25th International EUCARPIA Symposium Section Ornamentals

CROSSING BORDERS

PROGRAM BOOK OF ABSTRACTS

June 28th- July 2nd 2015

Institute for Agricultural and Fisheries Research Melle, Belgium

Welcome

Dear participant,

EUCARPIA aims to promote scientific and technical co-operation in the field of plant breeding in order to foster its further development. To achieve this purpose, the Association organizes on a regular basis meetings to discuss general or specific problems from all fields of plant breeding and genetic research. The section Ornamentals was founded in 1971 and a first meeting took place in Wageningen, The Netherlands. This year the twenty-fifth symposium is hosted in Melle, Belgium. Ornamental breeding is involved with a great number of species and a continuous demand for novelties. The importance of ornamentals cannot be underestimated as they contribute to the daily joy of life. They decorate our homes, landscapes and gardens, ameliorate climate, abate the harmful aspects of pollutions and much more.

"Crossing borders", the central theme of this symposium, expresses our intention to go beyond traditional ornamental plant breeding. Recent boosts in fundamental knowledge offers opportunities for ornamentals. Interaction and discussion between plant breeders and scientists create new ideas. We are excited that besides the lectures of leading experts also 130 scientific contributions from all over the world are presented. Parallel with the scientific sessions we scheduled two workshops. In these workshops active participation of breeding companies will be stimulated. A post-symposium tour gives you the opportunity to discover the dynamic and innovative ornamental plant breeding industry in Belgium.

It is my personal wish that the symposium can be the start of new longstanding collaborations and friendships.

Enjoy the meeting!

Johan Van Huylenbroeck Convener

Organizing and scientific committee

Organizing committee

-Johan Van Huylenbroeck (Institute for Agricultural and Fisheries Research, Belgium), convener
-Emmy Dhooghe (Institute for Agricultural and Fisheries Research, Belgium), secretary
-Jan De Riek (Institute for Agricultural and Fisheries Research, Belgium)
-Maurice De Proft (KU Leuven, Belgium)
-René Denis (Denis-Plants BVBA, Belgium)
-Danny Geelen (Ghent University, Belgium)
-Bruno Gobin (PCS Ornamental Plant Research, Belgium)
-Jan Oprins (Floraliën, Belgium)
-Isrik Van Bockstaele (Institute for Agricultural and Fisheries Research, Belgium)
-Marie-Christine Van Labeke (Ghent University, Belgium)
-Stefaan Werbrouck (Ghent University, Belgium)

Scientific committee

-Johan Van Huylenbroeck (Institute for Agricultural and Fisheries Research, Belgium)

-Emmy Dhooghe (Institute for Agricultural and Fisheries Research, Belgium)

-Jan De Riek (Institute for Agricultural and Fisheries Research, Belgium)

-Maurice De Proft (KU Leuven, Belgium)

-Danny Geelen (Ghent University, Belgium)

-Bruno Gobin (PCS Ornamental Plant Research, Belgium)

-Scott Hodges (University of California, Santa Barbara, USA)

-Monica Höfte (Ghent University, Belgium)

-Jochen Kumlehn (Leibniz Institute of Plant Genetics and Crop Plant Research, Germany)

-Chunlin Long (Minzu University of China and Kunming Institute of Botany Chinese Academy of Sciences, China)

-Andrea Mansuino (CIOPORA, Italy)

-Renate Müller (Copenhagen University, Denmark)

-Teresa Orlikowska (Research Institute of Horticulture, Poland)

-Erik Van Bockstaele (Institute for Agricultural and Fisheries Research, Belgium)

-Marie-Christine Van Labeke (Ghent University, Belgium)

-Jaap van Tuyl (Wageningen University and Research Center, the Netherlands)

-Stefaan Werbrouck (Ghent University, Belgium)

-Traud Winkelmann (University Hannover, Germany)

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General information

Congress Venue and Bus Shuttle

Institute for Agricultural and Fisheries Research (ILVO) Plant Sciences Unit Caritasstraat 21 9090 Melle, Belgium





A bus shuttle service will run between downtown Ghent (bus stop at the François Laurentplein) to the symposium and vice versa.

The bus stop will be at the François Laurentplein (at the map) in the city center of Ghent. The bus will leave at following hours:

Monday 29 June 2015:	departure from Ghent: 8h00 departure from Melle: 19h00
Tuesday 30 June 2015:	departure from Ghent: 8h15
	departure from Melle: 17h00
Tuesday 30 June 2015:	departure from Ghent: 18h45 Symposium Dinner
	departure from Symposium dinner: 23h00
Wednesday 01 July 2015:	departure from Ghent: 8h15
	departure from Melle: 17h00

Thursday 02 July 2015:

departure from Ghent: 8h00 Arrival at Ghent: 16h00







Catering

Coffee breaks and lunches are included in the registration fee.

Internet access

Internet is available at the symposium venue via a guest account.

Wifi/Wlan ssid: Eucarpia User: Eucarpia Password: 3uc@rp1a

Program

The official language of the symposium is English and no translation services are available. English must be used in all oral and poster presentations.

If you are presenting at one of the sessions, please hand over your slide show to our IT manager at the registration desk.

Speakers are requested to respect the allocated time given and should avoid any unnecessary delay of the program.

Oral presentations, except for invited speakers, are strictly limited to 15 minutes, followed by 5 minutes of discussion.

Short poster presentations should cover 5 minutes.

Only PowerPoint presentations (Windows or Mac) are accepted. The uploading of DVD movies is NOT supported by our system. No slide or overhead projectors will be available in the conference rooms. To allow smooth and quick transition from one speaker to the next, presentations will be uploaded on to a single computer (no personal laptops). Presentations will be removed after the sessions.

The use of CD-Rom's, USB pens or memory sticks is advisable to download presentations easily. The PowerPoint files with the presentations must be delivered at least two hours before the scheduled time for presentation. No presentation files will be accepted in the conference room.

Poster exhibition, set-up and removal

Posters are displayed in a separate room at the congress venue. Adhesive material to attach the posters will be available. Your poster has a number that can be found in the book of abstracts. Mount your poster only at the poster board that carries your poster number.

Posters should be mounted on Monday June 29th, 2015 from 8h00 onwards and should be removed on Wednesday July 1st, 2015 starting at 13h30 and before 17h00.

Keep your belongings safely with you

The venue does not have locker facilities. Coats etc. can be hung at the corridor near the conference room at own risk.

Liability and insurance

The organizers are not liable for any injury or damage involving persons and property during the symposium. Participants are advised to take out their own personal travel and health insurance for their trip.

Mobile phones

Delegates are asked to switch off their mobile phones when attending sessions.

Banking, currency and exchange

The official currency in Belgium is the Euro. Banking hours are Monday to Friday 9h00-16h00. Weekends closed. Automated cash dispensers are located outside every bank and other areas; cash can be withdrawn 24 hours a day using a credit card of European debit card (with chip). All major credit cards are accepted in most hotels, restaurants and shops. Foreign currency may be changed at banks, hotels, airports and in exchange offices.

Weather

Belgium weather is maritime and usually mild. Daytime temperatures in July range between 18°C and 23°C. Rainfall is possible.

Smoking

Smoking is not permitted in any of the symposium buildings.

Important and useful phone numbers

Emergency: 112 Taxi: 09/2222222

Other information

An information desk will be open at all times during the symposium activities. The people from the organizing committee all wear a green polo-shirt to be recognizable.

Program at a glance

Eucarpia Ornamentals 2015 – Program			
Sunday June 28 th , 2015	Welcome Reception with Registration		
	0.04	Regi	stration + Welcome
		Sessior	n Molecular Breeding I
Monday June 29 th , 2015			Session Flower
	РМ	Plenary Session	
		Belgia	In Evening Reception
Tuesday June 30 th , 2015			Registration
	AM	Session Plant Morphology	
		Session Stress	Workshop 'Future Perspectives for Ornamental Breeding'
		Session Biodiversity	Workshop 'Intellectual Property for Plants – Hot Topics'
	РМ	Visit Institute for A	gricultural and Fisheries Research
		Sy	mposium Dinner
Wednesday July 1 st , 2015			Registration
	AM	Session	Molecular Breeding II
		Session Breeding	
	РМ	Sessio	n Disease Resistance
		Closu	re and closure drink
Thursday July 2 nd , 2015		Post-Symposium Tour	

Program

SUNDAY 28 June 2015

16h00 – 18h00 WELCOME RECEPTION AND REGISTRATION (Location: Groot Vleeshuis, Groentenmarkt 7, Gent)

MONDAY 29 June 2015 8h00-9h00 Registration 9h00-9h15 Welcome SESSION MOLECULAR BREEDING I 9h15-12h10 **Chair: Renate Müller and Marie-Christine Van Labeke** Renate Müller (Denmark) 9h15-9h45 01. Natural transformation in plant breeding – A biotechnological platform for quality improvement of ornamental, agricultural and medicinal plants Joanna Gargul (Germany) 9h45-10h05 Molecular breeding of Petunia hybrida 'Famous Lilac Dark Vein' for 02. compact growth induction by MKS1 and GA_{2ox} constitutive overexpression Mohammed Bendahmane (France) 10h05-10h25 03. **Rose Genomics: Challenges and Perspectives** Ilya Kirov (Russia) 10h25-10h45 04. Development of cytogenetic chromosome markers in Rosa 10h45-11h15 BREAK Yuanjun Ye (China) 05. Development of Molecular Markers Related to Dwarf Traits of Crape 11h15-11h35 Myrtle Using SLAF-seq Method Victor Busov (USA) 11h35-11h55 06. Awakening the sleeping beauty in a poplar tree via activation tagging Barbara Ruffoni (Italy) 11h55-12h00 P1. Agrobacterium tumefaciens mediated transformation of Salvia spp Heike Molenaar (Germany) 12h00-12h05 P2. Evaluating production-related traits in *Pelargonium zonale* as a solid foundation for introducing marker-assisted selection Fadi Chen (China) Rapid genetic and epigenetic alterations under intergeneric genomic shock P3. 12h05-12h10 in newly synthesized Chrysanthemum morifolium × Leucanthemum paludosum hybrids (Asteraceae) 12h10-12h30 **ISHS and EUCARPIA** Margrethe Serek (Germany) 12h10-12h20 International Society Horticultural Science Richard Visser (The Netherlands)

12h20-12h30

Eucarpia

12h30-13h30		LUNCH
13h30-15h15		SESSION FLOWER Chair: Scott Hodges and Stefaan Werbrouck
13h30-14h00	07.	Scott Hodges (USA) The evolution and ecology of floral morphology in <i>Aquilegia</i> and the influence of horticulture on its emergence as a model system
14h00-14h20	08.	Olga Schulga (Russia) Chrysanthemum modification via ectopic expression of sunflower MADS- box gene <i>HAM59</i>
14h20-14h40	09.	Anne Behrend (Germany) <i>Calluna vulgaris</i> bud bloomers: Where have all the stamens gone?
14h40-15h00	010.	Rosanna Freyre (USA) Breeding and genetic studies of <i>Ruellia</i> at the University of Florida
15h00-15h05	P10.	Nobuo Kobayashi (Japan) AGAMOUS mutations of double flower cultivars in Japanese azaleas
15h05-15h10	P11.	Jiafu Jiang (China) <i>CmFTL2</i> is involved in photoperiod and sucrose control of flowering time in chrysanthemum Kente Heedenue (Japan)
15h10-15h15	P12.	Comparative expression analysis of five floral homeotic genes including newly isolated genes homologous to <i>PISTILLATA</i> and <i>SEPALLATA3</i> among four flowering cherries, <i>Prunus lannesiana</i> var. <i>speciosa</i> and three <i>P.</i> <i>lannesiana</i> cultivars
15h15-15h30		EUCARPIA BUSINESS MEETING
15h15-16h15		BREAK + POSTER SESSION
16h15-17h30		PLENARY SESSION Chair: Jaap van Tuyl and Johan Van Huylenbroeck
16h15-16h45	011.	Jaap van Tuyl (The Netherlands) Learning from breakthroughs in domesticating lily species: past present and future of ornamental breeding
16h45-17h15	012.	Anke van den Hurk (The Netherlands) Implementation of the Nagoya Protocol in the EU and the ornamental breeding sector
17h15-17h30	013.	Robert Wacker (The Netherlands) Use of new technologies in the graphical industry to protect intellectual property and to detect product piracy
17h30-19h00		BELGIAN EVENING RECEPTION

TUESDAY 30 June 2015

8h00-9h00		Registration
9h00-10h30		SESSION PLANT MORPHOLOGY Chair: Ottoline Leyser and Maurice De Proft
9h00-9h30	014.	Ottoline Leyser (United Kingdom) Hormonal control of shoot branching
9h30-9h50	015.	Camille Li-Marchetti (France) Effects of Genotype x Environment interaction on plant architecture in rose
9h50-10h10	016.	Marcus Linde (Germany) Allelic variants of strigolactone pathway genes shape the plant architecture: a study on the inheritance of horticultural traits in chrysanthemum
10h10-10h30	017.	Robrecht Dierck (Belgium) Hormonal and genetic regulation of axillary bud outgrowth in <i>Chrysanthemum morifolium</i> during floral initiation
10h30-11h00		BREAK
11h00-12h00		PARALLEL SESSION STRESS Chair: Teresa Orlikowska and Maurice De Proft
11h00-11h20	018.	Dália Carvalho (Portugal) QTL analysis for stomatal functionality in tetraploid <i>Rosa</i> × <i>hybrida</i> grown at high relative air humidity
11h20-11h40	019.	Geert van Geest (The Netherlands) Can phenotyping for water balance improve breeding for vase life?
11h40-11h45	P25.	Chrysanthemum WRKY gene <i>CmWRKY17</i> negatively regulates salt stress tolerance in transgenic chrysanthemum and <i>Arabidopsis</i> plants
11h45-11h50	P26.	Ji Hyun Kim (South Korea) Overexpression of AtNDPK2 enhanced abiotic stress tolerance in transgenic Chrysanthemum
11h50-11h55	P20.	Lin Zhu (China) The heritability and genetic correlation in diurnally and nocturnally flowering daylilies
11h55-12h00	P24.	Flávia Lais Gomes Fortunato (Brazil) Heritability and genetic parameters for size-related traits in ornamental pepper (<i>Capsicum annuum</i> L.)
		PARALLEL BUSINESS WORKSHOP
11h00-12h30		'Future Perspectives for Ornamental Breeding' Chair: Danny Geelen and Johan Van Huylenbroeck
		 Introducing biotech in your company (Rob Dirks, Rijk Zwaan) Get more out of your breeding data (Steven Maenhout, PROGENO) Plant breeding: what brings the future (Thijs Simons, Plantum)

12h00-13h30		LUNCH + POSTER SESSION
13h30-15h00		PARALLEL SESSION BIODIVERSITY Chair: Long Chunlin and Marie-Christine Van Labeke
13h30-14h00	020.	Chunlin Long (China) Biodiversity of Chinese ornamentals
14h00-14h20	021.	Yannick De Smet (Belgium) Biodiversity research and plant breeding, a mutually beneficial relationship
14h20-14h40	022.	Jon Suzuki (USA) Anthurium species identification in germplasm collections
14h40-14h45	P36.	Francine Cuquel (Brazil) Potential use of <i>Baccharis milleflora</i> DC. and <i>Baccharis tridentata</i> Vahl as
14h45-14h50	P37.	Phillip Wadl (USA) Evaluation of hybrids of the endangered <i>Pityopsis ruthii</i>
14h50-14h55	P38.	Katarzyna Kuligowska (Denmark) Wild <i>Kalanchoë</i> species for the development of new ornamental cultivars
14h55-15h00	P39.	Dariusz Kulus (Poland) Application of cryopreservation for chrysanthemum genetic resources conservation
		PARALLEL BUSINESS WORKSHOP
13h30-15h00		'Intellectual Property for Plants – Hot Topics' Chair: Andrea Mansuino, Edgar Krieger and Johan Van Huylenbroeck
		 General introduction on today's IP-situation Minimum Distance – how similar should varieties be? Essentially Derived Varieties – are all mutants and GMO EDV? Patents for plant innovations – evil or blessing?
15h00-15h30		BREAK
15h30-17h00		Visit Institute for Agricultural and Fisheries Research (ILVO)
19h00-23h00		SYMPOSIUM DINNER

WEDNESDAY 1 July 2015

8h00-9h00		Registration
9h00-10h30		SESSION MOLECULAR BREEDING II Chair: Jochen Kumlehn and Danny Geelen
9h00-9h30	023.	Jochen Kumlehn (Germany) Synthetic endonucleases: Novel tools for site-directed genetic modification of plants
9h30-9h50	024.	Nico De Storme (Belgium) Male apomixis: an alternative strategy to synthetically engineer clonal seed formation in plants
9h50-10h10	025.	Cytological investigations in Midday flowers (Aizoaceae) reveal high DNA contents in different somatic tissues and occurrence of unreduced male gametes
10h10-10h30	O26.	Yike Gao (China) Haploid culture of Chrysanthemum
10h30-11h00		BREAK
11h00-12h15		SESSION BREEDING Chair: Traud Winkelmann and Erik Van Bockstaele
11h00-11h20	027.	Traud Winkelmann (Germany) Interspecific hybridisation in the genus <i>Helleborus</i>
11h20-11h40	028.	Methodological basis and advances for ornamental breeding pepper program in Brazil
11h40-11h45	P54.	Yanchao Guo (China) New members of the Iridaceae family: Interspecific hybridization between Iris dichotoma and I. domestica
11h45-11h50	P55.	Interspecific crosses in <i>Passiflora</i> to obtain new hybrids with ornamental value
11h50-11h55	P56.	Agathe Le Gloanic (France) Interspecific hybridization in the tribe Genisteae with immature fruit rescue in vitro culture
11h55-12h00	P57.	Morphological aspects in <i>Heliconia chartacea</i> inflorescences for use as cut flower
12h00-12h05	P58.	Simas Gliozeris (Lithuania) New ornamental <i>Lophospermum</i> hybrids through interspecific hybridization
12h05-12h10	P59.	Selection of the best Black Mondo (<i>Ophiopogon planiscapus</i> 'Nigrescens') clone in tissue culture conditions for micropropagation
12h10-12h15	P60.	José Mejía-Muñoz (Mexico) Interspecific hybridization between <i>Dahlia dissecta</i> and <i>D. rupicola</i>

12h15-13h30		LUNCH
13h30-15h40		SESSION DISEASE RESISTANCE Chair: Monica Höfte and Bruno Gobin
13h30-14h00	O29.	Monica Höfte (Belgium) Basal and inducible disease resistance mechanisms in ornamentals
14h00-14h20	O30.	Gil Luypaert (Belgium) Opportunities to breed for broad mite resistance in <i>Rhododendron simsii</i> hybrids
14h20-14h40	031.	Harue Shinoyama (Japan) Transgenic chrysanthemum (<i>Chrysanthemum morifolium</i> Ramat.) carrying both insect and disease resistance
14h40-15h00	032.	Rusli Ibrahim (Malaysia) Induction of insect resistance in <i>Dendrobium mirbellianum</i> using ion beam irradiation
15h00-15h20	033.	Yiqian Fy (The Netherlands) Can a candidate gene approach bridge the gap between QTL analysis and marker-assisted selection for Botrytis resistance in Gerbera?
15h20-15h40	034.	Juliane Geike (Germany) Targeted Mutagenesis of MLO-Homologous Genes in the Rose Genome
15h40-17h00		CLOSURE AND CLOSURE DRINK

THURSDAY 2 July 2015

8h00 – 16h00POST-SYMPOSIUM TOUR
(Departure and Arrival: Ghent, François Laurentplein)

Events

Welcome Reception, Sunday June 28, 2015

The welcome reception, supported by the Provincie Oost-Vlaanderen, will take place on **Sunday 28th June 2015** from **16h00 till 18h00** in the 'Groot Vleeshuis' (Great Butcher's Hall) in the historical city centre of Ghent. Registration for the congress can be done during the reception.

The Groot Vleeshuis was designed by Gillis De Sutter and built between 1407 and 1419. Meat houses were covered markets where sales were centralised in order to check the meat's freshness and quality. Private sales at home were forbidden. Sixteen tripe stores were built onto the south-eastern side of the Groot Vleeshuis between 1542 and 1543. For reasons of hygiene, these separate stores had to be used for the sale of entrails, skin, and other remains from slaughtered animals.

The Groot Vleeshuis has a splendid and remarkable truss. A chapel with three large pointed arch windows was constructed in the building between 1446 and 1448. The chapel contains a 15th century wall painting which depicts a Worship Service.

From the end of the 19th century, it was also possible to sell meat at home. The Groot Vleeshuis was then used for various other purposes; it became a post office, storage depot, exhibition area, and a banquet hall in that order. It is now used as a covered market for regional tourism and products.

Address: Het Groot Vleeshuis Groentenmarkt 7 9000 Gent



Groot Vleeshuis - © Visit Gent

Symposium Dinner, Tuesday June 30, 2015

The Symposium Dinner will take place in Ghent in the 'Zebrastraat' building. It will start at **19h00**. A bus shuttle will be arranged from the Ghent city centre. There is also a parking possibility at the 'Zebrastraat' (New Zebra).

Departure of bus shuttle from Ghent city centre (François Laurentplein): 18h45.

The bus shuttle will leave the symposium dinner at 23h00 and bring the participants back to the city centre of Ghent (François Laurentplein).

Zebrastraat is a unique project: it bridges the gap between housing, culture and economy. The building was designed in 1906 as the first social apartments in Europe, but by the end of the 1990s the site was hopelessly run-down. The demolition hammer already hung over the Zebrastraat when the Foundation recognised it to be a pearl covered in dust. The result of the renovation is a modern site with an exhibition wing and a conference centre at the street side, with a multi-purpose lounge and 6 well-equipped seminar rooms. Behind it is an oval-shaped plaza sporting with a glossy water surface; around the plaza are 66 apartments, 8 guest rooms and several offices.

Since opening in 2005, the idea of a mixed function site (housing, economy and culture) has proven to be successful.

Address: Zebrastraat Zebrastraat 32 9000 Gent



Post-Symposium Tour, Thursday July 2, 2015

Busses leave at the bus stop (François Laurentplein) at 8h00 and will be back at 16h00.

Tour 1 Bloemisterij Leybaert:



Bvba Bloemisterij Leybaert specialises in the cultivation of *Azalea indica* and Rhododendrons, with some cultivation of *Chamaecyparis ellwoodii*. This large-scale (15 hectares) grower is highly automated. Recently an own breeding program in *Rhododendron* was started.

Deroose Plants:

Deroose Plants markets mostly bromeliads and nepenthes, all products of Deroose Plants' own breeding program. This tour features the greenhouse complexes were the breeding is done; although Deroose has its own in vitro labs, those will not be featured on the tour.



Gediflora:



Gediflora is a family company specialised in the breeding and propagation of pot chrysanthemums. The Gediflora range of products is marketed under the trade name Belgian Mums. This company has 3 hectares of greenhouses and 14 hectares of experimental and production fields. Tour 2 Denis-Plants:



Denis-Plants is a family-run company specialised in weaning young plants from tissue culture. The company has its own tissue culture laboratory. They breed in the Maranthaceae family.

Rudy Raes bloemzaden:

Although Rudy Raes started by producing and breeding *Begonia* and *Gloxinia* seeds, this company now has its own breeding programme for *Primula*. They specialise in the sale of seeds, power plugs of seeds and cuttings of annual and perennial bedding plants.



PCS Ornamental Plant Research:



PCS Ornamental Plant Research has the expertise and facilities to carry out applied scientific research and more practice-oriented research. At both levels the research is done thematically. PCS Ornamental Plant Research is situated in Destelbergen, right in the heart of the growing region for ornamental plants, which strengthens its function as a research and information centre for ornamental horticulture. Important research themes are the optimization of culture methods, growth and flowering regulation, use of energy, quality and post-harvest properties of ornamental plants and research for practical utility. In the present research, environmental protection has become a very important theme.

Exotic Plant:

The family company Exotic Plant has been in business for four generations. The current focus of Exotic Plant is to refine and produce new types of Bromeliads. In the past, this company has produced and multiplied young plants of Bromeliads, *Calathea*, *Musa*, Orchids, Anthuriums, *Helleborus* and many others.



Invited speakers

Mohammed Bendahmane (Ecole Normale Supérieure, Lyon, France)



My group's research mainly focuses on the investigation of the molecular and genetic mechanisms of petal development and function using the rose and *Arabidopsis* as models. In particular, we are interested in deciphering how cell proliferation and cell expansion are regulated during petal morphogenesis. In *Rosa* sp. we are interested in understanding flower development, petal number control and scent biosynthesis. We have also developed genomic, transcriptomic and biotechnology resources, to highlight the importance of the rose as a model ornamental species.

Scott Hodges (University of California Santa Barbara, USA)



Our laboratory primarily studies the genetic basis of floral characters in columbines (*Aquilegia*). We are particularly interested in characters such as flower colour, flower form, orientation and spur length. Differences in these characters between taxa promote visitation by different pollinators, such as bumblebees, hummingbirds and hawkmoths, resulting in reproductive isolation and speciation. We have led an international team to develop genomic resources for the genus including a high-quality, whole genome sequence.

Monica Höfte (Ghent University, Belgium)



Our fundamental research focuses on unravelling the mechanisms behind natural and inducible disease resistance against plant pathogens in some major crops. We use a molecular approach combined with a physiological study of the interaction between the bacterial/fungal attack and their host plant reaction. Special attention goes to the role of plant hormones in the plant pathogenic interaction.

Jochen Kumlehn (Leibniz Institute of Plant Genetics and Crop Plant Research, Germany)



The focus of our research is the plant reproductive biology (gamete development, fertilisation, early embryogenesis, apomixis) and alternative plant technologies as single cell dissection, genetic engineering and haploid technology. A more applied interest is genetic engineering towards crop improvement and molecular farming.

Ottoline Leyser (University of Cambridge, UK)



Our lab is interested in understanding the role of plant hormones in plant developmental plasticity, using the regulation of shoot branching by N availability in *Arabidopsis* as a model. Current areas of focus are on understanding the role of the auxin transport network in shoot branching plasticity and how other hormonal signals, such as cytokinin and strigolactone, are integrated into the system through their effects on auxin transport. This work involves cell biological and molecular genetic approaches to understand the mechanisms of action and interaction of these hormones, which are linked to whole plant phenotypes, physiology, and natural variation in branching response through computational modelling.

Chunlin Long (Minzu University of China and Kunming Institute of Botany Chinese Academy of Sciences, China)



My major interests cover botany and associated subjects, including biodiversity, plant genetic resources, economic botany, ethnobotany, ethnopharmacology and medicinal chemistry, plant phylogeny and taxonomy. I am interested in aroids (Araceae) and other groups, covering their biotechnology-based innovation and development. In the past decade I have made contributions to the studies on ornamental plants. The important groups include Magnoliaceae, Ericaceae, Aceraceae, Araceae, Orchidaceae, Amaryllidaceae, Primulaceae, and Theaceae.

Renate Müller (Copenhagen University, Denmark)



My research group covers important topics within flower physiology and reproduction biology of ornamental plants. Here, wide hybridisation techniques and tissue culture are essential approaches to improve the breeders' toolbox. For 15 years, my research has explored genetic and physiological factors that determine plant quality. I try to combine a strong horticultural background from industry with a scientific focus on ornamental plants.

Jaap van Tuyl (Wageningen University and Research Center, the Netherlands)



My areas of expertise include interspecific hybridisation, polyploidisation, in vitro pollination and embryo rescue techniques, chromosome painting, development of molecular marker techniques in lily and tulip, resistance breeding, flower longevity and genetic resources of bulbous plants. I have experience in the commercial ornamental industry as a flower breeder and researcher, and I am active as international consultant for flower breeding.

Robert Wacker (Floramedia Group, the Netherlands)



One of my interests is intellectual property and how to protect it in ways besides Plant Breeder Rights. Many new technologies are available to protect the copyright of brand owners across all industries. During this symposium, I will present customised solutions for the copyright protection of plants which have already been proven in other industries.

Traud Winkelmann (Leibniz Universitaet Hannover, Germany)



Our group's research mainly focuses on gaining a better understanding of plant propagation on the physiological and molecular level. This implies especially the use of in vitro culture techniques for propagation and breeding of horticultural crops. Protocols for regeneration, transformation, interspecific hybridization and haploid techniques are established and improved to be applied in fundamental research as well as in plant breeding.

ORALS



SESSION MOLECULAR BREEDING I





Session Molecular Breeding I

01.

Natural Transformation in Plant Breeding – A Biotechnological Platform for Quality Improvement of Ornamental, Agricultural and Medicinal Plants

Henrik Lütken, Josefine Nymark Hegelund, Martin Himmelboe, Uffe Bjerre Lauridsen, Renate Müller

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Abstract

Compactness is a desirable trait in ornamental plant breeding because it is preferred by producers, distributors and consumers. Presently, in ornamental plant production growth of many potted plants is regulated by application of chemical growth retardants, several of which are harmful to both the environment and human health. With the aim to develop an alternative to chemical growth regulators, a biotechnological approach without the use of recombinant DNA technology has been developed. In this method, the soil borne bacterium Agrobacterium rhizogenes inserts T-DNA, containing four root oncogenic loci (rol)-genes rolA, rolB, rolC and rolD among 18 ORFs, into the plant genome. Infection of plants by A. rhizogenes induces hairy roots, from which shoots containing rolgenes can be regenerated. Natural transformation with A. rhizogenes reveals very promising results in several plant species and can be useful in a broader range of application than ornamental breeding. One important aspect of this technology is that the hairy roots can be used directly in the selection process as a primary indicator of a successful transformation. Thus the technology avoids use of undesired antibiotic resistance marker genes. Noteworthy, in Denmark the authorities have confirmed that plant products developed using natural transformation by unmodified strains of A. rhizogenes are not considered as genetically modified (GM) plants according to the European legislation. Generally, rol-genes cause increased rooting, decreased plant height, short internodes, reduced apical dominance and changes in flower characteristics. Several of these traits improve ornamental plant quality and may also benefit characteristics useful in agricultural field crops. In addition, a number of regenerated plants derived from hairy roots contain higher contents of secondary metabolites compared to wild type plants. Hence, this method also has potential as a tool for boosting high value compounds in medicinal plants.

Molecular Breeding of *Petunia hybrida* 'Famous Lilac Dark Vein' for Compact Growth Induction by *MKS1* and *GA*₂*ox* Constitutive Overexpression

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Abstract

One of the top targets of recent breeding programs in ornamental plants is focused on developing an alternative way to the chemical growth retardants application in production of plants with compact growth habit. We describe work on Petunia hybrida 'Famous Lilac Dark Vein' plants with overexpressed MAP kinase 4 nuclear substrate 1 (MKS1) and gibberellin 2-oxidase (GA₂ox) genes. Petunia plants were transformed with pCAMBIA modified vectors in separate experiments, containing constructs either with the *MKS1* or the GA_2ox gene under the control of *Ca35S* promoter. The transformation was performed with the use of Agrobacterium tumefaciens strain GV3101. The integration and expression of the transgene was investigated using Southern blot and RT-PCR analysis. The mean internode lengths of the MKS1-transgenic lines were 2.5-fold shorter than those of the wild-type plants. The flowering delay in *MKS1*-transgenic lines was observed with a range of 6 to 11 days in comparison to the wild-type plants. The mean internode lengths of the GA_2ox transgenic lines were 6.5-fold shorter than those of the wild-type plants. The flowering delay in GA₂ox-transgenic lines was observed with a range of 7 to 12 days in comparison to the wild-type plants. The flower morphology either in MKS1- or GA₂ox-transgenic lines did not differ from wild-type plants. Constitutive overexpression of both MKS1 and GA20X genes results in compact phenotype but also lead to delayed flowering.

Rose Genomics: Challenges and Perspectives

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Abstract

Cultivated roses have a very ancient history. Artificial crossing led to what is today perceived as modern rose cultivars. Impressively, these modern rose cultivars were established from less than 10 species, which have contributed to the origin of the about 35,000 existing rose cultivars. Roses exhibit an extraordinary diversity of traits, both of economic and scientific importance. Several recent studies have been marked as important milestones on the journey towards deeply understand the molecular and genetic mechanisms that govern these traits, yet we still lack information on the genome sequences of rose species and cultivars, especially those that heavily participated to rose domestication and breeding programs.

Development of Cytogenetic Chromosome Markers in Rosa

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Abstract

Rosa is an important ornamental genus with more than 150 species. It is widely accepted that only 7-11 species are involved in the creation of more than 20,000 Rosa cultivars distributed worldwide. Although a better knowledge of the chromosome organization can accelerate plant breeding process, genome sequencing and phylogenetic analysis, the Rosa genus is poorly investigated by molecular cytogenetic approaches. Using tyramide-FISH on mitotic metaphase chromosomes, we could physically map several single-copy genes in Rosa wichurana (Kirov et al. 2014). Application of pachytene chromosomes allowed to perform high resolution physical mapping of those genes and to integrate the genetic and physical maps of Rosa wichurana (2n=2x=14) for chromosomes 1, 4 and 7. However, further progress in high resolution physical mapping and integration of physical and genetic maps of Rosa is depending on cytogenetic chromosome markers. Conserved tandem repeats (TR) such as 5S, 45S and telomere repeats are useful sources for chromosome markers. Physical mapping of these repeats on Rosa wichurana however allowed only two pachytene bivalents to be discriminated. Therefore, aiming at isolation and characterisation of repetitive DNA sequences useful for the development of more cytogenetic chromosome markers, we applied low-depth Illumina sequencing of four Rosa species including two tetraploid (R. foetida, R. gallica) and two diploid (R. wichurana, R. rugosa) species. Between 220,764 and 516,799 high quality pair-end reads were obtained with an average size of 150bp and a genome coverage between 0.08x and 0.23x. The reads were analyzed in RepeatExplorer (Novak et al. 2013). Several tandem repeats and retrotransposons were observed in the four Rosa species and their genome organization will be studied by molecular approaches. Their application as cytogenetic marker will be evaluated using FISH.

Kirov I, Van Laere K, De Riek J, De Keyser E, Van Roy N, Khrustaleva L (2014) Anchoring linkage groups of the Rosa genetic map to physical chromosomes with tyramide-FISH and EST-SNP markers. PlosOne 9(4): e95793

Novák P, Neumann P, Pech J, Steinhaisl J, Macas J (2013) RepeatExplorer: a Galaxy-based web server for genome-wide characterization of eukaryotic repetitive elements from next-generation sequence reads. Bioinformatics 29: 792-793

Development of Molecular Markers Related to Dwarf Traits of Crape Myrtle Using SLAF-seq Method

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Abstract

Plant architecture is an important trait which modulates both economic and ornamental values of plants. Dwarf crape myrtle cultivars with shorter internodes, compact crowns and abundant miniflowers have promising applications in urban landscape and as potted flowers. To determine the genetic inheritance of plant height and screen the genes modulating dwarf trait of crape myrtle, specific-locus amplified fragment sequencing (SLAF-Seq) method was employed to analyze the F_1 population of *Lagerstroemia fauriei* (standard) × *L. indica* 'Pocomoke' (dwarf) with 300 seedlings of different height. A total of 1221 polymorphic SLAFs were acquired and 12 specific markers related to dwarf trait were procured based on SNP-index association analysis. All the markers were validated by sanger sequencing and proved to be specific and stable to identify different phenotype in the populations of F_1 and BC_1 . Eight markers were verified successfully by allele specific PCR (AS-PCR) method. The results laid foundation for molecular marker-assisted selection (MAS) in crape myrtle breeding and cloning of dwarf genes.

Awakening the Sleeping Beauty in a Poplar Tree via Activation Tagging

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Abstract

We will report on our advances using activation tagging in conjunction with genomic resources available for Populus to identify genes of value to tree breeding. We generated a poplar activation tagging populations comprised of 5,000 unique events and observed a consistently high mutant phenotype rate of >7% based on greenhouse and field evaluations of morphology, with limited phenotypic and genetic overlap. Mutants affecting many aspects of tree biology were discovered, including phenology, cold tolerance, branching angle/density, stature, leaf size/form/color and tolerance to nutrient deficiency. The high mutation rate corresponds with the preferential insertion of the tag in the proximity of genes, likely because of more open chromatin configurations in these regions. Approximately in 70% of the lines, the tag is within 10Kb of a gene and can be activated as far as 13Kb. High efficiency of tagging was validated by recapitulation through retransformation of activated genes. These studies have discovered new gene-to-trait associations, as well as linked known genes to novel regulatory mechanisms and processes that are unavailable to study in Arabidopsis. For example we have recently shown that the tagged EARY BUD BREAK1 gene that affects bud phenology is a homolog of the DORNRÖSCHEN (The Sleeping Beauty) gene from Arabidopsis that regulates shoot apical meristem (SAM). These discoveries enable transgenic or conventional breeding approaches for modifications of important ornamental characteristics in woody plants.

P1.

Agrobacterium tumefaciens Mediated Transformation of Salvia Species

Laura Bassolino, Giada Baudino and Barbara Ruffoni

Poster

P2.

Evaluating Production-related Traits in *Pelargonium zonale* as a Solid Foundation for Introducing Marker-Assisted Selection

Heike Molenaar, Martin Glawe and Hans-Peter Piepho

Poster

P3.

Rapid Genetic and Epigenetic Alterations under Intergeneric Genomic Shock in Newly Synthesized *Chrysanthemum morifolium* × *Leucanthemum paludosum* Hybrids (Asteraceae)

Haibin Wang, Jiafu Jiang, Sumei Chen, Xiangyu Qi, Weimin Fang, Zhiyong Guan, Nianjun Teng, Yuan Liao and <u>Fadi Chen</u>

Poster

SESSION FLOWER




Session Flower

07.

The Evolution and Ecology of Floral Morphology in *Aquilegia* and the Influence of Horticulture on its Emergence as a Model System

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Abstract

The columbine genus Aquilegia has been grown as a garden ornamental for centuries. Because of its unusual floral shape with the sepals being petaloid and colorful and the petals forming slender nectar spurs, many collections were made and species have been available to scientists as well as horticulturalists. Given its widespread natural distribution throughout the northern hemisphere, this availability was essential for early studies establishing that species in the genus were often highly intercompatible and their hybrids could be established. This led to a number of studies showing the relatively simple genetic inheritance of many of the dramatic differences among flowers of various species. More recent work has established the phylogenetic relationships among most of the species and showing that the genus had a burst of diversification after evolving the distinctive nectar spurs. Other studies have shown that this burst of diversification was likely due to adaptation to different pollinators, especially in North America, with changes in flower color, orientation and spur length to match the preferences and tongue lengths of new pollinators. Further studies have dissected the genetic basis of some of these traits. Aquilegia has also been noteworthy for its contributions to our understanding of the evolution of floral organ identity and horticultural varieties have, and will continue, to aid in these efforts. Aquilegia has now been the subject of substantial effort to develop it as a new model genomic system with the establishment of a high-quality reference sequence, derived from a highly inbred horticultural line as well as other resources such as a functional assay to assess specific gene functions. These resources poise Aquilegia to continue to be at the forefront of plant evolutionary and ecological research.

Chrysanthemum Modification via Ectopic Expression of Sunflower MADS-box Gene *HAM59*

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Abstract

Flower and plant shape are important characteristics in ornamental plants. A possible strategy to modify flower shape is genetic manipulation of regulators of floral organ identity. Plant MADS-box transcription factors play key roles in this process. C-function MADS-box genes specify stamen and carpel development, the repression of A-class genes, and the floral meristem determinacy. This process is generally conserved. Previously, we have identified *AGAMOUS*-like cDNAs from *Chrysanthemum* (CDM37) and *Helianthus* (HAM45 and HAM59). In this work, *Chrysanthemum* flower shape was analysed after ectopic expression of HAM59. Twelve transgenic lines were isolated showing reduced male fertility. Two transgenic lines had modified floret structures. In disk florets, stamens were transformed into petal-like structures, resembling the double flower phenotype in *Arabidopsis agamous* mutants. In addition, ray floret petals were shorter than control, and ligule tips were divided. However, there were no homeotic changes of floral organs in ray florets. In conclusion we show that ectopic expression of sunflower *HAM59* gene modifies androecium to corolla-like tissues and leads to male-sterility.

Calluna vulgaris Bud Bloomers: Where Have all the Stamens Gone?

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Abstract

The bud bloomers boosted the market share of Calluna vulgaris in the sector of bedding plants due to their prolonged flower longevity. The bud bloomers are natural mutants of C. vulgaris that lack stamens and exhibit a second floral whorl of showy sepals instead of petals. In addition to the altered flower architecture, flower development of the bud bloomers arrests before anthesis. The closed flower buds make them especially attractive for customers. In a broad transcriptome study, bud bloomers turned out to rarely produce the transcript of the homeotic MADS-box gene PI. PI belongs in the class of B-genes and is important for the identity of stamen and petals. A B-gene mutant of C. vulgaris is supposed to look like the cultivar 'David Eason' with two whorls of sepals and additional carpels instead of stamens. This form of bud bloomer is called the polystyla subtype and clearly differs from the commercial most important bud bloomers which are classed as diplocalyx. In flowers of the latter type, the gynoecium is followed directly by two whorls of sepals. Histological studies in early developmental stages of the diplocalyx type spotted the stamens' primordia, but could not detect a development of male sexual organs in the flowers. However, in model plants the lack of Bgene PI is responsible for the homeotic transition of petals to sepals and form stamens to carpels, but it is not accountable for the loss of organs. Early works on B gene mutants in Arabidopsis came up with different phenotypes from flowers without visible stamen to stamenoid structures depending on the particular B gene mutant. Therefore, detailed morphological and histological studies in C. vulgaris where done to elucidate the fate of male sexual organs in C. vulgaris diplocalyx bud bloomers. The working hypothesis states the complete incorporation of stamens into the gynoecium.

Breeding and Genetic Studies of Ruellia at the University of Florida

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Abstract

Wild Ruellia simplex (Mexican petunia) is a popular landscape plant in the southern USA. This introduced plant is highly fertile, has escaped from cultivation and become invasive in natural areas. For several years 'Purple Showers' was the only existing sterile cultivar. Our breeding objective has been to develop sterile Ruellia cultivars with different flower colors and growth habits. Breeding approaches are ploidy manipulation and hybridization. Selected hybrids are evaluated in replicated trials in Florida. Female and male fertilities are determined by collection of fruits produced by open pollination, by manual hybridizations in the greenhouse, and by evaluation of pollen staining. From 2011 to 2013 the first three sterile hybrids named 'Mayan Purple', 'Mayan White' and 'Mayan Pink' were released, and are commercially available now. The 'Mayan' series was evaluated in several research and commercial trials in the USA demonstrating very good to excellent performance in terms of growth habit and flowering. In another research topic, genetics and anthocyanins responsible for flower color were studied in R. simplex. An F1 population was obtained from crossing a maternal individual with white corolla with purple throat (WP), and a paternal individual with pink corolla and dark pink throat (PK). All plants in the F_1 generation had purple flowers (P). The F_2 generation segregated 94P:30PK:24WP:5WPK (WPK is white corolla and pink throat). This data was separated into two groups: the corolla color fit a 9:3:4 recessive epistasis interaction (P = 0.22) whereas the throat color fit a 3:1 ratio (P = 0.54). HPLC analyses indicated that delphinidin derivatives conferred purple corolla color, while pelargonidin derivatives were responsible for the pink corolla color. Purple throat color resulted from delphinidin derivatives whereas the pink color was caused by peonidin derivatives.

P10.

AGAMOUS Mutations of Double Flower Cultivars in Japanese Azaleas

Nobuo Kobayashi, Kyeong-Seong Cheon, Sui Matsumoto, Akira Nakatsuka

Poster

P11.

CmFTL2 is Involved in Photoperiod and Sucrose Control of Flowering Time in Chrysanthemum

Jing Sun, Liping Ren, Jiaojiao Gao, Bin Dong, Yinjie Wang, Qike Fu, Sumei Chen, Fadi Chen and Jiafu Jiang

Poster

P12.

Comparative Expression Analysis of Five Floral Homeotic Genes Including Newly Isolated Genes Homologous to *PISTILLATA* and *SEPALLATA3* among Four Flowering Cherries, *Prunus lannesiana* var. *speciosa* and Three *P. lannesiana* Cultivars

Kento Hosokawa¹, Hironori Katayama² and Chiyomi Uematsu¹

Poster

PLENARY SESSION





Plenary Session

011.

Learning from Breakthroughs in Domesticating Lily Species: Past, Present and Future of Ornamental Breeding

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Abstract

In the history of ornamental plant breeding, polyploidization and interspecific hybridisation can be observed as one of the most important processes in domestication. In most of the ornamental crops the origin of the assortment traces back to diploid species, while through spontaneous interspecific crosses or applied polyploidization techniques the modern predominantly triploid or tetraploid cultivars were created. With lily as model crop, techniques for the development of interspecific polyploid crops are reviewed. A number of techniques are developed to overcome pre- and post-fertilisation barriers. Two ways of polyploidization can be distinguished, mitotic and meiotic. Mitotic polyploidization or artificial chromosome doubling can be induced by treatment with chemicals as colchicine, oryzalin or laughing gas. Artificial chromosome doubling induces autopolyploidization. There are 3 mechanisms of meiotic polyploidization through which 2n or unreduced gametes are formed e.g. FDR, SDR and IMR. When unreduced gametes are produced intergenomic recombination takes place during the meiotic divisions which can be proved by using molecular cytogenetic techniques as Genomic in situ hybridisation (GISH).

In many ornamentals these breeding technologies can be implemented. Looking to the future the development of molecular breeding technology will be the most challenging approach.

Implementation of the Nagoya Protocol in the EU and the Ornamental Breeding Sector

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Abstract

The Nagoya Protocol on Access and Benefit Sharing was finalized in 2010 and got into force on 12 October 2014. At the same time the European implementation of the Nagoya Protocol got into force. In the presentation the implications of the Nagoya protocol in general and the implementation in the EU in particular for the ornamental breeding sector will be provided. Furthermore, the issues that need to be further elaborated to fully understand the EU legislation in the plant breeding sector are dealt with. First of all in the EU legislation clarity should be obtained on the scope. Moreover, the breeding exemption of UPOV should not be effected by the definition of the scope. The plant breeding sector is of the opinion that the ABS legislation was developed for genetic resources that are found in the wild as well as landraces that have been developed through some natural and farmers selection. Therefore, varieties that have been/are or will be commercially available should be excluded from the scope. In addition, genetic resources that were already available in ex situ collections before 12 October 2014 should be left outside the scope. To further understand the scope it is important that the definition of utilization with the context of the Nagoya Protocol becomes clear. Some activities like crossing are within the definition while the breeders are of the opinion that the use of genetic resources as comparers or as tester for resistance, as well the screening of genetic resources should not fall into that definition. Genetic resources accessed through the multilateral system of the International Treaty on Plant Genetic Resources for Food and Agriculture are exempted from the EU legislation and due diligence obligations. For the breeding sector it is important to work with this system and even expand it to all breeding activities, including the ornamental breeding sector. Secondly clarity is required on the due diligence obligations within the EU legislation. This legislation determines that users of genetic resources have due diligence obligations on genetic resources they utilize according to the definition of utilization and falling within the scope of the legislation. This means that users have to be able to demonstrate for those genetic resources that they have the right to use the genetic resources and have an agreement to arrange benefit sharing if required by the provider. However, in many countries, it is not possible to get the necessary Prior Informed Consent (PIC) and Mutually Agreed Terms (MAT). The EU needs to indicate what this means for the implementation of the EU legislation. So the due diligence obligations and other requirements in the EU legislation may seem relatively simple, but they are not in practice. Therefore, further guidance is required. Important in this regard is to develop guidance that suits all type of companies and probably should be focused on the smallest ones. If they can follow the procedures, it will be also doable for medium-sized and large companies. The development of the guidance should be done with the sectors themselves. Lastly, guidance needs to be developed on checkpoints and monitoring. These items are also an important element of the EU legislation.

Use of New Technologies in the Graphical Industry to Protect Intellectual Property and to Detect Product Piracy

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Abstract

Plant breeders are constantly developing new varieties which are then protected by plant breeders rights. Despite active control illegal propagation is still going on. The emphasis of the article is to provide an overview about some of the the latest developments in protecting intellectual property. Hereby, we focus on the part in the supply chain where the products are ready for sale and shown in the market to consumers. Especially the graphical industry is able to offer solutions in the following areas: (I) detecting illegal use of images applied to protected varieties; (II) utilization of labels – possible visual technologies; (III) DNA analyses as a sophisticated method. Possible workflow procedures are introduced and practical samples are shown.

SESSION PLANT MORPHOLOGY



Session Plant Morphology

014.

Hormonal Control of Shoot Branching

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Abstract

Plants adapt their form according to the environmental conditions in which they are growing. This developmental plasticity requires the integration of multiple inputs into ongoing developmental programmes. We are using the regulation of shoot branching as a model system to investigate the mechanisms underlying developmental plasticity. Shoot branching is regulated by an environmentally sensitive network of systemically moving hormonal signals, providing a rich source of information that can be locally interpreted to determine branching behaviour. At the heart of this network is the auxin transport system. All active shoot apices export auxin into the polar auxin transport stream, which transports it rootward. Thus ultimately all shoot apices are in communication through their export of auxin into shared auxin transport paths to the root. Competition between apices for these transport paths can explain a range of phenomena in shoot branching, such as apical dominance. Furthermore, the modulation of the degree of competition, either locally or systemically, by environmental inputs can explain developmental plasticity in shoot branching. For example, strigolactones are transported from root to shoot and their production is up regulated when nutrient availability is low. Current evidence suggests that they act at least in part by reducing auxin transporter accumulation in the polar transport stream, increasing competition between branches for access to auxin transport routes in the main stem.

Effects of Genotype x Environment Interaction on Plant Architecture in Rose

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Abstract

The shape of an ornamental plant and, therefore, its architecture are critical for its visual quality and are dependent on genetic and environmental factors, such as water supply. The aim of the study was to analyze genotypic responses at the architectural level, in eight rose cultivars ('Blush Noisette', 'Baipome' Pink Gnome®, 'The Fairy', Hw336, 'Old Blush', Hw20, Hw154 and 'Perle d'Or'), to the alternation of water restriction (-20 kPa) and re-watering (-10 kPa) periods. Six architectural variables were measured at the plant and axis scales by 3D digitalization. Significant genotype effect was observed for all measured variables whereas significant environment effect was revealed only for the length and the insertion of long axes and the number of branching order. A strong Genotype x Environment interaction (GxE) was highlighted for most of the architectural measured variables. GxE interaction was mainly due to different response amplitudes between watering treatments according to genotype. Two groups of architectural responses of different intensities – weak and strong – were identified.

Allelic Variants of Strigolactone Pathway Genes Shape the Plant Architecture: a Study on the Inheritance of Horticultural Traits in Chrysanthemum

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Abstract

Shoot branching is crucial for the aesthetic value of a plant. Chrysanthemums (*Chrysanthemum indicum* hybrid) are important ornamental plants with abundant phenotypic diversity; however, there is only limited data about the inheritance of horticultural traits or corresponding marker-trait associations. We phenotyped and genotyped two types of chrysanthemum populations: a collection of 86 varieties and a biparental F1-population of 160 individuals. We identified 15 marker-trait associations with AFLP markers for the genotype collection using a genome-wide association study and 17 marker-trait associations for the population by applying a single locus analysis. Additionally, a candidate gene approach for strigolactone pathway genes identified marker alleles that were significantly associated with shoot branching in both populations. These genes described a large proportion of the variation in shoot branching in these populations. This study highlights the fundamental role of the strigolactone pathway and indicates that shoot branching in the chrysanthemum has a polygenic inheritance pattern, though other yet unknown factors are also likely involved. Although nearly all of the investigated traits were characterized by a continuous variation in phenotypic values, as was expected for the outcrossing hexasomic nature of the chrysanthemum, we identified informative marker-trait associations with important characteristics.

Hormonal and Genetic Regulation of Axillary Bud Outgrowth in *Chrysanthemum morifolium* during Floral Initiation

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Abstract

Shoot branching or the outgrowth of axillary buds is a crucial aspect in the production and breeding of *Chrysanthemum* sp. The outgrowth of axillary buds is inhibited by the shoot apex through apical dominance. Central to this regulation is the interaction of the plant hormones auxin, cytokinin and strigolactone. In this research we aim to study the hormonal and genetic regulation of apical dominance and axillary bud outgrowth in a cut flower cultivar of *Chrysanthemum*. For this we combined measurements of axillary bud outgrowth with UPLC-MS/MS quantification of auxin and cytokinin levels and RT-qPCR analysis of two genes involved in the regulation of branching. The cultivar used displayed apical dominance during vegetative growth. A release from apical dominance occurs during floral initiation with outgrowth and elongation of shoots under the apex. The outgrowth of axillary buds after release from apical dominance was preceded by decreased auxin levels in the shoot apex, axillary buds and stem and increased cytokinin levels in the axillary buds. RT-qPCR showed a decreased expression of the local bud outgrowth regulator *BRC1* preceding the release from apical dominance at the time of floral initiation.

SESSION STRESS



Session Stress

018.

QTL Analysis for Stomatal Functionality in Tetraploid *Rosa* × *hybrida* Grown at High Relative Air Humidity

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Abstract

In several plant species, high relative air humidity (i.e. $RH \ge 85\%$) throughout leaf ontogeny disturbs the capacity of the stomata to respond to closing stimuli. This leads to excessive water loss and consequently a negative water balance under conditions of high evaporative demand, resulting in short postharvest life mainly due to wilted and desiccated leaves. In roses, the magnitude of this problem is cultivar dependent, opening the possibility for breeding of cultivars with more responsive stomata. In this study, we aimed at (1) confirming that the relative water content after 4 h of leaflet desiccation (RWC_4h) is a reliable tool for predicting stomatal functionality, allowing to discriminate between tolerant and sensitive cultivars with regards to high RH; (2) identifying genomic regions associated with the control of water loss in plants grown at high RH. The F1 generation of a tetraploid cut rose population (108 genotypes) and the two parents grown at high RH (85%) were phenotyped to assess the RWC_4h. This phenotypic data was used to identify quantitative trait loci (QTL) associated with the control of water loss. The RWC_4h varied between 7% and 62% across the 110 genotypes. Stomata of the parent P540 were considerably more responsive to desiccation than stomata of the parent P867 (51% and 20% RWC, respectively). The F1 generation revealed transgressive segregation for this trait, however most of the genotypes (72%) showed a RWC_4h in between those of the parents. QTLs explaining approximately 30% of the variation in the RWC_4h were identified and positions of these QTLs on the parental linkage maps were determined. To the best of our knowledge, this is the first work identifying QTLs related to the control of water loss associated with post-harvest quality and longevity.

Can Phenotyping for Water Balance Improve Breeding for Vase Life?

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Abstract

Water stress is a main cause for early wilting of cut flowers during vase life. To prevent water stress, water uptake from the vase should compensate transpiration. Our goal is to identify parameters characterizing water balance that explain genotypic differences in vase life. This allows more precise phenotyping of vase life related traits. In two independent experiments 18 chrysanthemum (*Chrysanthemum x morifolium*) genotypes were characterized for three water balance parameters during their vase life: the maximum weight gain (MWG; % of weight increase after placement in water) and weight loss rate (WLR; % of weight loss per hour after MWG is reached), which together explain time to negative water balance ($t_{WB<0}$). As expected, MWG/WLR approximated $t_{WB<0}$. Genotypic variation in time to petal wilting was explained by $t_{WB<0}$. However, there was little correlation of genotypes in water balance parameters between experiments. In conclusion, fresh weight change explains vase life differences between genotypes, but there are major genotype by environment interactions

P25.

Chrysanthemum WRKY Gene *CmWRKY17* Negatively Regulates Salt Stress Tolerance in Transgenic Chrysanthemum and Arabidopsis Plants

Peiling Li^{1,2}, Aiping Song¹, Chunyan Gao¹, Linxiao Wang¹, Yinjie Wang¹, Jing Sun¹, Jiafu Jiang¹, <u>Fadi</u> <u>Chen^{1,2}</u> and Sumei Chen¹

Poster

P26.

Overexpression of AtNDPK2 Enhanced Abiotic Stress Tolerance in Transgenic Chrysanthemum

Ji Hyun Kim, Yeo Jin Youn and Nam-In Hyung

Poster

P20.

The Heritability and Genetic Correlation in Diurnally and Nocturnally Blooming Daylilies

Lin Zhu, Yike Gao, Yi Ren, Heyan Jia, Qixiang Zhang

Poster

P24.

Heritability and Genetic Parameters for Size-related Traits in Ornamental Pepper (*Capsicum annuum* L.)

<u>Flávia Laís Gomes Fortunato</u>, Elizanilda Ramalho do Rêgo; Mailson Monteiro do Rêgo, Cristine Agrine Pereira dos Santos and Michelle Gonçalves de Carvalho

Poster

SESSION BIODIVERSITY



Session Biodiversity

020.

Biodiversity of Chinese Ornamentals

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Abstract

China was dubbed as "the Mother of Gardens" by gardener and botanist Ernest Henry Wilson who collected and shipped about 2,000 species of plants from Asia to Europe and North America. Many ornamentals in western countries were originated from China. This paper presented the biodiversity of native Chinese ornamental plants at ecosystem, species and genetic levels, focusing on species and genetic resources with aesthetic potentials. The latest statistics reported 31,362 vascular species (taxa) occurring in China, of which about 6,000 species are with gardening values. The diversified ornamental plants become important components of different ecosystems from tropical rainforest to cryovegetation, from aquatic to arid, or from lowland to alpine vegetation, taking Yunnan as an example. As the dominant species, some ornamental plants established the plant communities in forests, grasslands, meadows or wetlands, which made the landscapes more colorful and beautiful. The breeding characteristics such as resistances and particular traits of some genetic resources were introduced in the paper. Famous representatives include orchids, Chrysanthemum, Camellia, Rhododendron, Rosa, Lilium and others. Lots of species are with multiple uses in addition to aesthetic purposes, like medicinal, edible, agricultural or daily life uses. The relationship between biodiversity and cultural diversity of ornamental plants was discussed. Biodiversity of Paeonia, bamboo, Osmanthus, lotus and many flowers endowed special meanings to traditional Chinese culture, which enriched cultural diversity. On the other hand, the traditional Chinese culture affected the diversity of ornamental plants. The authors recommended some unusual but potential ornamental plants, including Musella lasiocarpa (Musaceae), Paphiopedilum armeniacum (Orchidaceae); Camellia nitidissima (Theaceae), Tacca chantrieri (Dioscoreaceae), Leucocasia gigantea (Araceae), and Althaea rosea (Malvaceae). The strategies for conservation and development of Chinese ornamental plants were proposed in the present paper.

Biodiversity Research and Plant Breeding, a Mutually Beneficial Relationship

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Abstract

The genus *Hydrangea* became well known as a garden ornamental ever since its introduction into Europe from China and Japan in the 18th century. Since that time, numerous commercial cultivars became available, exhibiting a large range of colours and growth forms. However, the majority of these cultivars have been bred from the gene pool of the most well known *Hydrangea* representative: *H. macrophylla*. On a quest to introduce new desirable characteristics into this species, plant breeders have tried to cross this taxon with different *Hydrangea* species, or even species from related genera, such as *Dichroa*, with differing results. In this talk, we will present the progress made in by studying the biodiversity of *Hydrangea* and its allied genera, and how these results can be translated into new opportunities for plant breeders. Reversely, crossing experiments performed by breeders have the potential to provide a plethora of new insights into *Hydrangea* relationships and breeding systems.

Anthurium Species Identification in Germplasm Collections

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Abstract

Hybridization between Anthurium species has been a fruitful approach to incorporate and develop diverse, commercially important ornamental traits including various spathe colors and forms, plant habits and forms, scent and bacterial blight tolerance into modern Anthurium cultivars. Fertility may pose barriers to hybridization particularly between Anthurium species from particular subgenera. Thus, tools to augment proper species identification would greatly support thoughtful selection of parental material and insure integrity of accessions found in germplasm collections. Chloroplast gene sequences have previously been used extensively as species specific DNA barcodes in other plants and molecular phylogenetic analysis, contributing to recent revisions in phylogenetic relationships between species in Anthurium, the largest genus in the Araceae family. Current Anthurium cultivar improvement research includes development of molecular resources to characterize genetic differences between Anthurium species. This includes development and utilization of a DNA gene bank derived from a dried leaf collection of accessioned species, chloroplast molecular markers that potentially distinguish between incompatible, more distantly related species found in different subgeneric sectional groups as well as single nucleotide polymorphisms (SNPS) derived from comparison of whole Anthurium chloroplast genome sequences that may distinguish between closely related, interfertile species common in modern hybrids such as those of section Calomystrium.

P36.

Potential Use of *Baccharis milleflora* DC. and *Baccharis tridentata* Vahl as Ornamental Cut Foliage Plants

Grasiela Bruzamarello Tognon¹, <u>Francine Lorena Cuquel¹</u>

Poster

P37.

Evaluation of Hybrids of the Endangered Pityopsis ruthii

Phillip A. Wadl¹, Timothy A. Rinehart², and Robert N. Trigiano¹

Poster

P38.

Wild Kalanchoë Species for the Development of New Ornamental Cultivars

Katarzyna Kuligowska, Henrik Lütken and Renate Müller

Poster

P39.

Application of Cryopreservation for Chrysanthemum Genetic Resources Conservation

Dariusz Kulus¹, Monika Rewers², Agnieszka Abratowska³ and Anna Mikuła⁴

Poster

SESSION MOLECULAR BREEDING II





Session Molecular Breeding II

023.

Synthetic Endonucleases: Novel Tools for Site-directed Genetic Modification of Plants

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Abstract

Genome engineering is a breakthrough technology that facilitates the functional validation of genes and offers versatile novel possibilities of crop improvement through site-directed genetic modification. Aiming to establish genome engineering as a viable tool for cereals, we generated and expressed GFP-specific transcription activator-like effector nucleases (TALENs) in barley lines that harbour a single copy of GFP used as experimental target sequence. Mutant plants were produced via Agrobacterium-mediated transfer of a functional pair of TALEN constructs to pollen undergoing embryogenic development. When mutagenesis events occurred prior to spontaneous or artificially triggered duplication of the initially haploid pollen-derived tissue, non-chimeric and homozygous mutants were immediately obtained, as was indicated by non-segregating progeny. In an alternative approach, the two TALEN units required for site-directed cleavage activity were separately used to retransform GFP-transgenic barley. Crossings between obtained plants resulted in pair-wise combination of both TALEN units, which entailed the formation of mutations during early zygotic embryogenesis in the hybrid caryopses. While sequencing of target-specific PCR amplicons revealed multiple mutant alleles in each of the analysed seedlings, the GFP wild-type allele was only rarely detectable, suggesting an unprecedented efficiency of site-directed mutagenesis. In addition, we exemplified homology-dependent genome editing using a customized DNA-repair template. This approach allows to precisely create any DNA sequence of choice at any pre-defined genomic locus.

Male Apomixis: an Alternative Strategy to Synthetically Engineer Clonal Seed Formation in Plants

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Abstract

Apomixis is a specialized form of plant reproduction, in which clonal seeds are generated through a developmental reprogramming of the normal sexual pathway. Based on its ability to fixate genotypes over successive generations, apomixis is considered the holy grail for plant breeding, particularly in the perspective of hybrid fixation and polyploid stability. Although apomixis is a naturally occurring phenomenon, it has not been retrieved in major crop species. As a result, current research is focused on the genetic engineering of apomixis and hence on the identification of genes that underlie its major components, including female apomeiosis and parthenogenesis.

Here, we present an alternative method to genetically engineer clonal seed formation; namely 'male apomixis'. Basically, in this process, male meiosis is converted into a mitotic division and resulting clonal pollen are used to fertilize eggs that selectively eliminate their own genome input upon fertilization. By this way, clonal seeds are formed without the need for apomeiotic eggs. Recent studies have shown that parent-specific genome elimination (GE) can be obtained by uniparental changes in centromeric chromatin configuration (e.g. CENH3). Male apomeiosis, on the other hand, can be obtained by combining loss of meiotic recombination and reductional cell division. We here demonstrate that the combined loss of AtSPO11-1 and JAS, e.g. two proteins essential for meiotic recombination and reductional cell division into a mitotic one, hence yielding clonal 2n pollen. In addition, we show that the use of these pollen in the fertilization of a GE line yields diploid progeny plants that fully retain the genomic make-up of the original pollen donor. As such, these findings demonstrate that the combined loss of JAS and AtSPO11-1 is sufficient to confer male apomeiosis and hence provides a molecular basis for the synthetic engineering of 'male apomixis' in plants.

Cytological Investigations in Midday Flowers (Aizoaceae) Reveal High DNA Contents in Different Somatic Tissues and Occurrence of Unreduced Male Gametes

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Abstract

Breeding in the midday flowers (Aizoaceae) genera Lampranthus and Delosperma is of interest for developing novel drought tolerant bedding plants, possibly including polyploidization. However, cytological knowledge on these plants is scarce. Flow cytometric investigations on different genotypes of Lampranthus and Delosperma revealed that most organs of these plants predominantly consist of endoreduplicated cells with up to five different DNA contents (2C-32C). Endoreduplication (endocycling) is a process involving one or several rounds of nuclear DNA synthesis without chromosomal and cellular division. The highest proportions of endoreduplicated cells were detected in sepals, petaloid staminodes, and mature succulent leaves (up to 95 %), whereas only relatively low percentages were found in young leaves, internodes and roots (≈ 23-56 %). A comparison of greenhouse and in vitro grown plant organs in respect of their endoreduplication patterns only showed marginal differences. How endocycling affects in vitro shoot regeneration and/or chromosome doubling through antimitotic agents remains to be clarified. Further observations on midday flowers of these genera revealed that pollen grains of identical genotypes had various diameters, indicating spontaneous production of unreduced male gametes (2n pollen). In addition microscopic analyses showed that exceptionally large pollen grains also contained larger sperm nuclei. Flow cytometric analyses of isolated sperm nuclei at different dates revealed that all investigated genotypes shed varying percentages of gametes with somatic DNA content (\approx 6-32 %). Besides 2C sperm nuclei, in some cases also low proportions of triploid 3C nuclei were detected. Ongoing analyses of microsporogenesis as well as trials in climate chambers shall provide insights into the formation of 2n pollen and if it is affected by environmental conditions.

Haploid Culture of Chrysanthemum

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Abstract

Chrysanthemum is a highly heterozygous polyploid flower (2n = 6x). It is an important cut flower and potted plant. Inheritance in chrysanthemum is not well known. It has a complex genetic background and it is self-incompatible, which hampers the development of a homozygous line, which is essential for breeding progammes. The induction of haploid plants through anther cultures, unpollinated ovules and subsequent doubling the chromosome number to produce doubled haploids could be a valid alternative to the classic self-fertilization method, saving costs and time, since in a few months it is possible to produce many pure lines to be used both in conventional and unconventional breeding programmes. Five garden chrysanthemum cultivars were used as anther donor plants. The effects of the genotype and the addition of different growth regulators in MS medium were analyzed. Plants regenerated via anther culture were observed morphologically, through cell physiology and cytogenetics. A significant difference in callus formation in the anthers and in the regeneration rate was found among the cultivars. The most effective induction medium was basal MS plus 2.0 mg/L of BA + 1.0 mg/L of 2,4-D + 9% W/V sucrose. Haploid (n=3x=27) plants were obtained; they were similar in appearance to the hexaploid ones; however, they were somewhat smaller than their hexaploid counterpart. Moreover, the chloroplast number in stomata guard cells of haploids was 12, half of the number in the diploids. Callus was formed from unfertilized ovules incubated on Murashige and Skoog (MS) medium supplemented with 2.0 mg/L of BA + 0.5 mg/L of NAA + 0.6%W/V sucrose. Stomatal guard cell chloroplast counts and chromosome counts showed that the majority of 20 regeneranted plants were hexaploid (2n=6x=54) (80%) and haploid (n=3x=27) accounted for 5%. The production of haploids by unfertilized ovule culture opens a new prospect in chrysanthemum breeding.
SESSION BREEDING





Session Breeding

027.

Interspecific Hybridisation in the Genus Helleborus

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Abstract

The genus Helleborus comprises 22 species, which are allocated to six Helleborus sections. Helleborus species show differences with regard to leaf and flower morphology, scent, and sensitivity to different pests and diseases. Breeding programs aiming at these traits require the inclusion of a broader spectrum of Helleborus species in addition to the most popular species H. niger (Christmas Rose) and H. x hybridus (Lenten Rose), thus interspecific hybridisations need to be performed. Pollen viability was estimated for nine species by staining with thiazolyl blue (MTT) and varied between 67 and 99 % for fresh pollen and between 74 and 99 % for pollen dried for 24 hours. Crossing barriers had previously been localized in Helleborus species as predominantly post-zygotic. Therefore, embryo rescue techniques via ovule culture were established to overcome these barriers. Ovules were isolated from the maternal plants five to seven weeks after pollination and then cultured in vitro. Overall, from 661 crosses involving 13 Helleborus species, 40,885 ovules were obtained. In a combination with parents of low genetic distances between the parental species, up to 26.08 % of these ovules produced hybrid shoots, whereas in intersectional crosses this was the case in only 0.17 to 0.85 % of the ovules. In total, 217 hybrid offspring were verified by flow cytometry and molecular marker analysis (random amplified polymorphic DNAs), whereof 16 were derived from parental species belonging to different Helleborus sections. Hybrid plantlets of some cross combinations were transferred to the greenhouse and expressed intermediate leaf and flower morphology when compared to the parental species.

Methodological Basis and Advances for Ornamental Pepper Breeding Program in Brazil

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Abstract

The Federal University of Paraíba (UFPB) has been developing in the last two decades a breeding program for ornamental peppers. The goals are to evaluate and select breeding lines, to promote the hybridization among the selected lines and to distribute those genotypes among small farmers of Brazilian states. This pepper program is composed by three stages: 1) Germplasm bank maintenance, evaluation of genetic diversity and selection of superior genotypes as potted plant; 2) Mass selection with progenitor's selection and 3) Hybridization and evaluation of the single, triple and double hybrids and segregating populations. Parallel to the development of new cultivars by mass selection, intra- and interspecific hybrids were developed. The germplasm bank contains 500 accessions, 290 hybrids and 1100 lineages in advance generations. Using mass selection and selection in advanced generations (F₅) it was possible to select new commercial lines. Three new genotypes were created by mutation breeding: a dwarf, a variegated and a purple one. We also succeeded to obtain, by hybridization, plants with larger flowers and selected lines with increased shelf life as potted plant (72 days). The natural landraces and segregated populations showed variability to ethylene sensitivity. After applying ethylene, lines resistant to leaf and fruit abscission were selected.

P54.

New Members of the Iridaceae Family: Interspecific Hybridization between *Iris dichotoma* and *I. domestica*

Yanchao Guo, Yike Gao, Yanyan Shi, Lili Ruan and Qixiang Zhang

Poster

P55.

Interspecific Crosses in *Passiflora* to Obtain New Hybrids with Ornamental Value

Maurizio Vecchia¹, Laura De Benedetti², Ermanno Sacco², Antonio Mercuri², Luca Braglia³ and <u>Annalisa Giovannini²</u>

Poster

P56.

Interspecific Hybridization in the Tribe Genisteae with Immature Fruit Rescue in Vitro Culture

Agathe Le Gloanic¹, Joseph Belin², Valéry Malécot³ and Véronique Kapusta²

Poster

P57.

Morphological Aspects in *Heliconia chartacea* Inflorescences for Use as Cut Flower

Paula Guimarães Pinheiro de Araujo¹; Kessyana Pereira Leite²; Sueynne Marcella Santana Leite Bastos², <u>Vivian Loges²</u>

Poster

P58.

New Ornamental Lophospermum Hybrids through Interspecific Hybridization

Simas Gliozeris, Alfonsas Sigitas Tamosiunas

Poster

P59.

Selection of the Best Black Mondo (*Ophiopogon planiscapus* 'Nigrescens') Clone in Tissue Culture Conditions for Micropropagation

Esin Arı^{1,2}, Jeffrey Adelberg², Maria Delgado² and Mark Kroggel^{3,2}

Poster

P60.

Interspecific Hybridization between Dahlia dissecta and D. rupicola

<u>José Mejía-Muñoz</u>¹, Claudio Flores-Espinosa¹, Jerónimo Reyes-Santiago², Gisela Peña-Ortega¹, María Elisa Alvarado-Cano¹ and Ricardo Gaspar-Hernandez¹

Poster

SESSION DISEASE RESISTANCE





Session Disease Resistance

029.

Basal and Induced Disease Resistance Mechanisms in Ornamentals

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Abstract

Ornamental plants are challenged by a wide range of biotrophic, hemibiotrophic and necrotrophic microbial pathogens. Here, an overview is given how plants defend themselves against these pathogens via a range of constitutive and inducible defense mechanisms. Inducible defense is triggered when pathogens break through the first line of pre-formed defense and is based on recognition of pathogen- or damage associated molecular patterns and effectors by means of membrane or cytosolic receptors. Upon recognition a complex immune response is activated characterized by cell wall reinforcements, the induction of pathogenesis-related proteins, antimicrobial compounds and specific blends of plant hormones. Genomic strategies can help in identifying core effectors and corresponding receptors to obtain durable disease resistance. Induced resistance is a non-specific form of disease resistance in plants that can be triggered by a wide range of inducers of chemical or biological origin. We discuss how this knowledge can be applied to obtain disease resistance in ornamentals.

O30.

Opportunities to Breed for Broad Mite Resistance in *Rhododendron simsii* **Hybrids**

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Abstract

Broad mites (Polyphagotarsonemus latus Banks) are a key pests in many ornamental plants including pot azalea, *Rhododendron simsii* hybrid. These minute mites (< 200 μm) cause browning and curling of leaves and flowers which lead to economic losses as damaged plants are unmarketable. The development of resistant cultivars might lead to a sustainable solution in controlling this pest. First we screened a *R. simsii* gene pool for the presence of resistance or tolerance against this cryptic pest. On most genotypes, broad mites caused severe damage. However, 'Mistral' and its color sport 'Elien' showed to be tolerant towards the pest, as indicated by low damage rates and few mites found on the plants. Evaluation of leaf morphology using scanning electron microscopy revealed that different trichome types were present on leaves of R. simsii. As reported in other plant species trichomes might act as a limiting trait for the development of pest populations. We speculate that the presence of foliar glandular hairs on leaves of 'Mistral' and 'Elien' might be correlated with the observed tolerance against this pest. These trichomes occurred both on the adaxial and abaxial side of the leaves and were also present on the stem. Besides morphological features, the role of induced resistance in the defence strategy of plants against broad mites was evaluated. Controlled lab experiments indicated that induction of the jasmonic acid (JA) biosynthesis pathway resulted in a significantly lower rate of mite population increase compared to a mock treatment. Activation of the counteracting defence pathway regulated by salicylic acid (SA) did not significantly alter the rate of population increase. Transcript levels of JA marker genes were monitored by using RT-qPCR upon broad mite infestation. Preliminary results indicate induction of JA marker genes allene oxide synthase (AOS), allene oxide cyclase (AOC) and lipoxygenase (LOX) in R. simsii 'Nordlicht' by the presence of broad mites as compared to uninfested plants. The results presented here open new possibilities for future breeding for broad mite resistance.

Transgenic Chrysanthemums (*Chrysanthemum morifolium* Ramat.) Carrying both Insect and Disease Resistance

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Abstract

The chrysanthemum (Chrysanthemum morifolium Ramat.) is a very important ornamental flower worldwide. Damage due to both harmful insects and pathogenic microbes results in a loss of quality that leads to high control costs. To reduce the likelihood of such damage, we have developed genetically modified chrysanthemums by introducing a modified cry1Ab gene of Bacillus thuringiensis var. kurstaki HD-1 (mcbt) and a modified Sarcotoxine IA gene of Sarcophaga peregrina (msar) with or without the 5'-untranslated region of the alcohol dehydrogenase gene of Arabidopsis thaliana (AtADH-5'UTR, as ADH). The leaf discs were transformed using a disarmed strain of Agrobacterium tumefaciens, EHA105, carrying the binary vectors pBIK201BPS or pBIK201ABPS, which contain the mcbt and msar genes. The mcbt and msar genes were detected in all the regenerated plantlets by Southern blot analysis, with a transformation frequency of 6.8%. Northern blot analysis revealed that the density of the mcbt bands was influenced by the gene copy number in the GMchrysanthemum; however, the density of the msar bands was influenced by the presence of ADH regardless of the gene copy number. On the insect bioassay, the leaves of the GMchrysanthemums which expressed the level of Cry1Ab ICP exceeded 1.31 μ g per g of total soluble protein were showed a strong resistance against 4 kinds of lepidopteran larvae (Helicoverpa armigera, among others), they all died during the first instar stage. The GM-chrysanthemums showed a strong resistance against white rust (Puccinia horiana) infection, especially those possessing ADH, when the expression level of Sarcotoxin IA peptide exceeded more than 19.5 μ g per g FW of leaves. These GM-chrysanthemums will be very useful to reduce control costs for lepidopteran insects and white rust disease. For the practical use of these GMchrysanthemums, we will introduce male and female sterility traits to GM-chrysanthemums to reduce the environmental-risk via transgene-flow.

Induction of Insect Resistance in *Dendrobium mirbellianum* using Ion Beam Irradiation

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Abstract

Dendrobium orchids have been the major orchid cut-flower export for Malaysia as well as for other Southeast Asian countries like Thailand and Philippines. Although new orchid hybrids are consistently released every year to meet the current demand by customers, the only problem that remains unsolved in this industry until today is the problem of insect infestations. Insect infestations in the orchid flowers have caused a lot of losses not only to growers but also exporters due to strict quarantine regulations. The objective of this project was to produce new orchid mutants of Dendrobium mirbellianum that are resistant or tolerant to insect infestation, especially to mites and thrips. Tissue culture orchid materials (protocorm-like-bodies or PLBs) were irradiated with ion beams at doses of 0.4, 0.8, 1.0 and 2.0 Gy for mutation induction. Following irradiation, regenerated plantlets were randomly selected from each dose and subjected to in vitro infestation with mites and thrips, to analyze their resistance towards these pests. Potential insect resistant orchid mutants were then selected and subsequently planted in the glasshouse. Secondary screenings were carried out at flowering stage by challenge-infestation with the target insects. Results obtained showed that in vitro mite infestation study on regenerated D. mirbellianum orchids has showed an increase in resistance towards mite infestation on ion beam irradiated plantlets. The percentages of mite resistant mutant plantlets gradually increased with increasing irradiation doses. Through in vitro mite infestation study, a total of 50 potential mite tolerant plantlets were identified. Of these 50 plants, one plant was found tolerant to thrips when secondary screening was carried out at flowering stage (in vivo). All identified mutants were propagated to achieve large numbers of clones, and some samples were transferred to a private collaborator for pre-commercialization studies.

Can a Candidate Gene Approach Bridge the Gap between QTL Analysis and Marker-Assisted Selection for Botrytis Resistance in Gerbera?

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Abstract

Gerbera grey mould (*Botrytis cinerea*) causes substantial losses in quality and quantity during gerbera production and postharvest transportation. Developing varieties with resistance to Botrytis is therefore an important goal of gerbera breeding. In this study, we employed a candidate-gene (CG) approach to map potential candidate genes for botrytis resistance and screened those genes for colocalisation with QTL regions, which were mapped in two segregating populations. For example, the selected candidate gene 2-PS (2-pyrone synthase) codes for polyketide synthase which synthesise a putative precursor for two phytoalexins in gerbera. knocking out this gene show increased susceptibility to *B. cinerea* infection (Koskela et al., 2011). Using our recently developed transcriptome resource for the four parents of the two mapping populations, *2-PS* gene sequence variation was found and employed as single nucleotide polymorphisms (SNPs) markers. Segregation results in the populations showed that the *2-PS* gene is indeed strongly associated with Botrytis resistance. The SNP markers for the *2-PS* gene which is one of the genes controlling resistance to Botrytis can be introduced in gerbera breeding and have the potential to improve botrytis resistance using marker-assisted selection.

Targeted Mutagenesis of MLO-Homologous Genes in the Rose Genome

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Abstract

Powdery mildew caused by *Podosphaera pannosa* (Wallr.: Fr.) de Bary is one of the most severe diseases of roses. Especially for the production in the greenhouse it depicts a great problem. Biology and race structure of *P. pannosa* limit the effectiveness of race-specific, monogenic resistances making the application of fungicides indispensable. However, this application is not very desirable because of high costs, increasing legal restrictions and public concerns. An alternative to overcome this problem can be the mildew resistance locus o (*mlo*)-mediated resistance. The *mlo*-based resistance is so far characterized in barley, *A. thaliana*, tomato and pea where the loss-of-function of specific members of the *MLO* gene-family leads to a recessive broad-spectrum resistance. This resistance displays a high durability since it has been used in barley for over thirty years without being broken. The MLO proteins are heptahelical transmembrane proteins which probably manipulate or suppress the SNARE protein-dependent and vesicle-associated defense mechanisms of the cell and by that confer susceptibility towards the pathogen.

In rose four *MLO* homologs (*RhMLO1*, *RhMLO2*, *RhMLO3* and *RhMLO4*) closely related to the functional ones in *A. thaliana*, pea and tomato have been sequenced and mapped. The aim of this work is a reverse genetics approach to verify the functionality of the four mentioned rose *MLOs* regarding the mediation of susceptibility towards *P. pannosa*.

Therefore stable and transient knock-out mutants of the tetraploid *Rosa hybrida* cultivar 'Pariser Charme' for all four genes are produced with two different strategies: using RNAi and TALEN. For both strategies several shoots have been obtained and transferred to soil. First analyses to determine the transgenic character and the downregulation of the genes of interest have been performed. Moreover transient over-expression of the four *RhMLO* genes is analyzed in an assay with detached rose leaves as well as in heterologous expression systems.

POSTERS



Molecular Breeding

P1.

Agrobacterium tumefaciens Mediated Transformation of *Salvia* Species Laura Bassolino, Giada Baudino and Barbara Ruffoni

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Salvia L. is the largest genus of Lamiaceae family with more than 900 species known for high ornamental value and for active metabolite production. Among them S. dolomitica Codd, native to South Africa, is a very interesting multipurpose plant. This sage is drought resistant and works well in dry substrates; it can be cultivated in containers and used as groundcover, shrub border or edging. S. officinalis is a well known culinary herb with a large amount of healthy benefits. The increasing demand of aromatic pot plants for ornamental market justify the research efforts considering several field of action. The two species are good models to develop tissue culture and genetic transformation of leaf explants and callus due to their flexible management under in vitro conditions. Little is known about transformation protocols in aromatic plants mainly due to antibacterial metabolite content and difficulties in regeneration. Leaves from micropropagated S. dolomitica plantlets subcultured for 30 days and two-weeks old calli of S. officinalis were used as explants in order to set up a transformation procedure to increase secondary metabolite production. A key biosynthetic gene for Rosmarinic acid (RA) synthesis, SoHPPR, encoding a hydroxyphenylpyruvate reductase, was previously isolated in our lab from S. officinalis. RA is a powerful antioxidant phenolic compound widely used by pharmaceutical and food industry. Since we were interested in increase the concentration of RA in Salvia species by overexpressing the isolated gene, a binary expression vector for plant transformation in which the SoHPPR gene is under the control of the 35S promoter was obtained by using the gateway. The construct was then transferred into A. tumefaciens strain EHA105. Plant materials were submerged into the bacterial suspension for 20 min and transferred to Co-coltivation medium in dark for two days at 23 ± 1 °C. Afterwards leaf pieces and calli were moved to the CIM medium containing high levels of cefotaxime and kanamycin. Plates were incubated at 23 ± 1 °C, 16h lighting photoperiod and tissues were subcultured every 25 days. Putative T0 transformed calli of both S. dolomitica and S. officinalis, randomly picked, were analyzed by PCR leading to the production of 6 and 4, respectively, independent lines carrying the 35S:SoHPPR gene.

P2.

Evaluating Production-related Traits in *Pelargonium zonale* as a Solid Foundation for Introducing Marker-Assisted Selection <u>Heike Molenaar¹</u>, Martin Glawe² and Hans-Peter Piepho¹

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Rare phenotypes have always been of interest for ornamental breeders. With marker-assisted selection (MAS), rare phenotypes become even more important, because the phenotypes can be associated with their genotypic data and selection bases then on marker information so that breeders are able to select in early breeding stages, for example. Not unfrequently, putative rare phenotypes turn out to be merely a product of incorrect data analysis. This study was conducted i) to investigate production-related traits of 500 Pelargonium zonale genotypes, ii) to select rare genotypes based on mixed model analysis for generating genotypic data and iii) to associate phenotypic and genotypic data to find SNP based markers for production-related traits in the future work. Here, we focus on the phenotypic data collection and analysis. Genotypes were tested in a twophase experiment, where in phase one the number of stem cuttings per single plant were counted and in phase two, the root formation of harvested cuttings in phase one were evaluated by a newly defined scoring scale. We could estimate genotypic effects by accounting for the experimental design and were able to find medium ad hoc heritabilities for both traits. Additionally, bivariate modelling method was found to enable selection for both traits within one step. This study shows how to adhere to the principals of experimental designs as a basis for phenotypic data collection and analysis which are the foundations for introducing MAS.

P3.

Rapid Genetic and Epigenetic Alterations under Intergeneric Genomic Shock in Newly Synthesized Chrysanthemum morifolium × Leucanthemum paludosum Hybrids (Asteraceae)

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The Asteraceae family is at the forefront of the evolution due to frequent hybridization. Hybridization is associated with the induction of widespread genetic and epigenetic changes and has played an important role in the evolution of many plant taxa. We attempted the intergeneric cross Chrysanthemum morifolium × Leucanthemum paludosum. To obtain success in crossing, we have to turn to ovule rescue. DNA profiling of the amphihaploid and amphidiploid were investigated using amplified fragment length polymorphism (AFLP), sequence-related amplified polymorphism (SRAP), start codon targeted polymorphism (SCoT) and methylation sensitive amplification polymorphism (MSAP). Hybridization induced rapid changes at the genetic and the epigenetic level. The genetic changes mainly involved loss of parental fragments and gaining of novel fragments, and some eliminated sequences possibly from the non-coding region of *L. paludosum*. The MSAP analysis indicated that the level of DNA methylation was lower in the amphiploid (~45%) than in the parental lines (51.5%-50.6%), while increased after amphidiploid formation. Events associated with intergeneric genomic shock were a feature of C. morifolium \times L. paludosum hybrid, given that the genetic relationship between the parental species is relatively distant. Our results provide genetic and epigenetic evidence for understanding genomic shock in wide crosses between species in Asteraceae and suggest a need to expand our current evolutionary framework to encompass a genetic/ epigenetic dimension when seeking to understand wide crosses.

P4.

Using SSRs to Assess Genetic Diversity among Lagerstroemia Species

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The most recent taxonomic revision of Lagerstroemia occurred in 1969 was based on morphological characters and is the widely-accepted key for crapemyrtle species. As described, the genus is split into three sections including more than 50 species, several of which are grown for lumber in the asia and the philipines. Three species, L. indica, L. fauriei, and L. speciosa, are cultivated as ornamental trees in warm regions of the world. Crapemyrtle breeding in the United States began with L. indica but over the last 30 years new cultivar releases have been dominated by interspecific hybrids between L. indica and L. fauriei, mostly to improve resistance to powdery mildew. Recent work has described interspecific hybrids between L. indica and L. speciosa to increase flower size and verified the genetic backgrounds using SSR markers. Resulting progeny were sterile, suggesting a need to find bridging species or embryo rescue. While most Lagerstroemia species lack cold hardiness to be grown as ornamental in temperate zones and have small flowers with little ornamental appeal, there is potential for interspecific mating combinations to improve growth habit, disease resistance, and flower size. Here we used SSRs to assess genetic diversity and relatedness, and predict wide hybridization success.

P5.

Application of Transcriptome Sequencing in the Asteraceae Molecular Breeding

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The Asteraceae family has an advanced evolution in morphology and a strong adaptability to the environment. It is one of the largest families of the angiosperms and it has a tremendous ornamental and economic value. So far, there is no whole genome sequence available for species belonging to the Asteraceae. Due to the complexity of the genetic background, research on molecular breeding of Asteraceae is seriously limited. The emergence and development of transcriptome sequencing offer a ray of optimism; therefore this technique has been used recently in studies in many members of the Asteraceae family. This review focuses on the application of transcriptome sequencing in screening for key genes linked to specific processes, in developing SNP and SSR markers, and in studying the evolutionary processes within the Asteraceae.

P6.

The Importance of Salicylic Acid and an Improved Plant Condition in Determining Success in *Agrobacterium*-mediated Transformation

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A successful Agrobacterium-mediated transformation requires a balanced interaction between the plant and Agrobacterium. We hypothesize that blocking salicylic acid (SA) biosynthesis could increase the plant's susceptibility and gene transfer events. In this study, the influence of the SA biosynthesis on transformation efficiency was investigated. We tested the effect of increasing concentrations of SA inhibitors such as 2amidoindane-2-phosphonic acid (AIP) and paclobutrazol (PBZ) in *P. hybrida* and *N. benthamiana*. As a control, the effect of adding SA was examined. In addition, the effect of lipoic acid (LA) was studied. LA, an antioxidant, is considered to act as a general transformation enhancer by improving the plant's condition. Adding SA inhibitors should lead to improved conditions for Agrobacterium and adding LA should improve conditions for the plant. Explants from fresh leaves of P. hybrida and N. benthamiana were inoculated with A. tumefaciens strain AGL1(pBinGlyRed-Asc1) containing dsRed as a reporter gene. Transient dsRed expression was determined at 14 days after co-cultivation by counting red fluorescing spots and transformation efficiency was determined from the number of explants with calli surviving on selection media at 30 days. In P. hybrida, 10 µM AIP and PBZ resulted in the highest transient and stable transformation. However in *N. benthamiana*, only 50 µM AIP showed a significant increase and only in transient expression, and no evidence of any stimulatory effect of PBZ was observed. Adding SA always decreased the efficiencies in both plant species. Furthermore, 10 µM and 1 µM LA significantly increased transformation efficiency in P. hybrida and N. benthamiana, respectively, with less browning and necrosis. These preliminary results suggested that blocking SA biosynthesis promoted gene transfer but responses were variable and not equally successful between the two plant species. However, repeating these experiments providing us with more data might clarify the picture further.

P7.

Temperature Stress Induces Diploid and Polyploid Gamete Formation in *Arabidopsis thaliana* Male Sporogenesis

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In flowering plants, the reproductive cell lineage is extremely sensitive to adverse environmental conditions, with temperature constituting the predominant stress factor. Upon exposure to heat or cold, both male and female gametophyte development typically show cellular alterations that eventually lead to pre-mature spore abortion and gametophytic lethality. These alterations are highly variable and include irregularities in tapetal development, defects in sugar metabolism, altered auxin metabolism and oxidative stress response. However, despite these well-known effects on gametogenesis, little is known about the impact of adverse temperature conditions on the process of sporogenesis. In this study, we therefore aim to characterize the effect of both high and low temperature stress on meiotic cell division, thereby focusing on homologous chromosome pairing and recombination, reductional chromosome segregation and cytoskeletal figure dynamics. Cytological analysis of Arabidopsis male sporogenesis revealed that short periods of low temperature stress (1-40 hrs, 4-5°C) do not affect meiotic chromosome dynamics and segregation, but instead have a detrimental effect on the process of post-meiotic cytokinesis. More specifically, short periods of cold affect the formation of radial microtubule arrays (RMAs) which normally function as an internuclear phragmoplast-like structure required for the formation of the cell plate. As a result, cold induces the formation of syncytial bi- and polynuclear microspores that show subsequent nuclear fusion before PMI to yield di- and polyploid pollen grains. These gametes may form a basis for sexual polyploidization, yielding highly adaptive polyploid genomes. As such, we here postulate that, in contrast to the general detrimental effect on plant sporogenesis, environmental stress may alter the reproductive pathway in such a way that it drives plant evolution and speciation. In this perspective, state-of-the art findings on the impact of both cold and heat stress on Arabidopsis male sporogenesis will be presented.

P8. Molecular Mutation Breeding Dariush Ebadi Almas

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Advances in molecular genetics and DNA technologies have brought plant breeding, including mutation breeding, into a molecular era. With ever-increasing knowledge of molecular genetics and genomics and rapidly emerging molecular techniques, breeders can now use mutation techniques in breeding new varieties more wisely and efficiently than ever before. Plant molecular mutation breeding is here defined as mutation breeding, in which molecular or genomic information and tools are used in the development of breeding strategies, screening, selection and verification of induced mutants, and in the utilization of mutated genes in the breeding process. It is built upon the science of DNA damage, repair and mutagenesis, plant molecular genetics and genomics of important agronomic traits as well as induced mutations. Mutagenic treatment, super-mutable genetic lines, molecular markers and high throughput DNA technologies for mutation screening such as TILLING (Targeting Induced Limited Lesions IN Genomes), are the key techniques and resources in molecular mutation breeding. Molecular mutation breeding will significantly increase both the efficiency and efficacy of mutation techniques in crop breeding. A perspective molecular mutation breeding scheme is proposed for discussion.

Ρ9.

Genomics-based Approaches to Understand Control of Crop Timing Traits in *Petunia*

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Early flowering under non-optimal conditions such as cool temperatures is an important breeding target for greenhouse crop production. We are utilizing genomicsbased approaches to understand crop traits that regulate flowering time, including development rate, in Petunia. De novo transcriptome assemblies were constructed for three wild species (P. integrifolia, P. axillaris and P. exserta) and mined for molecular markers. A total of 89,007 single nucleotide polymorphisms (SNPs) were identified among the species. 15,701 of these SNPs were computationally converted into cleaved amplified polymorphic sequence (CAPS) markers. In addition, 2,949 SSR markers were identified from the P. axillaris transcriptome. The transcriptome was also mined for petunia homologs of genes known to influence development rate (plastochron). These plastochron-related transcripts were also converted to CAPS markers and mapped in a P. integrifolia × P. axillaris F_2 population. One of these genes, encoding an MEI2-like protein, co-localized with a previously identified QTL for development rate located on chromosome 5. Additionally, two interspecific hybrid Petunia recombinant inbred line (RIL) populations, P. integrifolia × P. axillaris, and P. axillaris × P. exserta were developed and genotyped with a GBS (genotyping-bysequencing) approach. High density linkage maps were constructed for the RIL populations with 6,291 SNPs placed on the P. axillaris x P. exserta map and 3,297 SNPs placed on the P. integrifolia x P. axillaris map. QTL mapping is being performed to study development rate and other traits related to flowering time. Furthermore, RNA-seq was performed on eight P. integrifolia × P. axillaris RILs varying in development rate. Initial analyses revealed over 200 differentially expressed genes between fast- and slow-developing lines. We are currently working to integrate these approaches to identify genes controlling development rate in petunia.

Flower

P10.

AGAMOUS Mutations of Double Flower Cultivars in Japanese Azaleas

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From the diversity of endogenous species and natural hybrids of evergreen azalea, many cultivars have been selected since the early 17^{th} century, the Edo era (1603 to 1867). Double flower; polypetalous form is one of the important floral traits in flower varieties also in evergreen azalea. For the purpose of application of double flower trait for evergreen azalea breeding, floral organ structure and MADS-box gene were investigated. Traditional double flower cultivars of R. macrosepalum, R. ripense, R. mucronatum and R. yedoense var. poukhanense, and wild species of each cultivar were used in this study. Sepals of whorl1 and petals of whorl2 are observed in both double flowers and wild types. Stamens of whorl3 are changed to petal-like organs, and pistil of whorl4 change to petal-like or proliferation organs in double flowers. Epidermal cells of petaloid stamens are similar to those of petals. In expression analysis of floral organs by RT-qPCR, AGAMOUS (AG) homolog transcription is reduced in petaloid stamens compare to normal stamen of wild type. In amplification of partial AG region in genomic DNA, 450-8000bp larger fragment bands were obtained in double flowers. The insert sizes and their positions were varied depend on each cultivars. These AG homolog mutations might induce petal-like organ in double flower cultivars. In the process of cultivars development, double flower form mutants which have original AG homolog mutations were selected from wild population of each species.

P11.

CmFTL2 is Involved in Photoperiod and Sucrose Control of Flowering Time in Chrysanthemum

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There are three FT-like genes in chrysanthemum, FTL1 may be a regulator of photoperiodic flowering under long day (LD) conditions, and FTL3 is a key regulator under short day (SD). However, the function of FTL2 is unclear. Here, CmFTL2 was isolated from both 'Jimba' (SD chrysanthemum) and 'Youxiang' (aLD chrysanthemum). The time and spatial expression patterns of CmFTL2 in the two varieties were similiar in the flowering period in a year. CmFTL2 was also extremely induced in chrysanthemum after sucrose treatment. Heterologous expression of CmFTL2 shortens the juvenile phase and promotes Arabidopsis mutation ft-10 flowering. These data indicate CmFTL2 may be a regulator of sucrose control of flowering time in chrysanthemum.

P12.

Comparative Expression Analysis of Five Floral Homeotic Genes Including Newly Isolated Genes Homologous to *PISTILLATA* and *SEPALLATA3* among Four Flowering Cherries, *Prunus lannesiana* var. *speciosa* and Three *P. lannesiana* Cultivars

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Since 1991, flower development is explained by ABC model in various plant species. Four types of floral organs are specified by three different classes of homeotic genes such as class A, B and C genes working solely or in combination of two genes in different classes. In Japan, there are so many flowering cherry cultivars showing divergent flower morphology. We tried to clarify whether this model can be applicable to flowering cherry or not. Especially we focused on three cultivars bearing double flowers, Prunus lannesiana 'Eigenji', 'Grandiflora', and 'Gioiko'. These are revealed to be bud mutations by means of SSR analysis (Oyama, 2013) even though 'Grandiflora' and 'Gioiko' bearing yellow or greenish flowers respectively. These three cultivars could be distinguished by stamen length and anther morphology. In this study, two genes homologous to PISTILLATA (PI), class B, and SEPALLATA3 (SEP3), class E additionally proposed, were cloned by RT-PCR and RACE from P. lannesiana var. speciosa wild flowering cherry bearing single white flowers. These genes were confirmed by high similarity in amino-acid sequence to those of other PI and SEP3 homologues. Furthermore three floral homeotic genes homologous to APETALA1 (AP1), APETALA3 (AP3) and AGAMOUS (AG) belonging to class A, B and C respectively were also investigated. Total RNAs extracted from separately collected floral organs of flower buds were subjected to an expression analysis performed by RT-PCR southern hybridization. As a result, an expression pattern obtained from P. lannesiana var. speciosa used as a control suited well to the classic ABC model. However, the other three cultivars showed unexpected expression patterns in class A and B genes. Further investigation should be required to confirm interactions among these genes and their functional activities, then it would be possible to reveal mechanisms underlying the floral organ specification and morphological changes in flowering cherries.

P13.

Photoperiod and Cold Night Temperature in Control of Flowering in *Kalanchoë*

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Kalanchoë species and cultivars are produced as ornamental plants by nurseries at many places in the world. The genus contains around 140 species comprising significant morphological variation. However, the number of species used for breeding is limited due to lack of knowledge of e.g. flower inducing factors. Having both parental plants flowering at the same time is vital for crosspollination; therefore, it is of significant importance to be able to control flowering time of the species of interest. The objective of this study was to control flower induction in Kalanchoë marmorata and K. longiflora. K. prittwitzii was used as control species to validate treatments that consisted of combining short day photoperiod (8h) and different night temperature (18°C, 12°C and 6°C). While K. prittwitzii had 100% flowering for all treatments, K. marmorata only flowered at 12°C (33% plants flowering) and 6°C (25% plants flowering), and K. longiflora did not flower in any of the treatments. The decrease of night temperature delayed the growth in all species and flowering in K. prittiwitzii and K. marmorata, but did not influence the number of flowers. The results demonstrate that there is a variation of the flowering stimuli in the Kalanchoë genus, and that short days are not the only factors controlling flowering. Even though short days are enough to induce flowering in easily-induced species (e.g. K. prittwitzii), cold night temperatures are required to flower-induce other species (e.g. K. marmorata). There are of course other factors limiting flower induction in K. marmorata and K. longiflora, which need to be addressed in further studies.

P14.

Petunia ×hybrida (Hook.) Vilm. with Bicolor Flowers, Obtained by Chemical Mutagenesis

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To achieve variability in flower coloration of ornamental plants, breeders use different approaches. However, bicolor cultivars, whose center and edge have different dyeing simultaneously in the same flower, are rare. In our work plants with bicolor corollas were obtained by chemical mutagenesis. Seeds of petunia cultivar 'Snow ball' with white flowers were treated with solutions of ethylmethansulfonat for 16 hours. To get M2 - M4 progeny the first flowers were isolated and artificially self-pollinated before bud dehiscence. The seeds were collected from individual plants. Anthocyanins are pigments conferring pink, red, violet and blue hues to flowers. For detection, the pigments were extracted with acid alcohol from fresh limbs, apart from the blue and pink parts. HPLC analysis was used for detecting anthocyanins and flavonols. Among the most significant results were the obtained mutant lines with violet-blue limbs with purple rose center (no. 33-9-7) and purpleblue limbs with lavender-rose center (33-5-1). In both cases the lobes and lobes fusion places have a "Blue" color. Biochemical analysis showed that in mutant plants corollas contain 15 to 20 different anthocyanins with various aglycones such as delphinidin, petunidin, malvidin and cyanidin. Thus, the line 33-9-7 contains delphynidin, petunidin and malvidin glycosides in the "rose" and "blue" part (on the whole 35 and 70 mg/l), respectively and corollas of line 33-5-1 contains the same compounds and even traces of cvanins (11 and 23mg/l). In the line 33-5-1 the corolla lobes have higher amount of delphinidin while methylated antocyanins are found mainly in the center of flower. However, flavonols as kaempferol and quercetin glycosides were found in large amount (590- 1100mg/l) in mutant flowers. The flavones concentration was 35 - 50 times higher than of anthocyanins in the "rose" part of the corolla. Thus, mutagens lead to changes in the flavonoid biosynthesis in the different parts of petunia corollas.

P15.

Flower Color Characteristics in a Mutation Group of Carnation

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The flower color diversity is an important horticultural trait of carnation. It is known that carnation is a genetically labile plant. A group of cultivars having different flower colors derived from bud mutation have been known in carnation. An advantage of sport cultivars is that they show same growth and flowering characteristic. The MINAMI series consists of several carnation genotypes with various flower colors. All were derived from a genotype 'Poliminia'. The order of flower color change occurrence is known; Poliminia (yellow) produce Orange MINAMI (pale orange with orange stripe) and Vanilla MINAMI (cream), then Orange MINAMI produced Feminine MINAMI (light pink), MINAMI (orange) and Lemmon MINAMI (deep yellow). Further bud mutation is often observed in this group. In this study, the flower color of six genotypes in MINAMI series was determined by colorimeter and their major pigment components were determined by HPLC. Major anthocyanin of the genotypes was pelargonidin-3,5malyl-digluciside and the majar flavonoid pigment was chalcononarigenin-2'-glucoside. Fluctuation of chalcone content and presence or absence of anthocyanin are the main factors of their flower color variation. The characteristics of flower color change suggests that multisite mutation including transposon are involved in MINAMI series color variation.

P16.

Cloning and Expression of Floral Organ Development Related Genes in Tree Peony (*Paeonia suffruticosa*)

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Tree peony (Paeonia suffruticosa) is a very famous traditional ornamental and medicinal plant in China, and is also appreciated internationally because of its large, showy and colourful flowers. Molecular breeding has been one of the hotspots in flower type modification. Till now, although many genes and cDNA clones for genes about floral development have been isolated and well characterized, little information, if any, is available concerning molecular aspect of floral organ development in tree peony. In this study, using degenerated primers derived from conserved sequences of previously cloned genes, five genes involved in floral organ development were first obtained from P. suffruticosa 'Zhao Fen' by RT-PCR and RACE, namely PsAP1 (GenBank No. HM14393), PsAP2 (GenBank No. HQ222889), PsPI (GenBank No. HQ878444), PsMADS1 (GenBank No. HQ222890) and PsAG (GenBank No. HQ222891), respectively. Sequence analysis showed that these genes all had MADS domains and the plant specific K domains, and belonged to MADS-box gene family except PsAP2. Then, the temporal and spatial expressions of these genes were detected using relative real-time PCR. The results indicated that all genes showed high tissue specificity. PsAP1 was expressed at the highest level at prophase and the lowest level at anaphase in the process of flower bud differentiation, whereas an opposite trend was observed in the expression pattern of PsAG. PsAP2, PsPI and PsMADS1 were all expressed at the highest level at metaphase and the lowest level at the initial stage in the process of flower bud differentiation. This work provides fundamental information for the flower type related genes in tree peony and may pave the way to elucidate the molecular basis of its floral organ development.

P17.

Characteristics of *Chrysanthemum seticuspe* f. *boreale* - a Model Plant of Chrysanthemum

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To develop the research on flowering physiology in the genus Chrysanthemum a model plant is required, because that greenhouse chrysanthemums are hexaploids and genetically highly heterozygous. Here we introduce Chrysanthemum seticuspe f. boreale (C. seticuspe) as a model plant in the genus Chrysanthemum. C. seticuspe is one of the 20 wild chrysanthemums in Japan. C. seticuspe has some advantages, e.g. diploid, both vegetative and reproductive propagation are possible, having rosette and juvenility characteristics as shown in greenhouse chrysanthemums and а stable protocol for Agrobacterium-mediated genetic transformation in C. seticuspe has been established. Both Florigen (CsFTL3) and Antiflorigen (AFT) of chrysanthemum has been identified in C. seticuspe. The only disadvantage of this species is its self-incompatibility (SIC). Recently, selfcompatible (SC) C. seticuspe line has found in a file of Japan. The SC-C. seticuspe is late flowering compared with other SIC genotypes of *C. seticuspe* under both 8h day length and natural short-day conditions. Considerable variations in leaf and inflorescence morphology were observed in C. seticuspe. Pollen of SC-C. seticuspe germinated well on both own and other SIC genotype's stigma. Seeds of SC-C. seticuspe selfpollination produced about 30% albino seedlings. Success of crossings of SC-C. seticuspe with greenhouse chrysanthemums were genotype depend. Characteristics of progenies are under investigation. CsFTL3transformed SC-C. seticuspe showed floral induction under non-inductive conditions.

P18.

Flower Color Variation in *Primula kisoana* and Reciprocal Interspecific Hybridization between *P. kisoana* and *P. obconica*

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To produce novel cultivars, interspecific hybridization is a famous methods to blend characters of both parent species. Many interspecific hybridizations have been performed between horticultural species and precultivated species to introduce new characters from wild species. Although flower color is one of the most important characteristics to produce novel cultivars in ornamental plants, heredity of flower color is difficult and expression of flower color could not be expected. The hybrid production is one of the ways to confirm flower color expression. Primula kisoana is a native Japanese Primula species. Variation of flower colors observed in P. kisoana was categorized into four kinds, red, pink, purple and white. These flower colors were summarized into three patterns by HPLC analysis. To extend flower color variation of *P. obconica*, reciprocal interspecific hybridization was conducted between P. kisogng and P. obconicg. 31 strains of P. obconicg and 48 strains of *P. kisoana* were used in the present study. A total of 412 reciprocal cross combinations, mostly consisting of legitimate combinations, were obtained between P. kisoana and P. obconica. Between 5-7 weeks after pollination, immature ovaries were collected, surface-sterilized. The placenta with ovules was excised from an ovary and put onto 0.25% (w/v) gellan gumsolidified half-strength MS medium (1/2 MS) supplemented with 5% (w/v) sucrose, 50 mg/l of GA₃, 0.1 mg/l of NAA and 0.1 mg/l BA. 17 Germinable seeds with hybridity confirmed by FCM were obtained from four cross combination, one of four was legitimate combination using P. obconica as maternal parent and the others were legitimate combination using P. kisoana as maternal parent. However, the color variation of four strains of P. kisoana, which produced hybrids, was only pink. More efficient hybridization system could be needed to introduce other flower color from P. kisoana.

P19.

Fifty Shades of Red: Flavonoid Analysis in *Rhododendron simsii* Hyrids

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Flower colour is still one of the most important features of pot azalea breeding (Rhododendron simsii hybrids). We already built a genetic linkage map of a population segregating for flower colour. The map includes a small set of functional markers for flower colour biosynthesis. The flower colour was in the first phase objectively phenotyped by means of RGB values. More recently also the CIEL*a*b* colour space was used on 3 replicates for every sibling to get an even more reliable parameter for QTL mapping of the petal colour phenotype. Gene expression data of key genes of the flavonoid biosynthesis pathway were generated by means of RTqPCR and were mapped as eQTLs (expression QTLs). To enlarge the amount of functional markers for flower colour, Illumina HiSeq PE-100 sequencing was performed on RNA of 3 pools of samples: red, pink and white flowers (10 genotypes per pool). A broad set of candidate genes will be filtered from this dataset for SNP development. Differential gene expression between flower colour groups will be validated by means of RTqPCR in order to determine what is behind differences in flower colour intensities. To enrich further the map value with mQTLs (metabolite QTL), we started to analyze pigment composition using LC-MS/MS. The quantity of five anthocyanidins and four flavonols known to be commonly present in azalea flowers was measured. The different results for the three flower colour groups will be discussed.

P20.

The Heritability and Genetic Correlation in Diurnally and Nocturnally Blooming Daylilies

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Daylilies are very valuable ornamental plants. Their various colors and resistance to undesirable conditions make them useful in perennial landscapes such as flower borders, gardens and road green belts. The natural distribution of daylilies covers East and Southeast Asia and mainly China. The nocturnal blooming species Hemerocallis citrina is not only used as Chinese traditional edible food but has also fragrance and produces large amount of flowers. Every year many new cultivars are released with different flower colors. shapes and sizes. However, the lack of knowledge on the heritability of traits limits the efficiency for the selection of cultivars with desirable characteristics. This study aimed to estimate genetic parameters and genetic correlations among diurnal and nocturnal daylily parents and their hybrids and to select new cultivars with fragrance and large amount of flowers. Crosses were performed among 3 nocturnal blooming (NB) species or cultivars (H. lilio-asphodelus, H. citrina, H. 'April flower') and 4 diurnal blooming (DB) cultivars. In total 386 F1 hybrids were obtained. In the third and fourth year. plant height, leaf length and width, number of flower buds per stem, stem length and the opening and closing times were recorded. High heritability estimates were obtained for plant height, leaf length and width and stem length. A new nocturnally flowering hybrid with fragrance has been selected and registered. The results indicated that the estimates of average heritability of families were superior to the estimates of individual heritability for most characteristics evaluated. The flower opening and closing time are regulated by different nucleus genes. This research will provide guidance for further hybridization in daylily.

Plant Morphology

P21.

Combining Ability For Morphoagronomic Traits In Ornamental Pepper

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The Capsicum genus contains agronomically important ornamental plants. Knowledge of the genetic effects is necessary for efficient plant selection in breeding programs. The objective of this study was to determine the effects of general combining ability (GCA), specific combining ability (SCA) and reciprocal effects of ornamental pepper plant traits. Manual crosses were made among six lines belonging to the germplasm bank of the Center for Agricultural Sciences at Universidade Federal da Paraíba (UFPB 131. UFPB 132. UFPB 348. UFPB 349, UFPB 358, and UFPB 449) in a complete diallel cross, forming 30 hybrids. Eighteen quantitative descriptors were characterized. Data were subjected to ANOVA. Diallel analysis was performed to estimate GCA and SCA effects, using a fixed model of Griffing's method I. The analysis of variance showed significant differences among genotypes for the evaluated traits. The $\frac{\phi^2 g}{\phi^2 s}$ ratio values were greater than 1 for height at first bifurcation, corolla length, and number of stamens, indicating predominance of additive effects controlling these traits. For seedling height, cotyledonary leaf length, cotyledonary leaf width, hypocotyl width, stem length, stem width, crown width, plant height, leaf width, leaf length, pedicel length, number of petals, petal diameter, anther length, and style length, the ^{¢2}g/^{¢2}s ratio values were lower than 1, indicating predominance of non-additive effects. The results suggest the possibility of exploitation of hybrid vigor for these traits.

P22.

Genetic Diversity and Importance of Morphoagronomic Traits in a Segregating F₂ Population of Ornamental Pepper

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Brazil has a broad diversity of species belonging to the genus Capsicum, which can be used in breeding programs for ornamental purposes. The objective of this study was to evaluate genetic diversity in an F2 population of ornamental pepper (Capsicum annuum L.) and to determine the importance of the evaluated traits. The experiment was conducted in a greenhouse in the Laboratory of Plant Biotechnology, at Universidade Federal da Paraíba (UFPB). Ninety-nine plants of an F₂ generation of ornamental peppers were used to evaluate the following morpho-agronomic traits: plantlet height, hypocotyl diameter, cotyledonary leaf length, cotyledonary leaf width, plant height, crown width, height at the first bifurcation, stem diameter, leaf width, leaf with petiole length, chlorophyll A, and chlorophyll B. The experimental design was completely randomized. Data were subjected to analysis of variance, with subsequent clustering of means for the Scott-Knott test (p<0.01). Tocher's method was used based on Mahalanobis distance to cluster the genotypes and the relative importance was evaluated by Singh (1981)'s method. There were significant differences at 1% for the F test for all evaluated traits, except hypocotyl diameter. Tocher's clustering allowed for the separation of genotypes into three groups, indicating the existence of genetic variability among them. Chlorophyll A (21.76%), hypocotyl diameter (14.95%), and stem diameter (14.21%) were the variables that most contributed to genetic divergence. Chlorophyll B contributed only with 0.057%, so it should be discarded in future studies. The phenotypic variability among the 99 genotypes can be used in the breeding program with selection within the family.

P23.

Compact Ever Flowering Pot Azalea by Constitutive Expression of a Cell Division Inhibitor Stefaan P.O. Werbrouck

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Cyclin-dependent kinase inhibitors, designated Kiprelated proteins (KRPs), control correct cell cycle progression and the tuning of developmental signals of the plant cell cycle machinery. By means of Agrobacterium tumefaciens mediated leaf disk transformation, KRP2 from Arabidopsis thaliana was introduced in Rhododendron simsii, 'Hellmut Vogel'. The regenerated plants show a moderate inhibition of cell cycle progression which results in a reduced shoot growth. As a consequence, the shoot apical meristems are rapidly converted into flower meristems. Then dormant lateral buds grow out and the flower induction process starts again on these shoots. This iterative process of branching and flower induction creates an ever flowering compact plant. Although flower and leaf size are reduced, length/width ratio of the leaves was not affected. Larger dimensions of particular cell types compensate the reduction in cell number. This is the first transgenic azalea with a potential horticultural value. Pinching and chemical growth reduction, which are necessary during classical commercial culture, are not required anymore. Nevertheless these plants show drawbacks. The production of cuttings is limited and the continuous presence of flower buds are ideal for overwintering of thrips.

P24.

Heritability and Genetic Parameters for Sizerelated Traits in Ornamental Pepper (*Capsicum annuum* L.)

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Peppers have increasingly been used as ornamental plants, because they show a wide varietion in foliage, fruit with intense color, and growth habits. The aim of this work was to study the heritability of quantitative size-related traits in ornamental pepper (Capsicum annuum). The experiment was conducted in the Laboratory of Plant Biotechnology of Universidade Federal da Paraíba, in Areia-PB, Brazil. Six lines of pepper belonging to the germplasm bank of CCA-UFPB (UFPB 346, UFPB 347, UFPB 348, UFPB 349, UFPB 355, and UFPB 356) were used as parents in a half diallel cross. Evaluated traits were plant height, canopy width, stem length, stem width, leaf length, leaf width, corolla length, petal width, anther length, and style length. The experimental design was completely randomized with five replicates. Data were first subjected to analysis of variance, and the diallel analysis was performed afterwards according to the methodology of Hayman. This the methodology is not based on previously established statistical models, but based on the knowledge of the nature of environmental and genetic statistics, such as means, variance and covariance, obtained from the diallel analysis. The effects of treatment (parents and F₁ hybrids) were significant for all traits. All traits were adequate in the test of sufficiency to the additive-dominant model. Recessive alleles were responsible for the increase in stem width and petal width. All other evaluated traits were increased by dominant alleles. The broad-sense heritability was high for all evaluated traits, indicating that most of the observed phenotypic variation is genetic and will be transmitted to the progenies. For most evaluated traits, with the exception of canopy width and leaf length, the narrow-sense heritability ranged from 0.93 to 0.59, revealing a greater contribution of additive effects on trait expression. Mass selection could be an appropriate method for improving these traits.

Stress

P25.

Chrysanthemum WRKY Gene CmWRKY17 Negatively Regulates Salt Stress Tolerance in Transgenic Chrysanthemum and Arabidopsis Plants

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WRKY transcription factors play regulatory roles as positive or negative regulators in response to various stresses in plants. In this study, CmWRKY17 was isolated from chrysanthemum (Chrysanthemum morifolium). The gene encodes a 227 amino acid protein and belongs to the group II WRKY family, but has an atypical WRKY domain with the sequence WKKYGEK. Our data indicated that CmWRKY17 was localized to the nucleus in onion epidermal cells and no transcriptional activity was detected in a yeast assay system. The expression of CmWRKY17 was induced by salinity in chrysanthemum, and a higher expression level was observed in the stem and leaf compared with the root, disc florets and ray florets. Overexpression of CmWRKY17 in chrvsanthemum and Arabidopsis increased the sensitivity to salinity stress. The activities of superoxide dismutase and peroxidase and proline content in the leaf were significantly lower in transgenic chrysanthemum than those in the wild type under salinity stress, whereas electrical conductivity was increased in transgenic plants. Expression of the stress-related genes AtRD29, AtDREB2B, AtSOS1, AtSOS2, AtSOS3, and AtNHX1 was reduced in the CmWRKY17 transgenic Arabidopsis compared with that in the wild-type Col-0. Collectively, these data suggest that CmWRKY17 may increase the salinity sensitivity in plants.

P26.

Overexpression of AtNDPK2 Enhanced Abiotic Stress Tolerance in Transgenic Chrysanthemum Ji Hyun Kim, Yeo Jin Youn and Nam-In Hyung

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Agrobacterium-mediated transformation using leaf explants of chrysanthemum (Chrysanthemum morifolium) cv. Jinba was conducted. Transgenic plants expressing the Arabidopsis nucleoside diphosphate kinase 2 (AtNDPK2) gene under the control of the oxidative stress-inducible SWPA2 promoter (SN plants) and the constitutive enhanced CaMV 35S promoter (CN plants) were generated and evaluated to develop plants with enhanced tolerance to various abiotic stresses. Among 11 CN plants and 9 SN transgenic plants, CN and SN plants were selected, according to the AtNDPK2 expression levels of transgenic plants. CN and SN plants demonstrated enhanced tolerance to oxidative, high temperature and salt stress on leaf disc and whole plant levels. When the leaf discs are treated in 30 mM H_2O_2 or 50mM NaCl, those of transgenics showed higher tolerance in oxidative or salt stress conditions than those of WT. On the treatment with 300 mM NaCl and high temperature (42°C) treatment to whole plants, both CN and SN transgenics showed enhanced tolerance due to the maintenance of water and chlorophyll contents, whereas WT plants were severely damaged. In addition, the CN and SN plants exhibited better plant growth under normal growth condition in greenhouse compared to WT plants. These results suggest that enhanced AtNDPK2 expression affects oxidative stress tolerance leading to improved plant growth in transgenic chrysanthemum.

P27.

Investigation of Morphological and Physiological Adaptation on Light Stress of Different *Impatiens* New Guinea Hybrids

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Impatiens New Guinea are sensitive against high light conditions, high temperature and low moisture. Consequently abiotic stress tolerance is one of the main targets of breeders. The aims of this examination were the characterization of light stress reaction on morphology and physiology of five Impatiens varieties and the development of a standardized light stress screening method. For the trials from 2012 to 2014 plants were grown in greenhouses with different light conditions. To produce plants with a higher light sensitivity, the light intensity was reduced by using common shading systems (70% shading) in the greenhouse. Further growing conditions were similar to commercial production. After 8 weeks of pre-treatment with different light conditions plants were transferred into balcony boxes. To determine the morphological reaction of the plants, leaves were harvested and used for histological investigations. The pictures taken of tissue sections illustrate that leaves developed under high light conditions have more densely packed palisade parenchym and more chloroplasts compared to the leaves developed in low light conditions. For the light stress treatment 50 % of the plants were placed outside in full sun radiation and 50 % under light protection by UV-permeable foil. After 12 h of full sun radiation several sun exposed leaves were already damaged. In addition, the light stress reaction of the plants was evaluated by measurements of chlorophyll fluorescence and the daily documentation of leaf damages. Within seven days of light stress treatment the plants with the low light pre-treatment had significant higher light stress reaction compared to the plants with high light pretreatment. However these reactions were not confirmed by the results of the chlorophyll fluorescence measurements. Surprisingly, all investigated varieties showed similar light stress reactions. Finally the results demonstrated that the conditions of plant cultivation have more influence on the light stress adaptation than the genotype.

P28.

Post-Production Longevity of Two Ornamental Peppers (*Capsicum annuum*) Affected by Ethylene Action

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Only a few varieties of potted ornamental peppers are available on the seed market. In this work we compared the post-production life of the commercial cultivar Calypso to a Brazilian variety MG 302 (EPAMIG-MG). Previous work showed that 'Calypso' drops all leaves in the presence of ethylene. This study evaluated the effects of ethylene and the inhibitors of ethylene action, 1-methylcyclopropene (1-MCP) and silver thiosulfate (STS) on the shelf life of potted 'Calypso' and 'MG 302'. Plants were grown in greenhouse and transferred to indoors when 30% the fruits were ripe. The experiment was conducted in under 8-10 μ mol s⁻¹ m⁻² white fluorescent light. Plants were treated with 10 μ L L⁻¹ of ethylene for 48 h, fumigation with 1 μ l L⁻¹ 1-MCP for 6 h, fumigation with 1 μ l L⁻¹ 1-MCP for 6 h followed by 10 μ L L^{-1} ethylene for 48 h, spraying with 2 mmol L^{-1} STS, spraying of 2 mmol L^{-1} STS followed by 10 μ L L^{-1} ethylene for 48 h and controls. The cultivar 'MG 302' showed intermediate sensitivity to ethylene action (38% of leaf abscission), while the cultivar 'Calypso' showed complete abscission of leaves when exposed to ethylene. For both cultivars, treatments with 1-MCP, 1-MCP + Ethylene, STS and STS + Ethylene slowed leaf abscission similar to control plants. The plants treated with STS showed longer durability when compared to the other treatments, about 6 days for the 'Calypso' and 15 days for 'MG 302'. Nevertheless, plants treated with 1-MCP also exhibited good shelf life, about 6 days for 'Calypso' and 10 days for the 'MG 302'. Although, treatment with STS although was more efficient compared to 1-MCP did not completely blocked the action of ethylene and exhibited some phytotoxicity.

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P29.

The Influence of NaCl on Growth and Nutrient Uptake of Young Anthurium Plants

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Anthurium andreanum is considered as rather sensitive to salt stress. We selected several adventitious shoots of 'Bolero' cv that were regenerated from leaves on NaCl containing medium. From present experiment, we would like to know a reaction of young pot plants of original 'Bolero' for NaCl aiming to develop a model conditions for testing of tolerance to NaCl of the above-mentioned regenerated plants. One-year-old plants propagated via axillary shoots in vitro were grown in the greenhouse. Plants were fertilized with complete nutrient solution containing NPK at 124; 28,8; 189 mg dm⁻³ + microelements. The experiment started after 7 months of cultivation. Three experimental treatments: I control only regular nutrient solution, II and III - regular nutrient solution containing 5 and 10 mmols of NaCl, were studied. Nutrient solutions, without and with NaCl, were supplied as sub irrigation (pots on saucers) without drainage. The evaluation taken after 16 weeks showed that NaCl reduced plant growth (leaf area, fresh and dry weight and side shoots number) and leaf gas exchange. The analyses of leaf and root samples taken after 8 weeks showed increased concentration of K, Ca, Mg, Fe, Mn, Cu, Zn, B and S SO₄ in leaves comparing to control plants. The concentration of Ca, Mg and S SO_4 in roots increased whereas, the concentration of P, K, Fe, Mn, Cu, Zn and B decreased. The concentration of Na after 8 weeks was 1.7 and 3.9 times higher in leaves, and 2.4 and 3.6 in roots, in treatments II and III respectively comparing to control. After 16 weeks, the concentration of Cl was 1.4 and 1.8 higher in leaves and 2.1 and 3.1 in roots in treatments II and III respectively than in control plants. Increasing tendency to accumulate Na and Cl in leaves and roots of anthurium as a result of continuous presence of low doses of NaCl in nutrient solution showed that in the subsequent months, the growth of plants can be steadily suppressed. The tendency to accumulate the Na⁺ and Cl⁻ ions in roots can be used as a method to select anthurium cultivars for their sensitivity to salt stress.

P30.

Different Cultivars of *Chrysanthemum* Have Intrinsic Differences in Water Losing Rate

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The genus Chrysanthemum contains different hybrids and thousands of cultivars with ornamental value. The potplant selections of *Chrysanthemum* x *morifolium* have a great economic value in Belgium. Through extensive breeding programs by Belgian companies, a continuous renewal of cultivars is introduced to the market. The different cultivars are not only phenotypically different, but will also have genotype differences which might result in different responses to abiotic stress. In order to cope with the seasonal nature of Chrysanthemum production and therefore the peak periods with a high demand of cuttings, cuttings can be harvested and stored for several days until the rooting phase can start. However, storage implies some drawbacks, one of which includes the loss of turgidity. In order to obtain an idea of the possible cultivar differences to maintain their water balance, we screened 25 cultivars. We optimized a standard protocol to determine the water losing rate (WLR). As expected, this WLR is cultivar dependent. Furthermore, the WLR does not seem to be related to the leaf surface of the different cultivars. However, it seems to be related to the number and size of stomata. Cultivars with a high density of small stomata have a lower water loosing rate. Therefore they will be able to hold turgidity longer during storage. The use of this WLR can be an easy tool in breeding programs for fast screening of the water holding capacity of cuttings.

P31.

Does Polyploidisation in Roses Result in Better Abiotic Stress Tolerance?

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Chromosome doubling is often used in ornamental plants to create variation and to induce interesting morphological traits. However, the effect of polyploidisation on abiotic stress tolerance in these crops is hardly investigated. We studied the potential effects of polyploidisation and drought stress on selected F1 genotypes of a crossing population of Rosa 'Yesterday' x R. wichurana. Of ten genotypes the diploid (2x) as well as the tetraploid (4x) counterpart, and their parental genotypes, were subjected to both a control and a drought treatment (irrigation stop). Thirty days post irrigation stop, the above ground biomasses of all plants were harvested and the fresh and dry weight per plant were determined. Subsequently, the calculations of several yield-selection criteria enabled the construction of a principal component analyse (PCA). The PCA analysis indicates on the one hand a distinct loss in above ground biomass among all studied rose genotypes as a result of polyploidisation. The effects of polyploidisation on the drought tolerance of the plants, however, is not unidirectional, with a clear distinction of three potential reaction groups, i.e. a group A with increased drought sensitivity, a group B with increased drought tolerance and a group C with unaltered drought tolerance. Accordingly, this study highlights the potential of using above ground biomass and yield-selection criteria in both polyploidisation-stress resistance research and ornamental selection studies as proxies for identifying the most optimal rose genotypes.

P32.

Screening for Cold Hardiness in Roses Lin Ouyang¹, Leen Leus², Marie-Christine Van Labeke¹

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Roses are widely used in landscaping and in gardens. Selection and breeding for different cold hardiness zones is a commercial important parameter especially if cultivars are introduced in other climate zones than the production site. Seventeen cultivars of roses belonging to different USDA hardiness zones (ranging from 2 to 6) were chosen to study cold hardiness. In a first approach stem tissue was sampled on the 3rd of February 2015, when night temperatures were below zero during one week. Subsequently, this stem tissue was submitted to controlled freezing from +2°C to -20°C at a rate of 6°C/hour and the injury index according to Flint et al. (1967) was calculated. The injury index determined at both -15°C and -20°C separated the cultivars in three groups where 'John Cabot', 'Dagmar Hastrup' and 'Moje Hammarberg' were the hardiest and 'Chandos Beauty', 'Red New Dawn' and 'Compassion' were the most susceptible to the imposed cold. As cold hardiness of plants is related to metabolic changes, soluble carbohydrates and starch content were analysed. The cryoprotective oligosaccharides were found in all cultivars but no clear difference in cold hardiness based on soluble sugars was found. Starch content in the stem tissue was still high and ranged from 4 g/100 g FW to 20 g/100 g FW indicating that stem xylem is a major storage organ in these cultivars. Next, dehydrins will also be analysed. Based on the injury index, carbohydrate and dehydrin analysis two genotypes with a different acclimation pattern are selected for more in-depth molecular studies.

P33.

Two-Phase Experiments: Vase Life Assessment in Carnations

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Good experimental design is necessary for the success of experiments and some comprise at least two phases, for example vase life trials in carnations. The first phase includes the cultivation in the greenhouse and the second the vase life assessment in the lab. In field or greenhouse experiments, it is common to use efficient experimental designs such as α -designs or row-column designs, while in the lab no particular experimental designs are applied. A vase life trial in carnations was conducted, in which it was not feasible to create an experimental design for the second phase in advance, because it was not predictable, which genotype reaches the lab phase at which time. To deal with the problem, two different approaches were established. First, in the lab all experimental units (148 blocks, each with 8 positions) were set up and defined. The first approach was based on the replicates as used in the greenhouse. At each day of harvest, the stems were grouped in the replicates and randomly assigned to free blocks. In the second approach, two random numbers for the block and the positions within the block were generated for each harvested stem, randomly selecting the positions. The data were statistical analysed with the procedure MIXED in SAS 9.3 by using linear, mixed models. By comparing the AIC (Akaike Information Criterion) of the linear models with and without using the random effects out of the lab, in both approaches the precision of data analysis could be increased.

P34.

Response of Two Iranian Damask Rose Genotypes to Exogenous Ethylene and Cloning of Expressed ACC Oxidase and Aquaporin Genes

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Damask rose (Rosa damascena Mill.) is one of the most important native species of roses in Iran that is used for ornamental and pharmaceutical purposes. To shed light on some physiological behavior of Rosa damascena Mill in response to exogenous ethylene treatment, this research was conducted and some ethylene-related genes were isolated during 2011 to 2012. After exposure of 40-cm cut flower stems of damask rose to ethylene concentrations (2, 4, 10µl/l) for 18h, ethylene-related postharvest characteristics were evaluated daily. Results indicated that relative fresh weight of two genotypes was reduced. This decreasing rate in 'Azaran' genotype was faster compared to 'Kashan'. Moreover, these results showed that 'Azaran' is more sensitive to ethylene than 'Kashan' based on leaf, petal and whole flower abscission rate. Also, in both genotypes, chlorophyll content was increased at first, and then reduced. Reduction in chlorophyll content in the more sensitive genotype, 'Azaran', was faster than in 'Kashan'. Extracting RNA from petals of 'Azaran' exposed to 10 µl/l ethylene concentration, cDNA was synthesized using oligo (dT) primer. Amplification of degenerated and specific PCR primers resulted in isolation of three partial mRNAs sequences from 'Azaran' genotype. These sequences showed a high degree of homology with plasma intrinsic protein (PIP), tonoplastic intrinsic protein (TIP1.1) and ACC oxidase (ACO) genes. These partial gene sequences revealed the highest similarity with their homologus genes from several species of the Rosaceae family.

P35.

Efficacy of Silver Nano-Particles as Anti-Ethylene Action Agent in Extending the Vase Life and Postharvest Quality of Carnation Cut Flowers

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Ethylene a well-known plant growth regulator promotes the fruit ripening and flower and leaf senescence. The responses of plant to ethylene vary among the plants individual genotypes and cultivars. Most carnation cultivars are sensitive to exogenous ethylene and their petals exhibit autocatalytic ethylene production during senescence. Compounds containing silver has been shown to act as anti-ethylene agent in improving postharvest characteristics of cut flowers. This experiment was conducted to evaluate the effect of silver nano-particles on postharvest quality and characteristics of cut carnation cultivar 'Miledy', applied in vase solution in a completely randomized design experiment with three replications. ICP-AES analyses showed that the highest amount of silver was absorbed in various tissues of plants treated with 5 mg L^{-1} , compared to other concentrations. Application of 5 mg L⁻ ¹ of silver nano-particles in vase solution of carnation cut stems of 'Miledy' cultivar resulted in the longest vase life and the lowest rate of ethylene production. The tissue accumulation of the Ag element was generally higher in basal stem ends and leaves treated with 5 mg L⁻¹ than other concentrations. The results of ICP and ethylene measurement showed that silver nano-particles reduced ethylene production as well as bacterial growth in vase solution resulting in improving the vase life of carnation.

Biodiversity

P36.

Potential Use of *Baccharis milleflora* DC. and *Baccharis tridentata* Vahl as Ornamental Cut Foliage Plants

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Identification of new ornamental plants among native species widens the offer of new products for the floricultural market. However, before indicating the use of a species as potentially ornamental, evaluation of its aesthetic features by possible consumers is of great importance. Thus, the aim of the study was to evaluate the potential as ornamental cut foliage of Baccharis milleflora and Baccharis tridentata. Ornamental potential of both species was determined basing on the methodology that considers qualitative and quantitative characteristics of ornamental importance for the consumer market. Each criterion was assessed by scores from ten (minimum) to 100 (maximum), thus the ornamental potential was established by averaging the scores assigned to each criterion. These two species showed high potential to be used as ornamental cut foliage, confirming adequate performance as complementary features in floral arrangements.

P37.

Evaluation of Hybrids of the Endangered *Pityopsis ruthii*

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Pityopsis ruthii (Ruth's golden aster) is an endangered, herbaceous perennial that occurs only along small reaches of the Hiwassee and Ocoee Rivers in Polk County, Tennessee, United States. The plants exist as rather inconspicuous basal rosettes of linear, silvery (hairy) leaves from March until August. In early to late July to August, elongated stalks are produced that support numerous delicate, yellow, daisy-like flowers, which will continue to bloom until a hard, killing frost. Pityopsis ruthii appears to be drought and flood resistant and will bloom within 1-2 years when grown from seed and in the first year when grown from stem cuttings. The desire for native plants is on the rise as awareness of the economic and ecological damage caused by invasive plants has increased. Additionally, because they are adapted to local soils and climatic conditions, native plants generally require less fertilizer and water input and are more tolerant to native pests and diseases. An informal survey administered to visitors at the University of Tennessee Gardens fall plant sale indicated interest of P. ruthii as an ornamental plant. The species has been noted as having potential as a year-round ornamental plant by landscape architects/designers and herbaceous perennial researchers. A breeding program has been initiated to develop ornamental selections of P. ruthii for landscape use. Hybrids (F_1) are being evaluated for flower structure, duration of bloom, habit, disease resistance, and tolerance to abiotic stress. Commercialization can benefit conservation of rare plants by providing information about breeding system, seed storage and germination, propagation and cultivation conditions, and increase public awareness in conservation of rare species.
P38.

Wild *Kalanchoë* Species for the Development of New Ornamental Cultivars

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The economic importance of ornamental plant industries requires constant development of novel varieties. Nowadays, the market of ornamental plants is determined not only by producers' selection and the needs of distributors but also by demands of customers. Development of new ornamental varieties has stimulated an increasing interest in utilization of wild species in breeding programs and as new cultivars. The Kalanchoë genus (Crassulaceae family) consists of around 140 species native mainly to Madagascar, Southern and Eastern Africa. The plants from the genus are perennial succulent shrublets or shrubs, rarely small trees. Kalanchoë blossfeldiana and its interspecific hybrids are commercially important as potted indoor and garden plants mainly due to abundant flowering and low demand of water and nutrients. Kalanchoë species are generally easy in cultivation and propagate readily from stem and leaf cuttings. Furthermore, due to their succulent habit Kalanchoë possesses outstanding postharvest quality. Wild Kalanchoë species have traits of potential commercial value. Flower characteristics are of great importance in ornamental production. Bellshaped flowers are a common feature of the Bryophyllum section of Kalanchoë that can be interesting in development of cultivars with new flower shapes. Several species in the genus is characterized by elongated growth habit and can potentially be used as cut flowers, a new direction in Kalanchoë breeding. A small number of species exhibit epiphytic growth habit. Species such as K. gracilipes, K. manginii and K. porphyrocalyx have potential for development of hanging cultivars. Plant fragrance can be obtained by hybridization with K. aromatica that has glandular aromatic hairs as well as K. thyrsiflora, K. petitiana or K. grandiflora that possess fragrant flowers. Here, we give an overview of selected Kalanchoë species that have potential in development of new ornamental flowering plants.

P39.

ApplicationofCryopreservationforChrysanthemumGenetic Resources ConservationDariuszKulus1, MonikaRewers2, Agnieszka Abratowska3and Anna Mikuła4

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The aim of the study was to determine the effect of sucrose concentration during preculture and the time of osmotic-dehydration on the efficiency of chrysanthemum 'Lady Orange' shoot tips crvopreservation by encapsulation-dehydration. In addition, the regenerated plants were verified at the phenotypic, biochemical and molecular levels. Shoot tips were precultured on MS medium supplemented with different sucrose concentrations of 0.09, 0.25 or 0.5 M for 14 days, encapsulated in sodium alginate and then osmotically dehydrated in sucrose gradient for 4 or 7 days. The best explant survival after cryopreservation reaching about 50% was obtained with the lowest (0,09M) sucrose concentration, and 4-day-long osmotic dehydration. It was found that higher sucrose concentrations slow down shoot growth, stimulate their vitrification and conduce to the regeneration via callus, while encapsulation inhibits rooting. Longer dehydration also led to increased formation of multiple shoots. Microscopic analyzes confirmed the protection of not only all of the initial meristem layers, but also of the leaf primordia. The analysis of the phenotype (inflorescences and leaf colour, diameter and weight, flowering time and plant habit) and biochemical activity (pigment content in ligulate flowers and leaves), as well as, cytogenetic analysis (DNA content, the number of chromosomes) and genetic markers (RAPD and ISSR) confirmed the stability of the plants obtained after liquid nitrogen treatment. However, it was noted, that the leaves of shoot tips cryopreserved-derived plants were smaller and had a reduced amount of chlorophyll, and their internodes were shorter when compared to the control. Furthermore, their inflorescences often opened slower. Finally, these phenotypic changes are positive from the horticultural production point of view. This confirms the validity of utilizing cryopreservation in the protection of valuable plant material.

P40.

Potential Ornamental Plants in Clusiaceae of China

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Based on field investigation of and literature study on family Clusiaceae (Guttiferae) in China, this paper dealt with the morphological characteristics in terms of aesthetic purposes, natural distribution, status of resources, and ethnobotany of species with potential ornamental values. There are 8 genera and 95 species in Clusiaceae occurring in China. They mainly grow in the tropical regions, except Hypericum and Triadenum, which are both mainly temperate in distribution. Some Clusiaceae species are commonly planted as street, parking lot, or garden trees, for their graceful shape, evergreen foliage, and large fragrant polyandrous flowers. With multiple uses, this family is considered as a very important group economically by the indigenous peoples in China. Except for aesthetic value, the plants of Clusiaceae can provide edible fruits, drinks, herbal medicines, dyes, oil-bearing seeds, valuable commercial resin or gum, and hard wood. Garcinia subelliptica is characteristically a coastal tree, which is often used as windbreaks, aesthetic trees and fences. In some ethnic minority areas, a few species even have unique cultural and religious significance such as Mesua ferrea. The plants we recommend as decorations comprise 27 species of trees, shrubs and herbs. Their development strategy and horticultural characters were analyzed, and proposals for their effective protection were also suggested.

P41.

Anatomical Characterization of Roots *Pilosocereus* pachycladus F. Ritter from caatinga of Paraíba, Brazil

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Pilosocereus pachycladus F. Ritter is a native cactus which is grown in the semi-arid area of Northeastern Brazil. The specimens have an ornamental value, yet studies on their anatomy are scarce. The root anatomy of P. pachycladus was studied in order to understand better the adaptation mechanisms. Specimens were collected in Areial, Arara and Boa Vista, State of Paraíba. Cross sections of roots were clarified with sodium hypochlorite (50%), stained with safranin and blue astra and examined under light microscopy. In general, the secondary structure of the roots was similar for the genotypes collected at the three sites. We observed the replacement of the epidermis by periderm, a cortex with few layers of parenchyma, collateral vascular bundles and fibers located in the parenchyma rays between beams. Druses were present in the cortical region. These were observed in higher numbers in roots collected in Areial and less frequently in roots from plants growing in Boa Vista. The presence of crystals indicate the adaptive capacity towards the xeric conditions, as a higher amount of druses was observed in specimens growing in warmer climate. This study contributes to the establishment of anatomical parameters, contributing to the taxonomy of the species.

P42.

Population Structure of *Pilosocereus pachycladus* F. Ritter in the Anthropized *caatingas* Areas from Westland of Paraíba, Brazil

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The Pilosocereus pachycladus F. Ritter is a native cactus, which grows in the semi-arid area of Northeastern Brazil. They have an ornamental value, yet studies on their ecology are scarce. The species is sensitive to human disturbance due to the low absolute growth rate, the restricted geographic distribution and the dependence of dispersal agents. We analyzed the population structure of *P. pachycladus* in disturbed *caatinga's* areas in the Westland of Paraíba. Structural surveys were made in the municipalities of Areial and Boa Vista, PB. We sampled all individuals and measured the height and diameter of each plant in a 1ha area. The Surfer® application was used to predict the spatial distribution of this species. In the Areial, 179 adults and 91 in natural regeneration (Absolute density, AD = 270 ind/ha) were sampled; while in the Boa Vista 169 in adulthood and 3 in natural regeneration (AD = 172 ind/ha) were found. The population of *P. pachycladus* in Areial showed a lower basal area (BA = 2.10 m^2) compared with the population of Boa Vista (BA = 3.82 m^2). In both *caatingas* individuals tended to cluster. It seemed that the populations were spatially present in small clusters within the studied environments. The specimens of Areial, area where a higher degree of human disturbance was observed, had a lower stem diameter and total height, in comparison to Boa Vista, PB, which is a more conserved land area. The populations showed similarity in terms of spatial distribution, yet structurally the anthropization affects significantly the development of P. pachycladus.

P43.

Characterization of *Paspalum* Accessions as Ornamental Lawn

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The Lawn industry involves hundreds of millions of dollars around the world, despite its recognized importance in landscaping, Brazilian lawns are formed mainly by Paspalum notatum. Brazil has extensive natural genetic variability between and within species of genus Paspalum, important source of genotypes with high potential for use as lawns. This study aimed to select the best Paspalum access to use as cover for ornamental purposes with rapid expansion and low need for pruning the conditions in northeastern Brazil. Five pre-selected Paspalum accessions (0102254, 043566, 023728, 010006 and 019178) were planted and field evaluations were made at fifthteen-day intervals for two years after establishment. Soil coverage rate, ornamental quality (general appearance) of the lawn, extension of lateral growth, color homogeneity and weed invasion were rated by means of a visual appraisal. Based on the results obtained at the end of the 2st year of cultivation, the accession 023728 had the higher scores and demonstrated ornamental quality equal to or greater than the control.

P44.

Selection of Alpinia as Cut Foliage

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Among the species of tropical flowers produced in Brazil, the genus Alpinia stand out as cut flowers, foliage and ornamental plants. Actually Alpinia purpurata cultivars, known as Gingers, are cultivated as cut flowers for their productivity, beauty, postharvest durability, transport and marketing facility and good acceptation in national and international markets. Recently the farmers started to cultivated Alpinia zerumbet and Alpinia sanderae for cut foliage due their excellent decorative effect, nevertheless it presents some problems of postharvest durability. The objective of this study was to evaluate the potential of Alpinia species, cultivated at 50% artificially shaded, to be used as cut foliage. The number of leaves and postharvest durably from six cultivars of Alpinia purpurata were analyzed. The leaves were cut from basal, middle and apex parts from the stems with inflorescence at the harvest point. The durability and adequacy of Alpinia purpurata cut leaves differ between the cultivars. The clump high, clump projection area, diameter, stem height, number of leaves from A. zerumbet and A. sanderae cultivated in full sun and at 50% artificially shaded condition were observed. The crop conditions of 50% artificially shaded contributed positively to the clump high, clump projection area, diameter and height of those two species. Leaves postharvest durability from A. zerumbet and A. sanderae cultivated at natural shade condition were observed too. The leaves were harvest from basal, middle and apex parts from the stems with and without inflorescences. Cut leaves from A. zerumbet stems without inflorescences present more than 30 days of postharvest durability.

P45.

Characterization Descriptors of Bearded Irises

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Irises are herbaceous perennial plants that belong to Iridaceae family. The Iridaceae has about 80 genera spread throughout tropical and subtropical climatic zones. Around the world approximately 300 iris species are known plus hundreds of subspecies, collected forms, and natural hybrids. All these plants come from the Northern Hemisphere and can be found growing as far north as Scandinavia to Japan, as well as in many parts of Europe. Their habitats range from mountainsides to deserts, open grasslands, dense woodland, and sandy coastal areas. Lithuanian cultivars of bearded irises are created by Petras Balčikonis, Jonas Auksuolis Liutkevičius, Jonas Tarvidas, Gintaras Klimaitis, Algirdas Gražys, Ona Griniuvienė and Dalia Žigarienė. Forty cultivars of Iris were included in the National List of Plant Genetic Resources of Lithuania. All the cultivars of bearded irises are assessed, and a big variety of their morphological characteristics are identified. This article presents the characterization descriptor of bearded irises used for a diagnostic purpose to describe the irises.

P46.

In Vitro Culture of South African Ornamental Bulbous Species Suitable for Cultivation in Mediterranean-type Climate Area of the Northern Hemisphere

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There are five geographical areas in the world that are characterized by possessing the Mediterranean-type climate: Chile, California, South Africa, south-west of Australia and the Mediterranean Basin. The presence of the same environmental and climatic characteristics allows species exchange and acclimatization. Several bulbous species (Aristea capitata (L.) Ker Gawl., Babiana, Bulbinella nutans (Thumb.) T. Duran & Schinz, Massonia depressa Houtt., Romulea hirsuta (Steud. Ex Klatt) Baker) have been selected for ornamental characters to explore the suitability of cultivation in the Mediterranean basin. Studies to verify the attitude to in vitro propagation and to set up a micropropagation protocol have been carried out to provide homogeneous selected material for a fast delivery on the market. The in vitro culture started from germinated seeds which were previously sterilized with a solution of sodium hypochlorite (1% of active chlorine) for 10 or 20 minutes depending on the species. Then the seeds were rinsed twice in sterile distilled water for 10 minutes each. The sowing was carried out in Petri dishes containing sterile medium composed of distilled water gelled with agar (7 g/L). After germination, the roots were discarded and the shoots were grown on MS base media containing salts and vitamins, sucrose (30 g/L) and agar (8 g/L). Three indole-3-butyric acid (IBA) concentrations (0.25, 0.5 and 0.75 mg/L) were added to the base media and the explants growth was compared against the plant growth regulators (PGR)-free control medium. The glass vessels were placed in growth chamber at 23±1°C with a photoperiod of 16 hours of light at 30 μ E m⁻² s⁻¹. After 30 days, explants height and weight, the multiplication rate, the rooting percentage and the explants quality were recorded. The plantlets were then transferred in the greenhouse for acclimatization under a mist system and subsequently grown in pot.

P47.

Assessing Vegetative Production Studies of 22 Native Plant Species Growing in Antalya, Turkey, Carrying Outdoor Ornamental Plant Potential

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Due to the global climate changes, developing environment consciousness, changing consumer trends and high adopted xeriscape approach laid out the growing use of native plants worldwide. Turkey has very rich plant genetic resources, some of which carry ornamental plant potential for landscape use. Their domestication is especially important for the assessment and exploitation of the underused genetic resources. This study was conducted in Batı Akdeniz Agricultural Research Institute in Antalya, Turkey, during 2007-2009, under TUBITAK-KAMAG-105G068-106G020 project. It was aimed to vegetatively propagate 21 native plant species including herbaceous, ivy, shrub, short and long tree types, some of which are endemic. Mainly adventitious rooting method via stem cuttings was used for the production of species which were Acer sempervirens, Amelanchier parviflora, Ampelopsis orientale, Arbutus andrachne, Cionura erecta, Clematis cirrhosa, C.vitalba, Daphne sericea, D.oleoides, Erica bocquetii, E.manipuliflora, E.sicula subsp. libanotica, Melissa officinalis, Myrtus communis, Ostrva carpinifolia, Pistacia terebinthus, Pyrus serikensis, Sedum sediforme, Sorbus umbellata var. umbellata, Tamarix tetrandra and Vitex agnus-castus. Additionally, stock division (for Carex pendula) and radial layering (for A.orientale and *C.cirrhosa*) methods were applied in the work. Regarding substrate, the stem cuttings were inserted in perlite, peat, peat:perlite (1:1), sand, sand:peat:perlite (1:1:1) or cocopite:sand (5:1). As the hormone, mostly commercial powdered rooting hormone was treated to the cuttings, beside the experimental IBA concentrations. 15 of the 22 plant species were propagated at different ratios ranging between 5% and 100%. The cuttings of A.orientale, C.erecta, C.cirrhosa, M.officinalis and S.sediforme were rooted at maximum rate (100%). Also, C.pendula was very regenerative for producing sister plants. Propagation results of this initial work were found very promising for the cultivation and domestication of the most of the targeted plant species. It is also possible to increase the propagation rates by different substrate and hormone applications in the future studies especially for non-propagated species.

P48.

Chinese Aceraceous Germplasm Resource Analysis: a Case from Kunming Tong Xin and <u>Chunlin Long</u>

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Most Acer species and cultivars are known for their graceful tree performance and the beautiful leaves with higher ornamental values. We surveyed and analyzed 15 parks in Kunming City, Yunnan Province, China. Based on SBE (Scenic Beauty Estimation Procedures) method, a quantitative analysis of 56 aceraceous sceneries were made . We also evaluated the germplasm resources and application of aceraceous plants in Kunming. In total 60 species of colored plants had been applied in Kunming parks, but only 7 species of aceraceous plants could be found in these parks, which implies a great potential to apply acerceous plants in Kunming landscape development. Some wild germplasm resources in the genus Acer were proposed for future uses in horticulture and gardening. Strategies for conserving aceraceous plants were also discussed, based on the status of some rare and endangered species.

P49.

Characterization of Senescence and Increased Durability of Harvested *Baccharis milleflora* dc. Stems

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Brazilian flora has several endemic plants that can be exploited as ornamental cut foliage, such as Baccharis milleflora DC., a species commonly found in the countryside landscapes of southern Brazil. It is an evergreen shrub that presents bright green cladodes that can be used to increase volume and serve as ornamental background in floral arrangements. However, studies about its durability and management are required to indicate its use as an ornamental cut foliage. This research focused on the characterization of the senescence process of stems, assessment of their durability when harvested in different seasons and on their management to increase this durability. Results are presented as a proposed grading scale of the senescence process, to be used in sensory evaluation. Furthermore, agricultural parameters and enzymatic characteristics of stems during vase life were analyzed. The minimum durability of stems was seven days (in spring) and maximum was 13 days (in autumn). Pre-cooling of stems in summer, to remove field heat, increased the durability of harvested stems. The applied management treatments in vase did not increase durability, in any of the four studied seasons.

P50.

Axonopus parodii and Paspalum spp. Ornamental Quality without Mowing

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The characterization of Axonopus parodii and Paspalum spp., both native species from Brazil, for their use as ornamental lawns started in 2012 by in the Paspalum Germplasm Bank from Embrapa (Brazilian Agricultural Research Corporation) localized in São Carlos-SP, Southeast Brazilian region. After a first evaluation of 40 accessions, eight accessions showed potential to be used as ornamental lawns: Axonopus parodii Vall, ined. (BRA 002658); Paspalum notatum (BRA 019178, BRA 023558, BRA 023566, BRA 023728, BRA 012254 and BRA 006301) and P. lepton (BRA 023591). Those accessions were cultivated in the condition of Rain Forest Zone -Pernambuco, Brazil during the period of October, 2013 to march 2014. The DBC design was adopted, with four replicates (one square meter). The accessions were characterized for following traits: period to be fully established; soil coverage rate and color homogeneity (by image analysis - program SisCob); general appearance (scale of notes: 1 - good appearance to 4 bad appearance); weed occurrence; height of the grass. The ornamental quality was variable between accessions, indicating that this is an aspect to be observed for the selection of these species as lawns.

P51.

Tissue Culture of Selected Plant Material from Dahlia brevis an Endangered Mexican Species

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The genus Dahlia is originated from the highlands of Mexico, and includes 41 species on four sections according to the growth habit. Dahlia brevis belongs to the section Dahlia and has an herbaceous habit, it is an endangered plant due to the human and livestock activities in the natural area of distribution. According to a National Program for collection and conservation of germplasm, accesions of D. brevis were collected and used for formation of half sib families, selection of the progeny was made for a new head flower color. Plants with white, orange and pink flower color were obtained from the wild light purple color population. Shoot tips of this plants were cultured on a Murashige And Skoog medium with mineral salts at 50% of their concentration and supplemented with PVP (Polivinilpirrolidone), benciladenine 0.5 mg/l⁻¹ and Naftalene Acetic Acid 0.5 mg/l⁻¹ for shoot multiplication. Rooting shoots were obtained in the same media with Indole Butiric Acid and Naftalene Acetic Acid $(0.5 : 0.5 \text{ mg/l}^{-1})$. Tuberous roots were observed on this media, and plants were acclimated in a greenhouse with a survival rate of 95%. Plants reached the flowering state after three months of cultivation. This method of in vitro mass propagation enables an efficient propagation for this endangered species of dahlia. Cloned plants were registered as new varieties for mexican horticulture.

P52.

Herbaceous Ornamental Perennials of the Main Botanical Garden Named after N. V. Tsitsin Russian Academy of Sciences

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The successful activity of the Main Botanical garden for 70 years has helped to create one of the largest collection of ornamental perennials. This collection is used to research on the morphology, anatomy, biochemistry, physiology and plant protection from pests and diseases. Nearly 15,000 taxa were tested. Now, the collection of the Main Botanical garden - 2956 taxa (2234 varieties and 772 species) included 277 genera belonging to 66 families. A significant part of the collection consists of ornamental species of the natural flora - 722 species. Cultural flora has been enriched by species of the genera Trillium, Hosta, Jeffersonia, Lysichiton, Chelone, Echinacea, Heuchera, Liatris, Rodgersia and etc. Analysis of the collection origin showed that the majority of species associated with meadow communities (Lilium, Geranium, Centaurea, Paeonia, Narcissus, Phlox), steppe plants (Tulipa, Iris, Heuchera, Crambe, Allium) and forest communities (Anemone, Asarum, Aruncus, Podophyllum, Pulmonatia, Trillium). Thus, in the collection there are species and varieties of ornamental plants, reflecting the biodiversity of ornamental perennials of temperate zone, which are able to decorate the flower beds throughout the growing season.

P53.

In Vitro Culture Initiation for *Lychnis nivalis* and *Silene dinarica*, Endemic and Rare Species

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Lychnis nivalis Kit. is an endemic species for Romania in Rodnei Mountains and it is considered rare or vulnerable. Silene dinarica Spreng. is endemic in the Fagaras Mountains (Romania) and it is considered a rare species. The ex situ preservation by in vitro collections of these species is a complementary method of in situ conservation for the endemic and endangered species. The in vitro culture was initiated from seeds collected from 2 different locations/each species. The main disinfectant agents used for the 3 methods of disinfection were: (1) hydrogen peroxide 4% and then 12%, (2) Domestos 10% and mercuric chloride 0.15% and (3) sodium hypochlorite 15%. The seeds were single inoculated in culture vessels and kept to light or dark. The disinfection rating was very high for all the 3 variants (97.5-100%). The percentage of germinated seeds after 20 days from inoculation was 25% for S. dinarica and 61.6% for L. nivalis. In case of L. nivalis, the disinfectant variants 1 and 3 led to similar results: 85% and 80% of germinated seeds in light and dark, respectively. For S. dinarica the best results were obtained in case of variant 1 - 30% and 25% germination in light and dark, respectively. The disinfection with mercuric chloride highly inhibited the germination. In conclusion, the disinfection with hydrogen peroxide is the most recommended method for the initiation of in vitro cultures for the 2 studied species. Keeping the seeds in light slightly stimulated their germination. The in vitro obtained plants were transferred for micropropagation culture media with hormonal balance on cytokinins/auxins 1/0.2.

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Breeding

P54.

New Members of the Iridaceae Family: Interspecific Hybridization between *Iris dichotoma* and *I. domestica*

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Iris dichotoma and I. domestica are Chinese native ornamental plants, both belonging to the Iridaceae family but different species. Iris breeding research is mainly focused on interspecific breeding, and intergeneric hybridization has been rarely attempted. Amplified fragment length polymorphism (AFLP) markers were employed to determine the genetic relationships among ten Iris species, including Iris dichotoma and I. domestica. The analysis indicated that Iris dichotoma and I. domestica were closely related, and it showed the possibility of interspecific hybridization between the two plants. Therefore, we selected I. domestica as male parent and transferred the pollen to Iris dichotoma, and interspecific hybrids were obtained. The offspring was identified as true hybrids by morphological identification and AFLP analysis. As interspecific hybridization between Iris dichotoma and I. domestica was proved to be feasible, the second filial generation (F2) and the first backcross generation (BC1) were obtained successively. Studies about the variation of hybrids got underway. The results showed that, F1 hybrids resemble their parents and most of them look like their female parent. However, the F2 offspring, unlike parents, have greater variation, especially in the flower colors and flowering time. In addition, all generations we obtained are fertile, showing that there are no reproductive barriers in parents and interspecific hybrids. This is consistent with our AFLP results that Iris dichotoma and I. domestica were closely related and they can breed with each other. With the progress of interspecific hybridization, there will be more variation in the following generations.

P55.

Interspecific Crosses in *Passiflora* to Obtain New Hybrids with Ornamental Value

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The genus Passiflora (tribe Passiflorae, Family Passifloraceae), believed to be originated in the Americas, comprises more than 560 species of vines, lianas and small trees. It is subdivided by Feuillet and MacDougal (2004) in four monophyletic subgenera: Astrophea (57 species), Deidamioides (13 species), Decaloba (214 species) and Passiflora (236 species). Passion flowers are considered ornamental plants because of the exuberant variant foliage and the attractive, colorful and exotic beauty of their flowers. Since their introduction to the Old World, around the seventeenth century, they have been used to decorate European glasshouses and gardens. Interspecific hybridization has been used to produce beautiful hybrids, with unique floral features. The present study describes the results of 17 years (1997-2014) cross breeding among Passiflora species and hybrids cultivated in Italy, to produce new varieties, for garden and pot plants, adapted to the Mediterranean climate. Pollinations were manually performed in warm greenhouses, in order to determine the reproductive compatibility between involved parents. Fruits were collected at ripening and the seeds were sown in controlled conditions. Several Passiflora hybrids have been obtained for ornamental purposes in the two most representative subgenera: Decaloba and Passiflora. Moreover, micropropagation protocols were applied to propagate and preserve the valuable plants and Tubulin-Based-Polymorphism (TBP) genotyping was used to produce a specific DNA barcode for each interspecific hybrid. Thus, the created TBP pattern, that reveals the genetic contribution of both parents in each cross, can supply an efficient tool for a rapid screening of the hybrids, promoting a clear identification and exploitation of new ornamental products.

P56.

Interspecific Hybridization in the Tribe Genisteae with Immature Fruit Rescue in Vitro Culture

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Since 2006, the Eurogeni GIE*, in cooperation with Joint research Unit IRHS (Institut de Recherche en Horticulture et Semences) has developed a breeding program on ornamental plants of the tribe Genisteae (Fabaceae). We have used interspecific hybridization associated with in vitro culture to produce innovative hybrids. The aim was to obtain hybrids with new flower color. To overcome post-zygotic incompatibility and embryo abortion we have previously developed an immature fruit rescue protocol using in vitro culture. Four different crosses between one male parent and four different female parents were achieved in greenhouse and in field. Young fruits were collected four to six days after pollination and cultured in vitro, until ovules extraction. Ovules were cultured until germination that occurred one to nine months later. Thirty-eight different clones were obtained, at least one for each different cross. Only twenty-nine survived until rooting in vitro, representing three interspecific crosses out of the four. Twenty-two clones are already acclimatized in greenhouse. Those clones were proven hybrid by molecular test using previously developed ISSR markers. Hybrids have also been characterized by flow cytometry. Based on this, some clones appeared to be tetraploid. We highlight the importance of parental genotype choice for breeding success in this group. Currently, the available clones are still waiting to flower.

* Eurogeni GIE is a group of European nurseries: Pépinières Minier, Pépinières André Briant Jeunes Plants, Pépinières Renault, Pépinières J. Dupont et Fils, Kordes Jungpflanzen, Pépinières Levavasseur, EARL Nicolandes.

P57.

Morphological Aspects in *Heliconia chartacea* Inflorescences for Use as Cut Flower

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Heliconia chartacea is native from Amazon Forest, South America. It is a herbaceous plant with pendulous inflorescence. The bright pink and light green bracts color of the inflorescence is very attractive. Those colors combination are unusual making the inflorescence reach high values as cut flowers. The farmers often use rhizome propagation. Nevertheless, because of the high value of the rhizome the farmers use seed propagation, promoting high variability in the plantings. The objective of this research was to characterize 19 distinct H. chartacea forms (clumps) identified in a farm in Goiana, Pernambuco – Brazil and to select plants with desirable aspects for cut flowers. The inflorescence length varies according to the number of open bracts and the inflorescence width range from 20 to 45 cm. The stalk length range from short (9 cm) to long (47 cm), with open angle in relation to the stem. The rachis differ in relation to the diameter (6.4 to 9.2 mm), color (pale pink to dark pink), shape (straight to very winding) and stiffness. The rachis twist is variable with the number of open bracts. The postharvest durability is higher than 10 days, depending on the number of open bracts. The presence of appendices leaf in the first bract in inflorescence from some clumps was observed. Inflorescence width larger than 35 cm, long peduncle, appendices leaf in the first bract and postharvest durability less than ten days were consider negative characteristics. Considering the characterization, the clumps present morphological variation and distinct forms. This variation will permit to select promising materials for crosses and those with more suitability characteristic for cut flower cultivation could be introduced to the cut flower market.

P58.

New Ornamental *Lophospermum* Hybrids through Interspecific Hybridization

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Lophospermum D. Don (Plantaginaceae Juss. formerly Scrophulariaceae Juss.) is a genus of plants with seven species that can be found in the area from Mexico to Guatemala. Some species of Lophospermum are grown in the greenhouses or in the open field for their ornamental flowers and leaves. Lately, the genus Lophospermum has attracted the attention only of few scientists. Particularly the species L. erubescens and L. scandens as ornamental plants for commercial use were hybridized and interspecific hybrids are patented. a limited number of cultivars are commercially available. Interspecific hybridization among species of Lophospermum offers the potential for new cultivars with desirable ornamental characteristics. Interspecific hybridization has been successful between some species of Lophospermum, viz., L. erubescens D.Don, L. scandens D.Don and L. purpusii (Brandegee) Rothm. The interspecific hybrids exhibited morphological intermediacy for various vegetative characteristics (trailing, climbing and erect plant habit; vigorous growth and freely branching; good garden performance, etc.) and produced flowers with distinct colours and shapes. Backcrossing of the F_1 hybrids resulted in a number of flower colours enhancing the ornamental value of the genus and new hybrids and cultivars were developed.

P59.

Selection of the Best Black Mondo (*Ophiopogon planiscapus* 'Nigrescens') Clone in Tissue Culture Conditions for Micropropagation

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Black mondo, Ophiopogon planiscapus 'Nigrescens', is an economically valuable ornamental grass creating a stunning contrast image with its almost black colour leaves. Conventional propagation by seed or division falls to meet current demand. In contrast, micropropagation presents an opportunity for the mass production in large quantities in a very short time. The major goal of this study was to choose the best black mondo clone, having the ability to simultaneously give superior shooting and rooting, among 114 genotypes for micropropagation. A kind of in vitro selection method consisting of the repeated 4 multiplication and 3 rooting cycles was performed on the in vitro germinated seedlings. Multiplication cycles were carried out in the agitated liquid system while the rooting procedures were executed in both solidified and liquid media to compare their efficiency. Meanwhile, the effect of higher concentrated sucrose on rooting ratio was examined. Our first selection criteria was to determine the genotypes, having the multiplication rate \geq 3 and rooting ratio more than 95%. Then the best three clones were selected among them. Accordingly, the multiplication ratios of the best three black mondo clones were determined to be 4.12, 3.65 and 3.27, respectively, while all of their rooting ratio was 100%. The findings revealed that Ophiopogon planiscapus 'Nigrescens' can be successfully micropropagated and additionally, this kind of *in vitro* selection method could be used efficiently for selection of the best clones either in black mondo, or possibly in other plant species.

P60.

Interspecific Hybridization between Dahlia dissecta and D. rupicola

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Dahlia is considered the Mexico's National Flower since 1963. It belongs to the genus Dahlia (Asteraceae) which consists of 41 species divided in four categories, according to the growth habit and leaf morphology. Dahlia dissecta and D. rupicola are in the Entemophyllon section and are distributed in the north part of Mexico. D. dissecta has a recurrent flowering habit whereas D. rupicola shows drought resistance. The aim of this work was to obtain interspecific hybrids, which shared characteristics of both species. D. dissecta was hand pollinated with D. rupicola, the obtained seeds were sown and grown up till the flowering stage, when morphological characteristics were evaluated. The progeny showed a morphology with intermediate characteristics of branching, height and leaf type. The flower heads had a diameter ranging from 10 to 16 cm with a pale purple color similar to the wild type. Phenotypes with branching and unbranching stems were observed. The flowering period indicated that some plants had the recurrent habit of D. dissecta. ISSR (inter simple sequence repeat) markers were used to confirm the hybrid state of the interspecific hybrids.

P61.

Intraspecific Cross Compatibility in Ornamental Pepper

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The production of Capsicum with ornamental purpose in Brazil increased in recent years. To support the activities of this promising market it is essential to develop new cultivars by hybridization. So, the knowledge of cross compatibility is important for plant evolution providing new genetic combination and to promote speciation. There are few records of intraspecific compatibility studies In Capsicum as well as their possible causes. The goal of this work was to evaluate and describing the cross compatibilities in Capsicum annuum. This experiment was carried out on the greenhouse of Universidade Federal da Paraiba. Nine lines of Capsicum annuum UFPB 45, UFV 46, UFPB 132, UFPB 134, UFPB 390, UFPB 392, UFV443, UFV 448 and UFV 449 belonging to the UFPB and Universidade Federal Visoca (UFV) Horticultural Germplasm Bank were chosen for the study, based on their broad genetic background and phenotypical diversity, and then they were crossed in a complete diallel way. The manual crosses were made in pre anthesis stage in emasculated flowers. The percentage of fruit set was estimated as number of formed fruits by the total number of crosses. The percentage of fruit set varied between 20 to 100%. The intraspecific compatibility varied both with the direction of the crossings as with the genotype used in the crossing. This percentage shows the intraspecific incompatibility and it depends on the genotypes. Parthenocarpic fruits and fruits with very few seeds were observed. Some fruits showed apocarpy, while some crosses showed irregular fruit shape and anthocyaninin into the placenta. These results indicated that knowing the cross compatibility is important to obtain intraspecific hybrids in breeding programs.

P62.

Intergeneric Hybrids between Species of *Hydrangea* L. and *Dichroa* Lour. - Germination in Vivo and in Vitro and Molecular Verification by RAPD Analysis

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Hydrangea macrophylla (Thunb.) Ser. is still very popular as a pot plant with a long breeding history. The genetic background is rather narrow although several hundred cultivars are known. The base of the most cultivars today goes back to few progenitors of H. macrophylla. In the past H. serrata (Thunb.) Ser. was used for widening the gene pool of *H. macrophylla* and to increase the genetic phenotypic diversity. Phylogenetic studies and demonstrated the close relationship of Dichroa and H. macrophylla and allies, potentially allowing further widening the gene pool and phenotypic variability through hybridization. Therefore, first crossings were realized between H. macrophylla as well as H. serrata cultivars with D. febrifuga (Lour.) or D. yunnanensis (S. M. Hwang.) Pollination by hand was done in late spring and summer 2013 and seeds were harvested in September 2013 until January 2014. The seed set varied depending on the parental partners. Macroscopically normal developed seeds and seeds where the seed number exceeded 30 per cross-combination were sown in peat soil in the first week of February 2014. Germination in vitro was applied for the few seeds obtained from eight cross-combinations. The best germination rate was observed with sterilized seeds after a treatment with 1.3 mg L^{-1} GA₃ and 1.0 mg L^{-1} KNO_3 followed by germination on 1/2 B₅-medium supplemented with 10 g L^{-1} sucrose and 0.13 mg L^{-1} GA₃. Plants originating from in vitro germination were successfully transferred to greenhouse cultivation. RAPD analysis performed on plants showing peculiar morphology confirmed that intergeneric hybrids between Dichroa and Hydrangea ssp. have been successfully generated by cross-breeding.

P63.

Tetraploid Male Fertile *Pelargonium crispum* Hybrids and their Use in Interspecific Hybridization

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Pelargonium crispum hybrids (section Pelargonium) are attractive ornamental plants with a long breeding history. Due to the domestication effect a low level of genetic and phenotypic variation is shown in seedling populations after cross-breeding with available cultivars. The genus Pelargonium offers with over 250 wild species in 16 sections a large unutilized genetic diversity. However, crossing barriers like different ploidy levels limit their use for hybridization. Here are shown preliminary results for polyploidization of diploid P. crispum hybrids (2n=2x=22) and their use for interspecific hybridization. Seedlings of P. crispum hybrids (cotyledon stage) were treated with a 200 μ M solution of amiprophos methyl on three consecutive days. Morphologically altered plants were investigated by stomata length measurement and flow cytometry. Genotype P615 showed a tetraploid DNA content, but longer stomata than the tetraploid standard. Genotype P616 indicated stomata lengths between diploid and tetraploid as well as a DNA content like diploids. Therefore, P616 was classified as mixoploid (4x/2x). To avoid mixoploid plants in further crossing programs, P615 and P616 were somatically segregated into homohistic diploid or tetraploid plants by formation of adventitious shoots in vitro. Regenerated plants were characterized morphologically, by flow cytometry and through genotyping. P615 produced exclusively 16 tetraploid regenerants. In contrast, 274 diploid and 17 tetraploid regenerants arose from P616. To determine the genetic variability, which potentially resulted from the in vitro culture or in the process of polyploidization, the genetic distance of P615 and nine tetraploid regenerants as well as P616 and eight tetraploid regenerants were investigated. The P615 group and the P616 group revealed a genetic distance of 0.1. We conclude, no further genetic variability, except the chromosome doubling, resulted from polyploidization and in vitro culture. Tetraploid regenerants were used in reciprocal cross-breeding with cultivars of P. ×domesticum (2n=4x=44).

P64.

Inheritance of Flower Color Characteristic in *Habenaria* Hybrid

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Inheritance of flower color characteristic in *Habenaria* was investigated by hybridization between *Habenaria rhodocheila* Hance, pink color, and *Habenaria xanthocheila* Ridl, yellow color. The hybridization showed nearly 100% seed capsule setting. There were 25 and 28 seed capsules in 2011 and 2012, respectively. In vitro seed culture in aseptic conditions showed that about 50% of seed capsules gave hybrids and reciprocal hybrids, which were around 104 and 127 plants, respectively. In 2014, flowers were observed from approximately 25 % of hybrid plants and most exhibited pale orange tone. The preliminary results from color characteristics of the hybrids showed that maternal effects were ineffective and an incomplete dominant genetic suppression was expressed.

P65.

Ethyl-Methane-Sulfonate in the Generation of Genetic Variability in *Capsicum*

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The genus Capsicum comprises economically important species around the world, among them is the most cultivated Capsicum annuum L., which has great ornamental potential. Methods to create new varieties of plants are targets of the studies of the breeders. A method that gets highlighted in several studies is the induction of mutation. Among the mutagens used Ethyl-Methane-sulfonate (EMS) is less phytotoxic and is reported as highly efficient. Given the above, the objective was to analyze the effect of the mutagen Ethyl-Methane-Sulfonate (EMS) in ornamental pepper (C. annuum L.). Seeds were soaked in distilled, deionized and autoclaved water for 12 hours, then were subjected to different treatments of Ethyl-Methane-Sulfonate (EMS), seven concentrations (0,0; 0,025; 0,050; 0,10; 0,15; 0,20 and 0,25%) and two exposure times (3 and 6 h), resulting in fourteen treatments. The seeds were inoculated in vitro on MS medium. Seedlings were acclimated to pots with substrate and then transferred to the greenhouse. There were no significant differences for the variables germination and seedling height. Of eighteen traits evaluated in plant stage (leaves, flowers and fruits), thirteen showed significant differences: petals diameter, stylet length, fruit weight, highest and lowest fruit diameter, pedicel length, thickness of the placenta, placenta length, number of seeds, fruit fresh matter, fruit dry matter and dry matter content, which indicates that the EMS contributed in generating of plant fruit variations arising of seeds treated with ethylmethane-sulfonate. In conditions of this study, the dose (0,1 %) and the exposure time of 3h EMS were responsible for major variations in traits, which can be used in breeding programs of this species.

P66.

Determination of Sensitivity to Gamma Rays and Mutation Induction in *Mandevilla* sp Cuttings

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Mandevilla genus, Apocynaceae family, has 151 known species that stand out for great ornamental and pharmacological potential. Despite the beauty of flowers, a few species of this genus are currently employed as ornamental plants. Mutation induction can generate hundreds of new ornamental cultivars. There are no reports in the literature on the use of mutation induction in Mandevilla. Cuttings of 10 cm length, with or without roots were irradiated with doses ranging from 7.5 to 52.5 Gy of gamma rays. Part of the 2 types of cuttings were protected at the plant base from radiation with a lead ring. After treatment, cuttings were planted in pots in a randomized block design in three replications. The already rooted cuttings, with or without protection were more sensitive to gamma rays (lower values of LD30 and LD50) than without roots. The protection of the cuttings decreased the sensitivity. For further work irradiation of unrooted cuttings with protection on plant basis should be applied, as this allows the use of higher doses of gamma rays: 24.7 Gy (LD30) and 41,2Gy (LD50) values obtained by linear regression. These doses were used on 2000 Mandevilla unrooted cuttings and protected by lead ring. This resulted in dwarfness and double coloured flowers.

P67.

Mutation Breeding Using Gamma-Rays to Search for New Traits and Increase Seed Germination Efficiency in *Rosa hybrida*

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Mutagenic agents, such as radiation and chemicals, are used to generate genetic variation and select the desired traits in commercially valuable plant species. Ionizing radiation, in particular, have been successfully exploited to develop new plant varieties with improved characteristics. When using gamma-ray-mutagenesis the optimal dose rate needs to be defined, based on radiosensitivity, which is dependent both on genotype and on physiological conditions of plant material. Modern rose (Rosa hybrida) breeding programs mainly focus on the introduction of new flower colour, shape and scent, thornless stem, higher production and good post-harvest performance. Protocols currently available need to be improved in order to increase mutation frequency and enhance commercial exploitation. The present work describes the effect of gamma-rays (0, 75 and 150 Gy) on cuttings of fifteen élite Rosa hybrida cultivars, provided by NIRP International, a leader Company in rose breeding and production, that after treatment were bud-grafted on the suitable rootstocks. On the other hand, rose propagation by seed is used for breeding new cultivars as well as for the production of rootstock plants. Seed germination is quite problematic due to endogenous and exogenous dormancy, requiring prolonged and expensive processing and a low germination rate (less than 20%) is still a major problem encountered in rose breeding programs. To avoid the risk of obtaining a small amount of seeds during hybridization, breeders use large quantities of pollen from a limited number of male parents chosen for their known fertility. Furthermore, exogenous treatments can be employed to improve the germination of seeds. For this reason, the present study also shows the effect of gamma-rays (0, 50, 100 and 200 Gy) on seed germination of the progenies coming from six different crossings of NIRP International Rosa hybrida cultivars.

P68.

Development of Ornamental Pinneapple as Potplant

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Ananas species (Bromeliaceae) are herbaceous monocotyledonous perennial tropical plants. Some of them are ornamental, for their beautiful, exotic and long-lasting inflorescence and foliage. Some hybrids of Ananas comosus var. bracteatus x A. comosus var. erectifolius are being selected for use as pot plants, landscaping and as cut flower because of their ornamental appeal. The objective of this study was characterizing the development of two ornamental pineapple hybrids as pot plants. Two accessions (A and B) of ornamental pineapple from Pineapple Active Germplasm Bank of Embrapa Cassava & Tropical Fruits were used. The plants acclimatization lasted 60 days and were then transferred to plastic 1L pots containing commercial substrate. They were grown under protected environment 80% shaded and additional fertilization was given monthly. Evaluations were made of the morphological characteristics, plant height, number of leaves and dry matter production and response to floral induction. Accession A with leaves predominantly purple-green, green at the base and apex had a greater development, height and number of leaves and mass accumulation compared to accession 'B' with light green leaves. Accession A had also more persistent old leaves and necessity of pruning. Both accessions are small-sized plant with no spines on the leaf margins. The slow growth until 90 days of cultivation indicates the greater need for fertilization after 150 days. Both were responsive to floral induction at 10 months and suitable for pot cultivation.

P69. Gladiolus Breeding in Korea

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In Korea, gladiolus is mostly used as cut flower. In many cases corms from foreign cultivars are imported. The initial development of our gladiolus research started with testing new cultivars from abroad on cut flower quality, environmental adaptability, etc. In 1986, a gladiolus breeding program was initiated. The first new cultivars were released in 1995 with the reddish 'Hongkwang', and the bright orange-based 'Hongeun'. During the period of 1998-2003, mainly red and white cultivars have been developed. Later on other colors and both very early flowering or very late flowering cultivars were introduced into the market. In 2012, the yellow colored cultivars with a lot of florets 'Yellow Rock' and 'Focus' have been developed. During this period breeding and selection of garden types started. A total of 49 varieties have been developed to date. Every year, on average two or three new varieties which are adapted to the local environmental growing conditions are released. Since 2002 research on resistance to thrips, which damages gladiolus leaves and flowers, is carried out. By crossing resistant genotypes, two new cultivars with improved thrips resistance could been developed. Finally in 2013 'Pink Smile' was selected which has a good resistance towards the flower stalk bending phenomena in the wet summer conditions. In future, hybrids between spring flowering cultivars and autumn flowering cultivars with many and small florets will be developed.

P70.

Analysis of Divergence and Correlation of Quantitative Traits in Ornamental Pepper (*Capsicum* spp.)

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This work aimed to estimate the genetic diversity among accessions of Capsicum spp. and to calculate the relative importance of different traits and the correlation among them. Morphologic characterization of 10 accessions was made using 24 descriptors. Grouping accessions was done by Tocher's method based on the Generalized Mahalanobis Distance. The lineages were separated in eight groups. The most explicative variables were fruit set/plant (29.57%), days to flowering (17.78%) and yield (12.62%). The physical fruit traits, in general, were positively correlated among them and with leaf length and width. They were negatively correlated with total soluble solids, acidity, fruit dry matter content, as with fruit set/plant and days to fructification. The accessions that belong to different groups can be used in hybridizations aiming to develop new pepper varieties for consumption and for ornamental purposes. The knowledge about existing variability can help to develop rational strategies to conserve Capsicum genetic resources.

P71.

Genetic Diversity in a Structured Family from Six Generations of Ornamental Chili Peppers (*Capsicum annuum*)

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The Capsicum plants have been used as ornamental plants, but there are only a few commercial varieties for this purpose in Brazil. The aim of this study was to analyze the genetic diversity in a family structured from six generations of ornamental pepper, based on quantitative and qualitative data, separately and integrated in the same analysis. These studies are part of the Capsicum breeding program developed at Federal University of Paraíba and Federal University of Viçosa. Six generations (P1, P2, F1, F2, BC1 and BC2) of ornamental pepper, belonging to the germplasm bank of the Universidade Federal da Paraíba were evaluated. Two accessions of ornamental pepper plants belonging to the germplasm bank UFPB (01, 132) were chosen based on an earlier diallel study. We studied 10 plants of each parent and F_1 , 72 plants of backcross 1 (BC₁), 75 plants of backcross 2 (BC₂) and 147 plants of the segregating F₂ generation. All generations were used for the analysis of genetic diversity. The quantitative data were submitted to cluster analysis by Tocher method. The multi category traits were analyzed based on the arithmetic complement of the index of simple coincidence. The sum of the matrices of quantitative and multi category characters was performed and the data were submitted to cluster analysis by the Tocher optimization method. The relative importance of the traits was evaluated by Singh Method (1981). A high genetic variability was determined in F₂ and backcrosses by the three cluster analysis, showing that parents 01 and 132 differ for the traits evaluated. According to the relative importance of characteristics, the traits that contributed more to genetic divergence were number of seeds per fruit, number of fruits per plant, days to flowering and days to fruiting.

P72.

Inheritance of Characters Related to Germination and Morphogenesis in Vitro in Ornamental Pepper

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The species C. annuum L. has a low seed viability and in vitro recalcitrance, requiring further studies on the genetic control of traits favorable to in vitro development obtained from a diallel analysis. In this context, the goal of this work was to study the inheritance of germination and morphogenesis in vitro in ornamental peppers and to calculate genetic values of genitors. Were used as parents four lines of ornamental pepper (UFPB 132, 134 UFPB, UFPB UFPB 137 and 390) and their crosses in the diallel, totaling 12 hybrids. The seeds were germinated in half strength MS medium (Murashigue Skoog). On the 7th day of culture, we analyzed the percentage of seeds germinated (SG). After 30 days, we analyzed the percentage of deformed seedlings (DS), the presence of root (PR), the length (LR) and number of roots (NR), the length (LH) and diameter of hypocotyl (DH), number of leaves (NL), length (LL) and width of leaves (WF). The data were submitted to analysis of variance and Hayman's diallel analysis. The effects of treatment (parents, F₁ hybrids and reciprocals) were significant at the 1% level for all traits, indicating the existence of genetic variability. In the sufficiency test of the model additive-dominant SG, NR and NL, variables showed non-significant values of the regression coefficients, demonstrating the validity of the assumptions imposed and indicating the feasibility of this model in the study of these genetic characteristics. The correlations $\hat{W}_i + \hat{V}_i$ and \hat{Y}_{ii} were negative for SG, PR, and LR ranging from -0.3137 to -0.9760, indicating dominant alleles and were positive for other traits, suggesting that the increase in the values observed in the characteristics are mostly provided by recessive alleles. Additive effects were more important than dominant effects for all traits, except for NL, ensuring that these characteristics will be transmitted to the next generations.

P73.

Genetic Control of Seed Germination and Physiological Quality in Ornamental Pepper

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The knowledge of the genetic effects of quantitative traits is important for the success of a breeding program. Pepper are spread by seeds and there is no information about inheritance of germination and seed physiological quality. The objective of this work was to study the inheritance of the germination and seed vigor in ornamental pepper (Capsicum annuum). For this two accessions UFPB 77.3 and UFPB 76 belonging to the germplasm bank of the Universidade Federal da Paraiba (UFPB) were used as parents. These accessions were crossed to produce $F_{1.}$ Thus the F_{1} hybrids were backcrossed with their parents to generate the backcross progenies (BC₁ and BC₂) and self pollinated to generate the F_2 generation. The six generations (P_1 , P_2 , F_1 , F_2 , BC_1 and BC_2) were sown in plastic boxes type gerbox, using moistened paper as a substrate. The evaluated traits were germination (G), first count at 14 days (FC); Germination Speed Index (GSI); radical length (RL) and shoot length (SL). The data were submitted to generation analysis. Genotypic variance was higher than the environmental variance only for FC and GSI. The strict sense heritability was lower than 40% for all characters. The degree of dominance indicates gene effect due to dominance and overdominance for all traits. This effects are unfavorable to selection. The additive-dominant model was sufficient to explain four traits G, GSI, RL and SL with highercorrelation values (70%). Only the trait FC was explained by full model indicating influence of epistatic effects.

P74. Using of Wild Species Germplasm in Breeding Ornamental Peach Varieties Larisa Komar-Tyomnaya

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Ornamental varieties of peach (Prunus persica (L.) Batsch) are excellent plants for gardens and parks. Their main advantage is very abundant and showy blossoms without leaves in mid-spring for 3 weeks. Varieties with double flowers of different colors and with an unusual (weeping, pillar, dwarf) shape of tree looks particularly beautiful. However, the sensitivity to fungal and bacterial infections is the reason why the ornamental peaches are infrequently used in landscaping. Creating varieties resistant to powdery mildew and leaf curl is the main target in our breeding work. In Nikita Botanical Gardens breeding programs are conducted based on the collection of 85 ornamental peach varieties, 62% of which are derived in this institution. Three natural species were used in this breeding program. As a result of crosses between ornamental peach varieties and Prunus mira Koehne, P. davidiana (Carr.) Franch., P. kansuensis Rehd were obtained F1- F2 generations. They were similar to wild species in many morphological and biological characteristics: height and habit of the tree, the strength of growth, the color of the barks stems and shoots, thickness of shoots, shape, size and color of the calyx, the length of the pedicle, the diameter and shape of the flower, the size and shape of the petals, the shape of the leaves, flowering period, relation to fungal diseases, the duration of the dormancy period and others. Following the results of the selection evaluation and variety trials we selected 8 new peach varieties of hybrid origin 'Zhisele', 'Solveig', 'Lel', 'Malenky Prince', 'Belosnezhka', 'Ruthenia', 'Freze Grant', 'Lyubava' that proved to be very showy and still tolerant to fungal diseases.

P75.

Rapid Generation Cycling Enhances Selection Rate of Geophytes: *Gladiolus* xhybridus

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Geophytic floricultural crops, i.e. those with underground storage organs, are primarily vegetatively (asexually) propagated. During breeding, domestication, and continued crop improvement, sexual cycles are necessary to study trait inheritance and select new genetic variation. The University of Minnesota Flower Breeding and Genetic program is breeding new phenotypes of *Gladiolus*, a cut flower and garden crop with corms. Typical generation cycles from seed to flower take 3-5 years. Our objectives were to create a rapid generation cycling process to select early flowering seedlings and enhance the rate of genetic improvement. Techniques under evaluation include germination (week) in the plug phase and correlation with flowering date, fast cycling of generations (2-3/year), along with selection of high leaf number and early stem stalk emergence in cycles 1-3. A total of 71 crosses and N=1,026 seedlings were evaluated in this study. Seedlings germinated primarily in weeks 2-4 after sowing (G2-G4) although some crosses took until G7. All seedlings had one leaf/plant in cycle 1; whereas in cycle 2, leaf counts ranged from one to seven. By cycle 2, 6% of the seedlings (primarily from six crosses) also had elongated stems with 1-4 leaves/stem. By cycle 3, those plants with elongated stems flowered, reducing generation time as much as 1-2 cycles. Stem elongation in cycle 2 is a highly heritable trait. As many as three cycles (generations)/year could be accomplished consisting of reduced greenhouse growth, dry down and cold storage treatments.

P76.

In Vitro Polyploid Induction of *Dendrobium draconis* Rchb.f.

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This study was carried out to find the optimal conditions for in vitro polyploid induction of Dendrobium draconis Rchb.f. Seed-derived protocorms of D. draconis were transferred to Murashige and Skoog (MS) medium containing different concentrations of colchicine (0-0.1%, in v/v) and maintained in the dark for 1, 2, 3 or 4 days at 25±2 ºC. The results showed that prolonged exposure (4 days) of the protocorms to high concentrations (0.1%) of colchicine produced a substantial reduction in their survival percentage and shoot regeneration capacity. Three-day exposure of the protocorms to 0.05% colchicine was most effective in polyploid induction. Only tetraploids were achieved, as suggested by squash technique. Levels of polyploidy induced in D. draconis were confirmed by DNA content and flow cytometry. The obtained polyploid plants had thicker, dark green leaves with larger guard cells when compared to diploid plants. The number of guard cells per unit area observed in the polyploid plants is however lower than that found in diploid plants, which might be due to the increase in the size of the guard cells.

P77.

Polyploidisation of Escallonia species

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Escallonia is a genus of about 50 flowering shrubs, native to South America. Current breeding efforts in *Escallonia* are low, as for most woody ornamentals. Interesting traits to introgress in *Escallonia* are a.o. winter hardiness and more compact growth habit. Chromosome doubling can result in changes in plant habitus as is seen in many plant species. Therefore we evaluated the effect of polyploidisation on plant morphology in different *Escallonia* species.

Actually we have a collection of 25 Escallonia species and cultivars. This collection is characterized for genome size, chromosome number, and leaf and flower parameters. Measured genome sizes of the collection varied between 1,04 pg/2C and 1,32 pg/2C. In vitro initiation of Escallonia rubra and Escallonia rosea were done on Murashige & Skoog medium (MS) and McCown Woody Plant Medium (WPM). Afterwards different chromosome doubling experiments were performed evaluating different mitotic inhibitors, concentrations and application methods. DNA-content was evaluated using flow cytometry. Results showed that for both E. rubra and E. rosea the mitotic inhibitors oryzalin and trifluralin were significantly more efficient compared to colchicine. We obtained up to 24% E. rubra tetraploids using 150 µM trifluralin and 17% E. rosea tetraploids using 150µM oryzalin, compared to only 2.8% E. rubra and 2.9% E. rosea tetraploids using 1000 µM colchicine. Experiments on E. rosea with acute (2,3 and 4 days) and chronical (6,8 and 10 weeks) treatments show a slightly higher number of ploidisation with chronical treatments compared to acute treatments. Chromosome doubled plantlets and diploid controls are acclimatized in the greenhouse. Phenotypical traits such as plant height, leaf morphology and flower size will be evaluated.

P78.

Breeding of a New Calla (*Zantedeschia* spp.) Cultivar 'Gagsi' for Cut Flower

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A new calla (Zantedeschia spp.) cultivar was bred by artificial crossing between female parent Zantedeschia spp. 'Super Gem' with rose-pink to lavender spathe, and male parent 'Best Gold' with yellow spathe at Gangwon Provincial Agricultural Research and Extension Services in 2003 in Korea. 200 seeds were harvested in October 2003 and sown in February 2004. The 'GWCL0548' with light pink (red purple 64B and Y4D color spathe) and vigorous characteristic was selected from the seedlings in 2006-2007. Selected tuber was proliferated by tissue culture. Growth and flowering characteristic tests were conducted from 2012 to 2013. Based on the evaluation of its characteristics, a new calla cultivar 'Gagsi' was developed in 2013 for cut flower. The spathe color is light pink (red purple 64B + Y4D) and the spathe diameter is 5.3 cm. Days to flowering are 70 days. Plant height is 57.9 cm and petiole length was 28.6 cm. The number of flower with 13.3g of tuber fresh weight was 1.6.

P79.

Breeding of Small-flowered Tuberous Begonia in Lithuania

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Botanists have described over 1000 species of Begonia, belonging to the Begoniaceae. In commercial production most of the tuberous begonia (Begonia x tuberhybrida Voss.) cultivars are complex hybrids of several species. Lithuanian gardeners have adopted the tuberous begonia as one of their favourites. In Lithuania largeflowered tuberous begonias (B. x tuberhybrida gr. Grandiflora) of different colours and various forms of flowers are the most popular ones. Small-flowered tuberous begonias (B. x tuberhybrida gr. Multiflora) being normally propagated vegetative by shoot cuttings are not widely spread because of disease problem. The number of small-flowered tuberous begonia cultivars is not high. At the Aleksandras Stulginskis University breeding of small-flowered tuberous begonia was started in 1980. The main objective was to generate many new small-flowered begonia hybrids. Thousands of various hybrids were created and grown during the period of thirty-four years. Three new small-flowered begonia cultivars (Irena Virkau, Vytautas Virkau and Jan Pawel II) were after DUS (distinctness, uniformity and stability) testing at The Research Centre for Cultivar Testing, Slupia Wielka, Poland included in the National List of Plant Varieties of Lithuania 2010.

P80.

Axonopus parodii and Paspalum spp. Inflorescence Production and Seeds Quality

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Brazil has been developing research for selection of native species of Paspalum notatum and Axonopus parodii for use as ornamental lawns. These species can be propagated sexually (seeds) or vegetatively (stolons and rhizomes). However, like most grasses, it can have low percentage of viable seeds. This study aims to evaluate the production of inflorescences and seed quality in P. notatum accessions and A. parodii with potential for use as utilities lawns in the condition of Rain Forest Zone - Pernambuco, Brazil. We adopted design in DBC, four replicates and eight accessions -Axonopus parodii Vall, ined. (BRA 002658); Paspalum notatum (BRA 019178, BRA 023558, BRA 023566, BRA 023728, BRA 012254 and BRA 006301) and P. lepton (BRA 023591). After stabilization of the lawn, the racemes number per square meter was recorded. The racemes were collected to make the analysis of seed quality. Means were compared by Tukey test at 5% probability. It was observed that the higher number of racemes per square meter were produced 93-108 days after planting (DAP), with a reduction of production after 121 DAP. The seed quality was variable between accessions, indicating that this is an aspect to be observed for the selection of these accessions as lawns.

P81.

Efficient Plant Propagation of Cosmos chocolate (Cosmos atrosanguineus)

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Cosmos atrosanguineus is an ornamental species presumably extinct from its natural habitat, the material used in horticulture does not produces seeds because not only for their sexual self-incompatible but also for the unavailability of pollen, this situation limits their reproduction to the vegetative form through in vitro culture. In vitro propagation in order to maintain and improve the available germplasm of this species uses Murashige and Skoog (1962) medium modified with myoinositol (120 mg) and thiamine (40 mg/l). We also improved the rooting stage through the use of NAA, IBA plus phloroglucinol as rooting cofactor (50mg/l), which shortens the rooting period in two weeks and increases the number of roots per shoot, facilitating the adaptation of the plantlets in the greenhouse. We are working on the breeding of this species to obtain new varieties to intensify the flower perfume; we collected seeds from Cosmos scabiosoides, which has similar characteristics to Cosmos atrosanguineus to initiate a program of interspecific crosses. Alternatively in vitro shoots were exposed to different gamma rays doses in order to calculate the LD50, the preliminary results indicate that Cosmos shoots survive gamma rays doses to 10 to 20 Gy.

P82.

In Vitro Tuberization of Cosmos chocolate (Cosmos atrosanguineus)

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Cosmos chocolate (Cosmos atrosanguineus) is one of the few species of the genus Cosmos, which has the capacity to produce tuberous roots, currently the populations of this species in the wild is low. The hypothesis arises that due to the medicinal properties of the roots and attractiveness of flowers, it was over-extracted from their natural environment which caused their almost total disappearance. In order to obtain tuberous roots and then conduct studies to determine the medicinal properties of tubers, we performed work of tuberization by micropropagation technique, this will also encourage the process of acclimatization and survival of plants from in vitro tuberization and have in the future the tools for commercial propagation of roots and obtaining the beneficial properties. The appropriate dose was determined for NAA and phloroglucinol as rooting cofactor (50 mg/l). The culture medium used was Murashige and Skoog (1962), modified with 120 mg of inositol, 4.0 mg/l thiamine. NAA concentrations (2-15 mg/L) were used in order to know it is effect on in vitro tuber development. We established 5 treatments plus control, we evaluated the variables number of swollen roots, thickened roots, length of root, number of total roots and length of shoots. The best treatment was 8 ml/l NAA, which developed on average 5-6 thickened roots, roots with greater thickening, with lengths of 5-6 cm and highest number of total roots.

P83.

Pollen Morphological Comparison of Natural Diploid and Triploid Tiger Lily (*Lilium lancifolium*) in Korea

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The self-compatible diploid and self-incompatible triploid tiger lilies (Lilium lancifolium) are not much different according to the morphological characteristics of the flowers and leaves, while the size of the bulbils and bulbs showed significant differences. In this study, pollen morphological characters which are pollen shape, size, aperture types as well as exine sculpture and structure, were investigated through the scanning electron microscopy (SEM). The length of polar axis (P) and the equatorial diameter (E) of grain, P/E ratio were measured and their variation was compared among studied taxa. Moreover, multivariate statistical analysis was carried out to distinguish morphometric information from measured parameters. All pollen grains were monocolpate, isopolar, large sized and resulted in many differerences. Triploid pollen of L. lancifolium was relatively larger than the other diploid, pollen type-Martagon, the shape-ellipsoid, and exine sculpturing was reticulate with the wide, thick muri formed by rectangular columellae. On the other hand, diploid pollen showed relatively smaller, pollen type-Callose, the shape-long-ellipsoid, and exine sculpturing was reticulate with the muri formed by separated rounded and polygonal columellae. At present the origin of the triploid is still unknown, this results suggest another grounds along with hypothesis that evolution of an exine sculpture shows pollen type trends from Martagon to Callose and smaller pollen grains appear to adapt better in habitats with extreme conditions.

P84.

Investigation of Micropropagation via Spore Culture Technique in Some Fern Species (Asplenium scolopendrium, Asplenium septentrionale, Asplenium adiantum-nigrum L., Asplenium trichomonas) Grown Naturally in Turkey

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The aim of this study was to propagate some fern species which grown naturally in Turkey (Asplenium scolopendrium, Asplenium septentrionale, Asplenium adiantum-nigrum L., Asplenium trichomonas) using spore culture technique and to observe the stages of germination, gametophyte emergence and sporophyte formation. In vitro regeneration experiments were conducted using spore explants from different fern species in order to develop micropropagation possibilities of this plant which is a valuable genetic resource of Turkey. In the experiment, different plant growth regulators; NAA (0, 0.5, 1.0 mg L⁻¹), BA (0, 0.5, 1.0 mg L⁻¹), KIN (0, 0.5, 1.0 mgL⁻¹) and IBA (0, 0.5, 1.0,mgL⁻¹) combinations and concentrations were used to optimize micropropagation protocol. As a result, ratio of the spores viability, ratio of germination, the growth and development of gametophyte and sporophyte stages were determined for all species.

P85.

Investigation of Somatic Embryogenesis in Some Crocus Species (*Crocus sativus* L., *Crocus ancyrensis, Crocus pallasii* ssp. *pallasii*) Grown Naturally In Turkey

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The aim of this study was to propagate some *Crocus* species which are cultivated and grown naturally in Turkey (Crocus sativus L., Crocus ancyrensis, Crocus pallasii ssp. pallasii) using somatic embryogenesis technique and to observe for *Crocus sativus* L. the stages of callus growth by histological analysis. In vitro regeneration experiments were conducted using corm explants belonging to different Crocus species to develop the micropropagation possibilities of this plant which is a valuable genetic resource of Turkey. In the experiments, different plant growth regulators; NAA (0, 0.5, 1.0, 2.0 mg L⁻¹), BA (0, 0.5, 1.0, 2.0 mg L⁻¹), 2 iP (0, 0.5, 1.0, 2.0 mgL^{-1}) and 2,4-D (0, 0.5, 1.0, 2.0 mgL^{-1}) combinations and concentrations were used to determine the growth and development of plants. As a result, conversion ratio of explants to the embryogenic callus, conversion ratio of embryogenic callus to embryo, investigation of embryo stages, survival ratio of explants were investigated for all Crocus species and the beginning stage of callus formation in C. sativus L. was determined via histological analysis.

P86.

Chrysanthemum indicum 'Golden Surfer' protoplast regeneration: a new horizon for somatic hybridization?

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Chrysanthemum indicum is one of the most important ornamental crops. Various breeding techniques have been explored to find tools to enlarge the commercial assortment. Interspecific hybridization is one of the most efficient techniques with regard to the introgression of new traits. Theoretically, somatic hybridization through protoplast fusion has a high potential to introduce new Chrysanthemum types. However, Chrysanthemum protoplasts, whether fused or not, have to our knowledge so far always been very recalcitrant in regeneration experiments, yielding only calli or roots. For our experiment, we initiated protoplasts from multiple chrysanthemum 'Golden Surfer' tissue types in MS based medium. In total we obtained 6781 calli derived from either mesophyll or callus. These calli served as explants in a series of regeneration experiments. Different circumstances were created based on phytohormone addition, light or dark culture, protoplast source and protoplast culture type preceding callus culture. Altogether, 5 calli (0.07%) started regenerating shoots up to 28 weeks after the start of the protoplast culture. All shoots regenerated from calli protoplasts in the dark, on an MS based medium supplemented with 0,3 mg/l BA. The plantlets are multiplied in vitro and will be rooted and acclimatized in order to compare their phenotype and growth vigor to the one of regular 'Golden Surfer' in vivo plants. In following experiments, we will test the protocol on several other genotypes; if protoplasts from multiple cultivars can be regenerated, it will renew prospects for somatic hybridization in this recalcitrant crop.

P87.

Progeny Selection of Elite Bigtooth Maples <u>Rolston St. Hilaire¹</u>

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Brilliant fall foliage color, small stature, disease and pest resistance, and morphological similarities to sugar maples could make bigtooth maples (Acer grandidentatum) a major player in the woody ornamental plant market. Furthermore, the contiguous geographic range of bigtooth maples which spans about 18 degrees latitude primarily in the semi-arid and arid regions of North America suggests that bigtooth maples are potential candidates for selecting drought-tolerant maples. Our research has established that progeny from wild-collected bigtooth maples tolerate stressful environments and have ornamental traits that are suitable for managed landscapes. In addition, physiological and growth traits of bigtooth maples might make them resilient to stressful environments. For example, bigtooth maples have a highly operational xanthophyll cycle and zeaxanthin levels differ among provenances. Using plant materials from a germplasm that was established over a decade ago, we have selected bigtooth maples that hold promise for use in managed landscapes of arid and semi-arid regions.

P88.

Phenotypic Plasticity in *Chrysanthemum* under LED Light

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Breeding and cutting propagation of mum chrysanthemum (Chrysanthemum x morifolium) is important in Belgium. Due to the high energy costs for chrysanthemum rooting, ways to reduce this production factor are investigated. One potential way might be to root and acclimate cuttings in multilayer systems using artificial LED light. Light quality will, however, impact plant characteristics as phenotypic plasticity is widely recognized as a potential adaptive trait under a wide array of environmental conditions (Gratani, 2014). Therefore we studied selected morphological and physiological parameters of 10 Chrysanthemum x morifolium cultivars under blue (460 nm), red (660 nm) and white (400-700 nm) LED. Plant morphology is cultivar dependent, but was also clearly affected by the light spectrum. On average, leaf surface was largest for plants grown under white light, while leaf thickness was lowest. White light also resulted in the largest stomata but the lowest stomatal density per leaf unit, while red light resulted in the most, but smallest stomata. Furthermore, red light resulted in less pigmentation (chlorophyll and carotenoids) in the leaves compared to white light. Yet, electron rate transport tended to be higher under blue and red light compared to white light resulting in a lower photochemical efficiency of photosystem II. Looking at plant metabolites, blue light enhanced leaf proline content in most cultivars. On the other hand, protein and total phenolic content was lowest in plants under blue light. The results showed also that there are cultivar exceptions on the global findings for the investigated parameters.

P89.

Are Tetraploid Roses More Resistant to Powdery Mildew Compared to Diploids?

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In roses morphological differences between tetraploids and diploids are well documented. In tetraploids thicker and larger leaves, etc. are described. However, little is known about stress resistance in polyploids. In our research we compared powdery mildew resistance in five diploid rose genotypes and their tetraploid counterparts. Four diploids derived from a well characterized cross between the diploid cultivar 'Yesterday' and the species Rosa wichurana were chosen for polyploidisation. The diploids differed in resistance level. Another diploid species hybrid 95/13-39 with known powdery mildew resistance was added to the test. The five selected rose genotypes were polyploidised through a treatment with the antimitotic agent oryzalin in tissue culture. Both tetraploids and diploids were acclimatized in the greenhouse and after six months powdery mildew inoculations were done on detached leaves. Two pathotypes of powdery mildew, R-E and R-P, were used. Disease development was evaluated on detached leaves after standardized inoculation using a sedimentation tower. A disease index was calculated from the scored leaves. In general the tetraploids showed an increased resistance to powdery mildew when compared to the diploids. Differences in resistance are discussed for the diploids and tetraploids tested.

Disease Resistance

P90.

Screening of Hot Pepper Accessions for Resistance against Cochineal (*Orthezia* spp.)

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Peppers belong to the Solonaceae family. They originated from the Americas and commercial varieties are sensitive to different types of plague, as cochineal (Orthezia spp.), which decreases the productivity. The most promising alternatives to control this pest would be the use of resistant varieties from the domesticated, semi-domesticated or wild species. So, this work aimed to screening different accessions of hot pepper for resistance against cochineal attack. The experiment was carried out at the Federal University of Paraíba, using twelve hot pepper accessions of Capsicum annuum L. and Capsicum chinense Jacq. from the germplasm bank of the Federal University of Paraíba, Northeast region from Brazil. We used a scale to evaluate the incidence of cochineal (0, absence of insect; 1, insect of presence) and the severity obtained according to the degree of incidence of the pest in the plant (0, highly resistant; 1, resistant; 2, moderately resistant; 3, moderately susceptible; 4, susceptible; and 5, highly susceptible). The means of the data and the diversity among accessions was estimated by Tocher's optimization method. Of twenty accessions evaluated, four accessions were totally susceptible while other two accessions were resistant. Fourteen accessions show segregation in severity level of cochineal attack. The Tocher's method formed five groups and separated the accessions according to level of severity of pest attack. The variable incidence of plague contributed 99.93% of the total variation among hot pepper accessions. The source of cochineal resistance found in hot pepper accessions of germplasm bank should be used in improvement breeding program of Capsicum species.

P91.

Selection and Propagation of Horse Chestnut with a Resistant Behaviour to the Horse Chestnut Leafminer in the Czech Republic

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The horse chestnut (Aesculus hippocastanum) is an important deciduous tree in horticulture (alleys, solitaires). The horse chestnut leaf-miner (Cameraria ohridella) is one of the most important pests of this species in Europe. In the Czech Republic the first occurrence of this pest was noticed in 1993. The moth adapted very quickly and regularly brought out damages of leaves in a different intensity. The damages caused the losses of ornamental value and weakening of trees. In 2014, the extremely high frequency of this pest was observed in the Czech Republic probably due to the weather, which resulted in a severe necrosis and very early defoliation (the beginning of August) of horse chestnut trees. In 1998, a search for genotypes with a resistant behaviour to horse chestnut leaf-miner in native populations of horse chestnut trees in the Czech Republic started. The results of our finding and selection during the period 1998–2014 were one registered cultivar 'Mertelík' and two new perspective clones (HPR2757, HSR6990) for next resistance breeding. The resistant behaviour in 'Mertelík' represents the creation of only small, atypical mines with a unfinished larvae development and overall damage of lamina up to 50 % compared to unresistant plants.

Development of propagation methods form an integral part of horse chestnut breeding program because they accelerate the breeding process. We focused either on the common asexual propagation (grafting) or plant tissue cultures (organogenesis and somatic embryogenesis).

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P92.

Segregation of Resistance to Calonectria pseudonaviculata in Interspecific Buxus Hybrids Katrijn Van Laere¹, Bjorn Gehesquière², Kurt Heungens², Johan Van Huylenbroeck¹

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In view of the economic importance of Calonectria pseudonaviculata (formerly known as Cylindrocladium buxicola/pseudonaviculatum) for the Buxus producing industry, the need for resistant cultivars is urgent. Maximizing the efficiency of a breeding programme towards more disease resistance depends on a good, fast and early screening method of resistant cultivars and interspecific F1 hybrid seedlings. Therefore, we optimised a bio-assay to evaluate the host susceptibility of Buxus hybrids using artificial inoculation with a C. pseudonaviculata conidial suspension. We applied this bio-assay to screen 4 hybrid populations made by controlled interspecific crossing between Buxus harlandii (2n=4x=56) as seed parent and B. microphylla 'Trompenburg' (2n=4x=56), B. sempervirens 'Latifolia Maculata' (2n=2x=28), B. sempervirens 'Suffruticosa' (2n=2x=28), B. sempervirens 'Angustifolia' (2n=2x=28) as pollen parents. Inoculation with a Calonectria *pseudonaviculata* suspension of 5.10^4 conidia ml⁻¹ was able to distinguish reliably between resistant and susceptible genotypes using percentage of diseased leaves and lesion size as good parameters to evaluate host susceptibility. By this, B. harlandii and B. microphylla 'Trompenburg' were confirmed to be very resistant to Calonectria pseudonaviculata, while B. sempervirens cultivars were very susceptible. The observed segregation patterns in the F1 hybrid populations indicate a complex, polygenic segregation of quantitative traits, in which most of the hybrid plants demonstrated intermediate susceptibility compared to the parent plants. But interestingly for breeding purposes, 8% of the B. harlandii x B. sempervirens 'Suffruticosa' and 2.8% of the B. harlandii x B. microphylla 'Trompenburg' hybrids showed a lower percentage of diseased leaves compared to B. harlandii. Moreover, 14.8% of *B. harlandii* x *B. sempervirens* 'Suffruticosa' and 10% of B. harlandii x B. microphylla 'Trompenburg' hybrids had a lesion size smaller than B. harlandii. Genotypes with improved resistance to Calonectria pseudonaviculata based on these two parameters of resistance will be used in further crosses aiming to introgress more disease resistance in Buxus sempervirens.

P93.

Is the Observed Differential Reaction of Rose Powdery Mildew on Rosa 'Yesterday' Correlated with Pathotype Groups?

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In rose powdery mildew two pathotype groups can be discriminated based on the differential reaction in rose and Prunus avium. Isolates from both groups can be identified by a one basepair difference in their ITS sequence. In previous research, the pathotype only infectious on rose was called R-E, while R-P infects both rose and Prunus. A specific resistance response characterized by a complete stop of development of germinated conidia was found on Rosa 'Yesterday' for pathotype R-E, while R-P could develop on 'Yesterday' by growing mycelium and the formation of new conidia. In current research we investigated whether the observed resistance response in 'Yesterday' could be correlated with the different pathotype groups. Therefore seven new isolates were collected and monoconidial cultures were established on tissue cultured roses. The pathotype groups were identified based on the ITS sequence. Three isolates belonged to the R-P type, while the other four were R-E type. Detached leaves of 'Yesterday' were inoculated with all new isolates and the fungal development was evaluated after lactophenol blue staining. The development of the conidia was defined by three different responses: not germinated, germinated and stop of further development and germination followed by normal development. For all seven isolates between 40 and 56% of the conidia didn't germinate after inoculation. Germination followed by a complete stop of further development was only seen in the isolates belonging to the R-E pathotype group, and not in the isolates belonging to the R-P pathotype group. However in the new isolates of the R-E type also normal conidia development was observed. These results indicate the specific response of conidia germinating, followed by a stop of development is specific for the pathotype group R-E although in most isolates this response is not absolute for all conidia.

P94.

Enhancing Quality of Ornamental Propagation Material: Optimization of Viruses Molecular Diagnosis Protocols

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The project QUALIMAPRO (Quality of propagation material: update and verification of control protocols) is funded by the Italian Ministry of Agriculture and Forestry (Mipaaf). It has the main objective in the establishment of protocols useful for a reliable, sensitive and fast diagnosis, which allows fast intervention for detecting and preventing the spread of the viral agents in propagation material. Such analysis permits a continuous control on crops and protect flower growers against the possible virus presence in import-export propagation materials. Virus affecting buttercup (Ranunculus asiaticus), carnation (Dianthus caryophyllus) and daisy (Agyranthemus frutescens) were taken into consideration in order to support the breeding activity of several italian local enterprises.

After the evaluation of the most important virosis occurring in these species, standard protocols for molecular detection by RT-PCR have been developed.

In particular for buttercup tests comparing the sensitivity and reliability of RT-PCR compared with conventional serological techniques have been carried out. Furthermore it was set up a multiplex RT-PCR system for the simultaneous diagnosis of the buttercup viruses more frequently detected in the samples analyzed.

In the same species, tests were carried out for the viruses eradication using the technique of the *in vitro* meristem tip culture obtaining virus-free clones. The project results will be useful for a more accurate certification of *in vivo* and *in vitro* propagation material with particular emphasis on exports where the qualitative competition is continuously growing.

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