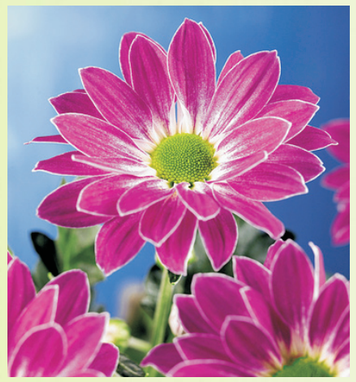
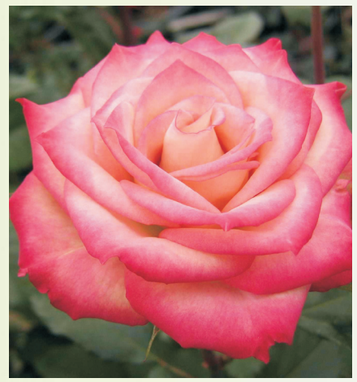
XXIIIrd International Eucarpia symposium, Section Ornamentals "Colourful Breeding and Genetics"

August 31 - September 4 2009
Leiden
The Netherlands





XXIIIrd International Eucarpia symposium, Leiden, The Netherlands

XXIII[rd] International Eucarpia symposium, Section Ornamentals "Colourful Breeding and Genetics"

Welcome

Dear Colleagues,

Welcome to the XXIII[rd] International Eucarpia symposium, section ornamentals "Colourful Breeding and Genetics", organized by Wageningen University and Research Centre in cooperation with Plantum NL and ornamental plant breeders of the Netherlands. This symposium aims to be a platform for and to exchange knowledge between scientists and plant breeders working on ornamentals from all over the world. This meeting has sessions with oral presentations on biodiversity, flower colour, interspecific hybridization, resistance breeding, plant breeder's rights, breeding and genetics, marketing and molecular breeding. In addition to the molecular breeding session there is a Molecular marker workshop to inform breeders and scientists on the prospects of molecular assisted breeding. In addition to the oral presentations 20 (out of 120) selected posters are presented in 5 minutes in two poster sessions. The historical location for the symposium is the beautiful just renewed Stadsgehoorzaal in Leiden, close to the ornamental breeding industry.

Such a meeting is only possible due to the over 250 participants from more than 35 countries, to the sponsors for their financial support and to the organizing committees for the many hours of work.

We hope that you will enjoy the XXIII[rd] International Eucarpia symposium, Section Ornamentals "Colourful Breeding and Genetics" as well as your stay in Leiden and the Netherlands.

Jaap van Tuyl
(Chairman of the section ornamentals of EUCARPIA)

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1. Organization of the XXIII[rd] International Eucarpia symposium, Section Ornamentals "Colourful Breeding and Genetics"

Convener

Jaap van Tuyl

Organizing Committee

- Jaap van Tuyl, Plant Breeding - Wageningen UR
- Sjaak van Heusden, Plant Breeding - Wageningen UR
- Sjoukje Heimovaara, Royal van Zanten BV
- Kees van ´t Hoenderdal, Dekker Chrysanten BV
- Johan Van Huylenbroeck, ILVO - Belgium
- Wendy ter Laak, Beekenkamp Plants BV
- Ronald Snijder, Royal van Zanten BV
- Thijs Simons, Plantum NL

Scientific Committee

- Jaap van Tuyl, The Netherlands
- Alain Cadic, France
- Thomas Debener, Germany
- Dik de Vries, The Netherlands
- Sjaak van Heusden, The Netherlands
- Johan Van Huylenbroeck, Belgium
- Antonio Mercuri, Italy
- Masahiro Mii, Japan
- Teresa Orlikowska, Poland

2. Sponsors

Main sponsor

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www.sandegroup.nl

www.royalvanzanten.com

www.bromelia.com

www.vletterdenhaan.nl

www.rijnplant.com

3. General information

Congress Venue

Stadsgehoorzaal Leiden
Breestraat 60
2311 CS Leiden
The Netherlands

Congress secretariat

The registration takes place at the reception desk at the entrance of Breestraat 60.
The persons dealing with the registration are Niek Botden (only the first day), Mariame Gada (tel 06 234 442 65) and Jelle van den Haak (06 242 401 80).

Catering

Lunches will be served in the Foyer of the “Aalmarkt” (also called “Kleine Zaal”).
The symposium dinner will be served in the “Nuon zaal “(also called “Breezaal”).

Speakers

Please deliver your presentation as soon as possible at the congress reception desk.

Lectures

All lectures will be given in the “Kleine Zaal”.

Poster sessions

Poster set-up will start on Monday, August 31, 2009 at 18.00 in the Foyer.
Please check your poster number in the author’s index.

Internet access

For the participants of the congress internet is available in a meeting room on the 3rd floor.

4. Excursion

A choice between the 4 tours should be made in advance, list at the reception desk.
All Tours from 05.45 till 07.00:
05.45 Bus leaves from Stadsgehoorzaal (very strictly!)
07.00 Auction

Auction Aalsmeer for tours 1 and 3
Auction Naaldwijk for tours 2 and 4
09.00 - 15.00 Companies (see below)
17.00 Hotel

Tour 1 Aalsmeer region

Hilverda De Boer (cash&carry)
Hilverda Kooij (Alstroemeria & Dianthus)
Florist de Kwakel (Gerbera)

Tour 2 Westland region

Fides (Chrysanthemum & pot and bedding plants)
Beekenkamp Ornamentals (pot and bedding plants)
NAKTuinbouw (Plant diagnostics & breeding rights)

Tour 3 Syngenta, North Holland region

Syngenta Flower (bedding plants)
Syngenta Seed (Vegetables)

Tour 4 Research, Wageningen region

Plant Science Group (Wageningen University and Research Centre)
Keygene (Molecular Markers)

5. Programme 23rd EUCARPIA SYMPOSIUM - Section Ornamentals "Colourful Breeding and Genetics"

Monday 31 August

Welcome reception and registration

16.00 - 20.00 Registration
18.00 - 20.00 Welcome reception

Tuesday 1 September

07.30 - 12.00 Registration

08.30 - 08.35 Opening by Jaap van Tuyl

Introduction and Biodiversity and Convention of Biological Diversity (CBD) treaty *Chair: Dik de Vries*

08.35 - 09.00 **Doeke Faber** (Chairman, Dutch Flower Auctions Association, NL) **L1**
Ornamental horticulture: where does it end?

09.00 - 09.30 **Orlando de Ponti** (President International Seed Federation, ISF) **L2**
Access to Biodiversity: New Rules of the Game

09.30 - 09.50 **Birte Lorenzen** **L3**
Access and benefit-sharing under the CBD - what consequences might an international regime have for the horticultural sector?

09.50 - 10.10 **Leila Samiei** **L4**
In search of genetic variation in *Rosa foetida* Hermann in Iran

10.10 - 10.30 **Qinglin Liu** **L5**
Biodiversity and ornamental plant breeding in China

10.30 - 11.00 Coffee/tea break

Flower Colour *Chair: Alain Cadic*

11.00 - 11.30 **Yoshikazu Tanaka** **L6**
The long, winding genetic modification path to more colourful flowers; blue, red and yellow

11.30 - 11.50 **Ellen de Keyser** **L7**
Flower colour as a model in azalea for integration of phenotype, genotype and gene expression

11.50 - 12.10 **Virginia Gitonga** **L8**
Inheritance of determinants of flower colour in tetraploid roses

12.10 - 13.30 Lunch

Interspecific hybridization and polyploidy *Chair: Jaap van Tuyl*

13.30 - 14.00 **Masahiro Mii** **L9**
Breeding of ornamental plants through interspecific hybridization using advanced techniques with a special focus on *Dianthus*, *Primula*, *Cosmos* and *Kalanchoe*

14.00 - 14.20 **Mark Bridgen** **L10**
Interspecific hybridization of *Alstroemeria* for the development of new, ornamental plants

14.20 - 14.40 **Tom Eeckhaut** **L11**
Morphological and anatomical characterisation of chemically induced polyploids in *Spathiphyllum wallisii*

14.40 - 15.00 **Gildas Gâteblé** **L12**
Advances in *Oxera* Breeding

15.00 - 15.20 **Valéry Malécot** **L13**
Polymorphic ITS as a tool to identify hybrids and their parents in cultivated Genisteae (Fabaceae)

15.20 - 15.40 **Ed Morgan** **L14**
Generating and delivering novelty in ornamental crops through interspecific hybridization: some examples

15.40 - 16.10 **Coffee/tea break**

Short presentations *Chair: Antonio Mercuri*

16.10 - 16.15 **Rodrigo Barba-Gonzalez** **LP1**
Chromosome identification on the genus *Lilium* using comparative genomic *in situ* hybridization (CGISH)

16.15 - 16.20 **Matteo Caser** **LP2**
Discriminating capacity of nucleotide binding site (NBS) and *MYB* gene profiling for genetic analysis of *Campanula* ecotypes

16.20 - 16.25 **Malgorzata Czernicka** **LP3**
Verification of the hybrid character of interspecific *Rhododendron* progeny by molecular tools

16.25 - 16.30 **Emmy Dhooghe** **LP4**
Production and characterization of intergeneric hybrids between *Anemone coronaria* and *Ranunculus asiaticus*

16.30 - 16.35 **Marisé Borja** **LP5**
Breeding system of *Glandularia* species native to Argentina

16.35 - 16.40 **Yoon-Jung Hwang** **LP6**
Library construction from micro dissection of chromosome #1 in lily (*L. lancifolium*)

16.40 - 16.45 **Agnieszka Marasek** **LP7**
Introgression breeding in genus *Tulipa* analysed by GISH

16.45 - 16.50 **Jiten Sharma** **LP8**
Morphological and molecular characterization of intergeneric hybrids between the orchid genera *Renanthera* and *Vanda*

16.50 - 16.55 **Anta Sparinska** **LP9**
Diversity in *Rosa rugosa* x *Rosa hybrida* interspecific varietie

16.55 - 17.00 **Werachai Tera-arisiri** **LP10**
Breeding for resistance and biocontrol of wilt disease in *Curcuma alismatifolia* Gagnep. by *Bacillus* spp.

17.00 - 17.15 **EUCARPIA business meeting**

Wednesday 2 September

8.00 - 10.00 Registration

Resistance breeding *Chair: Teresa Orlikowska*

08.30 - 09.00	**Thomas Debener**	**L15**
	Current strategies and future prospects of resistance breeding in ornamentals	
09.00 - 09.20	**Arwa Shahin**	**L16**
	Conversion of molecular markers linked to *Fusarium* and virus resistance in Asiatic hybrid lilies	
09.20 - 09.40	**Carole Koning-Boucoiran**	**L17**
	Specific mapping of disease resistance genes in tetraploid cut roses	
09.40 - 10.00	**Antra Balode**	**L18**
	Breeding for resistance against *Botrytis* in lily	
10.00 - 10.20	**Kullanart Obsuwan**	**L19**
	A dysfunctional CymMV movement protein gene confers resistance to CymMV in *Dendrobium* orchid	

10.20 - 10.50 Coffee/tea break

Plant Breeder's Rights *Chair: Lidwien Dubois*

10.50 - 11.20	**Judith Blokland**	**L20**
	Can we still take the breeder's exemption for granted?	
11.20 - 11.40	**Ben Vosman**	**L21**
	Essentially derived varieties in ornamentals	
11.40 - 12.00	**René Smulders**	**L22**
	Analysis of a database of DNA profiles of 734 hybrid tea rose varieties	
12.00 - 12.30	Discussion panel with Judith Blokland, Ben Vosman, Lidwien Dubois	

12.30 - 13.30 Lunch

Breeding and Genetics *Chair: Johan van Huylenbroeck*

13.30 - 14.00	**Riana Kleynhans**	**L23**
	Back to basics for new crop development	
14.00 - 14.20	**Neil Anderson**	**L24**
	Development of colored, non-vernalization-requiring seed propagated lilies	
14.20 - 14.40	**Dik de Vries**	**L25**
	Growth and development of cut rose clones; indirect selection for yield	
14.40 - 15.00	**Kell Kristansen**	**L26**
	In vitro mutagenesis of *Aster novibelgii* cultivars	
15.00 - 15.20	**Lazaro Peres**	**L27**
	Breeding the tomato micro-tom model system for ornamental value	
15.20 - 15.40	**Leen Leus**	**L28**
	Flow cytometry for plant breeding	

15.40 - 16.10 Coffee/tea break

Short presentations *Chair: Sjoukje Heimovaara*

16.10 - 16.15	**Pejman Azadi**	**LP11**
	A protocol for high rate *Agrobacterium*-mediated transformation of *Lilium*	
16.15 - 16.20	**Renate Müller**	**LP12**
	Transformation with *rol*-genes of *Agrobacterium rhizogenes* as a strategy to breed compact ornamental plants with improved postharvest quality	
16.20 - 16.25	**Antonio Mercuri**	**LP13**
	New genotypes of *Hibiscus rosasinensis* through classical breeding and genetic transformation	
16.25 - 16.30	**Youn-Hwa Joung**	**LP14**
	Investigation of the factors affecting cross-fertilization rate in rose	
16.30 - 16.35	**Supuk Mahattanapuk**	**LP15**
	Cloning of the ACC synthase gene from *Curcuma alismatifolia* Gagnep and its use in transformation studies	
16.35 - 16.40	**Mohsen Mardi**	**LP16**
	Assessing *Rosa persica* genetic diversity using amplified fragment length polymorphisms analysis	
16.40 - 16.45	**Norihiro Ohtsubo**	**LP17**
	Redesigning floral architecture: efficient modification of agronomic traits by CRES-T	
16.45 - 16.50	**Cristina Borghi**	**LP18**
	Kalanchoe x houghtonii: SSH and microarray analysis to screen genes involved in vivipary	
16.50 - 16.55	**Luca Pipino**	**LP19**
	Pollen characteristics affect seed production of rose cultivars	
16.55 - 17.00	**Hanneke Witsenboer**	**LP20**
	Application of crops® technology in a wide range of vegetable and field crops	

18.30 - 21.30 **Symposium dinner**

Thursday 3 September

Excursion day

Friday 4 September

Marketing *Chair: Thijs Simons*

08.30 - 09.00	**Susanne Lux**	**L29**
	Cooperative marketing – a way to stimulate sales and consumption of a plant?	
09.00 - 09.30	**Paul Roetenberg**	**L30**
	The brand: Frederique's Choice	

09.30 -10.10 **Coffee/tea break**

Molecular Breeding *Chair: Thomas Debener*

10.10 - 10.30 **Olga Shulga** **L31**
Early-flowering transgenic *Chrysanthemum* plants

10.30 - 10.50 **Phopgao Buddharak** **L32**
Isolation and transformation of *DFR* genes in *Curcuma alismatifolia* and *Clitoria ternatea* via *Agrobacterium tumefaciens*

10.50 - 11.10 **Frans Krens** **L33**
Oriental lily hybrids engineered to resist aphid attack

11.10 - 11.30 **Supatida Sirisawat** **L34**
DMMADS4, a *DEF*-like gene from *Dendrobium* is required for floral organ identity and flower longevity of orchid

11.30 - 11.50 **Marina Laura** **L35**
Over-expression and silencing of *KXHKN5* gene in *Kalanchoe x houghtonii*

11.50 - 12.10 **Marisé Borja** **L36**
Expression of an *Arabidopsis* aspartic protease in *Pelargonium*

12.10 - 13.30 **Lunch**

Workshop: molecular markers and their use in ornamentals **L37**

13.30 - 13.50 **Jan De Riek**
Overview of present use of molecular markers in Ornamental Breeding

13.50 - 14.10 **Paul Arens**
How to develop markers that can be used for identification of ornamental crops

14.10 - 14.30 **Sjaak van Heusden**
What do all the new developments in marker- and sequence technology mean for the use of markers in ornamentals?

14.30 - 14.40 **Hanneke Witsenboer**
The potential role of Marker Assisted Selection in breeding varieties

14.40 - 15.10 **Coffee/tea break**

15.10 - 15.40 **Round table discussion with all presenters of workshop**

15.40 - 15.50 **Closure by Alain Cadic**

6. Abstracts of lectures and short presentations

ORNAMENTAL HORTICULTURE: WHERE DOES IT END?

L1

Doeke Faber

Dutch Flower Auctions Association, Aalsmeer, The Netherlands
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Ornamental horticulture has developed over the past 50 years from a local industry to a global player. The horticultural sector was in the avant-garde of globalization. Already in the late 60's international trade became an important aspect of the horticultural sector. Because flowers and plants are time critical products, trade was initially limited due to transportation and temperature control. Present technology allows the movement of flowers across the globe by truck, train, boat and airplane.

The command of the supply chain reversed in the same period. The assortment of flowers and plants was largely determined by the growers till the late 90's, after that command in the chain was reversed; consumers determined what was to be produced.

Over time breeders and growers have learned how to interpret the wishes of the consumer; namely, learning from and working with the fashion industry, trend watchers, etc, the sector is continually trying to satisfy the ever changing tastes of the consumers. Over the past decade, it has been shown that consumer tastes are changing more rapidly as regards colours, shapes, types. At the same time, the consumer does not want to just buy flowers or plants, he wants to know the story behind the product, and he wants to buy sustainably and socially qualified produced flowers.

This changing behaviour has presented a challenge to breeders and growers alike! Innovation is therefore important; not only with regard to shape or colour or smell, but also with regard to the internal quality of the flowers or plants as regards disease resistance, temperature, growth, etc.

The marketing and sale of flowers and plants has also been subject to a real change; while the flower shop is still the most important outlet in many countries, the market share of retail stores, super markets, garden centres and DIY stores have increased significantly over the past decade and half!

Flowers and plants have been an important item in people's lives. We give flowers at birth, illness, marriage, death or other important occasions.

The challenge for the sector is to ensure that we enlarge the choice for consumers by offering a greater assortment of new products and at the same time create more occasions to give flowers!.

L2

ACCESS TO BIODIVERSITY: NEW RULES OF THE GAME

Orlando de Ponti (1)
Anke van den Hurk (2)

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(2) Plantum NL, Gouda, the Netherlands

Plant breeders, including plant breeders of ornamental crops, are dependent on genetic resources for the development of improved varieties. In the past the use of genetic resources was not regulated, access was free and genetic resources were considered a world heritage. Under the Convention on Biological Diversity CBD), ratified in 1993, genetic resources were no longer considered a world heritage, but were governed by the principle of national sovereignty. This resulted in entirely new basic rules for Access and Benefit Sharing (ABS), which still need to be further elaborated and implemented.

In 2004 the International Treaty on Plant Genetic Resources for Food and Agriculture (IT) was ratified. A so-called multilateral system for a limited list of plant species for food and agriculture is part of the IT. The ABS arrangements for the genetic resources that are part of the multilateral system are regulated through a Standard Material Transfer Agreement, which was approved in 2006. Through this agreement access is possible under reasonable standard benefit sharing arrangements. Most importantly, the breeders' exemption is recognized as a benefit on its own, and in that case mandatory payments to the IT for the use of the genetic resources are not required.

The species not falling under the multilateral system of the IT, including all ornamental species, fall automatically under the regime of the CBD. So, legal access to genetic resources of ornamental species can only be managed by bilateral agreements with the relevant national competent authorities of each specific country.

Legal access to valuable genetic resources for plant breeding not falling under the multilateral system of the IT is complex, if not impossible. However, both private and public breeders are responsible to take the necessary measures to have legal access to genetic resources, and possibly have to accept that some genetic resources are not (yet) available. As an international regime does not yet exist, ornamental breeders should strongly advocate for a workable ABS regime, which will re-establish efficient and fair access to these valuable resources.

ACCESS AND BENEFIT-SHARING UNDER THE CBD - WHAT CONSEQUENCES MIGHT AN INTERNATIONAL REGIME HAVE?

L3

Birte Lorenzen

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The international negotiations on an International Regime on Access and Benefit-Sharing are making progress; it is planned to adopt a system in 2010. Even though meanwhile at least part of the negotiators see a need for tailored solutions for different industries, horticulture and agriculture are held to have the same interests. But is this really the case? CIOPORA developed a position on the specialities and needs of breeders of asexually reproduced ornamental and fruit varieties.

We describe how access and benefit-sharing is carried out in the horticultural sector, what is different about the sector compared to others and show how a solution should look like for our sector. The basic assumption is, that by legal and practical means already without any additional administrative instruments sufficient benefit-sharing is executed and that a model like under the ITPGRFA might not be first choice for breeders of vergetatively reproduced ornamental and fruit varieties.

L4

IN SEARCH OF THE GENETIC VARIATION IN *ROSA FOETIDA* IN IRAN

Leila Samiei (1)
Roohangiz Naderi (1)
Ahmad Khalighi (1)
Ali-Akbar Bushehri (2)
Valiollah Mozaffarian (3)
Marinus JM Smulders (4)
Danny Esselink (4)

(1) Dept. of Horticulture, College of Agriculture and Natural Resources, Tehran Univ., Iran
(2) Dept. of Biotechnology, College of Agriculture and Natural Resources, Tehran Univ., Iran
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Iran is considered as one of the major centers of plant biodiversity. There are very diverse natural environments that lead to the high genetic diversity in plants. *Rosa foetida,* also known as Persian yellow briar, is one of the important species amongst 13 rose species that occur in Iran. It is a dense erect shrub (up to 2 m) with bright yellow or scarlet flowers with a yellowish reverse petal. It is most abundant in South West Asia. In Iran *R. foetida* occurs mainly in the north and west regions, notably in Tehran, West Azarbaijan and Kurdestan provinces, which are mountainous and contain very diverse habitats. In addition, some plants can be found in Eastern and Southern Iran. It is reported that this species is the strong yellow colour in modern roses. In this study we have used 10 microsatellite markers to determine diversity in *Rosa foetida* accessions collected across Iran. The dendrogram obtained using dice similarity coefficient resulted in only 2 genotypes. To our surprise, most of the samples collected showed the same genotype, even if they were collected at different sites, and only two accessions were representative the other genotype. The results are discussed in relation to breeding system, human influence and overall gene pool status.

BIODIVERSITY AND ORNAMENTAL PLANT BREEDING IN CHINA

L5

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Qinglin Liu

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China has a flower growing history of more than 2000 years, but the floricultural industry just began from the 1985 onwards. Floricultural industry not only contributes to enrich the people's spiritual life and to improve the living environment, but also is an important way to regulate the planting structure and to increase the farmers' revenue in China. Cultivars are the basic material of flower production. If only the new cultivars without Chinese breeder's rights were produced, it is no real sense to the Chinese floricultural industry. Although there are more than 5600 species of ornamental plants distributed in China, and 4400 cultivars bred in China, the commercial cultivated floricultural crops are mostly foreign species and cultivars. There is plenty of ornamental germplasm in China, but the often-used breeding materials are cultivars instead of species. Many ornamental species were bred in China, which mainly concentrated on some traditional famous flowers. However, pot plants, bedding flowers and woody garden plants are often involved. About 100 new flower cultivars were released annually, but few of them were used large-scale in floriculture or landscaping. Presently, the main problems to flower breeding in China are who should be the breeders and how to protect breeder's rights.

L6

THE LONG, WINDING GENETIC MODIFICATION PATH TO MORE COLOURFUL FLOWERS: BLUE, RED AND YELLOW

Yoshikazu Tanaka (1)
Steve Chandler (2)

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Flower breeding is constrained by the limited gene sources available within a target species. It is rare for a single species to have all possible flower colours, due to the lack all genes on the pigment biosynthesis pathway. Molecular breeding utilizing genetic engineering techniques has liberated breeders from this gene-pool constraint. For successful molecular breeding, it is necessary to isolate relevant genes, establish transformation systems, optimize expression of transgenes and obtain regulatory permission for both production and consumption. Though all these procedures are very often expensive and time consuming, the investment is long-term and a financial return is possible for transgenic flowers that meet the consumer's demand..

We have been developing transgenic flowers, with modified flower colour, for many years. Flavonoids and the coloured sub-class of compounds, anthocyanins, are dominant colour constituents of most flowers. The flavonoid biosynthetic pathway has been well studied and most biosynthetic genes have been obtained. It is feasible to generate white, yellow, red and blue flowers by engineering the pathway; both by over-expression of heterologous genes and/or down-regulation of endogenous genes.

The major cut-flower species rose, carnation and chrysanthemum, lack blue/violet cultivars because they do not accumulate the delphinidin-based anthocyanins that are present in most blue/violet flowers. Flavonoid 3',5'-hydroxylase (F3'5'H) is the critical enzyme for delphinidin biosynthesis and this gene is missing in species that do not accumulate delphinidin-based anthocyanins. An efficient transformation system was developed for rose and carnation and after introduction of the gene encoding F3'5'H transegnics were produced whose flowers accumulated delphinidin-based anthocyanins and an altered colour. Through careful choice of host cultivar and optimization of the expression of transgenes it has been possible to obtain flowers which accumulate delphinidin-based anthocyanins only. Such flowers exhibit an attractive colour change to blue/violet. Transgenic colour-modified carnation has been available commercially since 1996 and the first commercially available, colour-modified rose variety will be sold in 2009. From a biosafety perspective the transgenic flowers lines have no impact on the environment or health and pose negligible, if any, risk to biodiversity.

FLOWER COLOUR AS A MODEL IN AZALEA FOR INTEGRATION OF PHENOTYPE, GENOTYPE AND GENE EXPRESSION

L7

Flower colour is inherited as a semi-qualitative trait in azalea and is mainly determined by differences in anthocyanins and flavonols. A two-gene model is used to explain the phenotypic variation between white, brick red and carmine red colour: W in case the flower petals contain anthocyanins and Q if flavonols are present as co-pigments. However, the presence of flavonols in white flowers cannot be detected visually. Also, the existence of pink flowers is not explained by this two-gene model. Therefore, flower colour was determined on a crossing population using image analysis software and discriminant analysis was used for classification. Integration of the image analysis data as QTLs on a genetic map of the crossing population could be enlightening. A genetic map of 16 linkage groups was constructed. Besides anonymous AFLP and SSR markers also a set of functional markers were used. EST-markers were developed for four genes coding for key-enzymes in the flavonoid biosynthesis pathway. MYB-profiling, a sequence directed technique similar to NBS-profiling, generated in this crossing population fifteen dominant markers functionally related to the MYB gene family. In this way, phenotypic and genetic data were both integrated on the genetic map of azalea. In case both are located at the same mapping position, these genes are proven to be directly involved in the creation of the phenotypic variation of the trait. Nevertheless, it is very likely that not the genes themselves but transcription factors are the switches that regulate the phenotype of the trait. In that case, phenotype and genotype will be mapped at different positions, but phenotype is then expected to be mapped together with the true regulators, the transcription factors e.g. MYB markers. To confirm this theory, gene expression profiles of five flavonoid biosynthesis genes were generated in petals of a selection of flowers of the crossing population using qPCR and eQTL mapping again integrated these data with the genetic map.

Ellen De Keyser (1)
Peter Lootens (1)
Jan De Riek (1)
Erik Van Bockstaele (1,2)

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ellen.keyser@ilvo.vlaanderen.be

L8

INHERITANCE OF DETERMINANTS OF FLOWER COLOUR IN TETRAPLOID ROSES

Virginia Gitonga
Robert Stolker
Simon Ribot
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The choice of selection breeding for crop improvement in rose requires a better understanding of biological mechanisms and knowledge of the inheritance of the major target traits which can lead to new or improved screening methods. One of the problems in cut roses is that flower colour of some genotypes is not stable across growing environments. The dependency upon genotype suggests that this is a heritable trait. In general in rose, the genetic knowledge is still limited. Wageningen UR Plant Breeding together with international partners has produced one of the rose diploid maps and work is currently going on to create a highly dense tetraploid map using the progeny (181 individuals) from a cross between two tetraploid rose genotypes (P540 and P867) made available by Terra Nigra b.v.. The map will contain both phenotypical traits as well as molecular markers. The two parents were chosen to ensure sufficient genetic variation and segregation in the progeny for many morphological traits including colour, but also for disease resistance/susceptibility.

The current mapping population will be characterized for flower colour, by using colour charts such as the official chart of the Royal Horticultural Society, and additionally, by image analysis and measuring reflectance using a spectrocolorimeter. The genetics of flower colour will be determined by QTL analysis. In addition, flower petals of all genotypes will be analysed by HPLC to characterize secondary metabolic components that determine flower colour, such as anthocyanins. The inheritance of these components will also be assessed and compared to that of flower colour.

BREEDING OF ORNAMENTAL PLANTS THROUGH INTERSPECIFIC HYBRIDIZATION USING ADVANCED TECHNIQUES WITH A SPECIAL FOCUS ON *DIANTHUS*, *PRIMULA*, *COSMOS* AND *KALANCHOE*

L9

In ornamental plants, interspecific hybridization has successfully been used to produce novel cultivars with useful traits of both parents and to incorporate desirable traits of one species to another. Advanced breeding techniques like embryo rescue, polyploidization, protoplast fusion and molecular cytogenetic methods are used to produce and characterize interspecific hybrids in various taxonomic groups.. In this presentation, recent advances and problems in interspecific hybridization are described with special references to the use of embryo culture techniques for rescuing the abortive hybrid embryos and to the use of artificial polyploidization techniques for restoring the fertility of the interspecific hybrids. Some interesting topics related to interspecific hybridizations are also described such as the production of unexpected ploidy plants due to unreduced gamete formation and spontaneous chromosome doubling during in vitro culture in *Dianthus*, *Primula*, *Cosmos* and *Kalanchoe*. Some of the products of these interspecific hybridizations have successfully been commercialized directly or used as the germplasms for further breeding of novel ornamental crops.

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L10

INTERSPECIFIC HYBRIDIZATION OF *ALSTROEMERIA* FOR THE DEVELOPMENT OF NEW, ORNAMENTAL PLANTS

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Alstroemeria, the Inca Lily or Lily-of-the-Incas, is a popular cut flower plant because of the wide variety of flower colours that are available and the long postharvest life of its cut flowers. In recent years, cold-hardy introductions (USDA winter hardiness zone 5) by the authors have expanded the interest of this everblooming plant as a garden perennial. This research describes breeding procedures that have been used with the objective to breed novel, commercially valuable cold-hardy and fragrant flowered cultivars of *Alstroemeria*. Winter-hardy hybrids were developed by using the Chilean species, *Alstroemeria aurea* and fragrant hybrids were developed by using the Brazilian species, *Alstroemeria caryophyllae*. Interspecific hybrids were bred with the assistance of *in vitro* techniques such as *in ovulo* embryo rescue, micropropagation, and somatic embryogenesis. For embryo rescue, ovaries were collected 10-23 days after hand pollination and their ovules were aseptically excised. Ovules were placed *in vitro* on 25% Murashige and Skoog (MS) medium under dark conditions until germination. Three weeks after germination they were subcultured onto full-strength MS medium with 2 mg benzyl adenine (BA) per liter. Subsequently, plants were subcultured every three to four weeks onto a liquid MS medium with BA until they were large enough for rooting. After rooting and acclimation, plants were transferred to the greenhouse. Successful hybrids were evaluated under both greenhouse and field trials to determine winter survival and performance. As new hybrids and cultivars were developed, research was completed to develop production and propagation protocols for the plants. As a result of this research, the only fragrant cultivar of *Alstroemeria*, 'Sweet Laura', was developed. The new *Alstroemeria* cultivars 'Mauve Majesty' and 'Tangerine Tango' were recently patented and introduced by Cornell University. These plants are noteworthy by their winter hardiness, everblooming growth habit, and long flower stems. This project demonstrates the enormous potential for new plant development that exists.

MORPHOLOGICAL AND ANATOMICAL CHARACTERISATION OF CHEMICALLY INDUCED POLYPLOIDS IN *SPATHIPHYLLUM WALLISII*

L11

Tetraploids were induced in *Spathiphyllum wallisii* Regel (2n = 2x = 30) through in vitro application of mitosis inhibitors. Tetraploids were compared to the original diploid control plants. Polyploidization had a significant effect on plant anatomy and morphology. The stomatal area of diploids was smaller compared to the tetraploid plants. The leaf angle was smaller in diploids. The stomatal length and width, leaf thickness and angle and thickness of the spathum were positively correlated to the higher ploidy level. On the other hand, stomatal density, length/width ratio of leaf, spathum and spadix, number of shoots and leafs and length of the flower stalk decreased in tetraploids compared to the corresponding diploid controls. The leaf number of diploid plants was higher compared to tetraploids. Altogether, this study quantified the extended morphological changes of chromosome doubling in our model crop *Spathiphyllum wallisii*.

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L12

ADVANCES IN *OXERA* BREEDING

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The New Caledonian sub-endemic *Oxera* genus comprises several species of great ornamental interest. In 2006, we started to set up a vast interspecific hybridization program in order to create new cultivars with combined ornamental features. Twenty one of the twenty two endemic taxa (*O. neriifolia* subsp. *sororia* was not found during several field searches) were used in this breeding program and more than 7600 hand pollination crosses were performed. Pollinations were done with freshly collected pollen prior to bud opening and after immature anthers removal. 303 fruits containing 566 seeds were harvested and sown giving 361 viable plants. Limited by available plants and flower buds, 80 combinations out of the 420 theorically possible ones were trialled. Seeds were obtained in 37 combinations while 27 combinations gave viable plants. Only three taxa (*O. crassifolia*, *O. glandulosa* and *O. morierei*) involved in these crosses did not give any offspring. *Oxera sulfurea*, a species which flowers throughout the year, as the female parent, was involved in 3294 cross pollinations and has generated 284 offspring from 15 of the 20 taxa trialled. All the putative hybrids produced have intermediate morphological vegetative characters between the two parents and the ones that have already flowered also present intermediate flower forms and colours. This breeding program is still ongoing and cytology work is underway to determine the basic number of chromosomes of the genus *Oxera* and to learn if there are any different ploidy levels between the species.

POLYMORPHIC ITS AS A TOOL TO IDENTIFY HYBRIDS AND THEIR PARENTS IN CULTIVATED GENISTEAE (FABACEAE)

L13

Internal Transcribed Spacer (ITS) of ribosomal DNA is a classical sequence used for phylogenetic analysis. Usely, concerted evolution homogenise the numerous ITS of an organism in such way that a single sequence is amplified. However, for vegetatively propagated plants of hybrid origin, several ITS sequences can be obtained from a single individual. By cloning ITS sequences of various taxa assigned to tribe Genisteae within Fabaceae, we have been able to show such ITS polymorphism in *Cytisus* x *kewensis*, C. x *dallimorei*, C. x *praecox*, *C. "racemosus"* and *C.* 'Porlock' among others. For these taxa, the distinct ITS sequences can be compared with sequences of putative parents. We have then confirmed that *Cytisus* x *kewensis* is a *C. ardoinoi* X *C. multiflorus* cross. *C. "racemosus"*, commonly presented as a *C. canariensis* X *C. stenopetalus* and thus named *C.* x *spachianus* may in fact be a more complex hybrid involving also *C. monspessulanus*. We have also confirmed that *C.* 'Porlock' is a *C. monspessulanus* X *C.* "*racemosus*". We thus propose a way to asses the interspecific hybrid status of any vegetatively propagated plant, that also allows to clearly identify the putative parent taxa. This confirm a feature observed also in other wild and cultivated groups such as *Viola*, *Paeonies* and *Amelanchier*. Using additional data, mainly chloroplast microsatellites, we can further identify the maternal parent. These additional tools has shown that *Cytisus* x *kewensis* 'Niki' is a *C. ardoinoi* X *C. multiflorus* cross with *C. multiflorus* as maternal parent, while *Cytisus* x *kewensis* is a result of the same cross but with *C. ardoinoi* as maternal parent.

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L14

GENERATING AND DELIVERING NOVELTY IN ORNAMENTAL CROPS: SOME EXAMPLES

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The generation of novelty is a key focus for breeders of ornamental crops. This paper describes the application of a range of *in vitro* techniques to generate novel genetic combinations from which new varieties can be selected.

A program of interspecific hybridisation between *Limonium sinuatum* and *L. perezii* has resulted in a significant number of hybrids and back cross hybrids. All hybrids produced to date have had either very low or complete infertility and require chromosome doubling to restore fertility. A feature of the new hybrids is the increased visual impact of flowering stems conferred by branching angle and increased space between individual florets in the inflorescence.

Sandersonia is a flower crop well known to New Zealand growers. The single species has shown very little variation despite being entirely seed propagated. Attempts to increase variation in this crop have included hybridisation with related genera such as *Littonia* and *Gloriosa*. *Gloriosa superba* is a very variable species featuring individuals with ploidy levels from diploid to octoploid. There are considerable differences in the performance of hybrids between *Sandersonia* and *Gloriosa* with some lines almost impossible to grow and others extremely vigorous. Hybrids have all been infertile and to date chromosome doubling has not been successful in any of the lines tested.

Gentiana is a relatively poorly known cut flower crop. Ten years ago the colour range consisted of blues, pinks and whites. Red-flowered varieties are now available to commerce. A current challenge is to generate yellow and orange forms in order to provide a complete colour range. Yellow-flowered interspecific hybrids have been developed between *G. triflora* and *G. lutea*. These hybrids have proven difficult to grow out of *in vitro* culture. Chromosome doubling has been carried out to overcome the anticipated infertility of these hybrids, and work is underway to develop a strategy for producing back cross hybrids

CHROMOSOME IDENTIFICATION ON THE GENUS *LILIUM* USING COMPARATIVE GENOMIC *IN SITU* HYBRIDIZATION (CGISH)

LP1

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Single chromosome identification is of primal importance in the study of evolutive process in complex genomes, such as polyploidization and hybridization events, among others. Traditionally, chromosome identification is made by arranging the chromosomes by the length of the short (*p*) and the long (*q*) arms, identifying the centromeres and secondary constrictions. Furthermore, accurate chromosome identification can be performed trough chromosome differentiation techniques such as C-, N-, and Q-banding. In the last years, through the development of Fluorescent *In Situ* Hybridization techniques (FISH), even more accurate single chromosome identification has been accomplished, by the hybridization of highly conserved repetitive sequences, such as rDNAs. However, particular probes must be developed through laborious isolation and cloning molecular techniques. The comparative genomic in situ hybridization (cGISH) is a straightforward technique that allows the identification of single chromosomes by the generation of signals of conserved DNA regions along the chromosomes of different species. In this study we labeled total genomic DNA of *Triticum aestivum* and *Arabidospis thaliana* and hybridized it to chromosomes of different species of the genus *Lilium*. Different stringencies were applied to determine the optimum removal of cross hybridization, the 80% stringency showed to be the best, giving a clear signal and removing most of the cross hybridization. *Triticum aestivum* total genomic DNA exhibited six landmarks on three homologous chromosomes in the three different cultivars while *Arabidopsis thaliana* total genomic DNA exhibited six landmarks on three homologous chromosomes on *Lilium*, one of these signals being in a different chromosome of those of the *T. aestivum* signals. Together with the DAPI bands the total genomic DNAs landmarks allowed the identification of six out of 12 single homologous chromosomes.

LP2

DISCRIMINATING CAPACITY OF NUCLEOTIDE BINDING SITE (NBS) AND *MYB* GENE PROFILING FOR GENETIC ANALYSIS OF *CAMPANULA* ECOTYPES

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Two DNA-based marker methodologies, nucleotide binding site (NBS) and *myb* gene profiling, were evaluated with respect to their discriminating capacity and efficiency in genetically analyzing 11 *Campanula* ecotypes belonging to *C. latifolia* (Lat1 and Lat2), *C. rapunculoides* (Rap1, Rap2, Rap3 and Rap4), *C. spicata* (Spic1, Spic2 and Spic3) and *C. trachelium* (Trach1 and Trach2). Three restriction enzymes (*Mse*I, *Rsa*I and *Hae*III) were utilized with two NBS primers (GLPL6 and NBS2) and one *myb* primer (MYB2). Looking at the number of banding pattern, all two techniques discriminated the genotypes very effectively. On an individual assay basis, NBS profiling completely distinguished all the genotypes of the ecotypes Lat1 (*C. latifolia*) and Trach1 (*C. trachelium*). To assess the usefulness of the overall information provided by these marker data for establishing phylogenetic relationships, cluster analysis was performed. For the two markers a high similarity in dendrogram topologies was obtained although some differences were observed. In general, the dendrograms reflected the geographical diffusion of the ecotypes. The *myb* marker resulted to be more adept to differentiating species rather than ecotypes, while NBS profiling appeared to be more able to discriminate the ecotypes. Comparable population structuring was obtained with both marker systems. Two Bayesan assignment tests with the program STRUCTURE divided the accessions into groups agreeing with neighbour-joining trees. Both NBS and *myb* gene profiling data were demonstrated to be remarkably effective for group discrimination and genetic diversity studies. The use of these systems is discussed in terms of the choice of appropriate marker techniques for different aspects of local *Campanula* ecotypes evaluation.

VERIFICATION OF THE HYBRID CHARACTER OF INTERSPECIFIC *RHODODENDRON* PROGENY BY MOLECULAR TOOLS

LP3

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Progeny derived from five types of interspecific rhododendron crosses, i.e. (1) *R. aureum* x *R. brachycarpum*, (2) *R. aureum* x 'Catharine van Tol', (3) *R. aureum* x *R. yakushimanum* 'Koichiro Wada', (4) *R. yakushimanum* 'Koichiro Wada' x *R. aureum* and (5) 'Nova Zembla' x *R. aureum*, were investigated to confirm their hybrid character. The verification was based on genomic *in situ* hybridization (GISH) and supported by the RAPD-PCR method. Owing to the fact that GISH has never been used in analysis of rhododendrons, thorough optimization of the protocol was necessary. We assayed one to five plants representing each cross. Microscopic preparations of mitotic chromosomes of the tapetum and meiotic chromosomes of pollen mother cells (PMCs) were prepared by enzymatic digestion of anthers. Total genomic DNA from pollen donors was used as a probe in the ratio of 3 ng/µl and total genomic DNA from maternal plants was used as a blocking DNA (60-fold excess of the labeled genomic probe). The developed GISH protocol in rhododendron, has enabled the identification of parental chromosomes in genomes of the studied progeny. Hybridization signals, indicating the localization of the probes on chromosomes and on interphase nuclei, were detected in four interspecific rhododendron F1 hybrids. Obtained results were confirmed by RAPD-PCR analysis.

For this purpose DNA was amplified with 19 RAPD primers and we obtained RAPD markers which allowed to describe the relationship between parents and their progeny. None of studied plants representing the cross 'Nova Zembla' x *R. aureum*, was proven to be a hybrid. Our study confirmed that both genomic *in situ* hybridization (GISH) and RAPD-PCR markers were powerful tools for verification of the hybrid character of the rhododendron progeny derived from wide crosses.

LP4

PRODUCTION AND CHARACTERIZATION OF INTERGENERIC HYBRIDS BETWEEN *ANEMONE CORONARIA* AND *RANUNCULUS ASIATICUS*

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Ranunculus asiaticus L. and *Anemone coronaria* L. are common cut flowers which belong to the family of the Ranunculaceae. Between these species a high degree of variation can be found in leaves, flower shape and flower colour. Therefore intergeneric crossings between these genera might result in new interesting hybrids. Many of such wide hybridizations do not occur naturally because prezygotic and postzygotic barriers alter fertilization, embryogenesis and/or seed formation. However elaborate in vitro and in vivo work can overcome these barriers. The objective of this study was to produce and characterize hybrids between *Anemone coronaria* and *Ranunculus asiaticus.*

Crosses between *Anemone* and *Ranunculus* were performed in the greenhouse from January till May. Three to four weeks after pollination immature seeds were harvested and rescued in vitro. One year or two years later, parents and F1 progeny were grown in identical growth conditions in order to study possible morphological differences. To distinguish the plants on molecular level AFLP analysis was performed on F1 progenies and parents using the restriction endonuclease *Eco*RI as suggested by Nissim et al. 2004.

In most cases, crosses between *Ranunculus* and *Anemone* (and vice versa) were prevented because the pollen tube was not able to reach the ovules. The breeding experiments resulted therefore in a very poor seed set. The phenotype of the hybrids obtained following embryo rescue, showed a high similarity of that of the mother plant. However some novel characteristics were observed. Visual screening revealed variation in flower colour. Furthermore AFLP analysis demonstrated that some bands of the mother plant were deleted in the hybrids. Also some specific bands of the father plant and novel bands, absent in mother as father, were observed.

In conclusion, we were able to produce intergeneric hybrids between *Anemone* and *Ranunculus*. These hybrids have a similar morphology compared to the mother plant but the AFLP results showed some genomic reorganization. This is in accordance with other intertribal crossing studies, showing similar phenotypical and molecular phenomenons. To confirm these (preliminary) results, independent techniques such as chromosome spreads and GISH, analyzing chromosomal reorganisation, will be used in the future.

References:

Nissim Y, Jinggui F, Arik S, Neta P, Uri L, Avner C (2004) Phenotypic and genotypic analysis of a commercial cultivar and wild populations of *Anemone coronaria*. Euphytica 136:51-62.

BREEDING SYSTEM OF *GLANDULARIA* SPECIES NATIVE TO ARGENTINA

LP5

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The *Glandularia peruviana*, *G. platensis* and *G. glandulifera* breeding system was studied using controlled manual pollination. Self-pollination and cross-pollination experiments were conducted in selected clones under greenhouse conditions. Natural pollination was also studied as control. Pollen tube growth was analysed in self and cross- pollinated pistils to determine the existence of an incompatibility system.

Self-pollinated flowers produced no fruits in all species. Cross-pollinated flowers produced 46%, 8% and 80% fruits in *G. peruviana*, *G. platensis* and *G. glandulifera* respectively.

In the three species, pollen grains germinated normally in stigma in self and cross pollinated pistils, but further growth of the pollen tube was inhibited at different parts of the style in self pollinated pistils. Pollen tube growth rate is discused for both pollination conditions.

The behaviour of pollen tube growth and fruit set production experiments indicate the presence of a gametophytic self-incompatibility system which is characterized by the inhibition of the pollen tube growth at the style.

LP6

LIBRARY CONSTRUCTION FROM MICRODISSECTION OF CHROMOSOME #1 IN LILY (*L. LANCIFOLIUM*)

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Lilium lancifolium belongs to section Sinomartagon and has been known as polyploidy complex comprising diploid (2n=2x=24) and triploid (2n=3x=36). Chromosome #1 is the longest chromosome as a metacentric in the *L. lancifolium* metaphase complements. It was microdissected and collected from mitotic metaphase spreads of lily (*L. lancifolium*) by using PALM Robot MicroBeam System (Carl Zeiss AG, Germany), attached to an Axiovert 135 microscope (Carl Zeiss AG, Germany). Two experiments were performed using the chromosome. Firstly, DOP-PCR (degenerate oligonucleotide primed polymerase chain reaction) was conducted using a 22-mer degenerated primer. Secondly, chromosome was digested using *Sau3A*I, *Sua3A*I specific adaptor was ligated to chromosomal DNA, and adaptor mediated PCR was conducted. Southern hybridization results showed that the amplified products were homogeneous with lily genomic DNA, indicating that DNA from the dissected chromosome has been successfully amplified by DOP-PCR or adaptor mediated PCR. The PCR products were cloned using TOPO TA cloning kit (Invitrogen, USA) to create a chromosome-specific library. Evaluation of 200 randomly selected clones showed that the size of the cloned inserts varied from 300 bp to 2000 bp. These results suggest that microdissection and microcloning of lily chromosome #1 is feasible. The approach used here could be applied to the genetic mapping and isolation of chromosome #1-specific genes which are conveying resistance against environmental stresses such as cold, drought, diseases so on.

INTROGRESSION BREEDING IN GENUS *TULIPA* ANALYSED BY GISH

LP7

Interspecific hybridization is an important tool in *Tulipa* breeding to introgress some important horticultural traits into new cultivars. In our study, the main goal is the introgression of resistance against Tulip Breaking Virus (TBV), which is found in some cultivars of *Tulipa fosteriana* (F) to *T. gesneriana* germplasm (G), the commercial assortment. The diploid (2n = 2*x* = 24) F_1 interspecific hybrids between *T. gesneriana* × *T. fosteriana* (GF hybrids) were backcrossed to *T. gesneriana* and a number of BC_1 hybrids (GGF) have been analysed by genomic *in situ* hybridization (GISH) to evaluate the number of chromosomes derived from F and G genomes and the number of recombinant chromosomes. All of the BC_1 hybrids analysed were diploid (2n = 2*x* = 24). By GISH it was possible to distinguish chromosomes from both parental genomes as well as the recombinant chromosomes. Because the *T. gesneriana* parent was used for backcrossing, the number of G genome chromosomes (chromosomes which centromere was of *T. gesneriana* genome) predominated in the BC_1 progenies and varied from fourteen to eighteen whereas the total number of *T. fosteriana* chromosomes in hybrids ranged from six to ten. The number of recombinant chromosomes differed among hybrids from five to nine. For most genotypes there were two types of recombinant chromosomes. Those with a centromere of *T. fosteriana* chromosome with recombinant segment of *T. gesneriana* (F/G) and *vice versa* (G/F). Most recombinant chromosomes contained a combination of a single *T. gesneriana* and a single *T. fosteriana* fragment. The maximum number of recombinant segments per chromosome was two and their position ranged from distal to highly interstitial. For each genotype the percentages of each genome present in BC1 progenies was estimated. The percentages of *T. fosteriana* chromatin in hybrids ranged from 19.71 to 24.56%, while 25% was expected.

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LP8

MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION OF INTERGENERIC HYBRIDS BETWEEN THE ORCHID GENERA *RENANTHERA* AND *VANDA*

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The Indo-Burmese mega-biodiversity 'hotspot' houses a number of ornamental orchids belonging to rare, endangered, threatened and vulnerable species possessing highly attractive flowers. However, till date, many of these beautiful orchids growing in the pristine forests of north-east India have remained largely unexplored and unexploited. *Renanthera imschootiana* Rolfe is an IUCN red-listed rare and endangered orchid classified under Appendix-I of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). This orchid is reknowned for its bright crimson, long-lasting floriferous spikes which bloom during spring every year. *Vanda coerulea* Griff. ex. L., *Vanda testacea* (L.) Reichb.f. and *Vanda stangeana* Reichb.f. (endemic to Manipur) are simultaneously deeply coloured flowers with high aesthetic values. Using *Renanthera imschootiana* as the female parent, we have crossed it with the three *Vanda* species and intergeneric hybrids have been synthesized, viz., *Renantanda* Kebisana Shija (*R. imschootiana* x *V. coerulea*), *Renantanda* Prof GJ Sharma (*R. imschootiana* x *V. testacea*) and *Renantanda* Momon Shija (*R. imschootiana* x *V. stangeana*). All these hybrids have been registered with the Royal Horticultural Society, London. We have also developed *in vitro* propagation protocols for these parents and their hybrids keeping in view of their conservational and eco-restorative considerations. The synthesized hybrids produced beautifully coloured flowers with shelf-lives lasting from 1-1$^{1}/_{2}$ months. Morphological as well as molecular characterization of these hybrids have been made for confirmation of hybridity and other genetic information.

DIVERSITY IN *ROSA RUGOSA* X *ROSA HYBRIDA* INTERSPECIFIC VARIETIES

LP9

Modern roses represent a relatively narrow gene pool resulting in poor disease and abiotic stress resistance. Diploid *Rosa rugosa* have better disease resistance and higher tolerance for environmental stress conditions, however, their ornamental characteristics need improvement. Interspecific crosses between modern and *Rugosa* roses are difficult to obtain, but they could potentially yield resistant varieties with valuable ornamental properties. Eight Latvian varieties resulting from crosses with *Rugosa* type roses and six parents were analyzed with 21 microsatellite markers, to confirm the results of interspecific crosses and to identify presence of garden rose genetic material. Majority of varieties resulting from interspecific crosses showed strong presence of *R. rugosa* gene pool both in terms of genotype and phenotype. However, two crosses, 'Abelzieds' (*R. rugosa* 'Alba' x 'Poulsen's Pink') and 'Zaiga' (*R. rugosa* 'Plena' x 'Flammentanz'), exhibited presence of floribunda and climbing rose characteristics. Phenotypically only one interspecific variety 'Zaiga' showed garden type flower colour and leaf shape, but both cultivars could be used for breeding owing to good disease resistance, rigorous growth and ornamental characteristics.

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LP10

BREEDING *FOR* RESISTANCE AND BIOCONTROL OF WILT DISEASE IN *CURCUMA ALISMATIFOLIA* GAGNEP BY *BACILLUS* SPP.

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The objectives of this study were to breed for resistance of Curcuma alismatifolia Gagnep to wilt disease caused by Rastonia species, by screening and collecting virulent isolates of Rastonia Moreover, in order to preserve the value of these ornamental flowers, and to reduce the dependence on toxic chemicals required to combat this disease the biocontrol potential of bacteria antagonistic to Rastonia, , was investigated. Over 500 bacterial strains, isolated from soil, leaf surfaces of C. alismatifolia Gagnep. and hot springs in the Chiang Mai province in Thailand, were screened in vitro for antagonistic activity against R. solanacerarum. Three isolates providing growth inhibition in vitro , were identified as Bacillus licheniformis, B.amyloliquefaciens and B. subtilis. Subsequently, these isolates were used for biocontrol in planta. One of the isolates, B. subtilis was shown inhibition greater than 70% when compared with the control and provided a statistically significant growth suppression of the wilt disease on the curcuma. Moreover, all of the antagonistic bacteria isolates were selected to against Anthracnose caused by Colletotrichum sp. in planta. The highest levels of the Anthracnose disease suppression occurred using B. licheniformis and B. subtilis on curcuma flower. The present results indicate that B. licheniformis, B.amyloliquefaciens and B. subtilis could be used to inoculate and reduce the symptoms of disease on Curcuma alismatifolia Gagnep.

CURRENT STRATEGİES AND FUTURE PROSPECTS OF RESİSTANCE BREEDİNG İN ORNAMENTALS

L15

Ornamental crops pose several problems to breeding for disease and pest resistance. The large number of ornamental crops and the short turnover time of varieties limit the input invested to the individual variety. In addition many ornamental crops are polyploids hampering genetic analyses of resistance traits. In current breeding programms for most crops stringent selection for disease resistance is either omitted or performed at relatively late stages of the selection program. However, knowledge about the pathosystem including the genetic composition of both the host plant and the pathogen populations will allow efficient early selection for resistance in conventional breeding programs. These strategies can be significantly improved by the application of molecular diagnostic tools as e.g. molecular markers. Examples of improved selection schemes as well as marker applications will be shown for the pathosystem rose/blackspot. A limited number of ornamental crops are amenable to positional cloning (as e.g. roses) or Transposon tagging (as e.g. petunias) of genes allowing the de novo isolation of genetic factors important for disease resistance. Mid- and long term improvements can also be expected from current genome projects and the application of biotechnology. The identification of key factors with central functions in disease resistance via sequence homology and microsynteny will get easier with every completely sequenced genome. Here I present examples on how this information may be utilised in ornamental resistance breeding by using non transgenic strategies as for example Tilling and Ecotilling or by generating transgenic plants.

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L16

CONVERSION OF MOLECULAR MARKERS LINKED TO *FUSARIUM* AND VIRUS RESISTANCE IN *LILIUM*

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Lilies are one of the most economically important monocot flower bulbs. They are mainly cultivated in the Netherlands with a bulb acreage of more than 4000 ha. However, lily bulb production faces some challenges such as being susceptible to diseases like *Fusarium oxysporum* and Lily mottle Virus (LMoV). These two are the most important pathogens that cause serious damage to lily. Fortunately, some Asiatic lily hybrids showed high level of resistance to *Fusarium* and LMoV. The incentive for breeding resistances into the lily assortment is limited due to the relatively long juvenile phase (2-3 years) and many selection's cycles of breeding needed to place these desirable agronomic traits from different resistant parents into specific commercial cultivars. Therefore, using marker-assisted selection (MAS) could speed up the breeding process considerably. A genetic map of an intraspecific Asiatic backcross [Orlito (Pirate x Connecticut King) crossed with Connecticut King] was constructed using three different molecular marker systems (DArT, AFLP and NBS profiling). The disease tests for the two pathogens were carried out for four years on this Asiatic population [100 BC1].Four QTLs for *Fusarium oxysporum* and one for LMoV were localized on the map. The most tightly linked markers to the resistance will be converted into more robust PCR markers and use for breeding purposes. The most significant *Fusarium's* QTLs were successfully converted and specific primers for *Fusarium* resistance are developed.

SPECIFIC MAPPING OF DISEASE RESISTANCE GENES IN TETRAPLOID CUT ROSES

L17

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Control of fungal diseases is a major constraint of cut-rose cultivation in greenhouses and in transportation around the world. Therefore, development of resistant cultivars is a promising way to reduce the use of chemicals required for controlling the diseases. Genetic analyses and breeding for resistance, however, are hampered by a high degree of heterozygosity and the polyploid nature of cultivated roses.

Nucleotide-binding site (NBS) profiling of Van der Linden *et al*. (2004) was used as a tool enabling a more directed way of studying the genetics of resistance to pathogens responsible for diseases such as botrytis and powdery mildew.

NBS profiling is a multiplex screening technique, producing amplified resistance gene analogue (RGA) fragments by using degenerated primers based on the conserved motifs present in the NBS domain of resistance genes. Since NBS regions are abundantly distributed and highly polymorphic within the plant genome, they are very suitable as markers to identify resistance genes.

Twelve NBS degenerated primer/restriction enzyme combinations were used to genotype the whole rose tetraploid K5 population and its parents (Yan, 2005). To generate RGA profiles, the restriction enzymes: *Alu*I, *Hae*III, *Mse*I, and *Rsa*I were combined with primers NBS1, NBS3, and NBS5a6. The profiles were dominantly scored resulting in 135 polymorphic RGA markers which segregated in a 1:1 or 3:1 ratio.

The set of 135 markers, representing uni- and bi-parental simplex markers, were mapped on the two available parental AFLP/SSR K5 maps with Joinmap 4.0 (unpublished). This resulted in two parental maps of 1150 cM and 1160 cM with 203 markers and 198 markers, respectively. The tetraploid maps will be used to dissect the genetic variation for resistance to powdery mildew resistance.

Moreover, Rosaceae SSRs mentioned in the literature are currently tested on the K5 population to obtain allelic bridges between the tetraploid and diploid genetic maps in rose and related species in order to align them. These bridges will improve cross-ploidy comparisons in roses in order to strengthen cut rose breeding.

Van der Linden C. G. *et al*. Efficient targeting of plant disease resistance loci using NBS profiling. Theoretical and Applied Genetics, 109:384-393.
Yan Z. 2005. Towards efficient improvement of greenhouse grown roses: genetic analysis of vigour and powdery mildew resistance. PhD Thesis, Wageningen-UR, The Netherlands. 90pp..

L18

BREEDING FOR RESISTANCE AGAINST *BOTRYTIS* IN LILY

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The fungus *Botrytis* occurs worldwide and destroys various crops. In lilies, the strain *Botrytis elliptica* destroys leaves, flower buds and flowers. Reddish-brown spots, the first evidence of the disease, appear on the leaves. During wet weather, the spots eventually coalesce and the whole leaf collapses and decays. To avoid the use of chemical pesticides and to make growing of plants economically viable and ecologically safe, in lily breeding the current activities are directed towards the development of disease-resistant cultivars. Crosses between cultivars with different levels of resistance to *Botrytis elliptica* (study year 2002) have been obtained. Estimation of parent plants and the produced hybrids have been done, by two years of testing, on plants grown in natural environment (2004-2005). The level of *Botrytis* resistance was rated visually from 0-4 grades (0=healthy–4=very susceptible). When *Botrytis*-resistant parents were crossed, the hybrids were characterized by degrees of resistance to the disease; when *Botrytis*-susceptible cultivars were crossed with cultivars resistant to the disease, the hybrids were rated *intermediate* – their susceptibility to the disease was *dominant.* Under field conditions, 10 perspective hybrids have been tested (2006-2007) for resistance to the grey mould. After two years of testing, no significant differences have been found.

A DYSFUNCTIONAL CYMMV MOVEMENT PROTEIN GENE CONFERS RESISTANCE TO CYMMV IN *DENDROBIUM* ORCHID

L19

A *Cymbidium mosaic virus* movement protein gene with a site-specific mutation (*mut11*) under control of a ubiquitin promoter was inserted using biolistics into 2 *Dendrobium* varieties with the intention of creating CymMV-resistant orchids. Presence of the transgene in regenerated plants of *D.*x Jaquelyn Thomas 'Uniwai Mist' and *D.*x Jaq – Hawaii 'Uniwai Pearl' was confirmed by PCR using genomic DNA, and *mut11*-positive plants were potted ex vitro**.** Forty-two transgenic plants and 4 non-transgenic controls at the 4 to 6 leaf stage were inoculated with a 1: 1000 dilution of CymMV obtained from infected orchids. Plants were analyzed for systemic infection using tissue blot immunoassay (TBIA)**.** Seventeen plants from 6 independent transformations remained virus-free, whereas all control plants were infected with CymMV within 1 month. Further analysis by RT-PCR showed that the *mut-11* messenger RNA was detectable in only 2 of these 17 plants. All plants were challenged again with a second CymMV inoculation as above followed by TBIA analysis after 1 month. Thirteen of 17 plants remained free from virus. A third challenge of these plants with CymMV as above was followed by TBIA analysis at 1 week, 2 weeks, 1 month, 3 months, 6 months and 12 months after challenge. Results at 2 weeks post-inoculation showed that all 6 controls and 4 individual transgenic plants, including the RT-PCR-positive plants, became systemically infected with CymMV. Eight transgenic plants from both varieties remained free from CymMV 12 months after the third challenge. Lack of detectable *mut11* mRNA in these resistant lines suggests that a post transcriptional gene silencing (PTGS) mechanism may be conferring resistance to CymMV.

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L20

CAN WE STILL TAKE THE BREEDER'S EXEMPTION FOR GRANTED?

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Since the introduction of protection of plant varieties by plant breeders rights, the breeders' exemption has always been a crucial principle of our breeding industry. It means that breeders have the freedom to use without permission all existing varieties for further crossing and selection. This has lead to a flourishing industry in which many different companies have had the opportunity to start breeding activities and obtain a position on the market.

However, some developments might change this "freedom to operate". Within the Plant Variety Protection system the interpretation of Essentially Derived Varieties can limit the breeders' exemption to a serious extend. Second there is the increase of patents in the plant sector. In Europe varieties cannot be patented as such, but specific traits can be patented. All varieties that contain a patented trait fall under the scope of the patent which means one cannot use these varieties freely for breeding. Thirdly there is the biodiversity legislation which may limit the possibility to use varieties from certain countries without first closing agreements.

Discussions about these topics are mainly dealt with on a political or juridical level. Since these developments are likely to have a very big impact on breeding and research activities it is of great importance that also breeders enter into these discussions. In my presentation I will contemplate on the different positions that exist about the interpretation of the EDV-concept and about the effect of patents in the plant sector.

ESSENTIALLY DERIVED VARIETIES IN ORNAMENTALS

L21

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In ornamentals, natural or induced mutants are a common phenomenon. Such mutants or ‘sports’ may obtain Plant Breeders’ Rights (PBR) when shown distinct from all existing varieties. To protect the interests of the breeder of the original variety the International Union for the Protection of New Varieties of Plants (UPOV) has introduced the concept of ‘essentially derived varieties’ (EDVs). In the UPOV 1991 act several ways of obtaining an EDV are described, mutation being one of them. When a variety is shown an EDV, authorisation for commercial exploitation is needed from the breeder of the initial variety. There is considerable debate ongoing about which approaches to use for determining essential derivation and also which thresholds should be used in the different plant species. For determining whether a variety should be considered essentially derived from an existing variety two conceptually different approaches can be taken. The first one is based on genetic conformity, the second on a forensic approach. For the implementation of the EDV concept using the conformity approach it is important that similarities between unrelated varieties can clearly be separated from essentially derived varieties. In the forensic approach the high genetic similarity between original variety and mutant is taken as a starting point. The basic idea is to calculate the probability that a second, putatively derived, variety would have a profile identical to the initial variety, given an independent breeding history. Both approaches will be illustrated and ways to implement the EDV concept discussed

L22

ANALYSIS OF A DATABASE OF DNA PROFILES OF 734 HYBRID TEA ROSE VARIETIES

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Rose is the largest ornamental crop. Over 25,000 varieties of modern roses have been described, of which more than 10,000 hybrid teas. Such large numbers of varieties may cause problems in the DUS testing context. A major problem for all countries carrying out DUS tests is the requirement to compare new varieties to all other varieties in common knowledge. Clearly, strict adherence to this concept is logistically and financially impossible in a species such as rose, which is cultivated around the world. Another problem are the reference collections. Maintaining a living reference collection is unattractive because of the high costs associated and disease problems. When no reference collections are maintained, reference varieties need to be obtained from the breeders. It is important that the DUS examination office can quickly verify the identity of the material submitted. For this aspect of quality assurance, molecular markers are ideally suited, as they are highly discriminating and can be assayed rapidly and relatively cheaply.

DNA microsatellites (simple sequence repeats, SSRs) are highly polymorphic and have the advantage of providing a co-dominant marker system based on a PCR technology. When analyzed as sequenced-tagged microsatellite site (STMS) markers, they provide simple banding patterns that are easy to record and are especially suitable for automated and objective analysis. In addition, the resultant data can be readily stored in a database. New varieties or new markers can be easily added to an existing database. We used a set of 11 microsatellite markers developed for rose (Esselink et al., Theor Appl Genet 106: 277-286, 2003) to generate a database of molecular profiles of 734 entries of Hybrid tea varieties, including all new varieties of the period 2000-2005. Here, we report on the analysis of the molecular data in detail. Specifically, we have looked at discriminative power of the markers, reproducibility of the results, genetic (sub) structure in the set of varieties analyzed, as well as correlation between molecular and DUS characteristics.

BACK TO BASICS FOR NEW CROP DEVELOPMENT

L23

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Breeding of new ornamental crops is one of the most rewarding professions in the world. Yet researchers are also faced with challenges that require both new and innovative research, as well as the utilization of trusted classical breeding methods. Although new breeding methods like marker assisted selection and genetic modification opens up numerous possibilities, these methods can often not be applied to new (relatively unknown) crop development. First of all, there is a lack of basic information needed for application of these methods and secondly, the commercial value of these crops does not justify the use of such expensive methods. Breeders of new crops are, thus, often faced with a lack of basic information, requiring them to go “back to basics” and the utilization of more classical breeding strategies. The requirements for new crop development will be discussed on the basis of the experience acquired with the development of *Lachenalia* cultivars. When developing new crops, researchers work mostly with a number of species and the information required needs to be generated through researching market potential, genetic background and selection procedures. New crop development requires, first of all, the collection and characterization of germplasm, followed by the establishment of basic information on reproductive biology and market requirements, or the aim for breeding. Inter-specific hybridization and the establishment of specific selection criteria form the major part of breeding in new crops, and this is often complemented by the induction of polyploids. For the successful continuation of these processes it is necessary to establish a basic cytogenetic background for the crop. This becomes even more important when working with complex/diverse genera. The cytogenetic research can complement molecular systematic research to establish the relationships between the different species in the breeding program. The value of cytogenetic and molecular systematic studies in genetics and breeding are demonstrated through the research done on *Lachenalia* species. Mutation induction is another breeding strategy that requires basic background information for successful application. This includes the establishment of basic *in vitro* protocols and the selection of specific candidate plants. Only after the establishment of these basic information systems can the breeder move to advanced techniques.

L24

DEVELOPMENT OF COLOURED, NON-VERNALIZATION-REQUIRING SEED PROPAGATED LILIES

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Recent discovery of seed-propagated *Lilium x formolongi* hybrids that flower continuously in <1 yr. without vernalization (200-240d after sowing due to ≥1 dominant VER1, VER2 alleles) with frost-tolerance, day neutrality, and winter hardiness is an unprecedented combination in Lilium. Our objective to obtain coloured *L. x formolong*i was to use Class I lilies that initiate flower buds prior to a cold treatment as parents, e.g. Lilium martagon. Reciprocal interspecific crosses between fertile parents (1-*L. x formolongi,* 7-coloured *L. martagon*) were made in both directions to generate segregating hybrid (F_1) and inbred (F_2) progenies. A total of 8,826 F_1 seeds or embryos were generated. Embryo rescue was employed when *L. martagon* was the female, although viable embryos never germinated after ~1 yr. in culture. Mean germination ranged from 0% - 1.02% for in situ ripened seed. Hybridity for one successful cross (07L-14; *L. x formolongi* [00L-111-343 x 51-202-1] x *L. martago*n 'Cadense') was analyzed using morphological (flowering, leaf & internode number, leaf length:width ratios, compatibility, no. flower stalks & flowers, rosetting) and molecular markers (ISSR primers). Twenty-two 07L-14 genotypes were genetically similar to the female parent whereas 86 aligned closely with the male ('Cadense'). The number of shoots/plant was the only quantitative trait co-segregating with ISSRs. Flowering traits were intermediate to, but significantly different than, either parent. A majority of the plant height and inflorescence lengths were significantly greater than the parents. Two F_1s had slight flower colouration in the petals and tepals; one hybrid had an open-faced rather than trumpet flower (similar to an Oriental type).

GROWTH AND DEVELOPMENT OF CUT ROSE CLONES; INDIRECT SELECTION FOR YIELD

L25

The shoot yield of 177 large-flowered own-rooted cut rose genotypes, grown in a heated glasshouse, was recorded for 40 weeks in year # 1. These genotypes showed a normal distribution over 8 yield classes : 4<6,…..,18>20 shoots plant^{-1}. 5 Genotypes from each yield class (total of 40 genotypes) were propagated as cuttings and planted in the glasshouse early year # 2. The growth and development of these young plants were studied until each plant had 2 bottom-breaks (Bb). The mean number of days to the appearance of the 1st and the 2nd Bb, were 43.1 and 57.1 days after planting; days to flowering of these shoots took 35.5 and 34.2 days from their appearance; at flowering the shoot lengths were 77.5 and 83.4 cm; mean length increases were 23 and 26 mm.day^{-1}**.** Highly significant negative correlation (r = - 0.69^{+++}) between the shoot yield in year # 1, and the number of days to appearance of the 1st Bb of the same genotypes in year # 2, shows unique possibilities to indirect selection for future yield of clonal plants. The probable role of endogenous hormone action in the carbon partitioning in rose plants is discussed.

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L26

IN VITRO MUTAGENESIS OF *ASTER NOVI-BELGII*

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Aster novi-belgii is an important ornamental in the Danish pot plant industry with approximately 7 mill. pots produced annually in Denmark. The main commercial cultivars have been bred by Institute of Horticulture and are owned by the Danish Aster Association. During 15 years of conventional crossings more than 30 cultivars in two series (Viking and Victoria) have been granted plant breeders rights. The cultivars have since dominated the pot asters market worldwide. To increase breeding efficiency and explore new possibilities mutagenesis were investigated in two cultivars ('Fanny' and 'Jane') of the Victoria series. An *in vitro* culture system based on adventitious shoot formation was developed for a number of cultivars to reduce chimerism. The *in vitro* culture system used leaf blade sections from *in vitro* grown plantlets as explant source and adventitious shoots were produced within eight weeks on a MS based medium.

To generate mutants shoots from *in vitro* grown plants were irradiated with gamma rays prior to induction of adventitious shoots. The highest amount of radiation allowing adventitious shoot formation was initially determined to 18 Gy. Plants from the two cultivars was thereafter produced and evaluated in the greenhouse for mutants. Special interest was placed on flower characteristics, ie. colour, size and whorls of ray florets. The two cultivars differed with respect to mutations with 'Fanny' giving a higher number and more interesting mutants. Flower colours ranged from pink, to purple and dark violet. The number of whorls of ray florets in capitula varied from a single to numerous and plants only producing ray florets were found. Further variation in plant height, branching ability and time to flowering was observed among the regenerants. Despite adventitious shoot formation plants showing chimerism were found.

In vitro mutagenesis of *Aster novi-belgii* is a promising tool to improve the assortment; however, the potential of this technique depends on the cultivar used for mutagenic treatment and chimerism cannot be totally avoided.

BREEDING THE TOMATO MICRO-TOM MODEL SYSTEM FOR ORNAMENTAL VALUE

L27

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Taking advantage of its small size (8 cm tall) and short life cycle (70 days), the ornamental tomato cv Micro-Tom (MT) had been proposed as a model system for tomato genetics. Ever since, MT are being increased used for large scale mutagenesis and transgenic plant production, as well as the introgression of well characterized mutations and allelic variation already known in other cultivars and tomato wild species. Using such approaches we noticed that many mutations obtained in the MT background could be used to improve its value as an ornamental. Here we report the introgression of various mutations affecting fruit colour and morphology into the MT background. The introgression process consisted of series of crosses and backcrosses. In each cross, pollen was collected of the parent plants and used to fertilize emasculated MT flowers. The first cross F1 was selfed to obtain a recombinant F2 which was selected for small size and the mutation of interest. The selected plants were backcrossed with MT up to the sixth generation (BC6), with selfing every two generations to screen for homozygous recessive mutants. Plants were grown in 150 ml pots containing a 1:1 mixture of commercial pot mix and expanded vermiculite, supplemented with 1 g l^{-1} 10:10:10 NPK and 4 g l^{-1} lime in a greenhouse with mean temperature of 28 ^{o}C, 11.5 h/13 h (winter/summer) photoperiod, and 250 to 350 µmol photons m^{-2} sec^{-1} PAR irradiance obtained by reduction of natural radiation with a reflecting mesh. Different true type genotypes combining reduced size and fruit colour variation were obtained upon introgression of the mutations *Del*, *old gold* (*og*), *Beta* (*B*), *green stripe* (*gs*), pink fruit (*y*), *yellow flesh* (*r*), *tangerine* (*t*), *apricot* (*at*) and *green flesh* (*gf*) in the MT background. Four mutations affecting fruit format, i. e. *ovate* (*o*), *fascinated* (*f*), *sun* and *fs8.1*, were also introgressed in the MT. These mutations and the combination of them greatly extended the value of MT as an ornamental and introduced the possibility to explore the large diversity of tomato genetic for these propose.

L28

FLOW CYTOMETRY FOR PLANT BREEDING

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Flow cytometry is a well known and established technique for ploidy analysis in plant breeding. In general it is used to characterize parent plants, to screen offspring after interploidy crosses, to control ploidy levels during seed multiplication or to evaluate haploidisation or polyploidisation experiments. Recent developments in the apparatus itself as well as in automated sample preparation simplified the technique and made it more cost effective. This allows breeders to have an easier access and to apply the technique in their breeding programs.

Commonly for ploidy analysis DAPI staining is used to measure the relative DNA content in the cell. DNA content is then correlated to chromosome amounts. However no information is obtained on exact chromosome numbers. By flow cytometry more specific information linked to genome sizes can be obtained. Examples will be given on the use of genome sizes to chose parent plants for interspecific hybridization and to evaluate the hybrid nature of seedlings within *Buddleja* and *Hydrangea*. Besides flow cytometry as a tool to recognize unreduced pollen in *Begonia* and *Hibiscus* and to detect interesting modifications like e.g. sports in *Rhododendron* will be discussed.

A PROTOCOL FOR HIGH RATE AGRO-BACTERIUM-MEDIATED TRANSFORMATION OF *LILIUM*

LP11

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Lilies are economically important, mainly because of their large and attractive flowers. Low rate of transformation in Lilium is the main barrier for molecular breeding of this important plant. In this study, an efficient system for *Agrobacterium* mediated transformation of *Lilium* × *formolongi* and Acapulco was established using mersitematic nodular calli. The calli were inoculated with *A. tumefaciens* strain EHA101 containing the plasmid pIG121-Hm which harbors intron-containing β-glucuronidase (GUS) gene under the control of a 35S cauliflower mosaic virus promoter, hygromycin phosphotransferase (HPT) gene, and neomycin phosphotransfease (NPTII) gene as reporter genes. The effects of different concentration of calcium (0, 11, 22, 44 and 88 mg/l), different carbohydrates (sucrose, glucose and arabinose) and two types of MS media (MS full strength and MS modified) in inoculation and co-cultivation medium were considered. In all treatments, 10mM MES and 100 µM acetosyringone were used. In medium without calcium the rate of transformation slightly increased. Interestingly, the use of MS modified medium (without some macro elements) containing sucrose dramatically increased the transformation efficiency. The highest rates of transformation 25% and 23% were attained using MS modified medium containing sucrose in *Lilium* × *formolongi* and Acapulco, respectively. The hygromycin resistance calli were successfully regenerated into plantlets on hormone free MS medium. Transgenic plants were confirmed by GUS histochemical assay, PCR and Southern blot analyses.

LP12

TRANSFORMATION WITH *ROL*-GENES OF *AGROBACTERIUM RHIZOGENES* AS A STRATEGY TO BREED COMPACT ORNAMENTAL PLANTS WITH IMPROVED POSTHARVEST QUALITY

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The aim of the present investigation was to find and evaluate an alternative strategy to produce potted plants without using chemical growth regulators by transformation with *Agrobacterium rhizogenes*. In *Kalanchoe blossfeldiana*, root inducing (Ri) lines were regenerated from hairy roots produced by transformation with the natural occurring bacteria *A. rhizogenes*. Transformed plants displayed distinct changes in plant morphology,.A number of Ri-lines were analysed thoroughly in greenhouse trials and characterisation showed that both growth and development were affected. Several lines were compact and exhibited an increased number of lateral shoots. Time to flowering was the same as in control plants in one of the investigated lines and delayed by only three days in another Ri-line; other lines showed delayed flowering compared to control plants.

In ethylene free environment, transformants performed better than control plants and single flowers lasted longer. In response to exogenous ethylene, flowers of Ri-lines exhibited tolerance while chemically growth regulated and control plants were sensitive. Possible mechanisms behind the improved postharvest performance of plants transformed with *rol*-genes are presently investigated.

Crossing with established lines documented heredity of the traits of interest since dwarfism was obtained in the offspring. The presence of *rol*-genes was proved in the progeny exhibiting dwarfism. The heredity of the traits will be further investigated. A compact plant without delayed flowering, and improved postharvest performance is valuable for further breeding programmes.

NEW GENOTYPES OF *HIBISCUS ROSA-SINENSIS* THROUGH CLASSICAL BREEDING AND GENETIC TRANSFORMATION

LP13

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Hibiscus is the largest genus of the mallow family (*Malvaceae*), a group comprising 116 genera, many of which are economically valuable. Several *Hibiscus* species are used all over the world as ornamental plants. The most popular one, *H. rosa-sinensis* shows a high variety of flower colours and shape. However, its use as an ornamental plant is currently restricted by a few constraints: 1) most of the commercialised pot-plant varieties are obtained by applying growth retardants; 2) the range of colours and types in use is limited if compared with the available ones; 3) the present cultivars are not fit for the Mediterranean region in terms of propagation and growth rates, as well as flowering; 4) the market requires frequent introductions of novelties.

In 2006, a breeding programme was established at CRA-FSO with the aim of selecting specific cultivars suitable for pot-plant production and creating new genotypes well-adapted to the Mediterranean climate. A partial diallelic cross design (reciprocals without self-fecundations) was used to test the ability of the collected cultivars to produce valuable progenies. Only a limited number of cross progenies resulted to combine desirable characters with a reasonable degree of fertility. A range of very good female parents able to produce seeds in all cross combinations was found. The first selected cultivars are presented.

At the same time, another approach was followed, in order to investigate whether desirable morphological modifications (plant size reduction for pot plant cultivation) could be obtained by transforming *H. rosa sinensis* with *Agrobacterium rhizogenes. In vitro* seedlings were used as sources of explants for the transformation experiments. Two *A. rhizogenes* strains (ATCC 15834 and NCPPB 1855) were employed. Axenic hairy root cultures were established about 4 months after inoculation. Hairy roots grew vigorously on hormone-free medium whereas normal roots did not. Transformed roots displayed a typical hairy root phenotype characterized by fast growth, high lateral branching and lack of geotropism. So far, after more than one year of cultivation, a clone of hairy root deriving from a cotyledon formed a friable yellowish callus at root node level; several adventitious buds are spontaneously regenerating from it. Work is still in progress.

LP14

INVESTIGATION OF THE FACTORS AFFECTING CROSS-FERTILIZATION RATE IN ROSE

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Rose has most long history of artificial improvement by man-kind in ornamental plants. Due to their complex genetic constitution, rose is heavily self-incompatible and gives in low cross-fertilization rate by crossing genetically distant pairs. Consequently, low cross-fertilization rate results in a negative effect on breeding process. In this study, we investigated the factors that would influence cross-fertilization rate of roses. Correlation analysis was performed between cross-fertilization rate and genetic distances of their parents. Analysis of variance was also performed to study the paternal and maternal effects for the cross-fertilization rate. Reliable data for cross-fertilization rate was obtained from Jeollanamdo Agricultural Research and Extension Servies, Korea in 2006. Thirty two cross combination provided cross-fertilization rate, and their genetic distances were obtained by using RAPD marker analysis. In RAPD analysis, we used 16 primers and obtained clear 101 RAPD markers. Genetic distances were obtained by using Nei's coefficients. Relationship between cross-fertilization rate and genetic distance of parents was conducted by using correlation analysis. The correlation coefficient was as low as 0.058 that was no significant. Consequently, we determined that overall genetic distances do not influence the cross-fertilization rate by any means. The paternal and maternal effects for the cross-fertilization rate were determined by. The result of analysis of variance showed that only maternal side influences the cross-fertilization rate. This guide that rose breeder must have desirable materials that can be served as receiver plants. Moreover, we expect more complicated factors will influence on the cross-fertilization rate and more approaches should envision on this matter.

CLONING OF THE ACC SYNTHASE GENE FROM *CURCUMA ALISMATIFOLIA* GAGNEP AND ITS USE IN TRANSFORMATION STUDIES

L15

The goal of this work was to suppress the expression of the ACC synthase gene in the Siam tulip, *Curcuma alismatifolia* Gagnep. A cDNA fragment encoding ACC synthase from *C. alismatifolia* Gagnep. was isolated and expression studies were done. To isolate this gene a pair of primers was designed from the highly conserved motif of ACC synthases in various plant species. The PCR product of 600 bp. in *C. alismatifolia* Gagnep. was subcloned into the pGEM-T easy vector resulting in p*Ca-ACS*l. After sequencing, the sequence and its deduced amino acid sequence were highly homologous to the ACC synthases from other plants. To determine expression patterns of p*Ca-ACS*l, a Northern blot analysis showed that the expression of the p*Ca-ACS*l gene was in the bracts of curcuma, the highest expression was observed 2 days after cutting the flowers. *Ca-ACS*l was subcloned in pBI121 resulting in pBI121-*Ca-ACS*l and transformed into leaf tissues of *Torenia foumieri* and retarded shoots of *C. alismatifolia* Gagnep via *Agrobacterium tumefaciens* strain AGLO. Putative transformants, with the gene in an antisense orientation, were investigated by PCR analysis, GUS assays and Southern blotting. The transgenic plantlets were transferred to pots containing soil for cultivation in growth chamber. At present, the transgenic plants grow happily in the greenhouse and their phenotypic is under investigation.

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LP16

ASSESSING *ROSA PERSICA* GENETIC DIVERSITY USING AMPLIFIED FRAGMENT LENGTH POLYMORPHISMS ANALYSIS

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The genetic diversity among 128 Iranian *Rosa persica* (*R. persica*) accessions in the different populations was analyzed. Amplified fragment length polymorphisms (AFLP) technique was used to produce 171 polymorphic fragments. The number of polymorphic loci ranged from 101 to 147 and the polymorphism information content (PIC) varied from 0.289 to 0.073, with an average of 0.16. This shows extreme variability and genetic diversity among the studied *R. persica* populations. An indirect estimate of the number of migrants per generation (*N*m=0.376) indicated that gene flow was relatively low among populations of the species. Cluster analysis using the UPGMA method grouped all accessions into six clusters. The results did not show relative agreement with the genotypes' region of origin. Based on an analysis of molecular variance, 48% of the genetic variation of *R. persica* was within population and 52% was among populations. The present analysis revealed that Iranian *R. persica* genotypes are highly variable and genetically distinct from their origins. The apparent unique nature of the *R. persica* genotypes revealed by our results supports the case for the implementation of more intense characterization and conservation strategies, and provides useful information to address breeding programmes and germplasm resource management in *Rosa spp.*

REDESIGNING FLORAL ARCHITECTURE: EFFICIENT MODIFICATION OF AGRONOMIC TRAITS BY CRES-T

LP17

While many of the genetically-modified plants now benefiting our daily life, high costs for their development and commodification is still a major problem. One effective way to solve this problem is to increase productivity of useful transgenic plants by targeting transcription factors, because most of the known plant mutant phenotypes have been shown to be caused by the disruption of their function. From this perspective, we applied Chimeric REpressor gene-Silencing Technology (CRES-T) to various kinds of ornamental flowers. CRES-T is an efficient gene silencing system in which the chimeric repressors derived from various transcription factors dominantly suppress the expression of the respective target genes, and the resultant transgenic plants exhibited loss-of-function phenotypes specific for the transcription factors even in the presence of redundant transcription factors. More than 100 *Arabidopsis*-derived chimeric repressors driven by CaMV35S promoter were individually or collectively introduced into chrysanthemum, torenia, cyclamen, morning glory, lisianthus and gentian to evaluate the availability and general versatility of CRES-T for improving floral traits. We found that CRES-T functions in all the species tested, even in the hexaploid chrysanthemum. It is useful not only for producing novel petal colour patterns and shapes as well as leaf shapes, but also for controlling agronomic characters such as ethylene sensitivity or flowering regulation. In addition, we have succeeded to revive a lost garden variety of morning glory which only remained in some ancient texts. Now we are improving and applying this system also to major flowers such as roses and carnations to improve the commercial value. In this presentation, we show some latest data on the CRES-T-applied transgenic flowers and discuss how we should approach and handle this new technology. Some information including graphical contents of transgenic plant phenotypes is available to the public in our database “FioreDB” <http://www.cres-t.org/fiore/public_db/ >. This work was supported by the Program for Promotion of Basic and Applied Researches for Innovations in Bio-oriented Industry.

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LP18

KALANCHOE X HOUGHTONII: SSH AND MICROARRAY ANALYSIS TO SCREEN GENES INVOLVED IN VIVIPARY

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Vivipary, referred here as the formation of novel complete plantlets on mature organs, has been reported in many families as an asexual propagation strategy. In *K. xhoughtonii* (*Crassulaceae*), viviparous plantlets are formed on leaf margin notches in response to a long day photoperiod and their appearance follow a basipetal fashion. To identify genes involved in this process, suppression subtractive hybridisation libraries (SSH) were prepared. 200 c-DNA clones were classified and grouped into fourteen functional categories according to Goldberg database (http://estdb.biology.ucla.edu/PcEST/).

630 sequences (200 SSH library, 48 database Kalanchoe genus, 382 other species database genes) were used as probes for microarray analysis according to the CombiMatrix technology. A 4x2K Custom Array™ was synthesized .

RNA was extracted from margin of leaves at 7 stage of development before buds emission (5mm to 50 mm) during long-day photoperiod (permissive conditions) and one stage during short-day photoperiod (non permissive condition). Three replications for each sample were prepared. From double strand cDNAs antisense RNAs (a RNA) were synthesized and amino-allil-UTP incorporated and coupled with Alexa Fluor® 647. Microarray was hybridated according to CombiMatrix protocol. Data were extracted with CombiMatrix Microarray Imager software and exported into Microsoft Excel for computing of mean, median and standard deviation. Person's correlation was computed and data normalized. After background removal, probes were reduced to 484. “Fold change” method (FC=2) was used to compare levels of gene expression of samples in permissive conditions vs sample in non permissive condition. Significance Analysis of Microarrays Statistic (SAM method) generated 263 significant modulated genes with FDR of 5%.

POLLEN CHARACTERISTICS AFFECT SEED PRODUCTION OF ROSE CULTIVARS

LP19

Seed production and germination rates are important bottlenecks in rose breeding. Most cut roses are Hybrid Tea cultivars that are highly selected, typically tetraploid and highly heterozygous. Cut rose seeds do not germinate more than 50%, with hip contents usually ranging between one and 30 seeds. Therefore, during rose selection more attention should be paid to enhance the breeding efficiency itself. With the aim to characterize fertility markers in cut rose cultivars, a commercial breeding database (data 1994 -2007) was analyzed. From this database a high and a low fertile pollen donor and a high and a low fertile seed parent were selected and crossed in partial diallel. In this study, vitality, germinability and morphology analyses were carried out on dried and fresh pollen of the four parents, that were simultaneously cultivated in Belgium and Italy. Results suggested a correlation between the pollen diameter and the fertility index obtained from the crosses.

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LP20

APPLICATION OF CROPS® TECHNOLOGY IN A WIDE RANGE OF VEGETABLE AND FIELD CROPS

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KeyGene has developed the CRoPS technology®[1] (Complexity Reduction of Polymorphic Sequences) for high throughput polymorphism discovery in a wide range of species. CRoPS combines the power of AFLP® [2] as a robust genome complexity reduction method with the power of next generation sequencing of the Roche GS FLX. Since CRoPS is AFLP-based, no prior sequence information is required to enable polymorphism discovery by sequencing genome partitions of multiple samples in parallel. Recently we developed an export module for the CRoPS SNP mining pipeline to automate the design of ready-to-order Illumina BeadExpress assays for SNP genotyping.

We will present an overview of CRoPS runs performed in a number of species in terms of numbers of high quality putative SNPs obtained. These results show the efficiency of CRoPS as *de novo* SNP discovery technology and conversion rates of putative SNPs to high-throughput SNP genotyping assays. The fact that not much sequence information is yet available in ornamentals makes the CRoPS technology ideal for *de novo* SNP development in ornamental plants, thereby paving the way to marker assisted breeding. Being sequence based, the SNP genotyping platform will enable full integration of marker data with existing or future whole genome sequences. Sequencing of genomes of ornamental plants is currently within reach as sequencing costs are dramatically dropping with the development of new generation sequencing technologies.

[1] van Orsouw et al (2007) PLoS ONE 2 (11): e1172
[2] Vos et al (1995) Nucleic Acids Res 23: 4407-4414

COOPERATIVE MARKETING – A WAY TO STIMULATE SALES AND CONSUMPTION OF A PLANT?

L29

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While on many markets well known brands have been established, the flowers and plants sector developed a different approach to promote successfully the consumption of its products. In North and Western Europe cooperative marketing organisations have been set up which stimulated sales and consumption of flowers and plants. Usually, these activities did not focus on one product but the usage of flowers and plants in general, as decoration item for personal usage, as gift etc. More recently, product specific marketing campaigns have been set up, promoting in a cooperational approach the use of a certain plant or plant group.

Why cooperative marketing – and not for a brand but flowers and plants in general – or even a product? Cooperative marketing organizations for flowers and plants were set up because of the special characteristics of plants and their market. Given the number and versatility of products, not one product, not even a certain product range have a market share which enable them to spend budgets necessary for brand marketing. Brand marketing is very expensive. Big brands like Coca Cola spend every year hundred of millions euro for advertisement. That flowers and plants are generally not seen as brand has a big advantage: instead of paying hundreds of million euro for expensive advertisement, Public Relations is the way to approach the public. Consumer magazines and TV shows report on how to decorate with pot plants or on the welfare of green pot plants. Regularly, flowers are presented as valuable gifts. Via Public relations, cooperative plant marketing is very cheap – and very effective.

What presuppositions are there to conduct successfully cooperative marketing (for a product)?

Presupposition for establishing cooperative marketing is a “bottle” neck. This can be either by a law or by a group of companies deciding to collectively conduct marketing measures on a voluntary base. If marketing activities are based on voluntary cooperations between market partners, an important presupposition is the trust among the participants of the group. Old conflicts should have been solved. Furthermore, there should be a common idea, vision or goal as well as a common decision on how to raise funds.

Examples for successful marketing campaigns are e.g. the Plants of the month approach in the tree nursery sector, the Dutch Verrassend buiten, the European marketing campaign Cyclamen Colour Europe or Stars for Europe, the European poinsettia campaign. The goal of the marketing campaign for Cyclamen was to promote the outdoor usage for Cyclamen. The goal of the Poinsettia campaign is a change the old fashioned image and link the Poinsettia more intensively to Christmas as a time of extensive spendings for decoration items.
In my presentation I will focus on how to set up cooperative marketing campaign, talk about budgets necessary and the effects of such campaigns.

L30

THE BRAND: FREDERIQUE'S CHOICE

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Mission

Our mission is to create and grow a successful and powerful brand - a brand that stands for *Elegance,* for *Luxury,* for *Dynamism,* but also for *Approachability*.

We have adopted a new way of looking at traditional marketing processes. By shortening the chain between producer and consumer, we will control the product and guarantee a consistent quality. Our objective is to bring the best quality and design from the source to the consumer under the brand name Frederique's Choice.

Let's make flowers fashionable!

Philosophy

Frederique van der Wal is the personification of the Frederique's Choice brand. Products marketed under the brand name invite the consumer to share in the everyday glamour that Frederique embodies. Frederique will personally select and guarantee the style and quality of every product sold under the brand name.

A person who wants to be a discerning consumer of flowers and home products will only have to make one choice: FREDERIQUE'S CHOICE.

Consumers will identify with Frederique's approach to life, sense of style and her success. As one of the best-known faces in the world of lifestyle, Frederique is the ideal ambassador for her own brand. Her success in the world of fashion, design and beauty gives her the opportunity to represent her own brand: FREDERIQUE'S CHOICE, Life in full bloom!

The brand is built around the following core values:

- Quality
- European style
- Selection and control from the source
- Loyalty to our brand, our people and our environment
- Support of charities
- Creating a sense of everyday luxury

Products

In view of the enormous potential for a new brand in the flower sector and a new marketing approach, Frederique's Choice started in April 2008 by selling **fresh flowers, bouquets and flower bulbs**. There is currently no recognizable consumer brand for flowers. Introducing a flower brand that is identifiable and associated with quality and exclusive distribution is likely to have a larger impact and be of greater interest to media than introducing a general home and leisure brand. By starting with flowers, we maximize the impact of the new brand and create the right image from the start. Frederique's Choice also included other flower-related products. Eventually, we will create our own exclusively-produced home and leisure product lines to be supported by the brand and will launch them in the top end of the market.

EARLY-FLOWERING TRANSGENIC *CHRYSANTHEMUM* PLANTS

L31

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Chrysanthemum (*Chrysanthemum morifolium*) is one of the worldwide ornamental cultures. It is a short-day plant that blooms in autumn. When grown in greenhouse, they can be fooled into blooming any time of year by decreasing the amount of light they receive. Early-flowering cultivars are recommended to produce quality chrysanthemum cut flowers at lower cost of production. Flowering control should allow for shorter breeding cycles.

The transition to flowering is a complex process that is regulated by many different mechanisms. Extensive studies in model plant *Arabidopsis thaliana* have revealed genetic and molecular mechanisms of flowering. In Arabidopsis, *CONSTANS (CO)* and *FLOWERING LOCUS T (FT)* act in the photoperiod pathway. They up-regulate the floral meristem identity genes *LEAFY (LFY)* and *APETALA1 (AP1)*. These genes were demonstrated to be useful for shortening flowering time in certain plant species: constitutive expression of *LFY* and *AP1* cause early flowering of transgenic plants.

We had cloned three *AP1*-homologous genes from chrysanthemum (*CDM111*) and sunflower (*HAM75*, *HAM92*) and have become interested in the generation of chrysanthemum transgenic plants that over-expressed these genes. The coding region of each full length cDNA was fused to the CaMV 35S promoter into pGD121 binary vector. For *Agrobacterium tumefaciens* (strain CBE21) mediated chrysanthemum transformations, leaves from *in vitro* grown plants were used. Regenerating plants were selected on MS media containing 35 mg/l kanamycin. Transformation efficacy was about 15%. Totally 62 independently regenerated plants carrying integrated transgenes were produced and used for subsequent experiments on flowering induction.

We have demonstrated that over-expression of compositae *AP1*-homologs in transgenic chrysanthemum under long-day conditions had no effect on flowering time and vegetative development. In the same time, under short-day condition transgenic plants have started bud initiation two weeks earlier that non-transgenic control chrysanthemum plants. Later on, transgenic chrysanthemum plants showed colour earlier and resulted in earlier harvesting by 5 weeks compare to non-transgenic control plants.

These results open new possibilities for genetic improvement and breeding chrysanthemum cultivars.

L32

ISOLATION AND CHARACTERIZATION OF FLAVONOID 3' HYDROXYLASE (*F3'H*) GENE AND GENETIC TRANSFORMATION IN BUTTERFLY PEA *(CLITORIA TERNATEA LINN.)* VIA *AGROBACTERIUM TUMEFACIENS*

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The flavonoid 3' hydroxylase (F3'H) gene denoted as an AsF3'H was isolated and characterized from petals of Ascocenda sp. The partial cDNA encoded a 264 amino acid polypeptide which showed a high homology to known F3'H genes, especially, F3'H from Gerbera hybrida. F3'H is cytochrome P450 and it is a key enzyme in the flavonoid pathway leading to the production of the red coloured anthocyanins. The AsF3'H transcript was the most abundant in petals from flowers at an early stage of development and levels declined as the flower matures. No transcript levels were detected in leaves and stem.

In butterfly pea (Clitoria Ternatea Linn.) a genetic transformation system via Agrobacterium tumefaciens Was developped Therefore, the CuDFR gene was constructed in pBI121 and transformed into butterfly pea's leaves, petunia (Petunia axillaris) and tobacco (N. tabacum) by A. tumefaciens strain AGLO.. After 4 months in tissue culture, the transgenic shoots were detected on selective media and the efficiency of transformation was 20 %, 25 % and 23 % of GUS assay respectively. Moreover PCR technique reveals the positive bands of GUS gene and 35S promoter.

ORIENTAL LILY HYBRIDS ENGINEERED TO RESIST APHID ATTACK

L33

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Insects such as aphids are the major animal vectors for the spread of viruses in lily cultivation. Viral infections in lilies lead to a decrease in bulb and flower quality and have a significant negative impact on the economic value of the crop. Pyrethrins and mineral oil are the main chemicals used to fight viral infestations mediated by aphids. The availability of insect-resistant cultivars would provide the means to reduce the use of chemicals and to allow a more sustainable cultivation. One of the modern tools in breeding is genetic modification. Prerequisites are a gene transfer protocol for the crop of interest and genes coding for the desired traits. In our laboratory an *Agrobacterium*-mediated transformation protocol was developed based on Hoshi et al. (2004, Plant Cell Rep. 22:359). This protocol for the production of marker-free transgenic lilies proved successful on several *L.longiflorum* and on Oriental hybrid cultivars. Proteinase inhibitors have been used successfully in engineering insect resistance against feeding or sucking insects, such as aphids and thrips, after introduction of the appropriate genes. Genes coding for volatile repellents were also found to be effective by deterring insects. In a dual approach, a gene coding for a proteinase inhibitor will be combined with a gene coding for a monoterpenoid repellent in our marker-free binary vector, pMF2, and used for gene transfer to lilies. For this, a series of 22 lily cultivars, encompassing mostly orientals, but also longiflorums, OT and LA hybrids, have been used to induce callus on filaments and styles in order to test for regeneration ability and amenability to transformation. The first results on this will be presented.

L34

DMMADS4, A *DEF*-LIKE GENE FROM *DENDROBIUM* IS REQUIRED FOR FLORAL ORGAN IDENTITY AND FLOWER LONGEVITY OF ORCHID

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Two Class B MADS-Box genes were isolated from *Dendrobium moniliforme* i.e. *DMMADS4* and *DMPI*. *DMMADS4* encodes for protein with a predicted length of 224 amino acids; it shares 89% identity with *PeMADS4* at the amino acid level. *DMPI* encodes for a 210 amino acid protein that showed 94% identity to *PeMADS6* from *Phalaenopsis*. The results from RT-PCR analysis showed that *DMMADS4* was transcribed in petals, lip and column; however, *DMPI* was expressed in all four floral whorls. Expression pattern of *DMMADS4* is broader than that of *PeMADS4* which is expressed only in the part of the lip. Therefore, *DMMADS4* does not specify only lip development like *PeMADS4*. Interestingly, the expression of *DMMADS4* and *DMPI* were also detected during the ovule development. A high level of expression of both genes was found in pre-pollinated ovules of flowers. Yeast two-hybrid analysis demonstrated that *DMMADS4* and *DMPI* were able to form heterodimers. The phenotype of 35S::*DMMADS4* transformed *Arabidopsis* plants was indistinguishable from that of wild-type plants except plants size was reduced. However, in the 35S::*DMPI* plants, flowers have conversed sepals into a petaloid-like structure in whorl 1. The crossing between 35S::*DMMADS4* and 35S::*DMPI* resulted in the alteration of sepals into petaloid-like structures in whorl 1. Also, the plants maintained flower bud stage longer than wild-type plants. These results suggested that the B-function genes, *DMMADS4* and *DMPI*, are necessary for petal development and might be necessary for flower longevity of orchid.

OVEREXPRESSION AND SILECING OF *KXHKN5* GENE IN *KALANCHOE X HOUGHTONII*

L35

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K. x houghtonii (n=51), a triploid interspecific hybrid between *K. daigremontiana* Hamet & Perrier (n=17) and *K. delagoensis* Ecklon & Zeyher (n=34), forms epiphyllous buds on leaf margin notches in response to a long day photoperiod (vegetative vivipary). Several well known class 1 *knox* genes, as *Kn1* from maize, *STM*, *KNAT1* and *KNAT2* from *A. thaliana* and *Bkn3* from barley, play an important role in meristem formation and maintenance and a few reports suggest that this class of homeotic genes could be involved in vivipary. In attempt to characterize *KNOX* genes involved in vegetative vivipary in *K. xhoughtonii*, overexpression and post transcriptional gene silencing (PTGS) experiments of *KxhKN5* (EU240661) were performed. To accomplish over-expression, the complete cDNA sequence of the gene (1161 bp), overdrive by 35S promoter and NOS terminator, was cloned in the binary vector pGreen II (www.pgreen.ac.uk) that contain the NPTII gene, that confer resistance to kanamycin (pGreen II NPTII). PTGS construct was prepared by cloning in pJM007 (Schattat et al., 2004), a 326 bp fragment of the gene in sense and antisense orientation in the specific cloning sites located at the left and at the right of the PIV2 intron; silencing cassette was excided from pJM007 and cloned into the binary vector pGreen II NPTII; the derived vectors were then transferred to *A. tumefaciens.* Genetic transformation and selection on medium containing kanamycin, and cefotaxime were conducted; the regenerated shoots were isolated from the leaf explants and separately cultivated on propagation medium to establish plant clones some of which were acclimatized in greenhouse. In addition, to localize *KxhKN5* mRNA in early fase of *K. Xhoughtonii* epiphylly, *in situ* hybridization was performed with digoxigenin (DIG)-labelled RNA probe according to DIG RNA labelling KIT (Roche, Mannheim, Germany), with minor modifications. Overexpression and PTGS of *KxhKN5* affect plant architecture and leaf shape as reported for class 1 *KNOX* genes cloned from other species. Ectopic buds and shoots formation was not observed in transgenic plants overexpressing *KxhKN5*; furthermore the vegetative vivipary was reduced in the transgenic clones with extremely modified phenotype likely as a consequence of the leaf shape modification.

L36

EXPRESSION OF AN *ARABIDOPSIS* ASPARTIC PROTEASE IN *PELARGONIUM*

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Arabidopsis thaliana transgenic plants with constitutive over-expression of the aspartic protease gene At2g28010 (named CDS10) showed a bushy, multi-branching dwarf phenotype. In order to obtain compact plants of ornamental interest with an analogous phenotype in Pelargonium zonale, a tall cultivar (Boda Gitana Salmon) was transformed to over express the A. thaliana CDS 10 gene under the 35S promoter. Twenty seven transgenic lines were obtained with different levels of expression after gold particle bombardment and regeneration. Some of them showed indeed a bushy phenotype with a higher number of branches and a dwarf phenotype. However, an increase in the number of branches correlated with a decrease in the number of petals in the flowers. So the plants that were of interest from the compact habit point of view, had lost the double flower trait, and exhibited only 5 petals/flower which were also smaller than those from double flowers from the non transformed plants. Intermediate phenotypes with semi-double flowers and higher number of branches but without a compact phenotype were also observed. In order to determine if it was genotype related two other cultivars were transformed, Mirada Violet and Mirada Simple Pink double and single flower cultivars respectively. Transgenic plants showed indeed a higher number of branches and single flowers. Even if the busy phenotype was of interest in order to get a higher number of cuttings/plant and a compact phenotype, the pleiotropic effects of the over-expression of the A. thaliana CDS 10 gene on the flowers are too strong meaning it is only of interest in single flowered cultivars which are a small share of the market.

MOLECULAR MARKERS AND THEIR USE IN ORNAMENTALS

L37

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In the past 20 years the use of molecular markers has gradually expanded from the field of scientific genetic analysis towards the implementation in commercial breeding programs. Therefore the last afternoon of this Eucarpia congress on “Colourful Breeding and Genetics” will be dedicated to the use of molecular markers in ornamental breeding. A general overview of the use in research and practical breeding will be given with examples from a variety of ornamentals such as roses, lilies and tulip. Specific attention will be given to the development of markers that can be used for identification of ornamental crops, especially if no markers are known yet for a certain species. Another focus will be on recent advances in sequencing technology. These are changing the way molecular tools are used for breeding. Enormous amounts of data can be generated in a very short time, and most importantly, the costs have gone down to a level that now makes a 1000$ genome imaginable in the near future. High density genetic maps will be affordable tools not only for the major crops. Fast and cost-efficient discovery of markers and genes for important agronomical traits is possible only when genomics data is combined with expert crop knowledge and accurate and reliable phenotyping. How can we use it in research and breeding of ornamentals?

The company Keygene N.V. (http://www.keygene.com) will present its view on marker application in ornamental research and breeding. DNA technology combined with the laws of inheritance is being used by Keygene to develop procedures and methods that can elucidate the relationship between phenotypic variation and genotypic variation, thereby generating knowledge of the molecular control of valuable traits. Efficient and effective exploitation of this knowledge is the core business of the modern plant breeder. Many valuable traits have a complex inheritance in plant populations and therefore the molecular control of this type of traits has a complex nature as well. This presents serious challenges for the future and requires integration of different knowledge levels as well as breeding strategies and breeding schemes.

The afternoon will be closed by a round table discussion. During the congress it will be possible to submit specific questions to the organizers (a questionnaire will be provided in the congress bag). The questions will be discussed by participants and organizers.

7. Abstracts of posters

P1

ISOLATION OF FEMALE GAMETE CELLS FROM UNPOLLINATED OVULES IN *LILIUM*

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Ovules obtained from opened flowers of Oriental hybrid 'Casa Blanca', LA hybrid 'White Tycoon', *Lilium* × *formolongi* 'Hakuryu' and *L. formosanum* were wounded by a surgical blade, and then treated with an enzyme mixture. Afterwards, ovule sections were dissected using a handmade glass needle and the tip of a 30G short needle syringe under a stereomicroscope to remove the outer and/or inner integument. The isolated embryo sac with nucellar tissue was cut off by the 30G short needle syringe, and was then pushed off by a macromanipulating system to isolate female gamete cells. The isolated gamete cells could be easily captured and handled by a microcapillary system that was connected to an electric micropump. The combination of a surgical cutting with the use of an enzymatic treatment, shortened significantly the total operating time for the isolation of female gamete. However, the optimal incubation time for the enzymatic treatment differed between cultivars and species. It varied from 1 to 1.25 and 2.5 hrs for *L.* × *formolongi* 'Hakuryu', *L. formosanum* and *L.* 'Casa Blanca', respectively. No isolated gamete cells could be obtained from ovules of 'White Tycoon' due to the fact that the cut flowers were stored at low temperature for several days.

HERITABILITY OF COLD TOLERANCE IN *GLADIOLUS*

P2

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Gladiolus xhybridus are not cold tolerant (winter hardy) in northern temperate climates, such as USDA Winter Hardiness Zones 3-4, with corms capable of surviving -10°C to -12°C temperatures. The University Of Minnesota Gladiolus Breeding Program has an objective to screen germplasm (wild species, intra- and inter-specific hybrids) for use as parents to determine the extent of cold tolerance in the genus and its heritability for future release of winter hardy cultivars that do not require fall digging and overwintering in storage (>0°C). Wild species and hybrids were tested to quantify LT_{50} (the lethal temperature at which 50% of the progeny were killed) using controlled freezing tests with acclimated corms. Several hardy parents were found ($LT_{50} \leq$ -10°C). From these tests, interspecific crosses between non-hardy x non-hardy, hardy x non-hardy, and hardy x hardy parents (cross groups) were created to test the segregation of cold tolerance, the level of heritability (h^2), and inter-simple sequence repeat (ISSR) molecular marker segregation using multiple primers. The experimental design for Obj. 1 freezing tests was a completely randomized design (CRD) consisting of [5 temperatures x 3 groups x 5 freezing runs x 15 hybrid corms/group/temperature] for a total of 1,125 experimental units (corms). Corms were potted and acclimated at 2°C for 1,000 hours (6 wks) prior to controlled temperature freezing tests of 0°C, -3°C, -6°C and -10°C in a programmable freezer with varying ramp and soak times. Frozen corms were removed at each test temperatures and slowly thawed at +2°C for 24 hrs, then placed in a glasshouse at 18/16°C (day/night) and long day photoperiods (0600-2200 HR day lengths) for observation and forcing. Corms were examined for growth and vigor (1-5 scale), no. and length of shoots and roots, as well as visible damage to corm structures. In the hardy x hardy crosses, the mean number of live roots was 3.8 $\pm$ 2.5, with an average length of 5.9$\pm$ 4.4 cm, comparable to hardy x non-hardy crosses with an average number of 4.1$\pm$3.3 and a length of 6.2$\pm$4.8 cm. These differed from the non-hardy x non-hardy crosses, with 2.4$\pm$1.5 roots and 3.5$\pm$3.3 cm lengths. The average number of shoots in the hardy x hardy crosses was 1.1$\pm$0.3, with a mean length of 7.2$\pm$6.6 cm whereas hardy x non-hardy crosses had 1.1$\pm$0.3 shoots that were 6.9$\pm$7.4 cm in length. Comparatively, non-hardy x non-hardy crosses had only 1.0$\pm$0.0 shoots that were 2.2$\pm$1.8 cm long. Winter hardiness was heritable, although the h^2 were low (h^2<0.5) in most progenies. ISSR molecular markers showed that many of the winter hardy genotypes were similar to several wild species while most non-cold tolerant cultivars were more in separate principle component groups.

P3

INTERSPECIFIC HYBRIDIZATION OF BRAZILIAN *VRIESEA* SPECIES

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Bromeliaceae are endemic to Brazil, where forty percent of the species and seventy percent of the genus of this family are present. However, this important diversity has been threatened by extractivism. To generate new ornamental material, which can help to avoid excessive extractivism, hybridizations between different genetic accesses are important. In the present work, six species of bromeliads from the Brazilian Atlantic Forest and two commercial hybrids were hybridized. Pollen-receptor flowers were emasculated before anther dehiscence, following hand pollination with pollen from pollen-donor plants. Controlled crosses were done using plants from the same species as well as interspecific crosses and crosses between species and commercial hybrids. Capsule formation was observed after 60 days. Crosses between *V. carinata* x *V. paraibica* showed 100% capsule formation, independently of the pollen donor/receptor species. Reciprocal crosses using *V. friburguensis*, *V. incurvata* and *V. simplex* also presented high percentage of capsule formation. Seeds were collected when capsules matured and were placed in ¼ MS culture medium (Murashige & Skoog, 1962) for *in vitro* germination. Seedlings were grown *in vitro* and RAPD analyses were performed to confirm the hybrid character of the seedlings. The results indicate the efficiency of crosses with *Vriesea* species and their potential use for the production of new options for the ornamental bromeliad market.

KARYOTYPE ANALYSIS OF THREE IRANIAN NATIVE *IRIS* SPECIES

P4

There are several native species of Iris in Iran that has not been studied cytogenetically yet. Therefore, more cytological and molecular research is needed for their classification and identification. In this study, karyotypic analysis was carried out in three native Iranian *Iris* species: *I. caspica*, *I. spuria* and *I. meda*. *I. caspica* consisted of 22 pairs of chromosomes: 4 pairs of m-type, 8 pairs of sm-type and 10 pairs of acr-type. The pair chromosome 5 of this species had satellites. Total form percentage (TF%) in this species was 30.93%. In studied metaphase cell in this specie arm ratio of the chromosomes ranged between 1.10±0.04 and 5.30±0.88 and all of the chromosomes with arm ratio lower than 1.7 were metacentric. The mitotic metaphase cells of *I. spuria* consisted of 22 pairs of chromosomes; including 13 pairs of m-type and 9 pairs of sm-types without satellites. The TF percentage in this specie was 39.18%. In studied cells in this specie arm ratio of the chromosomes ranged between 1.18±0.03 and 2.94±0.7. The somatic chromosome complement of the mitotic metaphase cells of *I. meda* consisted of 10 pairs of chromosomes; including 1 pair of sm-type, 7 pairs of acr-type and 2 pairs of t-type without satellites. The TF percentage in this specie was 16.85% and arm ratio of the chromosomes ranged between 5.08±0.22 and 7.23±0.04. Regarding to karyotype formula of three iris species the polyploidy and chromosomal structure rearrangement have an important role in iris speciation procedure. Also the existence of many different chromosomes in individual species may imply the adaptation of this species with ecological circumstances. This study showed that *I. meda* species that was collected from mountainous zones had bigger chromosome as well as bigger genome than other species was collected from warmer climate.

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P5

EXPERIMENTAL RESULTS ON GENETICS AND BREEDING OF GILLYFOWERS (*MATTHIOLA* GENUS)

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Two species of *Matthiola* (*M. incana* L. and *M. longipetala* var. *bicornis*) as well as eight cultivars of *M. incana* were tested in three successive years (2003-2005) in Cluj-Napoca, Romania, concerning the homogeneity of their distinctive plant characters (plant height, no of inflorescences/plant, no. of flowers/inflorescence, flower diameter, start of blooming and persistence of flowering stage) which are considered of interest in breeding programs for ornamental purposes in this genus. On the basis of the obtained results, four cultivars of gillyflower (*M.incana* L.) were chosen as valuable genitors for the above mentioned characters and employed in an incomplete diallel crossing system in 2005, F_1 hybrids as well as their parental cultivars being grown in Cluj-Napoca in 2007 and 2007. Heritability both in wide (H) and narrow (h^2) sense was computed for the same characters as those analyzed in parents and phenotypic and genotypic correlations among all possible pairs of characters were evaluated and discussed in view of their efficiency on indirect/tandem selection. Additive and nonadditive effects of the poplygenes involved in the inheritance of the six analyzed characters were revealed by computing GCA and SCA values of the genitors involved.

The values of H = 0.55-0.84 were rather high suggesting a medium to very good phenotypic expression of the analyzed characters in F_1 hybrids of gillyflower. On the other hand, the medium and low values of the narrow sense (h^2) heritability for most the analyzed characters (except for start of blooming) revealed the fact that, in the case of these quantitative characters, most often high heritability in wide sense was accompanied by low or, at most, medium heritability in narrow sense. Quite a few of the analyzed pairs of characters were found significantly correlated at the phenotypic level, in the F_1 populations of gillyflower but out of these only in four pairs (plant height/diameter of flower, diameter of flower/no. of flowers in inflorescence, plant height/persistence of flowering stage, start of blooming/persistence of flowering stage) significant correlations were noted at the genotypic level as well. For all the analyzed characters, significant values of GCA effects were found this being true also for values of SCA effects, except for no. of flower/inflorescence in which the SCA effects were not significant.

THIN CELL LAYER SOMATIC HYBRIDIZATION BETWEEN *CALADIUM HUMBOLDTII* SCHOTT ‘PHRAYA SAVET’ AND *C. BICOLOR* (AIT.) VENT. ‘SUVARNABHUM’

P6

Characters of new clones from somatic hybridization between *Caladium humboldtii* cv. ‘Phraya Savet’) and *C. bicolor* cv. ‘Suvarnabhum’ using thin cell layer technique from *in vitro* culture were observed. Callus were induced from unexpanded leaf segments cultured on modified Murashige and Skoog medium (MS) supplemented with 2.69 μM 1-Naphthalene acetic acid (NAA) and 17.76 μM N^6-Benzyladenine (BA) for 4 – 5 months with subculturing every 6 weeks. Each thin cell layer of callus, about 1 mm thick, from both caladiums was alternately laid on the top of each other for 8 layers. Subsequently, the combination of thin cell layers was cultured on the same medium. The regenerated plantlets were grown in glasshouse conditions. From 3 morphological groups (leaf pattern, leaf colour and petiole), the regenerated caladium plants were found dissimilarly to both original caladiums at 85 percent with 8 types of different characters. Somatic hybridization between *C. humboldtii* cv. ‘Phraya Savet’ and *C. bicolor* cv. ‘Suvarnabhum’ gave rise to a number of most hybrids with all conserving *C. bicolor* characters. However, variations of each caladium from *in vitro* culture were compared to regenerated hybrids.

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P7

IDENTIFICATION OF CARNATION VARIETIES USING MICROSATELLITE MARKERS

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As in many ornamentals, also in carnation the number of varieties in common knowledge is large. Variety registration and protection in carnation is based on morphological characteristics, described in the UPOV Test Guidelines (document TG/25/8). Identification throughout the chain from breeder to consumer depends on the availability of methods that can use plant material from different stages and organs. Furthermore, in situations where a suspicion of an infringement of a PBR has been raised, there may be a need for a quick and reliable comparison of varieties before a lengthy trial for comparison of DUS characteristics is considered. Therefore, complementary approaches, such as the use of microsatellite markers, are being evaluated and molecular databases are being constructed. Microsatellites are highly polymorphic and have the advantage of providing co-dominant markers based on PCR technology. In a sequenced tagged microsatellite site (STMS) approach, they produce simple banding patterns, especially suitable for automated and objective description of plant varieties which are easy to store in a database. New varieties or new markers can easily be added to an existing database.

In this paper, we present the results of our study on the use of microsatellite markers from *Dianthus caryophyllus* L. for the characterization of carnation varieties as well as the construction and evaluation of a molecular database.

INTRASPECIFIC AND INTERSPECIFIC HYBRIDIZATION OF TROPICAL LILY (*LILIUM* SPP.)

P8

Lily (*Lilium* sp) is one of important cut flowers in Indonesia. Demands are increasing over the years. Lily has been utilized for flower arrangement, bouquet, and decoration in important events such as wedding ceremonies and International Conferences which are very often held in Bali. Production of Lily in Bali in particular, or in Indonesia in general are still limited and market are still dominated by imported Lily. here is an urgent need to produce superior varieties of Lily locally. Lily grown in Indonesia are mainly local cultivars which has limited variation in term of colour, shape and shelf life. However, local Lily has the benefit of well adaptation in tropical climate and produces more flowers in one stem compare to introduce cultivars. Therefore, research need to be done to increase Lily variation through breeding, so that Lily varieties with superior and preferable characteristics, such as increase flowering period, flower shape and size, more colour and resistance to disease can be obtained. This research project attempted to do intraspecific and interspesific hybridization of local lily. The short term objective of this project is to obtain viable crosses via of embryo rescue technique, while long term objective is to obtain new cultivars with unique and superior characteristics which are favored by consumers. This research is currently underway in Bali. Materials for crosses were obtained from collection of 'Eka Karya' Botanical Garden in Bali and flower growers. For intraspecific crosses, 3 cultivars of local lilies (orange, yellow and brown) were crosses reciprocally. For interspecific crosses, two cultivars of local lily (yellow and light brown) were employed as mother plants, while pollen from imported lilies were used as male plants. To avoid embryo abortion in interspecific crosses, embryo will be harvested before the seed mature and then transfer to tissue culture media. Crosses in currently underway. It is expected that viable plantlet could be obtained in the next year for characteristics evaluation.

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P9

GENETIC VARIABILITY IN *GLADIOLUS*

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Gladiolus or sword lily is commercially significant bulbous ornamental crop which is grown either as cut flower or garden display. Improvement in gladiolus required genetic variability, its heritability and genetic progress. Also, knowledge of association between yield and its component traits themselves can improve the efficiency of selection. The present study was undertaken to observe the phenotypic variability into its heritable and non-heritable components with suitable genetic parameters viz., phenotypic and genotypic coefficient of variation, heritability, genetic advance. Fifteen elite genotypes of gladiolus were collected from various places and conducted experiment at research farm, Department of Horticulture, Allahabad Agricultural Institute-Deemed University, Allahabad during 2005 in Randomized Block Design with three replications. High genotypic coefficients of variation (GCV) and phenotypic coefficient of variation (PCV) estimates were found for number of cormels per corm. High heritability with high genetic advance was observed for number of cormels per corm, days for spike emergence and spike weight. Number of florets per spike was significantly and positively correlated with number of shoots per corm and duration of flowering. Spike length and diameter of corm was significantly and positively correlated with spike weight but negatively correlated with duration of flowering.

PHYLOGENETIC ANALYSIS OF *ARBUTUS* SPP BY MORPHOLOGICAL CHARACTERISTICS AND MOLECULAR MARKERS

P10

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Morphological characteristics and the method of Random Amplified Polymorphic DNA Polymerase Chain Reaction (RAPD-PCR) was used to study the diversity of *Arbutus andrachne* and *A. unedo* individuals from two different regions of collection, Kalamos and Varympompi, prefecture of Attici (Greece), and individuals of an *Arbutus* sp. with intermediate morphological characteristics found in Kalamos. The morphological characteristics that were studied were: bark morphology, leaf shape, flowers and season of flowering, fruits and season of fruiting. The bark of *A. andrachne* plants and of those with intermediate characteristics was smooth and cinnamon-red in colour, peeling in long paper strips revealing a grey-green internal. Oblong areas of dark red colour were found on the bark of plants with intermediate morphological characteristics. *A. unedo* bark was rough and shreddy dull brown or ash-grey and occasionally peeling in small flakes revealing chestnut-coloured internal. The leaves of *A. andrachne* were ovate to oblong, entire, occasionally pointed, and of *A. unedo* elliptic to oblanceolate, toothed, pointed, while of the intermediate plants were elliptic to obovate, occasionally pointed and often toothed. The plants with intermediate characteristics fructified seldom contrary to the rich fructification of *A. andrachne* and *A. unedo*. Four 10-mer oligonucleotide arbitrary primers were used to amplify genomic DNA and 36 reproducible polymorphic fragments were generated. The degree of genetic similarity was calculated and the dendrogram of seven individuals was established. A genetic variation among individuals that bring intermediate morphological characteristics and those of *A. unedo* and *A. andrachne* was indicated, confirming the morphological variations observed. This allows the statement that it is another species, at least for the primers that were used, possibly the one reported in the bibliography as a natural hybrid between *A. unedo* and *A. andrachne*, named *Arbutus* x *andrachnoides*.

P11

BREEDING FOR NEW CUTFLOWER AND POTPLANT VARIETIES IN *ZANTEDESCHIA* (CALLA)

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Zantedeschia, also named Calla, belongs to the family of *Araceae* and is originally from South Africa. In this breeding programme we only use the tuber types of *Zantedeschia* section *Aestivae* and no root stock types of *Zantedeschia aethiopica* or *odorata* (Singh et al., 1996). The crop is increasing in popularity. The flowers of *Zantedeschia* distinguish themselves for flower form, colour, long vase life and can be transported under dry conditions. Tubers can be stored during a long period of time and therefore it is possible to produce flowers over a long period. *Zantedeschia* as a potplant shows a great promise due to the wonderful flower colours.

The poster will give a description of the pollination technique we use, technique for testing pollen germination and criteria we use for selecting crossing parents to make new varieties in cut flower and potplant types.

Singh, Y., H. Baijnath, et al., (1996). Taxonomic notes on the genus Zantedeschia Spreng. In Southern Africa., South African Journal of Botany 62(6): 321-324.

INDUCTION OF COLOURED CALLUS FROM *LAWSONIA INERMIS SYN. ALBA*

P12

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Tissue culture of *Lawsonia inermis*, *syn. alba* (henna) were carried out to induce coloured callus formation as well as the plant regeneration *in vitro*. Various explants from aseptic seedling were used such as leaf, stem, and root. These explant were cultured on Murashige and Skoog (MS) medium with different combinations and concentrations of hormones. The hormones used were 2,4-dichlorophenoxyacetic Acid (2,4-D), Napthalene Acetic Acid (NAA), Benzyl Aminopurine (BAP), Kinetin, and Indole acetic Acid (IAA). This studies focused on getting coloured callus from the explants which contain the important secondary metabolites such as lawsone and isoplumbagin. These secondary metabolite are useful for medicinal purposes. It can affect the body by slowing down the heart rate, reducing blood pressure,fever and pain, and also by acting as a sedative. Beside that, the economic importance of this herbal species is also used in cosmetic products such as hair conditioner, anti-dandruff, nail strengthening effects, and also as a sunscreen

Keywords: *Lawsonia inermis, in vitro*, medicinal plant, Murashige and skoog (MS) ornamental plant, 2,4-dichlorophenoxyacetic Acid (2,4-D), Napthalene Acetic Acid (NAA), Benzyl Aminopurine (BAP), Kinetin, Indole acetic Acid (IAA) and coloured callus.

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FLOWER TYPES IN THE ORNAMENTAL CROP *CALLUNA VULGARIS* – MORPHOLOGICAL AND MOLECULAR INVESTIGATIONS

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Calluna vulgaris is one of ~ 11,500 species in the *Ericales* family which display an enormous diversity of flower types (**Schönenberger et al. 2005**). *C. vulgaris* itself features several different flower types, one of which is the so-called 'bud-flowering' phenotype.

'Wild-type' flowers comprise four whorls of flower organs: one outer whorl of four coloured leaves, an inner whorl of four coloured leaves that are slightly grown together, eight stamens and four carpels. In contrast, 'bud-flowering' phenotypes display two identical whorls of four coloured leaves each that are not grown together. Moreover, the stamens are missing, which we assume to be at least one reason for the developmental arrest in the bud stage. Since an investigation regarding the flower organ identity of *C. vulgaris* (**McClintock 1986**) remained vague to the authors, we investigated the flower morphology in depth and started first analyses on the molecular basis of flower organ identity in *C. vulgaris*.

Histological analyses demonstrated the time-course of organ development in young flower buds and revealed that in 'bud flowering' phenotypes stamens are not only degenerated but totally missing. Scanning electron microscopy (SEM) was applied for analyses of the cell surface structure of the coloured leaf whorls. Here, we were able to identify differences of the cell structure between the two coloured leaf whorls of 'wild-type' flowers which is a strong hint to identify the outer whorl as sepals and the second one as petals. Analogous analyses of these organs in 'bud-flowering' phenotypes supports the hypothesis, that here both whorls of coloured leaves are to be classified as sepals. Thus, in this flower phenotype, not only a loss of stamens has occurred but also a change of organ identity in the second whorl.

Therefore, we are aiming at isolating MADS-box genes in *C. vulgaris* that might control these changes in flower architecture. 3'-RACE-PCR was applied in order to amplify MADS-box homologues from 'wild-type' mRNA in *C. vulgaris*. Until now, cloning and sequencing of RACE fragments led to the identification of B-gene homologues (DEF, GLO).

McClintock (1986) Acta Hort. 182:277-283
Schönenberger et al. (2005) Int. J. Plant Sci. 166(2):265-288

IN VITRO PLANT REGENERATION AS A TOOL TO IMPROVE ORNAMENTAL CHARACTERS IN *PASSIFLORA* SPECIES

P14

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The genus *Passiflora*, comprising about 500 species of vines, lianas and small trees, is the largest in the Passion flower family (*Passifloraceae*). Several species are grown in the tropics for their edible fruits (*Passiflora edulis* Sims.) and many others are grown either outdoors, in the warmer parts of the world, or in the glasshouses, for their exotic flowers. Passiflora exhibits several unique floral features, including multiple series of brightly coloured coronal filaments and elaborate floral nectary structures, which are of particular interest for the floricultural market. With the aim to exploit the ornamental value of some *Passiflora* species, a collection was settled at the CRA-FSO, in Sanremo (indoors). Nodal segments, floral buds and auxillary tendrils from greenhouse-grown plants were sterilized and *in vitro* propagated. Direct shoot regeneration was achieved from *P. 'Guglielmo Betto', P. x allardii* Lynch (*P. quadrangularis* x *P. caerulea* "Constance Eliott") and from *P. trifasciata* Lemaire tendrils cultivated on MS medium containing, either 4.4 μM 6-benzylaminopurine (BAP) and 11.42 μM indoleacetic acid (IAA). *P. foetida* L. cv. Hastata shoots were regenerated from immature flower organogenetic callus, developed on MS medium supplemented with 4.4 μM (BAP) and 11.42 μM (IAA). The *in vitro* regenerated plants were successfully acclimatized in the greenhouse. At flowering only *P. foetida* L. cv. Hastata regenerated plants showed morphological alterations in flower and fruit external bracts. Citological and molecular analyses of *in-vitro* cell clones and regenerated plants will be performed in order to test the existing genotypic variability. Immature flower shoot regeneration could be used in ornamental *Passiflora* for exploiting somaclonal variability in genetic improvement studies.

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ORNAMENTAL FOLIAGE POTENCIAL OF THIRTY FOUR *ANTHURIUM* ACCESSIONS

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The *Anthurium* genus comprises more than 600 species, most of them with ornamental potential. However, only *Anthurium andreanum* is remarkable in the floriculture industry, with an enormous commercial importance as cut flower. In the last few years, the commercialization of new foliage has been growing up. It is necessary to introduce new foliage crops in culture to reach market space and *Anthurium* species are an excellent option for cut foliage exploration, nevertheless, in Brazil, this is an incipient activity. The Brazil Northeast has great potential for these species cultivation, for local consumption or exportation, due to ideal climatic condition, excellent geographical localization. The aim of this work was evaluate the ornamental potential for cut foliage of 34 anthurium genotypes, from different Brazilian ecological regions, through morphological descriptors and phenological aspects. *Anthurium* collection has been carried out at Embrapa Tropical Agroindustry Corporation, located in Fortaleza-CE, Brazil. Plantlets from promising cut foliage accessions were obtained from sexual and vegetative propagation. The accessions were cultivated in plastic pots, containing commercial substrate under 80% of artificial shade. The accessions differ widely in leaf form, inflorescence size and stem length. It was also observed variation in: imature spadix colour (varing from light green to purple) and spadix diameter (ranging from 2,5 to 15,0 mm). Spathe length and width ranged from 3,0 to 25 cm and 1,5 to 5,cm respectly. The main nervure and pulvine were proeminent in all accessions. Peciolus colour was green in almost accessions, sometimes reddish, peciolus length ranging from 3,0 to 24,0 cm. Erect infructescences containing oblong to obvoid, white to purple berries were observed. About phenological aspects: accessions took 20 to 60 days to form complete developed leaves and 40 to 80 days for inflorescence emittion and complete development, and the total cycle from inflorescence emission to fructification took more than 100 days in all accessions. The peciolus length and inflorescence position in relation to the leaves are important atributtes to use indication. All accessions presented leaves with more than 40 days of shelf life. The descriptions obtained could indicate ten accessions with excellent foliage characteristics for commercial exploration and material for future breeding programs.

ANTHURIUM CONSERVATION STRATEGY USING 6-BENZILAMINOPURINA (BAP) FOR MULTIPLICATION

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Anthurium Schott. (Araceae) understand about 1000 species, usually herbs, epiphytes, natives of Tropical America. Most of them are ornamental, for its beautiful, exotic and long-lasting inflorescence and foliage. Just few species of anthurium are in cultivation, and many of them are vulnerable to antropic action. Obtained directly from the nature, sometimes just few plants are disponible or it is hard and slow to growth. A specific production system of plantlets, is a essential stage to the introduction of these species to evaluation, characterization and cultivation experiments. Anthurium could be sexually propagated, by seeds, resulting in heterogeneous populations and asexually, by stem section or shoot separation, but it's a slow process. Therefore, in vitro technology has considerable potential to plant proliferation, plantlets production in large scale, free of pathogens. The aim of this work was evaluated *in vitro* proliferation rate of *Anthurium plowmanii* and *Anthurium longipes*, in modified Pierik medium, with different concentration of BAP (0 - control; 2,22; 4,44; 6,66 e 8,88 µM). The stem segments explants, obtained from well established plants, were inoculated in flasks with 30 mL of culture medium, and maintained in growth room at 25 ± 1ºC temperature, at 30 µmol.m^{-2}s^{-1} light intensity and 16 hours of photoperiod. It was used a completely randomized design, in 5 x 10 factorial arranging, containing 2 replicates in a flask. After 28 days of inoculation, leaves and shoots numbers and proliferation.

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CROSSES FOR THE ADVANCED GENERATION OF TREE PEONIES AND HYBRID IDENTIFICATION BY AFLP MARKERS

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Reciprocal crosses between *Paeonia rockii* hybrids (RH), *P. ostii* 'Feng Dan Bai' (FDB) and *P.* ×*lemoine* 'High Noon' (HN) were made in 2006 and 2007 for breeding advanced generation tree peonies. The results from 25cross-combinations and 434 pollinated flowers showed that both RH×HN and FDB×HN were fertile with respectively 12.17and 4.45 seeds harvested in each flower averagely, but their reversed crosses were almost infertile completely, indicating that HN could should be very promising as pollen supplier in cross-breeding for the advanced generation of tree peonies. And 22 samples selected at random from the seedlings of 7 cross combinations of RH×HN were identified by AFLP makers to confirm the significance of this technique in early identification of the hybrids. Totally 1141 bands including 1051 polymorphic ones (92.7%) were amplified by 9 pair primers. The data of AFLP markers showed that the F_1 seedlings possessed some bands detected in both of the parents together, some specific to the maternal or paternal parents, but fewer bands didn't exist completely in the parents. Combined with UPGMA analysis, these results suggested that the seedlings should be true hybrids, most of which were more relative to the maternal parent than to the paternal one with the novel variation happened in a few cases, and AFLP makers were effective in early identification of tree peony hybrids. Therefore, based on a higher seed setting as cross compatibility and the most of F1 seedlings were identified as true hybrids by AFLP makers, the cross RH×HN were recommended as a promising combination for the breeding advanced generation.

AGROBACTERIUM-MEDIATED TRANSFORMATION OF DENDROBIUM ORCHID

P18

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A transformation protocol for dendrobium orchid was established by using immature protocorms as a target material for *Agrobacterium* inoculation. Seeds were obtained from the cross between two elite clones of *Dendrobium nobile*, 'Cinderella'×'True love', and germinated on New Dogashima medium containing 10 g/l sucrose without any plant growth regulators. Three-weeks-old protocorms were subjected to co-cultivation with *Agrobacterium tumefaciens* EHA101 containing plasmid pIG121-Hm that harbored genes for □-glucuronidase (*gus*), hygromycin phosphotransferase (*hpt*) and neomycin phosphotransferase II (*nptII*). More than 40% of the inoculated protocorms were recovered 2 months after selection of transformed protocorms on medium containing 20 mg/l hygromycin. Transformation efficiency was increased by prolonged period of inoculation (~3 h) with *Agrobacterium* suspension culture. Integration of transgenes was confirmed by PCR analysis and Southern hybridization. Stable expression of *gus* gene was indicated by histochemical GUS assay in the leaves and roots of transgenic plants.

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STUDY ON INCREASING ROSE SEED GERMINATION

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Rose is one of the most important cut flowers all over the world as well as Korea. Growers in Korea are exclusively relying on foreign cultivars. Rose breeding program started a decade ago, and lack of experience brought in series of topics that required attention from Korean researchers. One of the topics is the low germination rate in rose around 20%. High amount of substances that inhibit germination were found in the pericarp of the achene.

Four different treatments such as grinding (0, 5, 10min), UV-irradiation (0, 5, 10, 20, 30min), immersion in sulphuric acid (0, 5, 10min), and in microorganism (*klebsiella oxytoca* treatment for 0, 1, 48h), were applied to improve germination rate.

Only microorganism treatments (1 and 48h) were successful on improving germination rate by two times compared to the control. At the moment, we are testing microorganism in various ways together with temperature alterations.

REGULATION AND QUALITY OF FLOWERING IN BELGIAN POT AZALEA: INTERACTION BETWEEN GENETICS, PHYSIOLOGY AND CULTURE CONDITIONS

P20

Pot azalea (*R. simsii* hybrids) production in Belgium accounts approximately 40 million plants per year. The past decade, growers and their associations have made efforts to improve both the quality of the vegetative as well as the flowering plants; however, suboptimal flowering is often observed. The non uniform opening of flower buds at anthesis or flowers that do not entirely open at the consumers place are often observed. Problems related to flowering are detrimental for the good image of azalea as a quality product. Different potential causes have been quoted: year round production schemes, shortening of culture time, increased frequency of application of growth regulators, use of more persistent growth regulators or application of assimilation lights when forcing the plants. However, a clear cut direct cause is seldom found; interaction between several elements related to the culture conditions should be at the base.

The main objective of this research project is to identify influential factors related to flowering quality by an integrated approach focusing on the induction of processes at the level of RNA expression, on plant physiology and/or on experimental variation in culture conditions. In its practical implementation, we will start with the isolation of homeologous candidate genes for regulation of flowering, which will be transformed to expression markers using qPCR. Besides, relevant physiological, biochemical and morphogenetic parameters will be developed. Application of plant growth regulators to induce the transition from vegetative to generative growth under controlled cultured conditions is supposed to interfere with the later flowering process: need for cold, dormancy breaking of the flower buds, anthesis during forcing of the plants in the greenhouse and open flowering at the consumers place. Focused experiments based on the currently used culture conditions by the growers are designed for the 3 critical stages (transition, dormancy breaking and anthesis). In a later stage of the project, we want to try to validate the results on batches of plants taken from the azalea growing companies that display abnormalities in flowering.

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DIFFERENCES IN THE GROWTH AND DEVELOPMENT OF PRUNED AND UNPRUNED 'SONIA' CUT ROSE BUSHES

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4-w-old ‘Sonia’ combination plants, bench grafted onto *Rosa canina* ‘Inermis’ stocks, were planted in 40 L containers in a heated glasshouse and grown from February till December. Plants were treated, either as cut rose plants in the traditional plant architecture (pruned plants, PP), or were left completely undisturbed (UP). At seven pre-determined dates, 5 complete plants of each PP or UP category were dug up and the (fresh) weights of their shoots and roots recorded. Similarly, all fresh weights of the prunings (harvested parts) in the PP category were recorded. Both in absolute (AGR) and in relative terms (RGR), the PP plants grew slower than unpruned (UP) ones. The total fresh weights of PP plants (excluding the weight of the prunings) was reduced to about 40% of those of the UP, but including the weight of the prunings this was only 17% of the UP. Pruning affected neither the time of emergence nor the number of bottom-breaks, but in PP plants the diameter of these shoots was about 30% smaller. Similarly their root collars were about 30% thinner. At the end of the experiment the shoot/root (fresh) weight ratio of UP plants was about 2.5 times higher than of PP plants. In PP plants a functional root/shoot equilibrium of about 5.0 seemed to be established.

INHERITANCE OF 2N GAMETES FORMATION IN A F1 AND F2 POPULATION OF *BEGONIA* HYBRIDS

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In *Begonia* several 2n producing genotypes exist. Among these, *Begonia* 'Orococo', produces a high number of first division restitution (FDR) pollen as was shown by the formation of mainly diads during microsporogenesis. Crosses with *B.* 'Orococo' as father plant and *B. soli-mutata*, *B.* 'Art Hodes' and *B.* 'Orococo' as mother plants resulted in a total of 306 seedlings, of which 295 were triploid and only 11 diploid. In the reciprocal cross with *B.* 'Orococo' as mother plant and *B. soli-mutata* as father plant 50 diploids and only 3 triploids were detected, indicating that *B.* 'Orococo' produces also unreduced egg cells but in lower frequencies compared to unreduced pollen. In order to study the inheritance of 2n pollen formation, both pollen size and microsporogenesis aberrations in 50 triploid and 29 diploid F1 seedling were investigated. In all triploid seedlings monads or diads were observed. In several cases, also a high number of polyads were present. However the frequency such pollen was observed varied significantly between individual seedling. As a result, all seedlings produced large (2n) or only bad sterile pollen. None of them produced only normal pollen without 2n pollen. In contrast, only 13 of the 29 investigated diploid seedlings produced monads or diads. Six of these seedling produced no pollen mother cells at all. As a result, only a part of the diploid seedlings produced large 2n pollen, while several other seedlings produced only normal pollen or no pollen at all. The germination capacity of the F1 pollen was in general poor, although in some cases germination percentages reached above 20%. Some F1 triploids were used to produce tetraploid F2 progeny. A total number of 14 seedlings was obtained. Of the 8 investigated tetraploids, 6 produced monads or diads. The obtained results show that the heritability of 2n pollen production is very high.

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PHENOLOGY OF *LILIUM POLYPHYLLUM* D.DON EX ROYLE: IN GARHWAL HIMALAYA, INDIA

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Phenology is the study of periodically occurring natural phenomenon and their relation to climate and changes in season. Phenological progression in *Lilium polyphylum* of family Liliaceae was observed under natural habitats in temperate region of Garhwal, North West Himalaya, India. Considering the endangered status and medicinal values viz., anti ageing and aphrodisiac, species is prioritized for conservation and cultivation. However, this species shows distinct growth behavior as long gestation period follows short reproductive phase. Furthermore, it depends immensely on micro climatic conditions, therefore noticeable variation is observed in the commencement of different phenophases.

In nature, seed germinates during June- July after 80 days of dispersal when temperature reaches between 15 to 20°C and atmospheric humidity between 60-85%. Thereafter, it develops and remains in bulblet stage throughout the growth period (late September-October). First true leaf produces after 8 months of germination when winter season is over and climatic conditions are suitable during March – April (second year) immediately after new spouting. Shoot emerges from bulb during mid march and attain maximum height during June with bud maturation. Juvenile phase continues with the development of aerial parts as well as bulb. Once juvenile phase achieve its peak, reproductive stage instigate and flowering starts during mid June approximately after 5-8 years of seed germination. Flowering period remains for 15- 20 days and maximum blooming was observed when temperature ranges between 18-20°C with atmospheric humidity at 45-50%. Seed setting initiate during August followed by slow ripening of a capsule which takes nearly two months to mature. Seed shedding starts from mid October and continues till mid November simply by splitting of pods. During entire vegetative growth period bulb increases in size and biomass by adding scales and accumulation of reserve food material and during every winter, experiences a vernalization. Since phenophases are very good indicators of plant response to environment (climate in particular) and seasonal timing events can be critical for survival of life and reproduction. In general, phenological study provides background to functional rhythms of plant communities. Besides, the study will help to determine appropriate requirements for germination and seedling establishment for domestication purpose as well as will help in determining the best harvesting time for bulbs of the species for commercial use.

HAPLOID AND DOUBLED HAPLOID PRODUCTION VIA ANTHER CULTURE IN GENTIAN

P24

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Gentian has been used as ornamental cut- and pot- flowers in Japan. Many gentian cultivars have been established from two species (*Gentiana triflora* and *G. scabra*), and composed of F_1 hybrid varieties and clonal ones. Although many F_1 hybrid cultivars have been developed, it remains the problem that the difficulty to produce homozygous parental lines. The gentian is highly heterozygous and exhibits intense inbreeding depression by self-pollination. Production of doubled haploids from male and female gametophytic cells by *in vitro* culture has been reported in many crops. However, application of this technique to gentians has not been reported. In this study, we report successful production of haploids and doubled haploids of gentians by anther culture.

Embryos were obtained from anthers cultured on the 1/2NLN liquid medium with 13% sucrose after 2 months of culture. After these embryos were transferred to the regeneration medium, they could develop into plantlets. Although many embryos were produced in *G. triflora*, the genotypic variation on embryogenesis was observed among genotypes used. Flow cytometric analysis and count of chromosome number revealed that regenerated plants consisted of haploids, diploids and triploids, especially triploids reached to 70% of them. In *G. scabra*, a few embryos were produced on anther culture and only one plant regenerated.

These results indicate that anther culture is a useful method to obtain haploids and doubled haploids in gentians. Breeding programs and genetic studies using the doubled haploids obtained in this study are currently carried out.

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FABACEAE FROM NORTHWESTERN ARGENTINA AND THEIR POTENTIAL USE AS ORNAMENTALS

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The Fabaceae, one of the largest families of the Angiosperms, are amply distributed in both hemispheres, from wet tropics and across temperate zones. Many of them are characteristic of disturbed places, demonstrating a good adaptation to grow and to reproduce under unfavorable conditions, *e.g.*, soils poor in nitrogen, given their capacity to fix the atmospheric nitrogen by symbiosis with species of *Rhizobium*. Within this family, the subfamily Papilionoideae represents 70%, and most of the cultivated species of legumes belongs to this group. In addition to the crops used as food, the Papilionoideae are used also as ornamentals, as *Lathyrus odoratus* and *Spartium junceum.* As a first step in the characterization of native species of Papilionoideae from Northwestern Argentina, with potential use as ornamentals, we determined the floral characteristics and life cycle of the following species: *Centrosema virginianum* (L.) Benth; *Cologania ovalifolia* H.B.K.*; Crotalaria megapotamica* Burk.; *Crotalaria micans* Link; *Crotalaria pumila* Ort.; *Crotalaria stipularia* Desv.; *Desmodium cuneatum* Hook & Arn.; *Desmodium incanum* AD.; *Desmodium subsericeum* Malme; *Desmodium uncinatum* (Jacq.) DC.*; Galactia latisiliqua* Desv.*; Indigofera parodiana* Burk.; *Indigofera suffruticosa* Mill.; *Macroptilium erythroloma* (Benth.) Urban; *Macroptilium fraternum*; *Phaseolus vulgaris* L. var. aborigeneus (Burk.) Baudet; *Rynchosia edulis* Griseb.; *Vigna caracalla* (L.) Verdc. and *Zornia contorta* Mohl. *Desmodium* spp. have flowers contained in racemose inflorescences, they are pink coloured and present colour change toward violet-blue. *Crotalaria* spp, *Rynchosia edulis* and *Zornia contorta* have yellow flowers and racemose inflorescences. *Macroptilium* spp. and *Indigofera* spp. possess orange flowers. *Cologania ovalifolia* flowers are fuchsia, while the flowers of *Phaseolus vulgaris* and *Galactia latisiliqua* are pink. *Centrosema virginianum* and *Vigna caracalla* have large flowers, (2-5cm.), and they are violet-coloured. In the studied group, *Crotalaria micans, Crotalaria megapotamica* and *Vigna caracalla* produce a very intense fragrance. Considering the life cycle, 89% of the studied species are perennials. In relation to rewards, 72% of species produce nectar and this feature enhances their attractiveness because of pollinator's presence.

CHARACTERIZATION OF *VIGNA CARACALLA* FRAGRANCE

P26

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Vigna caracalla L. Verdc. (Fabaceae: Papilionoideae) produces large and beautiful flowers with an intense, pleasant fragrance and due to this fact, this species could be grown as an ornamental. The objective of this initial study was to identify, using headspace analysis, the major volatiles present in the floral bouquet of cut flowers. The eluted volatiles were analyzed using a gas chromatograph/mass spectrometer. Chromatographic separation was achieved using a PE-Wax column (50 m, 0.25 mm, 0.25 um). The predominant class of compounds was identified as monoterpene hydrocarbons which on average accounted for over 47% of the total volatiles. Within this group, the predominating acyclic compounds accounted for 41% of the total volatiles. Major monoterpene hydrocarbons were identified as trans-ocimen, α-farnesen and linalool. The former compound has previously been reported as the main constituent of floral scents from other leguminous flowers including sweet pea (*Lathyrus odoratus)* and faba beans (*Vicia faba*). One acyclic monoterpene alcohol (linalool) and one cyclic monoterpene alcohol (indol) accounted 21.6% of the total volatiles detected. The former compound has been detected in the floral scents of a wide range of plant species including alfalfa and sweat pea. A number of previously reported aromatic compounds were detected and together accounted for on average 21% of the total headspace. These included aldehydes, alcohols and esters, with phenethylalcohol being consistently the most abundant. Aromatic compounds have previously been shown to be present at high concentrations in the floral bouquet of other leguminous flowers such as white and red clovers. From this study it is clear that, as expected, the volatile profile of *V. Caracalla* flowers consists of a complex mixture, with a total of 32 compounds being detected in quantifiable amounts.

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OPTIMIZATION OF *CHRYSANTHEMUM* PROTOPLAST CULTURE FOR ASYMMETRIC HYBRIDIZATION

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Asymmetric (somatic) hybridization is an alternative for sexual hybridization and symmetric protoplast fusion. The latter two have to be followed by repeated backcrossing to limit the introgression of undesired genes, whereas asymmetric hybridization may instantly yield end products. However, both genome fragmentation of a donor parent and protoplast regeneration of an acceptor parent may create bottlenecks for the generation of such hybrids. The aim of this research was to optimize several parameters for successful culture of protoplasts of a selection of suitable receptor genotypes of *Dendranthema grandiflora*.

Altogether, 5 cultivars were selected. The effect of several parameters on both initial and sustained development (respectively yielding microcolonies and microcalli) was studied. As well growth conditions, media contents and culture systems were evaluated. More specifically, incubation time, enzyme mixture, agarose concentration, the evolution of the osmotic gradient, complex N-sources, ammonium content, sucrose level, presence of organic acids, the presence of nurse tissue and phytohormonal levels were the parameters whose effect was monitored.

Protoplast isolation could be optimised by very mild (10 rpm) overnight (16h) shaking in the following enzyme mixture: 0.5% cellulase, 0.3% macerase and 0.1% driselase. Initial protoplast divison was accomplished in NH_4 free medium enriched with both auxins and cytokinins and 20g/l sucrose, based on MS and supplemented with Nitsch vitamins. Sustained development up to microcalli (visible to the naked eye) was not yet observed.

Currently, experiments are ongoing in order to improve gaseous exchange in the culture containers. This would enhance the oxygen supply to the dividing protoplasts. Microcolonies would then have a higher chance to persist their division and yield microcalli. This appears to be the critical step in the regeneration process of chrysanthemum protoplasts. Bypassing this barriers would thus mean a major step forward in the development of asymmetric hybrids in this ornamental.

BACTERIAL WILT OF *PELARGONIUM*: DEVELOPMENT OF A SCREENING-METHOD FOR RESISTANCE

P28

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Pelargonium is one of the most important ornamental plants in home and garden. Cultivars are propagated vegetatively. Two bacterial pathogens (*Xanthomomas hortorum* pv. *pelargonii*, *Ralstonia solanacearum*) cause bacterial wilt and blight resulting in high economic losses. The first symptom of both diseases is the characteristic wilting of single leaves. After the invasion of the bacteria, infected stems become brown or black and the whole plant is dying. Symptomatically, these two bacterial diseases can not be distinguished but a microbiological differentiation is possible.

As the inoculation pathway is different for these species an aim of this project was to develop reliable inoculation methods for both pathogens. These were developed on plant material provided by Elsner pac® Dresden using bacterial strains of the collection of the Institute of Resistance Research and Stress Tolerance.

For the inoculation with *X. hortorum* pv. *pelargonii*, contaminated scissors were used, because in praxis the disease transmission occurs when preparing cuttings of plants during the propagation process. As the natural inoculation by *R. solanacearum* is via roots, the bacteria suspension was filled into pots and the roots of respective plants were cutted (wounded) with a knife to improve the bacteria invasion.

132 genotypes of *Pelargonium* were tested by both methods in the greenhouse. As a result, two genotypes could be identified as highly tolerant to *Xanthomonas* and one genotype as resistant against both pathogens. The resistant genotype will be used in the resistance breeding.

P29

OCCURRENCE OF PROGENIES WITH UNEXPECTED DNA CONTENTS OBTAINED FROM THE CROSSES USING UNREDUCED GAMETE-PRODUCING DIPLOID CULTIVARS WITH ONE F GENOME OF *CYMBIDIUM FLORIBUNDUM* (FF GENOME) WITH DIPLOID *CYM. EBURNEUM*

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In the breeding of *Cymbidium*, interspecific hybridization has frequently been conducted to produce novel cultivars. Although most interspecific hybrids did not show the hybrid sterility in *Cymbidium*, diploid cultivars, which have one genome of *Cym. floribundum* (FF genome), designated as diplo-1F cultivar, frequently formed unreduced gamete and yielded triploid and tetraploid progenies in the crosses with diploid and tetraploid partner plants, respectively. Since a large variation in DNA content between diploid *Cymbidium* species was detected in our preliminary study, DNA contents of unreduced gamete inherited were estimated from that of progenies. Flow cytometric analysis of DNA contents revealed that most progenies obtained by the crosses using diplo-1F cultivars as female parents were triploid or tetraploid depend on the ploidy level of partner plants. Moreover, calculated DNA contents of unreduced gametes produced were not exactly but nearly the same as the diplo-1F cultivars used as the parent. These results suggest that unreduced gametes of diplo-1F cultivars were FDR type.

In the present study, 10 progenies with tetraploid or over tetraploid DNA contents were obtained from the crosses of diplo-1F cultivars as a female parent with the pollen of diploid *Cym. eburneum*. In these plants, calculated DNA contents inherited from diplo-1F cultivars were far from that of FDR 2n-gamete. These results suggest that they were formed by natural chromosome-doubling of diploid embryos fertilized between n-gamete of diplo-1F cultivar and the haploid pollen of *Cym. eburneum* or by the fertilization between SDR 2n-gamete of diplo-1F cultivar and 2n-pollen of *Cym. eburneum*.

BIODIVERSITY CONSERVATION STRATEGIES OF RARE AND ENDANGERED LIGURIAN SPECIES

P30

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Ligurian botanical biodiversity is threatened by increasing urbanization of natural habitats. Efforts have been done to acknowledge the EU Community COUNCIL DIRECTIVE 92/43/EEC on the conservation of natural habitats and of wild fauna and flora (Natura 2000). However, little is known about morphophysiological and genetic variation in wild populations. The genus *Limonium* (*Plumbaginaceae*) has a worldwide distribution, with the largest number of species found in the Western Mediterranean basin. It includes species with a wide range of ploidies (mostly di-, tri-, and tetraploids) and reproductive systems (sexual and asexual through apomyxis), as well as a high proportion of hybrid taxa.. Many of these species inhabit salt-rich soils and their range has been reduced due to human pressure, resulting in increased isolation. In Italy, *Limonium avei* (De Not.) Brullo et Erben has been found only in a few Sicilian and Sardinian areas and one Ligurian site. *Erysimum burnati* Vidal (*Cruciferae*) is an endemic species of South-Western Alps. There are just a few populations in the Ligurian Alps, between 1400 and 1600 m a.s.l.. We are involved in the effort to preserve *L. avei* and *E. burnati* through *ex-situ* conservation methods. Tissue culture techniques have proved to be good and efficient methods for conservation of threatened species, because many plantlets can be obtained from a minimum quantity of original plant material and with low impact on wild populations. We have developed micropropagation and regeneration protocols for these two infrequent Ligurian plant species. We are developing strategies to assess the level of morphophysiological and molecular biodiversity among populations and to study the genetic variation within and among natural populations.

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INTERSPECIFIC HYBRIDIZATION AND POLYPLOIDY IN *MECARDONIA*

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Mecardonia Ruiz & Pav., of recent introduction in the ornament plants world market, is an American genre of Plantaginaceae (ex Scrophulariaceae) whose distribution covers an area from the east of the United States of North America to the north Patagonia and Central Chile (Roussow, 1987). It has around 9 herbal species, generally yellow flowers, five of which grow in Argentina (Greppi & Hagiwara, inéd.).

The commercial varieties found at present come from two species *M. tenella* (Cham. & Schltdl.) Pennell from Brasil and Argentina and *M. acuminata* (Walt.) Small from EE.UU.

Nevertheless, among the original argentinian species, besides the *M. tenella*, we can find some others of great ornamental value, such as *M. flagellaris* (Cham. & Schltdl.) Rossow and *M. reneeae* Greppi & Hagiwara.

The former, even tough is not so proper its plant in a flowerpot due to the fact that it has a postrate habit, with long stems, it is of ornamental interest, because it naturally displays a variability in the colour of its flowers, and they all can be yellow, rose or purple and white. On the other side, the M. renneae, has the particularity of being an upright and compact plant, and has flowers, though only yellow, bigger than the rest of the species of the genre. Also, *M.tenella*, is an upright or upward plant, with little yellow flowers, but with a greater degree of compactness than *M. reneeae*.

Given the morphological variability that these species display, it is of great interest the obtaining of interespecific hybrids. For that reason, a great number of crossbreeding between *M. tenella* and *M. flagellaris* and between *M.tenella* and *M. reneeae* were did.

In the first crossbreeding, viable seeds were obtained, which germinated normally, while in the second crossbreeding, no seed was obtained.

Also and so as to obtain a clue of the ploidy level of these species, the same were analyzed with a flow cytometer. The outcomes showed different levels of ploidy, being *M. tenella* and *M. flagellaris* diploids and *M. reneeae* tetraploids. Given this results, it was decide to diploid, by means of the treatment with colchicine, to *M. tenella*.

Eventually, poliploids invididuals of *M. tenella* were crossbreeded with *M. reneeae*, obtaining this time positive outcomes. The hybrids showed characteristics of both parents, and some of them result of ornamental interest.

MORPHOLOGICAL AND TISSUE CULTURE STUDIES OF *PLATYCERIUM CORONARIUM,* A RARE ORNAMENTAL FERN SPECIES FROM MALAYSIA

P32

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The genus *Platycerium* consists of about 18 species, commonly found in tropical and subtropical forest. Among the species found in Peninsular Malaysia are *Platycerium coronarium*, *P. platylobium*, *P. ridleyi* and *P. wallichi*. The most attractive is the the majestic *P. coronarium. Platycerium coronarium* is a gigantic, epiphytic fern native to tropical areas of South America, Africa, Southeast Asia, Australia and New Guinea. They nest on the upper branches of the tallest tree in the forest. Due to their unique-shaped fronds they are popular as ornamental plants which can be found in gardens, especially tropical gardens.

Records on detailed morphological studies of the species are scanty therefore it is one of the aims of the study to investigate the macro- and micro-morphological characteristics of the species. Studies were carried out on both intact and *in vitro* plants. Scanning electron microscope study revealed the presence of multicellular trichomes on the abaxial surface of both intact and *in vitro* leaves. Sunken, anomocytic-type stomata were also observed on the abaxial surface of the leaves. Propagation of the species through tissue culture using Murashige and Skoog (1962) media supplemented with hormones were used to obtain an efficient regeneration system as well as an approach for conservation. The most responsive regeneration of sporophyte leaves was obtained when the explants were cultured on MS medium supplemented with 0.1 and 1.5mg/l GA_3 and 30mg/l sucrose , at pH 5.6 in 16 hours light and 8 hours in the dark.

P33

A PRELIMINARY STUDY ON GENETIC DIVERSITY OF THE IRANIAN IRISES

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Iris is known as an important ornamental plant and Iran has been mentioned as one of its origins. A study has been carried out in order to analyze the genetic variation of Iranian *Iris* species. Initially, RAPD markers were used to determine the genetic diversity level and phylogenetic relationships among 16 genotypes of *Iris* species including wild material. A total of 14 random primers were used, 12 of which showed good amplification and polymorphism and the combination of these primers was found optimal for discrimination of the genotypes with very low values of cumulative confusion probability. Overall, 722 bands were produced, and 680 bands were polymorphic. Unweighted pair group method cluster analysis based on Jaccard's similarity values revealed 2 and 7 groups at the distance of 0.23 and 0.35 respectively. Preliminary results showed a broad genetic diversity among Iranian Irises. We consider a more intensive sampling across the western and northern parts of the country and using a more precise molecular marker in order to find a better understanding of Iranian Irises biodiversity for further analyses.

BREEDING WOODY ORNAMENTALS AT APR, LISSE

P34

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Applied Plant Research in Lisse has a long tradition of breeding ornamental nursery stock. The breeding of woody plants is characterised by the long generation time of the plants, which often take 7 years or more to flower for the first time after sowing. Some recent cultivars were the result of over 20 years of breeding and selection.

Pieris japonica 'Passion' is an evergreen shrub, characterised by its cherry red flowers in upright inflorescences. In 1980 *P.j.* 'Cupido' was crossed with *P.j.* 'Valley Rose'. The F1 had upright inflorescences, but all white flowers. Crossing the F1 with *P.j.* 'Valley Valentine' in 1988 finally resulted in the introduction of 'Passion' in 2008.

Female *Skimmia japonica* s.str. can have nice red berries, but since it is dioecious, it requires a male pollinator plant. Plants of *S. japonica* subsp*. reevesiana* are monoecious, so self-pollinators do occur in this species. In 1989 several *S. japonica* s.str. cultivars were crossed, and their offspring was screened for female plants which also had functioning anthers. After propagation and tests for cultivation properties, one monoecious selection will be introduced in 2009 as *S. japonica* 'Temptation'. It can self pollinate to produce berries, but still has the abundant bright red berries and good health typical of *S. japonica* s.str.

Hypericum x *inodorum* is a subshrub that suddenly became very popular for cutting in the 1990's. Both the cut branches and the garden plants of this hybrid are very susceptible to rust (*Melampsora hypericorum*). We screened many wild accessions of the hybrid's parent species *H. androsaemum* and *H. hircinum* for rust resistance. We crossed resistant plants with *H. x inodorum* 'Excellent Flair' in 1995, and in the offspring we selected a compact rust-resistant plant with an abundance of large berries. It was introduced in 2004 as H. x inodorum 'Arcadia'. It is excellent for containers, gardens and landscaping.

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KARYOTYPE ANALYSIS OF *LILIUM* SPECIES BELONGING TO MARTAGON SECTION BY FISH TECHNIQUE

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The genus *Lilium* which is consisting of over 100 species belongs to the family *Liliaceae*, and is mainly distributed in Northern Hemisphere. The genus *Lilium* is divided into seven sections, Martagon, Pseudolirium, Lilium, Archelirion, Sinomartagon, Leucolirion and Dauroliruon. The basic chromosome number of genus *Lilium* has been known as 2n=2x=24, with the exception of triploid (2n=3x=36) species, *L. lancifolium*. Martagon section consists of five species, such as *L. martagon*, *L. hansonii*, *L. tsingtauense*, *L. distichum*, and *L. medeoloides*. *L. hansonii* is one of the Korean native lily species, and originated from Ulleung island of Korea. *L. tsingtauense* is known as the only up-facing Martagon lily species and shows resistance against *Botrytis*. *L. martagon* has abundant flowers with dark purple-red coloured flowers and vigorous growth. *L. distichum* has orange-red coloured flowers and dark spot. *L. medeoloides* which is known as “wheel lily” is a common lily species in Japan. Fluorescence *in situ* hybridization (FISH), using 45S and 5S ribosomal DNA, clearly revealed nucleolar organizing region (NOR) and complements of each chromosome. We have constructed detailed karyotypes of *L. hansonii* (2n=2x=24), *L. tsingtauense* (2n=2x=24), and *L. distichum* (2x=2x=24). Seven pairs of 45S rDNA signals were in *L. hansonii*, four pairs in *L. tsingtauense*, and four pairs in *L. distichum*. Only one pair of 5S rDNA signal was detected in all speices of *L. hansonii*, *L. tsingtauense*, and *L. distichum*. The results clearly demonstrate that Martagon section could be discriminated from other *Lilium* taxa through karyotype analysis.

KARYOTYPE ANALYSIS OF SEVERAL SUBGENUS *ROSA* SPECIES BY FISH TECHNIQUE

P36

Rose is one of the most economically important ornamental crops in the world, due to its utlilization as pot or cut flowers and landscape shrubs. The genus *Rosa* is taxonomically divided into four subgenera, including *Hulthemia*, *Platyhodon*, *Hesperhodos*, and *Rosa.* Subgenus *Rosa* is comprised of 10 sections including *Pimpinellifoliae*, *Rosa*, *Caninae*, *Carolinae*, *Cinnamomeae*, S*ynstylae*, *Indicae*, *Banksianae*, *Laevigatae*, and *Bracteatae*. The basic chromosome number of them is seven and their ploidy level ranges from diploid (2n=2x=14) to octoploid (2n=8x=56). Because of its small chromosome size, cytogenetic analyses of genus Rosa were rarely performed until now, and similar chromosome morphology makes it difficult to discriminate their homologous complement. Fluorescence *in situ* hybridization (FISH) technique is an ideal method for discriminating chromosomes each other by observing specific markers, such as ribosomal DNA repeat. In this study, we have analyzed karyotypes of seven wild rose species, *R. multiflora*, *R. rubus*, *R. soulieana*, *R. chinensis*, *R. mulligani*, *R. indica*, and *R. gallica*, based on the chromosome length and FISH signals which are the result of hybridization with 45S ribosomal DNA. The number of somatic chromosomes was 2n=2x=14 in *R. multiflora*, *R. rubus*, *R. soulieana*, *R. mulligani*, *R. indica*, 2n=4x=28 in *R. gallica,* and 2n=6x=42 in *R. chinensis*. Two FISH signals of 45S rDNA were observed in diploid species of *R. multiflora*, *R. rubus*, *R. soulieana*, *R. mulligani*, and *R. indica*, four signals in *R. gallica,* and six signals in *R. chinensis*. All 45S rDNA was positioned on the terminal region of short arm of chromosomes in seven wild rose species. In conclusion, it was feasible to identify the genus *Rosa* by karyotype and physical mapping analyzed using ribosomal DNA.

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THE EFFECTOF IBA, NAA HORMONE ON STIMULATING ROOT GENERATION IN *MALVA CHINESIS*

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To study the effect of indole boteric acid(IBA) and naftalin ascetic acid(NAA) hormones on stimulating root germination in MALVA CHINESIS ,an experiment was carried out four replication through factorial in completely randomized. The hormones were of 0,1000,2000,3000 mgr/lit . Concentrations cutting of 1-1.4 centimeter diameter were put in the solutions for 60 seconds. Then they were put in three cultural media of gravel, sand, soil with decomposed manure. The results showed that in 3000 mgr/lit of IBA concentration more roots were generated(8.2). Though there was no statistical difference between 2000 and 3000 mgr/lit concentration. The longest root was seen in 2000 mgr/lit concentration of IBA and NAA. The most drought weight of root was obtained in 3000 mgr/lit concentration of IBA. The most percentage of root producing belonged to 2000 mgr/lit NAA ..The best cultural media were gravel and sand.

INITIATION OF ENDOPOLYPLOIDY IN SEED DEVELOPMENT OF *PHALAENOPSIS APHRODITE* SUBSP. *FORMOSANA* AND ITS APPLICATION TO POLYPLOID BREEDING

P38

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Endopolyploidy is the occurrence of cells with different ploidy in the same plant tissue as a result of chromosome DNA duplication without cell division. It is commonly found in the mature tissues of many plant species, including *Phalaenopsis* orchids. The objective of this study was to locate the critical stage in the development and germination of seeds as well as the position of the embryonic tissues by which the endopolyploidy was initiated in *Phalaenopsis aphrodite* subsp. *formosana*. The methods for this study included a combination of the analysis of DNA content by flow-cytometry and the cytological study by DAPI staining and fluorescence microscope equipped with Zeiss ApoTome Slider. It was shown that the growth of ovaries and the development of ovules began after pollination. By 43-50 days after pollination (DAP), 2C nuclei in the embryonic tissues were dominant over 4C nuclei. In addition, meiosis and fertilization took place at this stage. During seed development (50-85 DAP), 4C nuclei increased rapidly and became dominant over 2C nuclei as a result of intensive mitosis. After 90 DAP, 2C nuclei began to increase and reached the plateau level by 120 DAP. Seeds became mature and dormancy began. Endopolyploid cells with 4C nuclei occurred at the stage of embryo development. After sowing of seeds, 8C nuclei was observed 4 days after sowing (DAS) indicating the existence of endopolyploidy at the very early development stage of of protocorms. The optical sections of the protocorms between 10-30 DAS observed by using the Zeiss ApoTome Slider showed that large and prominent nuclei occurred at the basal region of the protocorms. These large nuclei increased as the DAS increased and was concurrent with the increase in 8C nuclei in the protocorm tissue at this stage. In this study, endopolyploidy in *Phalaenopsis aphrodite* subsp. *formosana* was found to occur in the embryonic tissues of the seeds before and right after sowing. The application of this finding to polyploid breeding in the orchid is discussed.

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FLOWERING OF DWARF IRISES DERIVED BY TISSUE CULTURE

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Plant regeneration by somatic embryogenesis and/or organogenesis has been achieved by zygotic embryo culture of two dwarf irises (*Iris pumila* and *I. reichenbachii*). Both regeneration processes are induced on MS nutritional media supplemented with only 2, 4-dihlorophenoxy acetic acid (2, 4-D). Concomitant somatic embryogenesis and organogenesis are achieved at concentration of 0.1-10 μM 2, 4-D for *I. pumila* and 0.5-1.0 μM for *I. reichenbachii*. Embryogenic calus is separated and cultured on medium with 2, 4-D and kinetin (0.5 and 5.0 μM, respectively) where somatic embryos are formed. Germination of somatic embryos (~70 %) is achieved on MS hormone free medium. At the surface of organogenic calli the shoots are formed. Afterwards, shoots are cultured on MS media for shoot multiplication supplemented with BAP and GA_3 (0.1 and 0.3 μM, respectively) where some plants flowered. *In vitro* formed flowers of *I. pumila* have the same colour and morphology as donor plants. Fully regenerated plantlets with both processes acclimatized in next flowering season. Plants of *I. reichenbachii* with changed shape and number of flower parts are observed. Altered phenotype was manly present among plants that are regenerated by organogenesis following described protocol. Some of *I. reichenbachii* plants have doubled all flower parts, which can be very useful for further improvement of irises since the investigated species are main parents for breeding of dwarf irises. Detail analysis of regenerants is in progress.

ASSESSMENT OF TULIPS (*TULIPA* L.) VEGETATIVE REPRODUCTION POTENTIAL

P40

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The principal aim of the research is assess vegetative reproduction potential of the whole mother bulbs cross-section according to special reproduction coefficient.

Numerous investigations on vegetative reproduction potential of tulips have been carried out worldwide, but until now more thorough studies have been fulfilled with comparatively little number of cultivars.

Vegetative reproduction capacity of the whole mother bulb spectrum (7 fractions) of 299 cultivars was ascertained according to special reproduction coefficients: total reproduction coefficient (TRC), generative bulb reproduction coefficient (GRC) and forcible bulb reproduction coefficient (FRC). Reproduction coefficients were calculated individually for each studied fraction of the investigated tulip cultivars. TRC is a quantitative indicator, specifying mean number of all daughter bulbs per clone. GRC is a qualitative indicator, specifying mean number of capable to blossom next year bulbs per clone. FRC is a qualitative indicator, specifying mean number of forcible tulip bulbs per clone. By modulating the data on TRC, GRC and FRC of the whole mother bulb cross-section, indexed reproduction coefficient (IRC) was deduced. IRC indicates comparative reproduction value of the whole mother bulb cross-section of the studied tulip cultivars. Empirical tulip cultivar dispersion analysis demonstrated that this coefficient most objectively reflects reproduction capacity of all fraction bulbs of the studied tulip cultivars. Basing on IRC, the investigated tulip cultivars were grouped into 5 grades of reproduction. According to IRC, most tulip cultivars were attached to 2nd – 4th grades (correspondingly 24, 30 and 30 %), whereas 8 % of the studied cultivars occurred in 1st and 5th grades.

P41

EFFECT OF ORYZALIN ON CHROMOSOME DOUBLING AND IN VITRO GROWTH OF *GERBERA JAMESONII*

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The possibility of in vitro tetraploid induction via oryzalin treatments and effect of this herbicide on growth in Gerbera (*Gerbera jamesonii*.) was studied. To assay the initial ploidy level of "Red Explosion" cultivar, the mother plants were determined by DNA Flow Cytometer. Five levels of oryzalin concentration (0, 30, 60, 120 and 240 µM) in modified liquid Murashigue and Skoog medium were used at various time of exposing (12, 24 and 48 hours) on shaker with 92 rpm. Then each plantlet divided into multiplication medium with 2 - 4 and 6 mg/lit kinetin. 10 plantlets per treatments were used as replication (random factor). The experiment was inducted as factorial based on completely randomized design for chromosome doubling and growth comparison. In order to peruse the effect of oryzalin on growth, fresh weight and number of leaves in days 22^{nd} and 44^{th} after oryzalin treatments were measured. Also 12 weeks after oryzalin treatments, ploidy level was evaluated by DNA Flow Cytometer. Minimum increase in fresh weight (0.17 g) was obtained in treatment containing 240 µM oryzalin, 2 mgL^{-1} kinetin and 48 hours exposure period. The lowest number of new leaves was produced on the treatment of 120 µM oryzalin for 24h on the medium containing 4mgL^{-1} kinetin whereas the minimum fresh weight was obtained on the treatment of 240 µM oryzalin for 48h on the medium containing 4mgL^{-1} kinetin. There was no observation of tetraploid plants in lower concentration (30 and 60 µM oryzalin) but in these treatments chimeras were obtained. Maximum percentage of tetraploid plant was recorded at 240 and 120 µM oryzalin treatments in 48 hours. The results show that however only in higher level of oryzalin concentration the tetraploid tissues can be obtained, effect of this antimitotic agent on growth is significant.

EVALUATION OF PRE-FERTILIZATION BARRIERS BY OBSERVATION OF POLLEN TUBE GROWTH AND ATTEMPTS FOR OVERCOMING POST-FERTILIZATION BARRIERS IN INTERGENERIC HYBRIDIZATION BETWEEN *ALSTROEMERIA* AND *BOMAREA* BY OVULE CULTURE

P42

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The genus *Alstroemeria* is a continuous demand for new cultivars as an ornamental flower. Many interspecific hybrids of *Alstroemeria* are utilized for commercial cultivars. However there is no report about intergeneric hybridization. For introducing novel characters into *Alstroemeria*, *Bomarea* species, which is related genus to *Alstoroemeria*, is selected in this study. We are investigating the possibility of creating intergeneric hybrids between *Alstroemeria* and *Bomarea*. Previously, we found that there was a post-fertilization barrier between *A*. *aurea* and *B*. *coccinea*. In the present study, we investigated species-dependant differences of the frequency of pollen tube entry into ovules after intergeneric pollination. Moreover, ovule culture conditions were examined for overcoming post-fertilization barriers after pollination of *Bomarea* pollen grains to *A*. *aurea*, *A*. *pelegrina* var. *rosea* and *A*. *magenta*.

The frequency of pollen tube entry into ovules were compared in the pistil of *A*. *aurea*, *A*. *pelegrina* var. *rosea* and *A*. *magenta* after pollination of *B*. *coccinea* pollen grains. Pollen tubes in *Alstroemeria* ovaries were observed with aniline blue staining 48 hours after pollination. The frequencies of pollen tube entry into ovules were 0.3%, 5.6% and 10.0% in *A*. *aurea*, *A*. *pelegrina* var. *rosea* and *A*. *magenta*, respectively. For the ovule culture, pollinated ovaries were harvested 3 and 7 days after cross pollination and the ovules were cultured on 2 g l^{-1} gellan gum-solidified MS medium with or without gibberellic acid and supplemented with sucrose at different concentrations (30, 60, 80 or 100 g l^{-1}). As a result, 3 plantlets were obtained in *A*. *pelegrina* var. *rosea* × *B*. *coccinea* cultured on MS medium supplemented with 80 g l^{-1} sucrose.

Although pollen tubes of *B*. *coccinea* were reached to *A*. *aurea* ovules, this combination might have stronger pre-fertilization barriers than those of *A*. *pelegrina* var. *rosea* and *A*. *magenta*. It supposed to be different intensity of pre-fertilization barriers among species. Our date also suggested that sucrose concentration at 80 g l^{-1} in culture medium was effective to obtain progenies after intergeneric pollination. Confirmation of hybrid natures for the plantlets is now in progress.

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DIFFERENCES IN PLOIDY LEVELS AMONG INTERSPECIFIC HYBRIDS OBTAINED FROM THE CROSS COMBINATIONS USING *PRIMULA SIEBOLDII* AS FEMALE PARENT

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Primula sieboldii (Section Cortusoides) is one of the traditional ornamental plants in Japan. In cultivars of *P. sieboldii*, 63 diploids, 8 triploids and 1 tetraploid were reported by somatic chromosome observation (Yamaguchi 1973).

Flow cytometric analysis of 200 cultivars also revealed that present population of cultivars in *P. sieboldii* was consisted with diploids, triploids and tetraploids. When we conducted interspecific crosses using *P. sieboldii* as a female parent, different patterns of ploidy levels, which were categorized into three types, were found among progenies according to interspecific cross combinations. 1) Both many diploids and few triploids were included in the same cross combinations, 2) Either diploids or triploids were obtained depending on the female cultivars of *P. sieboldii*, 3) All hybrids are triploid. Types 1, 2 and 3 were found from the intersectional wide crosses with the pollen of *P. obconica* (Section Obconicolisteri), from the intrasectional crosses with the pollen of *P. jesoana* (Sect. Cortusoides) and from the intrasectional crosses with the pollen of *Primula kisoana* (Sect. Cortusoides), respectively. Differences in contribution of unreduced gametes for fertilization and/or embryogenesis were discussed in relation to the interspecific cross combinations of *P. sieboldii* with different species as male parents.

MOLECULAR CYTOGENETIC ANALYSIS OF UNILATERAL AND BILATERAL SEXUAL POLYPLOIDIZATION IN RELATION TO INTERGENOMIC RECOMBINATION AND INTROGRESSION IN *LILIUM* SPECIES HYBRIDS

P44

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Longiflorum (L), Asiatic (A) and Oriental (O) lilies belong to section Leucolirion, Sinomartagon and Archelirion of genus *Lilium* respectively. These interspecific hybrids (LA and OA) are promising in lily breeding for various agronomical traits. Both LA and OA hybrids produce 2*n* gametes and have been used to develop sexual polyploids by backcrossing to Asiatic parents as well as by sib-mating of the F1 LA hybrids. The BC1 progenies were triploid, with few exceptions and the progenies from sib-mating were tetraploid or near tetraploids. Genomic *in situ* hybridization (GISH) technique was applied to assess the intergenomic recombination in the BC1 populations of LA and OA hybrids obtained after unilateral sexual polyploidization. It was found that in LA hybrids, LA × AA and in reciprocal crosses (AA × LA) plants were originated through the functioning of either 2*n* eggs or 2*n* pollen. Similarly the BC1 OA hybrids comprised of triploid plants which originated through functional 2*n* pollen from a diploid OA hybrid. In both type of crosses, a majority of the progenies originated through First Division Restitution (FDR) mechanism of functional 2*n* gametes either with or without a cross over with few exceptions where Indeterminate Meiotic Restitution (IMR) was the mechanism of 2*n* gamete formation. Based on GISH analyses it was found that most of the LA and OA hybrids exhibited recombination. Intergenomic recombination was also estimated in the progeny of sib-mated LA hybrid. In this case both parents had contributed gametes with the somatic number of chromosomes (i.e., 2*n*-2*n*). This population originated through bilateral sexual polyploidization. These allotriploid interspecific lily hybrids with recombinant chromosomes obtained from unilateral sexual polyploidization can be used for the selection of desirable traits at triploid level. However, allotetraploids obtained from bilateral sexual polyploidization can be fertile and used as parents repeatedly to produce triploid or tetraploid progenies.

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GENETIC VARIABILITY IN *CHRYSANTHEMUM*

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A huge quantum of variability exists in chrysanthemum with respect to shape, size, growth habit, flowering behavior, vase life etc. The phenotypic variability observed in chrysanthemum is further partitioned into different heritable and non-heritable component of variation with suitable genetic parameters such as genetic coefficient of variation (GCV), heritability, genetic advance, correlation and path analysis. The main emphasis in the present investigation was therefore, given to study the genetic variability in chrysanthemum which can be used in chrysanthemum improvement programme. An experiment was conducted at research farm, Department of Horticulture, Allahabad Agricultural Institute-Deemed University, Allahabad during winter season of 2006-07. Twenty genotypes of chrysanthemum were used for variability studies and replicated thrice. High genotypic coefficients of variation (GCV) and phenotypic coefficient of variation (PCV) estimates were found for yield of flower per hectare followed by duration of flowering and vase life. High heritability with high genetic advance was observed for yield of flower per hectare, duration of flowering and flower diameter. Positive and direct correlation of flower diameter with flower yield reveal that flowers production can be increased by directly selecting flowers with more diameter.

HYDRANGEA SUSPENSION CULTURE AS SOURCE FOR STABLE PROTOPLASTS

P46

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Hydrangeas as garden shrubs but also as potted plants and for floristry enjoy a truthful renaissance. There are more than 90 species of the genus *Hydrangea*. Most originate from Asia, some of them from the American continent. It could be assumed that this plentiful gene pool will be used for the development of new varieties, although up to now in breeding processes the use of biotechnological methods for hydrangeas is rare.

A protocol for the development and maintenance of suspension culture for *Hydrangea quercifolia* was elaborated. For primary callus induction explants from petioles were cultivated on ½ B5 medium supplemented with 10 g L^{-1} sucrose, 15 g L^{-1} glucose, 4 mg L^{-1} NAA and 0.09 mg L^{-1} TDZ. After about 5 weeks callus was transferred to a liquid medium with the same compounds as for the induction. The cultures were cultivated in the dark on a shaker with 100 rpm. All liquid media were changed by fresh ones every week. After two months without visible growth, the callus slices began to proliferate to round lumps. After a further three months the clusters were crushed using a spatula and transferred to liquid MS medium with 40 g L^{-1} sucrose and 1 mg L^{-1} 2,4-D. In that medium fine suspension was developed. Now, the subculture of the suspension will be performed by pipetting a small amount of suspension cells to fresh medium every week. The microscopic observation shows smooth cell clusters with many divisions. Therefore, the suspension cells could be useful as an unlimited source for protoplasts.

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EVALUATION AND APPLICATION OF FLOWER LONG-LASTING TRAIT (MISOME-SHO) OF AZALEA CULTIVARS

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The long-lasting trait of flower corolla with temporal change of colour exists in Japanese evergreen azalea. This trait called misome-sho has been found out in several species and cultivar groups from Edo era. For the purpose of application of this trait for breeding, we investigated several characteristics and gene expression of these cultivars.

Wild species of *Rhododendron kaempferi*, *R. indicum* and *R. macrosepalum* and flower long-lasting cultivars of each species were used in this study.

All flower long-lasting cultivars have smaller corolla compared to wild types and have stomata in corolla surface, while wild types do not have stomata in their surface. Pot planted flower long-lasting cultivars kept their corolla more than 100 days and corolla colour was changed from red or purple-red in anthesis to green in later. In contrast, wild type plants finished flowering in about 20 days. In microscopic observation of longitudinal section of flower, abscission layer in basal portion existed in wild type corolla are not observed in flower long-lasting cultivars.

Expression of two class B MADS genes: *AP3*- type and *PI*-type genes isolated from Kurume azalea was analyzed. *AP3*- type gene express in whole 2, 3 and 4 corresponding to corolla, stamen and pistil organ of wild species, but do not express in any whole of flower long-lasting cultivars. The long-lasting trait of corolla would be derived from sepaloid petal caused by homeotic gene mutation.

These cultivars have high pollen fertility and stabilized seed set ability. Inheritance of this trait is researching using cross progenies.

ENDANGERED ORNAMENTAL SPECIES

P48

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Recently estimation shows that about 3900 ornamental species (e.g. 13.9% of total ornamental species) are under threat and there is a weak positive correlation between number of threatened ornamental species and total number of species in related families. According to IUCN categories ” Endangered species ” are those that taxa in danger of extinction and whose survival is unlikely if the causal factors continue operating. Included are taxa whose numbers have been reduced to a critical level or whose habitats have been so drastically reduced that they are deemed to be in immediate danger of extinction. The application of this category was done for ornamental species using Geln’s book, Cultivated Plants of Southern Africa and IUCN’s red list of plants. Totally 92 endangered species is recorded. The highest number of endangered species found in the family of Palmae (21) followed by Bromiliaceae (16), Zamiaceae (14) and Cupressaceae (6). Most of species were presented here is used only for ornamental purposes but some have multi-purposes. For example *Juglans hindsii* (Jepson) Jepson ex R. E. Sm. (Cupressaceae), that is frequently used as rootstock for J. regia L. because of its resistance to disease. Another multi-purposes plant is *Malus hupehensis* (Pamp.) Rehd. (Rosaceae) that provide excellent resistant to scab and mildew and is used as rootstock for apples. From the roughly 20 species of endangered crops species already listed by Hammer and Khoshbakht in 2005, only 2 also appear here in the endangered ornamental category (ca. 10%). The overlapping between the groups of ornamental and crop plants will be discussed here.

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STRENGTHENING BREEDER'S INTELLECTUAL PROPERTY PROTECTION WITH THE CONCEPT OF EDV – CIOPORA'S POSITION

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Many misunderstandings still exist, when it comes to the concept of EDV. CIOPORA believes that only a clear interpretation of the EDV concept will provide fair and predictable solutions on this matter and protect the intellectual property of innovative breeders properly. In this regard the CIOPORA position paper distinguishes between two types of EDV: varieties which are solely based on the genome of the initial variety and where the genomic structure is highly conserved, e.g. spontaneous and induced mutants, GMO and apomicts on one side and "Me-too-varieties" on the other. For both groups CIOPORA developed clear rules.

The concept of "Essentially Derived Varieties" is a mixture of technical (describing) and legal aspects. It is a true extension of the breeders´ right and a temporary limitation of the breeders´ exemption. Taking into account Article 14 (5) (b) of the UPOV 1991 Act CIOPORA is of the opinion that an asexually reproduced ornamental and fruit variety shall be deemed to be *essentially derived* from another variety (the initial variety) if it

a) is clearly distinguishable from the initial variety,
b) is predominantly derived from the initial variety or from a variety that is itself predominantly derived from the initial variety and
c) except for the differences which result from the act of derivation, conforms to the initial variety in the expression of the essential characteristics that result from the genotype or combination of genotypes of the initial variety.

We explain how to deal with these prerequisites and with legal disputes in this respect.

For further details please find attached the CIOPORA position paper on EDV and the cover letter.

GENETIC VARIABILITY IN *DAVIDIA INVOLUCRATA* SPECIMENS GROWING IN POMERANIA

P50

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The aim of conducted studies was to determine genetic and morphological variability of selected *Davidia involucrata* specimens from Western Pomerania and Berlin using ISSR-PCR technique.

The studies were carried out on the *Davidia involucrata* var. ”*Vilmoriniana*” growing in Germany in the Botanical Garden in Berlin-Dahlen and in Poland, in Pomerania, nearby the Polish-German border: in the Dendrological Garden in Przelewice, Glinna and Central Cemetary in Szczecin.

ISSR technique made it possible to determine genetic variability of the examined specimens. This was done by means of 6 out of 30 ISSR primers used in the experiment. Six primers (802, 807, 810, 819, 839, 840) generated 64 visible amplification products, of which 11 were: monomorphic, 31 – polymorphic, 12 - genotype specific. On average 1 primer generated 10 amplification products which ranged from 2550bp to 266bp.The greatest number of ISSR-PCR products was observed in the range of 2550-266bp.

The analysis of phylogenetic tree showed that there was 60-87% genetic similarity between the examined specimens. The similarity between the youngest and the oldest tree in Przelewice Arboretum was 86,6%: between them and the davidia from Central Cemetary in Szczecin - 80% and 69.4% between the genotypes from Berlin and Glinna. Low genetic variability between the genotype from Glinna, Berlin, Central Cemetary and Przelewice may result from the fact that they are probably the descendants of the trees from a pre-war German nursery in Berlin. The davidia from Kornik, originated from English nurseries and introduced to Poland in 1931, turned out to be the most genotype specific .

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INDUCTION OF MUTATIONS IN CHRYSANTHEMUM (*DENDRANTHEMA GRANDIFLORA*) BY USING GAMMA-RAY IRRADIATION

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Chrysanthemum (*Dendranthema grandiflora*) is one of the important cut flowers all over the world as well as Korea. *Dendranthema grandiflora* cv. Beakma is a white standard-type cultivar developed in Korea and produce high quality cut flowers throughout the year. However, this cultivar shows vacant space in stem during high temperature in summer. The hardness of its stem is weakened and hard to handle by warping and bending.

The objective of this study was to induce mutant without vacant space of stem for improvement of quality in *Dendranthema grandiflora* cv. Beakma. In addition, the effect of radiation was investigated on the survival and growth rate of individuals. A total of 1,679 rooted cuttings were treated with different doses of gamma rays (10~50 Gy). All individuals survived regardless of irradiation-dose but the growth was decreased with an increase of radiation-dose. Particularly, plant height and internode length were remarkable diminished from 2 to 4 times at 40 and 50 Gy. In morphological characteristics of leaves, leaf length and width were increasingly decreased, and petiole length was increased as increase of dose.

In contrast the respiratory quotient was significantly increased about 3 times at 50 Gy in comparison with the control. The irradiated individuals were repeatedly propagated and an individual was selected as a mutant without vacant space inside the stem in a total of 7,109 individual stems. Cuttings from this individual were rooted for propagation and another selection.

We conclude that treatment of gamma-ray may be an effective way for inducing exclusive mutation of *Dendranthema grandiflora*. We will analyze by using RAPD markers and propagate from induced mutants in the future.

STUDY ON HEREDITY RULES OF *CYCLAMEN PERSICUM* COLOURS

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To identify the theoretic foundation for maintaining and improving high quality varieties and breeding new F1 hybrids, flower colour segregation occurred in the offspring in both self-pollination and cross-pollination of varieties of different colours were investigated. The main results indicate that the heredity of C*yclamen persicum* colour follows both quantitative and qualitative hereditary regularity. All genes can be classified as two groups of the throat-gene and coronal-gene occupying different spots on the chromosome. The coloured gene is the dominant gene, while the white gene is the recessive one. The gene groups and their quantity determine the colour of *cyclamen persicum*. And sometimes the throat- gene and the coronal-gene take a recombination, forming a new type of flower.

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INHERITANCE OF FLOWER COLOURS IN SWEET PEAS (*LATHYRUS ODORATUS* L.)

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As a result of biochemical studying structure of pigments and character of inheritance of flower colours at eight varieties, used in system diallelic crossings, and also at ten hybrids F1 of a sweet peas (*Lathyrus odoratus* L.) it has been established, that this attribute is controlled, at least, by four basic genes synthesizing anthocyanin pigments and three inhibitor- genes of these pigments.

HEAT TOLERANCE IN *PETUNIA* AS MEASURED BY AN ELECTROLYTE LEAKAGE TECHNIQUE

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Identification of heat-tolerant petunia (*Petunia ×hybrida* Hort. Vilm. Andr.) genotypes and techniques for rapid assessment of heat-tolerant plants during breeding programs for sub-tropical and tropical areas are desirable. The extent to which electrolyte leakage from petunia leaf discs at 50 °C for 20 min, measured using a test for cell membrane thermostability (CMT), could be related to the reduction in branch number induced by heat in the greenhouse-grown plants were determined. Heat-intolerant cultivars exhibited more reduced branches, but less reduced RI (relative injury) value than heat-tolerant cultivars with increasing mean growing temperatures from 16 to 27 °C. The cultivars with a high RI value are those with the lesser CMT and more reduction in branch number by high temperature at 27 °C. The relationship between the RI value occurring in leaf tissue discs of two seed-propagated cultivars and treatment temperature from 25 to 56 °C was sigmoidal. The RI values at the approximate midpoint of the sigmoid response curve occurred at 47 °C for ‘Primetime Carmine’ regardless of growing day/night temperatures, and at 47 and 49 °C for ‘Tidal Wave Silver’ grown at 25/20 °C and 30/25 °C, respectively. A high temperature at 30/25 °C resulted in reduced branch number in ‘Primetime Carmine’ but not in ‘Tidal Wave Silver’.

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ISOLATION AND EXPRESSION ANALYSIS OF A GENE ENCODING ACC OXIDASE IN *CURCUMA ALISMATIFOLIA* GAGNEP

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To understand the molecular functions of ACC oxidase genes in curcuma and to mean ethylene production knockout by recombinant technique, cloning and expression of ACC oxidase genes are essential for anti-senesce or gene silencing technique that reduces ethylene production, ultimately enhancing the storage life and quality of the harvested products. In this study, cDNA fragments encoding ACC oxidase from *Curcuma alismatifolia* Gagnep. were isolated and its expression analyzed. Highly conserved Primers were designed from ACC oxidase's various plants of GenBank database. The forward and reverse primers were derived from ENWGFFE and TNGKYKS amino acid segment, respectively. The PCR products had length of 600 basepair and were subcloned into pGEM T-easy vector resulting in p*Ca-ACO1*. After sequencing, the deduced amino acid sequence of the cDNA was highly homologouse to those of ACC oxidase gene isolated from other plant in NCBI database. The expression of the gene during postharvesting, organ different and wounding was investigated. Northern blot analysis shows that *Ca-ACO1* gene is expressed at petal and bract of curcuma and high accumulated at 1 day in petal and 3 days in bract at postharvesting of curcuma. This result was related the respiration and the ethylene production of open florets increased as they approached senescence of the curcuma flower. We suggest that a *Ca-ACO1* is one of ACC oxidase genes that relate in ethylene production and senescence in petal and bract of curcuma after harvesting.

HYBRID STATUS OF ‘ELIATOR’-BEGONIAS ANALYSED BY GISH

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Interspecific hybridization of various tuberous begonia species hybrids with *Begonia socotrana* results in so-called ‘Eliator’- begonias hybrids. In our study, karyotypical differences between parental genotypes was recorded regarding chromosome size and number. Somatic complement of *B. socotrana* comprised of 28 short chromosomes with a length of about 0.5 µm (2n = 28) whereas tuberous begonia had 52 chromosomes of a size ranging from 2 to 3 µm. A number of ‘Eliator’- begonias hybrids were analysed by genomic *in situ* hybridization (GISH) and flow cytometry for their mode of origin. For GISH, genomic DNA of tuberous begonia was sonicated to 1–10-kb fragments, labeled by nick translation with digoxigenin-11-dUTP and was used as a probe and *B. socotrana* DNA was autoclaved to 100 bp fragments and used as block. In hybrids two groups comprises short and long chromosomes were recorded. The long chromosomes of tuberous begonia were uniformly labelled green and their number ranged from 14 to 65 depending on the hybrids whereas the number of short *B. socotrana* chromosomes equaled 14. In case of two genotypes tested no *B. socotrana* chromosomes were recorded in putative hybrids. Recombination between genomes of tuberous begonia and *B. socotrana* was not observed. In addition, their hybridity was readily verified by flow cytometry. Thus, genomic *in situ* hybridization and flow cytometry analyses can be useful to identify the genome constitution of ‘Eliator’- begonia hybrids and thus gain an insight into the origins of these cultivars.

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INVESTIGATION OF THE GENETIC RESISTANCE OF *CHRYSANTHEMUM MORIFOLIUM* TO CHRYSANTHEMUM STUNT VIROID

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Stunting caused by chrysanthemum stunt viroid (CSVd) is one of the most damaging diseases of cultivated chrysanthemum (*Chrysanthemum morifolium*), the most important cut flower in Japan. This disease has been reported in many regions of the world. The symptoms are severe: stunting of plant height, reduction in flower size, and bleaching of the flower. It is difficult to cultivate viroid-free chrysanthemum plants, and CSVd-resistant cultivars have not yet been reported. The objective of our research was to find CSVd-resistant cultivars and to investigate their inheritance pattern. We screened 40 chrysanthemum cultivars for resistance to CSVd. Scions of the screened cultivars were inoculated with CSVd by grafting them onto CSVd-infected plant roots. Two months after the grafting inoculation, we analyzed the upper leaves of the scions by reverse transcription polymerase chain reaction (RT-PCR) in order to detect CSVd, according to the method of Hosokawa et al. (2005). CSVd infection occurred in all the cultivars except for 'Okayamaheiwa', which did not show any symptoms of infection. F_1 progenies were produced by crossing the resistant cultivar 'Okayamaheiwa' with a susceptible cultivar, namely, 'Sei-elza'. From approximately 200 seeds obtained from the F_1 progenies, 17 were randomly selected and grown; their seedlings were inoculated with CSVd by grafting. Of the 17 F_1 progenies inoculated with CSVd, 5 were not infected with CSVd, suggesting that the characteristic of resistance is inherited in *C. morifolium*.

FLOWER COLOUR AND PIGMENT COMPOSITION OF DIFFERENT ORCHID HYBRIDS

P58

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Orchid industry ia a multibillion-dollar business and it has become an important contributor to the country's economy. Orchid flower colour is an important factor in determining the marketability of a particular hybrid. There is a pressure to produce hybrids with new flower colour to meet the everchanging customers preferences. In order to achieve these objectives there is a need to understand the linkage between flower colour and the types of pigments involved. The information obtained is important in developing research strategies especially in selecting the right hybrids for colour manipulation and breeding purposes. A study, therefore, was carried out to establish the relationship between flower colour and pigment composition of different orchid hybrids. Flowers with colours ranging from white and shades of pink, orange and blue were studied. Different pigments which include anthocyanins, carotenoids and chlorophyll of the flower petals were analysed. The hybrids studied were *Mokara* Chark Kuan Orange, *Mokara* Chark Kuan Pink, *Aranda* Chark Kuan Blue, *Dendrobium* Sonia 17, *D.* Savin White, *Vanda* Mimi Palmer*, Vanda* white, *Phalaenopsis bellina, P. bellina* var alba, *Oncidium* Sharry baby and *Oncidium* Taka. The pigments of new hybrid *D.* Alya Pink and its parents (*D.* Tengku Anis x *D.* bigibbum) were also analysed. Generally the colourful hybrids *D*. Sonia 17, *V*. Mimi palmer, *P. bellina* and O. Sharry baby contain significant amount of anthocyanins as compared to white types *P. bellina* var alba, *D.* Sonia 17 and *V* White. *Oncidium* Taka*,* which is predominantly yellow in colour was found to contain a small quantity of anthocyanin but its β-carotene content is seven-fold higher than that of anthocyanin content. Three major anthocyanins were identified in the flower extracts. They were Petunidin, Delphinidin and Malvidin. The major anthocyanins in *D*. Sonia 17 is Petunidin and Malvidin, *O.* Sharry Baby and *V*. Mimi Palmer contain Delphinidin while Petunidin and Delphinidin are found in *P. bellina.* A small amount of anthocyanin was detected in the yellow and white flowers. Chlorophyll is present in all the hybrids including the white types. There is no clear variation in the pH values of the flower sap although there appears to have a lower pH value in the white types.

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OVULE CULTURE OF *HELLEBORUS* SPECIES

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Within the genus *Helleborus* about 21 species are found, part of which has been used for production of interspecific hybrids with new interesting characteristics. However, other interspecific combinations could result in new ornamentals and would be of commercial as well as scientific interest. In order to establish protocols for embryo rescue techniques, in a first step this study aimed at the identification of culture conditions for ovules from intraspecific crosses. The effects of dissection date after pollination and temperature during ovule culture were examined in intraspecific crossings of *Helleborus niger*, *H. argutifolius*, *H. x hybridus* and *H. foetidus*. For these species the period for seed ripening under natural conditions takes ten to twelve weeks depending on the climate.

Ovaries were harvested three to six weeks after pollination, surface disinfected in 70 % ethanol for 30 sec, 2 % sodium hypochlorite with one drop Tween for 10 min and rinsed in sterilised water three times. Ovules were dissected from ovaries and cultured on medium based on MS (Murashige & Skoog, 1962) solidified with 0.4 % Gelrite at a pH of 5.8. Two media supplemented with 2.5 or 5 % sucrose were compared. Ovules were cultured in darkness at 24 ± 1 °C or 16 ± 1 °C for twelve weeks. Thereafter, ovules of each temperature treatment were split and one half was incubated at 6 ± 1 °C for eleven weeks while the other half remained in the initial temperature. Afterwards the ovules were placed back to their initial temperature. During the following weeks germination was evaluated.

On average of all species, 2.0 % of the ovules dissected three and 0.6 % dissected four weeks after pollination germinated, while preparation after five weeks resulted in 3.4 % and after six weeks in 5.6 % germination, respectively. The intermediate cold treatment with 6 °C turned out to be very beneficial for later germination. Furthermore, 60 % of all germinated ovules were obtained on medium supplemented with 2.5 % sucrose, assuming that the sucrose concentration is not a crucial factor. The conditions identified in this study have now to be verified, if they also hold true in interspecific combinations.

REGENERATION OF MEDICINAL PLANT *CLITORIA TERNATEA* FROM MALAYSIA

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Tissue culture studies of medicinal plant *Clitoria ternatea* (Butterfly Pea) was carried out to investigate the regeneration potential this species *in vitro.* Various explants from aseptic seedling were used such as leaves and stems which were cultured on Driver and Kuniyuki Walnut medium (DKW) medium together with different concentrations and combinations of hormones such as Napthalene Acetic Acid (NAA), 2,4-dichlorophenoxyacetic Acid (2,4-D) and Benzyl Aminopurine (BAP) to achieve regeneration. This research also focused on getting coloured callus from the explants which has potential for coating technologies. The economic importance of this species includes anticonvulsant, antidepressant, indigestion, constipation and arthritis, eye ailments, as a cover crop and as an ornamental plant in Malaysia.

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AGROBACTERIUM-MEDITATED TRANSFORMATION OF STOCK (*MATTHIOLA INCANA*)

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Stock (*Matthiola incana*) is one of the important ornamental plants used as cut flowers and pot plants. In stock, insect damage by lepidopteran pests has been a serious problem, resulting in quality loss and high pesticide cost. In order to reduce the damage by pests, it is necessary to produce insect-resistant plants by introducing an endotoxin gene of Bacillus thuringiensis into stock cultivars. To achieve the goal, we established transformation methods of stock in this study. To establish a system of Agrobacterium tumefaciens-meditated transformation, we introduced β-glucuronidase (GUS) gene into stock cultivars. Genetically transformed plants of stock were regenerated after co-cultivating highly regenerable nodular calli with Agrobacterium tumefaciens strain EHA101 (pIG121-Hm) that harbored genes for GUS, hygromycin phosphotransferase (hpt) and neomycin phosphotransferase II (nptII). When calli of stock maintained in liquid Murashige-Skoog medium (MS) were inoculated with Agrobacterium, frequency of GUS-positive calli were increased with sonication and vacuum infiltration treatments. Adventitious shoots were regenerated from hygromycin-resistant calli after transfer onto agar-solidified MS medium containing sucrose and hygromycin. However, it was difficult to induce roots from these adventitious shoots. Transformation of the hygromycin-resistant calli and shoots were confirmed by histochemical GUS assay, PCR analysis and Southern hybridization. We have also established adventitious shoot regeneration system from leaf disk by using agar-solidified Woody Plant Medium (WPM) supplemented with zeatin. By utilizing this regeneration system, hygromycin-resistant shoots were successfully obtained through the same Agrobacterium tumefaciens-mediated transformation method. Transformation was confirmed by histochemical GUS assay, and PCR and Southern analyses.

GYNOGENIC HAPLOID INDUCTION IN *MIMULUS AURANTIACUS:* ESTABLISHMENT OF INDUCTION CONDITIONS AND CHARACTERIZATION OF REGENERANTS ACCORDING TO PLOIDY AND HOMOZYGOSITY

P62

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Mimulus aurantiacus Curtis is a promising ornamental plant native from North America. It is a self-fertile, hummingbird pollinated perennial shrub with tubular hermaphroditic flowers of a wide range of colours. The aim of our study was to establish an efficient method for gynogenic haploid induction in *M. aurantiacus* and to develop a reliable marker for homozygosity testing of regenerants.

In situ gynogenesis was performed using pollination with γ-irradiated pollen (600 Gy) and was followed by *in vitro* embryo rescue 11 to 35 DAP. Placenta attached and detached ovules isolated from 506 flowers were inoculated on several growth media. They consisted of modified Rangaswamy medium with sucrose concentrations ranging from 40 to 120 g l^{-1} and IAA concentrations ranging from 0.1 to 10 mg l^{-1}. Of 366 germinated embryos, ploidy of 189 was analyzed using flow cytometry. Ploidy evaluation revealed that 4 were haploids, 165 diploids, 3 triploids, 3 mixoploids, 2 aneuploids and 12 of undetermined ploidy level.

In order to identify the nuclear origin of the obtained diploid plantlets, a microsatellite marker specific for *Mimulus* species was developed using the cross-genera approach. The marker showed a high degree of polymorphism in *Mimulus* species and the heterozygous nature of most *M. aurantiacus* cultivars tested. The analysis of 64 diploid regenerants from haploid induction experiments revealed that 33 of them were homozygous for the locus tested while 31 of them were heterozygous and therefore hybrids.

This is the first report about the successful haploid induction in *M. aurantiacus*. The implementation of the technique could be a valuable tool for *M. aurantiacus* breeding. The developed codominant molecular marker for homozygosity testing is essential for *Mimulus* haploid induction research and applications. Early homozygosity determination enables efficient selection of doubled haploids from unwanted heterozyogotes at a very early stage of the breeding programme which saves time and funds needed for phenotypic evaluations.

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DIRECT BULBLET REGENERATION FROM BULB SCALE EXPLANTS OF *HYACINTHUS ORIENTALIS* CV CARNEGIE IN VITRO

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The effect of two plant growth regulator, Benzyl amino purine (BAP) and α-Naphthaleneacetic acid (NAA), on direct bulblet regeneration from bulb scale explants of *Hyacinthus orientalis* cv. Carnegie investigated *in vitro*. With upper scale explants, direct bulblet regeneration occur in Murashige & Skoog (MS) solid medium containing 1 mg/L NAA and 5 mg/L of BAP. Bulblet regeneration on lower scale explants occur in the presence of 0.5 mg/L NAA, only. Twelve weeks after culture regenerated bulblets were 1 – 1.5 cm in diameter. The maximum root formation observed in ¾ MS medium with 0.3 mg/L NAA. However, either in upper scale explants and lower scale explants, bulblets formed on abaxial side of scale explants. After rooting and adaptation, plants transferred to soil. Using this method about 200 – 250 hyacinth plantlet can produce from one bulb with 3 – 4 cm in diameter.

ISOLATION OF 3-DEOXYANTHOCYANIDIN GLUCOSYLTRANSFERASE GENE FROM *SINNINGIA CARDINALIS* FLOWERS

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Most anthocyanins are synthesized from 3-hydroxylated anthocyanidins, pelargonidin, cyanidin and delphinidin, etc. On the other hand, 3-deoxyanthocyanins are rare anthocyanin pigments in some plant species. They are pigments that provide orange to red colours. However, there have been relatively few studies on how the biosynthetic pathway produces 3-deoxyanthocyanins. Especially, there is no report on the modification of 3-deoxyanthocyanidin, which is important step to accumulate the pigments into vacuoles. In this study, we attempted to identify gene encoding glucosyltransferase (GT) for 3-deoxyanthocyanidins from *Sinningia cardinalis*, accumulating 3-deoxyanthocyanins abundantly in their petals. Degenerate primers were designed from the plant secondary product glycosyltransferase (PSPG) box, which was highly conserved among plant GTs. Five GT candidates, designated as *ScGT1 - 5*, were amplified using *S. cardinalis* flower cDNA as a template, and full-length cDNA for each *ScGT* fragment was cloned using RACE technology. Phylogenetic analysis showed that the deduced amino acid sequences of these *ScGTs* were classified into the UGT88 clade containing rose 5, 3-GT and snapdragon chalcone 4'-GT. Recombinant proteins of each *ScGT* candidate produced by *E. coli* expression system were used to investigate GT activity for various 3-deoxy and 3-hydroxyflavonoid substrates. As a result, ScGT5 could specifically transfer a glucosyl moiety to 3-deoxyanthocyanidin, apigeninidin and luteolinidin, but not other flavonoids including 3-hydroxyanthocyanidins, flavonols and flavones. Enzymatic properties of ScGT5 were also determined. *ScGT5* might be useful to modify flower colour by genetic engineering in floricultural plants.

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TRANSGENIC TORENIA EXPRESSING CHIMERIC *AGAMOUS* REPRESSOR EXHIBITS SERRATED PETALS AS THOSE INDUCED BY CYTOKININ APPLICATION

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Chimeric REpressor gene-Silencing Technology (CRES-T) is an efficient gene silencing system in which the chimeric repressors derived from various transcription factors dominantly suppress the expression of the respective target genes, and the resultant transgenic plants exhibited loss-of-function phenotypes specific for the transcription factors even in the presence of redundant transcription factors.

The homeotic protein AGAMOUS (AG) terminates floral meristem and promotes development of stamens and carpels in *Arabidopsis*. Disruption of its function or expression of the chimeric AG repressor (AGSRDX) results in redundant petals, known as double flower phenotype. We introduced this chimeric repressor into torenia (*Torenia fournieri* Lind.) to investigate whether CRES-T method is applicable to ornamental flowers to increase their horticultural value. Transgenic torenia plants expressing AGSRDX showed no redundancy in petal number, but exhibited serration in petal margins, anthocyanin accumulation and morphological change in stigma surface, formation of extra vascular bundles in petals and styles, and development of ectopic trichome-like cells in styles. Anatomical observation of petals and styles revealed that these phenotypes are highly similar to those of the treated torenia by a synthetic cytokinin analog CPPU in the derangement of vascular bundle. These serrated petals and extra vascular bundle were also observed when the chimeric repressors for torenia C-function genes *TfFAR* or *TfPLE1* were expressed. These results suggest that the morphological change in *AGSRDX* transgenic torenia plants is induced by the disruption of C-function, while the novel phenotypes might be caused by the modification of cytokinin-dependent regulation in vascular bundle formation and /or ectopic expression of the chimeric repressors in all whorls by CaMV 35S promoter.

EVALUATION OF RESISTANCE OF *GAULTHERIA* TO *COLLETOTRICHUM GLOEOSPORIOIDES*

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The genus *Gaultheria* consists of about 100 to 200 species. Most are found in North and South America, Canada, New Zealand, Australia, Asia and also in Great Britain. *Gaultheria procumbens* L. is an ericaceous perennial and winter hardy shrub and. It becomes an important species in Germany which is cultivated and used as an ornamental plant in autumn and winter. It is named by the Canadian medical scientist and biologist H. Gautier.

In the last years there are enormous losses up to collapses of the whole crop in German plant companies. Lesions on stems and sometimes on leaves, shoot wilting and dieback were observed. The causal agent of this disease is *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. (teleomorph*: Glomerella cingulata* (Stonemann) Spauld. & H. Schrenk). *C. gloeosporioides* is an important economical pathogen worldwide on legumes, strawberry, blueberry, citrus fruits, coffee, cocoa and *Hypericum calycinum* (St. John's wort). It is seed transferable and survives on plant remainders which serve as a source of inoculum. A prevention of the propagation of the pathogen is possible but only in the juvenile phase by frequent application of chemical fungicides. In older plants the application of fungicides only is preventively, since the fungus is colonizing the lower stem part, where fungicides do not arrive.

Due to the high inoculum pressure, the lacks of certain fungicides and the massive dispersion there plants resistant against *C. gloeosporioides* have to be developed. To evaluate the resistance we present practicable and reproducible pathogenicity tests.

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PRODUCTION OF BOUQUET-TYPE *LISIANTHUS* BY OVEREXPRESSION OF A RICE MADS BOX GENE *OSMADS1*

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Lisianthus is a very famous cut flower and many useful cultivars have been produced by conventional breeding. Here we attempted to produce new lisianthus with modified flowering traits by genetic engineering. Transgenic lisianthus (*Eustoma grandiflorum* Griseb. cv. Glory White) plants overexpressing a rice MADS box gene (*OsMADS1*) were produced by *Agrobacterium*-mediated transformation using *bar* gene as a selectable marker. The obtained plants showed remarkable shortened flower stalks and set bi or tri-flowers on one stem. Molecular analyses by Southern, northern and western blots confirmed that the foreign *OsMADS1* gene was stably integrated into the lisianthus genome and expressed, resulting in the accumulation of the OsMADS1 protein in the transformants. Because some other morphological and physiological changes such as dwarf, reduction of flower size and change of flowering time were also observed in primary transformants, self-pollination was performed to evaluate the traits in the next generation. Two representative T_1 progeny lines were selected and subjected to the cultivation experiment under a closed greenhouse condition. The results demonstrated that the phenotype with short flower stalks was inherited in both transgenic lines. Furthermore, they had statistically different plant height, flower size and number of flowers compared with untransformed control plants. Flowering time was also changed in the two transformant lines. The obtained transformants are very unique and might be useful as bouquet for flower arrangement. These results demonstrated that genetic engineering could successfully introduce new traits to lisianthus as is the case with horticultural crops.

POTENTIAL COMMERCIAL VALUE FROM MUTATED CLONES OF *CALADIUM HUMBOLDTII* SCHOTT 'PHRAYA SAVET' FROM *IN VITRO* CULTURE

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Mutated clones of Phraya Savet caladium (*Caladium humboldtii* cv. 'Phraya Savet') from *in vitro* culture were observed. Callus and small shoots were induced from unexpanded leaf segments cultured on modified Murashige and Skoog medium (MS) supplemented with 2.69 μM 1-Naphthalene acetic acid (NAA) and 17.76 μM N^6-Benzyladenine (BA) for 4 – 5 months with subculturing every 6 weeks. Shoots were transferred onto modified MS medium supplemented with 8.88 μM BA for shoot multiplication. Subsequently, roots were induced on MS without growth regulator for 2 weeks. The regenerated plantlets were vigorously grown in glasshouse conditions. From 3 morphological groups, leaf pattern, petiole and leaf colour were used to identify the mutated clones. The regenerated 'Phraya Savet' caladium plants were divided into 11 types. Variations were found in 10 types with multiple variants from 1 – 4 characters. The occurrence of variants was 34 percent. Leaf pattern variants were observed at the highest frequency of 28 percent while leaf colour variants and petiole variants were found 16.0 and 4%, respectively. The most significance for commercial value from mutated clones was round leaf (9 percent). Other interesting variations were discussed.

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IN VITRO SELECTION OF *ANTHURIUM ANDREANUM* FOR SALT STRESS RESISTANCE

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Salinity is a troublesome factor for plants growing in soil-less substrates, such as anthurium grown in greenhouse conditions. To overcome this problem, it is necessary to grow genotypes that are more tolerant to salt stress. *In vitro* selection of some crops for tolerance of salt stress has proven successful, so we attempted to apply this technique to anthurium. The high potential of anthurium for adventitious regeneration also supported this approach.

The youngest leaves, petioles and roots of micropropagated anthurium explants of the Bolero cultivar were *in vitro* incubated on media consisting of half-strengthMS (Murashige and Skoog, 1962) salts except ammonium nitrate lovered to ¼ concentration and different combinations of growth regulators. In one set of experiments, NaCl was included in the medium at concentrations of 0, 10, 20, 40, 80, 160 or 320 mM, and in the other at 0, 25, 50 or 100 mM

The most effective medium for shoot regeneration was that containing 0.5 mg/l thidiazuron (TDZ) and 0.5 mg/l ά-naphthalene acetic acid (NAA). The earliest regeneration was from leaves, then from petioles and roots. The highest NaCl concentration in which survival and regeneration occurred was at 40 mM when BAP and 2,4-D were used, and at 50 mM when TDZ and NAA were used. Shoot regeneration was usually preceded with globular callus, which was light green on the medium without NaCl, and light yellow with sporadic green points on the medium with NaCl. Green shoots on the media with NaCl regenerated 4-6 weeks later than those on NaCl-free medium. Their subculture on medium without growth regulators was performed for elongation, and with auxin for rooting, in both cases with the addition of the same concentration of NaCl on which the shoots had been induced. Most of these shoots yellowed and proved to be escapers, but some were still green after rooting. They will be tested for NaCl tolerance repeatedly before and after acclimatization in the greenhouse.

ESTABLISHMENT OF PLANT REGENERATION SYSTEM AND *AGROBACTERIUM*-MEDIATED GENETIC TRANSFORMATION IN DAHLIA

P70

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Dahlia (*Dahlia hybrida*) is a popular tuberous ornamental plant belonging to *Asteraceae* family. It has many cultivars with wide variations in flower colour, size and shape and grown for cut flower, and as pot and garden plants. For the cultivation, dahlias have many problems such as diseases and pests. Especially, viral diseases are the most serious since many cultivars are always facing to extinction because of the high susceptibility to some viruses such as dahlia mosaic virus. Although dahlia has high variations in flower colour and morphology, there have been constant demands for novel type of flowers such as blue flowers. To solve these problems, it is now expected to utilize genetic transformation methods. In this study, we aimed to develop an efficient protocol for *in vitro* plant regeneration and *Agrobacterium*-mediated genetic transformation. Calli were successfully induced from leaf on MS (Murashige and Skoog, 1962) medium supplemented with 10 mg/l TDZ and 30 mg/l sucrose and shoot formation was observed after transferring the calli onto hormone-free medium. Based on these result calli induced on MS medium supplemented with 10 mg/l TDZ were inoculated with *Agrobacterium tumefaciens* strain EHA101 (pIG121-Hm) harboring both β-glucuronidase (GUS) and hygromycin resistant genes. After 2 days of co-cultivation, the calli were transferred to a selection medium containing hygromycin with meropenem for bacterial elimination. Survived calli were successfully regenerated on hormone-free medium without hygromycin. The regenerated shoots rooted on hormone-free medium containing hygromycin. The hygromycin-resistant plants thus obtained showed histochemical blue staining for GUS. Transformation of calli were confirmed by PCR and Southern blot analyses.

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CHARACTERISTIC OF AN *ASTER SPATHULIFOLIUS* MUTANT DERIVED FROM Γ-RAY TREATMENT

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Aster spathulifolius is a multi-annual growing evergreen herb and can be easily found in the dry soil condition of the coastal area of Korea and Japan. It generally has heavily dense pubescences on leaves and shoots, grows up to 50cm height and flowers violet from late September to early December.

In an attempt to develop wild *Aster spathulifolius* into a pot plant with higher ornamental value and more suitable to flower bed of street, γ-ray was used for the mutation induction.

The proper treatment dosage of γ-ray (LD_{50}) seemed as 30Gy, which have shown 54% survival after the irradiation of γ-ray on the seeds.

However, from the continuous observations on the whole plant population of $M_1\mathbf{V}_1$ generation which have been irradiated in the process of dosage determination, we luckily selected one leaf mutant with golden coloured leaf margin among the 10Gy treated plant population.

This selected mutant has not showed coherent leaf pattern till $M_1\mathbf{V}_5$ generation but the characteristics of this plant could be kept by only clonal propagation successfully. The seeds from the mutant were segregated phenotypically in the following generation.

A DIGITAL IMAGE ANALYSIS SYSTEM (DIAS) FOR ASSESSMENT OF BIOASSAYS ON *RHODODENDRON SIMSII* AGAINST *CYLINDROCLADIUM SCOPARIUM*

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Cylindrocladium scoparium MORGAN belongs to the most important diseases of *Rhododendron simsii*. Successful controlling by breeding for resistance to this disease needs sensitive, practicable and reproducible screening methods. A research project aimed to develop effective screening methods for evaluation of plant resources for *Cylindrocladium* resistance in *Rhododendron simsii* will be presented.

Bioassays with detached leaves were established. Drop inoculation was applied on the injured leaf base with *C. scoparium* suspension in a concentration of 10^6 spores per ml. The leaves were placed in Petri dishes on filter paper and the incubation was carried out at 22 °C over a period of 14 days in climate chambers.

Development of disease symptoms could be quantified with a digital image analysis system (DIAS). All samples were photographed by a digital camera under defined illumination conditions 3, 7 and 14 days after inoculation. The software disperses and divides the image into colour grades according to the individual colour calibration. The calibration depends on the target of the bioassay and the variability of the symptom expression in the samples while users can define classes for their own discrimination needs. In some cases the differentiation between the healthy tissue and tissue infested by *C. scoparium* or the calculation of the disease severity ratio over the time series was highly problematic. As result of the analysis the proportional area of the respective colour grades were evaluated and transferred to a data base system. Afterwards the original data were transferred into the Excel programme and prepared for statistical analyses. This method allowed the accurate assessment of individual plant reaction to *C. scoparium*.

87 *Rhododendron* genotypes (65 *R. simsii* varieties and 25 *Rhododendron* species) were screened in the bioassays to *C. scoparium* respectively. The responses of the genotypes to *C. scoparium* were estimated by symptom scoring with the digital image analysis system (DIAS). The analyses of the disease symptoms showed significant differences regarding the susceptibility and disease progress to the fungal pathogen.

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INVOLVEMENT OF SPECIES *LILIUM CANDIDUM* IN BREEDING OF LILIES

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L. candidum is cultivated as ornamental plant from ancient time. However, despite high ornamental value and potency of vegetative propagation this species is limited by demand to environmental conditions and by sensitivity to fungal and virus infections. Reproduction isolation barriers are the main reason why *L. candidum* is rare involved for breeding of lilies. In this investigation *L. candidum* was involved in incongruous crosses with Asiatic Hybrids (AH), Trumpet Hybrids (TH) and *L. longiflorum.* The cut style pollination and pollination by mixed incongruous pollen were used as methods to overcome pre-fertilization barriers. Cultivation of isolated embryos in *vitro* allowed rescue hybrid progeny.

Neither native nor cut style pollination allow achieve fertilization in crosses *L. candidum* x AH or AH x *L. candidum*. However when pollen of *L. candidum* was used in mixtures with pollen of other incongruous species to perform pollination of AH female fertilization was successful. The progeny derived from such crosses were screened by inheritance of isozymes patterns and DNA markers. Several tens plants were selected as AH x *L. candidum* hybrids by superoxide dismutase (SOD) patterns inherited from *L. candidum*. Some progeny were received as descendant after pollination of TH with mixed pollen of *L. candidum* and *L. monadelphum* and several plants were received by cut style pollination of *L. longiflorum* with *L. candidum*. However it‘s hybrid nature must be confirmed.

Morphology and grow vigour of young hybrid plants AH x *L. candidum* grown in soil condition was in common with AH. They don’t form basal leave rosette which is characteristic to paternal species *L. candidum.* The flowers of solitary flowered young hybrid plants were something intermediate in form between maternal AH and parental *L. candidum*. Some of AH x *L. candidum* hybrids were converted in allotetraploid forms to involve them for backcrosses with AH and *L. candidum.*

SOMACLONAL VARIATION IN MICROPROPAGATED TULIPS AS A SOURCE OF NOVEL GENOTYPES – FIELD AND MOLECULAR CHARACTERISTIC

P74

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The aim of the study was to evaluate somaclonal variation (SV) in tissue-culture (TC) derived plants of three tulip genotypes: 'Giewont', 'Prominence' and a mutant 'Bs6'. The mutant 'Bs6' was selected from among the micropropagated plants of the cultivar 'Blue Parrot' in 2004. Compared to 'Blue Parrot' true-to-types the plants of this new genotype have flowers longer by 1.5-2 cm, stems longer by 5 cm and colour of flowers changed from purple-violet to red-violet. The plant material of all genotypes derived from the long-term cultures maintained *in vitro* for the period of 2-4 years for 'Giewont' and 'Prominence', and 6 years for 'Bs6'. The TC-derived plants were planted outdoors in an insect-proof tunnel in 1999-2004. During the subsequent years of cultivation 2003-2008 phenotypic evaluations were done when the plants were in full bloom. Juvenile plants were examined for leaf abnormalities. The reference plants for 'Giewont' and 'Prominence' were propagated conventionally while for 'Bs6' the reference plants were derived from *in vitro* culture.

SV frequency depended on genotype and time of culturing *in vitro*. In case of 'Prominence' the lowest SV frequency (5.3-9.2%) was observed for plants derived from the two-year cultures and the highest one (28.2-48.9%) for plants from four-year cultures. In case of 'Giewont' and 'Bs6' the SV frequency ranged from 6.7% to 13.8%. Most of the off-type plants out of all genotypes had minor changes. The colour of flowers was unchanged, however, the shape of flowers was slightly altered, e.g. in some 'Prominence' variants, tepals had acute tips or in 'Bs6' variants, a tepal goffering was atypical.

In all the studied genotypes, phenotypic evaluation showed a regular occurrence of variants with major changes such as highly malformed flowers. The colour of flower of these variants was unchanged, while tepals were irregularly notched and had white stripes. All the variants had leaves with thicken, vitreous venation. Such leaves were also found in some juvenile plants of all the genotypes. DNA analysis with an use of inter-simple sequence repeats (ISSR) carried out on the somaclonal variants with major changes of both juvenile and flowering plants confirmed that changes in leaf morphology resulted from genetic changes. The obtained results indicate that the trait of the leaf thicken, vitreous venation can be considered as the morphological marker for early detection of the major genetic changes within juvenile plant material.

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BUSINESS 2 RESEARCH: AN EFFICIENT SYNERGY

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Ornamental production is a very innovative sector. A prominent bottleneck however is the transfer and implementation of expertise present in research institutes to the hortibreeder business. On the other hand, introducing new cultivars in a successful manner is complex for public breeding institutes. In order to overcome this hurdle, a diverse range of long-term partnerships between ornamental entrepreneurs and research institutes was set up.

These close interactions can make the innovation process of companies feasible and more straight forward. Growers that take part in such collaborations between business and research pay therefore a yearly contribution, which is in turn directly invested in breeding activities and research. In this context, a successful project offering technological advice and service in horticulture, called SIETINET, has been running for already 5 years. Likewise, AZANOVA cvba and BEST-select cvba, involving innovative breeding in respectively pot azalea and woody ornamentals, are great examples of how research institutes can play a vital role in empowering a continuous strategy of innovation within hortibreeder business.

Offering expert technological advice constitutes a central component within SIETINET. Another interesting service of the network is the possibility to design testing services, defined by the grower, that require specialized scientific equipment. In the past, successful experiments have been set up for e.g. the fine tuning of the growing systems for new cultivars, the application of methods for ploidy alteration, mutation breeding, in vitro techniques to overcome interspecific crossing incongruities, study on rooting and acclimatization of candivars. In breeding activities of AZANOVA and BEST-select, the participating growers are involved at an early stage in evaluating new candidate cultivars. Equally important for these partnerships is the subsequent lucrative commercialization of the resulting product innovations. Consequently, efforts are made to attract media coverage and to improve access to markets.

As technological innovation and improvement play a crucial role in hortibreeder companies, services offered by these partnerships may have an important impact on members R&D investments and makes it possible for growers to get access to new cultivars.

MARKER ASSISTED BREEDING FOR NEMATODE RESISTANCE IN *HYPERICUM*

P76

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Root-knot nematode (*Meloidogyne* spp) is an important plant parasite in *Hypericum* L. So far, there are no acceptable cut flower or pot varieties with nematode resistance. Given that recently demand for *Hypericum* has risen mainly due to its versatility as a filler in mixed bouquets, there is an increased need for developing nematode resistant varieties. Introduction of nematode resistance will enable the production of varieties with improved performance and quality, without the use of dangerous nematicides and soil disinfection methods that are harmful to people and the environment. The aim of this project is to introduce nematode resistance in cultivated *Hypericum*, to identify molecular markers associated with nematode resistance and develop marker assisted selection in this crop.

Field assays with soil infested with root-knot nematode and evaluation of disease and pathogen levels have led to the identification of six resistance species. Out of these two resistant genotypes have been chosen for a crossing program. Four interspecific reciprocal crossings are in progress, between two cut flower varieties and the two nematode resistant genotypes. GISH will be used for verification of hybrid character of the crosses and assessment of the number of introduced chromosomes from the wild species and possible recombination between the species. For phenotyping, 100 F1 genotypes per crossing will be evaluated in an experimental field that will be uniformly infested by nematodes. For this purpose, a total of three replicates of 25 plants per genotype will be sown and the plants will be grown for two consecutive flowering periods. For genotyping, identification of associated molecular markers of nematode resistance will be performed with NBS-profiling, a molecular marker technique that efficiently produces markers in resistance genes by targeting towards conserved regions of these resistance genes.

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CYTOGENETICAL STUDY OF CHAMAN SHOUR SAHELI (*AELUROPUS LITTORALIS*)

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The Chaman Shour is plant that has growing in around of Erumeih pond, Iran. Whereas it's one of halophyte plant and also can use thereof for nutrition in ranch, therefore is important. In order to accustom with chromosome characteristic's its karyological studied in Islamic Azad University- Tabriz Branch in 2006. In this study have used from root apex meristematic cells. The results showed that basic number of chromosomal in this plant is fifteen (x = 15), kind of chromosomes are metacentric and its ploidy is diploid. The long of tallest Chromosome (number 1) is 3.0354^{+}- 0.12 micron and long of shortest Chromosome (number 15) have estimated 1.3684^{+}- 0.04 micron. The total long of genome have estimated 29.8619 micron.

ACQUISITION OF OTO, OA AND FO INTERSPECIFIC HYBRID LILIES AS A SUBSTITUTE FOR ORIENTAL HYBRIDS BY OVULE CULTURE

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Oriental hybrid lilies are popular cultivated in Korea and exported to Japan's flower market. However, it is very weak growth hot summer season, weak disease (ex. Virus and *Fusarium*) resistance, long breeding and bulb growth period and strong fragrance in Korea. To introduce new genetic variation and substitute for disadvantage of Oriental hybrids, it has crossed with *Lilium* OT interspecific hybrids; *L. x formolongi* hybrids and Asiatic hybrids by cut-style and stigmatic pollination method and ovule culture since 1997. For the first time, four new OTO (*Lilium* OT interspecific hybrid x *L.* Oriental hybrids) hybrids were bloomed in 2008. The parents of 'OTO-08-2' and 'OTO-08-2-2' are between 'Avocado' and 'Medusa'. The characteristics of 'OTO-08-2' have oriental flower shape, male sterile, strong growth and weak fragrance. Flowers are upward-facing and thicken red pink and ivory edge (RHS, 67P, W155B). The characteristics of 'OTO-08-2-2' have oriental flower shape, male sterile, and fragrance. Flowers are sideward-facing and reddish pink and ivory edge (RHS, R-51C, and W-155A). The parents of 'OTO-08-1' are between 'Avocado' and 'Cordoba'. The characteristics of 'OTO-08-1' have oriental flower shape, weak fragrance and male sterile. Flowers are upward-facing and white with ivory centered (RHS, W155B). The parents of 'OTO-08-3' are between 'Avocado' and 'Acapulco'. The characteristics of 'OTO-08-3' have oriental flower shape, male sterile, no pollen grains, and weak fragrance. Flowers are upward-facing and red with light salmon (RHS, R-46A, Y11G, and D). OA-05-1 was obtained between Oriental hybrid 'Casa Blanca' and Asiatic hybrid 'Sgl pepper'. Characteristics of OA-05-1 were upward-facing flowers, weak fragrance, purple flower colour (RHS, RP60B). FO progenies between 'Raizan' and 'Oriental O-54 line' are obtained FO-00-1, FO-00-3, FO-00-4, FO-00-6, FO-00-10, FO-00-12 and FO-00-16. Phenotypic characteristics of FO progenies were similar to that of Oriental hybrids such as diverse colour distribution from creamy ivory as female flower colour to deep pink as male flower colour, Oriental flower shaped and leaf shaped, flower size, weak fragrance and so forth. Among new diverse interspecific hybrids has been obtained, OTO hybrids will be adequate cultivars adapted to the Korean climate and environmental conditions.

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ESTABLISHMENT OF PROTEOME REFERENCE MAPS FOR SOMATIC AND ZYGOTIC EMBRYOS OF *CYCLAMEN PERSICUM* MILL

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Cyclamen persicum is a popular ornamental crop with a high economic relevance for the horticulture business. Within *Cyclamen persicum* somatic embryogenesis has been shown to be an efficient vegetative propagation system and the development of artificial seeds is an ultimate aim. For this approach, somatic embryos have to be produced which mimic their zygotic counterparts in protein composition. Moreover, the physiology of somatic and zygotic embryogenesis remains unclear in *Cyclamen* up to now. Initial analyses comparing the proteomes of somatic and zygotic embryos have been performed previously. Approximately 70 % of the proteins expressed in zygotic embryos also were present in somatic embryos in comparable abundance. ESI-MS/MS analysis led to identification of 20 proteins of both tissues.

Based on these analyses, further comprehensive proteomic characterizations were performed aiming to create proteome reference maps for somatic and zygotic embryos of *Cyclamen persicum*. Separation by two dimensional IEF-SDS gel electrophoresis leads to a resolution of more than 700 protein spots for each tissue. Consistent to the initial analysis, approximately

70 % of the spots likewise appeared in both zygotic and in somatic protein fractions. However, DIGE analysis revealed extremely high alterations in abundances for the majority of proteins present in both tissues. MS analysis for total 300 reproducible spots (260 of the zygotic embryos' protein fraction and additionally 40 spots appearing specifically in the somatic embryos' proteome) led to identify 261 proteins, whereof 33 specific for the somatic tissue.

Currently, identified proteins are clustered according to their physiological relevance and a digital proteome reference map is created. Additionally, shotgun proteomics are in preparation for both embryo tissues. Also proteomic analysis concerning the development and maturation of somatic embryos are in progress.

MUTATION FREQUENCY INDUCED BY CHEMICAL MUTAGENS IN M_3 GENERATION OF *PETUNIA X ATHINSANA* D.DON

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The seeds of cultivar ('Flash Red') of *Petunia x athinsuna* D. Don were used as the initial material for the studies. Mutations were induced by soaking these seeds for 1 hour in the solution with pH 4 of EMS (concentration 0.5 and 1.5 mM), MMS (1.5 and 2 mM), DES (0.5 and 1 mM) and AS (1 - 1.5 mM) at the presence of buffer orthophosphoric acid at the concentration 0.025 mM. In order to check the genetic background of the observed mutations in M_3 generation DNA samples were taken for analysis from the plants with the phenotype different from control plants. Variability on the DNA level was determined by ISSR-PCR technique.

In M_3 generation of mutants the most frequently occurring changes were white tiny spots or lines on corolla petals, flower colour change from red into pink, chlorophyll leaf changes and irregular corolla rim. The majority of M_3 phenotypic variation had already been found in M_2 generation. Chemical mutagens had a stimulating effect on the number of flower buds. The greatest number of flower buds in mutants resulted from the application of 0.5 mM of EMS and 0.5 mM of DES (520 – 530% of control) for mutagenesis.

In most cases phenotypic changes in M_3 generation were of genetic nature. Their genotype similarity ranged from 4.9- 25.5 %. Most changes of this kind were obtained after 0.5 mM EMS and 0.5 DES had been used for inducing mutation.

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FUNCTIONAL ANALYSIS OF *TORENIA* CLASS B GENES, *TFGLO* AND *TFDEF*: THEIR UNIQUE TRANSGENIC PHENOTYPES AND TARGET GENE REGULATION

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We are studying functions of floral organ identity genes using torenia (*Torenia fournieri* Lind. 'Crown Violet'), to establish efficient procedure for controlling floral traits by genetic engineering. In this study, we focused on *TfGLO* and *TfDEF*, the torenia class B genes, those have been supposed to play important roles in formations of petals and stamens.

To understand the roles of the two genes, we firstly generated a set of class B gene-overexpressing transgenic lines and -repressed lines. The latters were generated by introducing chimeric repressors with the EAR-motif repression domain (SRDX; Hiratsu et al. 2003). *TfGLO*-overexpressing lines showed the accumulation of anthocyanin pigments in sepals, and *TfGLO*-repressed lines exhibited distinctive serration in petal margins. In *TfDEF*-repressed lines, irregular pigmentation pattern along the vascular bundles was observed within the purple segment of petals, and no phenotypic change was observed in *TfDEF*-overexpressing lines.

We next examined the expressions of putative target genes of *TfGLO* and *TfDEF*, in accordance with previous *Arabidopsis* study. We isolated xyloglucan endo-1,4-beta-D-glucanase genes (*TfXEG1*, *TfXEG2* and *TfXEG3*) and chlorophyll *a*/*b* binding protein genes (*TfCAB1*, *TfCAB2* and *TfCAB3*) from torenia, then performed RT-PCR analysis using floral organs of the transgenic lines. As we have expected, the expressions of *TfXEG1*, *2* and *3* were up-regulated in class B gene-overexpressing lines and down-regulated in class B gene-repressed lines. On the contrary, the expressions of *TfCAB1*, *2* and *3* were not influenced by overexpressing the class B genes, while their expressions were up-regulated in class B gene-repressed lines.

These results suggest that both *TfGLO* and *TfDEF* participate in the developmental process of floral organs, while they seemed to have different functions. In addition, putative target genes of class B genes, such as *TfXEGs*, were up-regulated in torenia as same as in *Arabidopsis*. Now, we are focusing on the regulation of anthocyanin biosynthesis by class B genes because anthocyanin pigmentation in sepals was observed only in *TfGLO*-overexpressing lines. To understand further the individual functions of the two class B genes, we intend to examine the expressions of anthocyanin biosynthesis-related genes using the class B genes-overexpressing and -repressed lines.

INDUCTION OF 2N GAMETES AND 4N EMBRYO IN LILIUM (*LILIUM* × *FORMOLONGO* HORT. BY NITROUS OXIDE GAS TREATMENT

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The effect of nitrous oxide gas (N_2O) treatment on the induction of 2n gametes and 4n embryo in ***Lilium* × *formolongo*** hort. was examined. ***Lilium* × *formolongo*** hort. is an artificial hybrid between ***Lilium longiflorum*** Thunb. and ***Lilium formosanum*** Wall. In general the members in the genus *Lilium* is propagated vegetatively. However, ***Lilium* × *formolongo*** hort. is exceptionally propagated by seeds and blooms 6 - 8 months after sowing. Seeds of ***Lilium* × *formolongo*** hort. cultivars 'Raizan' and 'Kitazawa-Wase' were sown in a plastic tray with 200 holes on 5 February 2008 and seedlings bearing 2-3 leaves were transplanted in 10.5cm (in diameter) pots on 5 May, grown under ambient conditions in a green house. When these first flower buds of 'Raizan' plants were reached at 15 – 30 mm in length, these buds were sampled consecutively and observed by the aceto-carmine squash method to determine the meiotic stage of pollen mother cells. The buds ranging from 19 – 23 mm in length contained pollen grains at prophase I to tetrad. Immediately after the staging of pollen grain, plants with the buds in the same length were treated with N_2O gas for 48 h at 6 atm at room temperature. The diameter of mature pollen grains in metaphase, obtained from the plants with buds of 22mm in length at the time of exposure to N_2O gas, ranged from 76 to 169, although those from untreated ones ranged from 62 to 95μm. For the induction of 4n embyo, thirteen days after the pollination with untreated pollen grains of 'Kitazawa-Wase', ovaries of 'Raizan' were treated with N_2O gas for 72h at 6 atm. Plants with pollinated ovaries grew normally after the treatment with N_2O gas and these capsules were harvested 75 - 85 days after pollination. We obtained 25 seedlings from these capsules and ramdomly selected five seedlings. Of five seedlings, two were revealed to be tetraploid by flow cytometry. We could demonstrate that N_2O gas treatment was useful for the manipulation of both male gamete as well as zygote for the polyploidy breeding in ***Lilium* × *formolongo*** hort.

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EFFICIENT SCREENING OF TRANSGENIC TORENIA WITH NOVEL FLORAL TRAITS BY COLLECTIVE INTRODUCTION OF CHIMERIC REPRESSORS FOR *ARABIDOPSIS* TRANSCRIPTION FACTORS

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Molecular breeding enables us to produce novel floral traits of horticultural plants which could not be obtained by traditional breeding. Since a number of transcription factors, such as MADS-box and TCP family proteins, play a role in the control of flower development, transcription factors are useful tools for the manipulation of floral traits and generation of novel variation. Although we usually need sequence information of the gene that we try to use, horticultural plants have little information of genome and EST. By contrast, information of *Arabidopsis*, the model higher plant, is abundant and facilitates our molecular breeding. Accumulating facts that chimeric repressors of *Arabidopsis* transcription factors altered floral traits of some horticultural plant species may lead to an innovation of molecular breeding. On the other hand, it is necessary to develop an efficient screening method because we cannot find out useful genes for valuable floral traits without regenerating transgenic plants. To obtain new floral traits efficiently, we selected 42 transcription factor genes which highly expressed in *Arabidopsis* flowers and collectively introduced their chimeric repressors into torenia (*Torenia Fournieri* Lind. cultivar 'Crown Violet'). We screened phenotypically-altered 193 lines of 348 transgenic plants. We found that 82.4% of them had single transgene, and 39 of 42 constructs were introduced independently. One third of transgenic plants with single transgene induced recognizable phenotypes in petal colour and/or shape as expected, such as white margined petals, uniformed colour corolla, and partly opened flower. These results indicate that bulk collective introduction of chimeric repressors of *Arabidopsis* transcription factors makes possible the efficient production of novel horticultural plants with valuable traits. Some transcription factors used in this study seem to act in the same regulatory pathway because similar phenotypes were observed in some transgenic plants harboring different chimeric repressors. Most of selected 42 genes were functionally unknown, but phenotypes in transgenic torenias would provide information which cannot be revealed by analysis in *Arabidopsis*. Now we are transforming another set of 50 genes selected with different approach to explore further variation of floral traits.

ACCUMULATION, PRESERVATION AND INVESTIGATION OF LITHUANIAN ORNAMENTAL PLANTS IN THE BOTANICAL GARDEN OF VILNIUS UNIVERSITY

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Accumulation, preservation and investigation as well as conservation of genetic resources have become a prestigious task for all the countries of the world. By signing the Rio Convention on Biological Diversity on June 11, 1992, Lithuania has committed to conserve its genetic resources. In 1993 National Coordination Centre for Conservation of Plant Genetic Resources at the Lithuanian Agriculture Institute was established. Researches of the Floriculture Department of the Botanical Garden of Vilnius University joined the program of investigation and conservation of genetic resources in 1994. At present the gene fund collection consists of 900 flower taxa developed by Lithuanian plant breeders. The authors of Lithuanian flower cultivars (both amateurs and professionals) have created priceless national wealth i. e. flower cultivars and hybrids. Therefore, collection, preservation, investigation and evaluation of Lithuanian flower genefund is a new trend of scientific researches not only in the botanical gardens but also in the whole country.

Lithuanian flower breeders released a lot of new cultivars of *Crocus* L., *Dahlia* Cav., *Gladiolus* L., *Hemerocallis* L., *Iris* L., *Lilium* L., *Paeonia* L., *Primula* L., *Tulipa* L. and etc. The aim of this research is to study and evaluate ornamental properties of flower cultivars released by the Lithuanian breeders.

The investigations, descriptions and evaluations of morphological, bioecological and ornamental properties of gladiolus (*Gladiolus* L.), lily (*Lilium* L.), iris (*Iris* L.), primrose (*Primula* L.), dahlia (*Dahlia* Cav.), peony (*Paeonia* L.) cultivars were carried out in the period of 1998 – 2008 according to the requirements of the International Union for the Protection of New Varieties of Plants (UPOV) and methodologies used in neighbouring countries. The colour of the blossoms and leafs are determined according to the international R.H.S. (The Royal Horticulture Society) colour chart.

Having collected, accumulated, researched and evaluated the flowers created by Lithuanian breeders, in the future it will be possible to select the most valuable cultivars (genetic resources) and to make a system of the effective preservation and rational usage of the genetic resources. In general, Lithuanian cultivars are originals adapted to the local climate conditions, and it is urgent to conserve, investigate and foster it as a part of the land culture. The most distinguishing and attractive (unbelievable form, perfect display of blooms in the spike, colour harmony) Lithuanian gladioli cultivars, researched in the Botanical Garden of Vilnius University, are the following ones: ‘Fiji’ (author A. Lukosevicius), ‘Nu, Gromov, Pogodi!’ (P. Ciplijauskas), ‘Laimute’ (P. Ciplijauskas), ‘Merkurijus’ (A. Lukosevicius), ‘Norma’ (A. Lukosevicius), ‘Onute-3’ (P. Balcikonis), ‘Paparcio Ziedas’ (P. Ciplijauskas), ‘Saules Takas’ (P. Balcikonis), ‘Snieguole’ (P. Ciplijauskas), ‘Solveiga’ (A. Lukosevičius), ‘Spalvingas Sapnas’ (J.A. Liutkevicius). The results of *Paeonia* genus researches were summarized and 25 hybrids originated by E. and J. Tarvidas were suggested to the register of national gene fund.

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EXISTENCE AND FUNCTION OF PETAL COLOUR IDENTITY GENE IN APETALOUS WILD CHRYSANTHEMUM

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Chrysanthemum (*Chrysanthemum morifolium*) is one of the most important ornamental crops worldwide. In most chrysanthemums, the capitulum is composed of ray florets (petals) and disk florets. The ray floret colour in yellow-flowered cultivars is mainly due to carotenoids. Recently, Ohmiya et al. (2006) showed that the ray floret colour in white-flowered cultivars is due to the degradation of carotenoids by the expression of *CmCCD4a*, a gene that encodes carotenoid cleavage dioxygenase; *CmCCD4a* suppression by RNA interference (RNAi) give yellow colour to white ray florets.

In wild chrysanthemums, the ray florets are either white or yellow, indicating that ray floret colour probably results from crotenoid degradation in wild chrysanthemums, as in chrysanthemum cultivars. Southern blot analysis confirmed that wild chrysanthemum species with white ray florets had *CmCCD4a* homologues, while those with yellow ray florets did not. This suggests that carotenoid degradation also contributes toward producing ray floret colour in wild chrysanthemums.

CmCCD4a expression was observed to be strictly limited to ray florets. Southern blot analysis showed that *C. shiwogiku* has a *CmCCD4a* homologue, although its capitulum has only disk florets and no ray florets. To confirm the function of this *CmCCD4a* homologue, *C. shiwogiku* was interspecifically hybridized with a cultivar, ‘Squash’, which does not have *CmCCD4a* and produces yellow ray florets. The capitulum of all progeny individuals produced white ray florets. *CmCCD4a* homologue fragments were detected in all progeny individuals by polymerase chain reaction (PCR). These results showed that the *CmCCD4a* homologue present in *C. shiwogiku* performed an enzymatic function in the ray florets. Further, these findings suggest that *C. shiwogiku* may have originated from a species with white ray florets by loss of genes related to ray floret formation.

Similar to *C. pacificum*, *C. shiwogiku* produces numerous small capitula in each leaf axil, and this is a useful characteristic for breeding (De Jong, 1989). *C. shiwogiku* and *C. pacificum* have no ray florets, and therefore, breeders do not know the colour of ray florets in these species. Investigation of *CmCCD4a* homologue before crossbreeding can predict the ray floret colour of their progeny.

MICROPROPAGATION OF SELECTED ORNAMENTAL PLANTS

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In the present work, three (3) popular ornamental plants in Malaysia were selected for micropropagation studies using tissue culture system. The species utilized were *Begonia heimalis* Fotsch, *Gerbera jamesonii* and *Polianthes tuberosa.* The scope of the study covers investigation on the role of various plant hormones such as Naphthalene acetic acid (NAA), Indole acetic acid (IAA), kinetin, Benzyladenine (BA) etc on the *in vitro* morphogenesis of these species. The main aim being to regenerate these ornamental plants *in vitro* for mass propagation. Based on the results obtained, these species were very responsive in culture, they could form multiple shoots and roots quite readily and some could even produce *in vitro* flowering. The efficient regeneration systems were established for the three species on MS (Murashige and Skoog, 1962) medium supplemented with various hormones at optimum concentrations.

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P87

PLOIDY LEVELS OF DEGENERATED EMBRYOS IN THE CROSSES BETWEEN DIPLOID AND TETRAPLOID CYCLAMEN

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The formation of unexpected tetraploid progenies and inhibition of triploid-seed formation in the reciprocal crosses between diploid and tetraploid cyclamen was reported. In the crosses, it was also been suggested that the tetraploid progenies were formed by the fertilization between a reduced gamete from a tetraploid plant and an unreduced gamete from a diploid plant, and that the development of many zygotic embryos were inhibited. Ploidy levels of the degenerated embryos were, however, obscure. Therefore, embryo rescue by the ovule culture in the crosses were examined.

A lot of progenies were obtained in the 2x x 4x crosses, when the ovule culture by using medium with coconut water was done 28 days after pollination. Although almost all fruits were dropped 28 days after pollination in the 4x x 2x crosses, some progenies were obtained by the ovule culture at seven days after pollination.

Almost all progenies obtained by the ovule culture after the reciprocal crosses between diploids and tetraploids were triploids, suggesting that many triploid zygotes were formed in the crosses. Some tetraploid, pentaploid and hexaploid progenies were also obtained in the 2x x 4x crosses after the ovule culture. Origin of the pentaploids was suggested the fertilization between a reduced gamete from a diploid plant and an unreduced gamete from a tetraploid plant.

These results suggest that many triploid zygotes are formed in the crosses between diploid and tetraploid cyclamen, whereas development of the triploid embryo is inhibited without the embryo rescue. It is also indicated the possibility that pentaploids and hexaploid progenies as well as triploids and tetraploids are obtained in the crosses by using the ovule culture, depending on the cross combination. It should be suggested that development of the pentaploid and hexaploid embryos might be inhibited without the embryo rescue.

INTERGENERIC HYBRIDIZATION AND RELATIONSHIP OF GENERA WITHIN THE TRIBE ANTHEMIDEAE CASS. (I. *DENDRANTHEMA CRASSUM* (KITAM.) KITAM.×*CROSSOSTEPHIUM CHINENSE* (L.) MAKINO)

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An intergeneric hybridization has been made between *Dendranthema crassum* (kitam.) kitam. (2n=90; ♀) and *Crossostephium chinense* (L.) Makino (2n=18; ♂). Most of the hybrid embryos aborted at an early developmental stage. Using ovule rescue, totally 160 plump ovules (at 15d post pollination) were selected for *in vitro* culture, it was possible to establish a single intergeneric hybrid plant showing *2n*=54 chromosomes. The leaf length, leaf width and epidermal hair density of the hybrid were all intermediate between those of the parents. However the flower diameter, number of tubular florets, epidermal hair height and epidermal hair length exceeded those of both parents. A genomic *in situ* hybridization approach was able to distinguish between the parental genomes in the hybrid plant. Among the 54 chromosomes in the putative hybrid, nine were labelled by the avidin-FITC assay when the probe comprised genomic DNA of *C. chinense,* while the remaining 45 did not hybridize with the probe. To our knowledge, it is the first report of obtainment of intergeneric hybridization between *D. crassum* (kitam.) kitam. and *C. chinense* (L.) Makino.

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VARIATION IN RESISTANCE TO *FUSARIUM OXYSPORUM* F.SP. *LILII* FROM PROGENIES DERIVED FROM INDUCED 2N GAMETES IN ORIENTAL LILIES

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Fusarium oxysporum f.sp. *lilii* was isolated and identified from diseased bulb samples of oriental lilies collected in main production regions in yunnan province, China. Difference in resistance of different oriental lilies to *Fusarium oxysporum* f.sp. *lilii* was detected in a clonal test, and a high degree of resistance was found in genotype Cai-74. Cai-74 was derived from 4x × 2x crosses. The 4x material was derived from somatic doubling diploid cultivar, 2x is from sexual triploid which is derived from induced 2n gametes. Through karyotype analysis with Stebbins standard it was proved that significant differences were found in karyotype of Cai-74 icompared with the susceptible control. By analysis of saponins content in lily bulb with spectrophotometry, saponins content in Cai-74 was almost 30% higher than that in the susceptible control. This suggest a correlation between saponins and resistance to *Fusarium oxysporum* f.sp. *lilii*. The analysis of saponins of lily bulbs could be used for the evaluation of the resistance to *Fusarium oxysporum* f.sp. *lilii* in oriental *Lilium* cultivars.

IN VITRO CULTURE OF *CALADIUM BICOLOR* (AIT.) VENT. 'THEP SONGSIL' AND INCIDENCE OF VARIENTS

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Explants from the first fully expanded leaf of *Caladium bicolor* 'Thep Songsil' were cultured to determine the appropriate concentration of plant growth regulators in modified Murashige and Skoog medium for rapid micropropagation and the variation. From 11 in 16 combinations of 6-benzyladenine (BA) and ∝-naphthalene acid (NAA) or 2,4-dichlorophenoxy acetic acid (2,4-D) promoted callus formation. The combination of 17.76 µM BA with 2.69 µM NAA was the most effective for callus proliferation in *C. bicolor* cv. 'Thep Songsil'. Subsequently, plantlets were regenerated on MS medium containing 11.2 µM BA. The regenerated plantlets from every combination of plant growth regulators were randomly grown in greenhouse. From 11 combinations of BA and NAA or BA and 2,4-D, the regenerated plants were divided into 8 types. From each growth regulator combinations, 3 – 7 types of regenerated plants were observed. The occurrence of variants varied from 14.76 – 57.14 percent. The mode and incidence of variants were discussed. It was shown that the major influence on variation was not due to plant growth regulators in the medium. A few new regenerated variants were different from the original plants, potential for commercial value.

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SOMACLONAL VARIATION OF *CALADIUM BICOLOR* (AIT.) VENT. 'JAO YING' FROM *IN VITRO* CULTURE

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In vitro culture of *Caladium bicolor* by multiple shoot production via callus induction was established. Explants from the first fully expanded leaf of *C. bicolor* cv. 'Jao Ying', a commercial cultivar, were cultured to determine the appropriate concentrations of plant growth regulators in modified Murashige and Skoog medium for rapid micropropagation and variation. All 10 combinations of benzyladenine (BA) and ∞-naphthalene acid (NAA) provided callus induction. The combination of 8.87 µM BA with 2.69 µM NAA was the most effective for callus proliferation. Subsequently, plantlets were regenerated from calli on MS medium containing 11.2 µM BA. Most regenerated plants from *in vitro* culture, grown in glasshouse conditions, were more vigorous than the original ones. Within the 10 combinations of BA and NAA, the regenerated plants could be characterized into 13 types. From each growth regulator combination, 3 – 7 types of the regenerated plants were observed. The occurrence of variants varied from 22.41 – 87.32 percent. It was shown that the major influence on variations was not due to the plant growth regulators in the medium. The mode and incidence of variations were discussed. Many regenerated variants were totally different from the original plants, and tending to great possibility for commercial value.

GISH/FISH AS A TOOL TO CHARACTERISE HYBRIDS WITH SMALL GENOMES AND CHROMOSOMES

P92

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Fluorescence and Genomic *in situ* hybridisation (FISH and GISH) are useful tools to characterise chromosomes of a genotype and to analyse hybrid plants and natural polyploids as to their origin, genomic composition and intergenomic rearrangements. However, for plants with very small genomes and small chromosomes, GISH and FISH are difficult to perform. Visualisation of the morphology of small chromosomes is hampered and often only heterochromatin regions are labelled using GISH. In this study the *in situ* hybridisation technology was adapted for woody ornamentals, commonly characterised by small genomes, a high amount of small chromosomes and no sequence information.

Firstly, detailed karyotypes of *Hydrangea macrophylla*, *H. paniculata* and *H. quercifolia* were constructed on the basis of arm lengths and centromeric index, together with 45S rDNA FISH. The variability among 3 species was expressed by chromosome morphology and 45S rDNA signals. The chromosome portraits made in this study can be used to trace chromosome behaviour in interspecific hybrids resulting from breeding work between the 3 species.

Secondly, GISH was performed on *Buddleja* and *Hibiscus* hybrids resulting from an interspecific breeding program between different species of both genera. Using *B. globosa* as a probe, GISH analyses on *Buddleja* x *weyeriana* (an F2 selection of *B. globosa* x *B. davidii*) and F1 and F2 hybrids of *B. davidii* x *B.* x *weyeriana* crosses proved that all chromatin material of *B. globosa* was introgressed into the *B. davidii* chromosomes. Also F1 and F2 hybrids of *H. syriacus* x *H. paramutabilis* were analysed using GISH with *H. syriacus* as a probe. Also here recombinant chromosomes showing introgression of *H. syriacus* DNA in *H. paramutabilis* were detected.

The adaptation on the *in situ* hybridisation protocol for woody ornamentals consists of searching (i) the optimal probe/block ratio (not lower than 1/80) and (ii) the best labelling and detection system (biotin versus digoxigenin) for the probe.

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ORGANOGENESIS INDUCTION IN RESISTANT AND NONRESISTANT HORSE CHESTNUT (*AESCULUS HIPPOCASTANUM*)

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Horse chestnut (*Aesculus hippocastanum*) belongs to horticulturally valuable woody species. Successfulness of its planting in Europe is decreased due to a pest invasion, the horse chestnut leaf miner *Cameraria ohridella*. Research objective of this work was organogenesis induction in explants taken from grafted plants of resistant and nonresistant horse chestnut individuals. Explants (shoot apex of vegetative buds, petiole and shoot segment) collected in winter, summer and autumn season, were cultured on WPM and MS medium containing growth regulators in different concentrations. The endogenous bacterial contamination was showed to be limiting factor of regeneration induction. In spite of application of wide–spectrum antibiotics (Cefalotin, Cefotaxim, Timentin a Vancoccin), no viable *in vitro* culture was derived from winter buds. However summer and autumn buds were found as the most suitable and responsive explants with the lowest rate of bacterial infection (about 5%); regeneration of these isolated shoot apices was higher as compared to petiole and shoot segments. Lower shoot proliferation (30%) of autumn than summer explants (50–80%) was demonstrated. The presence of cytokinin BA (benzyladenine) in medium was an important factor of organogenesis induction.

BREEDING TROPICAL FRUIT CROPS FOR ORNAMENTAL PURPOSES

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Embrapa Cassava and Tropical Fruits has many germplasm collections under field conditions to give support to breeding programs carried out there. These programs were focused just to the development of new materials for fresh fruit consumption or processing. A new approach has begun in 2002 looking for ornamental genotypes in these gene banks, mainly in the pineapple, banana, citrus and acerola collections. The identification, characterization and selection of potential genotypes to be used as potted plants, cut flowers and landscape plants have been done. Initially, the morphological descriptors specific to each crop developed by IPGRI were adopted. Nevertheless, these descriptors are being adapted for use in the ornamental fruit crops aiming at cultivar protection and patenting. After the identification of interesting genotypes with ornamental potential, several controlled crosses were performed with pineapple, banana and citrus, resulting in the production of several promising hybrids. The resistance or tolerance against the most important diseases of these fruit crops have been considered in this work due to the economic importance they have in Brazil. Currently, new hybrids of pineapple, banana, citrus and some acerola genotypes identified in the germplasm collection are under field and greenhouse evaluations, depending on their intended use. In addition, a very interesting and novelty product was also generated: the minifruits. These are small, non-edible fruits whose appearance is similar to the regular ones but destined to a differentiated marked.

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TISSUE CULTURE OF LEAF EXPLANTS OF ORIENTAL *LILIUM* 'TIBER'

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Young leaves of *Oriental Lilium* 'Tiber' were divided into petiole and lamina, respectively as explant materials in tissue culture. The results showed that in MS media with different concentrations of NAA and BA, the differentiation ability of the petiole was stronger than that of the lamina. The combination of NAA and BA results petiole differentiation, while and the increase of BA reduced root formation. For the petiole, MS+NAA2.0mg.l^{-1}+BA0.10mg.$l^{-1}$was the best medium to induce shoots. MS+NAA0.50 and 2.0 mg.l^{-1}+BA2.0 mg.l^{-1} induced yellow and compact callus with successive shoot differentiation. The most appropriate medium for root induction was 1/2MS+NAA0.20 mg.l^{-1}. The concentration of sucrose in the medium affected the weight increase of the bulblets. MS media with every concentration of sucrose could make weight increase of the bulblets, however MS medium plus 60g.l^{-1} sucrose showed the highest increase. The results suggested that the leaf segments could be a good alternative as explants, which can provide material for physiological and molecular (breeding) research. .

RAPD MARKERS DEVELOPED FOR IDENTIFYING MALE STERILITY IN *TAGETES ERECTA*

P96

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Various experimental protocols for RAPD analysis of genes related to fertility in *Tagetes erecta* were systematically studied and an optimum program was developed for setting up a stable system.

CTAB method and SDS method were compared to extract DNA from different tissues of *Tagetes erecta*, the quality of DNA extracted was evaluated by spectrophotometry and PCR. Results showed that total DNA extracted from young leaves by CTAB method was suitable for the analysis of RAPD. The concentration of DNA template, MgCl2, buffer and dNTPs were examined and the procedure was redesigned for setting up the RAPD program. Amplifications were then carried out in 20 μL reactions containing 5μL diluted DNA template, 2 μL 10× PCR buffer, 2.5 mmol $MgCl_2$, 0.3 mmol dNTP, 0.3μmol primer and 1.50U Taq DNA polymerase. PCR reaction were operated under the following program conditions: 5 min at 94□ before amplification, 40 cycles of 94□ for 30 s, 37□ 1 min, 72□ 1 min, followed by one cycle of 72□ for 1 min.

By using this sytem, sterile and fertile plants of W205, a genic male sterile line in *Tagetes erecta* were tested. 288 bands were obtained from 64 RAPD primers screened, among which a band of 980bp derived from the primer G-02(5'-GGCACTGAGG-3') was coseparated with fertile plants. This RAPD marker named as G-02-980 could be used to identify male and fertile seedlings.

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GENETIC DIVERSITY AND SPATIAL AUTOCORRELATION OF GENETIC STRUCTURE OF *LILIUM REGALE*

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Lilium regale is a narrow distribute endemic specie in China. It made an important contribution to lily breeding. *L. regale* grows at dry valley in southwestern of China. It had been continually destroyed by human and earthquakes. To protect the species is quite pressing. However, there was seldom research on its genetic structure in past years. We research on the genetic diversity and spatial autocorrelation of genetic structure of *L. regale* base on ISSR molecular marker. The results indicated that, at the species level, the proportion of polymorphic loci was 97.7%, the effective number of allele (*Ne*) were 1.994 4, Shannon diversity index (*I*) were 0.3339. At the population level, the average expected heterozygosity was 0.664 0.Shannon diversity index (*I*) were 0.272 0.They showed that genetic diversity of *L. regale* was high. The result by AMOVA analysis indicated that the variation within population account 83.8% and variation among populations accounted for 11.849%, and the gene flow was 2.588. It demonstrated that the relationship of populations was closer. Gene differentiation was acute within population.

We analyzed the spatial autocorrelation of genetic structure of 4 populations with more than 35 samples. The Moran's *I* correlograms revealed no significant spatial structure within the 4 populations. It indicated that genetic variations of the most polymorphic loci within these populations were randomly distributed . However, there were gaps at the distance of 3 ~ 4m,5 ~ 6m and 8 ~ 10 m , and there were intrusion at a little polymorphic loci.

Base on these results, we regarded that the conservation approach of *L. regale* were to decrease disturbances, to preserve unique genetic variation , pay attention to individuals grown at the distance of 3-4m, 5-6 and 8-10m , to create lord to increase gene flow.

GENOME COMPOSITION OF INTERSPECIFIC HYBRIDS AMONG THREE GENOMES IN *LILIUM*: A MULTICOLOR GENOMIC *IN SITU* HYBRIDIZATION ANALYSIS

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In *Lilium*, the Longiflorum (L) hybrids have trumpet-shaped white flowers with distinctive fragrance and year round forcing ability, the Oriental hybrids (O) have mostly large pink and white flowers with a sweet fragrance and are resistant to *Botrytis elliptica*, the Trumpet hybrids (T) have trumpet-shape, pink or yellow flowers with a strong growth and are resistant to *Fusarium*. With the aim of combining the valuable traits of the three different genomes, crosses were made between a triploid LLO-hybrid and a tetraploid LT-hybrid. The tetraploid LT-hybrid originated from somatic doubling of a diploid F1-hybrid. From this cross, 71 plants were resulted from embryo rescue. 29 of them were tested for DNA-content using flowcytometry. Mc GISH (Multicolor Genomic *In Situ* Hybridization) was used to analyze these progenies. The results showed that:

1) the ploidy level of these progenies, based on DNA-content varied from almost triploid to tetraploid with a ploidy level from 3.1 to 3.8, and the chromosome number varied from 40 to 46.

2) the aneuploid progenies possessed chromosomes from all three genomes, indicating the hybrid character of the hybrids.

3) the hybrids owned 24 chromosomes of the Longiflorum genome, 12 chromosomes of the Trumpet genome and 4-8 originated from Oriental genome.

4) several genotypes showed the presence of 1 or 2 so-called B chromosomes.

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THE USAGE OF SPIRULINA ON ACCLIMATIZATION OF MICROPROPAGATED BEGONIA (*B. SEMPERFLORENS*) PLANTLETS

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Spirulina (*Arthrospira platensis*) is blue-green micro-algae. The usage of algae on acclimatization allows plants to tolerate greater levels of cold, reduce the amount of damage and take nutrient elements.

Micropropagated begonia plants acclimatized with Spirulina showed a greater leaf length and leaf width than the control plants without Spirulina. The leaf lengths in control plants and the plants with spiriluna were 2.63 and 2.97 cm, respectively. In addition, the leaf widths in the control plants and the plants with spiriluna were 1.93 and 2.1 cm, respectively.

Macro nutrient elements tested in control plants and the plants with spirulina showed similar results. However, Fe (108 mg kg^{-1}) and Zn (132 mg kg^{-1}) concentrations in control plants were lower than Fe (189 mg kg^{-1}) and Zn (186 mg kg^{-1}) concentrations in the plants acclimatized with Spirulina.

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