well. Starting from this, typical situations in plant breeding are reproduced with

the help of a computer simulation testing whether the BLUP breeding value prediction can be used in the selection processes of plant breeding.

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Plabsoft: Software for simulation and data analysis in plant breeding

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ABSTRACT: Plabsoft is a simulation and data analysis software for plant breeders. The functionality of Plabsoft comprises (1) population-genetic data analysis of molecular marker data and (2) simulation of individual breeding steps and entire plant breeding programs. In the area of population-genetic data analysis, routines for calculating genetic distance and diversity measures including their corresponding standard errors, as well as visualization tools such as dendrograms and principle coordinate analysis are available. Possible applications are illustrated for example by the study of Dreisigacker et al. (2004). In the area of simulation studies, the functionality of our previous software Plabsim (Frisch et al., 2000) was extended to simulate quantitative genetic models, which is illustrated in this paper. Currently we are extending the software for applications in association studies. We focus on tests for linkage disequilibrium, marker-trait associations, and algorithms for finding haplotype blocks. Plabsoft is written in C and implemented as a program library, for which we provide an interface to the statistical software R (Ihaka & Gentleman, 1996). The primary development platform is Linux, however, compilation is possible for a wide range of Unix operating systems. Licensing terms are available upon request.

Key words: Data analysis – Plabsoft – reciprocal recurrent selection – simulation – software

Introduction

In plant breeding and population genetics, computer simulations are a useful tool to investigate problems for which no analytical solutions are available. Until now, no software allowed one to simulate efficiently individual breeding steps and entire plant breeding programs under realistic genetic models. The objective of this paper is to illustrate the application of Plabsoft with a simulation study investigating the effect of gene action and breeding schemes on the long-term selection response in reciprocal recurrent selection (RRS).

Materials and methods

We investigated RRS (Comstock et al. 1949) with half sib family selection (Fehr 1993, p. 195) and a cycle length of three generations. This selection scheme can be applied in allogamous crops, where plants can be selfed and crossed at the same time. With RRS the general combining ability of two populations P1 and P2 with respect to each other can be improved simultaneously.

We simulated four scenarios. For scenarios I to III we used the following RRS scheme: All individuals of two populations P1 and P2 of size 90 were (i) selfed and (ii) crossed with five randomly sampled testers from the opposite population. Ten plants were selected on basis of the mean phenotypic value of the test cross progenies. One selfed progeny of each of the selected plants was used in a factorial crossing design. From each cross two plants were generated, which formed the base population of the next cycle. Scenario IV differed from scenarios I to III in that (a) the population size was 900, (b) 100 plants were selected, (c) five selfed progenies of each selected plant were generated, and (d) the base populations of the next cycle were generated by random mating the selfed progenies of the selected plants. For all scenarios 19 cycles of RRS were simulated and in each cycle, the mean genotypic value of populations P1, P2, and their hybrid population as well as the modified Rogers' distance (MRD) between population P1 and P2 were assessed. 200 simulation runs were conducted for each scenario.

Scenario I was applied in a simulation study of Cress (1967) and is characterized by the following genetic parameters: 40 independently segregating loci with two alleles per locus affecting one quantitative phenotypic trait in a diploid allogamous species. Overdominant gene action at all loci was assumed and the genotypic values of the three genotypes AA, Aa, and aa at a locus were 0, 2, 0. The maximum genotypic value of an individual was 80. The environmental effect was simulated with a Gaussian random variable with mean 0 and variance 120, modelling low heritability. The initial gene frequencies were f(A) = f(a) = 0.5 in population P1 and f(A) = 0.1, f(a) = 0.9 in population P2, both populations were in Hardy-Weinberg equilibrium. Scenario II was different from scenario I in the following two respects: (i) Gene action was assumed to be dominant, the genotypic values of the three genotypes AA, Aa, and aa at a locus were 2, 2, 0. (ii) The initial gene frequencies in populations P1 and P2 were f(A) = f(a) = 0.5. Scenario III was different from scenario II in that the environmental effect was zero, modelling a high heritability. Scenario IV was different from scenario III in that 20 independently segregating loci were assumed, and the genotypic values of the three genotypes AA, Aa, and aa at a locus were 4, 4, 0.

Results and discussion

Scenario I

Cress (1967) simulated scenario I with one simulation run, while we used 200 simulation runs. The results for the mean genotypic values of Cress (1967) are mostly within a confidence interval, created by $\mu \pm 2\sigma$ from the results of our simulation (Figure 1). After 19 cycles of RRS the hybrid population reached a mean genotypic value significantly below 80. The observation that the maximum value of 80 was not reached can be explained by the fixation of the same allele at many loci in both populations. The mean genotypic value of populations P1 and P2 decreased as expected, because gene action was overdominant. The genetic distance between the populations increased from 0.40 before cycle 1 to 0.95 in cycle 19 (Figure 1), which indicates that the populations were diverging.

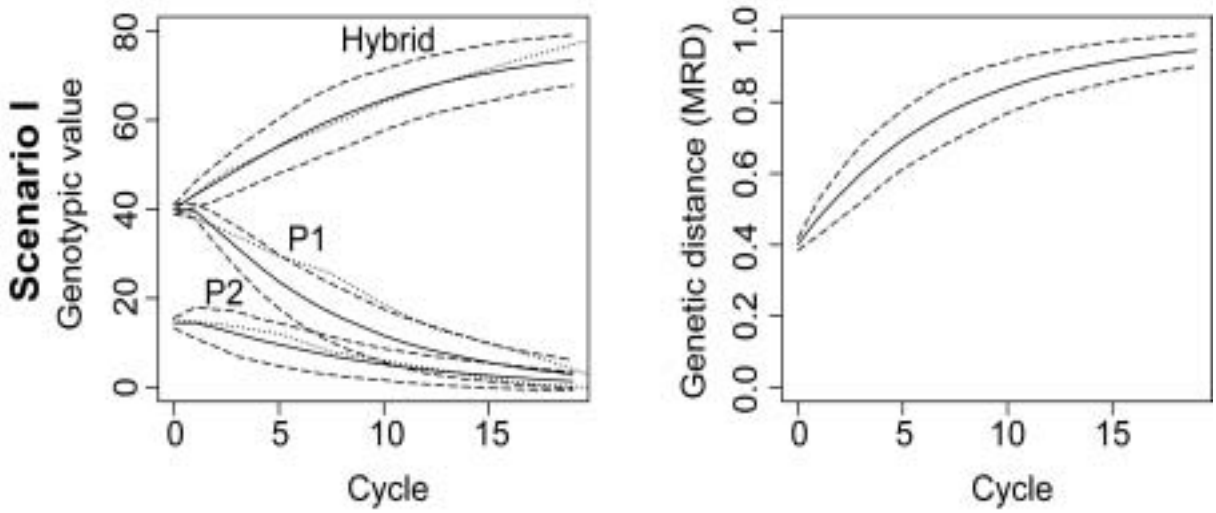

Figure 1. Scenario I. Left: mean genotypic value (solid line) under RRS of populations P1, P2 and their hybrid population. Results of Cress (1967, Figure 6) are presented with dotted lines. Right: Genetic distance (solid lines) between populations P1 and P2 under RRS. The dashed lines mark two times the standard deviation of the respective solid lines.

Scenarios II to IV

In scenario II the mean genotypic value of the hybrid population reached almost the maximum possible value of 80 after 19 cycles of RRS (Figure 2). The mean genotypic value of the populations P1 and P2 decreased slightly during the 19 cycles of RRS, however its standard deviation was quite high.

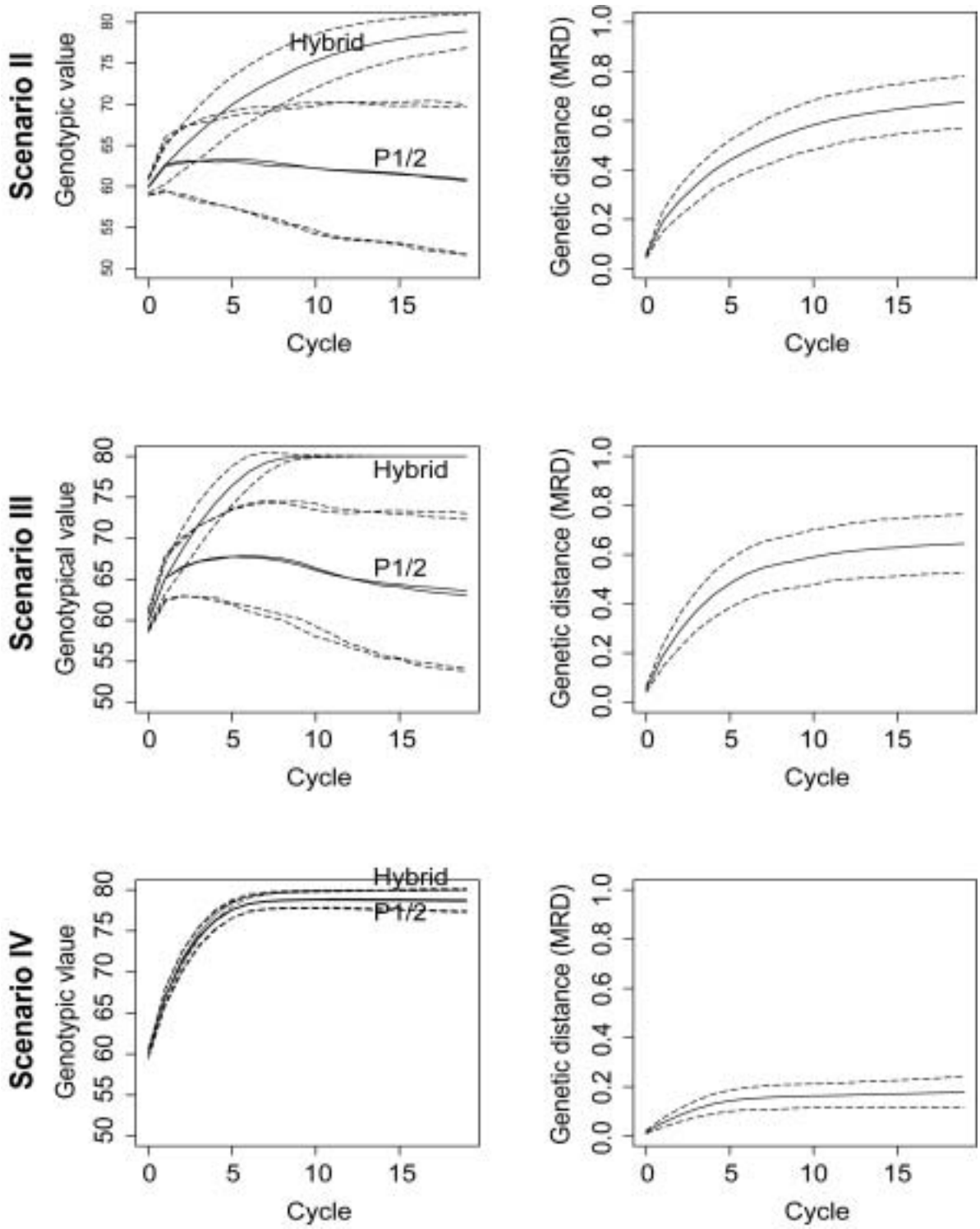

Figure 2. Scenarios II to IV. Left: mean genotypic value (solid line) under RRS of population P1, P2 and their hybrid population. Right: Genetic distance (solid lines) between the populations P1 and P2 under RRS. The dashed lines mark two times standard deviation of the respective solid lines.

According to Wricke and Weber (1986, p. 278), changes of gene frequency in recurrent selection in one population should be similar to those in RRS, if (i) initial gene frequencies are equal, (ii) population sizes are infinite, and (iii) gene action is additive or dominant. From this theory follows that an increase of the mean genotypic value to the maximum possible value is expected in the hybrid population as well as in the populations under RRS. Furthermore, the two populations P1 and P2 should not diverge genetically. However, these effects were not observed in our simulation of scenario I. This can be caused by (i) low heritability and/or (ii) drift due to finite population size.

In scenario III the mean genotypic value of the hybrid population reached after 8 cycles an mean genotypic value of 79.5 and the mean genotypic value of the populations P1 and P2 reached about two cycles later a plateau with an average value of 67.8. An explanation of this result is that in later cycles, the favorable allele is completely or almost fixed at least in one of populations P1 or P2 and, hence, selection is no longer effective. In such cases, random drift leads to a loss of favorable alleles in the opposite population. In consequence, the mean genotypic value in the populations under RRS decreases.

In scenario IV the mean genotypic value of the hybrid population reached a value of 79.5 after seven cycles of RRS, the mean genotypic values of the populations P1 and P2 reached a value of 78.9. The genetic distance after 19 cycles of RRS was 0.18. These results are in accordance with theoretical expectations: The mean genotypic values of populations P1 and P2 under RRS compared to scenarios II and III are higher and the increase of genetic distance over the cycles is reduced. Hence, the modifications (i) high heritability and (ii) larger population size were efficient to reduce drift.

This simulation study demonstrates that complete plant breeding programs can be efficiently simulated with Plabsoft. In addition to the features used in this study, the functionality of Plabsoft comprises (i) complex genetic models including linked genes, multiple alleles, and epistatic gene action, (ii) the possibilities to simulate arbitrary crossing schemes. The resulting parameters can be statistically analyzed and graphically visualized.

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Adaptability and performance of released bread wheat varieties evaluated at various environments in western Oromia, Ethiopia

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ABSTRACT: A selection of 17 wheat varieties released in Ethiopia was grown together with one local variety at four sites in two years. Compared to the local control, all varieties were earlier in flowering and maturity and shorter in plant height. In grain yield most of the improved varieties did not surpass the local check, however, the highest mean yields were obtained by the two new varieties 'HAR 1685' and 'HAR 604'.

Key words: Days to flowering – days to maturity – grain yield – plant height – *Triticum aestivum*

Introduction

Wheat is the major staple crop of the high lands of Western Oromia, Ethiopia. For centuries farmers did grow local varieties which were replaced more and more by new wheat selections. However, farmers still tend to cultivate local varieties covering about 50 % of the wheat growing area of the country. In order to get more information about the performance and adaptability of modern bread wheat, 17 varieties were grown at four sites in two years. As a control, one local variety was included in the experiments. Three field trials were performed in the Western Ethiopian high land (>2400 masl) at Arjo, Gedo and Shamb, representing typical wheat growing areas with cooler climate but one at Bako located at intermediate altitude (1650 masl).

Materials and methods

Seventeen nationally released bread wheat varieties (Tables 1 - 4) were grown together with one local variety, designated LOCAL at four locations (Arjo, Bako, Gedo and Shambu) during the main seasons of 2001 and 2002, except at Arjo where the experiment was conducted only during 2002. Sowing was made following the recommended dates of sowing wheat at respective sites. The field experiments were performed in a randomised block design replicated three times with a net plot size 3 m^2 (six rows of 2.5 m length and rows spaced at 20 cm distance from one another). The seed rate used was 160 kg ha^{-1} and a fertilizer rate of 100 kg/ha of DAP was applied at sowing at all sites.

Data were taken for days to heading, days to maturity, plant height (cm) and grain yield (kg ha^{-1}). The traits days to heading and days to maturity were not scored at Bako in 2001 and Arjo in 2002, respectively. Data analysis was made using MSTAT-C computer software.

Results

Statistically significant differences were observed among varieties (Tables 1 - 4) for the traits analysed, except for days to heading at Gedo (2001, 2002) and for grain yield at Shambu (2001). Compared to the local control, the released wheat varieties were on average at least 5 and 7 days earlier in heading and maturity, respectively (Tables 1 & 2).

Table 1. Mean days to heading at the four test locations

Entry name	Arjo	Bako	Gedo		Shambu		Variety
	2002	2002	2001	2002	2001	2002	mean
HAR 1685	71	58	78	76	66	68	70
HAR 1595	70	45	75	77	66	69	67
K-6295-4A	73	54	71	77	71	71	70
HAR 710	70	44	79	80	70	68	69
HAR 1407	68	54	69	70	65	67	65
ET 13	73	62	80	78	69	72	73
HAR 1522	73	53	77	78	73	72	71
HAR 1709	69	50	76	68	64	66	66
PAVON 76	72	55	76	70	66	68	68
DERESELIGN	69	50	77	68	57	58	63
6290 BULK	72	48	81	71	70	71	69
HAR 604	75	63	80	80	70	71	73
HAR 1899	71	55	70	72	65	68	67
HAR 1775	72	59	74	70	67	70	69
HAR 1865	72	58	89	79	69	69	73
HAR I889	70	55	65	69	65	68	65
HAR 1480	71	48	70	70	70	67	66
LOCAL	70	83	86	71	75	85	78
ANOVA	**	**	ns	ns	**	**	
CV (%)	2.5	10.4	14.7	12.0	2.5	2.5	
$LSD_{0.05}$	2.9	9.5	18.6	14.7	2.8	2.9	

**, P<0.01; *, P<0.05; ns, not significant (P>0.05); CV, coefficient of variation; $LSD_{0.05}$, least significant difference (P=0.05)

Table 2. Mean days to maturity at the four test locations

Entry name	Bako		Gedo		Shambu		Variety
	2001	2002	2001	2002	2001	2002	mean
HAR 1685	87	101	127	109	127	125	113
HAR 1595	86	94	128	113	126	127	112
K-6295-4A	86	99	136	114	132	129	116
HAR 710	80	95	128	112	128	125	111
HAR 1407	86	100	133	109	126	130	114
ET 13	90	106	135	116	133	139	120
HAR 1522	90	97	129	113	128	136	115
HAR 1709	85	98	132	114	127	129	114
PAVON 76	81	99	129	110	127	127	112
DERESELIGN	79	88	129	113	122	126	109
6290 BULK	80	93	130	113	129	125	112
HAR 604	95	104	132	115	129	129	117
HAR 1899	87	100	128	111	127	129	114
HAR 1775	90	106	127	112	129	127	115
HAR 1865	90	100	128	119	129	127	116
HAR I889	87	99	128	116	124	125	113
HAR 1480	90	97	129	117	129	130	115
LOCAL	121	116	134	120	136	136	127
ANOVA	**	**	**	**	**	**	
CV (%)	3.7	3.8	0.7	1.4	2.4	2.2	
$LSD_{0.05}$	5.47	6.3	1.4	2.7	5.1	4.7	

For plant height the varieties were on average at least 7 cm shorter then the local control (Table 3).

In grain yield the two varieties 'HAR 1685' and 'HAR 604' with the overall year by location means of 39.8 and 39.4 dt ha^{-1}, respectively, outyielded all the remaining varieties. However, most of the improved varieties did not surpass the local check (Table 4). Seasonal variation in grain yield was observed. During the 2001 crop season yield was generally lower compared to 2002 at Bako and Shambu sites. On the other hand, yield declined nearly by half at Gedo in 2002, mainly attributed to the occurrence of moisture stress that prevailed starting from sowing till the end of crop growth stage. The year 2002 was characterised by the late commencement, low and erratic rainfall distribution throughout the country. High location mean grain yields of 45 and 45.8 dt ha^{-1} were observed for Shambu 2002 and for Gedo 2001, respectively. These values of grain yields were nearly five folds of the location mean grain yield attained at Bako 2001 and by about twice the yield recorded at Gedo 2002 and Arjo 2002.

Table 3. Mean plant height (cm) at the four test locations

Entry name	Arjo	Bako		Gedo		Shambu		Variety
	2002	2001	2002	2001	2002	2001	2002	mean
HAR 1685	78	88	80	78	86	91	82	83
HAR 1595	84	87	78	78	87	103	88	87
K-6295-4A	88	87	102	90	101	119	101	98
HAR 710	79	82	90	78	86	96	85	85
HAR 1407	84	87	91	83	91	96	83	88
ET 13	90	91	84	105	98	120	112	100
HAR 1522	93	90	88	75	89	118	81	90
HAR 1709	88	86	107	92	104	116	107	100
PAVON 76	82	82	91	76	89	96	82	85
DERESELIGN	94	80	93	92	112	117	106	99
6290 BULK	92	81	101	96	109	100	102	97
HAR 604	92	95	98	90	85	104	102	95
HAR 1899	82	88	98	77	96	99	84	89
HAR 1775	74	91	90	72	84	98	77	84
HAR 1865	78	91	85	75	102	97	74	86
HAR I889	83	88	97	82	87	104	93	90
HAR 1480	76	91	88	80	100	106	78	89
LOCAL	72	120	117	87	83	133	134	107
ANOVA	**	**	**	**	**	**	**	
CV (%)	6.4	3.8	7.3	9.7	4.1	8.6	10.5	
$LSD_{0.05}$	8.9	5.6	11.3	13.4	6.5	15.1	16.1	

Table 4. Mean grain yield (dt ha^{-1}) at the four test locations

Entry name	Arjo	Bako		Gedo		Shambu		Mean
	2002	2001	2002	2001	2002	2001	2002	
HAR 1685	33.0	12.7	33.2	52.5	33.4	51.7	62.1	39.8
HAR 1595	22.5	7.2	27.5	40.8	20.5	38.3	44.4	28.7
K-6295-4A	24.9	8.3	27.8	48.9	18.1	26.8	46.1	28.7
HAR 710	20.6	8.5	22.5	40.1	26.8	27.2	48.3	27.7
HAR 1407	20.8	11.5	33.5	38.7	28.5	37.4	38.7	29.9
ET 13	21.5	6.5	24.1	46.0	19.4	30.9	45.4	27.7
HAR 1522	25.4	8.5	34.7	40.3	20.8	25.9	26.8	26.1
HAR 1709	26.7	9.0	34.3	48.6	25.3	39.2	47.1	32.9
PAVON 76	32.8	13.3	32.5	54.7	29.0	39.3	44.5	35.2
DERESELIGN	21.1	5.3	34.3	38.4	24.8	32.2	35.4	27.4
6290 BULK	18.4	7.2	32.2	50.1	17.6	30.2	43.4	28.4
HAR 604	32.8	8.9	27.9	66.3	30.6	53.0	56.6	39.4
HAR 1899	22.1	10.9	43.0	42.9	27.5	34.0	39.5	31.4
HAR 1775	17.2	8.6	28.5	40.0	27.2	26.6	46.1	27.7
HAR 1865	23.0	8.4	38.4	42.6	18.4	28.9	55.3	30.7
HAR I889	16.0	10.3	31.7	39.8	32.0	37.2	46.3	30.5
HAR 1480	14.0	8.6	27.7	43.3	18.2	23.7	39.4	25.0
LOCAL	19.9	-	13.3	50.0	25.1	37.3	45.4	31.8
Mean	22.9	9.0	30.4	45.8	24.6	34.4	45.0	
ANOVA	*	**	**	**	**	ns	**	
CV (%)	29.4	25.7	21.0	14.4	14.9	33.7	10.6	
$LSD_{0.05}$	11.2	3.9	10.6	11.0	6.1	19.3	7.9	

Discussion

Wheat, the number one cereal of the world is grown in most temperate and subtropical, but also in tropical countries (Onwueme and Sinha 1999). As a continent, Africa is not important for wheat production. The share of tropical Africa is very low. Only four countries (Zimbabwe, Ethiopia, Sudan and Kenya) produce this crop in large quantities of more than 0.1 million tonnes (Onwueme & Sinha 1999). Ethiopia is the second largest producer of wheat in Sub-Saharan Africa. Farmers tend to grow local varieties, although new material is provided by plant breeders. The data presented here clearly demonstrate, that new varieties are earlier in flowering and maturity and shorter in plant height. The two characters are important to avoid drought stress and lodging. In grain yield most of the improved varieties did not surpass the traditional ones, which seem to be better adapted. However, new selections are available now outyielding local varieties.

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Chromosome 1D as a possible location of a gene (*s*) controlling carbon isotope discrimination (Δ) in wheat (*Triticum aestivum L.)* under water-stress conditions

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ABSTRACT: In this study, F_2 back cross reciprocal monosomic crosses between varieties 'Falchetto' (low Δ) and 18 monosomic lines of 'Oxley' (high Δ) were used to identify chromosomal location of the gene (s) responsible for Δ. F_2 reciprocal monosomic families were initially assessed for dry matter production. F_2 families belonging to the chromosomes that indicated allelic variation for dry matter production were also assessed for Δ. The results revealed that reciprocal families belonging to chromosomes 1B, 7B, 1D and 5D indicated significant differences from which the family having chromosome 1D from 'Falchetto' had the highest difference from its relevant reciprocal. Assessing the reciprocals of this chromosome for ETE at the F_3 disomic generation indicated that the observed variation for Δ was translated into differences for ETE. These results indicate that chromosome 1D of 'Falchetto' is promising in reducing Δ and that the improvement of wheat varieties for ETE can be done by selection for Δ. When selecting for Δ, managing the experiment is easier and there is no need to monitor the water use during the growth cycle.

Keywords: Monosomic analysis – pre-anthesis – reciprocal crosses – *Triticum aestivum* – water stress

Introduction

When monosomic series are available for only one variety of the two varieties under study, backcross reciprocal monosomic analysis can be used to determine allelic differences between any two homologous chromosomes each belonging to one of the varieties. Backcross reciprocal monosomic analysis has been used as a method to identify chromosomes involved in various quantitative traits (Snape et al. 1983, Law et al. 1987, Buerstmayr et al. 1999). This method involves crossing a certain variety to an existing monosomic series and reciprocal crossing of F_1 hybrids with the initial monosomic variety. Comparison between the two F_2 backcross reciprocal families determines the allelic differences between the two homologous chromosomes and therefore the chromosomes involved in the character under study. Theoretically, F_2 reciprocal families for each chromosome have the same genetic background variation and differ only for the homologous chromosomes each originated from one of the parents if sufficient plants from different BC_1 hybrids are assessed in the replicated experiments (Snape et al. 1983). In this situation, any difference observed between the two F_2 backcross reciprocal families in fact reflects the difference between the two homologous chromosomes of the parents.

Data from a primary experiment (Figure 1) demonstrated that 'Oxley' showed clear differences from 'Falchetto' for carbon isotope discrimination. The aim of this experiment was to identify possible chromosomal variation between the two varieties for carbon isotope discrimination using backcross reciprocal monosomic analysis.

Material and methods

F_2 backcross reciprocal monosomic families were produced using a method proposed by Snape et al. (1983). The production of reciprocal families was successful for 18 chromosomes out of 21. Carbon isotope discrimination indicated negative significant relationship with dry matter (DM) in previous studies (Ehdaie & Waines 1996, Rebetzke et al. 2002). Because of

this relationship and also limitation in available funds for carbon isotope analysis, Δ was measured only for reciprocal lines, which produced large differences for dry matter production. At least 30 seeds from each family were grown in 10.6 cm pots. The pots were labelled randomly and transferred to a growth room (min 15ºC, max 23ºC; 16 hours photoperiod). The pots were irrigated evenly on a wet mat equipped with an automatic water supply. The plants were grown in this situation until at least 10 plants of each reciprocal line reached Zadoks stages 37-39. These plants were separated from the others and water-stress was imposed on them by withholding water for 7 days. The dried flag leaf from each plant was stored separately for measurement of carbon isotope discrimination. Leaves were finely ground to a powder to ensure homogeneity and to achieve greater accuracy in determination of carbon ratio. Only a small amount of plant material is required for the majority of combustion systems (Griffiths 1993), therefore samples were weighed in amounts of 1±0.05 mg. The carbon composition (δ ‰) of samples was determined using an elemental analyser isotope ratio mass spectrometer known as ANCA-SL (Automated Nitrogen Carbon Analysis unit for Solids and Liquids), PDZ Europe 20/20 mass spectrometer. Δ was calculated using the equation Δ (‰) = (δ_a-δp) / (1 + δp) assuming δ_a = -7.6 ‰ (Farquhar & Richards 1984). Vegetative water use efficiency (WUE_{veg}) and vegetative evapotranspiration efficiency (ETE_{veg}) were measured for reciprocals of chromosome 1D at F_3 according to Ehdaie (1995).

Results and discussion

From a primary experiment, a significantly higher carbon isotope discrimination (Δ) was consistently observed in 'Oxley' than in 'Falchetto' for the two treatments (Figure 1). Δ was significantly lower (P<0.01) in water-stressed than in well-watered plants of the two varieties. This indicates a negative effect of water-stress on Δ. The variation in Δ depends on differences in either stomatal conductance or photosynthetic capacity (Condon et al. 1987). It seems that stomatal resistance and photosynthetic capacity interact positively in the reduction of Δ under water-stress conditions. This is a possible explanation for higher differences between the varieties under water-stress conditions than under normal conditions. So, under normal conditions, where there is no significant difference between the two varieties for stomatal resistance, lower Δ in the variety 'Falchetto' can be accounted for by the higher photosynthetic capacity of this variety. But, under water-stress conditions, the lower Δ of 'Falchetto' is partly due to higher stomatal resistance and partly due to higher photosynthetic capacity in this variety.

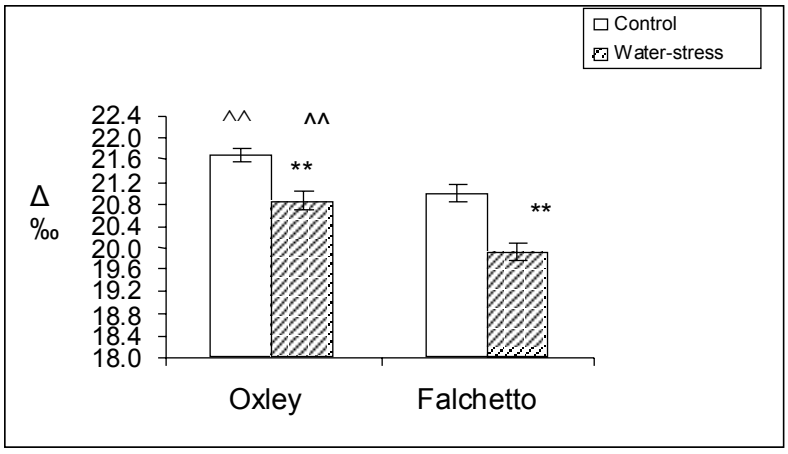

Figure 1. Comparison between treatments and varieties for Δ (standard errors of means are shown by vertical bars; **: significantly different from control at P < 0.01; ^^: significantly different from variety 'Falchetto' at P < 0.01)

Table 1. The mean performance and standard errors of each F_2 back cross reciprocal family for DM (g) together with the difference between each pair of reciprocal families

Chromosome designation	Chromosome origin		Difference between reciprocals	P value
	Oxley	Falchetto		
1A	1.27±0.14	1.25±0.12	0.02	0.90
3A	1.16±0.09	1.23±0.07	-0.07	0.52
4A	1.34±0.08	1.10±0.10	0.24	0.09
5A	1.16±0.07	1.25±0.08	-0.09	0.45
6A	1.25±0.06	1.04±0.09	0.21	0.33
7A	0.80±0.02	0.75±0.05	0.05	0.13
1B	0.54±0.06	0.77±0.08	-0.23	0.04
2B	1.22±0.12	1.58±0.15	-0.36	0.10
3B	1.37±0.12	1.21±0.14	0.16	0.42
4B	0.99±0.08	1.17±0.07	-0.18	0.10
5B	0.77±0.05	0.72±0.04	0.05	0.53
6B	0.91±0.09	0.67±0.05	0.24	0.06
7B	0.85±0.03	0.75±0.02	0.10	0.04
1D	0.68±0.10	1.29±0.06	-0.61	<0.001
3D	1.07±0.08	1.02±0.02	0.05	0.80
4D	0.98±0.05	1.13±0.06	-0.15	0.10
5D	0.69±0.06	0.92±0.06	-0.23	0.03
6D	0.90±0.07	1.28±0.25	-0.38	0.19
Euploid parents	0.55±0.04	0.72±0.05	-0.17	0.04

Table 2. The mean performance and SE of 4 pairs of backcross reciprocal families for Δ (‰) together with the difference between each pair of reciprocal families (n=10)

Chromosome designation	Chromosome origin		Difference between reciprocals	P value
	Oxley	Falchetto		
1B	27.08±0.20	26.54±0.16	0.54^{*}	0.04
7B	26.21±0.13	26.05±0.11	0.16	0.36
1D	24.94±0.16	23.61±0.13	1.33^{***}	4.3×10^{-6}
5D	25.85±0.30	24.93±0.12	0.92	0.011

*, ***: Significant at P<0.01 and P<0.001, respectively

Table 3. Mean and SE of F_3 backcross reciprocal disomic families of chromosome 1D for WUE_{veg} and ETE_{veg} and differences between the reciprocals

Character	Origin Oxley	Origin Falchetto	Difference	P value
WUE_{veg} (mg g^{-1})	1.38±0.04	1.68±0.08	-0.30^{***}	3.72×10^{-5}
ETE_{veg} (mg g^{-1})	2.86±0.06	3.73±0.14	-0.87^{***}	1.08×10^{-6}

Carbon isotope discrimination was measured for the reciprocals which had shown significant differences for DM. Comparison between reciprocal lines for 18 chromosomes revealed that reciprocals showed significant differences for dry matter production in chromosomes 1B, 7B, 1D and 5D (Table 1). In this case chromosomes 1B, 1D and 5D from 'Falchetto' and chromosome 7B from 'Oxley' had positive effects on this character. Therefore Δ was measured for these reciprocals only. The results with these lines are presented in

Table 2. Among 4 chromosomes, which were evaluated for Δ, reciprocals for 3 chromosomes showed significant differences for this character from which the line having chromosome 1D from 'Falchetto' had the highest difference from its relevant reciprocal. In the case of chromosome 1D, WUE_{veg} and ETE_{veg} were also measured at F_3 generation. According to the results presented in Table 3, reciprocals for this chromosome showed significant differences for ETE_{veg} and WUE_{veg}. This implies that allelic variation for both characters exists between the two varieties. A pleiotropic effect of chromosome 1D on dry matter, water use efficiency and Δ is expected due to physiological relationship between these characters. When stomata are closed and plants use water economically, water use efficiency is increased. On the other side, stomata closure causes low concentration of CO_2 in leaf cells and the capacity of carboxylase enzyme is sufficient to fix nearly all molecules of CO_2. When stomata are fully open, CO_2 concentration is too high to be totally fixed by carboxylase enzyme. In this situation, concentration of CO_2 molecules containing ^{13}C is depleted due to an unknown reason, and therefore Δ increases and reduces water use efficiency (Farquhar and Richards 1984).

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Role of varieties resistant to abiotic stress factors in reliable wheat production in Hungary

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ABSTRACT: The effects and interactions of extreme temperatures and water supplies on cereal species with different genetic backgrounds were studied in an artificial environment. It was concluded from the phytotron experiments that differences in the soil moisture content during freezing had little influence on the survival percentage of varieties with excellent frost resistance, while for varieties with moderate or poor frost resistance differences of as much as 50 % were observed between the survival rates of plants tested at different soil moisture contents. The optimum temperature for initial development and the response to extreme values were determined for each variety in an experiment set up in the gradient chamber. Although each of the 12 varieties exhibited different responses on the basis of phenological data, groups exhibiting characteristic development dynamics could be distinguished when all the traits were considered together. Changes in direct proportion to the environmental factors were characteristic of the phenological traits during the early stages of development, with the exception of changes in the biomass, which exhibited a logarithmic pattern. A reduction in the soil moisture content led to a significant decrease in biomass for all the genotypes tested. The extent of this biomass reduction as a response to unfavourable water supplies differed from one genotype to the other. There were also differences in the shoot-root growth dynamics. The shoots responded sensitively to unsatisfactory water supplies, while the root mass did not decrease proportionately with the drop in the water supply level.

Key words: Abiotic stress – cereals – drought tolerance – frost resistance

Introduction

The quantity and quality of cereal yields is the result of the effects exerted by numerous factors, one of the most important of which is the potential yielding ability of the variety. The extent to which this is achieved under various production conditions depends on the adaptability of the variety. Under the continental climate of the Carpathian Basin the most frequently encountered unfavourable environmental conditions are low or high temperature and water deficiency. The practical importance of this subject was most recently demonstrated by the weather during the 2002/2003 season, when the climatic extremes severely tested the adaptability of winter cereals. The unusually long, severe winter was followed almost immediately by a hot summer and a lack of rainfall. Consequently, all the unfavourable climatic effects characteristic of the Carpathian Basin were experienced together for a long period, leading to the lowest yield average for 30 years.

Of the many damaging effects of the winter, partial or complete frost damage was caused last year by the cold itself on areas with no snow cover, and by frost lifting on areas where the snow melted rapidly in spring and the wet ground was re-frozen. Of the abiotic factors, the development of frost resistance is influenced by both the quantity and distribution of rainfall, since the overwintering or survival of varieties with various levels of frost resistance, exposed to different hardening conditions, is determined to a great extent by the water content of the plant tissues (De Noma et al. 1989, Adak & Eser 1993).

In Hungary water is a decisive factor in the functioning, biomass yields and environmental effects of agroecosystems (Várallyay 2001). Water deficiency induces complex plant responses. The defence mechanisms developed by different varieties to combat drought stress include a mixture of stress-avoiding and tolerance-improving strategies. In

plants from the Mediterranean zone, avoidance mechanisms are dominant (Chaves et al. 2002), as evinced by various structural and metabolic changes which are now genetically fixed. Substantial differences can be observed between the wheat varieties in the magnitude of the stress response, which is manifested in the plant height, the dry mass of the straw, shoots and roots, and the proline, protein and chlorophyll contents, but also in the number, size and activity of the stomata and in the number of spikelets and grains/spike (Safaie & Ghadiri 1995).

Breeders are best able to judge drought tolerance on the basis of morphological traits. Both the below- and aboveground plant organs exhibit great genetic variability. Under adequate water conditions, root growth exhibits a close correlation with soil water content (Bchini et al. 2002), but is less sensitive to water deficiency than the shoot system (Chaves et al. 2002).

The present paper discusses the effect of soil moisture content on the frost resistance and development of cereals, and the influence of temperature and soil moisture content on genotypic differences due to their effect on biomass.

Materials and methods

The experiments were carried out under controlled conditions in the phytotron of the Agricultural Research Institute of the Hungarian Academy of Sciences in Martonvásár on varieties of winter wheat, rye and spring wheat chosen to represent the main wheat-producing zones of the world. The frost resistance of *T. aestivum* genotypes of different origin was studied at various soil moisture contents in a randomized block experiment with four replications. The plants were raised for six weeks on climatic programme M29 (for details see Tischner et al. 1997). In the sixth week and during the first phase of hardening five different soil moisture levels were adjusted. Freezing was carried out at -15°C for 24 hours. After thawing out for two days, the boxes were transferred to growth benches for three weeks of further growth, after which plants which had survived freezing and were beginning to develop could be easily distinguished from those which had been killed.

One of the most important factors influencing development is the temperature, the effect of which was determined in the gradient chamber. Pots containing plants of the different varieties tested were arranged on the growth bench in 12 rows (12 temperature levels) × 12 columns (12 varieties). Due to the border effect, evaluation was made only on plants from the inner 10 rows × 10 columns. The shoot length, number of tillers and number of leaves were recorded. After four weeks measurements were extended to include the fresh and dry mass of the shoots, roots and whole plants.

Early development was studied in 12 cereal varieties ('Bánkúti 1201', 'Mv Emma', 'Mv Mezőföld', 'Mv Martina', 'Mironovskaya 808', 'Bezostaya 1', 'Soissons', 'Lona', 'Mv 15', 'Motto', 'Thesee', 'NS Rana 2') which were bred in areas of the world with different rainfall supplies and were therefore expected to have different levels of water utilisation. Increasing levels of soil moisture were adjusted in the pots (30 %, 40 %, 50 %, 60 % and 70 % of natural water capacity). In the course of the experiment a record was made of changes in morphological traits (shoot length, tiller number, leaf number) providing a good reflection of the development of the seedlings. The results were statistically analysed using two-way analysis of variance.

Results and discussion

Correlation between soil moisture content and frost lifting

The mean mortality rates for *T. aestivum* varieties at various soil moisture contents exhibited significant differences (Table 1), lower rates being recorded at low soil moisture content and higher rates as the moisture content increased. The results indicate that under Hungarian

conditions the survival of wheat varieties with excellent frost resistance is not greatly influenced by the soil moisture content. For varieties with moderate or poor frost resistance, however, such as 'NS Rana 2', frost kill may be as high as 100 % in wet areas.

Table 1. Effect of soil moisture content on the survival of *T. aestivum* varieties

	Survival percentage			
Variety	Dry	Normal	Wet	Mean
Cheyenne	96.1	83.9	76.1	85.4
Martonvásári 4	98.8	89.3	90.9	93.0
Martonvásári 8	92.5	65.9	60.1	72.8
Bánkúti 1201	59.3	43.7	7.5	36.8
Martonvásári 2068fj.	40.3	21.6	5.3	22.4
NS Rana 2	20.9	11.9	0.0	10.9
Mean	68.0	52.7	40.0	53.6

Freezing temperature: –15ºC; Soil moisture content as a % of natural capacity: Dry=30%, Normal=45%, Wet=60%; $LSD_{5\%}$=14.9 between any two combinations; $LSD_{5\%}$=4.9 between mean values

Effect of temperature on the seedling development of cereals

Even at the beginning of the experiment (in the 4-week stage) varieties of different origin behaved significantly differently under differing environmental conditions. In some temperature ranges the differences in growth were in excess of 50 %. The shoot length and leaf number, characteristic of the seedling development of the variety, gave the widest range of values as a response to temperature conditions. The wheat varieties typically exhibited average or above-average intensive shoot development, in contrast to the rye variety 'Motto', which had slow shoot development, though the rate of change in the leaf surface was greater than the variety average for wheat. The greatest differences between the varieties were observed for fresh biomass (Figure 1). It is interesting to note that in this respect the varieties 'Mv Martina' and 'Mv Mezőföld' formed separate groups, the former being characterised by an intensive response to temperature and the latter by very little dependence on this environmental factor.

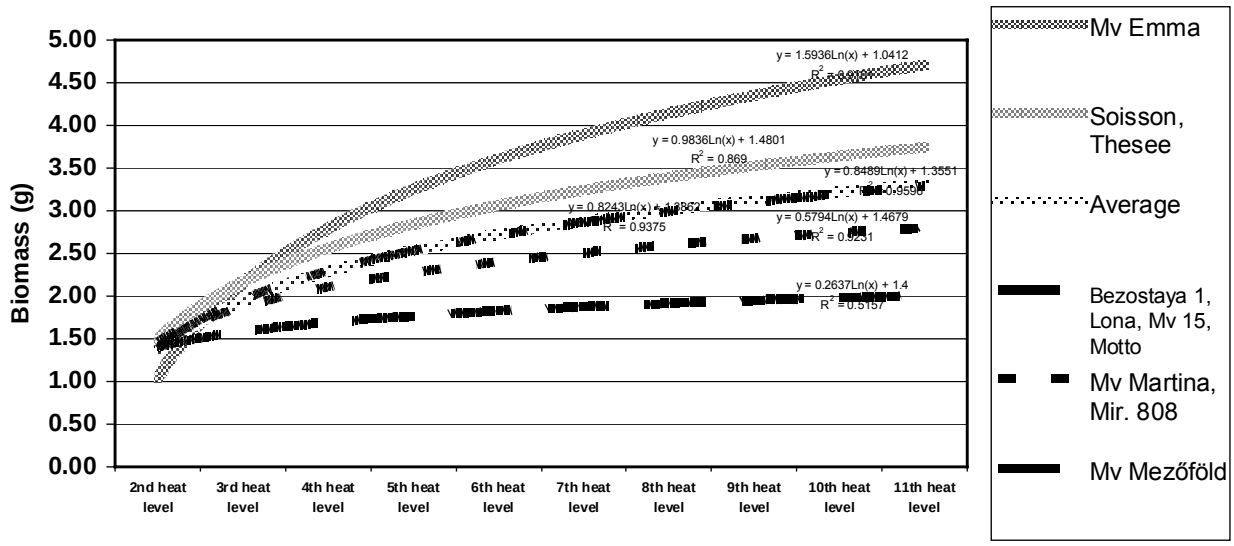

Figure 1. Changes in plant biomass at different temperatures

Genotype responses to water deficiency

The plants in the experiment responded to increasing levels of water deficiency by a clear reduction in biomass production. Depending on the sensitivity of the varieties this change differed in extent, but exhibited the same tendency. In some varieties ('Bánkúti 1201', 'Mv Martina', 'Mironovskaya 808') the decrease in biomass production was linear (Figure 2), while in 'Mv Mezőföld' and 'Mv 15' the biomass production at the highest water supply level was much greater than at the lower levels. The greatest reduction in total mass was observed for the wheat variety 'Mv 15', while that recorded for the rye variety 'Motto' was almost as great. The least damage was observed for 'NS Rana 2' and 'Thesee'.

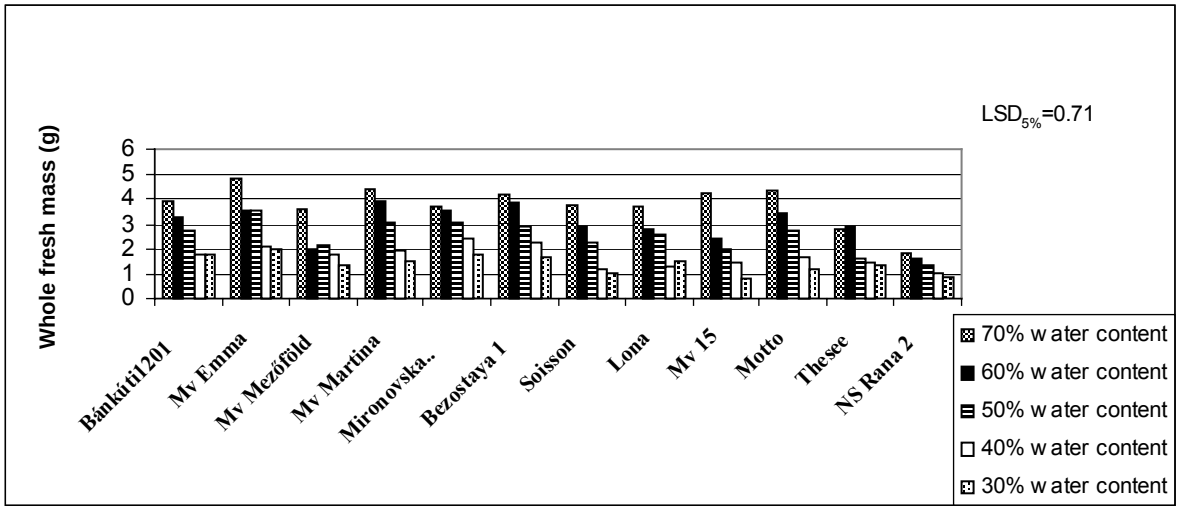

Figure 2. Change in the biomass of the varieties at different soil moisture levels

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Pre-breeding work in winter wheat (*Triticum aestivum* L.) for adaptation to Kosovan growing conditions

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ABSTRACT: Seventy-seven international winter wheat cultivars and/or breeding lines were grown in the year 2000 in Prishtina, Kosovo to test their adaptation to the local growing conditions. 49 genotypes were selected based on their yield, protein content and extensograph data for further tests in 2001 and 2002. The material was evaluated for yield, 1000 kernel weight, protein content, high molecular weight (HMW) glutenin subunit composition, SDS sedimentation volume, dough mixing and extension characteristics, and baking volume. Principal component analysis was performed on the single years' genotypic means, resulting in nine principal components with an eigenvalue >1.0. The scores of these nine principal components were subjected to Ward's minimum variance clustering method, resulting in three main clusters. The first cluster comprised Austrian winter wheats together with 'Bezostaya 1' and 'Balkan'. This cluster is characterised by high yields, highest 1000-kernel weights, highest protein contents and SDS sedimentation volumes, and excellent dough mixing and extension characteristics. The other two clusters consisted of eastern European genotypes and lines from diverse CIMMYT breeding programs. While the second cluster is characterised by the truly wretched genotypes in regard to both agronomic and quality traits, the third cluster comprises genotypes with highest baking volumes and HMW glutenin subunit scores, and good dough extension traits. It can be summarized that Austrian quality wheats represent a valuable source for winter wheat improvement programs in the Kosovo, especially in regard to quality traits.

Key words: Baking quality – common wheat – genetic diversity – protein – small-scale methods

Introduction

Approximately 80000 - 100000 ha are planted annually in the Kosovo with winter wheat. Seed for planting is mainly imported from neighbouring countries. So far no domestic cultivar is available for cultivation. The national wheat production with average yields of 2500 - 3000 kg ha^{-1} does not guarantee Kosovan self-supply. Hence, wheat for consumption has to be imported. There is an utmost need to establish a national wheat breeding program with the aim to create wheat cultivars specifically adapted to the prevalent growing conditions, which could enable the Kosovo to improve both wheat production and wheat quality.

The aim of the present study was to test international wheat cultivars and breeding lines for their adaptation to Kosovan growing conditions. These genotypes could serve as valuable genetic resources for the build-up of a Kosovan winter wheat breeding program in the future.

Materials and methods

Plant material and field trials

A total of 77 international winter wheat genotypes were grown in the year 2000 in a randomised complete block design in Prishtina. Based on yield, protein content and dough extension data 49 genotypes were selected for further growing in 2001 and 2002, while the truly wretched genotypes were discarded. The 2001 and 2002 trials were sown in row column designs in Shkabaj, 2 km north-west of Prishtina. Plot size was 5 m^2 (1.5 x 3.33 m; 15 cm row spacing). In all three years the field trials were fertilised according to the common Kosovan

standards for cereal production, i.e. 60 N, 60 P, and 60 K (kg ha^{-1}) applied at sowing, and 60 N (kg ha^{-1}) applied at tillering (Feekes' scale stage 3).

Quality evaluation

Crude protein content was determined by near-infrared transmittance spectroscopy (Infratec Food and Feed Analyzer 1255, Tecator AB, Höganäs, Sweden) using the calibration of RWA Raiffeisen Ware Austria AG. SDS-PAGE of high molecular weight (HMW) glutenin subunits and SDS sedimentation test were determined according to the protocols of the Saatzucht Donau breeding company. Scores for HMW glutenin subunit composition adjusted for the 1BL.1RS chromosome translocation were calculated according to Payne et al. (1984, 1987). Dough extension tests were carried out on a micro-scale using a texture analyzer. Analyzed parameters were maximum resistance, extensibility until rupture, and area under the curve until rupture (Grausgruber et al. 2002). Dough mixing characteristics were determined on 10 g samples using a Promylograph T3 apparat (M. Egger, St. Blasen, Austria). Recorded parameters were water absorption, length of the mixing curve, dough softening, and quality number (curve length from start until the decreasing curve falls below 450 units). Micro-baking tests were performed as rolls and pans using 20 g dough samples. Dough formula was: flour 50 g, salt 0.8 g, dry yeast 1.6 g, wheat malt flour 0.8 g, baking margarine 2.4 g and 55 – 65 % distilled water based on Promylograph water absorption. After mixing the doughs were formed by hand into rolls and pans. The latter one was given into Shogren typed baking forms (Shogren and Finney 1984). After a proofing time of 40 min at 30°C baking was carried out using a modified automatic home-bakery (Grausgruber et al. 2001). Baking volume was determined after cooling (2 hours after baking) by amaranth seed displacement.

Statistical analyses

The field trials were analysed according to their experimental design. In 2001 and 2002 yield and protein data were adjusted for nearest neighbour effects using post-blocking techniques as described by Vollmann et al. (1996). Single years' genotypic means of both agronomic and quality traits were subjected to a principal component analysis. Cluster analysis (Ward's method of minimum variance) was applied to the genotypic scores of principal components with eigenvalues >1.0. Analysis of variance and mean comparisons between the clusters were further performed using the genotypes within clusters as replicates. Analysis of variance and covariance, calculation of (adjusted) means, and principal component analysis were performed with SAS 8.2 software. Cluster analysis was performed with SPSS 8.0.

Results and discussion

Altogether three main clusters were distinguished within the set of 49 genotypes (Figure 1). Clusters I and III comprised genotypes with a generally better performance over all traits, whereas cluster II included the truly wretched genotypes in regard to both agronomic and quality traits. The first cluster, which included the Austrian cultivars and the well adapted standards 'Bezostaya 1' and 'Balkan' was characterised by high values for 1000-kernel weight (TKW), protein content (PROT), dough resistance (R), extensibility (E′), dough energy (A′), SDS sedimentation value, Promylograph quality number (PQN) and curve length (PCL) (see in parts Table 1). Cluster III included genotypes and breeding lines from eastern Europe and international breeding programs and had somewhat lower values than Cluster I for almost all quality traits but Payne score (HMW) and loaf volume (LV) in the year 2002. Cluster II also comprised genotypes and breeding lines from eastern European countries and international breeding programs, but was characterised by lowest values in almost all traits but TKW and loaf volume. The lowest loaf volumes, although seldom significantly different from the other two clusters, for Cluster I are astonishing considering the overall good

performance in indirect (protein content, SDS sedimentation, HMW glutenin score) and rheological (dough mixing and extension tests) traits. However, it has to be noted that the baking tests were performed on a micro-scale basis and correlation to standard baking tests using at least the 20-fold amount of flour has to be proved. Moreover, dough formulation and mixing in the present baking test was probably not optimal for cultivars with higher gluten strength.

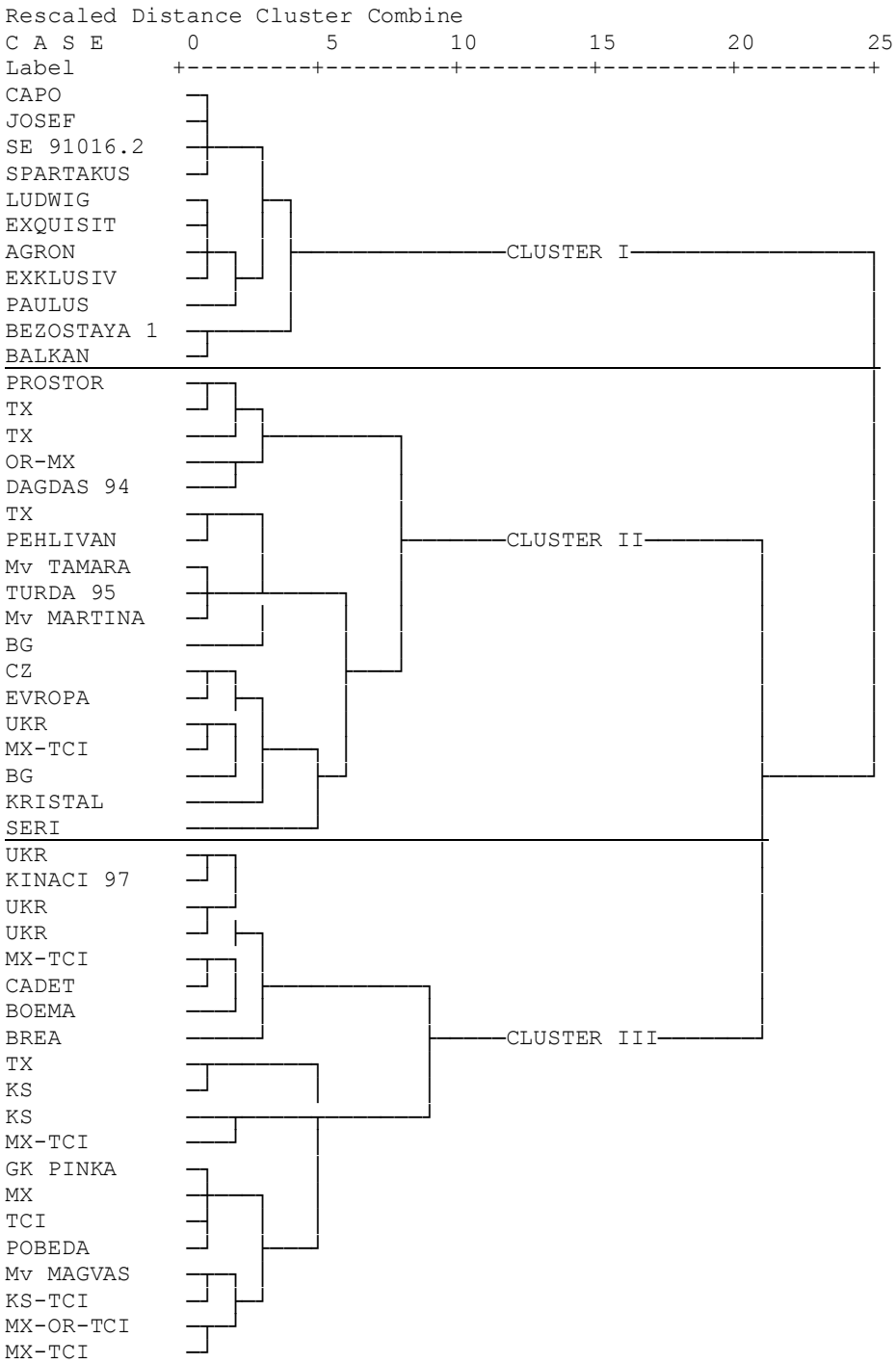

Figure 1. Dendrogram (Ward's minimum variance method) using genotypic scores of the nine significant principal components based on agronomic and quality traits. Cultivar names are written in full, origin of breeding lines is abbreviated as follows: BG, Bulgaria; CZ, Czech Republic; KS, Kansas; MX, Mexico; OR, Oregon; TCI, Turkey-CIMMYT-ICARDA; TX, Texas; UKR, Ukraine

Table 1. Cluster mean values for selected traits for the years 2001 and 2002

Cluster	YLD[1]		TKW		PROT		SDS	HMW
	2001	2002	2001	2002	2001	2002	2002	2002
I	806.0	595.2	42.3	42.6	14.5	15.0	68.4	8.3
II	687.4	543.9	37.5	40.4	12.9	13.9	48.5	6.8
III	758.4	566.3	38.4	38.5	12.2	13.3	52.2	9.0
	R		A′		PCL		PQN	
	2001	2002	2001	2002	2001	2002	2001	2002
I	17.5	23.0	817.1	1233.5	4.3	6.1	3.0	2.9
II	11.7	14.7	505.3	626.0	2.9	2.6	2.1	2.2
III	17.8	17.0	862.9	836.3	5.5	5.5	3.0	2.4

[1] YLD, yield (g m^{-2}); TKW, 1000-kernel weight (g); PROT, protein content (%); SDS, SDS sedimentation value (ml); HMW, adjusted Payne score for HMW glutenin subunit composition; R, micro-extensograph maximum dough resistance (g); A′, modified micro-extensograph dough energy (g mm); PCL, Promylograph curve length (cm); PQN, Promylograph quality number (cm)

In summary, it can be concluded that genotypes of Clusters I and III could serve as valuable wheat genetic resources for the build-up of a Kosovan winter wheat breeding program in the future. It has to be considered that the present material could not have been tested for disease resistance, since no diseases were recorded in the three years of investigation. During the parallel multiplication of the material in Austria, however, almost all breeding lines and cultivars of non-European origin, exhibited high susceptibility to the prevalent races of yellow rust and powdery mildew. Austrian cultivars with high baking quality (improver wheats) represent a valuable genetic resource both for quality and adaptation. Probably because of similar climatic conditions, e.g. hot and dry summers in eastern Austria and the Kosovo, and because of ancestors which are well known as donors of quality and adaptation, e.g. ‘Bezostaya 1’.

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Assessment for salinity tolerance through intergeneric hybridisation (*Triticum durum* × *Aegilops speltoides*)

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ABSTRACT: The development of more salt tolerant crop plants is a reasonable goal for plant breeding programs. For that purpose, a moderately salinity-tolerant *Triticum durum* accession was crossed with a moderately salinity-tolerant *Aegilops speltoides*. 500 hybrid seeds were produced, 95 % of which germinated. The triploid hybrid embryos produced from them were cultured on agar No. 1. Chromosome doubling was induced by using 0.05 % colchicine. The resulting hexaploid plants grew to maturity and produced a considerable amount of seeds. The synthetic hexaploid was tested for salinity tolerance, grown at 0, 100, 125, 150, 160, 170, 180, and 200 mM NaCl in a standard nutrient solution for two weeks. The amphidiploid materials showed greater salinity tolerance than either parent, suggesting the presence of different genes for tolerance in the parents.

Key words: Amphidiploid – durum wheat – hybridisation – salinity tolerance

Introduction

Salinity is an increasing problem in many areas of the world. It is now well recognised that selection and breeding of crops for salt tolerance is an economic and efficient method of overcoming soil salinity problems (Epstein et al. 1980).

Screening and selection can, however, only increase salt tolerance in wheat to some extent (Dvorak & Ross 1986). Hence, researchers have been using other sources of variability. The further source of variability that can be and has been exploited with significant success in the breeding for disease and pest resistance, is variability that is present in wild relatives of cultivated species (Mahmood & Quarrie 1993). The work described here was carried out to produce salt tolerant synthetic hexaploid wheat using the intermediate salt tolerance of diploid *Aegilops speltoides* and an intermediate salt tolerant tetraploid *Triticum durum*.

Materials and methods

Plant material

1) *Triticum durum* line 9245 (2n = 4x = 28; AABB; origin Jordan) from ICARDA, Aleppo, Syria. This accession had intermediate salt tolerance in 100 mM NaCl of 29 accessions from different origins (Sadat Noori & McNeilly 2000) and was used as the female parent. 2) *Aegilops speltoides* subsp. *speltoides* (2n = 2x = 14; SS; origin Turkey) obtained from the Genebank of the Institute of Plant Genetics and Crop Research (IPK) Gatersleben, Germany. This accession also had intermediate salt tolerance in 150 mM NaCl of 12 accessions from different origins (Sadat Noori & McNeilly 1999) and was used as the male parent.

Seeds were placed in a cold room (4°C) for one week, were surface sterilised for 1 minute with 80 % ethanol and for 6 minutes in 5 % sodium hypochlorite solution, and were germinated in petri dishes containing 0.1 mM gibberellic acid (GA3). This treatment was carried out to break dormancy for quick germination of seeds.

Seeds of the parental lines were planted at six one-month intervals between sowing to ensure concurrence of flowering. Flowering of the male parent *Aegilops* began 6 months from sowing. Emasculated *T. durum* florets were pollinated in the early morning or late evening 3

to 4 days after emasculation. Each spike was covered with a non moisture-proof glassine bag until seed setting was completed. Embryo rescue was performed following King et al. (1993) and treatment with colchicine was as described by Laurie & O'Donoughue (1994).

Seeds from the doubled triploid plants (amphidiploid) were collected from all spikes, and each line was bulked in order to produce more seeds (C2). All selfed progenies of the amphidiploid were uniform. Chromosome counting was carried out following the methods according to Darlington & La Cour (1962).

Salinity tolerance assessment

The salinity tolerance of the synthetic amphidiploid and its parents was assessed in eight solutions, control (0), 100, 125, 150, 160, 170, 180, and 200 mM NaCl, prepared in 1/10 strength nutrient solution (Hewitt 1966). Ten seeds per genotype were sown on 3-layer deep rafts of black alkathene beads floating on 300 cm^3 solution plastic beakers, arranged in a completely randomised design with three replicates in a growth room. Longest root lengths after two-weeks of seedling growth were measured.

Results and discussion

Five thousand pollinated florets resulted in ≈500 embryos (3 to 6 per spike). All embryos were collected and placed on culture medium in tube culture under sterile conditions. Before colchicine treatment, the root tips of the hybrid plants were excised for somatic chromosome counting. The number of chromosomes in hybrid plants which were triploid was 2n = 3x = 21 (ABS) and of the amphidiploid 2n = 6x = 42 (AABBSS).

The root length of the synthetic hexaploid *T. durum* × *Ae. speltoides* and its parents was reduced significantly ($P < 0.001$) by increasing NaCl concentration. Amphidiploid relative NaCl tolerances are shown in Figure 1, and it is clear that variation for salinity exists in the hybrids produced, and tolerance was present up to 200 mM NaCl. Exploitation of variability in these two species appears to be a valuable means of developing highly tolerant lines. The wild and cultivated relatives of bread wheat (*Triticum aestivum* L.) form a large pool of genes of value to wheat breeders for yield improvement, disease resistance and adaptation to extreme, stressful, or changing environmental conditions (Porceddu et al. 1988).

Tillering of synthetic hexaploid was moderately. Jauhar (1991) considered the expression of the tillering habit and the perennial growth habit from the male *Thinopyrum* parent in hybrids between *T. durum* and *Th. bessarabicum*. The amphidiploid produced here had tough rachis. Lange & Jochemsen (1992) have reported a tough character of rachis for hybrids which derived from *T. turgidum* (fragile rachis with breakage at the top) × *Ae. squrrosa* (fragile rachis with breakage at the bottom). They concluded that it is a new character for hybrids. The expression of genes for disease resistance from *Ae. speltoides* in hexaploid wheat was demonstrated, but the expression of (a) gene(s) for salt tolerance from *Ae. speltoides* was not. Genes for salt tolerance from other wild species are expressed in amphiploids with hexaploid wheat (Forster et al. 1987).

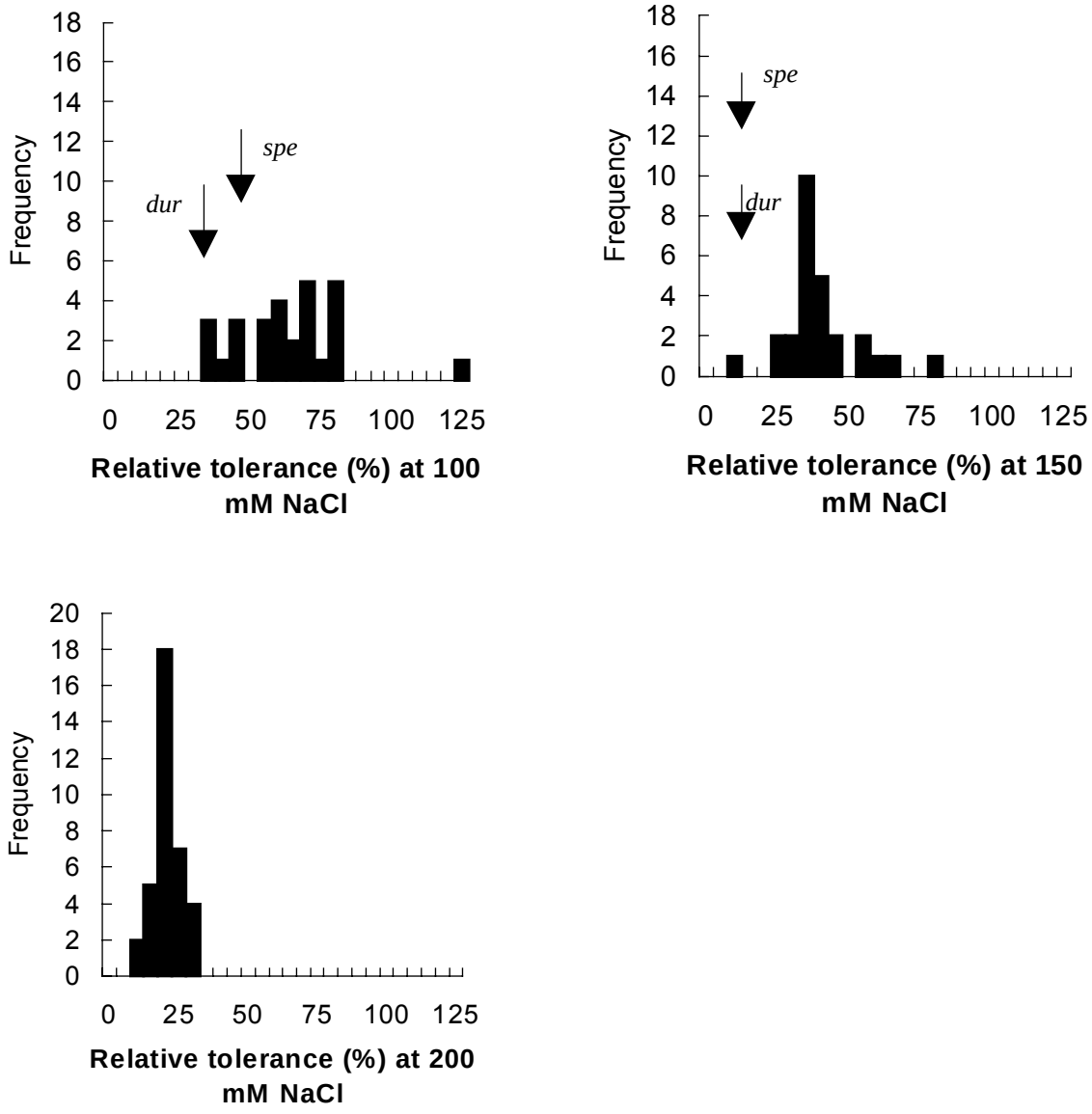

Figure 1. Frequency distribution of relative root length (%) of synthetic hexaploid (*T. durum* × *Ae. speltoides*) and its parents under 100, 150 and 200 mM NaCl (*spe* and *dur* indicate *Ae. speltoides* and *T. durum*, respectively)

The expression of (a) gene(s) for salt tolerance was demonstrated in the present study by comparing the synthetic hexaploid hybrid produced here (AABBSS) with their parents *Ae. speltoides* (SS) and *T. durum* (AABB) using relative root length (mean root length in salinity / control) in different NaCl treatment. Considering the relative root length of this hybrid, it is shown that variability exists for NaCl tolerance and that the synthetic hexaploid was more salt tolerant than its parents. This increased salt tolerance might be due to the addition of nuclear genes from *Ae. speltoides* and *T. durum* caused by increases in ploidy level and/or the interaction between genes on the A, B and S genomes derived from the parents. The third assumption is accumulation (combination) of different gene response to NaCl tolerance in hybrid plants.

Comparing the above synthetic hexaploid (*T. durum* × *Ae. speltoides)* with existing salt tolerant hexaploid wheats, such as 'Lu26.s' and 'Ho2' based on absolute root length, plants

grown in 200 mM NaCl showed a similar degree of salt tolerance (unpublished data). This amount could perhaps be increased using the existing variation (Figure 1) by recycling the selection under high NaCl levels. However, no evidence was found for specific crossing barriers and on the other hand, the amount of salt tolerance was greater than its parents and equal to existing salt tolerances of hexaploid wheats. Thus, the present amphidiploid could be used for further studies and for breeding common wheat. The information gained in the work described here shows that the tolerant lines from *T. durum* and *Ae. speltoides* carried salt tolerance genes and that it was possible to transfer these genes to the hexaploid hybrid. These results indicated that NaCl tolerant lines are available within *T. durum* and *Ae. speltoides*. A greater variability may be yet found searching different populations with diverse origin.

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Influences of drought and salt stress on grain quality of durum wheat

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ABSTRACT: Drought and salinity are the most significant abiotic stresses for plant agriculture that influence quantity and quality of plant production. This work aimed to assess the influences of drought and salinity stresses on grain quality characteristics of selected salt-tolerant genotypes of durum wheat (*Triticum turgidum* L. subsp. *durum* (Desf.) Husn.), derived from a direct (field) and indirect (*in vitro*) assessment methods for salinity tolerance. Eight durum wheat genotypes comprising 3 salt-tolerant genotypes and 1 salt-sensitive genotype derived from each of the methods were used. This study was conducted under controlled, drought and saline field conditions using three separate randomized complete block designs replicated three times. Protein content, sodium dodecyl sulfate (SDS)-sedimentation volume, wet gluten content, dry gluten content, 1000-grain weight and test weight were evaluated. The results showed a significant genotypic effect on the grain quality traits. There was a significant genotype by stress interaction for SDS sedimentation volume, 1000-grain weight and test weight. Both drought and salinity stresses significantly ($P<0.01$) influenced the grain quality characteristics. Comparison to control, drought and salt stress increased protein content by 12 % and 18 %, SDS sedimentation volume by 31 % and 66 %, wet gluten content by 20 % and 3 %, and dry gluten content by 20 % and 8 %, respectively. On the other hand, 1000-grain weight and test weight were reduced significantly under both stress conditions. Base on combined data analysis, protein content had a significant and positive correlation with wet gluten, dry gluten and SDS sedimentation volume, whereas it showed a strong and negative correlation with 1000-grain weight and test weight.

Key words: Quality – salinity – *Triticum turgidum*

Introduction

Recent world markets demand a high durum wheat quality. Hence breeding and production for acceptable end-use quality has become an important issue for breeders and farmers of durum wheat. Protein content and gluten quantity and quality are the most important variables in determining pasta quality. Protein composition, wet and dry gluten content and SDS-sedimentation volume are used to predict some of these quality characteristics (Ames et al. 1999, Troccoli et al. 2000). The SDS sedimentation test is commonly used as an indicator of gluten quality (strength). High test weight and large grains are important traits in world markets because they generally indicate sound grain with high flour yield. These are affected by genotype and environment and their interactions (G × E) under either rainfed (Rharrabti et al. 2003) or salinity (Francois et al. 1986) conditions. Drought and salinity are major constraints in the durum wheat production area, particularly arid and semiarid areas of world. A field study was contacted to determine the influences of drought and salinity on grain quality traits in durum wheat.

Materials and methods

Plant materials

Eight durum wheat (*Triticum turgidum* L. subsp. *durum* (Desf.) Husn.) genotypes including 3 salt-tolerant genotypes and 1 salt-sensitive genotype derived from *in vitro* assessment (Arzani & Mirodjagh 1999) and 3 salt-tolerant genotypes and 1 salt-sensitive genotype derived from a two-year field assessment under saline conditions were used in this study. *In vitro* salt tolerant genotypes (ITGs) comprised 'Dipper 6', 'Prion l' and PI40100, an *in vitro* salt sensitive genotype (ISG) was 'Massara 1'. Field selected salt-tolerant genotypes (FTGs) included

Ajaia/Hora/Jro/3/Gan (Aj/.../Gan)", Srn/Vic, PI40098, and a field selected salt-sensitive (FSG) genotype was 'Lund 6'.

Field experiments

The study was conducted in three separate field experiments including non-stress, drought stress and salt stress using three separate randomized complete block designs, each of which replicated three times. Drought stress exercised with ceasing the irrigation at the flowering. Saline soil (described as a Haplic Solonchaks) irrigated with saline water having EC of 10 dS m^{-1} was used for salt stress experiments. Weight per hectolitre (test weight), 1000-grain weight, protein content, wet and dry gluten content and sodium dodecyl sulfate (SDS)-sedimentation volume were determined.

Statistical analysis

Separate analysis of variance (ANOVA) was done for each of the field experiments and a combined ANOVA was carried out using data from the three experiments. Analysis of variance was carried out using PROC GLM of SAS software. Contrast of ITGs vs. FTGs and ISG vs. FSG were done by orthogonal (independent) comparisons. Mean comparisons were conducted using Fisher's Least Significant Differences (LSD). Parametric correlations were used to determine the relationships among the various quality tests at the different field experimental conditions.

Results and discussion

The results showed a significant genotypic effect on the grain quality traits. There was a significant genotype by stress interactions for SDS sedimentation volume, 1000-grain weight and test weight. Rharrabti et al. (2003) reported a significant G×E interaction for quality traits in durum wheat. Tables 1 and 2 indicate mean values of the grain quality traits under non-stress field condition (control) and their variation as production percentage of control at drought and salt stress field conditions. Wide ranges in each quality parameter among genotypes were detected in each condition. Protein content ranged from 12 % (Lund-6 (FSG)) to 13.8 % (Massara-1 (ISG)) at control site. Both drought and salt stress increased protein content with an average of 12 % and 18 %, respectively. Lund-6 (FSG) showed the highest increase in protein content due to both drought and salt stresses. The greatest change to the grain quality traits due to stress was related to SDS sedimentation volume, where drought and salt stress increased SDS sedimentation volume by 31 % and 66 %, respectively. Rharrabti et al. (2003) tested 10 durum wheat genotypes at different field trials. They reported higher protein content and SDS volume under rainfed conditions compared to those produced under the irrigated condition. Francois et al. (1986) showed that salinity stress significantly increased protein content of durum wheat genotypes. In the present study, genotype means for wet and dry gluten percent were increased by both stresses, while genotypic responses varied especially at salt stressed field conditions. Thousand grain weight and test weight were reduced under both field stressed conditions (Table 2). Drought stress affected 1000-grain weight and test weight with lower impact than salinity stress. Correlations between the tested traits in this study were not consistent among the three sites (data not shown). This may be due in part to different effects of non-stress and stress conditions on the traits. Protein content showed a significantly positive correlation with wet gluten, dry gluten, SDS sedimentation volume and strong negative correlation ($P<0.001$) with 1000 grain weight, and test weight using combined data of the three locations (Table 3). Thousand grain weight only showed strong positive relationship with test weight ($r = 0.80^{***}$), and these two traits showed negative correlation with the other quality traits, however their correlation coefficient was not significant with wet gluten content and low with dry gluten content. Wet and dry gluten

content are indices for the magnitude of gluten. Gluten strength has been assessed by SDS sedimentation volume (Ames et al. 1999). SDS volume was slightly correlated with dry gluten contents ($P<0.05$), and there was no association between SDS volume and wet gluten content.

Table 1. Means of protein content, SDS sedimentation volume, wet gluten percent and dry gluten percent under non-stress field condition (control) and their values for drought and salt stress field conditions as percentage of control (non stress)

Genotype	Protein content (%)			SDS volume (ml)			Wet Gluten (%)			Dry Gluten (%)		
	Non stress (%)	Drought stress (% of non stress)	Salt stress (% of non stress)	Non stress (m)	Drought stress (% of non stress)	Salt stress (% of non stress)	Non stress	Drought stress (% of non stress)	Salt stress (% of non stress)	Non stress	Drought stress (% of non stress)	Salt stress (% of non stress)
Prion-1 (ITG)	13.1	110	118	25	168	188	44	135	93	16	128	103
Dipper-6 (ITG)	12.8	116	115	31	107	135	42	112	85	17	104	89
PI40100 (ITG)	13.6	113	120	25	143	149	54	122	100	19	121	110
Massara-1 (ISG)	13.8	107	111	19	149	207	41	135	125	15	126	119
Srn/Vic (FTG)	13.6	103	118	32	113	150	44	103	124	17	101	122
PI40098 (FTG)	13.6	110	114	26	109	167	45	123	99	16	131	108
Aj/…/Gan (FTG)	13.2	112	118	23	166	214	52	114	97	18	121	105
Lund-6(FSG)	12.0	129	131	27	108	144	43	120	100	15	126	107
Mean	13.2	112	118	26	131	166	46	120	103	17	120	108
Means SE	0.08	0.97	0.75	0.49	3.32	3.75	0.59	1.41	1.76	0.15	1.39	1.27

Table 2. Means of 1000-kernel weight and test weight under non-stress field condition (control) and their values for drought and salt stress field conditions as percentage of control

Genotype	1000-kernel weight (g)			Test weight (kg hL^{-1})		
	Non stress (g)	Drought stress (% of non stress)	Salt stress (% of non stress)	Non stress (kg hL^{-1})	Drought stress (% of non stress)	Salt stress (% of non stress)
Prion-1	45	67	37	78	88	80
Dipper-6	41	69	53	80	90	83
PI40100	39	83	70	81	87	85
Massara-1	44	66	40	78	88	73
Srn/Vic	48	67	50	79	92	85
PI40098	42	69	51	74	92	94
Aj/…/Gan	48	65	47	77	90	82
Lund-6	49	78	41	81	91	80
Mean	45	70	48	79	90	83
Means SE	0.42	0.81	1.30	0.29	0.24	0.74

Table 3. Simple correlation coefficients between the quality traits based on combined of the three location data [A]

	TW	WG	DG	SDS	PRO
TGW	0.80***	-0.06[ns]	-0.19*	-0.78***	-0.66***
TW		-0.18[ns]	-0.29**	-0.68***	-0.63***
WG			0.91***	0.12[ns]	0.32**
DG				0.27*	0.43***
SDS					0.59***

ns, non significant; * P< 0.05; ** P< 0.01,*** P< 0.001.
[A]TGW = Thousand grain weight (g); TW = test weight (kg hL^{-1}); WG = wet gluten (%); DG = dry gluten (%); SDS = SDS volume (ml) and Pro = protein content (%).

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Improvement in the yellow index of winter durum wheat

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ABSTRACT: Winter durum wheat genotypes have been selected in Martonvásár on the basis of the yellow colour of the semolina since 1995. During this period the yellow index of 1686 durum wheat varieties and advanced lines was tested using a Minolta CR-300 chromameter. The average yellow index values of the advanced lines and of Hungarian and foreign varieties fluctuated over a wide range (19.9 - 24.4 b*) in different years. Correlation analysis confirmed previous observations on spring durum wheat varieties, which indicated that no correlations could be demonstrated between the yellow pigment content and other pasta quality parameters even at a medium level of significance. Before selection was begun, the average yellow index of these lines was 99.2 % compared with that of the control variety 'Parus', while this value had risen to 119.4 % by 2003. The yellow index of the best lines exceeded that of the control varieties by more than 30 %, while the ratio of lines with yellow index values lower than that of 'Parus' declined from 60 % to 0 %. The mean yellow index values recorded for state registered Martonvásár varieties and advanced breeding lines over the last seven years confirm that the technological quality of winter durum wheat genotypes can be substantially improved by selection.

Key words: Breeding – quality – winter durum wheat – yellow index

Introduction

The yellow pigment content is one of the most important technological quality parameters of semolina, the ground durum wheat product used in pasta-making (Irvine 1971). The pigment, consisting mainly of lutein and the esters of this compound (Lepage & Sims 1968), has little or no influence on the pasta-manufacturing and cooking properties (Dexter et al. 1981), but it leads to a considerable improvement in the aesthetic value, storability and marketability of pasta made without the addition of eggs.

The yellow pigment content is a genetically determined trait (Braaten et al. 1962). Data in the literature suggest it is influenced chiefly by additive gene effects. The high heritability index of this trait also indicates that it is oligogenically inherited and determined by only a few alleles. Earlier studies suggested that genes for yellow pigment quantity were located on chromosomes 2A and 2B (Joppa & Williams 1988), but in analyses carried out at the DNA level, Elouafi et al. (2001) identified regions responsible for this trait on chromosome arms 7BL and 7AL, among which the QTL on 7B was found to explain 53 % of the genetic variance. In bread wheat QTLs influencing the carotinoid content were identified on chromosomes 3A and 7A (Parker et al. 1998) and on 3B and 7A (Mares & Campbell 2001).

Traditionally, yellow pigment content has been measured by solvent extraction (ICC Standard No. 152), but it can also be determined indirectly using a Minolta chromameter. In recent years this rapid, easily replicated method, which requires only small samples and no chemicals and can also be used for the direct determination of semolina colour, has gained ground throughout the world (Borrelli et al. 1999). The instrument uses three stimuli to record three values for each sample: L* = brightness (dark-light axis); a* = red or green colour; b* = yellow or blue colour. On the basis of these three data all shades of all visible colours can be plotted on a three-dimensional diagram. The Minolta b* index, known in the literature as the yellow index, is of most significance for the technological quality of durum wheat. This index is in very close correlation with the quantity of yellow pigment in the semolina. In experiments carried out by Borrelli et al. (1999) the correlation coefficient between the two

traits was found to be 0.96, while previous measurements on winter durum wheat varieties and advanced lines in Martonvásár gave a value of 0.99.

In Hungary, the growing area of durum wheat is sown almost exclusively to winter varieties, which have favourable gluten quantity and quality traits, but whose yellow pigment content often leaves much to be desired. Winter durum wheat genotypes have been selected on the basis of the yellow colour of the semolina since 1995 in a search for potential quality sources. The present paper discusses the variability observed in the breeding stock and the results of selection. The experiments were aimed at studying the correlations existing between the yellow index and other technological traits.

Materials and methods

Since 1995 the yellow index of 1686 durum wheat varieties and breeding lines has been recorded in the Quality Analysis Laboratory of the Agricultural Research Institute of the Hungarian Academy of Sciences using a Minolta CR-300 chromameter (Minolta Camera Co. Ltd., Osaka, Japan). The grain was combine-harvested at full maturity.

The semolina required for the analyses was prepared according to the Hungarian standard (MSZ-08-0700-84) from durum wheat samples with a moisture content of 16 %. These were ground using a Brabender Junior mill converted as suggested by Vasiljevic et al. (1977). The removal of the bran and separation according to particle size was carried out on a Retch sieve series. The 160 - 315 μm fraction free of bran contamination was used to measure the yellow index.

The thousand kernel weight, grain diameter and grain hardness were measured using a Perten SKCS 4100 instrument (AACC Approved Method 55-31) and the gluten content using a Perten Glutomatic 2200 instrument (ICC Standard Method 137/1). Vitreousness, protein content and gluten spread were recorded using the MSZ 6383 Standard, while a Soltek SDS System was used to measure the SDS sedimentation volume.

Results and discussion

The statistical data on the yellow index for winter durum wheat varieties and breeding lines harvested between 1995 and 2003 are presented in Table 1 for the individual years and the overall period. The correlation matrix for the traits examined is shown in Table 2.

Table 1. Yellow index of winter durum wheat genotypes

Year	Mean	Range	CV%
1995	23.8	21.2-28.3	5.0
1996	20.0	16.5-26.0	8.9
1997	20.3	17.4-24.8	8.2
1998	20.6	15.6-28.0	10.3
1999	19.9	16.6-24.2	9.6
2000	23.3	19.2-29.0	11.7
2001	22.1	15.5-28.5	9.0
2002	24.4	19.0-30.3	8.2
2003	24.1	17.8-30.2	11.0
1995-2003	19.9-24.4	15.5-30.3	5.0-11.7

The lowest value recorded in the laboratory was 15.5 (in 2001) and the highest 30.3 (in 2002). The yellow index values exhibited a wide range during the 9 years of investigations. The yellow index of the majority of the genotypes was close to average (small annual CV values), but in each year it was possible to identify and select for winter durum

varieties and advanced lines with an exceptionally high yellow pigment content, close to that of spring varieties.

Correlation analysis (Table 2) on the winter durum wheat varieties and lines examined in Martonvásár confirmed the observations of Dexter et al. (1981), who found no correlation of even moderate strength between the yellow pigment content and other pasta-making quality traits. Only a moderate negative correlation was observed with milling traits (thousand kernel weight and Perten hardness index), indicating that lines with a high yellow index could be found even among the large-grained genotypes favourable for milling purposes.

Table 2. Correlation of the yellow index with other technological parameters

Parameter	Yellow index
Thousand kernel weight	-0.414***
Kernel diameter	-0.012
Perten hardness index	-0.451***
Vitreousness	-0.306***
Protein content	0.161***
Wet gluten content	0.061*
Gluten spread	0.258***
Gluten index	-0.226***
SDS sedimentation volume	0.127**

*, **, *** = correlation significant at the 0.05, 0.01 and 0.001 levels, respectively

As the result of selection there has been a substantial increase in the yellow index of winter durum wheat lines awaiting state registration. The data for the lines are compared with those of 'Parus', an Ukrainian winter durum variety with favourable agronomic traits, excellent winter hardiness and moderate yellow pigment content (Figure 1). This variety has been sown as a control ever since the breeding programme was commenced. When selection was begun in 1996 the average yellow index of the lines compared with the control variety was 99.2 %, while by 2003 this value had increased to 119.4 %.

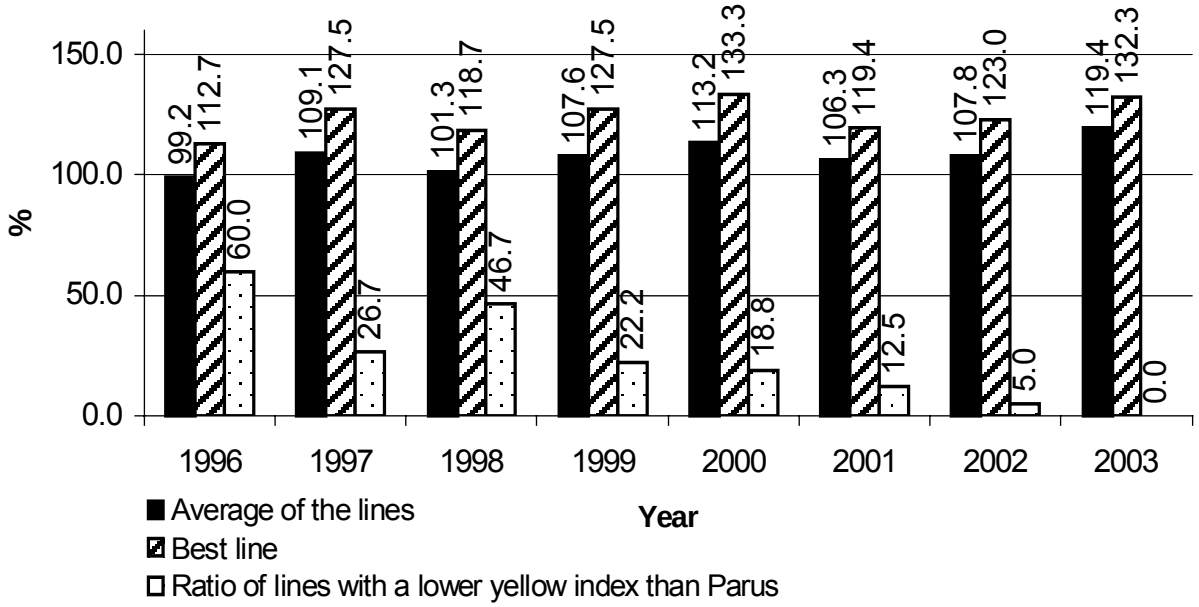

Figure 1. Yellow index of winter durum wheat lines compared with that of the control variety 'Parus', and the ratio of lines with yellow index values lower than that of the control variety

The yellow index of the best lines exceeded that of the control variety by more than 30 %. Selection also led to a substantial reduction in the ratio of lines with a yellow index smaller than that of 'Parus'. In the first year after the purchase of the Minolta CR-300 instrument this ratio was still 60 %, while in 2003, after several years of selection, it had dropped to 0 %. The mean values recorded for the yellow index of registered varieties over the last seven years (Table 3) prove that the technological quality of winter durum wheat genotypes can be substantially improved by selection.

Table 3. Yellow index of registered Martonvásár winter durum wheat varieties (Martonvásár, 1997-2003)

Variety	Year of registration	Yellow index
'Martondur 1'	1996	19.40
'Martondur 2'	1996	22.39
'Martondur 3'	1999	22.04
'Mv Maxi'	2001	23.32
'Mv Makaróni'	2001	25.65

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Avena genetic diversity for plant breeding

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ABSTRACT: This research presents the field results of studying wild oat species with different ploidy level. The evaluation was targeted at agronomic traits and resistance to the most widespread diseases: crown and stem rusts, and barley yellow dwarf virus (BYDV). Moreover, a representative collection of all species has been analyzed for groat protein content, amino acid composition, groat oil content and fatty acid composition.

Key words: Agronomic character – *Avena* sp. – disease resistance – oat oil content – oat protein

Introduction

In most cases the cultivated species of cereals have lost numerous traits initially inherent in their wild ancestors. Resistance to unfavourable environmental factors, wide range of adaptation to different edaphic and climatic conditions, resistance to pathogens, a number of characters including increased productivity and quality – all of these present unique breeding sources for crop improvement. The vast area of distribution of *Avena* species provides for the formation of extensive intra-specific diversity of the characters. Comparative analysis of the whole specific diversity of crops were incited by the profound interest to the use of these forms in breeding practice, enforces by the late development of genetics, molecular-biology, genomics and other researches. Practical importance of interspecies hybridization lies in combining properties of different species that drifted apart in the process of evolution. Fusion of high yield and different valuable parameters in one cultivar has been the goal of breeding in the past years. According to many researchers, utilization of intra- and inter-specific hybridization of conventional breeding together with various modern breeding techniques may raise the plant adaptation and percentage of qualitative kernels components in the crop to a very high level.

As to the practical significance of inter-specific crosses, they allow transfer of agriculturally important traits from wild and weed species to cultivated ones. Crosses between species of equal or different ploidy levels have long attracted the attention of researchers. The genus *Avena* L., which includes many species, has three ploidy levels and is represented by di-, tetra-, and hexaploids. All *Avena* L. species are subdivided into two groups according to their crossability: all weed hexaploid species readily crossable with cultivated oat; diploid and tetraploid species either not directly crossable, or yielding sterile hybrids in subsequent generations, or requiring tissue culture methods for hybridization. Cross incompatibility presents the greatest difficulty in transferring genes from diploids and tetraploids to hexaploids. This can be overcome by the use of back-crosses, mutants, genetic intermediates and methods of biotechnology. These breeding methods allow many agriculturally important traits to be transferred into cultivated oat for extending its gene pool as well as the gene pool of the entire genus.

Material and methods

At the same time, further search for and utilization of new sources for breeding purposes is one of the objectives pursued by Vavilov Institute of Plant Industry (VIR) in studying its global germplasm collections. For this reason, it was started investigating the gene pool of

wild species to extend the genetic potential of cultivated oat species. At present, the VIR possesses a rich collection of *Avena* L. species, which contains about 2000 accessions of twenty-two wild species. The wild accessions represent numerous morphological variants reflecting their wide geographic distribution in the Mediterranean countries. A representative collection was made in Transcaucasia, where wild oat species are the most diverse in the CIS countries.

A decade of study of the representative set of 2000 accessions of genus *Avena* L. with different ploidy levels has made it possible to disclose intra-specific diversity of all characters involved in the research, which will contribute to a targeted search for the best breeding sources and broadening the genetic base of the released oat cultivars. The research was based on the International Descriptors of *Avena* L. (1984) and Oat Descriptors List (IBPGR 1985).

Results and discussion

For breeding purposes some agronomic characters were evaluated in oat species. The results of our field researches reported great diversity in the structure and separate elements of panicle. Variation of these descriptors was insignificant throughout the years of study. Analyzing the panicle structure on the species level certified that such parameters as panicle length, number of spikelets and panicle density varied greater in the diploid wild species that in other groups of species.

Evaluation of wild oat species cast light on the rich diversity in kernel characters. On the whole, it was ascertained that diploid species had the highest values of huskness percentage and the lowest of kernel size. The least percentage of husk (43 - 46 % on the average) was observed in *A. damascena*, *A. wiestii* and *A. hirtula*. The size of kernel was notable in the forms of *A. longiglumis* with 1000-grain weight over 14 g. Two tetraploid species *A. magna* and *A. murphyi* were also distinguished for a large size of their kernels, since their 1000 grain weight (23.5 - 23.8 g) reliably exceeded maximal average values of all species studied (Loskutov 2003).

The result of the field study, when crown rust resistance had been assessed on the level of species, it was observed that most diploid wild species missed this character. Among the tetraploid species (*A. barbata, A. magna, A. murphyi, A. insularis, A. macrostachya*), resistance was observed in most species. Resistance was most expressed in the hexaploid accessions from Spain, Italy, Turkey, Israel and Iran. Resistant forms for all groups of species came mostly from North Africa, such as Tunisia, Algeria and Morocco.

While assessing stem rust resistance, variation of responses in the species studied was wider than in cultivated oats. At the same time, among few diploid species (*A. pilosa, A. longiglumis, A. hirtula*) medium resistance to this disease was identified only. Tetraploid wild species were characterized as strongly susceptible to this pathogen, with the exception of some forms of *A. barbata, A. magna, A. insularis* and *A. macrostachya*. All hexaploid wild species, on the average, demonstrated medium resistance to the agent of stem rust. Resistant forms were identified among the accessions from Italy, Iran, Iraq, Israel, Tunisia, Algeria, Morocco and Ethiopia. Group resistance to major obligate fungal diseases (crown and stem rust) was observed in the forms belonging to species *A. longiglumis*, *A. canariensis*, *A. hirtula*, *A. barbata*, *A. agadiriana*, *A. magna*, *A. insularis*, *A. macrostachya*, *A. occidentalis* and *A. sterilis* (Loskutov 2002).

Medium tolerance to BYDV was observed in the diploid species with A genome variants. A majority of tetraploid species (*A. barbata, A. vaviloviana, A. magna, A. macrostachya*) with different genomes had medium tolerance to this virus. All hexaploid species basically demonstrated medium tolerance to BYDV, with *A. occidentalis* having the highest percentage of resistant accessions. The strongest and medium tolerance was typical of the oat forms from Greece, Turkey, Syria, Israel, Morocco, Algeria and Tunisia. Comparing the data of BYDV resistance and strong aphid colonization ascertained identification of

BYDV resistant accessions belonging to diploid species *A. clauda*, *A. pilosa*, *A. damascena*, *A. canariensis* and *A. hirtula*.

Resistance to powdery mildew (caused by *Erysiphe graminis* D. C. f. sp. *avenae* Em. March.), oat Victoria blight (caused by *Bipolaris victorae* Shoem.), oat leaf blight (caused by *Septoria avenae* Frank.), oat necrotic mottle (caused by *Mirothecium verrucaria* Ditmar. ex Fr.) were demonstrated by the accessions collected in various regions and belonging to the different ploidy level.

Besides, the data of biochemical research on wild and weedy field oat species showed the highest groat protein content (over 20.0 %) in the accessions of diploid *A. longiglumis* and *A. atlantica*, tetraploid *A. magna* and *A. barbata*, and hexaploid *A. sterilis*. Potential sources of high protein content would be *A. murphyi* and *A. occidentalis* (over 19.0 %). High nutritive value of protein was notable in tetraploid *A. barbata* (5.6 % of lysine in protein). Hexaploid species appeared to have the percentage content of lysine and other essential amino acids in protein comparable with the level of cultivated *A. sativa*. Noteworthy for high groat oil content (7 - 10 %) were accessions of diploid *A. pilosa* and *A. canariensis*, tetraploid *A. murphyi* and *A. magna*, hexaploid *A. fatua, A. ludoviciana* and *A. sterilis* (Loskutov 2000). The quality of oil in oat may be determined by the content of monounsaturated fatty acids, such as oleic acid capable of prolonging oil preservation time during storage. The highest content of oleic acid (over 46 % of the sum of acids) was detected in the forms of diploid *A. hirtula, A. longiglumis* and *A. wiestii*, tetraploid *A. barbata, A. vaviloviana* and *A. magna*, hexaploid *A. fatua* and *A. ludoviciana*. At the same time, biological activity of such oil is determined by the correlation between linoleic and oleic acids that should be equal to one. This correlation was observed in the accessions of diploid *A. ventricosa*, *A. clauda*, *A. pilosa* and tetraploid *A. vaviloviana*. This research resulted in mapping the geographic distribution of intra-specific diversity with regard to all oat species and forms. It appeared that accessions with high groat protein content had originated mainly from Israel, Morocco and Azerbaijan, while those with high groat oil content from the Ukraine, Azerbaijan, Georgia and Morocco (Loskutov 2002).

These studies confirmed that species *A. sterilis* and *A. ludoviciana* are the most promising and important both in terms of grain quality and in terms of transferring this trait onto cultivated oat. The research resulted in finding intraspecific variation in biochemical parameters under study, which opens a possibility to search for forms with a complex of commercially valuable properties and high grain quality.

The wide diversity in response to photoperiod and vernalization illustrated the level of polymorphism for these characters within all wild genepools of the genus *Avena* L. Moreover, forms differing in the sensitivity to photoperiod and vernalization were found (Loskutov 2001). The results of evaluation of aluminium tolerance have shown that the wild species (diploid and tetraploid ones) carrying a C genome had low level of resistance to the edaphic factors, while the carriers of A and B genomes were more frequently characterized as having high alum resistance (Loskutov et al. 2001).

The comprehensive study of the entire range of species in the genus *Avena* L. with different ploidy levels made it possible to display intraspecific diversity on all of the characters involved. The diploid and tetraploid species and especially the hexaploid ones were identified as sources of the assessed descriptors and may be included in the conventional and other breeding process for disease resistance, agronomic traits, and grain quality for feed and food. Use of the diverse wild oat species with regard to their morphological traits, geographic occurrence, and ecological preference is the most promising method for reducing genetic erosion of cultivated varieties.

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Estimate of heterosis and combining ability in maize (*Zea mays* L.) using diallel crossing method

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ABSTRACT: A diallel cross between 10 early maturing inbred lines of maize (*Zea mays L.*) was carried out and evaluated to estimate genetic parameters for yield, some yield components and other traits. The F_1 and reciprocal crosses were evaluated in 2002 in a triple lattice design. Significant differences were observed among genotypes for all studied traits. Therefore, Griffing's Method 3, Model 1 was used to partition the genotypic effect into general combining ability (gca), specific combining ability (sca), and reciprocal effects. Variances due to gca and sca, and also the reciprocal effects were significant for all traits studied. The ratio $2*\sigma^2_{gca}/(2*\sigma^2_{gca}+\sigma^2_{sca})$ was calculated for each trait to detect the relative importance of additive and non additive gene effects. Additive gene effects were more important than non additive gene effects for all traits. General combining ability effects were significant for most of the parents and all the traits. Specific combining ability effects were significant in a few crosses for all traits. Yield and plant height were significant in more than 50% of the crosses. High heterosis was observed in more than 50 % of the crosses for all studied characteristics. The highest and lowest amounts of heterosis were observed for grain yield and number of ear row, respectively. Number of days from planting to tassel emergence and maturity showed negative heterosis. Although high broad-sense heritability estimates (0.85 to 0.95) were observed for most of the traits, the estimates of narrow-sense heritability were relatively low, and its lowest values belong to number of kernel row and grain yield (0.23 and 0.38, respectively). Number of days from planting to tassel emergence and maturity had the highest narrow-sense heritability estimates (0.75 and 0.74).

Key words: Combining ability – diallel analysis – grain yield – heritability – heterosis – *Zea mays*

Introduction

The improvement of maize yield depends on the knowledge of the type of the gene action involved in its inheritance and also the genetic control of the related traits such as yield components. Also the choice of the most efficient breeding program depends on that information (Liao 1989, Pal & Prodham 1994). Whereas dominance gene action would favour the production of hybrids, the additive gene action indicates the standard selection procedures would be effective in bringing about advantageous changes to the character. Different genetic analyses have been used extensively to obtain information about the genetic control of quantitative traits. Diallel cross analysis has been used most extensively by breeders (Jinks & Hayman 1953, Stuber & Moll 1966, Walters & Morton 1978, Liao 1989, Pal & Prodham 1994) to obtain this type of information.

Materials and methods

In 2001, ten Iranian maize (*Zea mays* L.) inbred line of early maturity, named K86/8, K1264/1, K1250/3, K1291/2, K1298/1, F.C393, K2558, K561, K1263/1and K12/8 (herafter called number 1 to 10), were crossed using a full diallel fashion. In 2002, F_1 progeny and reciprocal crosses were evaluated in a triple lattice design at the Research Farm of the College of Agriculture, Tehran University. A three row plot of 2.5 m length and 70 cm width, with a

within row spacing of 20 cm was used. Fertilizer treatments were 200 kg/ha of ammonium-phosphate applied prior to planting plus 200 kg/ha of N top dressing at thinning and tassel emergence. Eight different traits including grain yield (kg/ha, 14 % moisture), yield components, and some others traits such as days to tassel emergence, plant and ear height were determined. Analysis of variance was performed based on triple lattice design and its relative efficiency was calculated over randomized complete block design. Griffing's (1956) Method 3 Model 1 and Hayman (1954 a, b) analyses were performed to estimate genetic parameters, such as general combining ability (gca) and specific combining ability (sca) variances, and broad-sense and narrow-sense heritability. According to Baker (1978), the ratio of $2*\sigma^2_{gca}/(2*\sigma^2_{gca}+\sigma^2_{sca})$ was calculated for each trait to detect the relative importance of additive and non additive gene effects. Heterosis and heterobeltiosis were obtained based on deviation of F_1 mean from mid parent and the best parent, respectively for each trait and hybrids.

Results and discussion

For all the traits studied, the efficiency of lattice design was less than one. Therefore, data were analyzed as a randomized complete block design. The variance components according to Griffing (1956) are given in Table 1. Significant ($P < 0.01$) differences were observed among genotypes for all the studied traits. General and specific combining ability mean squares were statistically significant for all traits studied. Also reciprocal crosses were significant for all traits (Table 1). According to Baker, the ratio of $2*\sigma^2_{gca}/(2*\sigma^2_{gca}+\sigma^2_{sca})$, revealed that additive gene effects were more important than non-additive gene effects for all the studied traits. This ratio ranged between 0.62 and 0.88 for ear height and number of days to tassel emergence, respectively. The highest and the lowest values of average heterosis were observed for grain yield and number of days to maturity. The heterosis was negative for number of days to maturity and number of days to tassel emergence, respectively (Table 2). Broad-sense heritability (h^2_b) estimates ranged from 85 % for number of days to maturity to 95 % for grain yield. Narrow-sense heritability (h^2_n) estimates ranged from 0.23 for number of grain per row to 0.75 for number of days to tassel emergence (Table 2). The effects of gca for parents are given in Table 3. It was considered that in general more than seven cases of gca effects for all traits were significant. The parental lines with significant gca can be used to improve different traits. In this investigation nearly 50 % of hybrids showed heterobeltiosis for all traits. For example, for grain yield from 90 crosses, about 77 percent of them showed heterosis. Also 35 F_1 progenies had positive heterosis and only two crosses (K86/8 x K1291/1 and K86/8 x K1298/1) showed negative heterosis. The highest and lowest amounts of heterosis for grain yield were observed for K1264/1 x K1250/3 and K1298/1 x K561 crosses that were 139.5 % and 24.6 %, respectively.

Table 1. ANOVA F-values according to Griffing's Method 3 Model 1 for grain yield and others traits

Sources	DF	Grain yield	Number of days to tassel	Plant height	Number of tassel branches	Ear height	Number of rows per ear	Number of grain per row	Days to maturity
Replicate	2	1.2 $^{n.s}$	179**	1394**	42.8**	2436**	28**	3.7 $^{n.s}$	691**
Genotype	89								
gca	9	32.6**	347**	4316**	313**	1267**	36.2**	327**	495**
sca	35	2.9**	11.1**	576**	31**	190**	2**	26.7**	31.5**
Reciprocal	45	1.9*	15.6**	502**	24**	210**	2.6**	14.1**	27.2**
Error	178	0.5	4.1	111	4.4	49	0.8	8.2	12.7

*,**, ns: significant at 0.05, 0.01 probability levels, and not significant, respectively

Table 2. Baker ratio, heritability and heterosis for grain yield and others traits

Parameters	Grain yield	Number of days to tassel	Plant height	Number of tassel branches	Ear height	Number of rows per ear	Number of grain per row	Days to maturity
$2*\sigma^2_{gca}/(2*\sigma^2_{gca}+\sigma^2_{sca})$	0.75	0.88	0.65	0.72	0.62	0.81	0.75	0.79
Average of heterosis	80.1	-5.6	16.9	23.9	26.3	12.4	27.8	-1.2
h^2_b	0.95	0.91	0.90	0.87	0.86	0.91	0.92	0.85
h^2_n	0.38	0.75	0.44	0.56	0.44	0.53	0.23	0.74

Table 3. The gca effects of parents for grain yield and others traits

parents	Grain yield	Number of days to tassel	Plant height	Number of tassel branches	Ear height	Number of rows per ear	Number of grain per row	Days to maturity
K86/8	0.72**	1.68**	10.3**	-2.3**	3.6*	-0.24 n.s	-1.8**	2.17**
K1264/1	0.3*	2.58**	2.2 n.s	-1.7**	2.2 n.s	-0.9**	5.47**	1.15 n.s
K1250/3	-0.2 n.s	-2.3**	-3.3 n.s	2.7**	-3.16 n.s	0.24 n.s	-2.3**	-2.4**
K1291/2	-0.87**	-3.0**	12.8**	3.3**	-5.35**	-1.4**	-0.65 n.s	-5.1**
K1298/1	-1.14**	-4.99**	-5.5*	3.9**	-2.4 n.s	-0.55**	0.35 n.s	-5.7**
F.C393	0.13 n.s	0.87 n.s	-9.9**	-0.8 n.s	-3.3*	0.1 n.s	-3.7**	1.2 n.s
K2558	-0.1 n.s	-0.7 n.s	-10.3**	-2.95**	-7.4**	0.43*	2.18**	1.25 n.s
K561	-0.48**	1.99**	10.3**	-2.4**	1.93 n.s	-0.2 n.s	-0.17 n.s	2.83**
K1263/1	0.8**	0.78 n.s	8.7**	1.0 n.s	4.72*	1.0**	1.62**	1.77*
K12/8	1.37**	3.18**	-5.8*	-0.76 n.s	9.15**	1.15 n.s	-0.9 n.s	2.87**
Std. err.	0.26	0.48	2.5	0.64	1.66	0.21	0.68	0.84

*,**, ns: significant at 0.05, 0.01 probability levels, and not significant, respectively

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The use of indigenous germplasm in maize breeding

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ABSTRACT: The investigation was associated with the improvement of the resistance of the maize germplasm against the northern corn leaf blight (NCLB) (*Exserohilum turcicum* (/Pass./ K.J. Leonard et E.G Suggs)) which was based on crossings between tolerant inbreds and inbreds with other useful agronomic traits. The segregating progenies were artificially infected with the fungus under field conditions each year in two different terms. Disease severity was estimated twice, at the time of flowering and 4 weeks later. The resistance was determined visually and the plants were divided in R (resistant) and S (susceptible). The continuous selection combined with selfing of R/RS plants resulted (in the last year, 2003) in 29 families which had at least one R/RS plant. These families will be used for further breeding to recombine the genotypes with good agronomic traits with those that are resistant against to NCLB.

Key words: Disease resistance – *Exserohilum turcicum* – genetic improvement – gene bank – *Zea mays*

Introduction

One of the most important factors influencing the plant breeder‘s success is variability of the available genetic material. Among the variable traits, resistance against the most frequent plant diseases and pests is extremely important and for this reason, it is essential to have an appropriate germplasm collection (a gene bank) with reliable sources of genes for controlling these traits. The use of the genetic resistance is probably the best way to insure quality of yield (without disease) and to reduce the pollution of the environment. The success of breeding, however, does not depend only on the genetic material, but also on the breeding method and genetic and biological characteristics of the pathogen.

Maize has several diseases, and some of them are causing serious problems in agricultural production. One of those diseases is caused by the fungus *Setosphaeria turcica* Leonard & Suggs, with its conidial state *Exserohilum turcicum* (/Pass./ K.J. Leonard et E.G Suggs), known as northern corn leaf blight (NCLB). It causes wilt of plants in many tropical and temperate environments (Welz & Geiger 2000, Renfro & Ulstrup 1976). Grain yield losses can surpass 50 % when the disease appears before flowering (Pedersen & Bradenburg 1957, Pratt et al. 1993, Tefferi et al. 1996).

NCLB is characterised by several physiological races (Prat et al. 1993, Schechert et al. 1999, Welz et al. 1999, Welz & Geiger, 2000) because the pathogen appears to be dynamic and fast changing. It is able to respond to the selection pressure of the environment much faster than the host, maize. For this reason, the best solution is attempting a horizontal resistance, which is durable and efficient against all races. This type of the resistance is complex and it is difficult to achieve it. Because of this, most of the breeders are focussing on the vertical type of resistance and in this way concentrating on individual prevalent races.

One of the main objectives of our breeding program is to improve the genetic resistance (both horizontal and vertical) of the existing maize genebank materials against NCLB, using the genetic sources which exist within this genebank.

Materials and methods

Based on our earlier investigations (Rozman et al. 1998, Rozman & Kragl 2003), we recombined several inbred lines taken from our maize genebank (which are characterised by good morpho-agronomic traits) with the inbreds, tolerant to NCLB. In this presentation, we will discuss about one of the hybrid combinations, a cross of the NCLB tolerant line with another line, which was not tolerant to NCLB but very useful regarding to other agronomic traits.

The field trials were conducted at the Biotechnical Faculty in Ljubljana. After forming the F_1 generation (in the first year), we selected and selfed 100 plants which were used for forming 100 F_2 families. In the following years, we evaluated the tolerance to NCLB in field trials. Each family was represented by 10 plants, planted in a row. We evaluated families and individual plants within them.

For spreading the disease we used the very susceptible maize hybrid ‚Minnesota 706', which was artificially infected with the spore suspension, and from this susceptible hybrid, the spores of the fungus spread to the investigated materials. The inoculation took place twice; at the stage of 4 - 6 leaves, and 7 - 10 days later. In each generation, the main selection criterion was the resistance/tolerance against NCLB, combined with other desired morpho-agronomic traits. Disease severity was estimated twice: at the time of flowering and 4 weeks later. The resistance/tolerance was determined visually and the plants were divided in R (resistant/tolerant) and S (susceptible). In addition to this, the disease severity in % was determined.

Results and discussion

The selection started in 2000, in the F_2 generation. Each year we selected only the most tolerant plants which were in the following year represented by their progenies (individual families). In each F_2 (S_1) generation, we selected and selfed 100 plants, and formed 100 S_2 progenies. Based on only one hybrid combination, the evaluation of F_2 material showed that there were 64 R/RS progenies with at least one desired R/RS plant (Table 1). Of the altogether 91 selfed families, 64 had at least one R/RS plant. In the following year, 10 plants (out of 273 F_3 progenies, including the 64 selfed R/RS families from the previous year) were planted in separated rows and we selected 74 R/RS progenies with at least one desired R/RS plant (altogether 188 plants). In the last year (2003), 29 families (out of 60, some of them represented the subfamilies from the previous year) with at least one R/RS plant were selfed and utilised for the further selection.

Table 1. Numbers of investigated families with at least one plant tolerant to NCLB

Year	Generation	Families	Families with R/RS plants	Selfed families	Selfed R/RS families
2000	F_2 (S_1)	100	64	91	64
2001	F_3 (S_2)	273	188	91	74
2002	F_4 (S_3)	88	56	57	40
2003	F_5 (S_4)	60	34	58	29

Some of the families, which had more R or RS plants (out of altogether 10 plants), are shown in Tables 2 and 3. The investigation indicated that two types of the resistance, R and RS, occurred. The results showed that in Slovenia not only race 1 is present, as suggested by Rozman et al. (1998), but also race 2, and this is confirming the findings of Palaveršić et al. (2001). The families with only the R type resistance are resistant against both races, 1 and race 2. In the future, more attention should be paid to the individual plants with R type. The race 2 of NCLB is also present in the neighbouring Croatia (Palaveršić & Lendler 1996).

The results of this investigation suggest that the research should continue in this direction. In further investigations we plan to include additional gene bank material, increase the number of hybrid combinations and concentrate on horizontal resistance.

Table 2. Families with resistant/ tolerant progenies in 2002

Plot	Families	R/RS plants (%)	
		R type	RS type
1	p2-01-1r	20	50
2	p2-01-7r	20	30
3	p3-01-6r		50
13	p11-01-7r		60
14	p15-01-2r	30	
15	p15-01-6r	20	50
17	p16-01-1r		70
18	p17-01-2r		80
20	p154-01-8r		60
21	p25-01-3r	10	30
22	p180-01-4r		80
23	p180-01-5r	10	30
24	p29-01-2r		100
25	p29-01-3r	50	50
27	p269-01-1r	20	40
30	p248-01-2r		90
37	p38-01-6r	10	40
42	p45-01-5r		70
47	p97-01-2r		80
48	p54-01-7r		60
49	p55-01-4r	20	70
52	p59-01-10r	20	30
54	p60-01-4r		100
55	p229-01-1r		90
66	p77-01-5r		90
67	p77-01-6r		90
68	p170-01-8r		90
73	p135-01-1r	50	40
74	p135-01-10r	10	80
75	p85-01-9r		80
82	p139-01-9r	10	70

Table 3. Families with resistant/tolerant progenies in 2003

Plot	Families	R/RS plants (%)	
		R type	RS type
2	p14-02-1r	30	30
4	p15-02-1r		40
5	p15-02-2r	10	30
6	p16-02-1r		60
7	p16-02-8r	10	30
8	p18-02-5r	10	40
9	p19-02-1r		50
11	p19-02-6r		20
13	p24-02-6r		20
14	p28-02-3r	20	30
15	p30-02-9r		20
16	p31-02-1r		40
17	p31-02-2r	10	20
18	p40-02-5r	10	50
19	p40-02-8r		60
25	p50-02-4r		70
26	p52-02-9r	10	10
30	p58-02-2r		60
31	p58-02-8r		30
36	p66-02-4r	10	80
37	p66-02-5r		20
38	p68-02-4r		30
42	p73-02-1r	10	80
43	p75-02-3r		60
45	p76-02-7r	20	70
47	p78-02-4r		50
52	p86-02-3r		70
54	p87-02-2r	50	30
57	p89-02-8r		30
59	p92-02-3r	10	80
60	p100-02-7r	10	80

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Quantitative genetic analysis of full-sib family recurrent selection in an F_2 maize population

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ABSTRACT: Seven cycles of full-sib family recurrent selection were conducted in an source F_2 population derived from a cross of maize lines D145 and KW1292. The objective of this study was to evaluate the effects of selection on the population mean, genetic variance and inbreeding coefficient. A pseudo-factorial crossing scheme was used to generate 144 full-sib families in each cycle, which were evaluated in three locations from 1994 to 2003. The selection intensity was 25 %. Grain yield increased 9.7 % per cycle and dry matter content 1.7 % per cycle. Inbreeding coefficient increased from zero in the source population to 0.12 in cycle 7. Estimates of additive and dominance variance were significantly different from zero, except for dry matter content in cycle 3 and cycle 6. No significant decrease in additive and dominance variance could be shown for both traits after seven cycles of selection. The results indicate that full-sib family recurrent selection has been effective in increasing the per se performance of the population while maintaining the genetic variance.

Key words: Recurrent selection – selection response – variance components – inbreeding coefficient

Introduction

Recurrent selection (RS) is a long-term breeding procedure aimed at gradually increasing the favourable allelic frequencies at loci controlling quantitative traits. Maize (*Zea mays* L.) has been intensely subjected to RS and the populations most frequently utilized as sources have been open-pollinated and synthetic varieties (Hallauer & Miranda 1981). F_2 populations with a more restricted genetic base have been employed only in a few instances (e.g. Genter 1982; Moll 1991). The breeding potential of F_2 populations could be enhanced by a few cycles of recurrent selection because the intermating of selected genotypes favours the recombination among linked loci, thereby increasing the chance of attaining superior recombinants.

Mostly the recombination of selected individuals in a RS-program is made by random mating. Cockerham & Burrows (1980) proposed an alternative, where from the s selected individuals the s_1 best where used twice and the $s - s_1$ once. This pseudo-factorial crossing scheme results in a higher selection response by the same selection intensity. The success of a RS program is determined by evaluating improvement in the mean of the target population. The RS program should also maintain the genetic variability within the population, to facilitate improvement in future cycles of selection.

The objectives of our study, therefore, were to evaluate the changes in (i) population means, (ii) inbreeding coefficients, and (iii) the additive and dominance genetic variance.

Materials and methods

Plant materials

Two early maturing homozygous European flint lines D145 and KW1292, subsequently referred to as C and D, were used as parents. The F_2 generation, consisting of 240 plants, was developed from the cross C×D. The F_2Syn3 generation was randomly derived from the F_2 generation by three times chain crossing the 240 F_2 plants, i.e., 1 × 2, 2 × 3, ..., and 240 × 1.

Selection procedure

In 1994 the F_2Syn3 generation was grown and pairs of plants were crossed to produce 120 full-sib (FS) families. In the following year they were tested in field trials and six selfing ears were produced in each FS family. The 36 families with the highest selection index (2 × dry matter content + grain yield) were selected and divided into two sets, consisting of the even and uneven numbers. The six selfing ears of the 18 FS families in each set were intermated according to the pseudo-factorial crossing scheme of Cockerham & Burrows (1980). The resulting 144 FS families were tested in a trial similar to that in the previous cycle and again 36 families were selected by means of the selection index. In this way, seven cycles of recurrent FS selection were carried out for population C×D between 1994 and 2003. The seven field trials were conducted at three environments, each with three replications. The experimental design was an α-lattice (10×15) with 6 check entries. Each plot consisted of one row, 4.75 m long and spaced 0.75 m between rows. Plots included 25 plants. The usual field techniques were adopted.

Statistical analyses

Analyses of variance for grain yield (GY) and dry matter content (DMC) were performed for each experiment and environment using PLABSTAT (Utz 2001). Adjusted entry means were then averaged over the environments and the means for both traits, relative to the check entries (F_2), were calculated for each cycle.

With the known pedigrees of all entries, the inbreeding coefficients were calculated, using the PROC INBREED procedure in SAS and assuming an initial inbreeding coefficient of 0 in the F_2Syn3 generation.

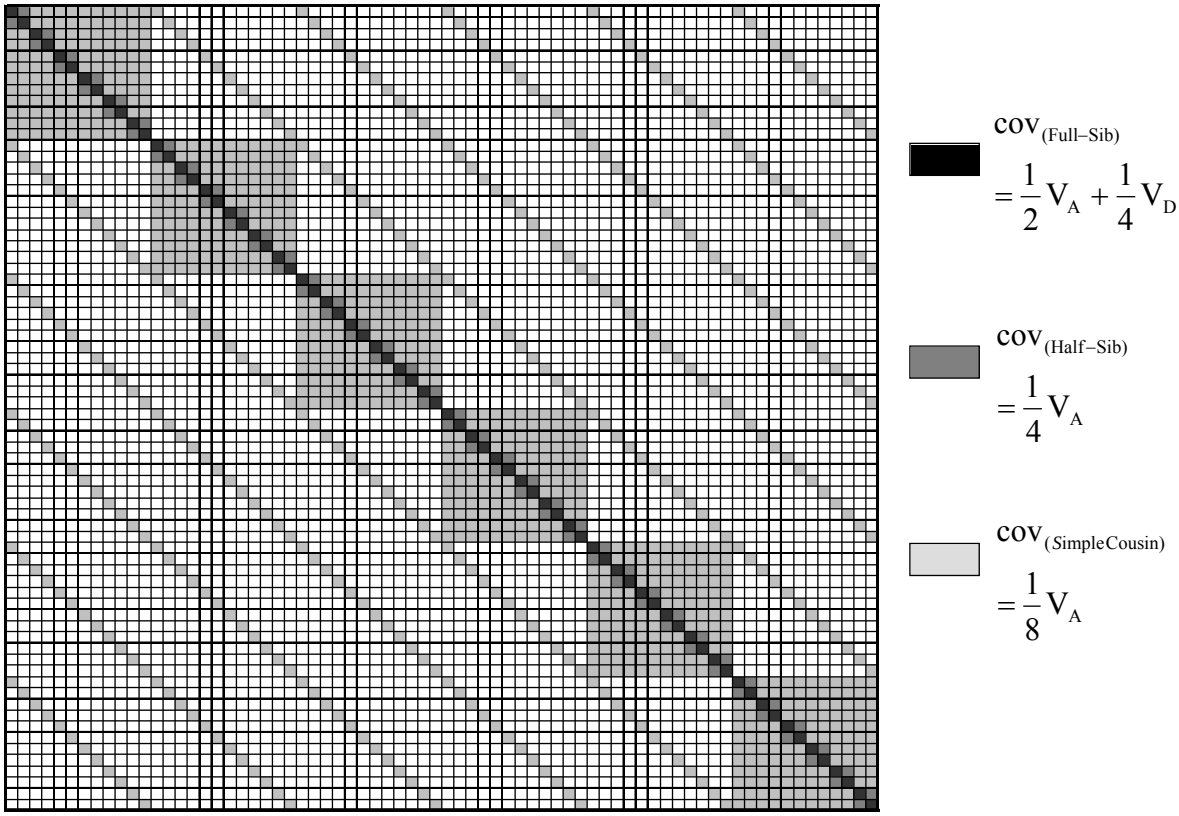

Figure 1. Schematic diagram of the variance-covariance-matrix V for one set consisting of 72 entries and corresponding covariances between relatives in terms of additive (V_A) and dominance variance (V_D)

For calculating the likelihood we used the model described by Lynch & Walsh (1998, p. 746 ff) and the variance-covariance matrix V (Figure 1). Additive and dominance variance were estimated by a restricted maximum likelihood (REML) method using PROC MIXED in SAS. For the estimation mean values over environments and replications were taken. Additionally the assumption was made, that there is no relationship between the F2Syn3 plants (f_{XY}=0). Furthermore the slightly increasing coefficients of coancestry between the entries after Cycle 0 (C0) were not included in the model.

Results and discussion

Mean GY relative to the check entries increased from 98 % in C0 to 158 % in C7, corresponding to an average rate of 9.7 % per cycle. For DMC the average selection response was 1.7 % per cycle. The lowest value was calculated in C2 with 99 % and the highest value in C7 with 107 % (Figure 2). The estimated rate of gain for grain yield was higher then the estimates obtained by Schnicker & Lamkey (1993) of 6.46 %, Keeratinijakal & Lamkey (1993) of 6.95 %, and Landi & Frascaroli (1993) of 7.3 %. The higher rate of gain in our study can be a result of the use of a pseudo-factorial crossing scheme instead of a random mating design applied by these authors.

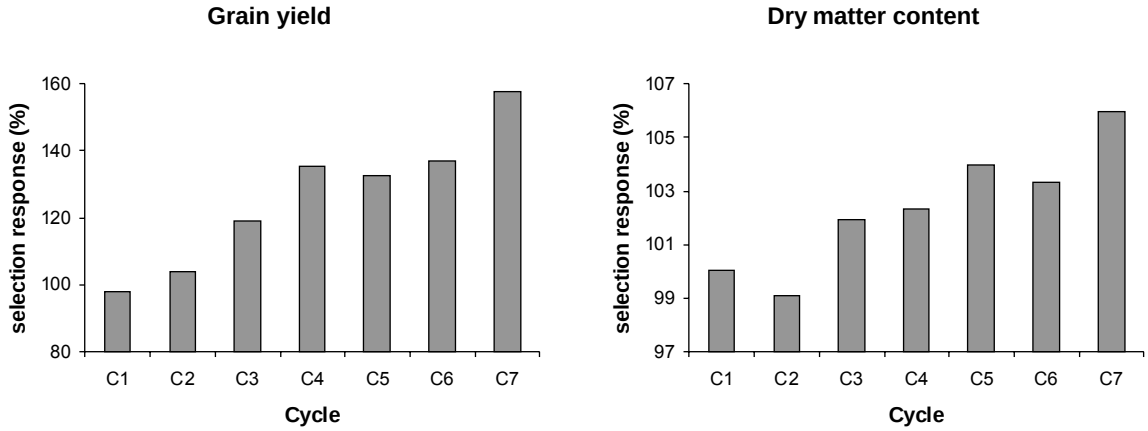

Figure 2. Mean selection response relative to check entries in the different cycles of population C×D regarding grain yield and dry matter content

The mean inbreeding coefficient, which was assumed to be zero in the F_2Syn3 generation, raised in C7 to 0.12 regarding all 144 entries and 0.11 in the selected 36 entries. The selected entries had particularly in the later cycles a slightly lower mean inbreeding coefficient than the whole trial, which indicates that the 36 best entries were partly selected owing to a low inbreeding depression.

Estimates of additive variance for GY and DMC in all seven cycles were significantly different from zero. Estimates of dominance variance were significantly different from zero, except for DMC in C3 and C6. Although additive and dominance variance for GY were lower in C7 than in C1, there was no significant trend over all cycles. For DMC additive variance increased from C0 to C7, while dominance variance remained on a low level except for cycle C7 (Figure 3). These factors indicate that full-sib family RS has increased the mean of the population without a significant loss of genetic variation. Hallauer (1984) examined genetic variance among full-sib progenies between BS10 and BS11 and similary found no significant decrease after seven cycles of RS.

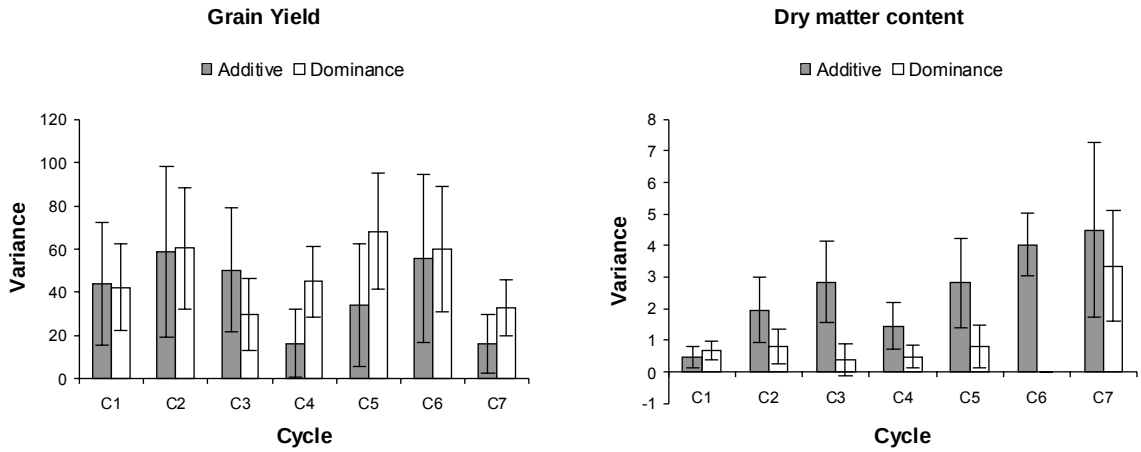

Figure 3. Additive and dominance variance (2 × standard error) for grain yield and dry matter content from cycle C_1 to cycle C_7

Conclusions

RS has been effective for improving the mean performance of the population. There was no evidence for a reduction in the genetic variance for GY and DMC. These results suggest that future selection response should be maintained at or near current rates of progress.

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Stalk strength of maize synthetics grown at different plant densities in Hungary

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ABSTRACT: The stalk strength of four maize populations representing the major heterotic groups in Hungary was evaluated in the experiment together with that of standard hybrids at three plant densities (40,000, 70,000 and 100,000 plants/ha). In addition to the original variant of *Mindszentpusztai Yellow Dent (MYD)*, two cycles of this genotype improved by recurrent selection were also examined. Stalk strength was evaluated on the basis of two parameters: lodging percentage and rind resistance. At all the plant densities, *MYD* had the highest lodging percentage. On the basis of rind resistance values, *Mv Synt 2* had the weakest stalks at the lowest plant density, while in stands with 70 and 100 thousand plants/ha *MYD* and *Mv Synt 2* did not differ from each other, but were both weaker than any of the other genotypes tested. Recurrent selection did not result in any significant change in the stalk strength of the improved cycles of *MYD*.

Key words: Puncture test – stalk lodging – stalk strength – *Zea mays*

Introduction

In maize, an increase in plant density leads to a significant increase in lodging (Stringfield & Thatcher 1947, Zuber & Loesch 1962, Perry 1983). Lodging resistance can be improved by breeding if the breeding stock possesses sufficient genetic variability. When the *Iowa Stiff Stalk Synthetic (BSSS)* population was created (Sprague 1946) the parental lines were selected on the basis of their resistance to stalk rot. Due to the outstanding importance of stalk strength, breeders sought for a reliable method by which selection could be made more efficient. Zuber & Grogan (1961) reported on the use of the crushing test, while Zuber (1973) and Colbert & Zuber (1978) applied the puncture test.

The values recorded in the puncture test correlate well with other parameters of stalk strength (Colbert et al. 1983, Berzonsky & Hawk 1986, Zuber & Kang 1978). According to Twumasi-Afriyie & Hunter (1982), the measurement of rind resistance is the best method for selecting for resistance to stalk lodging, because it is extremely simple and is well correlated with lodging resistance. A similar opinion was expressed by Anderson & White (1994). In the present experiments the stalk strength of the populations was thus evaluated on the basis of both lodging percentage and rind resistance.

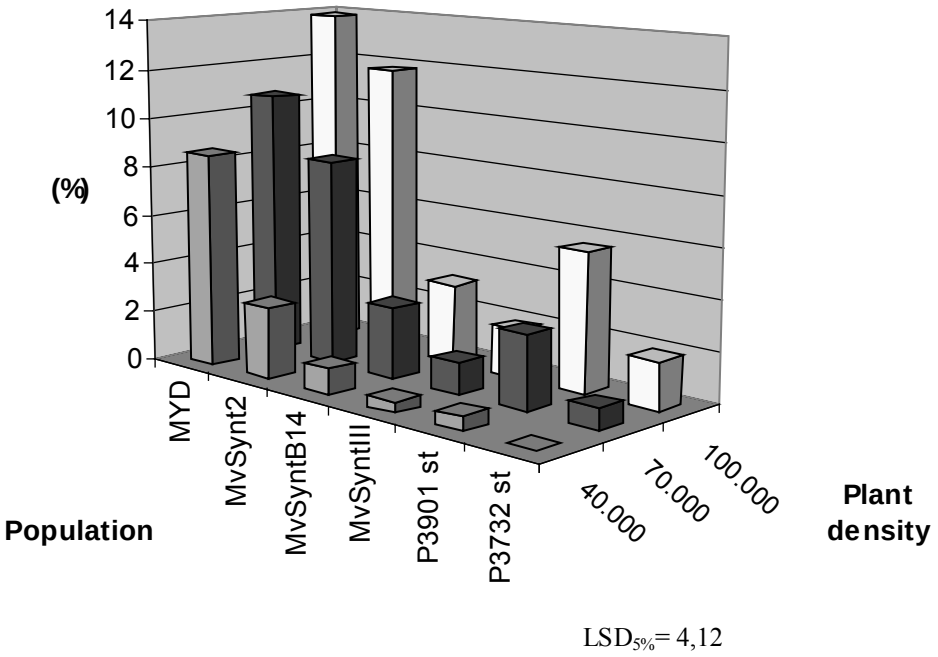
Materials and methods

In the experiments evaluations were made on the stalk strength of four populations representing the heterotic groups of most importance in Hungary *(Mindszentpusztai Yellow Dent (MYD), Mv Synt 2 Lancaster, Mv Synt B14, Mv Synt III Iodent)* and of the standard hybrids *(P 3901, P 3732)* in two years at three planting densities (40, 70 and 100 thousand plants/ha). In addition to the original variant of *MYD*, two cycles improved by recurrent selection were also evaluated (Herczegh et al. 1986).

Stalk strength was characterised using two parameters: 1: stalk lodging (%), the frequency of plants lodged to an angle of over 30° below the ear, 2: puncture test (kp/mm²), recorded using the method elaborated by Zuber (1973) in the middle of the first elongated internode.

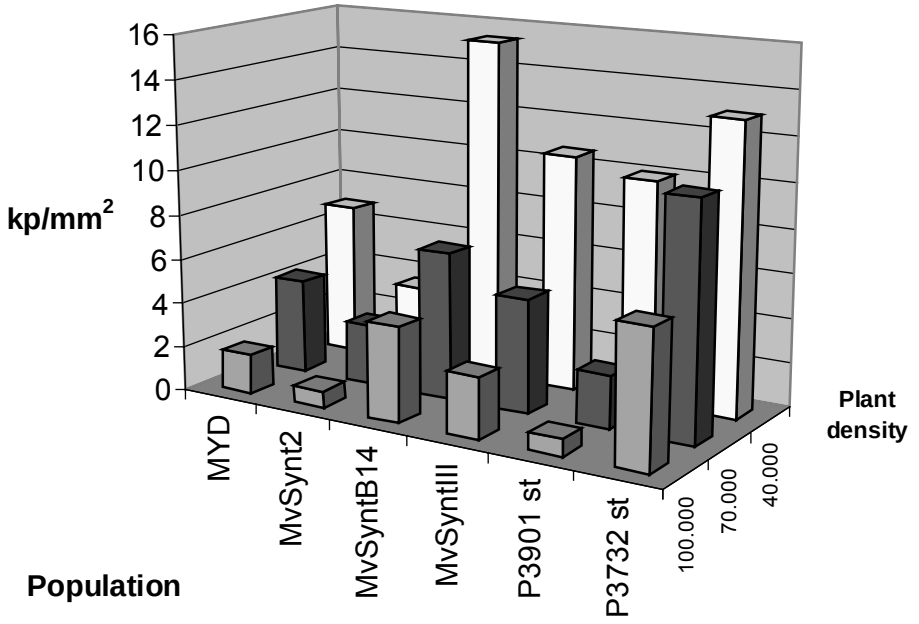
Results and discussion

At all the plant densities *Mv Synt III Iodent* exhibited the lowest lodging values, even lower than that of the standard *P 3901*, and *MYD* the highest (Fig. 1). The values recorded for *Mn Synt III Iodent* were similar to those of the standard *P 3732*, which had the strongest stalks, in all the plant density treatments. At the lowest plant density the rind resistance of *Mv Synt B 14* was the greatest, exceeding even that of the standard hybrids (Fig. 2).



$LSD_{5\%}$= 4,12

Figure 1. Effect of plant density on the lodging percentage of the populations



$LSD_{5\%}$= 2,114

Figure 2. Effect of plant density on the rind resistance of the populations

At greater plant density (70 or 100 thousand plants/ha) the standard *P 3732* had the best rind resistance. The rind resistance of *MYD* was better at the lowest plant density than that of *Mv Synt 2*, but was worse than that of any other plant population. In the 70 and 100 thousand plants/ha stands *MYD* and *Mv Synt 2* had the weakest stalks. There was very little difference in lodging percentage between the original population of *MYD* and the improved cycles (Fig. 3). A similar picture was obtained for the rind resistance of the three *MYD* cycles at the three plant densities (Fig. 4).

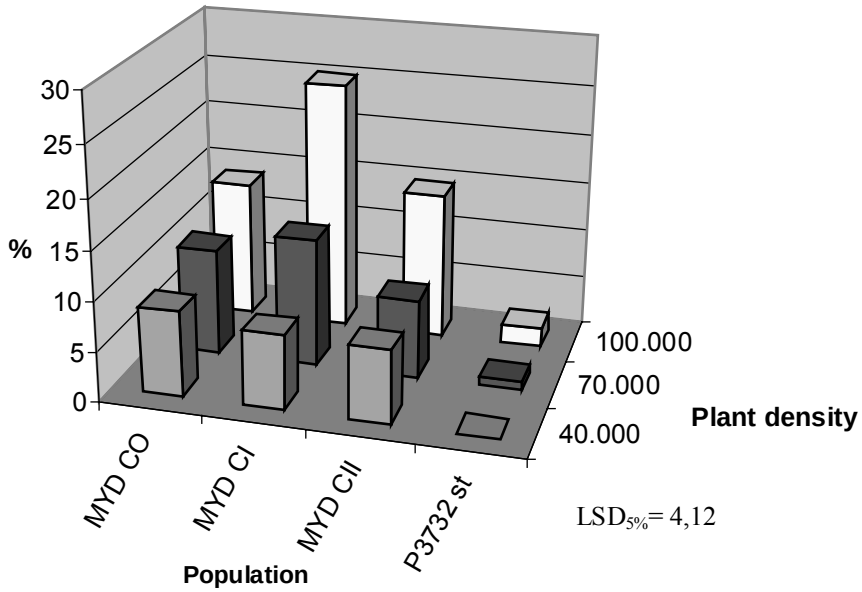


Figure 3. Effect of plant density on the lodging percentage of improved cycles of *MYD*

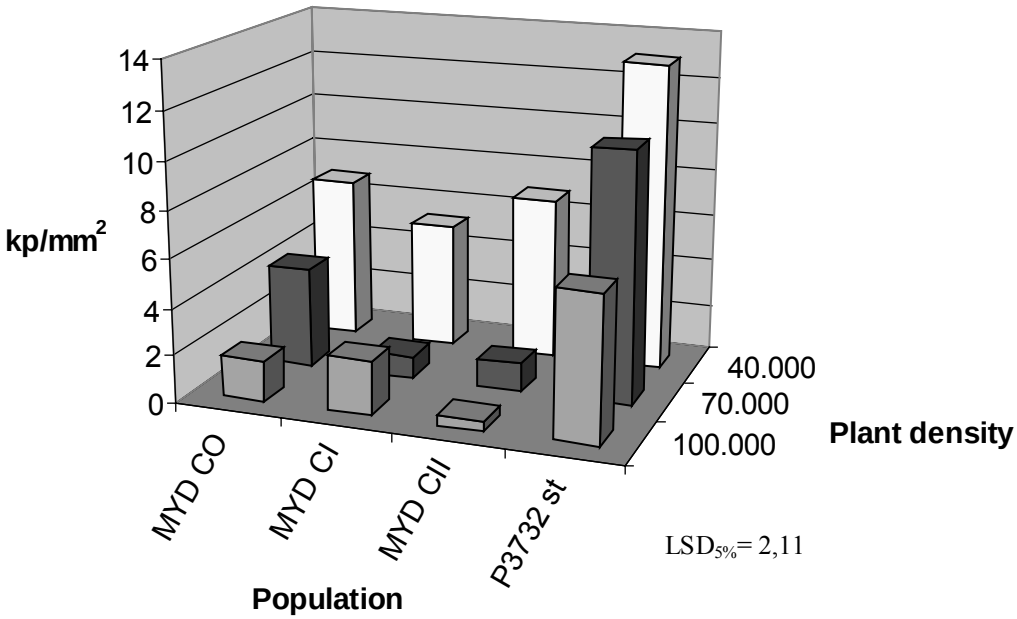


Figure 4. Effect of plant density on the rind resistance of improved cycles of *MYD*

A comparison of the lodging percentages and rind resistance values of the cycles did not demonstrate any trends. It can be seen that the first two cycles of selection, aimed at improving major traits, did not result in any change in the stalk strength parameters, which exhibited extremely poor values in *MYD* compared with the other populations.

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Genotypic response of sorghum (*Sorghum bicolor* L. Moench) to drought stress

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ABSTRACT: This study aimed at assessing the genotypic variability for drought tolerance in sorghum (*Sorghum bicolor* L. Moench). Eight genotypes were selected out of a set of 96 which had already been analysed by SSRs. Three field experiments were conducted in Sudan at two locations (Medani and El Rahad) in two seasons (2002-2003) under two water regimes, and a pot experiment was carried out at Giessen, Germany. In field tests drought was imposed naturally, while under pot conditions drought was imposed at 40 % and 70 % water hold capacity. In the pot trial and field experiments, genotypes differed significantly in most of the traits measured. In the pot experiment grain yield under drought stress ranged from 28 - 61 g plant^{-1} and relative yield ranged from 30 - 56 % with an average of 47 %, while in the field, the yield varied from 26 - 53 g plant^{-1}, with an average of 41 g plant^{-1}, and the relative yield ranged from 59 - 85 % with a mean of 75 %. The yield under field stress condition was moderately but significantly correlated with yield under pot stress condition (r = 0.52*). Based on yield, genotypes 'Wad Ahmed', 'ICSR91030' and 'SAR 41' turned out to be the best under both stress conditions, and based on the relative yield the best genotypes were 'Arfa Gadamak' (85 %), 'Wad Ahmed' (84 %) and 'Gadamballia' (81 %) under field conditions, and 'SAR 41' (56 %), 'Wad Ahmed' (55 %) and 'Red Mugud' (53 %) in the pot experiment.

Key words: Drought stress – genotypic variability – *Sorghum bicolor*

Introduction

Sorghum (*Sorghum bicolor* L. Moench) is widely grown in the semi-arid tropics (SAT) of Africa, where various types of stress exist, i.e. drought and high temperatures. Drought is one of the major limiting factors of agriculture in this area and has to be considered as the most important cause of yield reduction in crops (Boyer 1982, Sari-Gorla 1999). Some of the devastating effects of drought stress on crops could be overcome by exploiting genetic variation for drought tolerance (Sari-Gorla et al. 1999). Breeding drought tolerant sorghum varieties and hybrids which cope with water stress in a most suitable manner (Sari-Gorla et al. 1999) is an urgent issue in this crop, since sorghum as an important crop in SAT is widely grown under drought stress conditions (Blum et al. 1989). Genetic variation for drought tolerance in sorghum is depending on divergent mechanisms involved. Identification of physiological mechanisms involved in plant responses to drought stress will provide the basis for breeding plants with improved drought tolerance (Sanchez et al. 2002). Therefore, particular physiological or morphological traits that are separately inherited and positively correlated to yield under stress should be identified and introduced into high yielding lines to improve drought tolerance (Blum 1979). The objective of this study is to assess genotypic variability for drought tolerance in sorghum and to obtain information on physiological differences among the genotypes tested.

Materials and methods

Plant material

Eight genotypes i.e. 'Red Mugud', 'Wad Ahmed', 'Tabat', 'Gadamballia', 'Arfa Gadamak', 'SAR 41', 'PI 569695' and 'ICSR 91030' from different morphological groups and geographical origins were selected out of a set of 96 genotypes based on genetic diversity estimated by SSR analyses (Abu Assar et al. 2004).

Pot experiment

A pot experiment was carried out in the greenhouse on movable trays (under rainout shelter) during May-September 2003 using a randomised design with four replications. Plants were sown at a density of 10 plants pot^{-1}. The soil of each pot (11 kg per Mitscherlich pot) contained 26 g fertilizer (N 12 %, P_2O_5 12 %, K_2O 17 %, and SO_2 15 %) well mixed with the soil before sowing. Pots were pre-irrigated by distilled water (500 ml/pot) in order to get equal soil moisture conditions. After sowing pots were covered by polyethylene net to reduce evaporation during germination. At the three- to four-leaf stage the number of plants was reduced to 2 plants pot^{-1}. Further on, pots were irrigated with distilled water 70 % and 40 % maximum water holding capacity for normal and stress treatments, respectively, throughout the experimental period. The daily mean temperature during the experiment period ranged from 14.5°C in May to a maximum of 22.5°C in August. Dry weight of shoots and roots were determined by drying the plants in an oven at 80°C for 48 h. Seven different traits were considered in the pot experiment and field trials: days to 50 % flowering (days), plant height (cm), growth rate (cm day^{-1}), grain yield/plant (g), 1000 grain weight (g), biomass weight/plant (g) and harvest index (%).

Field evaluation

In a field trial the same eight genotypes were evaluated in Sudan at two locations (Medani and El-Rahad) in two seasons (2002 and 2003) under two water regimes (normal and stress). A split-plot design with 3 replications was adopted, with water treatments assigned in main plots and genotypes randomised in sub-plots. Statistical analysis was performed using SPSS 11.5 software. Tukey's multiple range test was used to separate the means. The genotypes' performance under stress and normal conditions were used to estimate drought parameter index (relative performance) for the studied traits as follows:

$$P_{rel}(\%) = \frac{P_{stress} \times 100}{P_{normal}}$$

In order to obtain useful information on selection criteria usable in breeding programs for drought tolerance a correlation analysis (Pearson' correlation coefficient) among traits was performed.

Results and discussion

Performance of reproductive and vegetative traits under drought stress conditions

Significant differences were found among the genotypes for all of the traits measured in the field and greenhouse, with the exception of relative harvest index under pot stress condition. Genotypes suffered drastically under the water stress in both environments, showing reduction on means of all measured traits (Table 1). The effect of drought stress on grain yield of each genotype was assessed as absolute yield under stress and as relative yield under stress in percent of the controls. Regarding the grain yield in response to drought, a clear reduction was registered in both trials. In pot stress condition, yield ranged among genotypes from 28 - 61 g $plant^{-1}$ with an average of 45 g $plant^{-1}$, corresponding to a relative reduction of 70 - 40 % (mean 53 %). Evidently, water stress reduced yield to more than half on average. However,

Table 1. Environmental means, variation and significance for water stress-related traits and relative performance under pot drought stress conditions in Germany and in field trials under stress conditions across three environments over two seasons in Sudan

Trait	Mean		Minimum		Maximum		Significance[2]	
	Pot	Field	Pot	Field	Pot	Field	Pot	Field
YLD[1]	45	41	28	26	61	57	**	**
YLD_{rel}	47	75	30	59	56	85	**	**
DF50	82	72	71	60	94	80	**	**
$DF50_{rel}$	105	107	97	105	117	108	**	**
PH	95	153	75	123	125	210	**	**
PH_{rel}	61	85	52	82	68	93	**	**
GR	1.2	2.2	0.8	1.6	1.5	3.4	**	**
GR_{rel}	58	70	53	73	66	87	**	**
TGW	25	24	17	16	29	28	**	**
TGW_{rel}	93	87	91	82	97	90	**	**
BMP	121	126	102	93	173	186	**	**
BMP_{rel}	45	75	35	66	53	84	**	**
HI	37	36	28	28	39	45	**	**
HI_{rel}	106	105	57	79	156	123	NS	**

[1] YLD, yield per plant (g); DF50, days to 50 % flowering (d); PH, plant height (cm); GR, growth rate (cm d^{-1}); TGW, 1000 grain weight (g); BMP, biomass $plant^{-1}$ (g); HI, harvest index (%); relative performances are indicated by a appended and subscripted 'rel'

[2] **, significant at 0.01 probability level; NS, not significant

genotypes differed very markedly in their response to drought stress. The highest yield under pot stress condition was achieved by the cultivar 'Wad Ahmed' (61 g), followed by 'SAR 41' (55 g) and 'ICSR 91030' (54 g). On the other hand, in field trials, the yield/plant ranged from 26 - 57 g with an average of 41 g, while the relative yield varied from 59 - 85 % with an average of 75%. It is interesting to note that the same three genotypes that revealed high productivity in the pot experiment showed the same trends in field trials, i.e. 'Wad Ahmed' (57 g), 'ICSR 91030' (52 g) and 'SAR 41' (44 g). Based on the relative yield, the best three genotypes were 'Arfa Gadamak' (85 %), 'Wad Ahmed' (84 %) and 'Gadamballia' (81 %). Mean relative 1000-grain weight and biomass were reduced to 93 % and 45 % in pot experiment and to 87 - 75 % in field trials, respectively. Harvest index under pot stress varied in a wide range from 28 - 39 % among genotypes. Regarding the relative HI, a clear trend was determinable among genotypes ranging from 57 - 156 % in the pot experiment to the range of 79 - 123 % in the field as compared with controls. Days to 50 % flowering under water stress was delayed up to 117 %, depending on genotypes. Genotypic differences with regard to days to 50 % flowering in the pot experiment may be due to an interaction with European climate conditions regarding differences in day length, humidity and temperature, while in field conditions, the days to 50 % flowering was delayed by up to 107 %. The high yield of 'Wad Ahmed' and 'SAR 41' under drought stress was not associated with escape mechanism as they were slightly later heading than 'Gadamballia' and 'PI 569695'. Plant height under drought stress varied from 75 to 125 cm in pot experiment and from 123 cm to 210 cm in the field experiment according to the genotypes. There was a relative reduction of plant height ranging from 52 - 68 % with a mean of 61 % in the pot experiment and from 82 - 93 % with a mean of 85 % in the field in comparison to the controls. The mean growth rate was 1.2 cm day^{-1} ranging between 0.8 - 1.5 cm day^{-1} in the pot experiment and was 2.2 cm day^{-1} varying between 1.6 - 3.4 cm day^{-1} in the field experiment. Relative growth rate in pot ranged from 53 - 66 % with a mean of 58 %, while in the field, it ranged from 73 - 87 % with a mean of

70 %. These data point out a large variability among the tested genotypes in phenology and morphology. The use of the relative indices revealed effective in estimating different genotypic responses to drought stress.

Association among grain yield and related traits

The grain yield per plant under field drought stress condition was moderately but significantly correlated with yield under pot stress condition (r = 0.52*). Grain yield under pot stress was positively correlated with relative yield (0.89**), total biomass (0.56*) and harvest index (0.81**), but negatively correlated with 1000-grain weight. From the positive and significant association between total biomass and harvest index, it appears that selection would be effective to simultaneously improve the yield under stress. This finding is in agreement with Blum et al. (1992), who reported the association of harvest index and above-ground dry mass of sorghum suggesting that further improvement might be possible if higher harvest index, larger dry mass and early flowering could be combined in sorghum under drought conditions.

Based on the results obtained, it seems that the semi-controlled environment (pot condition) used to stimulate water stress was effective and could be used in further plant breeding research to develop tolerance to drought stress. Based on drought tolerance parameters the best three genotypes under drought stress in the pot experiment with regard to the relative yield were: 'SAR41' (56 %), 'Wad Ahmed' (55 %) and 'Red Mugud' (53 %). Regarding the field trials, 'Wad Ahmed' (84 %) was again one of the three best along with 'Arfa Gadamak' (85 %) and 'Gadamballia' (81 %). On drought stress productivity (absolute yield/plant), three superior genotypes identified in both experiments were: 'Wad Ahmed' (61 and 57 g plant^{-1}), 'SAR 41' (55 and 43 g plant^{-1}) and 'ICSR 91030' (54 and 52 g plant^{-1}). Therefore, these cultivars should be good candidates for improving sorghum for drought tolerance.

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Characterization of two short root mutants selected from mutant panel in rice (*Oryza sativa* L.)

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ABSTRACT: Two short root mutants RMM5 and RMM6 (root mutant derived from mutant panel) were screened from tissue culture-derived R_1 lines in rice (*Oryza sativa* L. cv. 'Nipponbare'). Both mutants had significantly shorter seminal and crown roots as compared to wild type. The total number of crown roots reduced significantly in RMM5 and RMM6 compared to wild type. Seminal root diameter increased significantly in RMM6 compared to wild type, but remained unchanged in RMM5. To know the cause of altered root character of RMM5 and RMM6, we performed histological observations of longitudinal section of seminal root tips of wild type and mutants. Histological observation revealed that both mutants had shorter cortical cells than the wild type. The cortical cell width and the number of cell files in cortex were similar in RMM5 but increased significantly in RMM6 as compared to wild type. Finally, to determine the mode of inheritance of the mutants, we carried out genetic analysis. The segregation of wild type and mutant phenotypes in R_1 and R_2 population fitted a 3:1 ratio, indicating that the mutants are monogenic recessive.

Key words: Cortical cell – mutant panel – rice – root length

Introduction

Roots have several important roles such as absorption of water and nutrients, support of a plant body, and reservation of amino acids and assimilation products. Furthermore, roots are deeply connected to the growth, productivity and resistance to the soil-stress conditions. However there is much less knowledge about the root than the aerial part. The same situation is true for rice, which is considered as an important model in monocotyledonous plants. It is important to isolate root mutants in rice to study the genetic and the physiological mechanism of root development.

In recent years, the gene knockout method is drawing attention as one of the effective methods for investigating the gene functions in plants. In rice, the retrotransposon, *Tos17* is used for gene knockout (Hirochika 1997). *Tos17* is activated by tissue culture and inactivated by redifferentiation. Moreover, the number of *Tos17* copies increases over culture period, and they transpose copies at random around chromosomes. The mutation induced by *Tos17* insertion is stable and inherited to next generations. Using such features of *Tos17*, about 50,000 mutant panel lines have been produced in the 'Nipponbare' background and are being used for analysis of gene function. It was reported that the percentage of mutants caused by *Tos17* insertion was 5 - 10 % (Hirochika 2001). In this paper, we describe the characteristics of two short root mutants selected from the mutant panel in rice.

Materials and methods

Plant materials

Screening was performed using R_1 generation of 768 mutant panel lines, made available by Laboratory of Gene Function at the National Institute of Agrobiological Sciences (Tsukuba, Japan). The mutagenesis with *Tos17* has been described by Hirochika et al. (1996). Seedlings

were grown in water (pH 5.2) at 25˚C. After 2 weeks we selected lines segregating plants with altered root morphology.

Morphological observation
Seeds of R_2 generation were used. Seedlings of wild type and mutants were grown in Kimura B nutrient solution (pH 5.2) for thirty days, and then morphological data were recorded.

Histological observation
Seminal roots of 4-days-old seedlings were cut from the root tip in the position of 1 cm and were sectioned longitudinally.

Genetic analysis
Segregation of phenotypes was observed in R_1 and R_2 generations of the mutant lines, F_1 generation of mutants crossed with wild type and F_1 generation of the cross between mutants.

Results and discussion

Mutant screening
We selected two root mutants, RMM5 and RMM6. To know whether the mutation was caused by *Tos17* insertion, Southern hybridization with *Tos17*-specific probes and linkage analysis were carried out. The results showed that these two mutations were independent of *Tos17* insertion. These mutations are probably caused by other factors that occurred during tissue culture.

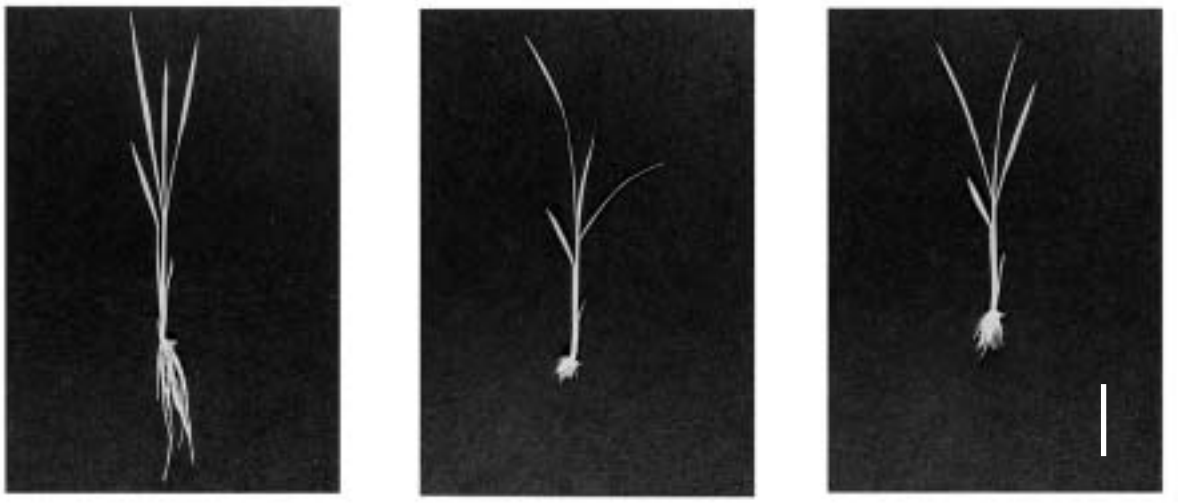

Figure 1. 30 days old seedlings wild type (‘Nipponbare’), RMM5 and RMM6 (from left to right; bar = 5 cm)

Morphology of mutants
Morphological characteristics were investigated on 30-days-old seedlings of wild type and mutants. The seminal root length in RMM5 and RMM6 were about 20 % and 50 % of wild type, respectively (Table 1 and Figure 1). Plant height and crown root length were shorter in both the mutants than wild type. The number of crown root reduced in RMM5 and RMM6 compared to wild type. Seminal root diameter of RMM6 was thicker than wild type.

Table 1. Morphological characteristics of wild type, RMM5 and RMM6 seedlings

Character	Nipponbare	RMM5	RMM6
Plant height (cm)	31.2 ± 0.9	20.6 ± 0.3**	27.2 ± 0.3**
Seminal root length (cm)	9.2 ± 0.1	1.9 ± 0.0**	4.9 ± 0.2**
Crown root length (cm)	14.7 ± 1.6	1.8 ± 0.1**	4.5 ± 0.3**
Crown root number	51.1 ± 0.8	49.2 ± 1.0*	38.9 ± 0.3**
Root diameter (mm)	0.46 ± 0.01	0.46 ± 0.02ns	0.53 ± 0.01**

30-day-old seedlings were investigated; **, *: significant different from 'Nipponbare' at 1 % and 5 % level, respectively; ns, not significant

Histological characterization of mutants

We carried out histological observations. It is known that shortening of root length is due to reduction of either cell size or cell number. In order to investigate the cause of the short root phenotype in the mutants of this study, longitudinal sections of seminal roots were made and then the cortical cells were observed in a position of 7 mm from root tip of the seminal root (Figure 2). The cortical cell length in RMM5 and RMM6 was about 50 % and 60 % of wild type, respectively (Table 2). These results indicate that the short root phenotype in RMM5 and RMM6 was due to reduced cortical cell length. Cortical cells in RMM6 were much wider as compared to wild type. Furthermore, in RMM6 the number of cortical cell files increased than wild type. These results suggest that the larger seminal root diameter in RMM6 is due to both wider cortical cells and an increased number of cell files in cortex.

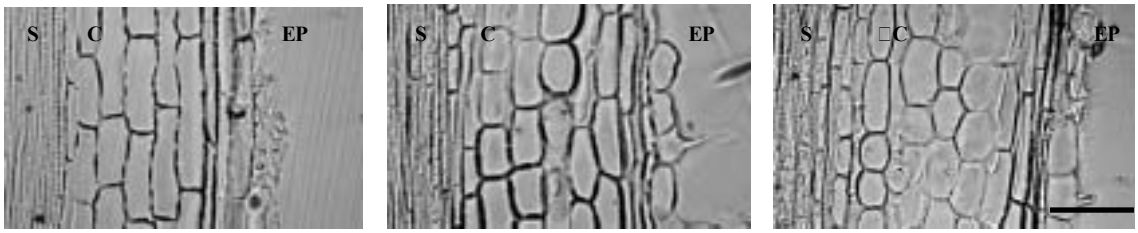

Figure 2. Mature cortical cell morphology of seminal root tips of 4-day-old seedlings. 'Nipponbare' (left), RMM5 (center) and RMM6 (right). S, stele; C, cortex; and EP, epidermis (bar = 100 μm)

Table 2. Cortical cell character of the seminal roots of 4-day-old seedlings

Character	Nipponbare	RMM5	RMM6
Cortical cell length (μm)	100.8 ± 6.8	53.0 ± 0.7**	63.1 ± 7.0**
Cortical cell width (μm)	15.8 ± 1.7	17.3 ± 3.0ns	26.3 ± 2.8**
Number of cell files in cortex	7.0 ± 0.0	7.0 ± 0.0ns	8.5 ± 0.6**

About 7mm from the root tip was sectioned; abbreviations for significances see Table 1

Genetic analysis

We performed genetic analysis. We observed the segregation in R_1 generation and R_2 (next generation of R_1 heterozygous plant) in RMM5 and RMM6. Segregation of wild type and mutant phenotype fitted to the expected 3:1 ratio (Table 3), indicating that a single recessive gene is controlling the short root phenotype in RMM5 and RMM6. Furthermore, all the F_1 plants of the cross between RMM5 and RMM6 showed wild type phenotype, indicating that these two mutations are not allelic.

Table 3. Segregation in R_1 and R_2 generations and F_1 hybrids among short root mutants and 'Nipponbare'

Line	Phenotype		χ^2	P (3:1)
	Wild type	Mutant		
Nipponbare	20	0		
RMM5 R_1	14	5	0.02	0.9<P
RMM5 R_2[1]	416	120	1.81	0.1<P<0.25
F_1 (RMM5×Nipponbare)	5	0		
RMM6 R_1	16	2	1.19	0.25<P<0.50
RMM6 R_2[2]	239	89	0.69	0.25<P<0.50
F_1 (RMM6×Nipponbare)	3	0		
F_1 (RMM5×RMM6)	5	0		

[1] Plants were derived from 10 heterozygote R_1 plants
[2] Plants were derived from 13 heterozygote R_1 plants

In summary, we isolated two short root mutants, RMM5 and RMM6. Although several short root mutants were reported in rice (Yao et al. 2002), the present mutants are unique in having a reduced crown root number for both of the mutants and a thicker seminal root for RMM6. The short root phenotype in RMM5 and RMM6 was controlled by a single recessive gene, but the mutants were also associated with changes in other characters such as plant height. Therefore, it needs to be analyzed whether the changes of multiple characters are due to pleiotropy or independent linked mutations.

Acknowledgements
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Variability in the texture of Catalan landraces of common bean (*Phaseolus vulgaris* L.): sensory and chemical approach

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ABSTRACT: Superior organoleptic characteristics of some traditional common bean landraces (*Phaseolus vulgaris* L.) have been pointed to as the reason for their persistence in cultivation, despite the advance of new commercial varieties. Nevertheless, as consumers become more demanding, more objective information is needed to enhance the position of these landraces through protected denominations or geographic indications of origin. Obtaining reliable information on sensory traits is not easy due to the need for panels of tasters and the weak relationship between sensory and chemical traits found thus far. Both sensory and chemical approaches were used here to characterize the variability in texture among four Catalan landraces of common bean and the variability within one of these ('Ganxet'). The results show that texture differences exist among Catalan landraces and that these can be detected by sensory and chemical methods. On the other hand, although chemical variability for the main traits presumably responsible for texture is found within the 'Ganxet' variety, these differences are not perceived by panellists because it is very difficult to discriminate when dealing with the extremes of the scale (very high creaminess and very low perception of the seed-coat). Therefore, although breeding for increased creaminess and lower perception of the seed-coat in 'Ganxet' on the basis of chemical data seems possible, this appears unlikely to have an impact on the consumer preference. In general, it seems advisable to perform intra-variety selection only after sensory tests on the degree of differences shown by extreme genotypes for the significant molecules involved in the organoleptic expression.

Key words: Catalan landraces – chemical composition – organoleptic quality – sensory analysis

Introduction

Autochthonous traditional vegetable landraces, clearly out-yielded by modern improved varieties, remain in commercial cultivation due to a supposedly superior organoleptic quality. Many of these varieties present a distinctive morphological trait that has probably contributed to their persistence as commercial crops, but little is known about their objective culinary value. Therefore, a clear description based on scientific data is needed if these materials are to be properly promoted for their sensory qualities.

Texture characteristics are very important in determining the organoleptic value of dry beans (*Phaseolus vulgaris* L.), while taste is mainly provided by accompanying products in the cooked dish (like in Italian "pasta"). There is little information available on texture differences in grain pulses (Wasimi et al. 1990, Sanz-Calvo & Rey 1999). Moreover, although some correlations between texture and chemical composition of the seed have been reported (Pujola et al. 2004), the use of sensory panels is still compulsory.

In this context our aims are: i) to establish objective differences in the texture of four Catalan landraces of common bean, both from the sensory and chemical points of view, ii) to apply the same approach to investigate the variability within the 'Ganxet' variety, the most promising Catalan landrace from an organoleptic point of view, and iii) to evaluate the most effective way to proceed in breeding common bean landraces for improved organoleptic values.

Materials and methods

Plant material

For the inter-variety study the four most appreciated Catalan landraces of common bean ('Ganxet', 'Genoll de Crist', 'Tavella Brisa', 'Castellfollit del Boix') and a well-known market class ('Canela') were used. For the intra-variety study 23 inbred lines representing the agronomic and chemical variability among 'Ganxet' (Casañas et al. 1999) were chosen.

Field trials

Field trials were performed in a single location where all the germplasm had previously proved to grow satisfactorily. A randomized block design (two blocks) with 80 plants per plot was used.

Sensory and chemical analysis

Beans were cooked in low mineralized water. Twelve trained panellists evaluated the cooked seeds in a multi-comparison trial, performed in duplicate sessions. Seed-coat and whole seed chemical analyses were performed in duplicate on uncooked material from each plot.

Results and discussion

Inter-variety analysis

1. Sensory approach. The three texture traits considered presented significant differences between varieties (Table 1). 'Tavella Brisa', 'Castellfollit del Boix' and 'Genoll de Crist' showed the smoothest surface of the seed-coat, while 'Ganxet' was the creamiest and had the least perceptible seed-coat (Table 1). Consumers associate high quality with low perception of the seed-coat and high creaminess, and this set of landraces encompasses a wide range of organoleptic variation for these traits.

2. Chemical approach. Protein and/or amylose/amylopectine ratio, previously reported by Pujolà et al. (2004) to be related with creaminess, also showed significant variability (Table 2). The chemical and sensory approaches were consistent as the creamiest varieties were those with the highest protein content, although the role of amylose/amylopectine ratio was not clear. Regarding seed-coat perception, the content of uronic acid seems to be a better indicator than Ca^{++} or Mg^{++} content (Tables 1 and 2), as previously reported (Casañas et al. 2002).

If this consistency were general for the common bean, as much variability in protein content has been reported in a wide scope of landraces throughout the world, it would seem reasonable to make a first approach to organoleptic value starting from the analysis of some selected chemical components.

Table 1. Mean values of the texture traits on cooked seeds in the varieties measured by panellists on a scale from 0 to 5; values followed by the same letter are not significantly different ($P \leq 0.05$)

Variety	Seed-coat surface roughness	Seed-coat perception	Seed creaminess
Ganxet	3.4 b	1.1 a	3.7 c
Canela	2.6 b	3.0 b	1.0 a
Castellfollit	1.4 a	1.2 a	2.4 b
Tavella B.	1.4 a	2.6 b	2.1 b
Genoll C.	1.3 a	2.1 ab	0.8 a

Table 2. Mean values of the chemical traits in the varieties in g kg^{-1}; values followed by the same letter are not significantly different (P≤0.05)

	Whole seed				Seed - coat		
Variety	protein	amylose	amylopectine	aml/amp	Uronic ac.	Ca^{++}	Mg^{++}
Ganxet	257a	94.2b	265.4b	0.36a	190.3a	4.7c	4.3a
Genoll C.	219b	105.8a	343.3a	0.31ab	147.3bc	4.7c	4.2a
Tavella B.	216b	93.4b	326.7a	0.29b	-	-	-
Castellfollit	223b	102.5a	333.8a	0.31ab	198.5a	11.6a	3.4a
Canela	221b	101.9a	352.1a	0.29b	129.6c	6.7bc	2.4a

Intra-variety analysis

1. Chemical approach. 'Ganxet' was chosen to study intra-landrace variability for sensory related traits, as it had the best organoleptic qualities among the varieties considered (Tables 1 and 2). The 23 inbreds selected showed significant variability for both seed-coat and whole bean compounds (Table 3); therefore, the lines with extreme values for the traits (Table 4) were submitted to the sensory analysis.

2. Sensory approach. Panellists found no significant differences between 'Ganxet' lines for the texture traits (Table 5), but chemical analysis revealed significant differences in all compounds considered (Table 4). If we assume that high creaminess is related with high protein content and/or low amylose/amylopectine ratio, some lines should have been considered creamier. It is likely that panellists failed to discriminate between lines due to the extreme creaminess of 'Ganxet', i.e. panellists are unable to perceive consistent differences in creaminess within this range of chemical variation (from 242 to 279 g kg^{-1} protein content and from 0.26 to 0.31 aml/amp ratio, Table 4). Likewise, the absence of differences in seed-coat perception (low) could be due to the very high uronic acid content in the seed-coat of the 'Ganxet' variety.

The results indicate that texture differences exist among Catalan landraces and that they can be detected by sensory and chemical methods. On the other hand, although there is variability in the main chemical compounds responsible for texture differences within the 'Ganxet' variety, these differences are not perceived by panellists because it is very difficult to discriminate at the extremes of the scale (very high creaminess and very low perception of the seed-coat). Therefore, although it seems possible to use chemical data in breeding for increased creaminess and lower perception of the seed-coat in 'Ganxet', the obtained improvements are likely to go unnoticed by the consumer.

In view of these results, it seems reasonable to screen existing information on the chemical composition of common beans to identify promising varieties to be tested in sensory trials. Selection within varieties should be performed only after sensory tests on the degree of differences shown by extreme genotypes for significant molecules.

Table 3. Mean values (g kg^{-1}), standard error of the mean, and maximum and minimum values recorded for the chemical traits studied in the set of 23 inbred lines representing agronomic and chemical variability in 'Ganxet'

	Whole seed				Seed - coat	
	protein	Amylose	amylopectine	aml/amp	Ca^{++}	Mg^{++}
Mean	256	93.4	341.6	0.27	5.7	3.5
Std. error	2.5	0.6	0.0	0.00	0.2	0.0
Max.	280	98	380	0.32	7.2	3.9
Min.	239	88	298	0.24	4.0	3.3

Table 4. Mean values (g kg^{-1}) of the chemical traits studied in the five 'Ganxet' inbreds considered extreme for the recorded traits; values followed by the same letter are not significantly different (P≤0.05)

	Whole seed				Seed - coat	
Line	protein	Amylose	amylopectine	aml/amp	Ca^{++}	Mg^{++}
103	242c	93.2b	352a	0.26bc	5.3ab	3.5b
12103	274b	97.7a	316c	0.31a	5.0b	3.4b
20103	279a	93.2b	360a	0.26c	5.7ab	3.6b
40103	246c	90.4c	336b	0.27bc	6.6a	3.9a
42103	254c	93.4b	334b	0.28b	6.8a	3.5b

Table 5. Mean values of the texture traits in the 'Ganxet' lines measured by panellists on a scale from 0 to 5; values followed by the same letter are not significantly different (P≤0.05)

Line	Seed-coat surface roughness	Seed-coat perception	Seed creaminess
42103	2.7 a	1.4a	3.4 a
103	2.9 a	1.5 a	2.8 a
12103	2.9 a	1.3 a	3.0 a
40103	3.6 a	1.8 a	3.1 a
20103	2.6 a	1.6 a	2.2 a

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Understanding floral display and design traits to assess the feasibility of new breeding strategies in partially allogamous crops

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ABSTRACT: This research attempts to evaluate the feasibility of increasing heterozygosity in open-pollinated varieties by promoting cross-pollination by selecting for pre-mate floral traits. Using an approach that combines allozyme markers, multilocus likelihood-based estimation of outcrossing and multivariate regression analysis we examined the relationship between floral traits and outcrossing in *Vicia faba* L. and the contribution of each of the floral characteristics to the outcrossing level. For faba bean increasing the level of allogamy by selection of floral display and design traits could be a suitable choice.

Key words: Increasing heterozygosity – multilocus outcrossing – multivariate regression – open population – synthetic varieties – *Vicia faba*

Introduction

From the point of view of sustainable agriculture the allogamous crop improvement approach has been encouraged not simply for yield but for yield stability and for maintaining or increasing diversity (Cooper et al. 2001). Improved open populations or synthetic varieties are the mainstay of breeding efforts for partially allogamous crops. Sufficient amount of heterozygosity in these varieties is required to maintain the desired levels of hybrid expression. Any genetic mechanism that would improve outcrossing potential would increase the chances of success with recurrent selection schemes as well as with the development of synthetics.

Considering that the mating patterns depend greatly on flower design and display (Barret 2003), cross pollination could be promoted by the introduction in the cultivar of floral traits that promote outcrossing. The necessary breeding must be guided by an understanding of the role of floral traits in governing the mating system. The recent advances in the mating system estimation (Ritland 2002) and the availability of hypervariable markers (Parker et al. 1998) in combination with multivariate regression analysis allow the plant breeder to understand the mating system with unprecedented resolution and to identify the specific characteristics of the flower that control its variation. Therefore, these recent advances in biometrical models and genetic markers provide the exciting opportunity of building populations where the level of heterozygosity is maintained thanks to the floral behaviour. This way of exploiting the advantage of heterozygosity should certainly be preferable to conventional hybrids from the sustainable standpoint. Besides, through exploiting the heterosis by manipulation of genes controlling floral traits responsible for the level of allogamy, breeders will not be confronted with the dangers of genetic uniformity. The first real challenge would be to obtain empirical estimates of the mating patterns and the links between floral display and design and outcrossing.

Using an approach that combines allozyme markers, multilocus likelihood-based estimation of outcrossing and multivariate regression analysis we have examined the quantitative variation in floral traits in *Vicia faba* in order to determine whether or not a relationship exists between variation in floral traits and outcrossing rate so as to assess the relative importance of each of the floral characteristics in their contribution to variation in the

outcrossing. Our study was centred on *Vicia faba* as a model plant, because of its genuine mixed mating, although the results would be relevant to other partially allogamous legumes such as lupin, vetch, clover or cowpea. The research was focused on an animal pollinated plant because wind-pollinated plants have a strongly bimodal distribution (Aide 1986) of outcrossing rate.

Materials and methods

Seven lines belonging to the four faba bean botanical groups were selected to develop two experimental synthetic populations, syn-4 and syn-5 as described in Maalouf et al. (1999). The two experimental syn populations have undergone 5 years of multiplication in open-pollination conditions with no artificial selection before having been used for this experiment.

Field experiments were carried out at Cordoba, southern Spain in two seasons. In season 2001/2002 plots of syn-4 and syn-5 were sown. Seeds for outcrossing estimates were randomly harvested from fifty families from each one of the two experimental synthetic populations over that season. 15 seeds from each family were genotyped for codominant loci and data used to estimate the outcrossing rate. Seeds from these collected maternal plants were also used to generate a breeding experiment that included 50 maternal half-sibs. In season 2002/2003, fourteen seeds of the same families (50 half-sib families) were grow in a completely randomised design with two replications to be evaluated for flower traits.

Outcrossing rates were estimated for the syn populations as a whole and for individual maternal plants using the multilocus estimation program MLTR (Ritland, 2002). A total of seven isozyme polymorphic loci were scored. Protocols for enzyme extractions, PAG electrophoresis and stain recipes were followed as described by Suso et al. (1993).

Floral traits were classified into three classes: (1) phenology, (2) design and (3) display. Floral design traits chosen for the analysis were nectar volume, sugar concentration and different single and composite measures of the standard and keel petals and ovary. To estimate the size and shape of flowers, scanner images were recorded and analyzed by a image tool program (UTHSCSA Image Tool; http// www.uthsca.edu/dig/itdesc.html). Floral display traits, number and arrangement of open flowers in a plant were recorded weekly during the flowering period, once the first flower opened.

Variation in floral traits was analyzed using both univariate and multivariate techniques. Forward stepwise multiple regression was used to investigate whether or not a relationship exists between outcrossing and floral traits, to describe the nature of this relationship and to determine which traits could account for the large proportion of the variation in outcrossing among the plants. Statistical descriptions are based on mean values per family. Floral trait averages were used as independent variable and outcrossing as a dependent variable. The dependent variable outcrossing rate was always subjected to arcsine square root transformation. When necessary, the other variables were transformed to conform to the assumptions of analysis. Multiple regression analysis was carried out using the program Statistica v. 6 (StatSoft Inc.).

Results and discussion

Plant breeders recognise the importance of high levels of crossing in recurrent selection schemes and in the development of synthetic varieties that express heterotic effects on yield and yield stability. Although breeders have been using male sterility and self incompatibility there is a need to identify new and more suitable systems, with minimal dependence of chemical hybridising agents and maximum use of biological power, to increase the amount of heterozygosity. Given the potential association among floral features and mating patterns in wind and animal pollinated plants, breeders should consider this association to increase the level of outcrossing. Some researchers have advocated for the use of pre-mate floral traits to

generate open varieties with a high level of heterozygosity in wind-pollinated plants (Virmani 1996, Ghani et al. 2003), but this seems to have received less attention for animal–pollinated crops. Besides, recent advances in molecular techniques and biometrical models have made it feasible to investigate directly the relationship between floral characters and outcrossing. Knowledge about the floral traits related to outcrossing and its underlying genes might open new ways for exploitation of heterosis in open populations and synthetics.

Table 1. Summary of regressions of outcrossing (dependent variable) (t_m± SE) against floral display and design and phenology (independent variables) in *Vicia faba* synthetic populations (only significant variables are shown)

Population t_m±SE	R^2	Independent variable	Standard regression coefficient	t statistic	P value
Syn-5	0.94	LT/ANE	-0.55	-5.73	0.000
0.48±0.04		LTB	-0.84	-6.43	0.000
		LT/LQ	-0.59	-5.06	0.000
		NECT	-0.26	-2.99	0.012
		NFA3	1.44	5.12	0.000
		NFA1	-1.51	-4.85	0.000
		NNFA1	1.90	5.00	0.000
Syn-4	0.80	NFA3	-0.58	-3.48	0.002
0.51±0.08		LT/ANE	0.77	2.38	0.028
		CICLO	0.88	2.69	0.015
		OVUL	0.43	2.75	0.013
		NNFA1	1.19	3.02	0.007
		NFA1	-1.39	-3.35	0.003
		AZU	-0.35	-2.27	0.035
		LTB	-0.31	-2.19	0.042
		LQ	0.67	2.74	0.013
		PERE	-0.84	-2.39	0.028

Floral display: open flowers (NFA1, NFA2, NFA3) and inflorescences (NNFA1, NNFA2) at first, second and third week of flowering; total open flowers NTFA and inflorescences NTNF. Phenology: duration of flowering (CICLO). Floral design: (A) Advertising: standard length (LTC), width (ANE) and perimeter (PERE), tube length (LTB); (B) Fit to pollinator: keel length (LQ); (C) Female sexual dimension: (OVUL); (D) Reward: nectar volume (NECT) and sugar concentration (AZU).

The present investigation was undertaken to generate information about key floral traits, which help to assess the possibility of selecting for pre-mate traits to increase the level of heterozygosity in faba bean. In our study, both syn populations showed about equal amounts of selfing and outcrossing (Table 1) and large amounts of phenotypic variation among plants for floral traits. Since many features of flowers affect the level of outcrossing it is necessary to address the problem of how well the presence of floral features can be used to increase the outcrossing. By studying multiple regression models (Table 1), it becomes evident that the pattern of variation in outcrossing is determined by floral features ($R^2 > 0.80$). Examination of the t statistics and the associated P values for the individual regression coefficients reveals that most of the variation in outcrossing was explained by variation in floral display size. Differences in floral design, though statistically significant, had a lower influence on the variation on outcrossing. Thus, additional traits such as flower size, shape, reward tube length,

were also important. Large tube length decreases the level of outcrossing in faba bean. Contrary to that usually accepted by breeders, low nectar reward and low sugar concentration in nectar increase the level of outcrossing. The role of the flower size and shape in outcrossing is less predictable and depends on the syn population, so its usefulness as a generalized indicator of allogamy is questioned. Our results contrast with the perception of phenology being important in determining the level of outcrossing. There was no evidence of any relationship between outcrossing level and first and last flowering dates. Only duration of flowering period significantly and positively affects the level of outcrossing in one of the populations. These results showed that: 1) floral display and design plays an important role in determining the level of outcrossing, 2) outcrossing in faba bean might be enhanced by modifying floral display size and design and 3) provide information to be used in developing synthetics or in selection recurrent schemes to increase allogamy without the use of male sterility or self-incompatibility or chemical hybridising agents.

Breeding programs of partially allogamous crops requiring effective cross-pollination should begin to incorporate pre-mate floral traits. In order to include pre-mate floral traits it is necessary first to gather detailed information concerning the role of floral traits in determining the mating system in different environments. Secondly, the genetic association of these traits with other agronomic characters needs to be measured. Depending on the genetic association, the manipulation of floral characteristics could either be facilitated or prevented. The ability of the mating system to respond to selection depends on the amount of additive genetic variation for those floral traits which determine the mating system. So, thirdly, genetic diversity for these traits must be sufficient and expectations of QTLs of major effect high enough to allow effective genetic manipulation.

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Potato breeding – exploiting the Commonwealth Potato Collection

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ABSTRACT: The use of the Commonwealth Potato Collection in potato breeding is set in the context of the evolution of the crop and the need to widen its genetic base by introgression and base broadening. Future use is discussed given increasing knowledge of biochemical pathways and genomics in potato.

Key words: Base broadening – biochemistry – cultivated potato gene pool – genomics – introgression

Domestication and evolution in the Andes

A few closely related and inter-fertile tuber-bearing *Solanum* species were domesticated in the Andes of southern Peru and northern Bolivia over 7000 years ago. The result was diploid *S. stenotomum*, also referred to as a form of *S. tuberosum* (Group Stenotomum), from which other cultivated species were derived, including diploid *S. phureja* (or Group Phureja), tetraploid *S. tuberosum* subsp. *andigena* (Group Andigena) and tetraploid *S. tuberosum* subsp. *tuberosum* (Group Tuberosum). Andigena potatoes became the most widely grown form in South America. Tuberosum potatoes were selected from Andigena types for tuber production in long days in Chile and are referred to as Chilean Tuberosum. Phureja potatoes were selected from Stenotomum for lack of tuber dormancy and faster tuber development so that up to three crops per year could be grown in the lower, warmer, eastern valleys of the Andes. Tetraploid by diploid crosses give tetraploid offspring as a result of a 'triploid block' and the diploid producing *2n* unreduced gametes. Hence Andigena, Tuberosum, Stenotomum and Phureja can be regarded as an inter-fertile cultivated gene pool.

Introduction to Europe and the rest of the world

Andigena potatoes were introduced into the Canary Isles around 1562 and from there to mainland Europe in the 1570s. As the growing of potatoes spread north eastwards across Europe, the potato became adapted to the long summer days of northern Europe and evolved sufficiently to be classified as subspecies *tuberosum*, albeit, with Andigena cytoplasm. Starting in the 17th century, potatoes were taken from Europe and cultivated in many other parts of the world. Today, potatoes are grown in 149 countries from latitudes 65 ºN to 50 ºS and at altitudes from sea level to 4000 m, and the potato is the fourth most important food crop after wheat, maize and rice.

Potato breeding from a narrow genetic base

Potato breeding in the modern sense began in 1807 in England when Knight made deliberate hybridizations between varieties by artificial pollination. It flourished in Britain and elsewhere during the second half of the 19th century when many new cultivars were selected and propagated by farmers and hobby breeders. A single Chilean Tuberosum cultivar, 'Rough Purple Chili', was introduced into the USA in 1851, and its descendents were widely employed as female parents in crosses with European Tuberosum at the end of the 19th century, following losses from the late blight epidemics in Europe and North America during the 1840s. Hence Chilean Tuberosum cytoplasm predominates in modern cultivars. The Tuberosum form of cultivated potato was thus founded on a narrow genetic base compared with that available in its centre of origin and in wild species. As a consequence, it lacked

genes for adequate levels of resistance to a number of pests and pathogens which became problems once it had assumed its role of a staple food. Furthermore, by the beginning of the 20th century, the narrow genetic base was starting to impede more general progress in potato breeding.

Germplasm collections

Recognition of Central and South America as the centres of origin and diversity of the tuber-bearing members of the genus *Solanum* resulted in numerous collecting expeditions, from those pioneered by the Russians in the 1920s to the more recent ones of the 1990s. These in turn led to the establishment of a number of potato germplasm collections worldwide, including the world collection at the International Potato Centre (CIP) in Lima, Peru, and the Commonwealth Potato Collection (CPC) which is now held at SCRI, Dundee, Scotland.

The CPC dates back to the expeditions to Mexico and South America which were commissioned in 1938 and 1939 by the Imperial Agricultural Bureaux of the United Kingdom government and which were led by E.K. Balls and J.G. Hawkes. Since the mid 1960s the entire collection has been maintained and stored in botanical seed form. During the 1990s it was expanded by the incorporation of the personal collection of wild and cultivated potatoes amassed by Professor J.G. Hawkes and held at Birmingham University. Today it comprises about 1300 accessions of which two thirds are wild and one third are cultivated species. The collection includes 77 of the 228 recognised tuber-bearing *Solanum* species. It has been comprehensively screened for the absence of true seed borne diseases and a substantial part of the collection meets the most stringent of European quarantine standards for plant health.

Use of the CPC to widen the genetic base of Tuberosum

Introgression

Disease and pest resistance have been introgressed from wild and cultivated species but, until recently, relatively few species had been used to any extent in the breeding of successful modern cultivars, and there had been mixed fortunes with the durability of resistance. The main sources of resistance to late blight, viruses PVX and PVY, and potato cyst nematodes used at SCRI were: (1) Late blight: major dominant *R* genes and quantitative resistance both from *S. demissum* (6x, EBN4, i.e. endosperm balance number 4 which is effective ploidy); (2) Potato cyst nematodes: major dominant *H1* gene for resistance to pathotypes *Ro1* and *Ro4* of *Globodera rostochiensis* and *H3* genes for quantitative resistance to *G. pallida*, both from *S. tuberosum* subsp. *andigena* (4x, EBN4), and quantitative resistance to both species from *S. vernei* (2x, EBN2); (3) Virus PVX: major dominant Rx_{adg} gene for extreme resistance from *S.tuberosum* subsp. *andigena* (4x EBN4) and a different major dominant gene Rx_{acl} for extreme resistance from *S. acaule* (4x EBN2); (4) Virus PVY: major dominant Ry_{sto} gene for extreme resistance from *S. stoloniferum* (4x EBN2) and major dominant *Ny* (*dms*, *chc*) genes for hypersensitive resistance from *S. demissum* (6x EBN4) and *S. chacoense* (2x EBN2) (gene also present in *S. microdontum,* 2x, EBN2).

It usually took five or six generations (four or five backcrosses) to transfer a major dominant resistance gene from a wild species into a successful cultivar, but only four generations (three backcrosses) from cultivated Andigena potatoes. For example, 'Pentland Dell' was released in 1960 and has three late blight *R* genes (*R1*, *R2* and *R3*) transferred from crosses made with *S. demissum* in 1932 (*S. demissum* x 'The Alness' (4x)) and in 1937 (*S. phureja* (2x) x *S. demissum*). In contrast, 'Pentland Javelin' was released in 1967 and has the *H1* gene for resistance to the golden potato cyst nematode (*G. rostochiensis*) from a cross made with Andigena in 1952. The transfer of quantitative resistance has taken longer and has proved more difficult to incorporate into successful cultivars. The major gene resistances to PVX and PVY have proved durable and the *H1* gene has remained effective against

G. rostochiensis in Britain because *Ro1* is still the main pathotype, but its widespread deployment has not prevented the spread of *G. pallida.* The *R* genes for resistance to late blight have been anything but durable, and recent breeding efforts have concentrated on quantitative resistance.

Base broadening

The resistance to *G. rostochiensis* and PVX mentioned previously derive from Andigena and are not found in Tuberosum varieties. These are likely to be two of many valuable traits found in Andigena germplasm yet which are not readily accessible to breeders. Tuber traits found in Andigena include diverse skin and flesh colours and patterns, and tuber shapes, tastes and textures. To make this diversity more readily available to breeders, beginning in 1959 a gene pool of Andigena with origins approximately 45 % Bolivian, 35 % south Peruvian, 10 % north Peruvian and 10 % Colombian was subjected to recurrent mass selection in outdoor plots. A range of traits distinguished this material from a panel of Tuberosum varieties, including greater blight resistance (probably maintained by some of the selection being performed under high blight pressure in Cornwall), diverse flesh colours, improved mean crisp colours, higher specific gravity and PVX and PVY resistance. The products of this exercise were subjected to pedigree selection for various traits in the 1970s, and they were then incorporated into commercial breeding schedules. The original mass-selected population was retained and a bulk seed harvest taken for long-term storage. This biodiverse population has now been recovered, tested for current quarantine diseases, and is entering research programmes using the latest molecular approaches.

A population deriving from Group Phureja with a small contribution from other diploid cultivated material has also been developed as a resource for breeding, genetics studies and other uses. The collection of clones contains a range of useful traits including high levels of tuber carotenoids, improved flavour, reduced cooking times and resistance to *Erwinia.* These clones are now widely used experimental material at SCRI, and are the subject of the first major innovation in commercial potatoes in the UK for many decades.

Future use of the CPC in the genomics age

In all crops, the development of molecular tools and the deeper understanding emerging of gene structure and function has driven the reawakening of an interest in exploiting germplasm collections. Potatoes are in a particularly advantageous position to benefit from these advances, as there is a particularly wide range of diversity in both cultivated and cross-compatible wild relatives. Using molecular markers it has been shown that not all of the groupings of species as described by traditional taxonomy are valid. For example, the species in series Megistacroloba fall into three groups interlaced between other diploid species. Furthermore, determination of the origin of the different genomes within allopolyploid groups is now possible, opening the prospect of more refined searches for novelty amongst the available germplasm.

Introgression

The technology has much to offer at the level of improving understanding of the genetics of traits in exotic germplasm, as well as providing tools for the more effective selection of desired individuals in segregating populations. New sources of late blight and PCN resistance, to both *G. rostochiensis* and *G. pallida*, are under active research using molecular markers to understand and assist the selection of the genes involved. With simply-inherited traits and by using molecular markers to select the most appropriate segregants for further rounds of crossing, we anticipate reducing the introgression from closely related wild species to commercial material to three backcross generations and six years.

Biochemistry and genomics

The last few decades have seen great improvements in our understanding of biochemical pathways in plants and in the accessibility and throughput of biochemical analytical techniques. End-user needs in potato production are influenced increasingly by tuber biochemistry, and new market opportunties exist for potatoes with enhanced nutritional quality. Examples of these biochemical traits include carbohydrate metabolism, affecting cooking and crisping quality, glycoalkaloid levels, and tuber flesh anthocyanin, carotenoid and ascorbate levels and stability. Linking together genomics and biochemistry offers great benefits for potato improvement. One example of this is our current effort to identify the genes and alleles responsible for elevated levels of carotenoids. Here, we can exploit natural variants for the trait by looking for an association in sets of germplasm with DNA sequence variants in candidate genes for biosynthesis and turn-over. By this means we can compare alternative candidate positions in pathways and directly determine the gene involved. We expect that this approach will become a useful method for directly determining crucial steps in pathways using existing sets of germplasm and without the need to create traditional segregating populations. The challenge will then be to get desirable alleles into new cultivars and hence farmers fields as quickly as possible.

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The least square method application for parent choice in potato breeding

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ABSTRACT: The aim of potato breeding was to obtain progenies for three kinds of processing: starch, crisps and chips. The choice of parents was evaluated with least square method and progeny assessment was done by using the same traits for each kind of processing. The evaluation of progenies proved that genotypes were found most acceptable for each kind of processing, when parents were identified to be the most acceptable couple by least square method.

Key words: Breeding – parent choice – *Solanum tuberosum*

Introduction

Parent varieties for hybridisation could be chosen differently. Usually parents with complementing traits have been chosen according to the breeding goal. The assessment of phenotype gives information of trait expression. One of the methods for parent choice based on polygenic traits phenotypic expression is least square method (Smiryayev et al. 1992). This method has been used in cereal breeding. The application of the method in potato breeding is described. Four couples of parent varieties chosen for obtaining progenies suitable for potato processing into starch, crisps and chips were evaluated with this method. The obtained progenies suitability to three kinds of processing was assessed. The results of progenies assessment and results of parent's evaluation with least square method were compared.

Materials and methods

Four couples of parent varieties and obtained progenies were investigated (Table 1). The parent choice and progenies suitability for potato processing into starch, crisps and chips were assessed. Suitability to processing into starch was evaluated with two traits, suitability to processing into crisps - nine traits, and suitability to processing into chips - eleven traits (Table 2).

Table 1. Description of hybrid populations

Parent varieties / Origin[1]	Year of assessment	Number of progenies
Hertha (NL) / Ausonia (NL)	1998	44
Hertha (NL) / Impala (NL)	1998	55
Zarevo (UKR) / Ausonia (NL)	1998	39
Verba (BY) / Certo (PL)	1997	23

[1] BY, Belarus; NL, The Netherlands; PL, Poland; UKR, Ukraine

The purpose of parent choice with least square method is to obtain hybrid population, which average values of genotype traits are between the traits optimal value and acceptable deviation. Only additive function of genes for polygenic traits has been exploited. The parent genotypes and their interactions are evaluated by sum of each traits parent's average value and optimal value deviation ratio with acceptable deviation. The average traits value in the hybrid population is calculated as average of parents' traits value, considering parents genome density in hybrid population (1):

$$F_j = \sum_{i=1}^{m} p_i x_{ij}$$

where F_j – average traits value in hybrid population,
m – number of parents,
p_i – parents genome density in hybrid population,
x_{ij} – parent's traits value.

The sum of square ratio for each potential parents combination is calculated:

$$Q = \sum_{j=1}^{N} \frac{(I_j - F_j)^2}{D_j^2}$$

where Q - sum of square ratio,
N – number of traits,
I_j - trait's optimal value,
F_j – average traits value in hybrid population,
D_j – acceptable deviation from optimal trait's value.

The most acceptable parent combination from compared all expected combinations is one with the least sum of square.

Table 2. The optimal trait's value and acceptable deviation

Trait[1]	Usage	Optimal value	Acceptable deviation
STARCH (%)	starch	20.0	3.0
SMALLGRAIN (%)	starch	25	25
TSI	crisps	1.2	0.2
STARCH (%)	crisps	20.0	4.0
COL (pts.)	crisps	9	2
CRISP (pts.)	crisps	9	2
TSI	chips	1.7	0.3
STARCH (%)	chips	17.0	3.0
DARK (pts.)	chips	9	1
COL (pts.)	chips	9	2
COLCOOK (pts.)	chips	9	1
TEXT (pts.)	chips	9	2
EYE (nos.)	crisps, chips	6.3	2.8
EYEDEP (mm)	crisps, chips	0	1
GLUC (mmol l^{-1})	crisps, chips	5.0	5.0
RES (%)	crisps, chips	80	30
DARKPEEL (pts.)	crisps, chips	9	1

[1] STARCH, starch content in tuber; SMALLGRAIN, small grain percentage in starch; TSI, tuber shape index; COL, colour; CRISP, crisp crispiness; DARK, tuber flesh darkening after boiling; COLCOOK, chip colour after pre-cooking; TEXT, chip inside texture; EYE, number of tuber eyes; EYEDEP, tuber eye depth; GLUC, glucose content in tuber juice; RES, tuber resistance against mechanical damage; DARKPEEL, tuber flesh darkening after peeling.

Results and discussion

Comparison of the sum of square ratio (Q) calculated for acceptable traits for processing into starch, crisps and chips, shows the most acceptable parents for each kind (Table 3). The parent couple 'Zarevo' / 'Ausonia' was stated as the most acceptable for obtaining progenies suitable for starch production. The Q_s for this couple was the smallest. The choice of varieties 'Hertha' / 'Impala' was successful for obtaining progenies acceptable for processing into crisps and chips.

Table 3. Sum of square ratio for parent couples

Parent varieties	Q_s – Starch processing	Q_{cr} – crisps processing	Q_{ch}– chips processing
Hertha / Ausonia	10.5	18.1	20.8
Hertha / Impala	11.0	12.8	11.3
Zarevo / Ausonia	2.9	17.5	28.6
Verba / Certo	12.5	24.8	31.5

The majority of the progenies (96 %) with acceptable starch content in tubers and small grain percentage in starch for processing into starch were found in hybrid population 'Verba' / 'Certo'. 75 % of progenies with both acceptable traits for starch processing were established in hybrid population 'Zarevo' / 'Ausonia'. The assessment of progenies proved that both choices of parents were successful for obtaining progenies acceptable for processing into starch. There were only 7 % of 'Hertha' / 'Ausonia' progenies and 2 % of 'Hertha' / 'Impala' progenies with both acceptable traits (Table 4).

Table 4. Evaluation of progenies suitability to starch processing

Parent varieties	Progenies with acceptable traits for starch production (%)		
	with 2 traits	with1 trait	without
Verba / Certo	96	4	0
Zarevo / Ausonia	75	15	10
Hertha / Ausonia	7	47	46
Hertha / Impala	2	42	56

The progenies' suitability for crisp production was determined by testing nine traits. Acceptance of all traits was found for one progeny in hybrid population 'Hertha' / 'Impala'. The choice of those parents was successful: eight acceptable traits were found to 4 % of progenies, but seven acceptable traits to 20 % of progenies. The hybrid population 'Hertha' / 'Ausonia' was not adequate for obtaining suitable progenies for processing into crisps. Only 5 % of clones were found with seven traits acceptable (Table 5).

Eleven tested traits for suitability for processing into chips (Table 6) were proper to one progeny in hybrid population 'Hertha' / 'Impala'. More than one third of the progenies had ten and nine traits suitable for processing. Hybrid population 'Hertha' / 'Impala' was successful for obtaining progenies suitable for processing into crisps and chips. Nine acceptable traits were established for 11 % clones in hybrid population 'Hertha' / 'Ausonia' and for 15 % in 'Zarevo' / 'Ausonia'. The comparison of results of testing progenies in hybrid populations with the assessment of the choice of parents using the least square method proves the suitability of varieties 'Zarevo' / 'Ausonia' for obtaining progenies for starch production. The choice of 'Verba' / 'Certo' was successful, too, which was proved by progeny assessment.

Table 5. Evaluation of progenies suitability to processing into crisp

Parent varieties	Progenies with acceptable traits for crisp production (%)			
	with 9 traits	with 8 traits	with 7 traits	2-6 traits
Hertha / Impala	2	4	20	74
Zarevo / Ausonia	0	36	43	21
Verba / Certo	0	17	13	70
Hertha / Ausonia	0	0	5	95

Table 6. Evaluation of progenies suitability to processing into chips

Parent varieties	Progenies with acceptable traits for chips production (%)			
	with 11 traits	9-10 traits	7-8 traits	2-6 traits
Hertha / Impala	2	35	38	25
Zarevo / Ausonia	0	15	64	21
Hertha / Ausonia	0	11	50	39
Verba / Certo	0	0	11	89

The investigation of progenies proved the prognosis with the least square method, that hybrid population 'Hertha' / 'Impala' would be suitable for obtaining clones for processing into crisps and chips. The least square method is acceptable for application in potato breeding for parent choice.

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Forage quality of 'Perenne', a new perennial rye variety (*Secale cereale* x *Secale montanum*)

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ABSTRACT: 'Perenne' is a perennial rye variety of interspecific origin (*Secale cereale* x *S. montanum*) registered in Hungary. The green yield, dry matter, crude protein and fiber content of 'Perenne' were determined. Green mass at the early stem extension was 7.52 t ha^{-1} and at the late one 19.66 t ha^{-1}. Maximum green weight was measured at heading (23.91 t ha^{-1}) and started to decrease later. Dry matter content was found to be 1.35 t ha^{-1} at the early stem extension, whilst 3.91 t ha^{-1} at the late stem extension phase. Dry weather in spring (2003) explains slow rate of increase in dry matter content and the substantial decrease in green weight following heading. Fiber content was found to be 22 % in the tillering and the early stem extension stage, which was followed by a steady increase until heading (34.3 %) and a gradual decline following ripening stage. Crude protein changed more significantly than fiber content. Early stem extension had the highest value (30.7 %). A rapid decline was observed during late developmental stages. For grazing purposes the early stem extension stage seems to be the optimum ones, when the plant height is approximately 30 cm with the highest crude protein (30.7 %) and low fiber (22.2 %) content. For cropping purposes the late stem extension phase was shown to be the optimum one because this provides high green mass along with relatively high crude protein (23.2 %) content.

Key words: Forage quality – perennial rye – *Secale cereale* – *Secale montanum*

Introduction

The cultivated rye (*Secale cereale* L.) has been used as a grain crop and also for green forage mainly in the northern regions of Europe. In Hungary it is commonly grown under poor soil conditions which are insufficient for other cereals. The perennial mountain rye (*S. montanum* Guss.) is a native wild species in southern Europe, Morocco, Iran and Iraq (De Bustos & Jouve 2002). The value of *S. montanum* as a pasture crop has been tested successfully in the United States (Robert et al. 1988), Australia and New Zealand (Oram 1996). Remarkable efforts were made to cross these species although the hybrids had reduced fertility (Stutz 1957, Reiman & Gordon 1984) or weak perenniality (Cox et al. 2002).

In 1998, a perennial rye variety ('Perenne') of interspecific hybrid origin (*S. cereale* x *S. montanum*) has been registered in Hungary. 'Perenne' has acceptable fertility and good perenniality (Kotvics et al. 2001). Considering the strong tillering and plant height of 140 - 150 cm, the cultivar's advantages as a forage crop are obvious. The objectives were to analyse the quantity and quality of the green mass in 1st, 2nd, and 3rd years and various phenophases as well as to determine the optimum cutting period. In this paper yield, protein and fiber content in different phenophases are presented.

Materials and methods

The field experiments were carried out at the Experimental Station of the St. István University in Gödöllő (Hungary) on a brown forest soil (sandy loam physical type). 'Perenne' was sown in mid-September 2001 at a sowing rate of 2.5 billion seeds ha^{-1} with 24 cm row space and fertilized with 30 N, 30 P, and 30 K (kg ha^{-1}) in 2001 and 2002. Samples were collected in 2002 and 2003. The amount of precipitation was 515 mm and 385 mm in 2002 and 2003, respectively. Green mass and dry matter, crude protein and fiber content were determined in 7

phenophases (Table 1). Crude protein content was measured by the standard Kjeldahl method and the fiber content was analysed by the standard Henneberg-Stohmann method (Church & Pond 1988).

Table 1. Plant height and sampling date of 'Perenne' in different phenophases

	Phenophase						
	A[2]	B	C	D	E	F	G
DATE[1]	10/10/02	25/04/03	03/05/03	13/05/03	20/05/03	30/05/03	02/07/03
PH	10	30	60	90	110	155	155

[1] DATE, sampling date (DDMMYY); PH, plant height (cm)
[2] A, tillering; B, early stem extension; C, late stem extension; D, early heading; E, late heading; F, flowering; G, ripening

Result and discussion

Green mass varied between 1.58 t ha^{-1} at tillering and 23.91 t ha^{-1} at ripening. It increased significantly till heading, and it started to decrease later (Figure 1/I). Dry matter content between tillering and ripening increased continually, 1.35 t ha^{-1} measured at tillering and 7.45 t ha^{-1} at ripening. Following heading the rate of increase became moderate (Fig. 1/II). Dry weather in the spring of 2003 accounts for the moderate increase in dry matter content and a marked decline in green weight after heading. The amount of precipitation was 40 mm during the months of March, April and May. Fiber content was 22 % in the tillering and the early stem extension, increased until heading (34.3 %) and began to decrease at the ripening stage (Fig. 1/III). Crude protein content changed more substantially than fiber content. Early stem extension had the highest crude protein value (30.7 %), although it showed a rapid decrease during late developmental stages.

For grazing the highest crude protein (30.7 %) and low fiber (22.2 %) content, the early stem extension phase seems to be the optimum one, when plant height is approximately 30 cm and green mass is 7.52 t ha^{-1}. Grazing is feasible until heading. For cropping purposes the late stem extension phase appears to be the best because this ensures a rather high green yield (19.66 t ha^{-1}) with relatively high crude protein (23.2 %) and low fibre (26.2 %) content. The optimum cutting season for most forage crop is at heading. Cutting perennial rye at heading is not recommended due to the low crude protein and high fiber content. Dry weather in the experimental year did not allow for second cropping.

Accurate characterization of the forage quality requires the analysis of a wider range of traits. Crude protein and fiber content together with ash, fat and polysaccharide content provide enough information for farmers to the optimal forage portion.

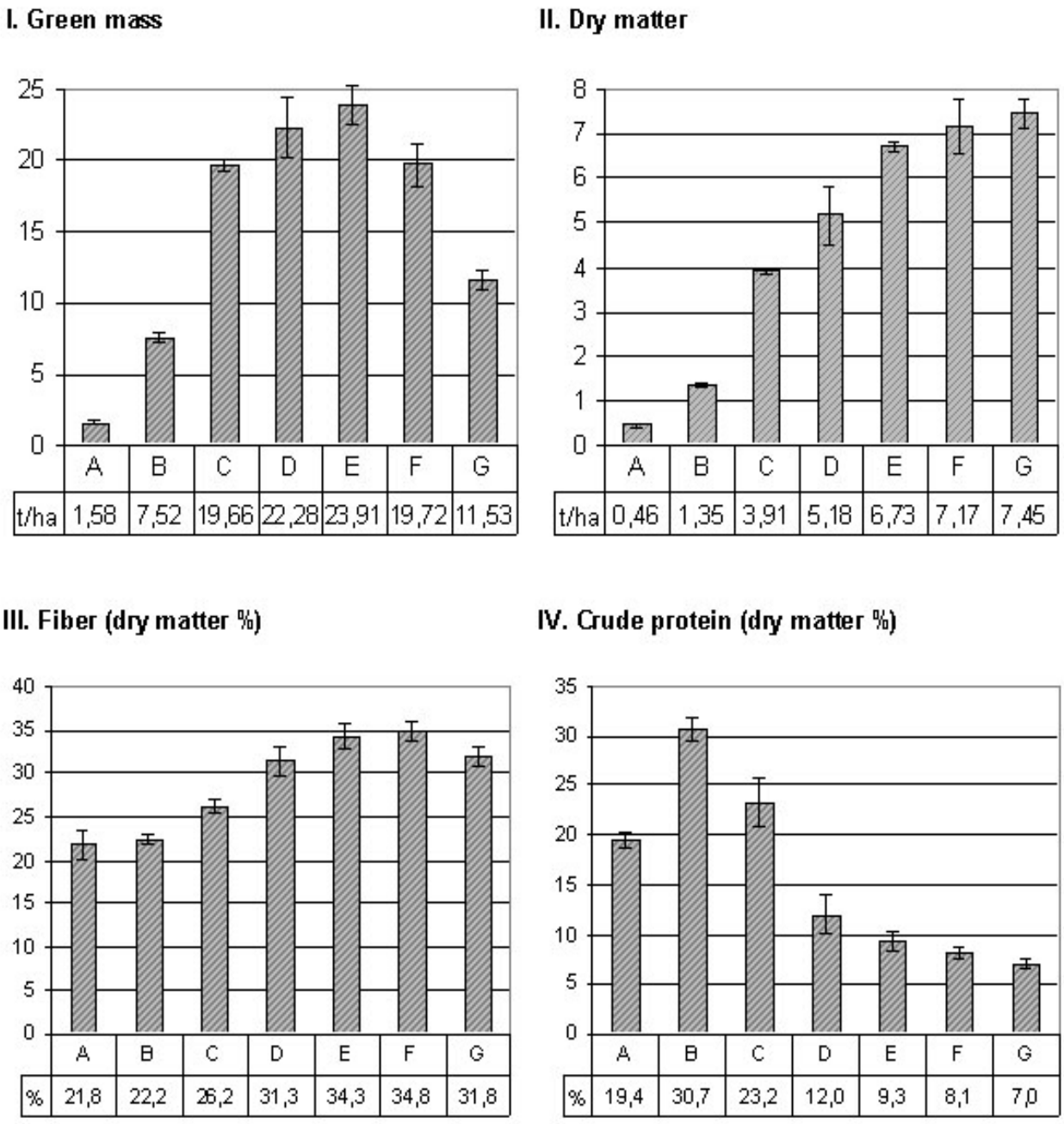

Figure 1. Effect of developmental stages on green mass (I), dry matter (II), fiber (III) and crude protein content (IV) of 'Perenne' (abbreviations for phenophases: see Table 1)

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Apple breeding: Exploitation of genetic variation via recurrent selection

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ABSTRACT: A recurrent selection programme using a broad-based exotic apple germplasm, was set up at the Horticulture and Food Research Institute of New Zealand limited (HortResearch), with the aim to create and maintain genetic diversity for sustainable genetic improvement in apple. We have estimated genetic parameters with a subset of HortResearch's apple germplasm in the gene bank, and breeding populations in the recurrent selection programme, to assist us in making informed decisions on management and breeding strategies in the recurrent selection programme. Gene diversity indices including F variance ratio test, F-statistic (F_{st}) and N_m (gene flow measure), showed significant genetic variation within the germplasm, most of which was found within sub-populations, indicating a low level of genetic differentiation between sublines in the recurrent selection programme. The estimates of narrow-sense heritability for both tree and fruit traits were generally high, while between-trait genetic correlations were mostly high for tree traits (up to 0.95). The implications of these results are discussed in the context of recurrent selection in apple.

Key words: Apple breeding – genetic diversity – genetic parameters – recurrent selection – sublines

Introduction

Breeders of long-lived perennial fruit and nut crops do not have the luxury of short generation cycles and/or intervals due to prolonged juvenile periods. To facilitate quicker genetic gains, apple breeders tend to use parents which by experience, are known to produce high quality progenies. This usually involves inter-crossing the most recent cultivars or elite selections from their own or other breeding programmes. The practice however, has led to increasing levels of co-ancestry and relatedness in modern apple cultivars (Noiton and Alspach 1996), as only five cultivars form the bulk of parents used for new variety development. To increase genetic diversity in order to facilitate sustainable genetic improvement, a recurrent selection programme using a broad-based apple germplasm, was set up at the Horticulture and Food Research Institute of New Zealand limited (HortResearch). The objectives were to, 1) increase genetic variability in breeding populations, 2) enhance the mean performance of populations for future commercial development and, 3) study the genetics of horticulturally important tree and fruit characters to improve selection efficiency.

In this report, we present data on gene diversity indices (from the entire HortResearch's germplasm collection), and estimates of narrow sense heritabilities and genetic correlations from the breeding populations. The future direction of the recurrent selection programme is discussed in the light of these results.

Materials and method

Establishment of breeding populations

The plant materials used to establish the breeding populations in the recurrent selection programme were imported mostly in form of open-pollinated (OP) seed, from different parts of the world including USA, Canada, UK, Europe, Australia, and South Africa. Seed of wild species of *M. sieversii and M. kirghisorium*, were also collected in Kazakhstan (the presumed centre of origin of *M. sieversii*). This germplasm consists of old cultivars, cider apples and

Malus species, some of which are putative sources of pest and disease resistance. In total, there were about 520 maternal sib-families, and these were divided over four sublines; 91, 92, 93 and 94 (number denoting the year seed were sown). The families were replicated on 3 sites namely, Havelock North (39° 40'S 176° 53'E), Riwaka (41° 04'S173° 00'E) and Clyde (45° 12'S 169° 18'E), and planted in the orchards in randomised incomplete blocks of 20 trees each at a distance of 3 x 0.75 m. There were 213 (ca 10,320 trees), 149 (ca 12,800 trees), 72 (ca 4860 trees) and 86 (3700 trees) families in sublines 91, 92, 93 and 94, respectively.

Assessment of genetic diversity within and between sub-populations

To assess the level of diversity within the entire HortResearch's apple germplasm collection to assist us in making informed decisions on management procedures, as well as on the choice of breeding strategies, we took a random sample of old apple cultivars and commercial cultivars in our apple genebank, as well as from the breeding populations that form part of the recurrent selection programme. Altogether, 155 genotypes were sampled and these were subdivided into 4 populations. Subpopulation 1 comprised 50 old cultivars/commercial cultivars maintained at the genebank, and subpopulations 2, 3, and 4 consisted of sublines 91 (30 genotypes), 92 (30 genotypes), 93 and 94 combined (45 genotypes), respectively. These genotypes were screened with 9 RAPD markers and a total of 43 bands resulting from these markers were subjected to AMOVAR (analysis of molecular variance), to obtain gene diversity indices for all individuals within and between subpopulations (see Oraguzie et al. 2001a).

Selection of parents for the next cycle of recurrent selection

Following tree and fruit evaluations, the following model was fitted for every quantitative variate to assist in the computation of family means,: $y_{ijk} = \mu + S_i / B_j + F_k + \varepsilon_{ijk}$, where y_{ijk} was the observation for a tree of the k^{th} family from the j^{th} incomplete block at the i^{th} site. Generally, sites and blocks were treated as random effects while family was set as a fixed effect. For each trait, the difference between family mean and the general mean was standardised by dividing by the standard error (to account for the differing number of trees in each family). Generally, families with a mean more than 3 standard errors from the general mean were identified. The standardised residuals of each individual were computed and used to select extreme individuals from each chosen family as parents for the next cycle (Oraguzie et al. 2001b). The frequency distribution of the base population versus selected individuals was plotted to give an indication of how genetic diversity has been maintained following selection.

Computation of narrow sense-heritabilities and genetic correlations in subline 91

Additive genetic and residual variance components were estimated using the restricted maximum likelihood method (REML) in ASREML software (Gilmour et al. 1998). Generally, the following mixed effects model was fitted for the estimation of variance components:

$$y_{ijk} = \mu + S_i + S_i/B_j + F_k + S_i*F_k + \varepsilon_{ijk}$$

where y_{ijk} was the value for the tree from the k^{th} family F planted in the j^{th} incomplete block B within the i^{th} site S. The sites covered the main apple growing areas of New Zealand and were modeled as fixed effects, while blocks and families were designated as random effects. The residuals (ε_{ijk}) were checked against the fitted values for lack of homogeneity, and plotted to check for deviations from the Normal. Transformations were undertaken where necessary.

Individual narrow-sense heritability was estimated from univariate data for each site and a univariate estimate from across sites with the following formula:

$$\hat{h}^2 = \frac{\hat{\sigma}_{family}^{\ 2}}{C\left(\hat{\sigma}_{family}^{\ 2} + \hat{\sigma}_{e}^{\ 2}\right)}$$

where σ_{family}^2 is the family variance, σ_e^2 is the error variance and C is the coefficient of relationship. $C = 0.25 + 0.25/n$ for n pollen donors (see Alspach and Oraguzie, 2002) which is 0.5 when n=1 (full-sibs) and tends to 0.25 as n tends to infinity. We assumed an average of 3 pollen donors per OP family and hence, set C at 0.33.

Genetic correlation between traits was estimated from bivariate analysis of traits taken pair-wise at one site. These were calculated with the following formula:

$$r = \frac{\sigma_{a,b}}{\sqrt{\sigma_a^{\ 2}\sigma_b^{\ 2}}}$$

where r was the correlation between traits a and b, $\sigma_{a,b}$ was the covariance between a and b, and σ^2_a and σ^2_b were the variances of a and b respectively. Standard error was calculated with Pearson's approximation of the variance of a ratio (Gilmour et al. 1998).

Results and discussion

Analysis of molecular variance showed that about 95% of the variation in the germplasm collections was found within subpopulations (Table 1). Although, the F test shows weak evidence for variation among sub populations (a P value of 0.0381), the between subpopulation variance component shows that this difference is not major. Furthermore, the N_m value (an estimate of gene flow) of 3.54, obtained using the formula: $0.25(1-F_{st})/F_{st}$ (our calculated F_{st} was 0.066, see Table 1), is rather too high to suggest otherwise. In fact, it is an indication of a high level of gene exchange and interaction among the sub-populations consistent with the life history and sexual biology of apples (since apples are natural out-crossers and have no barriers to cross-fertilization). These results have two implications in the recurrent selection programme. First, since the breeding populations were divided into 4 sublines with large families (ranging from 72 to 213 families) to control inbreeding (Oraguzie et al. 2001a), it will be possible to minimise inbreeding using appropriate mating designs in subsequent generations to allow for a reduction in the number of families within a subline. Secondly, any subsequent rise in inbreeding levels can be managed by introducing new germplasm or by crossing between sublines to produce commercial cultivars. The high levels of genetic variation within sublines and the low level of genetic variation between sublines suggests that reducing the number of sublines would have only a small impact on genetic diversity.

Table 1. Analysis of molecular variance (AMOVAR) based on 43 RAPD bands for 155 op seedling selections/cultivars of apples grouped into 4 sub-populations. The estimated within subpopulation variation (%) is 0.244 for subpopulation 1, 0.245 for subpopulation 2, 0.242 for subpopulation 3, and 0.242 for subpopulation 4

	DF	SS	MS	%Var	F	1-Pr(F)	Vcomp	F_{st}
Between sub-populations	3	2.1	0.699	0.054	2.88	0.0381	0.012	0.066
Within sub-population	151	36.7	0.243	0.946	NA	NA	0.243	NA

From Oraguzie et al. (2001a)

More than 80 % of families were selected in all but subline 91 (Table 2) for advancement to the next generation. The lower proportion of progeny selected in subline 91 is a reflection of both the lower proportion of fruiting trees and the smaller number of fruit traits evaluated compared to other sublines. Subline 91 was the first population established and was particularly useful for the optimisation of our phenotyping and data management techniques.

Plots of the frequency distributions of the base population versus the selected families (figures not shown) showed that diversity has been maintained following selection and that the populations were slightly improved.

Table 2. Number of families selected and family-based selection intensity per subline in the first generation

Subline	Total no. of families evaluated	No. of families selected	Selection intensity (%) (family basis)
91	213	148	68
92	149	125	84
93	72	63	88
94	86	74	86

From Oraguzie et al. (in press)

Univariate analyses within sites and across sites were used to estimate variance components for calculation of narrow-sense heritability for subline 91 (Table 3). Flowering and harvest dates, fruit size, fruit aspect ratio and leafing out date all had very high heritabilities (>0.7), moderate heritabilities ($0.2 < h^2 < 0.5$) were estimated for fruit sugars, fruit conicity and fruit squareness and low heritabilities ($h^2 < 0.2$), were observed between tree growth habit and fruit acidity. Analyses at each site confound the additive variance estimate with the genotype-by-environment variance, so further analysis with pooled data from both sites was needed to produce unbiased results. The pooled-sites heritability was similar to the average heritability for each site, suggesting that GxE was minimal. High between-site genetic correlations previously reported for majority of these traits (Oraguzie et al. 2003), support the evidence for low GxE. The low GxE suggests that the ranking of individuals will be unchanged at each site and that breeding and initial selection is necessary only at one site. Although, ranking may not change, the performance of a genotype in each environment may be different and therefore testing selections at different sites is still necessary to ensure the right genotype-environment combination is selected. An example might be fruit maturing later at one site changing the optimum selections to meet a market window.

The generally high trait heritability estimates suggest that selection based on phenotype for the traits concerned would be efficient. Stricter measurement protocols for acidity and fruit sugars may increase their heritability, otherwise progeny testing would be necessary for gain in these traits. Further estimates with all three sites, other sublines and after at least one generation of random mating would strengthen confidence in these conclusions.

Table 3. Univariate narrow-sense heritability (standard error in brackets) for apple traits estimated separately for Havelock North and Nelson, and across both sites

Trait	Havelock North	Nelson	Combined
Vigour	0.62(0.06)	0.28 (0.05)	0.42 (0.05)
Tree growth habit	0.41 (0.05)	0.20 (0.04)	0.19 (0.03)
Leafing out date	0.72 (0.07)	0.83 (0.08)	0.60 (0.06)
Powdery mildew incidence[1]	0.40 (0.05)	—	—
Flowering date[1]	0.92 (0.09)	—	—
Harvest date	0.82 (0.12)	0.86 (0.11)	0.66 (0.09)
Fruit size	0.90 (0.12)	0.91 (0.12)	1.01 (0.10)
Fruit acidity	0.17 (0.08)	0.22 (0.08)	0.19 (0.06)
Fruit sugars	0.26 (0.08)	0.25 (0.08)	0.26 (0.06)
Fruit firmness	0.44 (0.12)	0.53 (0.10)	0.47 (0.09)
Fruit aspect ratio	0.82 (0.15)	0.89 (0.16)	0.74 (0.12)
Fruit conicity	0.46 (0.13)	0.36 (0.12)	0.32 (0.09)
Fruit squareness	0.43 (0.12)	0.35 (0.12)	0.32 (0.09)

[1] Assessed only at the Havelock North site

Phenotypic and genetic correlations between traits were estimated from bivariate analysis of traits across both sites (Table 4). High positive genetic correlations ($r_A > 0.65$) were observed between leafing out and flowering dates ($r_A = 0.95$), harvest date with fruit firmness ($r_A = 0.92$), and leafing out date with harvest date ($r_A = 0.69$). The largest negative correlations were found between vigour and fruit squareness ($r_A = -0.82$), and tree growth habit with leafing out date ($r_A = -0.53$). Large positive genetic correlations indicate that selection for one trait will also have a correlated response to selection in the other traits, which enables either indirect selection or rapid gain in multi-trait selection. Subsets of individuals within the breeding population with high between-trait genetic inter-correlations could be formed to make rapid gains in certain directions. For example, rapid gains in this population could be made by selecting for late maturing, firm, and low acid varieties. Large negative genetic correlations such as those found between vigour and fruit squareness (-0.82) and tree growth habit with leafing out date (-0.53) indicate that gain from these multi-trait combinations could be slow.

There was little genetic correlation between vigour and the traits: tree growth habit, leafing out date, flowering date, mildew resistance, harvest date, fruit acidity, fruit sugars, fruit firmness, fruit aspect ratio and fruit conicity. Although, vigorous trees tended to produce fruit that was rounder, larger and firmer. Powdery mildew incidence was not genetically correlated to any trait. Fruit size also was not genetically correlated to any trait apart from a small positive correlation with vigour ($r_A = 0.34$) and a small negative correlation with firmness ($r_A = -0.31$). The lack of genetic correlation indicates independence between the traits and provides opportunity to select in both directions with similar gain (eg: for large and small apples).

These genetic correlations should be interpreted with caution as there may be a bias due to linkage disequilibrium. Linkage disequilibrium is the loss of certain gene combinations through selection so that genetically-independent traits may appear to be correlated. Each generation of random mating halves the linkage disequilibrium between independent traits, quickly returning the population to linkage equilibrium (Falconer 1989). The apple breeding population in this study was founded on OP families (random mating) so the effect of linkage disequilibrium may be small, but these parameters need to be re-estimated after further generations of random mating.

Table 4. Bivariate genetic correlations (max S.E. = 0.20) in lower triangle and phenotypic correlations above the diagonal between apple traits on individual trees with combined sites data

Trait	1	2	3	4	5	6	7	8	9	10	11	12	13
1. Vigour	1	0.00	-0.13	-0.02	-0.06	0.04	0.17	0.01	-0.06	0.08	-0.04	0.04	-0.16
2. Tree growth habit	-0.11	1	-0.08	0.00	-0.16	0.00	-0.03	-0.06	-0.07	-0.02	0.02	-0.01	-0.03
3. Leafing	0.04	-0.53	1	-0.03	0.64	0.26	0.12	0.05	0.01	0.07	0.00	0.02	0.03
4. Powdery Mildew[1]	-0.07	0.06	0.04	1	-0.01	-0.01	-0.06	0.03	0.05	0.03	0.00	0.00	0.00
5. Flowering date[1]	0.18	-0.51	0.95	-0.03	1	0.17	0.16	0.06	-0.03	0.02	0.00	0.02	0.01
6. Harvest date	0.16	-0.01	0.69	-0.01	0.41	1	0.12	0.11	0.18	0.16	0.00	0.00	0.00
7. Fruit size	0.34	-0.11	0.29	-0.13	0.29	0.10	1	0.02	0.00	-0.31	0.00	0.12	0.08
8. Fruit acidity	0.16	-0.23	0.28	-0.07	0.22	0.24	-0.12	1	0.22	0.01	0.02	0.09	0.02
9. Fruit sugars	-0.05	-0.19	0.06	-0.27	0.25	0.06	0.00	0.29	1	0.08	0.07	0.00	-0.02
10. Fruit firmness	0.31	-0.16	0.41	0.04	0.17	0.92	-0.31	0.61	0.37	1	0.03	-0.04	0.00
11. Fruit aspect ratio	-0.03	0.36	0.00	-0.01	0.00	0.00	0.00	0.06	0.09	0.18	1	0.02	0.05
12. Fruit conicity	-0.06	-0.01	0.16	0.00	0.20	0.00	0.09	0.15	0.32	-0.07	0.00	1	-0.05
13. Fruit squareness	-0.82	-0.20	-0.28	0.00	-0.04	-0.28	-0.09	-0.12	0.16	-0.01	0.19	-0.20	1

[1] Powdery mildew incidence and flowering date were assessed only at the Havelock North site

The estimated parameters to date suggest that the breeding programme could be re-structured for more efficient use of resources. The future programme could have the breeding population at only one site, with just the commercial selections propagated in other sites for site-specific testing. The breeding population could have fewer sublines and fewer families within sublines and still manage inbreeding and maintain genetic diversity. Selection for traits needs to be built on an understanding of heritabilities and genetic correlations and these parameters need to be re-estimated to continue to refine selection strategies.

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Broadening the genetic base for better-adapted varieties for organic farming systems: participatory characterisation, evaluation and selection of onion accessions for new base populations

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ABSTRACT: As organic farming refrains from high and chemical inputs it needs varieties better adapted to organic conditions to improve the yield stability and quality of crops. In order to make gene bank accessions more accessible for the utilisation in organic breeding programmes, a participatory research project with farmers was carried out in 2002 to 2003. From the Dutch genebank collection 37 onion accessions, divided into five different groups (according to their market use), were selected and planted at a commercial organic farm. Farmer participation in characterisation and evaluation of the material resulted in including additional plant traits for genebank characterisation as well as new selection criteria for breeding. It also provided researchers insight into how farmers evaluate and value certain plant traits. Variation for important properties was found within and between the five groups. To establish base populations, the farmers, in collaboration with the researchers, selected the best genotypes within the five groups of onion accessions. The new base populations may be exploited in order to achieve better-adapted material for organic farming systems.

Key words: *Allium cepa* – characterisation – gene bank – organic plant breeding – participatory selection

Introduction

Organic farmers depend on varieties bred for conventional high-input farming systems. Although organic farmers in north-western Europe benefit from the improvements made by modern breeding activities, the fact that most of them use modern varieties does not imply that these are the best varieties for optimising their cropping system. As organic farming refrains from high and chemical inputs it needs varieties better adapted to organic conditions to improve the yield stability and quality (Lammerts van Bueren et al. 2002). For this type of varieties additional characteristics are required such as nutrient efficiency, intensive root architecture and weed-suppression. Exploiting genebank material can be helpful, as such additional properties might have disappeared by selection under modern, high input conditions. De Melo (2003) found that relatively old onion cultivars showed a higher total root length (or higher root density) than modern cultivars. Establishing base populations from old open pollinated onion cultivars with a broad genetic diversity can therefore offer new gene pools for breeding focussed on low-input and organic farming systems.

In order to make genebank accessions of onions more accessible for the utilisation in organic breeding programmes, a participatory research project was set up to characterise and evaluate 37 old open pollinated onion accessions under organic farming conditions and to establish new base populations. The partners in this project were the Centre for Genetic Resources (CGN), the Louis Bolk Institute (LBI, specialised in research for organic farming) and three experienced organic farmers, specialised in the cultivation and selection of onion.

Materials and methods

The 37 accessions were nearly all selected from the CGN collection (Boukema 1999) and divided into 5 different groups according to their market use: *Round Rijnsburger* group (13),

Flat Rijnsburger group (7), *Red flat onion* group (4), 'Zeeuwse Bruine' group (3) and a group of old Eastern European varieties (12). In 2002 these CGN accessions were sown and seedlings were planted on a commercial organic farm. The farmer carried out all cultural practices and after harvest managed the storage of the onions. Researchers of CGN characterised the populations in the field at full leaf stage, shortly after harvest and after storage using their internal standard descriptor list based on the IPGRI Descriptors for *Allium* (IPGRI et al. 2001). At the same time the farmers assessed these accessions using criteria important for organic farming systems such as leaf quantity, field tolerance to pest and diseases, yield potential, bulb characteristics and storability. To establish several base populations, the farmers, in collaboration with the researchers, selected the best genotypes within the five groups of onion accessions. The researchers of LBI supported the farmers in conducting documentation on the characterisations and selection process. To produce seeds of the base populations, selected bulbs of six separate groups were planted in the greenhouse facilities of CGN; it included the best selected genotypes of each of the five original groups and an extra group selected from the most promising *Round Rijnsburger* accessions.

Results

Based on the ideotype for the organic onion crop, which is developed by the farmers in an earlier research project (Lammerts van Bueren et al. 2003), new plant traits were added to the genebank characterisation (Table 1). Most traits added by the farmers are quantitative traits with a strong genotype X environment interaction supporting yield stability. For instance, next to disease resistances, early maturing types are required to gain sufficient yield before downy mildew falls in. They also considered the appearance of dead leaf ends as an indication for sensitivity to stress conditions.

Table 1. List of traits used by CGN and the farmers/LBI

Growth phase	CGN traits	Common traits	Farmers traits
Leaf	Leaf length Foliage cranking Leaf waxiness	Leaf quantity Foliage attitude Leaf colour	Dead leaf ends *Botrytis squamosa* *Peronospora destructor* Uniformity Maturity type
Harvest	Bulb size Bulb shape Bulb length Bulb top shape Bulb base shape Bulb skin colour intensity Bulb splitting tendency Bulb root disk position	Bulb colour Bulb uniformity	Ideal farmer's onion type Yield potential Neck thickness General impression
Storage			Bulb hardiness Amount of bulb skins Skin strength

The joint characterisation and evaluation also gave researchers insight into how farmers evaluate and value certain plant traits. And as some traits were assessed both by the farmers and the researchers of the gene bank, the results could be compared, as shown in Table 2. Obviously, the focus of the two groups was somewhat different. CGN characterised the

accessions according to their standard descriptor list of onion taking the large variation in the existing (world) collection into account. The farmers focussed particularly on traits to be used for further selection, not merely characterised but assessed the accessions with their farmer's eye. Therefore they tended to maximise differences in scores. The differences in minimum and maximum scores of the farmers were therefore greater than those of the CGN scores (e.g. foliage attitude). With a trait such as leaf quantity, the scores of the farmers were sometimes lower than those for the CGN-researchers, because the farmers were more critically looking for those types that produce a larger quantity of leaves under organic conditions. The same applies for leaf colour. The farmers assessed the criterion bulb uniformity more roughly, because they were merely interested in the uniformity of size, shape and the occurrence of deviations, such as thick-necked onions or double centres. CGN researchers included more aspects, such as the variation in the bulb base and top shape.

Table 2. Mean, minimum and maximum scores of traits characterised by farmers and CGN (recorded on a 1 - 9 scale; 1 = bad/unfavourable; 9 = good/favourable), 2002

Group	Variation	Foliage attitude		Leaf quantity		Leaf colour		Bulb uniformity	
		Farmers	CGN	Farmers	CGN	Farmers	CGN	Farmers	CGN
Round	Mean	4.1	6.9	6.1	6.1	4.6	5.8	7.1	5.3
Rijnsburger	Min.	3.0	6.0	4.0	4.0	3.0	5.0	5.0	4.0
	Max.	6.0	7.0	7.5	7.0	5.5	6.0	9.0	6.0
Yellow flat	Mean	3.7	6.7	6.2	6.6	4.6	5.9	6.5	5.6
Rijnsburger	Min.	3.0	6.0	4.0	4.0	4.0	5.0	4.0	5.0
	Max.	4.0	7.0	7.0	8.0	5.0	6.0	8.0	6.0
Red flat onion	Mean	5.0	7.0	6.3	5.3	4.3	6.7	8.0	6.0
	Min.	4.0	7.0	6.0	5.0	4.0	6.0	8.0	5.0
	Max.	7.0	7.0	7.0	6.0	5.0	7.0	8.0	7.0
Zeeuwse Bruine	Mean	3.7	6.7	7.3	7.3	5.0	6.0	6.5	5.3
	Min.	3.0	6.0	7.0	6.0	4.0	5.0	5.0	5.0
	Max.	5.0	7.0	8.0	9.0	6.0	6.0	8.0	6.0
Eastern Europe	Mean	5.2	6.8	5.1	5.3	5.4	6.0	5.0	5.2
	Min.	3.0	6.0	3.0	4.0	4.0	5.0	4.0	4.0
	Max.	7.0	7.0	8.0	7.0	6.0	7.0	7.0	6.0

With respect to the purpose of evaluating the accessions for further exploitation for organic farming systems, it was noticed that within these open pollinated varieties there was a substantial diversity. Variation for important properties was found within and between the 5 groups. However, the selection procedures differed very much per group and per accession. In Table 3 an overview is given of the percentages of selected bulbs in the different phases of the selection process. Of some accessions from the Eastern European group no plants were selected for the establishment of a new base population. The five best performing accessions within the *Round Rijnsburger* group were selected to establish an extra base population, out of which one can rapidly select a marketable variety. The other populations can offer interesting traits for further breeding for organic agriculture. The healthiness of the leaves in the Eastern European group was notable.

Seeds of six new base onion populations are now available for organic breeding programmes and will be used in further selection. The group also defined 6 highly performing accessions with a score on the general impression of 8 or higher, which may also be exploited on their own in order to achieve improved genotypes for organic farming systems.

We learned from this project that participation of organic farmers in the evaluation of the accessions is a good method in order to make genebank accessions of onions more accessible for the utilisation in organic breeding programmes. However, it should be noted

that not all traits, which are important for organic farming, are easy to evaluate by the genebank itself. CGN combines characterisation and conservation (regeneration) in one procedure in order to conserve the potential genetic diversity within the accessions and to guarantee enough quantity and quality of seeds. This standard procedure includes the use of agro-chemicals against pests and diseases and therefore disease resistance cannot be evaluated. Usually storage ability is not assessed because of lack of storage facilities. Without extra financing a genebank cannot provide the extra information on accessions important for the organic sector. So far CGN has not established base populations. It may be possible that in the future the demand from breeding companies for genetic base broadening will increase and that the establishment and maintenance of base populations will fulfil this need. It certainly serves the needs of the organic agriculture to select better-adapted varieties from a broad genetic base.

Table 3. Overview of the amount of selected bulbs (%) in different phases of the selection process, 2002 (adapted from Lammerts van Bueren et al. 2004)

Group	ACC[2] (n)	NPF (n)	SBH (%)	SBS (%)
Round Rijnsburger	13	1117	62	15
sel. Round Rijnsburger[1]	5	452	62	8
Yellow flat Rijnsburger	7	718	74	23
Red flat onion	4	387	66	6
Zeeuwse Bruine	3	298	65	30
Eastern Europe	12	970	56	11

[1] Group selected after storage within the original Round Rijnsburger group 1
[2] ACC, number of accessions; NPF, number of plants in the field (counted in June); SBH, selected bulbs after harvest (in % of the original number of plants); SBS, selected bulbs after storage for establishing new populations (in % of the original number of plants)

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Carrot haploid production through induced parthenogenesis

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ABSTRACT: The aim of this study was to find the most efficient conditions to produce carrot haploid plants. Nineteen genetically different carrot stocks were used. Donor plants were grown in the growth chamber. For parthenogenesis induction, carrot flowers were pollinated with pollen of other species. Four induction media were used. Two years of research showed that callus and embryos were formed with the frequency of 0 to 3.87 % and 0 to 2.08 %, respectively, depending on the genotype, pollen source, and induction medium composition. 'Nantejska' produced embryos with the highest frequency (2.08 %), while 'Karlena' gave the highest frequency of callus (3.87 %). Parsley was the best pollen source in the experiment. H medium supplemented with 0,01 mg/dm^{-3} IAA was the most efficient for embryo development (1.46 %), while A12 medium supplemented with 2 mg/dm^{-3} 2,4-D and 6-BA was the most suitable for callus development (3.41 %). Plants were regenerated from callus and embryos. Ploidy level analysis of the obtained regenerants revealed the presence of diploids (98 %), no haploids were observed. Among diploids 52 % were homozygous, as proved by the Pgi isozyme test.

Key words: Carrot – haploid – induced parthenogenesis – *in vitro* culture

Introduction

The production of carrot inbreds for hybrid programs is a slow and often difficult process, because of long generation cycles, demand for isolation and inbreeding depression. Most often, carrot lines are selfed for only three or four generations, which leads to lack of their complete homozygosity. Induction of haploid plants by means of induced parthenogenesis may be an alternative path to shortening the breeding process. In the case of carrot, there has been no established protocol for induction of haploid plant development, and only a few reports can be found (Andersen et al. 1990, Hu et al. 1993, Matsubara et al. 1995, Rode & Dumas de Vaulx 1987).

Materials and methods

In the present study, the research was carried out in 2002 and 2003. Nineteen carrot open pollinated cultivars and breeding lines were used. Donor plants were grown in a chamber with controlled temperature of 25°C and a 16 h day with irradiance of 100 µmol m^{-2}s^{-1}. Donor plants were selected on the basis of the isozyme glucose-6-phosphate isomerase (Pgi) test (Barański 2000). Only plants, which were heterozygous at the Pgi locus were used as ovule donors. Four pollen sources, i.e. parsley, celery, parsnip and cabbage were used in the experiment. After pollination, each umbel was sprayed with the water solution of 2,4-D (10 mg 2,4-D / 100 ml water). Non-pollinated umbels sprayed with 2,4-D were used as a control. Umbels for *in vitro* culture were harvested 15 days after pollination, sterilized with 70 % alcohol for 5 minutes, 10 % chloramine T for 20 minutes, and washed with sterile distilled water three times for 5 minutes. Ovules were isolated from enlarged ovaries in sterile conditions and plated on agar media. Four types of media were used, i.e. H (0.5 MS, 0,01 mg dm^{-3} IAA), P (MS, 0.1 mg dm^{-3} kinetin), K3 (B5, without growth regulators) and A12 (B5, 2 mg dm^{-3} 2,4-D and 6-BA). All media were supplemented with 20 g dm^{-3} sucrose. Twenty ovules per Petri dish were plated and sealed with parafilm. Dishes were placed in temperature of 25°C in light with 16 h day and irradiance of 55 µmol m^{-2}s^{-1}. Developing calli were transferred on the R medium (MS, 3.0 mg dm^{-3} glycine, 20 g dm^{-3} sucrose), embryos

were regenerated on the same medium supplemented with 1.0 mg dm^{-3} zeatin. *In vitro* cultured plants were kept in 25°C in the same irradiation conditions as cultured ovules. Single plants or clones were evaluated with regard to their ploidy level using flow cytometry. All diploid plants were verified for their homozygosity using the Pgi isozyme test.

Results and discussion

In the present study we investigated the possibility of haploid production by means of parthenogenesis induced by pollination of carrot plants with pollen of other species (Table 1). Cabbage pollen stimulated ovules to callus formation (1.93 %), parsnip and parsley pollen induced embryo development with the frequency from 0.1 up to 1.5 %. Celery pollen did not induce parthenogenesis. In the control combination K1, no embryo or callus development was observed.

Table 1. Effectiveness of induced parthenogenesis in carrot ovule cultures, depending on pollen source

Year	Pollen donor	Number and frequency of ovules				
		Plated	Producing embryos		Producing callus	
		No.	No.	%	No.	%
2002	Parsley	1327	20	1.51	6	0.45
	Parsnip	2034	2	0.09	0	0.00
	Celery	414	0	0.00	0	0.00
	K1	80	0	0.00	0	0.00
2003	Parsley	9047	93	1.02	112	1.23
	Parsnip	2451	31	1.26	9	0.36
	Cabbage	3921	14	0.35	76	1.93
	K1	2599	0	0.00	0	0.00

Differences in the ability for embryo or callus production were found between cultivars and lines used in the experiment (Table 2), which confirmed the opinion that the effectiveness of haploid production highly depended on genotype (Lelu & Bollon 1990).

In total, embryos and callus were formed from ovules in fourteen genotypes with a frequency of 0 - 3.87 %. Five carrot cultivars did not respond during the culture. Embryos were relatively frequently produced by 'Nantejska' (2.08 %) and 'Kreta' (1.92 %), three genotypes 'Berlo', 'Karlena' and 'DC 84022' produced embryos with the frequency close to 1.5 %. The highest frequency of callus production was observed for 'Karlena' (3.87 %) and 'Berlo' (2.37 %). Also, in three genotypes ('Fatima', 'Jawa', 'Vita Longa') callus was present from 1 to 1.8 %. The remaining genotypes produced embryos or callus from 0 - 1 %.

Media composition had a great effect on ovule development in the culture (Table 3). The highest callus formation (3.41 %) was obtained on A12 medium, while the highest amount of embryos appeared on H medium. 1 % embryos and 0.3 % callus was obtained on the K3 medium, while on the P medium efficiency of callus and embryo formation was the lowest (0.16 and 0.31 %, respectively).

Table 2. Effect of genotype on embryo or callus formation in the isolated ovule cultures of carrot (only genotypes showing positive reaction during the culture are included)

Year	Genotype	Number and frequency of ovules				
		Plated	Producing embryos		Producing callus	
		No.	No.	%	No.	%
2002	Koral	3120	2	0.06	0	0.00
	DC 84022	1775	20	1.12	6	0.33
2003	Berło	1390	21	1.51	33	2.37
	Danvers	677	0	0.00	6	0.88
	Fantazja	1986	1	0.05	2	0.10
	Fatima	945	1	0.10	17	1.79
	Flacoro	1072	1	0.09	4	0.37
	Flamanka	1564	6	0.38	5	0.31
	Jawa	3628	35	0.96	38	1.04
	Karlena	2455	28	1.14	95	3.87
	Koral	1860	2	0.10	7	0.37
	Kreta	1927	37	1.92	5	0.25
	Nantejska	527	11	2.08	3	0.56
	Vita Longa	1494	0	0.00	20	1.33
Total / mean frequency		26751	165	0.61	241	0.90

Table 3. Effect of medium on embryo or callus formation in the isolated ovule cultures of carrot

Year	Medium	Number and frequency of ovules				
		Plated	Producing embryos		Producing callus	
		No.	No.	%	No.	%
2002	H	4895	22	0.45	6	0.12
2003	A12	5158	6	0.11	176	3.41
	H	4920	72	1.46	34	0.69
	K3	4892	48	0.98	16	0.32
	P	5345	17	0.31	9	0.16

Ploidy investigation of 348 plants originating from the isolated ovule cultures showed that 98 % plants were diploids, 2 % were polyploids (3x or 4x), while no haploid plants were observed. 300 of diploid plants were tested using the isozyme Pgi test. The test indicated that half of the plants obtained from isolated ovule cultures were homozygous at the Pgi locus (Figure 1), thus confirming their gametic origin. Heterozygous plants most probably developed from somatic tissue.

Similar results were reported previously in carrot by other authors. Andersen et al. (1990) obtained 70 % of diploid plants from anther cultures. Matsubara et al. (1995) reported that 96 % of plants obtained were diploids. Górecka and Krzyżanowska (2001) also suggested that spontaneous diploidization occurred in carrot. High percentage of diploids might be caused by a spontaneous diploidization during cultures, which was observed in other species, e.g. cabbage (Rudolf et al. 1999).

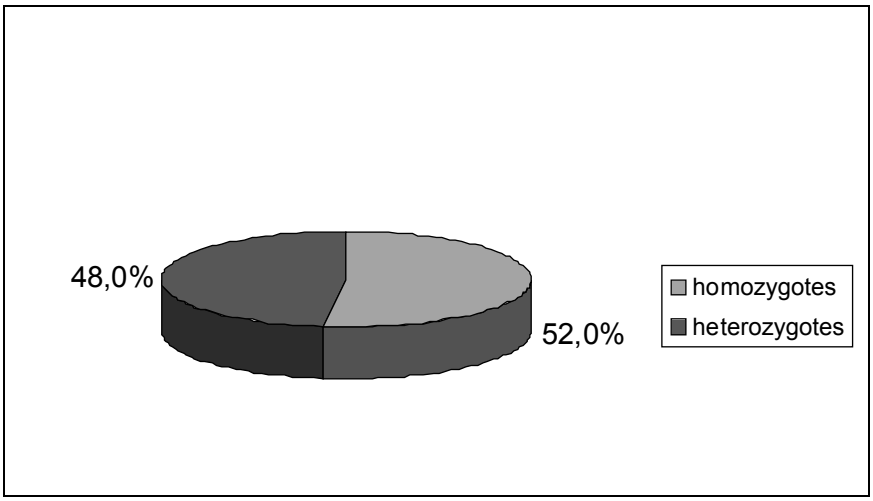

Figure 1. Homozygosity of carrot plants regenerated from ovule cultures (Pgi test)

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Genetic variation in *Capsicum frutescens* L. as a result of an SSD method modification

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ABSTRACT: The main objective of the research was evaluation of the modified SSD (single seed descent) method in breeding of *Capsicum frutescens* L. The change consisted in an attempt to maintain high genetic variation due to SSD by means of limited selection of plants. The research material was a heterozygous breeding population of *C. frutescens* L. Within this population half of individual plants were selected to be represented by two descendants next year. Thus, limited selection oriented to qualities of high importance was performed and high genetic variation characteristic of SSD was intentionally narrowed. However, high differentiation of the level of observed qualities and high variation coefficients indicate that a population managed in this way maintained a satisfactory genetic variation level. Moreover, a negative correlation coefficient was found between the weight of seeds and the biological performance of fruits, understood as the share of the edible part of the pericarp in the fruit weight. The modification of SSD method makes it possible to maintain high, but intentionally oriented genetic variation.

Key words: Correlation coefficient – single seed descent – coefficient of variation

Introduction

Pedigree selection including evaluation of issue is an effective method of breeding of self-pollinated plants. The selection of plants in any generation is generally limited to a small number of genotypes. Such activity leads to quick reduction of genetic variation, since it denotes giving up of genetic information of plants that are not accepted. The SSD method (single seed descent) is contrary to the above-described one. It consists in the use and evaluation of individual plants, being the issue of individual plants from the previous generation. This procedure enables maintenance of the maximum genetic variation. It does not pose the dilemma of choice to the breeder either, because all issues are used, although they are represented by individual plants. Casali and Tigchelaar's (1975) research into tomatoes is a practical proof of assumptions presented above.

Capsicum frutescens L. is one of the few species of *Capsicum* that are of significant consumer value. It is used as wild form and as materials being an effect of breeding. Results of research show that *C. frutescens* L. is an interesting source of many important characteristics and genes conditioning the same (Greenleaf 1986). A characteristic feature that should be mentioned is soft flesh combined with different content of capsaicinoids. Our research (Nowaczyk & Nowaczyk 2004a, 2004b) suggests that both *C. frutescens* L. genotypes as well as hybrids with *C. annuum* L. may be the raw material for production of new foodstuffs, including biologically active food or nutraceuticals. The main objective of research was the evaluation of the modified SSD method in breeding of *C. frutescens* L. The change consisted in an attempt at maintenance of high genetic variation due to SSD with limited selection of plants.

Material and methods

The research material was a heterozygous population of breeding materials of *C. frutescens* L. In 2001 research was carried out on 100 plants. Based on observations, half of them were selected as the source of issue subject to research in 2002. Each of the plants selected in 2001

was represented by two descendants. As a result of research and selection carried out in 2002, 50 plants were chosen and their issue, represented by two plants each, was subject to observation in 2003. In every year of research, the plants were cultivated in unheated foil tents according to agrotechnical principles for *C. annuum* L. The biometric analysis comprised the average weight of a ripe fruit, biological weight of the fruit, biological performance and the weight of the placenta and seeds. Biological weight of the fruit is the weight of the edible part of the pericarp after the placenta and seeds are removed. The percentage share of the biological weight in the weight of the fruit was determined as the biological performance of the fruit. In a statistical approach to clarify results variation and correlation coefficients were determined for the characteristics subject to research.

Results and discussion

In the analysis of data relating to fruit characteristics (Table 1), big differences between extreme values are noticeable. The maximum level was from a few to several tens of times higher than the minimum level, depending on the observed characteristics. Concurrently, big differences were found in successive years of research. This comparison must exclude biological performance expressed in percents, thus in relative values. However, it must be added that big differences were observed here as well. They were larger than in experiments carried out by Wang and Wang (1996). Values observed by them oscillated from 62 - 86 %.

Table 1. Variation of *Capsicum frutescens* fruit traits

Trait[1]	Year	Min	Max	Mean	CV %[2]
FW (g)	2001	3.10	35.2	14.5	75.7
	2002	2.15	27.6	11.2	57.0
	2003	1.59	45.8	18.5	65.3
BFW (g)	2001	2.30	29.9	12.1	77.6
	2002	1.04	24.6	8.4	63.9
	2003	0.98	35.6	15.5	67.1
BP (%)	2001	69	96	82.8	5.9
	2002	48	89	72.5	13.6
	2003	62	94	83.0	8.8
PW (g)	2001	0.34	8.28	2.48	90.5
	2002	0.36	6.18	2.01	67.1
	2003	0.17	10.2	2.96	70.8
SWF (g)	2001	0.46	2.49	1.29	45.3
	2002	0.33	2.51	1.12	41.3
	2003	0.38	2.86	1.29	41.3

[1] FW, fruit weight; BFW, biological fruit weight; BP, biological performance; PW, placenta weight; SWF, seed weight per fruit

[2] CV%, coefficient of variation (%)

The above-discussed characteristic is significant for industry because it determines effective processing of the raw material. We should not convince anyone that genotypes with fruits in which the edible part of the pericarp constitutes more than 90 % are more valuable than those in which the weight of inedible parts is half of the fruit weight. The results presented point to high differentiation in populations subject to research. And this provides a possibility to produce genetically stable cultivars with different level of the characteristics. This observation is confirmed by data for variation coefficients. The high level of the same indicates that plants representing a much differentiated level of the fruit weight, biological weight of the fruit, and the weight of the placenta occur. The range of variation in fruit weight was higher than in

populations of wild forms of *C. frutescens* L. examined by Sreelathakumary and Rajamony (2003). The seed weight variation coefficients are worth mentioning. Their values were relatively lower in comparison with the above-mentioned characteristics, except for biological performance expressed in percents, and simultaneously stable within three years of research.

Effective selection within selected groups is determined to a great extent by mutual relations within the same. The correlation coefficients determined (Table 2) provided interesting information. In every year of research the coefficient was highest and very stable for the total weight of the fruit and for its edible part, that is, for the biological weight. Therefore, it can be suggested that selection aiming at increased fruit weight will also result in a directly proportional growth in the biological weight. A similar relation with reference to the non-edible part, that is the placenta, came as a surprise of some kind. However, as expected, we have to note negative correlation between biological performance and the weight of seeds. The results gathered indicate that the increasing weight of the fruit, due to the increase in number of the same, results in a change in weight of the placenta tissues, and thus, negatively affects the share of the edible part of the pericarp in the total weight of the fruit.

Table 2. Correlation matrix between *C. frutescens* fruit traits

Trait	Year	BW (g)	BP (%)	PW (g)	SWF (g)
FW (g)	2001	0.996**	0.176	0.964**	0.716**
	2002	0.983**	0.492**	0.877**	0.522**
	2003	0.994**	0.206	0.889**	0.617**
BW (g)	2001		0.236	0.964**	0.685**
	2002		0.610**	0.782**	0.419**
	2003		0.289	0.840**	0.570**
BP (%)	2001			0.163	-0.033
	2002			0.126	-0.085
	2003			-0.034	-0.037
PW (g)	2001				0.650**
	2002				0.629**
	2003				0.628**

For abbreviations see Table 2.
**, highly significant ($P<0.01$)

The experiments made use of a modified SSD breeding method. The change consisted in the use of two plants as the issue of half of plants selected in the previous generation instead of individual plants representing every plant of the previous generation. During vegetation selection was performed in every pair, leaving the issue of one of them for reproduction that was in turn represented by two plants. This procedure contributed, without doubt, to narrowing of genetic variation as compared with the basic version of the SSD method. Selection performed within one pair, however, had an effect of intentional limitation of variation. However, it aimed at a higher level of characteristics significant in terms of usability. Can the compromise that consists in narrowing of genetic variation from the high level which is adequate to SSD method and introduction of selection be considered satisfactory from the practical point of view? Taking into consideration results of the above-presented results of experiments, a reply to such a question may be positive. The level of compromise used may be subject to discussion. The method in which we combine two types of selection slightly deviates from SSD. It provides no solution for the breeder's problems related to the choice-making decisions. In our opinion, however, it is an interesting solution making it possible to maintain high, but intentionally oriented variation.

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Breeding of energy effective tomato varieties

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ABSTRACT: Yield efficiency of assimilation system and peculiarities of manifestation of characters of fertilizer use effectiveness on different agrophones by tomato hybrids were studied. Higher stability of yield characters, effectiveness of assimilation system work and harvest index was revealed in most hybrids in comparison with their parents. Differentiation of the material under study according to ecological stability signs and fertilizer use effectiveness was shown, when genotype with maximum yield was selected on different agrophones. Responsibility to fertilizer and unstable genotypes are distinguished on rich phone, on poor phones - universality in relation to different levels of mineral nutrition and stable genotypes.

Key words: *Lycopersicon esculentum* – stability – yield

Introduction

Increase of energy-effectiveness in agricultural production is an highly actual question especially in vegetable growing where low energy value of products and high production expenditures lead to negative balance between the quantities of the energy spent on it and obtained from it. In present energy analysis is used both in evaluation of technology and selection. Creation of highly adaptive varieties of vegetables, tomato in their number, is an important reserve in increasing energy-effectiveness of their cultivation.

Graham (1984), Klimashevsky (1991), Fageria (1992) and some other authors widely use such terms as energy-effective variety, varieties of low and high input in their works. They point out the genetic determination of energy effectiveness characters. Nevertheless, inheritance of those characters is still unexplored. Study of connection between energy-effectiveness and stability of forms is an actual question as well. With knowledge of these questions, breeders will be able to create forms that can assimilate to maximum all available energy sources (natural and anthropogenic). This will lead to increase of cultivation effectiveness in crops, tomato in particular. In connection with this, the aim of this work is a genetic analysis of energy effectiveness characters for elaboration of methods of tomato genotype selection, which will combine high yield capacity, ecological stability and energy effectiveness.

Materials and methods

Object of the investigation were 28 genotypes of tomato, created by diallel scheme hybridization without reciprocal crosses (7 varieties and 21 hybrids). All experiments were held in the experimental field of the Department of Agricultural Biotechnology and Ecology of Belarussian Agricultural Academy. The genotypes were tested on two phones of mineral nutrition – control (without applying fertilizers) and fertilizer (60 N, 120 P, 120 K). Study of specificity of growing and development of plants during vegetative period was conducted with use of different methods: estimation of photosynthetic efficiency (net assimilation rate - NAR, relative growth rate - RGR, leaf area ratio - LAR) (Laaman et al. 1996); cultivar specification of assimilates re-distribution (harvest index - H_I) (Fageria 1992); response to fertilizer application (index of energy efficiency - I_E, yield efficiency coefficient - C_E, agronomic efficiency - A_E) (Gerloff et al. 1983, Graham 1984); energetic efficiency of

resource use (energy efficiency of factor - E_{EF}) and bioenergy coefficient - C_{BE}) (Babak 2002). Genetic methods were used for analysis of data: dispersion analysis (Hayman 1954), determination of genetic parameters of characters and estimation of ecological stability of varieties and hybrids (Kilchevsky & Khotylyova 1989).

Results and discussion

As a result of Hayman analysis, parameters of yield characters and assimilation apparatus work effectiveness, degree of dominance on fertilizer effectiveness characters and attraction of assimilants into fruits the following peculiarities of the characters manifestation in different varieties and hybrids were found out: (1) inheritance of net assimilation rate in tomato occurs as over-dominance towards increasing of the sign irrespective of cultivation conditions. The type of inheritance of leaf area ratio of a plant and relative growth rate is overdominance, the direction of which is changing from positive to negative depending on weather and agro-technical conditions. Alteration of the conditions to the optimal ones contributes to dominance towards decreasing of LAR; (2) heterosis of harvest index depends both on weather conditions and agrophone. Deterioration of the attraction conditions leads to manifestation of positive overdominance on harvest index in hybrids, alteration of the attraction conditions to optimal ones changes the direction of dominance to the opposite; (3) there is a weak and medium inverse dependence (from -0.45 to -0.06) between the manifestation of traits which characterize the effectiveness of assimilate accumulation (NAR, RGR) and their redistribution into reproductive organs (H_I) in the tomato varieties under study; (4) heterosis effects of responsiveness on the additional application of nutrition elements and yield (commodity and total) have different directions; (5) the main type of inheritance of fertilizer-use efficiency index in tomato hybrids is negative overdominance.

Yield effectiveness coefficient (C_E) takes a special place among the parameters under study, which characterize the effectiveness of mineral fertilizer utilization. Selection on this sign permits to combine at the same time selection according to yield capacity and effectiveness of fertilizer use on different agrophones. Genotypes that have high yield effectiveness coefficient can be regarded as universal ones, the cultivation of which is effective on different levels of mineral nutrition. The type of inheritance of yield efficiency coefficient is incomplete dominance and positive heterosis which coincides with manifestation of commodity and total yield domination in the majority of samples. Heterosis selection appears to be an important method of creating highly productive forms of tomato which will use both high and low doses of mineral nutrition elements.

Study of peculiarities of stability parameters in tomato genotypes on the groups of traits under study permits to choose the right strategy in breeding energy effective forms. Analysis of relative stability of genotypes, coefficient of environmental regression of the genotype, general adaptability permitted to find a higher stability of productivity traits, effectiveness of assimilation apparatus work and harvest index in the majority of hybrids in comparison with their parents. A close correlation between I_E on commodity yield and parameters S_{gi} and b_i ($r = 0.77$ and 0.82, respectively) was found, which points out combination of responsiveness of fertilizers and weather in one genotype of tomato. Information about changes of parameters while choosing the best forms taking into consideration the most important traits - commodity and total yield on different agrophones - is to be of some interest for working out methods of selecting energy effective varieties. For this purpose an analysis of average meanings of the traits under study on both phones in the 5 best forms of tomato taking into consideration their commodity and total yields was made (Table 1).

Table 1. Means of commodity yield in the five best genotypes of tomato on the fertilized and control phones

Genotype	Yield	I_E	C_E	A_E	C_{BI}	E_{EF}	S_{gi}	b_i	SGV
	Fertilized phone (60 N, 120 P, 120 K)								
Talalikhin	819.0	2.03	1.27	105.0	5.34	2.97	36.3	1.00	396.8
Talalikhin x A 39	760.0	3.18	1.09	106.2	5.08	3.0	51.7	1.22	238.2
Dokhodny x Radek	748.4	2.84	1.07	107.6	5.04	3.04	51.1	1.36	263.6
Linia 7 x Radek	774.9	2.97	1.13	91.4	4.94	2.58	42.8	1.18	361.5
Talalikhin x Dokhodny	779.4	2.38	1.26	88.2	4.91	2.49	37.3	1.04	410.7
Mean	776.3	2.68	1.16	99.7	5.06	2.88	43.8	1.16	334.2
	Control phone								
Talalikhin x Povarek	745.7	1.62	1.21	34.3	3.99	0.97	34.6	1.03	452.1
Dokhodny x Linia 7	825.9	1.91	1.42	69.3	4.87	1.96	34.1	1.04	468.4
Talalikhin x Radek	761.4	1.44	1.21	34.3	4.28	1.39	38.8	0.90	372.6
Linia 7 x Povarek	780.5	2.18	1.25	71.3	4.78	2.02	34.0	0.96	443.4
Povarek x Radek	683.8	1.59	1.06	53.7	3.98	1.52	23.3	0.67	493.1
Mean	759.5	1.75	1.23	52.6	4.38	1.57	33.0	0.92	445.9

The peculiarities found of the best forms selected under different agrochemical conditions are shown on Figure 1.

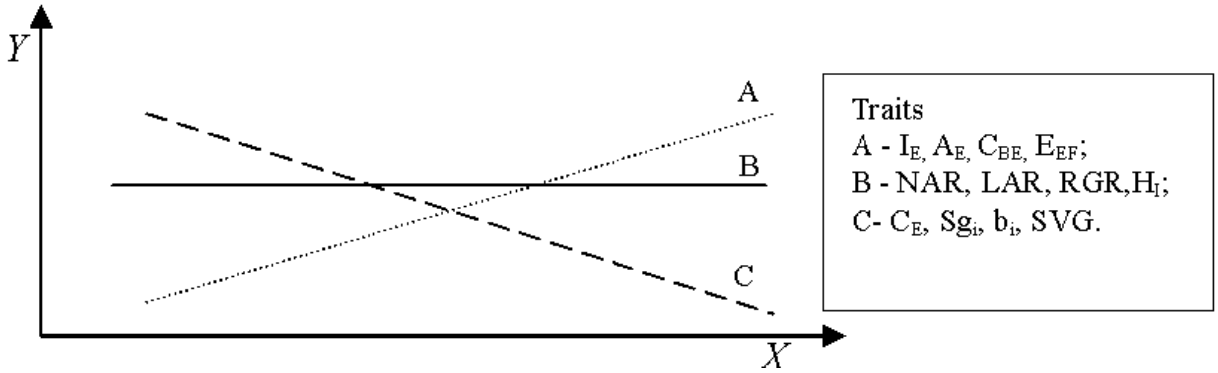

Figure 1. Peculiarities in manifestation of energy effectiveness index and ecological stability while choosing highly productive genotypes on different agrophones (X - dose of mineral fertilizers; Y- value of traits under study)

The x-axis shows increasing of agrophone fertility, while the y-axis shows the meaning of the traits under study. Line A shows character of changes in fertilizer use traits (I_E, A_E, C_{BE}, E_{EF}), while line B shows changes in traits which characterize the effectiveness of accumulation and distribution of assimilates (NAR, LAR, RGR, harvest index) and line C shows the reaction of genotypes on different agrochemical conditions (C_E), selection value of genotype (SVG) and yield stability (Sg_i, b_i). This picture shows how the selection of the highest yielding forms on different agrophones differentiates the samples under study according to the fertilizer use efficiency and yield stability. Genotypes with maximum productivity on highly fertile phone have at the same time high responsibility on additional application of mineral nutrition elements, agronomic effectiveness and recoupment of energy input; the best yielding forms chosen on low fertility phone are of higher productiveness stability, higher universality with regard to different levels of mineral nutrition.

Together with analysis of traits, changes in the 5 best genotypes under different agrochemical conditions, their manifestation in the 5 worst genotypes according to their commodity and total yield was under study. Choosing of the best genotypes on their commodity yield in comparison with the worst samples on both agrophones ensured rising of

commodity yields, RGR, NAR, C_E, C_{BE}. At the same time the importance of early yield went down. Differently-directed character of selection was found out on traits I_E, A_E, S_{gi}, b_i, SVG. So, the 5 best commodity yield genotypes had higher means of I_E, A_E, S_{gi}, b_i and lower SVG at the phone with fertilizer application in comparison with the 5 worst genotypes. At the phone without fertilizers, a reverse reaction could be seen: the worst genotypes had higher means of I_E, A_E, S_{gi}, b_i and lower SVG. The obtained data confirm the rules by Jinks and Pooni (1991). The authors showed that selection in 'rich' environment (which ensures a higher mean of a trait) in positive direction (towards increasing of the trait) and in 'poor' environment in negative direction brings an increasing environmental sensitivity (loss of ecological stability). Selection of the best forms according to total yield in comparison with selection of the worst forms on both phones brings to rising of such traits as total, commodity yield, RGR, NAR, C_E, C_{BE}, E_{EF}, SVG. At the same time the importance of early yield, LAR, harvest index is decreasing. The tendency towards increasing of I_E, A_E, S_{gi}, b_i, (rising of responsibility and loss of stability) was found out during selection of the best forms on the fertilized phone and the worst ones on the non-fertilized phone. This is one more confirmation of the rule by Jinks and Pooni (1991).

Conclusions

Heterosis selection is an important method of creating highly productive forms of tomato which will use both high and low doses of mineral nutrition elements as well as solar energy. Hybrids have higher stability of productivity traits, higher effectiveness of assimilation apparatus and harvest index in comparison with their parents.

Heterosis manifestation of response to additional applying of nutrition elements and yield (commodity and total) are differently directed. Heterosis on 'yield efficiency coefficient' coincides with the same on commodity and total yield in the majority of genotypes. Combination of a wide norm of reaction on agrophone changes and weather conditions in one genotype could be seen in the tomato forms under study.

Choosing of the best yielding forms on other agrophones differentiates the material under study according to the effectiveness of the fertilizer application and yield stability. Genotypes with maximum productivity on highly productive phone have higher responsibility to the additional application of mineral nutrition elements and lower ecological stability. Selection of the best yielding forms in the low mineral nutrition conditions at the same time leads to choosing more productivity stable forms, rising their universality towards different levels of mineral nutrition.

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Use of near infra-red spectroscopy for screening total and individual glucosinolates in cabbage leaves

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ABSTRACT: The potential of near infra-red spectroscopy (NIRS) for screening the total glucosinolates (t-GSL), gluconapin (GNA), gluconasturtiin (GNAST) and neoglucobrassicin (NGBS) contents of cabbage (*Brassica oleracea* L.) leaf, was assessed. Leaves of this species were analysed by NIRS, and their reference values of glucosinolates obtained by high performance liquid chromatography, were regressed against different spectral transformations by modified partial least squares (MPLS) regression. The coefficients of determination in the cross-validation (R^2_{cv}) for t-GSL, GNA, GNAST and NGBS were 0.83, 0.70, 0.62 and 0.60, respectively. The standard deviation to standard error of cross-validation (SECV) ratios were, for these constituents, as follow: t-GSL: 0.42; GNA: 0.54; GNAST: 0.61; NGBS: 0.63. An examination of the MPLS loadings used in modeling the t-GSL equation, suggests that O-H groups, C-H combinations of the methylene molecule and also N-H groups of amides, were those molecular associations more used in developing the first three terms of this equation. The results reported in this work have demonstrated the successful application of the NIRS technique to the screening of glucosinolates in plant leaf, showing the lower cost and considerable reduction of the time required in contrast to those of the standard techniques of analysis.

Key words: *Brassica oleracea* – cancer chemoprevention – HPLC – human nutrition – NIRS

Introduction

Over the past three decades, *Brassica* production has increased to become one of the most important sources of oil and protein of plant origin for human and animal nutrition, respectively. In addition, some species of the genus are highly consumed as green leafy vegetables all over the world. In the Iberian Peninsula, the high consumption of *Brassica* crops is reflected by a large use of flower buds and leaves of several of these species, including cabbage (*Brassica oleracea* L.). However, the information available on the glucosinolate (β-thioglucoside-*N*-hydroxysulphates) composition of this species is scarce (Rosa et al. 1997).

Glucosinolate-containing plants in the *Brassicaceae* family represent a potential source of control for different soil-borne pests (Fahey et al. 2001) and as cancer chemoprevention agents (Shapiro et al. 2001). However, the toxic and anti-nutritive effects of glucosinolates have limited the use of *Brassica* species for human and animal feed (Sorensen 1990). The determination of the glucosinolate content by high performance liquid chromatography (HPLC) is expensive and time-consuming, and specialised personal is needed. In contrast, the use of fast analytical techniques such as near infra-red spectroscopy (NIRS) results in many advantages, since analysis can be carried out with a considerable saving of time, at a low cost and without using chemicals. The purpose of this work was to test the potential of NIRS for screening the total glucosinolate content (t-GSL) as well as some major glucosinolates exhibiting different molecular forms of those reported in cabbage leaf, i.e., gluconapin (GNA, aliphatic), gluconasturtiin (GNAST, aromatic) and neoglucobrassicin (NGBS, indole).

Materials and methods

Plant material and HPLC analysis

The plant material used in this work is widespread in Portugal and north Spain. Freshly harvested leaves of cabbage were collected at different maturity stages to increase chemical and physical variability in the samples. Then samples were taken to the laboratory for immediate analysis. Desulphoglucosinolates were determined by HPLC at the Field Crops Department at the Universidade de Trás-os-Montes e Alto Douro, Vila Real, Portugal, following the method proposed by Spinks et al. (1984).

NIRS procedure: recording of spectra and processing of data

Freeze-dried and ground samples of leaves of cabbage were analysed in an NIR spectrophotometer (NIRSystems 6500, Foss-NIRSystems, Inc., Silver Spring, USA) in the reflectance mode. Sample spectra were registered as an individual file in the range from 400 to 2500 nm, at 2 nm intervals. In the second step, the individual and total glucosinolate contents, as they were determined by HPLC, were added to the file of spectra, thus each spectrum having an associated value for each glucosinolate.

Developing calibration equations

Using the application GLOBAL v. 1.50 (WINISI II, Infrasoft International, LLC, Port Matilda, USA), different calibration equations for t-GSL, GNA, GNAST, and NGBS were developed on the whole set. Calibration equations were computed using the raw optical data (log 1/R, where R is reflectance), or first or second derivatives of the log 1/R data. To correlate the spectral information and the content of the different glucosinolates, modified partial least squares (MPLS) were used as a regression method. In addition, standard normal variate plus de-trending transformations (SNV-DT) (Barnes et al. 1989) were used to correct baseline offset.

Validation of the equations

The performances of the different calibration equations obtained were determined from cross-validation. The method is carried out by splitting the calibration set into *M* segments and then calibrating *M* times, each time testing about a (1/*M*) part of the calibration set (Martens & Naes 1989). The prediction ability of the equations obtained was determined on the basis of their coefficient of determination in the cross-validation (R^2_{cv}) (Shenk & Westerhaus, 1996) and standard deviation to standard error of cross-validation (SD_{ref}:SE_{cv}) ratio (Williams & Sobering 1996).

Results and discussion

Glucosinolates in the samples

Glucosinolate composition of the samples used in this work is summarised in Table 1. The t-GSL and individual glucosinolate contents found in the samples used in this work covered a wide range of values, similar to those contents characteristic of this species, as it has been previously reported (Rosa 1999).

Equation performances

The validity of cross-validation to evaluate the performance of an NIR equation has been supported by different researchers (Shenk & Westerhaus 1996, Williams & Sobering 1996), having been applied successfully by the authors of this work to the analysis of glucosinolates in a previous report (Font et al. 2004). In the present work on the basis the R^2_{cv} values, the four equations for glucosinolate composition showed from good quantitative information (t-

GSL and GNA) to correct separation of the samples into low, medium and high groups (GNAST and NGBS) (Table 1, Figure 1) (Shenk & Westerhaus 1996).

Table 1. Calibration and cross-validation statistics for the different equations developed (n=100) (μmol 100 g^{-1} dw)

Glucosinolate	Calibration					Cross-validation		
	Variation	Mean	SD_{ref} [1]	SE_{cal}	R^2_{cal}	SD_{ref}:SE_{cv}	R^2_{cv}	N_t
t-GSL [2]	48.78-3492.65	741.16	621.99	196.23	0.90	2.38	0.83	8
GNA [3]	0-1321.67	205.83	268.02	124.29	0.78	1.85	0.70	9
GNAST [3]	0-118.50	47.99	30.30	14.51	0.77	1.63	0.62	8
NGBS [3]	0-166.15	41.80	34.71	13.98	0.80	1.58	0.60	9

[1] SD_{ref}, standard deviation of the reference chemistry data (HPLC); SE_{cal}, standard error of calibration; R^2_{cal}, coefficient of determination of the calibration; SD_{ref}:SE_{cv}, ratio of the standard deviation of the reference chemistry data to the standard error of cross-validation; R^2_{cv}, coefficient of determination of cross-validation; N_t, number of terms of the equation

[2] Second derivative transformation (2,5,5,2; SNV+DT) of the original spectral data

[3] First derivative transformation (1,4,4,1; SNV+DT) of the original spectral data

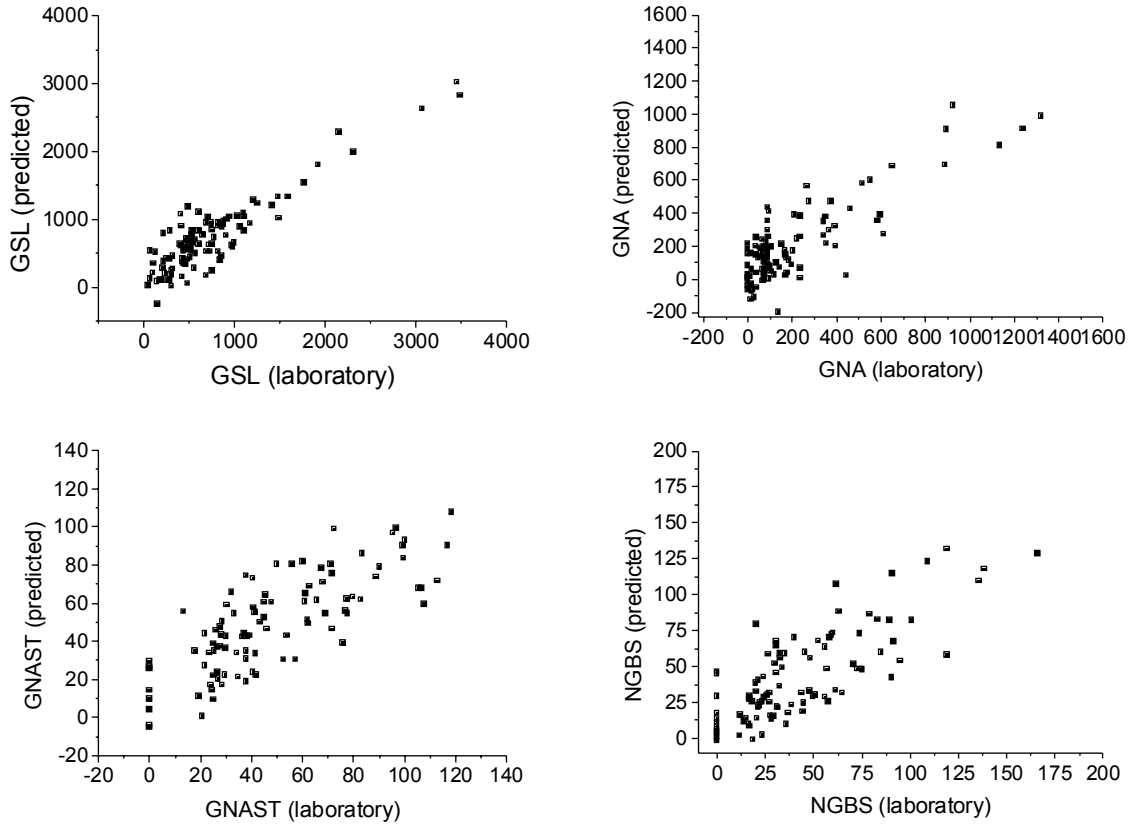

Figure 1. Cross-validation scatter plots for total glucosinolates, gluconapin, gluconasturtiin and neoglucobrassicin

In spite of the low concentrations in which glucosinolates are found in the leaves, in comparison to those contents of the seed of *Brassica*, the different equations showed R^2_{cv} and SD_{ref}:SE_{cv} values that were similar or slightly lower than those obtained previously by us (Font et al. 2004) over the intact seed of Indian mustard. On the other hand, the decrease of the correlation existing between the spectral and chemistry data that is observed in Table 1, from the highest of t-GSL to the lowest exhibited by NGBS, is explained here on the basis of the mean concentrations shown by these molecular forms in the leaf samples.

The results reported in this work show that NIRS is able to predict the t-GSL and GNA in the leaves of cabbage with good precision. In addition, the GNAST and NGBS equations could be used to identify samples with low, medium and high contents of these glucosinolates. Then, a proper selection of the individuals of interest can be made for more accurate chemistry values. The use of this methodology supposes and important reduction of time and cost of analysis in routine work. Each sample of leaf that we analyzed by using the NIRS method took us approximately 1.5 min, and prediction results for the individual and total glucosinolate content were monitored simultaneously. The development of these calibrations will allow researchers to identify quickly those individuals of interest in the leaves of cabbage without the need of doing HPLC analysis.

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Creating light environment for genotype screening in plant breeding

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Abstract

Light plays a prominent role throughout the life cycle of photosynthetic organisms. Plants have evolved a number of photosensory systems that allow them to sense neighbors, compete for light, or rely on photoperiodic signals to trigger their seasonal responses. These systems influence every major developmental transition. The analysis of growing form and some quantitative characters within plant species or varietal populations under marginal photoperiodic and photomorphogenetic conditions (analyzing environments) shows significant variation in genotype developmental rhythms. It is important to recognize both genetic variation in the performance and the environmental constraints within which this variation is expressed. Some of the genotype by environment interactions can be ascribed to the differences in the length of juvenile phase, critical day-length, civil twilight sensitivity, light spectral quality response. These important determinants must be taken into consideration for effective breeding program design and population screening. Analyzing environment application provides good background to effective selection of genotypes with the desired rhythms of growth and development, productivity or bolting resistance. Therefore, creation of specific marginal (analyzing) photoperiodic/photomorphogenetic environments capable of inducing maximal variation of genotype response traits within populations for subsequent screening of genetic material is of great importance for the breeder. Under the controlled conditions, it is possible to decrease environmental variance so that genetic variation within populations will be clearly revealed. Our studies on photoperiodic and photomorphogenetic control of growth and development in vegetable crops representing various life-forms and life strategies made it possible to determine analyzing environments that induce maximal variation of many important characters. Experiments were conducted under natural light conditions and in the phytotron using artificial light sources that simulated various red : far red ratios, day-lengths and threshold dim light extending short photoperiod. Sets of mustard self-pollinated lines were developed for environmental value evaluation and testing of each environmental capability to segregate populations. Using of the analyzing environments with special light regimes improves the performance of the breeding process significantly.

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Advanced backcross QTL analysis for drought tolerance in DH-line-populations derived from crosses between Iranian and European wheat genotypes

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Abstract

Five Iranian wheat genotypes, chosen for their drought tolerance, were crossed with two European spring wheat varieties in altogether 10 combinations. After two to three backcrosses with both parents, doubled haploid (DH) lines through crosses with maize will be developed. Cross combinations for final evaluation will be selected based on: (1) genetic distance of the parents determined by 400 wheat SSR markers (number of polymorphic SSR loci), (2) their seed storage protein banding patterns (SDS-PAGE and A-PAGE), and (3) a drought tolerance test of the parents carried out under controlled conditions.

Primary aim of the study is to map genes (QTLs) controlling tolerance to drought using DH-line progenies of specific crosses. Special attention will be paid to group 5 and 7 chromosomes, which are known to play a major role in adaptation especially to drought. Furthermore, new genetic variation will be introduced into European and Iranian breeding material for a mutual benefit of breeding for the two geographic areas.

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The effect of homoeologous group 5 chromosome substitutions on phenology, yield components and winter survival in wheat

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Abstract
The effects of homoeologous group 5 chromosomes, carrying different major genes for vernalization, on the growth and development of hexaploid wheat (Triticum aestivum L.) were analysed using a set of substitution lines. Chromosomes 5A, 5D and 5B from the spring cultivars 'Zlatka', 'Chinese Spring' and the alternative variety 'Česká Přesívka', respectively, were substituted into different winter wheat backgrounds ('Košutka', 'Vala' and 'Zdar') and shown to change growth habit from winter to spring type due to the expression of dominant alleles at the Vrn-1 loci.

The effect of the dominant genes Vrn-1 on the duration of phenological stages was analysed in two contrasting recipient genetic backgrounds of 'Zdar', sensitive to photoperiod, and 'Košutka', insensitive to photoperiod from early and later spring sowing. The results showed considerable differences between the early and late sowings, which was probably connected to the response to day length. The biggest effect on shortening the phenological stages was found in the lines with substitutions of chromosome 5A, probably due to the epistatic action of Vrn-A1; while the lines with a substituted 5B (Vrn-B1) showed the least reduction of the phenological stages; the effect of 5D (Vrn-D1) was intermediate.

Statistical analysis of yield components revealed big differences between the early x late sowing in lines carrying Vrn-D1 while the substitution lines for chromosome 5A were the least sensitive to the date of sowing. This suggests the over-riding influence of Vrn-1 loci on yield components, correlating with the influence of vernalization response.

Winter survival was evaluated using plants grown in pots placed at different heights above the ground over the winter by the so-called 'provocation method' under natural conditions. Differences in winter survival were found with substitutions of 5B, 5A and 5D, showing lower survival by 20 %, 36 % and 41 %, respectively, compared to the original cultivars.

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Improving Fusarium head blight resistance in durum wheat: Two different approaches

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Abstract

Improving Fusarium head blight (FHB) resistance in wheat is a major challenge world-wide. Particularly durum wheat is known to be highly susceptible against FHB. As durum wheat is mainly used in direct human consumption, the contamination with Fusarium mycotoxins needs strict control. We applied two different approaches:

The first was the transfer of two QTL for FHB resistance already mapped in the hexaploid cultivar Sumai-3. The resistance QTL *Qfhs.ndsu-3BS* (Anderson et al. 2002) and *Qfhs.ifa-5A* (Buerstmayr et al. 2003) have been introduced into three adapted durum cultivars. To restore the specific durum traits, the F_1 was continuously backcrossed (up to 6 times) with the recurrent durum parent. A screening of BC_4, BC_5 and BC_6 derived lines for their FHB resistance in the field under artificial inoculations is ongoing (2003 and 2004).

The second approach is the mapping of novel FHB resistance QTL in tetraploid wheat. Two *T. dicoccoides* accessions showing moderate resistance (Buerstmayr et al. 2003) were crossed and backcrossed one time with the recurrent durum parent. Two BC_1F_4 derived populations of 130 and 90 plants, respectively, are investigated. The same procedure is carried out with a BC_1F_3 population (130 plants) derived from a cross *T. durum* with *T. dicoccum*. The final goal is to pyramid the most promising QTL in adapted durum cultivars.

Acknowledgments

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Assessment of cultivar differences in response to Fusarium head blight infection and fungicide treatment in winter wheat

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Abstract

Results are based on field experiments performed over three years at Prague-Ruzyně. Nine winter wheat cultivars treated with fungicide (tebuconazole) after artificial infection with *Fusarium culmorum* were evaluated for DON content in grain, percentage of Fusarium damaged kernels (FDK), symptom scores and reduction of grain yield components. Genotypic response to the infection and fungicide treatment was also evaluated with the use of quantitative real time PCR analysis. Analyses of variance showed significant effects for year, genotype and treatment with fungicide, and interactions between these factors on all traits. Moderately resistant cultivars 'Arina' and 'Petrus' exhibited in all environments lower DON contents and lower affection of other traits by the disease.

DON content was closely related to parameter C_T Fus (transformed) obtained from quantitative real time PCR analysis. From other traits percentage of FDK appeared to be the best predictor of DON content and performance in examined disease severity traits. Correlation analyses showed differences in trait relations between years and due to fungicide treatment, but all traits were interrelated on a genotypic base, which indicates that resistance to DON accumulation in a cultivar was accompanied with resistance to head blight and affection of kernels by the disease.

The developed PCR system enabled to specify clearly cultivar response to infection and fungicide treatment. Both the examination for DON content and C_T Fus (transformed) showed significant cultivar and year differences in response to treatment with fungicide. While in 2003, that could be characterized by rapid disease progress, all cultivars responded to application of fungicide (efficacy ranged between 41 and 89 %) in 2001, which promoted slow disease development and high DON content, fungicide treatment was not effective particularly in late maturing cultivars (efficacy 0 - 9 %). Due to complicated character of the disease and inconsistent effects on various traits, PCR assays, that enables to identify causal organism, can be reckoned as very helpful in evaluating genotype resistance and deciding on effective differential control measurements.

Acknowledgements

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Resistance gene pyramiding in common wheat using recurrent mass selection

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Abstract
A field-scale recurrent mass selection (RMS) programme for common wheat, based on dominant male-sterility (*Ms3*) and hydroponic culture of cut tillers, is being conducted (Marais & Botes 2003). Male sterile F_1 plants are used as female parents whereas male-fertile plants are inbred (single seed descent) and field selected for three seasons before being used as male parents in the F_7 (fourth season). Inbreeding and marker-assisted selection (MAS) of the male parents can rapidly shift the frequencies of target genes. As the frequencies of disease resistance genes increase, the programme is expected to yield a correspondingly growing proportion of inbreds with pyramided genes. Variability can be sustained by controlled introgression using 'recurrent backcrossing'. In order to test the concept and at the same time study possible interactions involving species-derived resistance genes, a RMS sub-population segregating for numerous leaf, stem and stripe rust resistance genes of known and unknown identity is being established. Genes known to occur in the population include *Lr34/Yr18* (7DS), *Sr2* (7BS), a recombinant of the *Sr31/Lr26/Yr9/Pm8* translocation (1BS) without the *Sec1* locus (Lukaszewski 2003, personal communication), a recombinant of the *Lr19* translocation (*Lr19*-149 on 7BL) lacking the yellow pigment and *Sr25* loci (Marais et al. 2001), and the *Lr37/Sr38/Yr17* translocation on 2AS. The translocations can be detected making use of PCR marker systems. The adult plant resistances, *Lr34/Yr18* and *Sr2*, are selected for making use of, respectively, the morphological markers leaf tip necrosis and pseudo black chaff. For the purposes of the experiment, the selection cycle is reduced to two years and the male parents inbred to the F_5 only. Field selection for agrotype, yield, quality and disease resistance is supplemented by marker-based selection in the F_1 (female plants) and the F_5 (male plants). Marker based recurrent backcrosses to introgress a further species-derived adult plant resistance gene, *Lr35* (2B) were initiated. Gene frequencies, agronomic variation and the frequencies of F_5 inbreds with different gene combinations will be measured annually.

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Prehaustorial resistance of *Triticum monococcum* – a source for durable resistance to *Puccinia triticina* in *T. aestivum* and *T. durum*

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Abstract

Up to now only few examples are known for the successful use of *Triticum monococcum* (genome AA) as a source for resistance, although it exhibits effective resistance to a number of important leaf diseases of wheat. Extensive evaluations of *T. monococcum* germplasm collections (457 accessions) were carried out to study the variation of disease severity of leaf rust (*Puccinia triticina*) in both climate chamber and field tests, each with three replications. A three-stage selection scheme was used to identify the most resistant genotypes comprising different microscopic techniques, scoring of seedlings at different times after inoculation and scoring of adult plants at anthesis after artificial inoculation via spreader rows in the field. None of the *T. monococcum* genotypes was as severely infected as were the standard cultivars (*T. aestivum*). In the case of susceptibility of *T. monococcum* only flat pustules with a low spore production were detected. Most of the genotypes showed necrotic and chlorotic spots with varying intensity. They are the result of hypersensitive reactions that followed the successful penetration of the fungus and the forming of haustoria. There was a continuous variation with regard to the frequency of haustorium mother cells, haustoria and the degree of leaf flecking. In 13 genotypes no or only very few haustoria were detected, although the fungus has developed haustorium mother cells. Visually the plants looked completely green without leaf spots. This type of reaction was found in replicated tests and using leaf rust isolates of different virulence. By microscopical analysis it turned out that the selected genotypes express a prehaustorial type of resistance that causes a cease of fungus growth before haustoria are formed. Prehaustorial resistance is characterized by unspecific reactions to fungus isolates carrying different virulence genes and a broad genetic base. Therefore, it may be assumed to be durable. Resistance of this type is not yet used in actual wheat breeding. The project aims at the development of molecular markers and the marker assisted transfer of prehaustorial resistance to tetraploid and hexaploid wheat.

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Breeding for resistance to orange wheat blossom midge

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Abstract
Orange wheat blossom midge (OWBM), *Sitodiplosis mosellana* Géhin is present in temperate zones across the northern hemisphere. Wheat growing areas recording recent economic infestations include North America, the UK and China. Active resistance breeding programs are underway in all three zones. OWBM larvae hatch from eggs laid on emerging heads of wheat (*Triticum aestivum* L.) and feed on and severely damage developing kernels. Both yield and quality are affected. Two distinct types of cultivar resistance have been detected. Some cultivars deter egg laying (antixenosis) while others inhibit larval development (antibiosis). Data is presented that highlights our improved understanding of the expression and genetics of resistance, the search for new resistance genes, the use of markers vs. trait-based selection and progress in cultivar development.

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Doubled haploid (DH) technology in wheat breeding

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Abstract
The first question, which was raised simultaneously with the publication of first promising paper on *in vitro* haploid methods, was how could we produce large-scale pure lines in laboratory by applying tissue culture methods. Our first experiments confirmed the differential response of genotypes in anther culture and the superior induction result of segregating breeding materials (F_2) over the parental lines or varieties. These results prompted the breeders to opt for the anther culture method. Each breeding program is based on a large germplasm collection. Collection of breeding material is tested for response to induction and regeneration of the varieties. In the design of breeding programs the best responsive varieties are integrated into crossings.

Breeding from early generation (F_1 - F_3) via DH lines: 'GK Délibáb'
F_2 segregating populations were screened for agronomically ideal donor elite individuals. These were used for haploid induction. DH lines obtained via colchicine treatment were multiplied and tested further in nursery. After 3 years of official tests for registration, the DH line 773 named 'GK Délibáb' was registered and released as a winter wheat DH cultivar in Hungary. Benefits of this breeding approach are perfect homogeneity, shorter breeding time, easy and simple variety maintenance, whereas the disadvantages are tedious laboratory processes, relatively high number of unusable DH lines because of lack of selection for agronomic characters.

Breeding from late generation (F_4–F_6) via DH lines: *'GK Szindbád' and 'GK Tündér'- Use of DH lines in disease resistance programs*
The main dream of the breeders is to produce and release an ideal variety, which bears a long cultivation time. To reach this ideal situation, we need a lot of testing under the selection. When we know from the nursery and laboratory tests about what are the best populations or individuals for breeding, the haploid and DH induction can be an effective support in the process of breeding. In our resistance breeding program, after the most important resistance and preliminary yield tests from F_4 and F_6 generations, 2 varieties were produced. 'GK Szindbád' has an excellent fungus resistance to the most important Hungarian diseases. The high level of resistance combined with good yield and bread quality are combined in 'GK Szindbád'; the variety was released in 1996. Another new variety, 'GK Tündér', was evolved from a special disease resistance program, which was similar as in the case of 'GK Szindbád', but the tests were supplemented with head blight (*Fusarium* spp.) in early generation test. This new variety had broad resistance to most of the important wheat diseases in Hungary. The 'GK Tündér' is a promising modern variety, which bears important agronomic characters in itself. Benefits of the above breeding approach are perfect homogeneity, more useable regenerants in breeding compared with the early generation (F_1–F_3) regenerants, simple variety maintenance; the disadvantage is that there is no significant time saving in the breeding process.

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Agronomic performance of doubled haploid lines and lines derived by single-seed descent in triticale

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Abstract
Primary triticale, synthesized from wheat (*Triticum aestivum, T. durum*) and rye (*Secale cereale*) can be used to broaden the genetic basis of a breeding programme of secondary triticale. However, crosses between primary (hexaploid or octoploid) and secondary (hexaploid) triticale often lead to reduced performance of the offsprings due to cytological imbalances. The use of doubled haploids (DH) in plant breeding has the potential to shorten breeding cycles by producing homozygous lines from a segregating population more rapidly than most classical breeding methods. The aim of the study was to compare the performance of DH-lines and lines derived by single-seed descent (SSD) from the same crosses. Three groups of reciprocal crosses were investigated i) secondary × secondary triticale, ii) secondary × primary hexaploid (6x) triticale, and iii) secondary × primary octoploid (8x) triticale. In total, 18 crosses were used to develop DH-lines and SSD-lines. From each of the 18 crosses up to 16 DH-lines and SSD-lines, resulting in a total of 295 lines, were evaluated in three environments. Results for agronomic traits will be presented and the consequences for triticale breeding discussed.

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Exploring the genetic diversity in European winter triticale

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Abstract

Knowledge of the genetic diversity of a species is important for the choice of crossing parents in line and hybrid breeding. Our objective was to investigate European winter triticale cultivars and advanced breeding lines using SSR and AFLP markers and pedigree data with regard to genetic diversity and grouping of germplasm. The material originated from 13 breeding companies and research institutes from seven central and east European countries. Three to five primer pairs for each of the 42 chromosomes and ten *Pst*I/*Taq*I AFLP primer combinations (PC) were selected to analyse 128 European winter triticale cultivars. SSR analysis of a total of 93 SSR markers resulted in 657 alleles within triticale with an average of 6.8 alleles per primer pair. The average polymorphism information content (PIC) for polymorphic markers was 0.59 and the marker index (MI) was 0.55. AFLP analysis revealed 344 polymorphic fragments, an average PIC per PC of 0.25 and a marker index (MI) of 8.56. Genetic similarity (GS) calculated according to Dice averaged 0.61 for AFLPs in comparison with 0.43 for SSRs and 0.06 for Malecot's coancestry coefficient *f*. More than 85 % of the 8128 pairwise comparisons had an *f*-value of $f<0.1$. Correlation between *f* and GS estimates of molecular markers was low, probably due to (1) partially incomplete pedigree data and (2) unrealistic assumptions in the calculation of *f*. The correlation of GS_{AFLP} and GS_{SSR} was 0.70 for the entire dataset and 0.81 after discarding all unrelated genotypes ($f<0.1$). A broad range of genetic diversity was observed in the material under study, however, no clear grouping of the germplasm could be detected by principal coordinate analysis or cluster analysis. One possible explanation for the lack of distinct groups might be the exclusive use of triticale in Europe for one end-use purpose, namely grain feed. Hitherto, no management of germplasm with regard to different end-uses (e.g. feed, bread-making quality, technical uses) or establishment of heterotic pools for hybrid breeding has taken place.

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Line selection for exploiting durum wheat (*Triticum turgidum* L. subsp. *durum*) local landraces in a modern variety development program

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Abstract

Pure line selection for grain yield applied within four durum wheat local landraces (*Triticum turgidum* L. subsp. *durum*) was studied in an effort to determine: (1) the agronomic performance of selected oligogenotypic line bulks and their value as potential new cultivars, (2) the effectiveness of pure line selection for yield within the local landraces and (3) the correlated selection response in yield components and kernel quality traits. Four local durum wheat landraces were used. Two of them, *Mytilini 1* and *Mytilini 2* are still cropped in some rural areas of the island Mytilini where the other two, *Limnos* and *Mavragani*, are maintained in the Greek Gene Bank. Following a preliminary evaluation, 100 individual plants from each landrace were randomly selected. Selected plants (pure lines) were evaluated head to row during 1997-98 and 1998-99 growing seasons, and bi-directional selection for high vs. low yield was practiced within each landrace. Five high yielding selected lines and their corresponding five low yielding ones from each landrace were bulked to form the high and low yielding oligogenotypic line mixtures, respectively, securing the necessary seed quantities for wide testing. The selected line bulks along with the four source landraces and two commercial varieties, 'Mexicalli' and 'Simmeto', used as checks entered field-testing in three environments. Data for yield and yield components indicated that at least three of the landraces studied were line mixtures and responded to line selection resulting in isolating oligogenotypic line mixtures, which seem to be promising either as potential new varieties or as germplasm sources. Especially selected line bulk performance of *Mytilini 2* landrace provided evidence that selection of lines combining high yield with high protein content could be effective. In conclusion, pure line selection within landraces was effective in differentiating among genotypes and isolating lines to form oligogenotypic line bulks, candidates of direct utilization as newly derived varieties. Data provided evidence that within landrace selection of lines combining high yielding ability with desirable yield components and good kernel quality traits seems feasible and worth of further breeding effort.

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Studies on breeding for resistance to ergot (*Claviceps purpurea*) for organic farming in self-incompatible winter rye populations

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Abstract
Ergot (*Claviceps purpurea* [Fr.] Tul.) is best known as a disease of rye but it also affects most other cereals and grasses. An infection during the flowering time results in dark wintering organs (sclerotia) that are developed instead of kernels. They contain alkaloids harmful for humans and animals. The maximum content of ergot in cereal grain yields must not exceed 0.05 % (for humans) and 0.1 % (for animals), as regulated by the German law. Toxicity of sclerotia causes reduced productivity and health problems in animals. The objectives of this study were (1) to test the resistance to ergot of widely grown rye population varieties and rye genetic resources, such as landraces and old rye varieties, by applying artificial inoculation, as well as (2) to estimate genetic variation within and among rye populations by full-sib families (FSF).

All populations were tested for resistance to ergot in two locations under the conditions of organic farming. The inoculation was performed by spraying 3 x 10^6 conidia/ml of an aggressive mixture of *Claviceps purpurea* isolates three times.

First results indicated significant genotypic differences among population varieties. Depending on the genotype, 0.3 to 1.5 % sclerotia by weight was detected in the harvested grain. To estimate genetic variation within populations, five rye populations each consisting of 50 FSF were screened. Significant genotypic variance was detected among FSF within each population. The proportion of sclerotia varied between 1.7 and 14.5 % in the total grain yield. The mean of parents did not differ from the mean of progeny, suggesting an additive inheritance of resistance to ergot. The significant difference indicated the possibility to improve resistance to ergot by breeding.

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Breeding of rye (*Secale cereale* L.) as raw material for industrial use

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Abstract

Rye is a starch-producing plant to be considered as candidate for industrial use. This crop can be grown on large areas in Germany, especially in the eastern part, but the amount of rye necessary for human consumption is limited. So far the traditional use of rye was for food and feed. A high pentosan content is wanted for bread rye, while feed rye should have a high protein and a low pentosan content. For industrial use a high starch content will be important. At present the German variety list contains only open-pollinated and hybrid varieties with green-coloured grains, but grain colour is still a register trait of variety licensing in rye. Light-coloured rye strains are used in rye breeding programmes for crosses. In future, light-coloured rye may be of interest for specific end use purposes like paper production.

In our experiments F_2 seeds were produced from five crosses between light- and green-coloured self-fertile inbred lines of the Lochow-Petkus GmbH. They were visually sorted in two subpopulations (SP), the light- and green-coloured SP, multiplied and grown in F_3 and F_4 at two locations differing drastically in soil quality (high: Bergen near Celle, low: Petkus) with two replications as block design in two consecutive years (2002 and 2003).

The yield potential is the main breeding goal independent of the end use purpose. As expected, there were large differences between the two locations. A considerable genetic variation could be detected for yield, but no important interactions with the environments. Significant differences were found between the populations and, within a population, between the green- and light-coloured SP. On average the light-coloured SP realized a higher plot yield, thousand kernel weight as well as starch and protein content. There is a strong correlation between yield and starch yield ($r = 0.99$), but also a positive correlation between yield and starch content ($r = 0.42$). This shows the breeding potential of rye given for bioethanol production. A negative correlation could be observed between starch and protein content. While a high protein content hinders bioethanol production, the role in production of biodegradable materials is not clearly investigated. No other trait was correlated with the total pentosan content. Pentosans slow down bioethanol production time, but they protect the starch kernels from enzymatic destruction.

The material was taken from an actual breeding programme to develop seed parents for hybrids. Is is expected that there will be heterosis for starch yield as for yield. However, data for specific starch hybrids in rye are missing until now.

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Variation for resistance to Fusarium head blight in spring barley (*Hordeum vulgare* L.)

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Abstract

Fusarium head blight (FHB) is a fungal disease of barley and other cereals, causing substantial yield and quality losses, mainly due to the contamination of the harvest with mycotoxins. We evaluated genetic variation for resistance to FHB and its association with other plant characters in diverse barley germplasm in order to identify useful lines for resistance breeding. The 143 barley lines consisted of 88 current European spring barley lines and cultivars, 33 accessions from the genebank at IPK Gatersleben, and 22 lines obtained from North American institutions. We conducted artificially inoculated field experiments with *Fusarium graminearum* Schwabe during two seasons. FHB severity was evaluated by repeated assessment of visual symptoms. On a set of 49 lines the content of the mycotoxin deoxynivalenol (DON) was analyzed.

Variation for FHB severity was quantitative. The lines with lowest FHB severity were *CIho 4196* and *PI 566203*, both lines were obtained from North American colleagues but originate from China. Also within the European spring barley collection variation for FHB severity was highly significant. The lines with the relatively lowest FHB severity were 'Hellana', 'Pixel', 'Secura' and 'Thuringia'. From the genebank accessions 'Misato Golden' and 'Lubicki' were those with relatively low disease severity. There was a significant negative correlation between plant height and FHB severity ($r = -0.55$). FHB severity assessed in the field and the amount of deoxynivalenol in the harvested grains were positively correlated ($r = 0.87$). Several lines with a useful level of FHB resistance were found or confirmed and are recommended as crossing partners. A full paper describing all relevant results in detail has been published by Buerstmayr et al. (2004).

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Sources of resistance to barley yellow dwarf virus and their utilization in barley breeding

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Abstract

The resistance to barley yellow dwarf virus (BYDV) in barley cultivars, breeding lines, and resistance sources has been tested in field trials in RICP Prague-Ruzyne since 1992. Most spring and winter barley cultivars grown in Central Europe were found susceptible or very susceptible to infection with PAV strain prevalent in this region. Among spring barleys, only the cultivars 'Malvaz', 'Atribut', and 'Madras' were found to be moderately resistant to BYDV. Moderate resistance was also detected in the winter cvs. 'Perry' from the USA and 'Sigra' from Germany. High resistance levels were associated with the presence of *Yd2* gene in spring and winter barley. PCR diagnostic markers YLM and Ylp were used to identify this gene. The results of field tests and genetic analyses in winter barley corresponded with marker analyses only when the Ylp marker was used.

We have also concentrated on the exploitation of BYDV resistance sources in barley breeding. In spring barley significant levels of resistance to BYDV were obtained by combining the resistance gene *Yd2* with genes detected in moderately resistant cultivars. The developed lines have not yet shown desirable parameters from breeding aspects. However, winter barley lines with moderate resistance to BYDV derived from 'Sigra' reached good yield levels and could be included into the official trials. Also resistant lines derived from 'Perry' after backcrossing to modern cv. 'Luxor' are a perspective for utilization in breeding.

Microsatellite markers of genes *ym4* and *ym5* (Bmac 29) conferring resistance to BaYMV and Ylp marker of *Yd2* gene were effectively used to detect lines possessing combined resistance to BYDV and BaYMV in the cross between cv. 'Nelly' (*ym4* gene) and cv. 'Wysor' (*Yd2* gene).

The possibility to combine resistance to BYDV with tolerance to abiotic stresses in barley (drought and low temperature stresses) was studied in BYDV resistant doubled haploid lines (of spring and winter habit) obtained from the cross 'Igri' / 'Atlas 68' (*Yd2*).

Acknowledgements

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Characteristics of spring barley varieties for organic farming

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Abstract

Modern spring barley varieties have been developed with the aim of combining high productivity and standardised product quality under high-input conditions. The organic growing system is a system where pesticides and inorganic fertilizers are generally not allowed. Hence, biotic and abiotic stresses have to be overcome by growing appropriate varieties and by practising good farm management. An important question is whether modern spring barley varieties possess the right combinations of characteristics to ensure a stable and acceptable yield of good quality when grown under different organic growing conditions. We know that varieties often perform and yield differently in different environments due to genotype-environment interactions, so it may be important to evaluate characteristics of varieties in organic as well as in conventional farming systems. However, it remains unclear to date whether the differences between the conventional and the organic growing systems are large enough to justify breeding and testing of varieties in both environments. Despite quite intensive testing of varieties, predictions of future performance of varieties when grown on specific locations are known to be nearly impossible; this especially within organic growing systems, where no pesticides and fertilizers can help to stabilize yield. Therefore, using mixtures of appropriate varieties might be a way to obtain more stable and acceptable yields. Variety mixtures have so far been studied especially in relation to effect of single diseases and under conventional farming conditions. The potential added value of variety mixtures adapted to local growing conditions ('farm varieties') has been studied very little. These aspects of cereal production are considered in a Danish project on organic spring barley (BAR-OF) as well as in a European Network on varietal characteristics and crop diversity (COST 860).

Results from field trials with about 120 varieties and variety mixtures grown in different growing systems at three locations in two years will be presented with focus on plant height (full data available on internet). Plant height is one of the important characteristics of spring barley for organic farming being much related to competitiveness against weeds. Grain yield was found to correlate differently to plant height under mainly organic compared to mainly conventional growing conditions. Further, in some variety mixtures natural selection under organic growing conditions implied an increase in the average plant height whereas in others a decrease. In conclusion, complex patterns of genotype-environment and genotype-genotype interactions were found.

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Estimating quantitative-genetic parameters of European maize populations to optimize hybrid breeding methods by model calculations

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Abstract

Quantitative-genetic model calculations allow to assess the relative advantages of alternative recurrent selection and line development methods in crop breeding. Computer programs are used to vary the number of entries, environments and replications in order to determine an allocation which maximises the expected gain from selection under the restriction of a given budget and available technical resources. Precise and accurate estimates of population parameters (genotypic variance, genotype x environment interaction variance, error variance, variance of the general and specific combining ability, heritability and genetic correlation between lines *per se* and testcross performance) are needed to obtain reliable predictions of the genetic gain. The size of the parameters depends on the kind, the coefficient of inbreeding, and the genetic structure of the breeding material in question. Further, the estimates depend on the test units (e.g. type of line or testcross progeny) on which they are based. In hybrid breeding a specific problem exists due to the limited possibilities of relating estimates obtained at the inbred level to those from non-inbred test units and vice versa. The goal of the present study was to estimate population parameters for major performance traits of European maize breeding materials, which later on will be used to optimize various line development procedures. On the one hand, population parameters were estimated from field trial data provided by collaborating breeding companies and from studies carried out at the University of Hohenheim. In these trials, *per se* and testcross performance of lines is assessed in various inbreeding generations, including doubled haploid lines. Datasets from different sources were pooled hierarchically and variance components were estimated using REML procedures. On the other hand, appropriate parameter estimates from the literature and from statutory trials (provided by the *Bundessortenamt*) were collected and included in the study. The combination of data sets from different experiments by means of the application of REML procedures allowed to furnish the accuracy and precision needed for the model calculations. Preliminary results show that, for given breeding materials, the relative sizes of parameters obtained from the assessment of line *per se* performance may differ from those derived from the evaluation of testcross performance. Hence, different standard sets of population parameters are necessary to estimate the selection gain for line value and general combining ability in model calculations. Parameter sets will be presented for major performance traits of grain and forage maize in the most common maturity groups.

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Inheritance of mineral concentrations in kernels of elite maize inbred lines

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Abstract
Mineral deficiency has been recognized as the most important nutritional problem especially in many developing countries. One sustainable agricultural approach of solving the problem is to enrich major staple food crops with minerals through plant breeding. Although maize lacks some minerals, humans and animals can obtain at least part of their nutritional requirements from maize grain. There is existence of sufficient genetic variation and workable heritabilities to improve mineral values in maize kernels. It was demonstrated that genetic background of inbred lines played a central role in the concentration level of some minerals (Fe, Zn, B, Cu and Mn) in maize varieties. Diallel analysis of the F_1 generation suggested a simple inheritance of concentrations of the six minerals due to consistently non-significant effects of specific combining ability. However, results of generation mean analysis across several populations of the same genetic material showed inconsistent genetic effect estimates. Therefore, further quantitative genetic as well as marker-based studies are needed to elucidate the inheritance of mineral concentrations in maize kernels.

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Utilisation of genetic diversity in grain amaranth (*Amaranthus cruentus* L.)

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Abstract

Grain amaranths (*Amaranthus* spp.) were an ancient crop in Mexico, during Aztec Empire it was considered as staple food and ceremonial plant. Together with maize, beans and chili, grain amaranths were cultivated in several regions of Mexico. After discovering of the new world, many crops in Mexico were neglected and disappeared due to different reasons. Recent studies have shown that great variability is present in the amaranth cultivation in several states of central and south of Mexico. Transplanting is one of the most important agronomic practices in a surrounding area of Mexico City. These practices are known as Chinampas-system or floating gardens. Direct sowing into soil is a common practice in state of Morelos Tlaxcala, Puebla. Slash and burn is an agricultural system where amaranth is cultivated in Oaxaca and Guerrero State. Due to climatic conditions in several states grain amaranth is cultivated in two dates of sowing during the year.

In recent years CIIDIR DURANGO IPN has been focused attention to preserve genetic variability and utilize it in breeding programmes in order to develop new cultivars or selections for other states, where ecogeographical conditions allow modern agriculture and obtain better yields for increasing consumption and uses of amaranths.

Exploration and collection of plant germplasm was conducted in most of the places where grain amaranth culture is still preserved by farmers. A plant breeding programme was initiated in 2003 using plant genetic material from *A. cruentus* from regions of Morelos, Puebla and Guerrero. A combined family selection method was chosen for improving germplasm. After first cycle of selection, response to selection and heritability were rather low.

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Soybean breeding program at the Agricultural Institute Osijek (Croatia): Achievements and challenges

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Abstract
Soybean (*Glycine max* (L.) Merrill), a 'wonder crop' of the 20th century, is a major source of vegetable oil and protein in the world. Current world soybean area is about 83.6 million hectare with the average grain yield of 2.3 t/ha (FAO STAT, 2004). In Croatia, soybean production has had a positive trend both in surfaces and unitary yields, particularly through the last five years. Horizontal expansion of soybean as a crop and yield increasing resulted from improved cultivars as well as advances in cultural practices and general management, respectively. The contribution of the soybean breeding program at the Agricultural Institute Osijek to the development, stability and improvement of soybean production in Croatia has been fundamental and very significant as it has permanently developed high-yielding cultivars with genetic yield potential of 5 - 6 t/ha, satisfactory grain quality (protein and oil concentration) and high tolerance to the principal diseases as well as resistance on lodging and pod shattering. Besides yield level, cultivars ought to have good yield stability and adaptability to different agroecological conditions of production as well. The genetic improvement has been accomplished through the use of conventional breeding methods and outlines valid for self-pollinated crops such as soybean. Domestic and foreign genotypes have been used as parental components in hybridization programs. The pedigree selection method and single-seed-descent (SSD) method as well as combinations of these methods are applied to the inbreeding generations (F_2 - F_5) for population improvement towards homozygosity. At homozygosity, preliminary tests with parents are carried out, and then selected lines are subjected to the exact experiments with the standard cultivars into the particular maturity group over different environments. The main testing as a part of the breeding program is done on the grain yield and grain quality (protein concentration, quantity and quality of oil). Moreover, the testing covers other important production traits. In the frame of the breeding activities, a program on application of molecular markers for determination of soybean genetic diversity as well as for detection of fungal pathotypes has been initiated. The result of the previous work on soybean breeding is 31 registered cultivars in the frame of 00 to II maturity groups which are released to the Institute (two cultivars are released in Hungary as well) in a period from 1976 to 2003. These cultivars are sown on large areas in the wide production (at present, about 10 Institute's cultivars cover more than 60 % of total area under soybean in Croatia) and significantly contribute to the increasing and improving of soybean production in the country. Further genetic improvement of soybean cultivars will be able by the application of conventional and molecular breeding tools.

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Is there a genetic influence of the growing faba bean seed on its own size ?

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Abstract
The growing and maturing seed connects two generations. It is a constituent of the mother plant's phenotype for traits like yield, it may express its own phenotype for some traits (xenia), and it represents the next generation. The question arises whether and inasmuch the genotype of the growing and maturing seed has an impact on its growth, e.g. speed of development and ultimate size and weight. Genetic differences between mother plant and seed can be created in two ways: either the seeds are segregating, or they have different male parents, e.g. some being selfed, others being cross-fertilized. A potential heterotic impact of the growing seed on its own size is an important aspect here. For this situation to be analyzed, it is advantageous if there is no complication by a triploid endosperm, hence, if the embryo constitutes the seed, and if there is a very large genetic variation for speed of seed growth and for ultimate seed size. Therefore, faba bean was taken as model species. The hypotheses to be tested are: (1) The growing and maturing seed has a significant genetic impact on its own developmental speed and ultimate size, (2) the genetic relationship between mother plant and pollinator is more important for the pollinator's effect on mature seed size than the alleles for seed size inherited by the pollinator, and (3) hybrid seeds become larger and grow faster than inbred seeds.

These hypotheses will be tested under more and less competitive situations, i.e. selfed and crossed seeds growing in the same pod, in different pods on the same plant, on different plants, and with or without drought stress conditions. Preliminary experiments (Figure 1) showed that the genotype of the seed has indeed a significant heterotic influence on its own ultimate weight. The project started in 2004 (DFG SPP 1149), major results of the first experimental season will be presented.

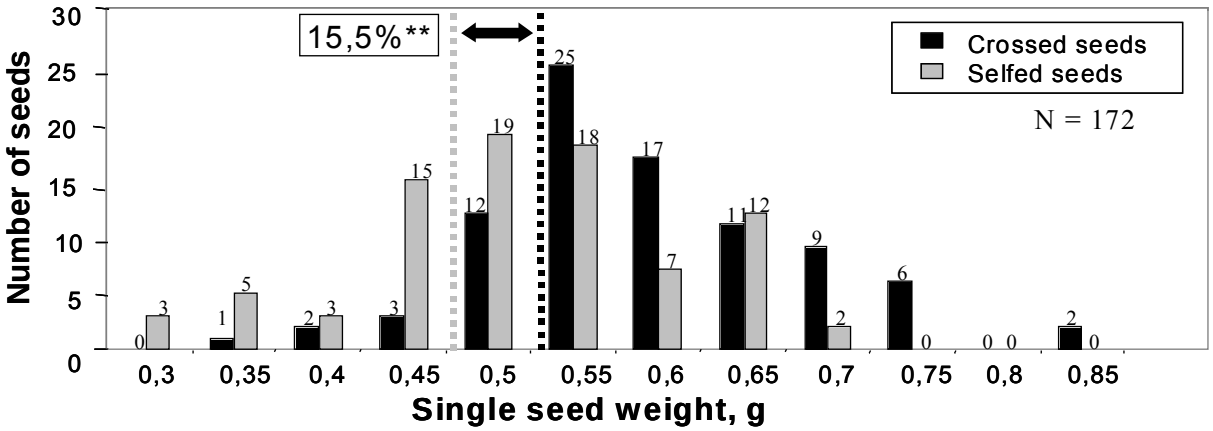

Figure 1. Distribution of single seed weight of faba bean for two groups of seed: selfed vs. crossed (mean values given as dashed line). A number of six inflorescences per plant (N = 21 plants; inbred line 'Gobo/1') produced two pods per inflorescence, one pod manually self-fertilized and one pod manually cross-fertilized (pollinator was F_1 ('Maya' / 2* 'Maris Bead/1')). Data from 2003, isolation cage Reinshof, Göttingen.

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The study of vitamin C effects on flowering, yield production and quality in *Vicia faba*

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Abstract

Vitamin C or ascorbic acid is a water soluble vitamin which can be found in all compartments of plant cells and affects metabolism in different ways. Faba bean (*Vicia faba*) belongs to the *Fabaceae* and is one of the most important annual winter crops in the Middle East and other arid and/or semiarid areas with mild winters. *Vicia faba* has many uses, e.g. human nutrition, stock feed, etc. In human diet it can be used as a substitute of meat and it represents a rich source of protein. In the present study, seeds of *Vicia faba* cv. 'Saraziri' were cultivated at the Dezfoul farms, Khozestan, Iran. Plants were treated with different concentrations (0, 10, 50, 100, 200 $mg\,L^{-1}$) of vitamin C. Different growth factors were measured at several developmental stages. Samples were prepared using histo-cytological methods by means of L.M. and T.E.M. The results showed changes in meristem structure and ultra structure. In treated plants more flowers were produced (+35 %), number of pods and pod length increased (+27 % and +32 %, respectively), and seeds had a larger size and weight (+30 %). Studies on the chemical composition of seeds using cyto-chemichal methods revealed an increased amount of proteins (+10 %) and carbohydrates (+18 %), whereas the amount of lipids remained constant. Thus, from the obtained results it can be concluded that vitamin C applied on plant level can cause better performance in regard to yield production.

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The impact of genotype by environment interactions on agronomic traits of chickpea (*Cicer arietinum* L.)

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Abstract

Present investigations were taken up to understand the impact of genotype by environment (G x E) interactions on the performance of different agronomic traits, interrelationship between various traits and to identify the adaptation and yield stability of different groups of chickpea cultivars for different agro-ecosystems. For these investigations 90 genetically divergent genotypes were selected to investigate various aspects. These 90 genotypes belonging to different groups of genotypes *desi* bold seeded, *desi* medium seeded, *kabuli* bold seeded and *kabuli* medium seeded group of cultivars. The results showed that the performance of seed yield and other major traits like number of branches, number of pods and biomass production of desi bold seeded group of cultivars was superior under rainfed environments in comparison to other groups of cultivars. This indicated that this group has the inbuilt capacity to withstand well under water limiting environments. This may be due to desirable genepools present in this group. On the other hand, *kabuli* bold seeded group of cultivars showed superior performance over the other groups of cultivars under non-stress environments under irrigation plantings. This showed that this group is suitable for irrigated conditions due to presence of specific genepools suitable for non-stress environments. The study of phenotypic correlations between seed yield and other agronomic traits indicated that the stable interrelationship of seed yield was obtained only with number of branches, number of pods and biomass under both the environments. On the other hand, the associations of seed yield with other trials like plant height, days to flowering, days to maturity and seed size was unstable and changing from rainfed to irrigated environments and vice versa. It shows that selection for seed yield, number of branches, number of pods and biomass can be exercised simultaneously during generation advance. It also shows that these traits collectively playing important and crucial role in the management of drought stress. The stability analysis provided very useful information regarding adaptation and stable yield performance of different genotypes. It is interesting to mention that most of the cultivars showed poor adaptation to both the environments, four cultivars showed good adaptation to non stress or rich environments and four cultivars to unfavorable environments. It is also interesting to mention that out of 90 genotypes only 7 showed stable yield performance over the years and environments. Based on these findings it was suggested that the identification of stable genotypes, correlation pattern and yield contributing traits are important and essential for breeding programme for the development of drought tolerant populations and for input responsive genotypes.

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Breeding strategies for improving productivity, multiple resistance and adaptation in chickpea (*Cicer arietinum* L.)

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Abstract

Chickpea is an important crop for the developed as well as underdeveloped countries in general and specially to Indian sub-continent which contributes more than sixty percent of both area and production at global level. Harsh environmental conditions under which it is generally grown impose restrictions on the full expression of genetic yield potential and overall plant performance. Present investigations were undertaken to evaluate different breeding approaches to develop genotypes possessing multiple resistance to different biotic and abiotic stresses coupled with enhanced productivity. The experimental material consisted of 90 genetically diverse germplasm lines of both *desi* and *kabuli* types, different promising donors used in the hybridization and 32 improved new genotypes including checks for multi location testing during 2000 - 2002. The evaluation of 90 germplasm lines consisting of 35 *desi* medium seeded, 35 *desi* bold seeded, 10 *kabuli* medium seeded and 10 *kabuli* bold seeded was carried out under stress and non-stress environments. The experiments were conducted in randomized block designs with three replications in four row plots at New Delhi during 1996 - 1999. The evaluation of these germplasm lines revealed that *desi* bold seeded genotypes showed superior performance under moisture stress environment over other groups of cultivars. The second important finding that emerged from this assessment was that *kabuli* bold seeded genotypes are suitable for non-stress environments as they out-yielded other cultivars under irrigated plantings. In the second part of the investigation multiple hybridization was carried out between desirable donors and F_1's for the development of high yielding *kabuli* types, to incorporate multiple resistance and to introgress alien genes for higher productivity and adaptation. From this investigation more than 25 genotypes were identified as promising genotypes which were then tested for yield and adaptation at various locations in India. From the second part of the investigation 6 *kabuli* high yielding genotypes with wide adaptation having drought tolerance were identified. The approach of pyramiding genes for multiple resistance proved very useful in identifying 10 lines possessing multiple resistance. The approach of introgression of wild genes was also found to be very useful in producing promising genotypes which showed high yield potential, resistance to soilborne diseases and adaptation to moisture stress environments. From these experimental approaches, it was concluded that high productivity, multiple resistance and wide adaptability can be achieved simultaneously by using potentially complementary approaches.

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Rapeseed as a model to analyse 'fixed' heterosis in allopolyploid plants

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Abstract
The spontaneous hybridisation of related species by combining their genomes (allopolyploidy) has played a prominent role in plant evolution. An important reason for the success of allopolyploids are the favourable interactions between genes on their homeologous chromosomes ('fixed' heterosis), analogous to the interactions between homologous chromosomes in heterozygous plants of diploid species ('classical' heterosis). Fixed heterosis may also play a prominent role in diploid species because even in many diploids large genome segments are duplicated. *Brassica napus* (AACC genome) is a very suitable model system to analyse 'fixed' heterosis because artificial 'resynthesized' lines can easily be developed from the diploid parental species *Brassica rapa* (AA) and *Brassica oleracea* (CC). Therefore a material will be created, which is completely balanced in the allelic contributions, but differs in the amount of heterozygosity (homozygous vs. heterozygous), ploidy (diploid vs. tetraploid) and genomic diversity (autotetraploid vs. allotetraploid).

In a first step 'resynthesized' rapeseed lines have been produced from double haploid lines of *B. rapa* and *B. oleracea* using embryo rescue. *B. rapa* was used as mother in all crosses. The crossability (number of cultivated embryos/number of crossed buds) showed large variation between the genotypes with a coefficient of variation of 70,5%. The influence of the father genotype was low and can mainly be explained by poor pollen quality. A diallel of eight *B. rapa* and eight *B. oleracea* parents and the corresponding 64 resynthesized lines is now available for investigation. In parallel tetraploids of the used *B. rapa* and *B. oleracea* lines are produced.

In a next step intraspecific crosses will be done and finally all genotypes will be analysed phenotypically for vegetative biomass production to study the importance of polyploidy, 'fixed' heterosis and 'classical' heterosis due to heterozygosity.

The following hypotheses will be investigated:
- 'fixed' heterosis plays an important role in performance of allopolyploids,
- 'fixed' heterosis is larger under stress conditions,
- 'fixed' heterosis is larger when parents are combined which show large classical heterosis,
- 'classical' heterosis on allopolyploid level is low when the allopolyploids show large 'fixed' heterosis.

This material will also provide unique possibilities for a detailed analysis of the molecular, biochemical and physiological causes of "classical" and "fixed" heterosis.

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The European *Brassica napus* core collection – opportunities of utilization from a breeder's point-of-view

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Abstract
Core collections are being established with the intention of providing coverage of the maximum possible variability available within existing gene bank material in a representative set of well-characterised genotypes. In order to improve the utilisation of the *Brassica* gene pools in Europe by plant breeders and growers, the EU genres project CT99 109-112 was seeking to increase the knowledge about the genetic resources available within the four important *Brassica* species i.e. *B. oleracea*, *B. rapa*, *B. napus* and *B. carinata*. The species *B. napus* primarily represents oilseed rape (ssp. *oleifera*) with spring and winter types. Additionally, it comprises leafy fodder and green manure types (ssp. *napus*) of spring and winter forms. Swedes or rutabagas (ssp. *napobrassica*) are cultivated as a vegetable and for forage purposes and finally, a mixed group of 'exotic' material primarily is used as vegetables, e.g. leaf rape, hakuran or Siberian and Asparagus kale types.

In the course of pre-evaluation field trials a basic differentiation of about 1100 *B. napus* gene bank accessions was achieved with regard to morpho-agronomic characters and seed quality traits. Together with the information on country of origin and breeding pedigree a preliminary *B. napus* core collection of 150 - 200 accessions was created being evaluated regarding resistance to clubroot disease (*Plasmodiophora brassicae*) and important pests including flea beetles (*Psylliodes chrysocephala*), stem weevils (*Ceutorhynchus* spp.) and field slugs (*Deroceras* spp.). Additionally, the seed quality was analysed comprising parameters such as oil-, protein- and glucosinolate content as well as fatty acids composition of the seed oil. The final core collection of 150 accessions is equally covering the wide variation observed for the evaluated traits and representing the extreme values of all the resistance screenings as well as the high and low values of the seed quality analyses. The detailed results of the screenings are available from the ECP/GR central *Brassica* crop database (BrasEDB) website (http://www.cgn.wageningen-ur.nl/pgr/collections/brasedb/) and may be used directly for selecting accessions for breeding and research.

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Utilization of various self-sterility systems in hybrid breeding of winter oilseed rape

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Abstract
There are several pollination control systems being developed and used for hybrid seed production in *Brassica napus*. Breeding of winter oilseed rape hybrid cultivars in the Czech Republic is based on three self-sterility systems including self-incompatibility (SI), CMS ogu-INRA and CMS Shaan 2. The original lines with recessive determined self-incompatibility were derived from some older cultivars and breeding materials with a high glucosinolate (GSL) content. Stabilization of SI character and improved seed quality has been achieved by crossing SI lines with self-compatible (SC) donors of double low quality and subsequent deriving of doubled haploids. This method is also utilized for improving agronomic traits, mainly yield parameters of produced SI lines. Molecular markers have been developed for screening of self-incompatible genotypes. Two different CMS systems were chosen to investigate the possibility of their practical application in breeding hybrid cultivars. Initial CMS Ogu-INRA lines and particularly their fertility restorers (Rf) from France showed a high GSL content due to the introgression of *Raphanus* genome. Improved CMS lines were obtained by repeated crossing with donors of quality. It is possible that the decrease of GSL content in Rf lines could be attained by development of doubled haploids from perspective F_1 combinations of CMS lines and fertility restorers. CMS Shaan 2 and Rf lines originating from China are considered to be related to Polima CMS system which is characterized as less stable under European climatic conditions. The lines possessing reliable sterility were obtained by means of repeated selection. All home breeding materials used showed to be maintainers of sterility. Sufficient seed quality of CMS and Rf lines was achieved by six backcrosses with double zero quality DH lines derived from various hybrid combinations.

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Outcrossing frequencies and distribution of transgenic oilseed rape (*Brassica napus* L.) in the nearest neighbourhood

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Abstract
Pollen dispersal from transgenic oilseed rape (OSR) can lead to unintended transmission of the transgenic DNA sequence to neighbouring non-transgenic OSR crops. This is of major concern especially if it causes quality losses in conventional crops by exceeding the actual EU labelling threshold of 0.9 % for transgenic contamination in food and feed. According to the new EU regulations, the co-existence of gmo, non-gmo and organic production must be guaranteed by appropriate measurements.

In order to determine the outcrossing frequencies from transgenic OSR in non-transgenic OSR within short distances, a two year field experiment was carried out. 6 m x 6 m plots with plants of the herbicide-tolerant transformation line 'Falcon GS40/90' containing the *pat*-gene for resistance towards the broad-range herbicide glufosinate-ammonium were surrounded by eight plots with the non-transgenic, isogenic variety 'Falcon'. In the second year, we also examined the outcrossing rates from plots with different ratios of transgenic plants (1.0 % and 0.1 %) in order to simulate transgenic seed contaminations.

120,000 seeds from defined sampling points were tested for the the *pat*-gene in 2002 and 300,000 seeds in 2003, respectively, by spraying the seedlings at the four leaf stage with a 1 % solution of BASTA®. Putative transgenic seedlings were verified in subsequent PCR reactions with *brassica*- and *pat*-specific primers. The replicated average outcrossing rates within a distance of 3 - 11 m from 100 % transgenic plots were consistent and ranged from 0.25 % - 0.29 %. The transgenic contamination in neighbouring OSR crops was therefore below the EU labelling threshold of 0.9 %. The 'seed contamination' with 1 % and 0.1 % transgenic plants resulted in outcrossing frequencies of 0.01 % and 0.0065 %, repectively.

The distance from the central 100 % transgenic donor plots was proved to affect the outcrossing rates significantly. We observed an exponential decline within a distance of 3 - 11 m. In contrast, no statistical correlation was found between the wind distribution during the whole flowering time and the distribution of the outcrossing events. Outcrossing rather seemed to be undirected and caused mainly by insects like honeybees or bumblebees. The decline of outcrossing events with increasing distance from the transgenic plots could be described by a well-fitting exponential curve.

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Predicting the flowering cycle of Indian mustard plants through the glucosinolate analysis of the seed by near-infrared spectroscopy

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Abstract

Indian mustard (*Brassica juncea* Czern. & Coss.) is a plant species characterised by the presence of a high concentration of erucic acid and a large variability in the glucosinolate pattern of the seed. Whereas *B. juncea* genotypes of European or North American origin contain from 150 to 200 μmol g^{-1} of sinigrin (2-propenyl glucosinolate) (oil extracted, air-dried meal), genotypes from the Indian subcontinent contain variable amounts of sinigrin and gluconapin (but-3-enyl glucosinolate). In addition, other minor glucosinolates are also present in the seed, such as 4-hydroxyglucobrassicin. The glucosinolate pattern exhibited by *B. juncea* seed makes it one of the most promising species as a potential source of variability for glucosinolates.

The Department of Agronomy and Plant Breeding (Institute of Sustainable Agriculture, CSIC, Córdoba, Spain) holds a germplasm collection of *B. juncea* from Europe and the Indian subcontinent, showing a large variability in its oil and meal composition, including glucosinolates. This germplasm collection has been the basis for developing near-infrared spectroscopy (NIRS) research in recent years (Font et al. 2003).

We have found through experience in field trials performed at IAS, Córdoba, that the particular pattern of glucosinolates found in the seed of this species, is related with the flowering cycle of the plant. According to this, genotypes containing mainly sinigrin show a larger flowering cycle than those having gluconapin as the main glucosinolate. This correlation allows us to infer the flowering date from the glucosinolate pattern of the seed.

In order to support this fact, glucosinolate determinations have to be done through their normal occurrence ranges, which implies the analysis of hundreds of seed samples to cover the whole distribution of these compounds in the plant. Thus, the success of the experiment implies the use of fast analytical techniques that allow accurate results. In a previous work (Font et al. 2004), we reported the use of near-infrared spectroscopy (NIRS) as a suitable technique for predicting individual and total glucosinolates in *B. juncea* seed.

The purpose of this work was to study the relationship existing between flowering cycle and glucosinolate composition in the seed of *B. juncea* in field assays. To do this, NIRS was used as a rapid, cheap and sufficiently accurate technique for the analysis of glucosinolates and for predicting the flowering cycle in *B. juncea*.

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On the genetics and histology of the hull-less seed character of Styrian oil-pumpkin (*Cucurbita pepo* L.)

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Abstract

The genetics of the hull-less seed character was studied in progenies derived from three crosses between oil-pumpkin varieties as the hull-less seed and two zucchini and one crock-neck varieties as the hulled parents. All F_1 plants were selfed to produce F_2 populations. In one case F_3 and F_4 generations were already obtained. The seed coat of the F_1 was of a completely hulled type and segregated in the next generation 3 : 1, hulled vs. hull-less seeds. However, for some F_2 plants the hull-less seeds were not completely hull-less exhibiting a residual lignification. Following a visual examination of the hull-less progeny, the seed coat types were classified in 3 categories. They did not fit into any simple Mendelian segregation pattern. Histological observations did not correspond to the three visually established categories, instead giving a more or less continuous variation with respect to the degrees of the residual lignification. Segregation of the hull-less categories in F_3 showed varying levels of residual lignification independent of the F_2 category from which the F_3 progeny was derived.

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Introducing resistance genes against zucchini yellow mosaic virus (ZYMV) into Styrian oil-pumpkin with classical and molecular selection methods

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Abstract

A catastrophic virus epidemic in 1997 has shown that Austrian oil-pumpkin varieties (*Cucurbita pepo* var. *styriaca*) do not possess any genetic protection against the zucchini yellow mosaic virus (ZYMV). The loss of half of the pumpkin harvest in this particular year prompted the initiation of a 'Pumpkin-Project' with the purpose of introducing resistance genes into Austrian pumpkin breeding material and developing molecular markers to aid selection. Financial support for the project was granted by the federal government, by the mostly affected provinces of Styria, Burgenland and Lower Austria, as well as by the private plant breeding company Saatzucht Gleisdorf. In the past five years the project resulted in the introduction of two different resistance genes, both originating from *C. moschata*, a closely related species to *C. pepo*. Several RAPD markers were developed for one of the resistance genes. Two of them are now routinely being used in selection and in combination with resistance reaction of the plants for pyramiding resistance genes. Interspecific crosses were carried out successfully between *C. pepo* and *C. moschata* genotypes, thereby introducing new genetic variation into the former including further resistance genes against the virus and against mildew.

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Genotyping of released safflower (*Carthamus tinctorius*) varieties by DNA fingerprints

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Abstract
Carthamus tinctorius (2n=2x=24) (family *Asteraceae*), commonly known as safflower, is widely cultivated in various agricultural production systems of Asia, Europe, Australia and the Americas as a source of high quality vegetable and industrial oil, and as a feed for livestock. India ranks first in the production of safflower oil. Inspite of safflower being one of the major oil crops in the world, it has received very little attention from geneticists and cytogeneticists alike. More notably, in comparison to other oil yielding crop species, safflower has been the focus of relatively little, almost nil, research with respect to characterization by molecular markers. In the past few decades, India has released 14 high oil yielding varieties suited for various agro-climatic regions of the country. For reasons more than one, these varieties have been comprehensively fingerprinted by RAPD, ISSR and AFLP markers utilizing 36 and 21 primers, and 4 primer combinations, respectively. RAPD and ISSR markers with individual primers were not able to discriminate more than 4 varieties at a time, whereas, one primer combination in AFLP fingerprinting could distinguish 13 out of 14 varieties. Two primer combinations have been recognized in the present study which were able to individually fingerprint all the 14 varieties explicitly. Our results also show that AFLP is the most suitable marker for measuring genetic diversity as well as fingerprinting the world safflower germplasm resources.

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Oliv-Track - A European project for olive oil traceability

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Abstract
Olive oil is economically very important for Mediterranean countries and its use is considered positively for human consumption and health diet. Therefore, safety issues related with purity (non adultered oils), quality and traceability of this product are of most relevance. Traceability is particularly important in the case of monovarietal and DOP olive oils. A molecular approach is foreseen in order to correlate DNA extracted from olive oils with DNA molecular markers of the cultivars from which the oil was produced.

Oliv-Track is a European project that involves 14 partners from 6 countries. The co-ordinator is Nelson Marmiroli from Parma University, Italy. In order to achieve the general goals, the 11 working groups will concentrate their objectives on: feasibility study and genomics of olive oil; molecular markers for olive cultivars; genomic and metabolic profiling; development of a technological platform; forensic analysis of olive oils; and dissemination and exploitation of the results. Access by internet to an integrated information on European cultivars, their characterisation, agronomic performance, regional distribution, oil composition, etc. is also available for general and specialised public.

The Centre of Genetics and Biotechnology of the University of Trás-os-Montes and Alto Douro (CGB-UTAD) is one of the two Portuguese groups envolved in OLIV-TRACK, and it is responsible for cultivar database and collaborates in the development of DNA markers. Data obtained by one of the Portuguese teams (CGB-UTAD) are presented in 'Variability analysis between different Portuguese *Olea europaea* L. cultivars by RAPD and ISSR'.

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Varietal response of potato, bean and corn to intercropping

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Abstract
This study aimed to evaluate the varietal response of potato and bean as they are intercropped with corn and with each other. Three varieties of potato ('Sponta', 'Agrico' and 'Alaska'), three varieties of bean ('Bronco', 'Matadore' and 'Lolita') and one variety of corn ('Jubilee') were used. Light interception and leaf area were measured in order to find out their effect on yield of crops. The results revealed that only two potato varieties ('Sponta' and 'Agrico') gave significantly higher yields when they were intercropped with corn and with the three bean varieties as compared with their pure stands. However, bean varieties were more beneficial to potato 'Sponta', while corn was more beneficial to potato 'Agrico'. On the other hand, potato 'Alaska' grown with bean varieties gave an increase in yield of 17 % - 32 %, while with corn gave a reduction in yield of 61 % as compared with its pure stand. This reduction was related to a significant decrease in light interception and leaf area values. Therefore, not all varieties of the same crop behaved similarly with the associated crop due to their microenvironment requirements such as light interception. The significant reduction in values of both light interception and leaf area obtained by bean 'Lolita' as it was grown with corn gave the highest yield. In contrast, the yield of bean 'Matadore' grown with corn, decreased significantly due to a significant decrease in values of light interception only, as compared to its sole crop, while the reduction of light interception obtained by bean 'Bronco' intercropped with corn did not affect its yield. In addition, yield of the different bean varieties was almost not affected by the associated potato varieties. Corn planted with either bean or potato varieties gave significantly higher yields, and it was not affected by their varieties. Regardless of the variety used, the land equivalent ratio values indicated clearly the superiority of intercropping over the sole cropping system.

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Androgenesis as a means of dissecting and selecting useful gene combinations for breeding stress tolerant grasses

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Abstract

A novel approach to plant breeding using androgenesis to enhance gene expression and thereby to aid selection of genes for complex traits is described. *Lolium multiflorum* x *Festuca pratensis* (2n = 4x = 28) amphiploid cultivars have undergone considerable genome reorganisation during their development which can be visualised using genomic in situ hybridisation (GISH). One outcome of this was genomic imbalance in favour of the *Lolium* genome. Large populations of primarily dihaploid plants (n + n = 14) derived from a single parent plant may be regenerated following anther culture from such amphiploid *Festulolium* cultivars. These comprise genotypes with a range of gene expression for traits such as drought resistance or freezing-tolerance, frequently in excess of that found either in the parent species or the parent cultivar from which they were derived. Many of the dihaploid lines have male and/or female fertility and can be backcrossed onto either *Lolium multiform* (2n = 2x = 14) in order to enhance the abiotic stress resistance of the *Lolium* cultivar, or onto *Festuca pratensis* (2n = 2x = 14) in order to enhance the forage quality of the *Festuca* cultivar. By applying molecular markers associated directly with genes transferred between *Lolium* and *Festuca* chromosomes, breeders' toolkits can be designed for subsequent use in the development of new cultivars harnessing desirable *Lolium* and *Festuca* traits. Furthermore, by recurrent backcrossing onto *Lolium* or *Festuca* species, complex traits derived from one or the other donor species are effectively 'dissected' into their component parts. Using these procedures different mechanisms found in *L. multiform* and *F. pratensis* for photosynthetic acclimation to low temperatures were elucidated and these were subsequently correlated with differences in freezing tolerance and winter survival.

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Cytogenetic characterization of tetraploid *Bromus ciliatus* genome

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Abstract
Tetraploid *Bromus ciliatus* L. is a North American bromegrass that has been placed in the *Pnigma* section of *Bromus*. The objective of this study was to characterize the genome of tetraploid *B. ciliatus* by cytogenetic methods and compare it to the genomes of the other species included in the section *Pnigma*. All plants of accession PI 232214 selected for chromosome counting were tetraploids (2n = 28). The mean 2C nuclear DNA content for tetraploid *B. ciliatus* was 19.13 ± 0.07 pg as determined by flow cytometery which is significantly greater than the tetraploid DNA content of *B. inermis* (11.74± 0.16 pg). C-banding procedures were used to identify individual mitotic chromosomes and to develop a karyotype for *B. ciliatus*. The genome of the tetraploid *B. ciliatus* consisted of 16 median chromosomes, 8 submedian chromosomes and 4 chromosomes with satellites including one pair with a large satellite and one pair with a small satellite. The general pattern of the distribution of constitutive heterochromatin in *B. ciliatus* was quite different than the other bromegrasses that have been analyzed to date including *B. inermis*, *B. riparius*, *B. erectus*, and *B. variagatus*. Except two pairs of chromosomes, all chromosomes in tetraploid *B. ciliatus* had telomeric bands on one or both arms. Some of the chromosomes with telomeric bands had centromeric bands situated at one or both sides of the centromere and intercalary bands which were generally absent in the other bromegrass species. It was possible to identify all chromosomes of tetraploid *B. ciliatus* and to match the pairs of homologous chromosomes by using chromosome lengths, arm length ratios and C-banding patterns. The results of this study indicate that tetraploid *B. ciliatus* has different genomes than the European species evaluated to date in the section *Pnigma*.

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Development of symbiotic systems between pasture legumes and soil microbes for restoring fertility of polluted and arid soils

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Abstract

Plant populations of the species *Psoralea bituminosa*, *Medicago ciliaris*, *Lotus edulis*, *L. ornithopodioides*, *Scorpiurus muricatus*, *Astragalus hamosus* (legume species), *Oryzopsis miliacea* and *Rumex* sp. (non-legumes), originated from Iglesias region (South Sardinia) and contaminated by heavy metals, were screened for their tolerance to cadmium, zinc and lead using hydroponics culture. Plant populations of the same species derived from seeds collected on unpolluted Asinara Island (North Sardinia) soils were included in these experiments for comparison. The root length, which indicates the growth rate of plants, was measured in the presence and absence of stress caused by heavy metals.

P. bituminosa, M. ciliaris, L. edulis and *L. ornithopodioides* leguminous species were most tolerant to the heavy metals studied in this work and selected for subsequent experiments. The resistance of these plant species to cadmium, zinc and lead is most probably determined by species genotype and did not result from the environmental conditions of the plant growth. Pot experiments have been performed to study the effect of inoculation of *P. bituminosa, M. ciliaris, L. edulis* and *L. ornithopodioides* plants with root nodule bacteria and plant growth-promoting rhizobacteria (PGPR) on the growth, nodulation efficiency and uptake of nutrient elements (N, P, K, S, Ca, Fe) as well as accumulation of heavy metals. The aim of the pot experiment was to study the capacity of symbiotic and non-symbiotic microflora to stimulate the growth of *M. ciliaris, L. edulis, L. ornithopodioides* and *P. bituminosa* plants under stress conditions caused by heavy metals.

Strains of root nodule bacteria were isolated for the inoculation purpose during this work from the nodules of mentioned legume plants growing in contaminated soil of Iglesias region. The PGPR strain having high tolerance to heavy metals was also used for the inoculation of plants. This strain was previously isolated from the soil of Iglesias region and identified as *Variovorax paradoxus* using sequences of 16S DNA gene.

The most efficient plant-bacteria associations having ability to promote plant growth and nodulation activity under stress conditions will be used in subsequent field trials. The biomass of inoculated and uninoculated plants will be compared, as well as the nodule formation, uptake of nutrient elements and accumulation of heavy metals.

Thus, it seems that *M. ciliaris, L. edulis, L. ornithopodioides* and *P. bituminosa* legume species are the most tolerant ones among the investigated plant species, chosen from those growing in Iglesias region and studied in this work. These legumes have high biomass and should be effectively stimulated by the inoculation with specific symbiotic bacteria and PGPR as well, and can be good candidates for the phytoremediation of soils polluted by heavy metals.

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Results and prospects of fruit crops breeding in Georgia

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Abstract
According to N. Vavilov's theory, Middle West Asia, which also includes Georgia, is recognised as one of the primary centres of origin of fruit crops. Proceeding from that fact, Georgia is characterised by a broad diversity of autochthonic varieties of fruit crops.

Georgia has rich traditions of targeting breeding to fruit crops. The controlled crossings with the utilisation of local varieties were initiated since the 20th century, but starting from 1930-1940 the basic broad scale scientific-research breeding activities are done at the Georgian S/R Institute of Horticulture, Viticulture and Wine making. The main purpose of fruit crops breeding was the development of fruits with high productivity, maturity time of wide spectrum, having taste abilities and resistance against diseases. The process of crossing mainly included local as well as introduced universal donor varieties.

As a result of long-term breeding programmes of this research Institute the following number of varieties of different fruit crops were bread: apple - 14, pear - 3, quince - 4, peach - 18, plum - 2 and cherry - 2 varieties. These varieties cover the significant areas in production assortment of Georgian fruits.

At present, due to the lack of funding assistance of breeding research activities, the institute maintained the breeding programmes only in direction of apple, peach and pear. During recent years the following varieties were released: two high desert fruit quality varieties of apple: 'Delisi' and 'Khorumi', 5 varieties of peach: 'Tsedisuri Tsiteli', 'Tsedisuri Kviteli', 'Mariami', 'Atenuri Yellow' and 'Khidistavis sakonservo' (Bobokashvili & Dzeria 2001, Kvaliashvili 2002). Despite of this, some advanced high-quality selections of pear, peach and apple are the last field testing stage, after which the Institute is supposed to register some new varieties.

As a priority aim for future Georgian fruit crops' breeding is defined the breeding for good fruit quality and high productivity, long storability with resistance to the key diseases of fruits, along with some other important horticultural traits.

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Genetic diversity in the 4H chromosomal region carrying *Vrn-H2* vernalization response gene in barley (*Hordeum vulgare* L.)

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Abstract
Vernalization response is one of the most important key components of both winter hardiness and heading date in cereals. Recently the candidate genes of two vernalization response loci have been identified and published (Yan et al. 2003, PNAS 100: 6263-6268; Yan et al. 2004, Science 303: 1640-1644). The wheat *Vrn2* gene - and its homologue *VrnH2* gene in barley - encode repressors of the *Vrn1* and *VrnH1* loci, respectively. The *Vrn2* and *VrnH2* loci are down-regulated by vernalization. We mapped the *VrnH2* gene, together with the reportedly linked *HvSnf2* transcriptional regulator gene, on the most distal part of chromosome 4H. For mapping, we used a doubled haploid population derived from the cross of 'Dicktoo' x 'Kompolti korai' (Karsai et al., unpublished). We characterized 56 barley varieties of different geographic origin and that represent different growth habits and inflorescence types for alleles at *VrnH2* and *Hvsnf2* and two additional loci that map to this region of chromosome 4H: OPS3-430 (RAPD) and HvM67 (SSR). These marker loci are located 6.7 and 8.8 cM upstream from *VrnH2*, respectively. The presence or absence of the *VrnH2* gene was in high correlation with growth habit (r = 0.764***). The gene is missing from three of the 27 winter barleys ('Scio' and 'Dicktoo' (USA) and 'Botond' (Hungary)). The gene is present in three of the 29 spring barleys ('Maresi' and 'Bitrana' (Germany) and the CIMMYT selection *LBIran*). *VrnH2* showed a weak, but significant correlation with heading date under autumn sown and spring sown field conditions (r = –0.293* and r = 0.261*, respectively). The *VrnH2* allele state was highly correlated with the difference in heading date between the two planting seasons (r = –0.612***). The two upstream markers were correlated with head type, but not with growth habit. The larger amplicon allele for HvM67 and the presence of the 430 bp band for OPS3 were characteristic of 25 of the 29 two-rowed varieties, while smaller amplicon and the absence of the 430 bp band for OPS3 were characteristic of 21 of the 27 six-rowed varieties. No genes determining inflorescence type are reported in this region of the genome.

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Genetic engineering of β-glucan contents of oat

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Abstract
The health benefits of oat (*Avena sativa* L.) are mainly associated with its mixed-linked β-glucan. Mixed-linked β-glucan is not metabolised by digestive enzymes. It lowers the cholesterol levels of blood and balances the glucose and insulin contents of serum after meals. These physiological effects reduce the risks of cardiovascular diseases. Our aim is to increase the β-glucan content of Finnish oat cultivars through genetic engineering. The ultimate aim is to use plant-derived genes to elevate the β-glucan content of oat to levels not obtainable through traditional plant breeding methods.

Embryogenic cell cultures were started from mature embryos of oat cultivars 'Aslak', 'Veli' and 'Kolbu'. Microscopic- and HPLC-analysis of β-glucan of seeds, apical meristems and cell cultures were carried out. Gene transfer of microbial 1,3-β-glucan synthases by particle bombardment was performed.

The β-glucan was mainly localized in subaleurone layers of oat seeds by Calcofluor staining. The molecular weight of oat seed β-glucan was ca. 2 000 000 and the amount varied from 40 to 60 g/kg. In apical meristems trace amounts of β-glucan were observed. In cell cultures the molecular weight of the β-glucan was ca. 200 000 and the amount varied from 2 to 3 g/kg. Gene transfer experiments with microbial 1,3-β-glucan synthase genes have been started in order to evaluate their effect on β-glucan contents of oat cell lines. The cloning of plant β-glucan synthase genes is on the way.

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Genetic resources for the creation of an undersown and perennial oilcrop

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Abstract

The future agricultural crop production faces the challenge of producing high yields with reduced energy inputs and reduced environmental effects. Cropping systems with undersown and/or perennial crops save energy by fewer sowings and reduced tillage. They also prevent leaching of plant nutrients to the ambient aquatic environment by reduced tillage and vegetative growth in periods critical for leaching. The wild biennial *Lepidium campestre* has a life cycle well suited for undersowing in a spring cereal. It has a good agronomic plant type, high seed yield, good winter hardiness and an oil composition that can be adapted to an industrial quality. Close relatives show a perennial life cycle. This means that *L. campestre* should be possible to domesticate as a new undersown and perennial oilcrop. Such a crop plant would mean the establishment of a cropping system with lower fuel consumption and less leaching of plant nutrients compared to the present systems with only annual oilcrops. Efforts to domesticate *L. campestre* have been started. Work on shattering resistance, oil composition and perenniality is reported.

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Studying apple biodiversity: opportunities for conservation and sustainable use of genetic resources

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Abstract

The genetic resources of *Malus sylvestris*, the apple tree native to Western and Central Europe, are endangered. Forest decline and forest fragmentation have caused a reduction of suitable habitats. Moreover, the biodiversity and genetic identity of *M. sylvestris* is threatened due to possible hybridisation with the omnipresent cultivated apple (*M. x domestica*). For the development of conservation strategies, besides a thorough analysis of the extent and geographical distribution of genetic diversity in wild populations, it is of paramount importance to discriminate between 'genuine' wild genotypes and genotypes derived from or closely related to cultivated varieties.

Genetic diversity in *Malus* is also of great importance for the apple breeder; the amount of genetic diversity available for breeding will determine the progress and success of apple breeding programs. At present however, the genetic base used by apple breeders is limited and genetic diversity in modern apple cultivars is possibly decreasing. To avoid future problems resulting from inbreeding and to introduce new traits, genetic diversity in apple breeding programs has to be renewed. Old *M. x domestica* varieties, *M. sylvestris* and other *Malus* species from the region of origin may be suited genetic resources to expand genetic diversity in modern apple.

In this study we apply different types of molecular markers in combination with morphological traits to study different levels of organisation of genetic diversity. The vast majority of wild apple trees in Belgium has been localised and leaf and graft material collected for ca 700 trees. Material from 500 old regional varieties and 100 modern varieties are included in the study. In order to place the Belgian *Malus* diversity in a European context, we also included wild apple trees from France, Germany and Denmark and cultivated apples from France, UK and Denmark. The phenotypes of all trees will be described, including morphologic parameters and disease resistance traits. An in-depth study of the Belgian *Malus* gene pool (both the wild gene pool as the regional cultivated gene pool) will be enabled through use of neutral molecular markers (microsatellites and SSAP, an AFLP-like method to visualise retrotransposon insertions), study of functional diversity (with markers derived from sequences of gene families involved in disease resistance and establishment of the self-incompatibility genotype) and investigation of phylogenetic origins.

This study will thus result in a extensive description of the biodiversity of the Belgian *Malus* gene pool, reveal information on the present distinctness between wild and cultivated apples and give insights into past and present hybridisation events. Based on these findings, conservation guidelines will be devised together with stakeholders and end-users. The usefulness of wild apple trees and regional varieties to expand the genetic basis of current apple breeding programs will be determined. Finally, the applied techniques and designed strategies will be evaluated for use in conservation programs for related *Rosaceae* species (*Pyrus pyraster*, *Prunus spinosa*, *Prunus avium*).

The project is coordinated by KU Leuven, CLO and CRA are partners. Subcontractors that delivered apple material are IBW, CRNFB and NBS.

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