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Yoshioka Y., Iwata H., Fukuta N., Ohsawa R. and Ninomiya S.
The paper provides information on floriculture and ornamental plant production in developing countries. Flower and ornamental plant production have become a component of improved food security and better livelihood in developing countries. Within the trade liberalization process, developing countries can seize the opportunities to develop their ornamental plant industry as means to create employment and generate income. The paper illustrates examples where FAO has been instrumental in exploring and developing initiatives where developing countries have taken advantage of its biodiversity and comparative advantage to supply local demand or to compete on export markets. By providing assisting to its member countries in developing the floriculture sector, FAO supports the global endeavor for the conservation of plant genetic resources and the implementation of the global plan of action as adopted at the Leipzig conference in 2002.

**ORAL PRESENTATIONS**

**Session 1: EXPLOITATION OF BIODIVERSITY**

**O1. GENETIC RESOURCES AND BREEDING ORNAMENTALS IN BRAZIL**

Tombolato A.F.C.

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Brazilian flora is known all over the world for its riches, but the main flower and ornamental plants prevailing in the inner market are still the traditional exotic species, e.g. roses, chrysanthemums and saintpaulias. Floriculture business produces very few for export, besides some government efforts for exportation, the inner market buys almost all Brazilian flower production. The new international wave for the use of tropica has influenced the country consumption, and then the costumer slowly opens his eyes for the natives too. Many new research projects have been established aiming to improve the quality of the tropical ornamentals and to rescue the native germplasm and transform it in a commercial product. Actually this is a long way and it is also a difficult goal to reach because the wild species, in many cases, deserve of domestication, pre-breeding and breeding. In this presentation some results are shown, as the cultivars of *Hippeastrum* and anthuriums selected by the Instituto Agronômico – IAC, and also the list of ornamental natives from the Center-West prepared by the Project Plants for the Future from the Ministry of Environment.

**O2. ENDENMIC ORNAMENTAL PLANTS RESOURCES IN CHINA**

Chen L. and Chen D.

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Located in East Asia, on the western shore of the Pacific Ocean, the People's Republic of China (PRC) has a land area of about 9.6 million km². In China, abundant plant resources exists due to its varied and complicated terrain and great variety of temperature belts. However, many wonderful species with valuable characters have not been exploited and utilized till now. This article focuses on some endemic plants of China with potential exploitable value in industrial floriculture. Many wild genus and breed variety such as *Osmanthus*, *Lilium*, *Lycoris*, ornamental ferns and rare species of *Orchidaceae* are described in this paper. There are 157 cultivars with different tree shape, flower color and florescence in natural habitat. Some endangered ferns with special beauty appear hopeful as indoor foliage plants. These resources are described.

**O3. DIVERSITY STUDY AND BREEDING OF BROOMS (TRIBE OF GENISTAEAE).**

Bellenot-Kapusta V., Pesteil C. and Cadic A.

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A new program of plant breeding and genetic studies has begun in 2005 on brooms (*Genistaeae* tribe – *Fabaceae* family). This program is included in the framework of an association between nurseriesmen and the Joint Research Unit UMR GenHort (INRA). The aim is to combine characters of horticultural interest (habit, perfume, colour...) which have are never been associated in present cultivars. Preliminary studies were carried out on 25 clones from the genera *Cytisus*, *Genista*, *Argyrocytisus*, *Lembotropis* (respectively 17, 6, 1 and 1 taxa). The aim was to obtain informations on cytogenetic characteristics, floral biology, and on self- and cross- compatibility. DNA amount of the different taxa was evaluated by flow cytometry, and in some cases chromosome counts on root tips. The taxa have been classified in 3 groups according to the DNA quantity: high, medium and low. High and low groups include only few taxa, from *Genista* and *Argyrocytisus* species; at the opposite the medium group include various species which cannot be statistically distinguished. For compatibility studies we observed pollen tubes growth in styles, using fluorescence microscopy. The styles were sampled after self- or cross-pollinations; cross pollinations included intra-specific, inter-specific and inter-generic crosses. Despite self-incompatibility mentioned in the literature, most of the pollen tubes in self-pollinations had a good progression and reached the ovules. The fruit set of the self-pollinations ranked from 0 to 9.6 million km².
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25 % with some seeds in each pod. Some of these seeds have been rescued by embryo in vitro culture. For cross-pollinations, pollen tubes often stopped in the style, but in a third of the cases some pollen tubes reached the ovary; we compared the progression of pollen tubes between the different types of crosses and could rank them on a decreasing compatibility scale: intra-specific, inter-generic, inter-specific. In all cases, no pod have developed. Morphological observations of the stigmas by electronic microscopy indicated differences between species which could partly explain the difficulties for emasculation and pollination. Based on these data, we established an efficient method of pollination on Cytisus scoparius (stages of castration and pollination), which will be tested now on various species. These preliminary results will be complemented by further studies on cytogenetic, genetic and botanical diversity and heredity of horticultural characters.

O4. CHARACTERIZATION OF THE ORNAMENTAL VALUE OF CALIBRACHOA SPP. NATIVE TO ARGENTINA

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Calibrachoa La Llave & Lexarsa (Solanaceae) is an American genus with high ornamental and economic value due to the existence of successful commercial varieties for pot plant and landscaping. However, most of the native argentine species or genotypes have not been used for breeding purposes yet. The objective of this study was to characterise the ornamental value of the Calibrachoa collection of the Institute of Floriculture. 41 accessions were evaluated: C. calycina (6), C. helianthemoides (7), C. linearis (6), C. kleinii (6), C. variabilis (1), C. parviflora (1), interspecific hybrids (8) and 5 commercial varieties. The UPOV DUS descriptor with 26 characters was used for morphological characterisation. Some other characteristics such as: number of flowers in full-bloom, number of branches and pot cover, both after 3 months culture in full-bloom and one month after pruning, were recorded. Accessions were propagated by cuttings and cultivated in greenhouse during March to August (Autumn in Argentina, Summer in Spain), to evaluate precocity. In September plants were pruned and sprouting capacity was after pruning, were recorded. Flowering precocity was detected in C. kleinii, C. linearis and their interspecific hybrids. Shape variability was observed in C. kleinii: since erect, semi-erect and trailing plants were characterised. Genotypes suitable for pot plant development were selected. After 3 months culture, C. parviflora and C. kleinii covered more than 80 % of the pot, while in C. calycina and C. helianthemoides this percentage was less than 40 %. Most of the Calibrachoa accessions showed sprouting capacity after pruning, except C. kleinii, were none was observed and C. Helianthemoides, in which only one accession sprouted after 80% pruning. On the other hand, the fastest pot covering capacity one month after pruning was observed in C. parviflora and C. variabilis (91-97 %). Variability in number of flowers was observed among accessions in Autumn and Spring periods. C. helianthemoides was sensible to insects. This study allowed us to obtain valuable information for breeding programs.

O5. RESULTS OF A BREEDING ACTIVITY ON LIMONIUM SPP.

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Limonium is grown for use as a cut flower for both fresh and dry flower arrangements. A breeding activity on Limonium was carried out at the Experimental Institute for Floriculture in Sanremo and Pesca since 1998. Wild ecotypes, botanical species and commercial varieties were utilized in an incomplete diallelic cross design with the aim to obtain new varieties suitable to the cultivation in Mediterranean conditions with low energy requirements. A first group of selected progenies derives from crosses among L. latifolium, L. gmelinii, L. caspia, L. bellidifolium, L. otolepis and L. serotinum. The first inter- and intra-specific hybrids and progeny deriving from open- and self-pollinations were evaluated since 2001 in different environments. These genotypes require low-energy for cultivation, minimum tillage and low input of fertilisers and pesticides. In these conditions, these new I.S.F. varieties show a productivity and commercial quality comparable and also higher than the commercial control cultivars. A second group derives from the cross L. bundwii x L. sinuatum (Statice). L. bundwii is a Mediterranean wild species that needs to be improved in relation to productivity, flower colour variety (flowers are only yellow) and stem architecture for commercial production. The first interspecific hybrids were selected in 2001. The genotypes of three selected populations (the hybrids L. bundwii x L. sinuatum and the selected progeny deriving from the parents) were evaluated in comparison with commercial cultivars of Statice in Pesca. The selected hybrids resulted to be characterized by a production significantly higher, a stem significantly shorter and harder than L. bundwii and a number of branches per stem significantly higher than L. sinuatum and commercial varieties. Furthermore, the flowers show new different colour combinations of the calyx and the corolla: ‘Pink, yellow, violet, white, etc.’. A third group derives from selected progenies of L. tataricum. This species could not be crossed with any other species, so only one new variety was obtained by selection from free pollination. This variety (I.S.F. SOI’50) is characterized by a good production, a high number of flowers per stem, a very attractive architecture and a white colour of both the calyx and the corolla. In L. tataricum the corolla is commonly pink or light-violet. The last group derives from a new breeding program started in 2001 with crosses among L. aureum, L. sinensis, L. tetragonum, L. fortunei and the commercial cultivars ‘Lemon Star’, ‘Yellow Star’ and ‘Superlady’. The first hybrids were obtained in 2002: they show the good agronomical and ornamental characteristics of the parental cultivars and the stress tolerance of the parental botanical species.

O6. BREEDING FOR TROPICAL MINIATURE POTTED DENDROBIUM ORCHIDS

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In the orchid industry, miniature potted orchids are becoming popular throughout the world. In view of its potential, in 1976 an active orchid breeding programme was initiated in MARDI. The main objective is to generate many new tropical quality orchid hybrids, for both the potted and cut flower orchid industry. Miniature Dendrobium was one of the orchid genus that was given emphasis in MARDI’s breeding programme. A successful breeding programme depends very much on the selection of suitable
O7. **IMPROVING THE EFFICIENCY OF HERBACEOUS PEONY BREEDING METHODS**

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Peony cultivated for the cut flower market is an increasingly popular crop. Cultivars used, are generally old varieties or derived hybrids belonging to the *Paeonia* lactiflora group. The lack of cultivars specifically adapted to cut flowers might be balanced by an efficient breeding effort. In this aim, we developed a soil less method in perlite bags, combined with a sequence of several environmentally controlled stages. This method allowed to drastically reduce necessary time between sowing and the first flowering. This artificial combined approach affecting the biological cycle of peony made it possible to obtain first flowers sprouting less than 27 months after seeds sowing. By this experimental way, environmental requirements of peonies were more precisely defined. These results could contribute to breed herbaceous peony more efficiently than traditional methods. They could also have a significant impact on Peony growth and propagation activities and methods under mediterranean conditions.

O8. **THERMOSENSITIVITY OF THE RESTORATION OF MALE FERTILITY AND GENOTYPIC DIFFERENCES IN THE FORMATION OF ABBRANT FILAMENTS AND PISTILS AMONG THREE MALE-STERILE CULTIVARS OF ASIATIC HYBRID LILY**

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Three Asiatic hybrid lily cultivars, ‘Akita Petit Cream’, ‘Akita Petit Lemon’, and ‘Akita Petit Gold’ are male sterile. ‘Akita Petit Cream’ has pollenless anthers and the others has antherless stamens. In our field trial of these cultivars to delay flowering time and expand harvesting time of cut flowers in autumn, intact anthers of ‘Akita Petit Cream’ were unexpectedly observed in less than 10% plants, using bulbs which had been frozen for storage. The purpose of the present study is to elucidate the factors controlling the expression of undeveloped anther phenotype of these three cultivars. Three cultivars each were grown in three temperature regimes in growth cabinets, namely high temperature regime (32/25°C), middle temperature regime (25/18°C), and low temperature regime (18/11°C). Among these, expression of undeveloped anthers of three cultivars examined was stable and no stamens had anthers in low temperature regime. In middle temperature regime, the anthers of ‘Akita Petit Cream’ were restored and dehisced. However, the anthers of other two cultivars had not been restored. In high temperature regime, intact anthers were restored in three cultivars and these anthers of ‘Akita Petit Cream’, ‘Akita Petit Lemon’ were dehiscence. However, the anther of ‘Akita Petit Gold’, were coloured in brown at the tip of anther and were not dehiscence. Though the pollen in restored anthers of ‘Akita Petit Cream’ and ‘Akita Petit Lemon’ in high temperature regimes were stained in blue by lactophenol-cotton blue, the pollen of ‘Akita Petit Gold’ were not stained. Pollen grains of ‘Akita Petit Gold’ were also observed by scanning electron microscope and were revealed to be immature morphologically. The possible role of temperature on the induction of anther as well as pollen formation and anther dehiscence will be discussed.

O9. **BREEDING STRATEGIES TO INCREASE GENETIC VARIABILITY WITHIN HIBISCUS SYRIACUS**

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*Hibiscus*, belonging to the *Malvaceae*, is a polymorphic genus of some 200-250 species of trees, shrubs and herbs. They are widely distributed in mainly tropical and subtropical regions. Three species, *Hibiscus syriacus* L., *Hibiscus sinosyriacus* Bailey and *Hibiscus paramutabilis* Bailey, are natural to temperate regions of the world. Among these species, breeding work is mainly done in *Hibiscus syriacus*, a well-known winter hardy ornamental shrub. Compared to *H. syriacus*, *H. paramutabilis* and *H. sinosyriacus* grow more vigorously, with larger leaves and flowers. In this work, the aim was to introgress more growth vigour into *Hibiscus syriacus*. Therefore, two breeding strategies were followed. A first focus was on interspecific hybridization between tetraploid (2n=4x=80) and octoploid *H. syriacus* cultivars with *H. paramutabilis* (2n=4x=82) and *H. sinosyriacus* (2n=4x=80). When used as a seed parent, *H. paramutabilis* and *H. sinosyriacus* failed to set fruits. However, when pollinated by *H. paramutabilis* or *H. sinosyriacus*, *H. syriacus* ‘Oiseau Blue’ and *H. syriacus* ‘Red Heart CV’ reacted by fruit setting. Most fruits aborted in an early stage of development, but of the surviving fruits, embryos could be transferred to an *in vitro* germination medium (‘embryo rescue’). Two or three weeks after *in vitro* initiation a lot of them (50% for the *H. syriacus* x *H. sinosyriacus* cross combination was between *Dendrobium* Peewee and *Dendrobium* Chao Praya Gem. The potential miniature progeny selected, which was registered as *Dendrobium* Tuanku Fauziah is free flowering, producing many small white coloured flowers with purple coloured interior lip. The third potential miniature progeny which was registered as *Dendrobium* Doctor Sharif, had all the good features for a miniature potted orchid plant. The plant structure is small with attractive sprays of small but many attractive purple coloured flowers. The second cross combination was between *Dendrobium* Peewee and *Dendrobium* Chao Praya Gem. The potential miniature progeny selected, which was registered as *Dendrobium* Tuanku Fauziah is free flowering, producing many small white coloured flowers with purple coloured interior lip. The third potential miniature progeny which was registered as *Dendrobium* MARDI, was derived from cross between *Dendrobium* Spellbound Compactum and *Dendrobium* Chao Praya Gem ‘alba’. The selected hybrid is free flowering, with attractive and slight curly white to greenish white flowers and purplish coloured interior frilled lip. The details of each cross combination will be further discuss in the paper. All the developed miniature *Dendrobium* hybrids are easy to handle as an indoor potted plant and the most rewarding orchids to grow. They are exotic, unique, attractive and showy potted plant, suitable as gifts for loved ones or the individual flowers can be also crafted into beautiful accessories, such as gold or silver plated rings, pendants or bracelets.
Session 2: IN VITRO MANIPULATION

O10. MUTATION-ASSISTED BREEDING FOR IMPROVING ORNAMENTAL PLANTS
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Genetic variation is necessary in any plant breeding program for crop improvement. Induced mutations are highly effective to enhance natural genetic resources and have successfully assisted in developing improved and new cultivars among both seed and vegetatively propagated crops. So far, among more than 2300 officially released mutant varieties worldwide, 566 represent ornamental plants (http://www-mvd.iaea.org). Some of the selected traits of the mutant ornamental plants are flower colour, flower morphology, plant architecture, compact growth, flower type, and variegated leaves. Among mutants, gamma rays have been commonly used effectively for mutation induction. Recently, Heavy-ion beam (HIB) has attracted increasing interest in floriculture for mutation induction. Some of the important ornamental plants, both cut and potted plants, that have been used for mutation breeding for example are: chrysanthemum, orchids, rose, pelargonium, canna, and carnation. The growth of floriculture industry has taken long strides worldwide, especially in the developing countries as a result of outsourcing, which is limited to a few F1-hybrids. A new and promising method is developed in which 2n-gametes can be induced by the application of laughing gas. The method proved to work and in lily the occurrence of homoeologous recombination was detected. In tulip interspecific hybrids enormously.

O11. INDUCTION OF 2N-GAMETES FOR OVERCOMING F1-STERILITY IN LILY AND TULIP
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For overcoming F1-sterility in interspecific hybrids, mitotic and meiotic polyploidisations are applied in lily and can result in fertile allopolyploids. The mechanism of viable pollen production of mitotic and meiotic polyploidisation is quite different. Mitotic polyploids are obtained by artificial chromosome doubling and result in a pair of homologous chromosome sets, which enables chromosome paring during the meiosis. Meiotic polyploidisation occurs in rare cases by irregular chromosome division resulting in unreduced gametes. In contrast to mitotic doubling homoelogous recombination can occur in these gametes. Genomic in situ hybridization (GISH) has been used to discriminate parental chromosomes and to detect homoelogous recombination.

Mitotic polyploidisation showed no homoeologous recombinations between the parental genomes whereas in meiotic polyploids it can be detected frequently. The use of 2n-gametes is therefore the most promising way for the introgression of desirable characters in the breeding with interspecific hybrids. In both cases, the frequency of viable gametes appeared to be low and limited to a few F1-hybrids. A new and promising method is developed in which 2n-gametes can be induced by the application of laughing gas. The method proved to work and in lily the occurrence of homoelogous recombination was detected. In tulip where the production of mitotic polyploids takes at least 5 years, laughing gas treatment will speed up breeding with interspecific hybrids enormously.

O12. FLOW CYTOMETRIC DETECTION OF UNREDUCED POLLEN IN BEGONIA
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In ornamentals, interspecific hybridization efforts are one of the main source of genetic variation. In F1-hybrids, fertility is often restored by the application of mitosis inhibitors like colchicine, oryzalin or trifluralin. Nevertheless, this does not allow introgression in the F1-generation. For that reason, unreduced gametes are a valid alternative. Our objectives are (i) to elaborate a proper screening method for the detection of unreduced gametes, (ii) to use unreduced gametes in a breeding program to induce polyploids and (iii) to evaluate the practical use of meiotically vs mitotically obtained polyploids. Because of the short reproduction time and the ability to produce pollen throughout the year, Begonia is used as a model plant for the detection of unreduced pollen. Chemical, enzymatical, mechanical and osmotical assays have been used for the isolation of pollen nuclei from Begonia species, cultivars and interspecific hybrids. Nuclei were isolated from germinated pollen exclusively.
and their DNA content was measured using flow cytometry. Flow cytometric analysis usually showed 2 peaks, associated with vegetative (1C) and generative (2C) nuclei. The presence of unreduced gametes was deduced from the ratio between the 4C and 2C peaks. Clear peaks were obtained, even when germination was only 5%. Other ornamentals as *Rosa*, *Hibiscus*, *Rhododendron* were evaluated in the same way. By using unreduced pollen, higher ploidy levels (up to 2n = 6x) were reached in *Rhododendron* through simple crossing between 'Starlight tetra' and 'Casablanca tetra' (both 2n = 4x). Efficiencies of up to 2 hexaploids / 40 seedlings were attained. At this moment, the natural frequency of 2n gametes, and the influence of genotypic and environmental stress upon their biosynthesis is being defined. Evidently, 2n gametes will also be applied for breeding programs in other ornamental crops, whenever suitable.

O13. **BREEDING FOR POLYPLOIDY IN BELGIAN AZALEA (RHODODENDRON SIMSII HYBRIDS)**

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Evergreen pot azalea hybrids belong to the main ornamental crops produced in Belgium. Throughout their breeding history, numerous efforts have been performed to induce new morphological properties. Few attention has been dedicated to the creation of polyploid cultivars. As a result, nearly all commercial genotypes are diploid. In our work, polyploid azaleas have been induced using three different protocols. Through the use of mitosis inhibitors, meristematic cells of seedlings were tetraploidized and could be regenerated into solid tetraploids, although ploidy chimeras frequently occur. Seedlings were more efficient than stock cultures. Oryzalin and trifluralin (0.3 mM during 3 consecutive days) were both effective and are preferable over colchicine. The best moment for treatment was immediately after the unfolding of the cotyledons. A second method was based on the regeneration of tetraploid flower margins in picotee chimeras in vitro. This could be done for several cultivars, like ‘Marcella’, ‘Starlight’ and ‘Koningin Fabiola’. The drawback of this system is that the number of cultivars with tetraploid petal margins is rather low. On the other hand, this system offers the advantage of the absence of ploidy chimeras. A third solution is the application of unreduced gametes. At this moment, induction and screening methods for unreduced azalea gametes are being developed. The production of 2n pollen has yet been demonstrated. Mixploidy does not occur within the offspring; on the other hand aneuyploids are possible. Plants obtained from either treatment exhibit aberrant flower shape, resulting in thicker petals, especially in the flower centre. Other ‘typical’ polyploidy characteristics noted were reduced fertility (poor anther development and anthesis), brittle wood, a significantly reduced length/width index and an increased leaf basis angle. In a next step, the commercial value of polyploid azaleas will be evaluated. This will be performed by a further characterization of polyploidy-driven morphological consequences and post-harvest quality. A thorough knowledge of both the induction process and the consequent polyploidy will allow to efficiently integrate polyploidization in pot azalea breeding programs.

O14. **USE OF HOMOZYGOSITY IN THE RANUNCULUS ASIATICUS BREEDING PROCESS**

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The in vitro androgenesis method is mainly goalied to produce homogeneous diploid genotypes (HD) of *Ranunculus asiaticus* originated from cultivated heterozygous diploid genotypes. This presentation shows four important uses of the homozygosity in the breeding process for ranunculus : The fundamental use of in vitro androgenesis to produce homozygous HD progenitors. The realization of a serial plan of crosses in order to increase the level of ability to produce HD plants directly from pollen grains. The use of HD as intermediate contributors in crossing plans between F1 hybrids (from combinations HD X HD) and uninefficient genotypes, to increase the haploid induction ability for the progeny. At last, the use of androgenesis to multiplicate male fertile HD which pointed out some limits of the method. In conclusion, the whole of this work is globally aimed to the production of high value F1 hybrids suitable for growers of ranunculus varieties.

Session 3: **MOLECULAR METHODS IN GENETIC IMPROVEMENT**

O15. **AGROBACTERIUM-HOST INTERACTIONS: BIOLOGY AND BIOTECHNOLOGY**

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Agrobacterium-mediated genetic transformation of plant species is the most commonly used technique for the production of genetically modified plants. While research in the past several decades has revealed much of the bacterial molecular machinery and processes by which the bacterium delivers a portion of its DNA into the host cell, we have only recently begun to understand the roles played by host proteins during the transformation process. Several studies have revealed how Agrobacterium hijacks basic cellular processes and uses various plant factors for the transport of its DNA through the host-cell cytoplasm and nuclear membrane and for its integration into the host genome. These studies hold great promise for the future of plant biotechnology, as they can potentially be used to develop new techniques and methods which will expand Agrobacterium's host range to recalcitrant plant species.
O16. NAVIGATING THE NETWORK OF FLORAL SCENT PRODUCTION

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Flower fragrance is a composite character determined by secondary metabolites of diverse biosynthetic origin. Together with other traits, such as flower color, it is used by plants to lure pollinators and seed dispersers, thus ensuring plant survival.

Research into the regulatory mechanisms leading to floral scent production/emission is still in its infancy and even less is known regarding flow within and cross-talk between secondary metabolic pathways leading to floral scent production. Using transgenic plants modified in anthocyanin production, we revealed an intriguing interaction between the branches of the phenylpropanoid pathway leading to the production of anthocyanins and volatiles. Specifically, we recorded five- to sevenfold higher levels of the volatile phenylpropanoids methyl benzoate and 2-hydroxymethyl benzoate in flavanone 3-hydroxylase (F3h)-suppressed carnation flowers with dramatically reduced anthocyanin levels, as compared to control non-transgenic flowers. Furthermore, overexpression in petunia flowers of the transcriptional regulator Pap1 (production of anthocyanin pigment 1), which activates the phenylpropanoid pathway, led to increases in both anthocyanin accumulation and volatile phenylpropanoid emission. Using virus-induced gene silencing (VIGS) for large-scale identification of floral scent genes, we further characterized metabolic flow within the pathway. The advantages of VIGS and of petunia as a model plant create a solid infrastructure for the future isolation of regulatory factors involved in floral scent production/emission. Knowledge gained from an understanding of mechanisms leading to floral scent production/emission should provide us with better insight into nature's way of ensuring evolutionary success, as well as with advanced tools for the metabolic engineering of fragrance.

O17. SCREENING OF VIIVIPAROUS PLANTLET FORMATION-RELATED GENES IN KALANCHOE DAIGREMONTIANA BY SSH ANALYSIS

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Kalanchoe daigremontiana propagates asexually forming plantlets from epiphyllous buds on leaf margin notches. To identify genes involved in this process, suppression subtractive hybridisation libraries (SSH) were prepared. RNA was extracted from tissue forming buds (tester) and, separately, from tissue not forming buds (driver). cDNAs were synthesized and selectively amplified to enrich differentially expressed sequences. PCR products of subtracted tester library were inserted in a plasmid vector. These clones were differentially screened by hybridization using either tester or driver libraries as probes. The expressed or silenced cDNA clones were sequenced to identify the genes potentially involved in the bud formation. On the basis of tblastx algorithm and after correcting for redundancy, 135 cDNA clones were classified and grouped into fourteen functional categories according to Goldberg database (http://estdb.biology.ucla.edu/PcEST/). Metabolism (16 cDNA), Energy (22), Cell Growth/Division (2), Transcription (10), Post-transcription (6), Protein Synthesis (4), Protein Destination and Storage (7), Transports (6), Intracellular Traffic (1), Cell Structure (4), Signal Transduction (10), Disease/Defense (6), Secondary Metabolism (1), and Unknown (31) or Unclassified Proteins (9). The normalised quantitative RT-PCR technique were adopted to weigh up the role of selected genes on the epiphyllous bud formation, using both the cDNAs utilised for the SSH library preparation and cDNAs prepared from leaf sectors at different growth phase. Preliminary expression profiling of these genes will be presented.

O18. PROTEOMIC ANALYSES OF SOMATIC AND ZYGOTIC EMBRYOS AND ENDOSPERM TISSUE OF CYCLAMEN PERSICUM MILL.

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In the horticulturally important ornamental species Cyclamen persicum Mill. somatic embryogenesis is an efficient vegetative propagation method and interest is given in the development of artificial seeds. Previous studies have shown that cyclamen somatic embryos can successfully be encapsulated and desiccated, but need the external supply of nutrients for germination.

Objectives of the present study were to systematically compare the proteomes of zygotic embryos, somatic embryos grown in liquid medium containing 30 or 60 g l-1 sucrose, germinating embryos of both types, and endosperm in order to obtain novel insights into seed and germination physiology. Using high resolution two-dimensional isoelectric focussing/sodium dodecylsulfate polyacrylamide gel electrophoresis (2D IEF / SDS PAGE), 74 % of the proteins expressed in zygotic embryos were found in similar abundance in somatic embryos grown in 60 g l-1 sucrose. Among the differentially accumulating proteins, four enzymes of the primary metabolism involved in glycolysis (UDP-glucose pyrophosphatase, fructose bisphosphate aldolase, triosephosphate isomerase, glyceraldehyde-3-phosphate dehydrogenase) were specifically induced in somatic embryos. Proteins of high abundance in embryos and endosperm and comparatively low abundance in germinating embryos represent candidates for seed storage proteins, which so far were unknown for C. persicum. 115 globulin proteins identified by mass spectrometry were present in high levels in somatic embryos, zygotic embryos and endosperm, whereas 75 globulins were detected in endosperm and zygotic embryos mainly. Xyloglucans are known to be another group of seed storage compounds in C. persicum, while their assembly was not studied so far. Interestingly, xyloglucan endotransglycosylases, one enzyme group playing a role in xyloglucan biosynthesis but also degradation were found to be highly expressed in endosperm tissue.
O19. **REGULATORY GENES IN CREATING FLOWER COLOR PATTERNS**

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Differences in structural gene expression are responsible for a wide range of responses from human cancer to patterned flowers. Gene silencing is one of the ways in which gene expression is controlled. We have developed a model system to study gene silencing using a gene silencing mutation in *Petunia* (Star mutation) and the ability of certain viruses to reverse the silencing mutation. This model system was used to characterize how the Star color flower pattern was controlled.

O20. **COMPARISON OF TWO TRANSFORMATION METHOD EFFICIENCY USING PELAGRONIUM X HORTORUM 'PANACHE SUD' PROTOPLASTS AND LEAF DISCS AS EXPLANTS**

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Because water resources must be preserved by both plant producers and consumers, the use of ornamental plants that maintain aesthetic qualities under drought stress is of great interest. We have tested *P. x hortorum* cultivars and found that resistance was related to sugar accumulation. Since drought resistance is a complex phenomenon, we choose to face the problem via genetic transformation, using a construct harbouring 1-SST gene isolated from onion and coding for fructan synthase. First of all, plant regeneration from leaf protoplasts and leaf discs was optimized and genetic transformation protrial was performed. Then, the process was set up. Differences in structural gene expression are responsible for a wide range of responses from human cancer to patterned flowers. Gene silencing is one of the ways in which gene expression is controlled. We have developed a model system to study gene silencing using a gene silencing mutation in *Petunia* (Star mutation) and the ability of certain viruses to reverse the silencing mutation. This model system was used to characterize how the Star color flower pattern was controlled.

O21. **INSECT RESISTANT TRANSGENIC CHRYSANTHEMUM [DENDRANTHEMA X GRANDIFLORUM]**

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Chrysanthemum *Dendranthema x grandiflorum* (Ramat.) Kitamura is now one of the important ornamental flowers of their wide range of flower color, shape and form. Insect damage by lepidopteran pests is very serious problem, resulting in quality and high pesticide cost. In order to reduce the damage, we introduced a modified endotoxin gene of cry1Ab of *Bacillus thuringiensis var. kurstaki* (named as mcbt), into chrysanthemum cultivars. The five popular chrysanthemum cultivars were transformed using a disarmed strain of *Rhizobium radiobactor* (Agrobacterium tumefaciens), EHA105, carrying a binary vector, pBIK201mcbt harbouring a mcbt gene. Leaf discs were co-cultured with *Rhizobium* and thereafter cultured on the regeneration medium containing G418 for the selection, according to the procedure of Shinoyama et al. (2002). The regeneration frequency was 9.2 to 25.5% from inoculated leaf discs of five cultivars. The *mcbt* gene was detected in all the regenerated plantlets by Southern blot analysis. In Northern blot analysis, no degradation of the mRNA was detected in the *mcbt* transformants. On the other hand, the transformants by wild type *cry1Ab* gene showed a heavy mRNA degradation. The accumulation of *Cry1Ab* insecticidal crystal protein in 20 transformed lines, selected at random, was confirmed by Western blot analysis. The level of accumulation of *Cry1Ab* ICP (molecular size was 58 kDa) ranged from 9.3 ng to 82.5 ng per 50 µg total soluble protein. Feeding leaf area was under 1% by five larvae. All larvae of *S. litura* tested died during the second instar supplying the leaves of the *mcbt*-transformants whose expression level of *Cry1Ab* ICP exceeded 48.9 ng per 50 µg total soluble protein. Feeding leaf area was under 1% by five larvae. All larvae of *S. littura* tested died during the second instar supplying the leaves of the *mcbt*-transformants whose expression level of *Cry1Ab* ICP exceeded 62.3 ng per 50 µg total soluble protein. Feeding leaf area was 3.5 to 5.2% by five larvae. The transformants are very useful to reduce costs for lepidopteran insects control and/or to reduce working hours. The environmental pollution by the scatter of pesticides might also be avoided. In the future, a safety assessment of these insect-resistant transgenic chrysanthemum should be carried out to be proven their benefits.
developed. The aim of the study was to verify this method with respect to the genetic stability of the plants produced. The new protocol for tulip micropropagation by cyclic multiplication of adventitious shoots, in the presence of thidiazuron, was developed. Further improvement is desired, and there are still open questions on how to achieve reliable and reproducible results at reasonable time and cost. To find answers to these questions scientists engaged in ornamental research can contribute significantly. In the field of vegetative reproduced ornamentals EDV’s play an important role. To find a commonly accepted interpretation of the concept of EDV and its limits will be one of the major challenges in the future of the ornamental breeding world.

O22. Molecular Biology Approach for Improving Chrysanthemum Resistance to Virus B

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Chrysanthemum is an important cut flower group and is ranked third in international market. The chrysanthemum have been propagated vegetatively from a long time so the viruses and viroids which infect this group, have also perpetuated. Chrysanthemum virus B (CVB) is a member of the carlavirus group and is widespread throughout the world in cultivated varieties of chrysanthemum. There are some strategies for creating virus resistant plants. The most important based on expression of coat protein genes, antisense sequences and on post transcriptional gene-silencing. For creating virus resistant chrysanthemum we used the disarmed super virulent strain CBE21 with plasmid pBi121, containing the gene CVB coat protein. The nucleotide sequence ORF5 encodes the CVB coat protein was cloned in All-Russian Agriculture Biotechnology RAAS. Three constructs had CVB open reading frame 5 (ORF5) in sense, antisense and double sense orientation. The constructs had also double 35S promoter and kanamycin-resistance selectable marker gene. Total 12 transgenic lines with antisense cDNA sequence, 7 lines with sense cDNA sequence and 20 line with double sense cDNA sequence were produced. The transformation frequency at kanamycin selection varied from 2.3 to 6%. Presence of coat protein gene was detected by PCR. New vector for chrysanthemum transformation create on a base of a generic vector, PHANNIBAL. In this case virus RNA degradation can be activated by introducing DNA sequences that are homologous to expressed genes (RNAi technologies).

Session 4: Breeders’ Rights: Molecular and Phenotypical Characterization

O23. New Developments in the International Union for the Protection of New Varieties of Plants (UPOV)

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UPOV, which continues to be the only internationally harmonized, effective sui generis system of plant variety protection, is continuing to expand. In December 2005, UPOV published a report on the impact of plant variety protection according to the UPOV Convention, some of the findings of which are summarized in this paper. With the expansion of UPOV in both geographical terms and in terms of the number of genera and species for which protection is sought, there are increasing demands for general information on the UPOV Convention. This paper explains some of the initiatives taken by UPOV in recent years to meet those needs, including the launch of a distance learning course and the development of new guidance documents on the examination of distinctness, uniformity and stability (DUS). One aspect of the UPOV Convention explored in this paper is the provision for essentially derived varieties. The relationship between initial varieties and essentially derived varieties is explained and the role of the authorities in matters concerning essentially derived varieties is considered. An overview of the current situation with regard to the possible use of molecular techniques in the DUS examination is also presented by reference to proposals considered within UPOV.

O24. Implications of Essentially Derived Varieties (EDV’s) for Ornamental Plant Breeders

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The incorporation of the concept of Essentially Derived Varieties (EDV’s) in the UPOV 1991 Act has been a remarkable improvement in the protection of plant varieties. However, the respective article in the UPOV Act unfortunately is worded in very unclear and partly redundant language, which makes it difficult to understand and, a fortiori, to interpret the provision in a uniform way. Several questions remain open. One of the most important is whether the existence of an EDV has to be determined on the basis of the phenotype or the genotype. This question is especially important in the field of ornamental varieties. Out of the examples named as act of derivation in Article 14 (5) (c) UPOV 1991, only the following are used in the practise of breeding ornamentals: selection of a natural or induced mutant or of a somaclonal variant, the selection of a variant individual from plants of the initial variety and transformation by genetic engineering. All of these acts of derivation result in mono-parental varieties that are genotypically nearly identical with their mother variety. The most important practical examples for EDV’s are mutants. In this regard it must be taken into consideration that mutants – being the main reason for incorporating EDV’s in the UPOV 1991 Act – often differ significantly in phenotypic characters from their mother variety in their phenotypic characteristics. Focussing too much on the phenotype a mutant often would not qualify as an EDV. Consequently there are strong indications that the genotypic relationship between the initial variety and an EDV thereof rates high and is of major importance to identify an EDV. Although the tools for determining genotypic relationship between two varieties are quite developed, further improvement is desired, and there are still open questions on how to achieve reliable and reproducible results at reasonable time and cost. To find answers to these questions scientists engaged in ornamental research can contribute significantly. In the field of vegetative reproduced ornamentals EDV’s play an important role. To find a commonly accepted interpretation of the concept of EDV and its limits will be one of the major challenges in the future of the ornamental breeding world.

O25. Somaclonal Variation in Micropropagated Tulips Determined by Phenotype and DNA Markers

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The new protocol for tulip micropropagation by cyclic multiplication of adventitious shoots, in the presence of thidiazuron, was developed. The aim of the study was to verify this method with respect to the genetic stability of the plants produced. The
genetic fidelity of the micropropagated plants was evaluated by phenotypic observation and DNA analysis with RAPD (Random Amplified Polymorphic DNA) and ISSR (Inter-Simple Sequence Repeat) techniques. Genetic relationships among the cultivars and micropropagated plants was estimated on the basis of created DNA patterns. At first, several distant and closely related genotypes were used for analysis: ‘Blue Parrot’, ‘Prominence’, their sports and ‘Giewont’. In reaction with 27 RAPD and 90 ISSR primers 56 polymorphic bands were obtained. The distant related cultivars were easily distinguished in both reactions. But within the closely related cultivars, either ‘Prominence’ or ‘Blue Parrot’, each was distinguished only from its two sports among the three ones analysed. Generated DNA markers were assessed to detect genetical changes in ‘Blue Parrot’ somaclones which were selected within the micropropagated plants on the basis of phenotypic observation. The plants derived from the long-term cultures, lasted from two to eight years. Within the flowering plants, no phenotypic off-types were noted in the progeny derived from a 2-year-old culture, but in the progeny derived from a 4-year-old culture, all plants had a changed colour of flowers, from purple violet to red purple. Within the juvenile plants, derived from 4-7-year-old cultures, the off-types with variegated or deformed leaves occurred. A genome analysis was performed on 23 genotypes, including original cultivar, as a control, and micropropagated off-types and randomly selected true-to-types. Using 32 RAPD and ISSR primers, 1935 bands were obtained and 42 of them were polymorphic. The phenotypic true-to-type plants, derived from a 2-year-old culture, did not reveal polymorphism or it was very low. DNA of all plants micropropagated for four and more years showed the changes in genome sequence (confirmed by RAPD and ISSR reactions). Besides, the rate of genetic changes increased along with the duration of micropropagation. In conclusion: 1) both types of DNA markers are suitable for analysis of genetic stability/instability of micropropagated tulip plants, 2) shoot multiplication can not last more than two years, 3) some somaclones seem to be an interesting material for tulip breeding.

O26. MORPHOLOGICAL CHARACTERISTICS AND AFLP MARKERS FOR CLASSIFYING AN ITALIAN GENEPOOL OF EVERGREEN AZALEAS

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Morphological and genetic characterisations were carried out to investigate origins, classification and diversity of evergreen azaleas grown in historical gardens and nurseries of the Lake Maggiore area (Northern Italy). Ninety three genotypes (locally classified in the groups Indica, Japonica and Amoena) were described by means of 10 variables referring to flower and leaf morphological characteristics and were DNA-typed by means of the AFLP technique. Forty species and cultivars, chosen among distinguishable groups within the evergreen azaleas (Belgian, Hirado, Kurume and Satsuki), were also included in this study as a reference for classification to reveal the origins of the Italian accessions. Similarities of the fingerprint patterns were evaluated as an estimate for genetic conformity and for relatedness performing ordination analyses (UPGMA clustering and PCO). Morphological and AFLP data were compared by means of Mantel’s test. Assignment tests both on the level of the groups as on individual plant level for the reference and Italian genotypes were applied to evaluate further the relatedness of the groups or individual accessions. Results demonstrated that the AFLP technique together with morphological characterisation could be an useful tool for clarifying the origin and classification of evergreen azalea gene pools. Comparing the reference and Italian genotypes, conclusions about the classification of the evergreen azalea cultivars located in the Lake Maggiore area could be drawn. In more detail, the Indica group appeared as a distinct entity from the reference ones from which it derives. The Amoena azaleas seemed to be a subgroup of the Kurume group while the existence of the group Japonica appeared doubtful.
P1. **Breeding Program on Woody Ornamental Plants in Angers – France: A Collaboration of 32 Years Between INRA and SAPHO**

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The request for novelties is very important in the field of ornamental horticulture. Since 1972, INRA (National Institute of Agronomic Research) and SAPHO (Association for the improvement of horticultural plants) have cooperated to propose innovative varieties. These innovations include aesthetic traits (colour, shape), new uses (ground cover, compact habit) and rusticity (disease and frost resistance). SAPHO is now acting for the edition of these new varieties in different countries (Europe, USA). Several techniques have been developed to increase the genetic variability in different species bred during those last 32 years: intra- and interspecific hybridization, in vitro culture (somaclonal variation, embryo rescue, micro-propagation of selected hybrids), mutagenesis to modify flower colour and plant habit and growth. Up to now, this collaboration has produced 25 varieties that have been protected through breeders’ rights and spread in Europe. Some varieties have also been propagated in North America under licence. Some have received an award in professional trade shows. The success of this project shows that collaboration between public institutes and private firms is essential to improve the quality of plants and regularly propose new varieties to the market.

P2. **General (GCA) and Specific (SCA) Combining Ability for Quantitative Characters of Ornamental Value in F1 Interspecific Morning Glory Hybrids**

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Complete diallel crosses were performed, in 2001, involving four Ipomoea species: I. tricolor, I. violacea, I. purpurea and I. nesheracea. F1 hybrids were grown in two location in 2002 and 2003 and, based on results obtained, general (GCA) and specific combining ability (SCA) effects were estimated for the main quantitative characters considered as important breeding objective in these ornamental species: plant height, length of inflorescence, no. of flowers/inflorescence, length of open corolla, diameter of open corolla, earliness of flowering and persistence of open flowers. The great majority of the studied characters, except for earliness of flowering and persistence of open flowers, showed significant additive effects, revealed by the high values of GCA effects. These results suggest that, in ornamental species of Ipomoea genus, for the quantitative characters taken into consideration, there might be found distinct groups of polygenes with positive effects and other groups with negative ones upon these traits. On the other hands, the SCA effects have been found significant only for two characters: length of inflorescence and earliness of flowering. Considering these results it might be stated that, with the four ornamental morning glory, species under study, there are poor chances to obtained simple hybrids which would exhibit a significant heterosis of the main quantitative traits of interest in breeding programs.

P3. **The Development of New Products Through the Collection of Old Cultivars and Wild Species of Ranunculus Genus**

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The commercial production of ornamental plants, and thereby their introduction into economic and cultural daily life, depends upon the availability of “new” introductions and novelty is an important consideration in selection of ornamental crops. Therefore, news designs, colours and uses are goals to create new varieties. In developing new products, scientists, plant collectors and gardeners introduce new cultivars better adapted to the wishes of mankind and search for ornamental plants previously unknown in their region. The ornamental species Ranunculus asiaticus L. is a leader product at the Flower Market of Sanremo and a high percentage (about 84%) of dealers is involved in its commercialisation. This species is widely used as cut flower, but dwarf types can be forced as potted and border plants. R. asiaticus has a long and distinguished history in cultivation. Its popularity began in the 17th century and most of the old named cultivars are apparently now extinct, few of them being still present in botanic gardens or nurseries. A propagation scheme for this crop through in vitro techniques has been outlined (Beruto & Debergh, 2004) which was shown to be an attractive alternative for rapid clonal multiplication bringing novelties (clones) onto the market. In this work, the collection of old cultivars of R. asiaticus and wild species of genus Ranunculus was undertaken. The choice of botanic wild species was made by taking into consideration particular aesthetic traits (foliage, flowers or sepals colour, habitus, number of flowers per stem, etc...) and the different local ecopedological conditions. In vitro techniques have been applied in order to save, collect and propagate the different wild genetic resources which will be evaluated under in vivo conditions as garden and cut flower products. Historical researches were performed too, in order to report the names and sometimes the description and the date of introduction of old cultivars of R. asiaticus and, when possible, the in vitro culture of some of them was undertaken.
P4. **INTERSPECIFIC HYBRIDIZATION BETWEEN Kalanchoe blossfeldiana AND SEVERAL WILD Kalanchoe species with ornamental value**

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Kalanchoe blossfeldiana is one of the most important ornamental indoor plants during the winter season. Breeding of K. blossfeldiana has been restricted to intra- and inter-specific hybridization with allied species. To enlarge further variations, reciprocal crosses between K. blossfeldiana and several wild Kalanchoe species were carried out and ovule culture technique was applied to rescue the hybrids. Reciprocal cross compatibility to K. blossfeldiana was observed in K. spathulata and K. pumila which yielded normal seeds. Unilateral cross compatibility was observed in K. citrina, K. farinacea, K. garambiensis, K. nyikae and K. pubescens which yielded normal seeds, whereas K. daigremontiana and K. laxiflora needed ovule culture technique to obtain the seedlings when K. blossfeldiana was used as seed plant. Hybridity of these F1 plants was confirmed by flow cytometric and RAPD analyses. Among these species, K. pubescens, K. daigremontiana and K. laxiflora have pendent flowers and are classified into section Bryophyllum, whereas the others including K. blossfeldiana have erect flowers and are classified into section Kalanchoe. Consequently, three combinations of intersection hybrids were obtained in the present study. Flower morphology and color of these intersection hybrids were almost intermediate between both parental species, but direction of flower was almost upwards. Since these F1 hybrids were sterile, we tried to produce amphidiploids by utilizing spontaneous chromosome doubling in tissue culture. As the results, amphidiploids of K. blossfeldiana × K. pubescens and K. blossfeldiana × K. pumila were successfully obtained by leaf tissue culture. These amphidiploids are expected to restore fertility and to produce F2 generation.

P5. **GREENHOUSE SELECTION FOR BLACK SPOT RESISTANCE IN ROSES**

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Black spot (Diplocarpon rosae) is the main fungal disease on outdoor grown roses worldwide. In garden rose breeding, disease resistance to this pathogen can only be evaluated from the moment greenhouse selected seedlings are transferred to open air. Because disease resistance is an important trait in new rose cultivars, methods are needed to screen black spot resistance during the first year of selection in the greenhouse. Procedures to inoculate rose seedlings with black spot in the greenhouse were established. Since black spot needs a prolonged period of leaf wetness for conidia germination and humid conditions for further development, greenhouse circumstances were adapted. Seedlings were covered with a plastic tent after inoculation and plants were watered on the leaves to increase humidity. Optimal conidial concentrations were defined and the effect of repeated inoculations was tested to optimize the screening procedure. Also the ideal inoculation moment during the growing season was determined. Different experiments on groups of seedling populations led to the development of a protocol useful for greenhouse inoculation and resistance screening in the first year of selection.

P6. **GENETIC IMPROVEMENT AND COMMERCIAL DEVELOPMENT OF Lupinus havardii Wats. (big bend bluebonnet) as a new specialty cut flower crop**

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Lupinus havardii/Wats. is native to a narrow geographical range along the Rio Grande River in southwestern Texas, and produces attractive tall blue flowered racemes having great potential as a specialty cut flower crop. A research project, initially focusing on improving crop uniformity and developing novel flower colors, was initiated in 1991. Other traits used for recurrent phenotypic selection breeding included low shattering and long display life of flowers on the raceme to improve vase life. It was discovered that flower abscission and senescence, which are related to sensitivity to ethylene, were the key components that postharvest vase life of cut racemes. Therefore, the relative response of the promising germplasm to ethylene was evaluated following treatment with 2-chloroethylphosphonic acid in the holding solution. Over the years, we have developed several blue (Blue Select, ‘Texas Sapphire’), white (White Select, ‘Texas Ice’) and pink flowered (Pink bulk, Light Pink, Dark Pink, and Coral Pink) lines and cultivars of L. havardii with reduced ethylene sensitivity and extended vase life. Based on evaluation, test marketing and limited commercial production, two cultivars ‘Texas Saphire’ (blue flowers) and ‘Texas Ice’ (white flowers) have been released. Our results clearly establish the important role of selection and breeding strategies in the improvement of bluebonnet as a specialty cut flower.

P7. **EXPERIMENTAL RESULTS ON BREEDING FOXGLOVE (Digitalis spp.) FOR MEDICINAL AND/OR ORNAMENTAL PURPOSES**

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In spite of the fact that out of the countless “medicine plants”, probably foxglove is the most beautiful flower, very little has been done so far in breeding cultivars of high ornamental value, most efforts being directed toward breeding and releasing cultivars with high and very high content in cardiotonic glycosides. In a rather limited breeding program, four foxglove cultivars, three belonging to D. purpurea and one to D. lanata, were used as genitors in complete diallel crosses performed in 2000. The F1 hybrids as well as their parents were grown in two locations and two consecutive years (2001 and 2002). Based on results obtained, heritability (both in wide and narrow sense) as well as general (GCA) and specific combining ability (SCA) were computed. We declared intention of understanding the genetic determinism of several quantitative traits which are
considered valuable medicinal and/or ornamental breeding objectives: plant hight, length of inflorescence, no. of flowers/inflorescence, flower size, no.of leaves/plant, leaf size, weight of dried leaf and content of cardiotonic glycosides. Most of the quantitative characters taken into consideration have had high values (H = 0.70-0.95) of wide sense heritability but very seldom these were accompanied by comparable values of narrow sense heritability, suggesting a rather low efficiency of phenotypic selection for such traits. Additive effects, emphasized by high GCA values for several quantitative characters, including cardiotonic glycosides content, suggest that, in foxglove, for most of the characters under study, there might be found distinct groups of polygenes with positive effects and other groups with negative ones. SCA showed significant values for most plant and flower characters which could be a proof that, in foxglove, there are fair chances to develop commercial hybrids exhibiting an obvious heterosis in the expected direction (negative or positive). There has been concluded that such data could be efficiently used in breeding ornamental foxglove cultivars with low and very low contents of cardiotonic glycosides vs. cultivars destined only to medical purposes, with low or no ornamental value but high content of cardiotonic glycosides.

P8. EVALUATION OF PROGENIES OF LILIUM BY SOME TRAITS
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The genus Lilium (Lilium L.) is one of the six major genus of vegetative propagated bulbous plants. Recently, the acreage of bulb cultivation for cut flower production has increased in Holland and Italy. Imported material is generally developed through selection programmes (Grassotti et al., 1990) in environments very different from local conditions in Latvia. Every year breeders have to make selections among and do analysis for several thousands of plants. The main objective in the evaluation of different traits is to obtain true criteria for selection, determine most essential factors effecting productivity and evaluate traits of progenies of Lilium. In order to obtain Lilium selections suitable in Latvia, seedlings of 14 populations were tested for morphological traits: plant height, number of flowers per stem, diameter of a flower during two development years. Significant differences between and within populations were detected. Hybrids were characterized by low to medium variability of the diameter of a flower (coefficient of variation 6.8 –17.8%), medium variability of the plant height (9.7 –20.4%) and high number of flowers per stem (34.2 – 71.3%). In the second year of development, a significant increase in the plant height was observed in 85.7% combinations, in the number of flowers per stem in 64.3%, and in the diameter of a flower in 21.4% combinations compared to seedlings of the first development year. Positive correlation was determined between the plant height and the number of flowers per stem. No significant coherence was found between the plant height and diameter of a flower, between the number of flowers per stem and diameter of a flower.

P9. MODERN HUNGARIAN ROSES - THE BEST VARIETIES FOR PUBLIC PARKS
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In Hungary there are many good results in outdoor rose breeding. Gergely Márk hybridist has been working for decades, the number of his rose varieties is about 600. Two of his roses has been awarded medals in international competitions. A scientific evaluation of the G. Márk bred varieties has been going on since 2004 in the exhibition garden in Törökbálint to selecting the best roses for public parks. 547 varieties were evaluated. Because of the high number of the items, ranking examinations were carried out. The ranked values were corrected by own made formulas (X3/35 at flowering capability, 3.8 at density of foliage) and averages were calculated. By means of the upper and lower quartiles and upper and lower 1/8th of the varieties’ data, 1, 2 and -1, -2 points were given to the best and the poorest roses respectively. Then weighted sum was made, were the weight numbers were the followings: blooming: 4, foliage: 3, fragrance: 3, spring: 1, late autumn: 1, after the flowering: 1, strength: 2, health: 2. The most important features are the blooming ability and the mass of the foliage, those were assessed several times. In spring 2005 the growth intensity of the shoots, glitter and colour of the foliage were ranked. In late autumn 2004 blooming ability, showy foliage and attractive hips were ranked. After the blooming period three rankings were made: attractiveness of the old flowers, and plant habit after a cloudy period. In the autumn of 2005 the health of the varieties was observed: green colour of the foliage, falling of infected leaves and the seriousness of the leaf-disease (Diplocarpon r., Phragmidium m., Sphaerotheca p., Sphaceloma r.). The strength of the woody parts of the bush was evaluated also. The series of data was completed with the intensity of the fragrance. According to the total scores the best Hungarian varieties are the followings: Hybrid Tea: ‘Teleki Blanka emléke’, ‘Marscika’, ‘Ruttkai Eva emléke’, ‘Fra Diavolo’, ‘Tisza’, ‘Áprily Lajos emléke’; Floribunda: ‘Laborfalvi Róza emléke’, ‘Bem apó emléke’, ‘Báthory István emléke’, ‘Erzsébet királyné emléke’, ‘Katona József emléke’, ‘Királyhelmec’, ‘Wilma Holder’; Polyantha: ‘Játóka Sándor emléke’, ‘Savaria’, ‘Petőfi Sándor emléke’, ‘Emese’; Miniatures: ‘Ermye’, ‘Libán’, ‘Lippay János emléke’, ‘Szépcseck’, ‘Somogy’, ‘Modern Shrub: ‘Fehér Picurka’; ‘Csíkcsőröska’, ‘Panca’, ‘Tündér Ilona’, ‘Anikó’. The most floriferous were Polyantha and Miniature group although Hybrid Tea had the strongest scent. Foliage and bush of Modern Shrub is the healthiest, but Miniature had the densest foliage. The previous two classes were the best in spring and late autumn, but after the main blooming wave Floribunda and Hybrid Tea were the most decorative. ‘Fehér Picurka’ has got the highest score among all the Hungarian varieties.
new species to this cultivation technique demands selection criteria for certain plant attributes such as dwarfism, precocity, plasticity, and rusticity. In Pernambuco state, Northeast region of Brazil, the traditional nurseries have been growing some fruit bearing plants in containers, mainly species like jaboticaba (Myrciaria jaboticaba), star fruit (Averrhoa carambola), sapota (Manilkara zapota), mango (Mangifera indica), orange (Citrus sinensis), lemon (Citrus limon), and pomegranate (Punica granatum). When plants are commercialized with 2 years old, the growers obtain higher profits because of the high prices, especially if some fruits are already present. Ornamental attributes were observed in potential species: canopy shape, foliage habit, colors and texture of flowers, fruits, leaves and presence of scent. Among the native species, the ones with greater potential for selection and breeding for ornamentals in containers are members of the Myrtaceae, Leguminosae, Sapotaceae, Annonaceae, and Anacardiaceae families.

P11. **ORNAMENTAL ATTRIBUTES FOR LANDSCAPE DESIGN WITH HELICONIA PLANTS IN BRAZIL**


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Heliconia family has ornamental species with extremely exotic appearance, exuberant green foliage and hot colored inflorescence, characteristics of tropical garden plants. They are used in floriculture industry either as cut flower or pot plant, but the use of heliconias in landscape design is not restricted to outdoor gardens, they are also used in bay window, and indoor garden with low luminosity. Despite the esthetic function, these plants can aggregate fauna such as bats, hummingbirds, and even small primates. The objective of this study was the evaluation of ornamental attributes of Heliconia Germplasm at the UFRPE Collection in Pernambuco-Brazil. The following 28 genotypes were observed: H. bihai (L.) L. cv. Kamehameha; H. bihai (L.) L. cv. Nappi Yellow; H. pendula Wawra; H. episcopalis Vellozo; H. collinsiana Griggs; H. rostrata Ruiz & Pávón; H. caribaea Lamark x H. bihai (L.) L. cv. Carib Flame; H. stricta Huber; H. psittacorum L.f x H. spathocircinata Aristeguieta cv. Golden Torch, and H. bihai (L.) L.; H. psittacorum L.f x H. spathocircinata Aristeguieta cv. Golden Torch Adrian; H. psittacorum L.f x H. spathocircinata Aristeguieta cv. Alan Carle; H. psittacorum L.f cv. Strawberries & Cream; H. psittacorum L.f cv. Suriname Sassy; H. psittacorum Red Opol; H.pseudoaemygdiana L. Em. & Em; H. psittacorum Red Gold; Heliconia x nickeriensis Maas & de Rooij; H. latispitha Benth (orange.); H. latispitha Bentham cv Yellow Gyro; H. rautulliana; H. bihai (L.) L. cv. Fire Bird; H. pendula Wawra; H. episcopalis Vellozo; H. collinsiana Griggs; H. rostrata Ruiz & Pávón; H. caribaea Lamark x H. bihai (L.) L. cv. Carib Flame; H. stricta Huber; H. psittacorum L.f x H. spathocircinata Aristeguieta cv. Golden Torch, and H. bihai (L.) L. The hybrid Golden Torch presented better shoot production with 143.7 shoots per clump on average.

P12. **NUMBER OF SHOOTS AND BLOOMING OF HELICONIA CULTIVATED UNDER PARTIAL SHADE**

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The area cultivated with heliconia for cut flowers is increasing in North and Northeast regions of Brazil. The environmental conditions are suitable for this tropical plant and there is a great potential for commercialization of its beautiful inflorescence. Several research institutes have been working hard to improve the performance of many aspects of the cut flower production chain in Brazil. Agronomic aspects such as production of shoots, number of inflorescences and production season are very important for selection of material to support breeding programs. Ten genotypes of Heliconia were evaluated during 18 months in Pernambuco state, Northeast region of Brazil: H. bihai (L.) L. cv. Kamehameha; H. bihai (L.) L. cv. Nappi Yellow; H. stricta Huber cv Fire Bird; H. pendula Wawra; H. episcopalis Vellozo; H. collinsiana Griggs; H. rostrata Ruiz & Pávón; H. caribaea Lamark x H. bihai (L.) L. cv. Carib Flame; H. stricta Huber; H. psittacorum L.f x H. spathocircinata Aristeguieta cv. Golden Torch, and H. bihai (L.) L. The hybrid Golden Torch presented better shoot production with 143.7 shoots per clump on average. The genotypes H. pendula, H. episcopalis and H. bihai cv. Nappi Yellow produced the smallest number shoots per clump (31.7 to 41.3, on average) and also the smallest number of inflorescence, explained by the correlation between these two factors and, from H. pendula, the seasonal production. The longer time needed between the shoots emission and the inflorescence flowering period, development speed, clump basal area, growth habit, inflorescence and leaves color.

P13. **MORPHOLOGICAL AND TIMING PARAMETERS FOR HARVESTING HELICONIA INFLORESCENCES**

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Heliconia inflorescences are becoming very popular because of the great variety of color, shape and size. In Brazil, the tropical cut flower industry is expanding rapidly with the increase of heliconia commercialization in the domestic and international market. Pernambuco state, in Northeast region, is leading the production of heliconia inflorescences and has been developing several programs to support research initiatives and farmers training. Consumers are mainly demanding high quality and long shelf life of the inflorescences, which are aspects directly related to the morphological and timing parameters for harvesting the inflorescences. The main criteria used by the farmers are the number of open bracts and the length of the inflorescence stem. This paper presents the results of a 18-month evaluation of ten genotypes from the Heliconia Germplasm Collection of Federal Rural University of Pernambuco State: Heliconia bihai (L.) L. cv. Kamehameha; H. bihai (L.) L. cv. Nappi Yellow; H. stricta Huber cv. Fire Bird; H. pendula Wawra; H. episcopalis Vellozo; H. collinsiana Griggs; H. rostrata Ruiz & Pávón; H. caribaea Lamark x H. bihai (L.) L. cv. Carib Flame; H. stricta Huber; H. psittacorum L.f x H. spathocircinata Aristeguieta cv. Golden Torch, and H. bihai (L.) L. The inflorescences were harvested when they presented between 2 and 4 bracts, depending on the...
species. It was accounted the number of days between the shoot emission and the inflorescence emission (DBSI). The shortest DBSI was 105.45 days and the longest DBSI was 126.93 days. The average harvesting interval (interval between emission of inflorescence bud and the harvesting day) varied from 14.4 days (Hybrid Golden Torch) to 27.9 days (H. bihai cv. Nappi Yellow). All the genotypes produced the inflorescence stems longer than 74 cm, meaning that all of them satisfy the standards for this trait.

P14. EFFECT OF GIBBERELLIN (GA₃) AND CHLORMEQUAT (CCC) ON GROWTH AND CHEMICAL COMPOSITION OF FICUS BENJAMINA L. PLANT

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In greenhouse trials, Ficus benjamina L. plants were subjected to one of the following treatments: GA₃ at 250, 500, 1000 p.p.m. and CCC at 0, 1000, 1500, 2000 ppm. GA₃ and CCC treatments were added to the growth medium (Sphagnum peatmoss + sand 1:1 v/v) drenched, 25 ml/plant bimonthly alternately. A fertilizer mixture (NPK 19:19:19) at 5 gm/plant were supplied bimonthly. The results revealed that using GA₃ at 250 p.p.m. increased leaf measurements and their content of N, but decreased internodes length, fresh and dry leaves/branches ratios total carbohydrates percentage in branches & root and K % in branches. GA₃ at 500 p.p.m. led to an increment in length and number of internodes, plant height, No. of branches and leaves, stem diameter, total leaf area, plant canopy, root length, fresh weight and dry weight of leaves, branches, shoots, roots and whole plant. GA₃ at 1000 p.p.m. increased chlorophyll (chl.) a, b, & total chls., phenols and amino acids in leaves. But, it decreased internodes & roots length. CCC at 1000 ppm increased No. of internodes and their length, plant height, No. of leaves and branches, leaf measurements, total leaf area, plant canopy, root length and FW&DW of leaves, branches, shoots, roots and whole plants. Oppositely, it decreased stem diameter, dry leaves/branches ratio and K% in roots. CCC at 1500 ppm caused an increment in internodes length, carotenoids and phenols contents in leaves, N% in branches & roots and P&K% in branches. Whereas, it decreased stem diameter and T.C. % in roots. CCC at 2000 ppm increased dry leaves/branches ratio. T.C.% in branches, NBP% in branches & roots and K%, indoles, phenols & amino acids in leaves. While, it decreased stem diameter and DW of leaves, branches, shoots, roots and whole plant.

P15. INTEGRATED PROCESS FOR TESTING POWDERY MILDEW RESISTANCE IN ROSE BREEDING

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Powdery mildew is the major economic depreciatory foliar disease for production of cut roses. The responsible of the symptoms is an external fungus: Sphaerotheca pannosa var. rosae. During the process for selection of the new cultivars, breeders need to estimate the level of resistance inside their plant material, either for parents or for new genotypes. This paper describes the search for tools of screening resistance and sensitivity. In the one hand, the preliminary steps of the method showed the necessity to carry out suitable technics for the isolation of the inoculum and following for the obtainment of mono-conidial isolates. In the other hand, the approach led to a biological test performed in Petri dishes on excised rose leaflets. Finally, to improve the efficiency of the test, comparisons were made between in situ inoculations and inoculations on excised leaflets.

P16. NEW ORNAMENTAL PEACH TREE VARIETIES – PRESENT AND PERSPECTIVE IN ROMANIA

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In the last 6 years, at Fruit Research Station Constanta, located in south eastern of Romania 6 peach varieties with ornamental characters were homologated. These genotypes were selected through hybridisations, self-pollinations and selections in hybrid fields of different ages as well as clonal selections. Four of these homologated genotypes have standard habitus and two of them dwarf size. The standard cultivars were studied in experimental trials in different zones of the country conforming with the methodology normaly used in plant tree breeding (Cociu, 1989; Ardelean, 1986). The aspects studied are the following: the phenological stages especially the blossoming time; the diameter, the form and the number of the petals and the colours of them; the resistance of the cultivars to pests and diseases; the yields of the fruits per tree; the dry matter content and the acidity of the fruits. Also, as an important element of this study, the decorative effect of the peach trees was observed. These ornamental peach varieties named Giuvaer, Alizeu, Purpuriu, Zefir are very attractive, with double flowers—red and pink coloured and with red foliage (Purpuriu). The blossoming time is for each of them of 10-14 days, covered almost one month of decor in spring and its can be used single or in combinations in landscapes or in gardens.

P17. SELECTION COMMERCIAL CLONES FOR ITALIAN LILY GROWER. A BREEDING PROGRAM AND DETECTION OF VIRUS ON LILY IN ITALY

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In Italian floriculture, ornamental bulbs are very popular for cut flower production; among them, in the last 20 years Lilies were the most important. Every year every year more than one hundred million lily bulbs are imported from abroad, in particular from Holland. In order to reduce the bulb cost, it is strategic for the national Italian Lily growers should have Italian Lily varieties and bulbs enlarged in the Italian environment. A preliminary study was started in 1983 at the Experimental Institute for Floriculture in Pescia. The breeding program involved crosses between 19 commercial varieties in order to select material to employ in a selection project. In 1984, 5 cvs of ilies were exposed to irradiation of X-rays, to obtain interesting mutants lilies. In 1986, on the basis of a previous work, another important breeding programme started includig non commercial ilies varieties.
P18. **Production of inter-section hybrids with different ploidy levels in reciprocal crosses between tetraploid *Primula denticulata* (section Denticulata) and diploid *P. rosea* (section Oreophoromis)**

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Inter-section hybrids with two types of genomic combinations were successfully obtained by the reciprocal cross between tetraploid *P. denticulata* of section Denticulata and diploid *P. rosea* of section Oreophoromis. When *P. denticulata* was used as maternal parent, the hybrids were all triploid with two genomes of *P. denticulata* and one genome of *P. rosea*. When *P. denticulata* was used as maternal parent, two genome type hybrids were obtained; one was triploid hybrid with the same genome combinations as those obtained when *P. denticulata* was used as maternal parent and the other type was tetraploid hybrid with two genomes of each species. These results suggest that unrandomized 2n gametes were formed only in female side of *P. rosea*. Among these hybrids, only triploid flowersed with two types of flower size; one type had many small flowers at the top of floral stalk similar to *P. denticulata*, and the other produced a small number of large flowers at the top of floral stalk similar to *P. rosea*. All the triploid hybrids showed low pollen fertility and did not yield the progenies by self-pollination. In contrast, tetraploid hybrids obtained when *P. rosea* was used as maternal parent showed poor growth and they have not yet attained to flowering stage.

P19. **Fifty years of woody landscape plant breeding at the University of Minnesota**

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The Woody Landscape Plant Breeding Program (WLBP) at the University of Minnesota has been in existence for 52 years. In that time over 40 woody landscape plant cultivars have been released by the program including seven large stature shade trees, eight small stature ornamental trees, eight shrubs, thirteen deciduous azaleas, and nine garden roses. The primary breeding objective for the program has always been to develop plants capable of surviving winter low temperatures equivalent to USDA Plant Hardiness Zone 4, -28.9 to -34.4°C. Secondary objectives have included selecting for elevated pH tolerance and drought tolerance in selected taxa. Recent efforts have included screening for disease tolerance, dwarf stature, and recurrent bloom in selected taxa. In 1990, the program reinitiated an effort to develop USDA Zone 4 cold-hardy, recurrent blooming shrub roses with a wide range of flower color and black spot resistance. The first three releases from this effort, small-stature polyantha types, will be available to the public in 2007. The program has undertaken efforts to understand the nature of the rose black spot disease, incited by the fungal organism *Diplocarpon rosae* Wolf, the most important rose disease in the world. A race differentiation test of black spot isolates collected from across eastern North America revealed the existence of 5 races of the black spot pathogen. Work is underway to identify race specific genes and loci associated with components of partial resistance to the pathogen in Rosa germplasm. Identified genes will be incorporated into cold hardy rose germplasm with the intent to develop durable resistance to black spot in garden rose cultivars. An effort has been made to identify sources of resistance to powdery mildew disease, *Microsphaera sp.*, in deciduous azalea germplasm. A replicated field evaluation of 41 deciduous azalea cultivars revealed seven genotypes to be completely free of symptoms in 2 locations (Minnesota and Ohio, USA) over the course of three field seasons. A survey of 112 deciduous azalea accessions in five botanic gardens and arboreta in Ohio and Minnesota revealed 17 cultivars and four species accessions with no disease symptoms in any location over three seasons. North American species and cultivars derived from these species showed a high degree of resistance to powdery mildew. *Rhododendron molle*, an Asian species was the most susceptible species evaluated, suggesting it to be the source of susceptibility to powdery mildew disease in modern cultivars. Additional breeding efforts include identifying sources of dwarfing and recurrent bloom in *Weigela* germplasm, resistance to golden canker, *Cryptodiaporthe corni*, in Pagoda dogwood, *Cornus alternifolia*, germplasm, and sources of cold hardiness in small-stature Asian maples including Korean maple, *Acer pseudesieboldianum*.

P20. **Interploid crosses in *Anemone coronaria***

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On *Anemone coronaria* L., the triploid level, obtained by crossing 4x X 2x, combines the majority of the advantages of the diploid and of the tetraploid but its commercial exploitation is delicate because the weak rate of germination of triploid seeds. On the other hand, the male fertility of these hybrids appears to be relatively high (30 to 70% of viable pollen). The crosses including these 3x plants (used as male) gave progenies with variable ploidy level according to ploidy of the other relative progenitor used. This work reports the range of ploidy level obtained from various interploid combinations and illustrate the interest of these results by an example of application of interploid crosses in breeding for flower colors. As a conclusion, triploid
plants, used in crosses, plays a central role in the breeding process of anemone by their ability to easily create a new genetic variability in an autoploid or polyplid context.

P21. **TRIPLOID HYBRIDS FORMATION IN INTERSPECIFIC CROSSES USING PRIMULA SIEBOLDII AS FEMALE PARENT**

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Primula sieboldii is one of the unique traditional ornamental plants developed in Japan and has many cultivars with wide variations in petal morphology and color pattern. Most cultivars of P. sieboldii are diploid and several cultivars, which have vigorous nature, are known to be triploid, but tetraploid cultivars are very few. For breeding of novel cultivars in P. sieboldii, there have been no reports on the use of interspecific crossing. In the present study, therefore, we tried to produce interspecific hybrids of this species with two wild species belonging to the same Cortusoides section, P. kisoana and P. jesoana, which are native to Japan with different distribution area each other. In the cross between P. sieboldii as maternal parent and P. kisoana as pollen parent, 16 cross combinations were made. Interspecific hybrids were obtained from three cross combinations of two maternal cultivars, and they were all triploids. Whereas, two ploidy levels, diploid and triploid, of interspecific hybrids were found in crosses between P. sieboldii as maternal parent and P. jesoana as pollen parent. In this interspecific cross, 63 cross combinations were made and interspecific hybrids were obtained from 15 cross combinations of six maternal cultivars. Differences of ploidy levels were depended on female cultivars of P. sieboldii in this interspecific cross combination, and two of six cultivars produced only triploid hybrids and others produced only diploid. Throughout this study, one cultivar, ‘Miyuki’, of P. sieboldii produced only triploid hybrids in both interspecific crosses. However, in intraspecific crosses using cv. Miyuki as maternal parent, only about 5% progenies were triploid and others were diploid. These results suggest that some specific cultivars of P. sieboldii partially produced unreduced female gametes always or under specific condition. These unreduced female gamete might be a causal factor for the spontaneous production of triploids in the crosses among diploid plants in P. sieboldii.

P22. **MORPHOLOGICAL CHARACTERISTICS FOR SELECTED INDIVIDUALS IN CORNUS KOUSSA BURG**

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The purpose of the research is to establish basic database on Cornus kousa by way of investigate, analyze and compare characteristics of leaf and flower, then foster good cultivar on each morphological characteristics - leaf length, leaf width, petiole length, left lateral vein, right lateral vein. Leaf length and leaf width in Mt. Jiri shows big tendency which is 83.5mm, 52.4mm each in comparison with the whole mean of 72.5mm, 41.2mm, whereas populations of Suwon and Mt. Halla has leaf length of 66.0mm, 65.7mm ~ 9.8%, 10.4% lower value in comparison with seven mean population; leaf width is 38.4mm, 35.3mm ~ 7.3%, 16.7% lower than whole mean and shows lowest tendency among seven selected populations. Long width of flower and short width of flower in Boeun shows big tendency which is 99.9mm, 96.5mm each in comparison with the whole mean of 76.0mm, 73.6mm, whereas populations of Mt. Halla has 50.1mm, 48.2mm which shows lowest tendency. On petal length, petal width and length of flower petiole, Boeun populations have bigger and Mt. Halla shows little tendency. The measurement result of flower colors on each population by using Spectrum Color Mater shows followings: populations of Mt. Duckyoo and Mt. Halla shows lower lightness than any other populations, but wholly shows higher lightness which is refers brightness in seven selected populations. Thus it shows peculiarities of white flower color.

P23. **THE IMPROVEMENT OF ORNAMENTAL PEACH VARIETIES IN THE CRIMEA**

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From extensive peach genefund, obtained and collected in Nikita Botanical Garden as a result of the selection program, the forms having ornamental importance were selected. They have made the basis of ornamental peach collection, which was formed in the early eighties of the last century. The forms and varieties of the collection are characterized by a significant range of variations of habitus, form of crown, number of petals in flower, their form and colouring, taste of fruits, blossom time, frost resistance, damage by fungous infection. The overwhelming majority of varieties with double-flowers were very sensitive to fungus pathogens, that limited their use. Taking into account the importance of ornamental peaches for gardening as resistantce, damage by fungous infection. The overwhelming majority of varieties with double-flowers were very sensitive to fungus pathogens, that limited their use. Taking into account the importance of ornamental peaches for gardening as
Cut flower production of heliconia is becoming an important agribusiness in Brazil. Selection of genotypes to support the production of inflorescences in quality and quantity is strategic for this expanding industry. Number of days from inflorescence emission to harvesting (DIH), fresh weight of stem (FWS), number of leaves in the stem at inflorescence emission (NL), diameter stem 20 cm under the inflorescence (D), stem length (SL) and inflorescence length (IL) are some important traits to be considered for genotype selection. Eighteen genotypes from the Heliconia Germplasm Collection of Federal Rural University of Pernambuco State (H. psittacorum x H. spathocircinata Aristegueta cv. Golden Torch Adrian; H. psittacorum x H. spathocircinata Aristegueta cv. Golden Torch; H. psittacorum x H. spathocircinata Aristegueta cv. Alan Carle; H. psittacorum x H. spathocircinata Aristegueta cv. Strawberries & Cream; H. psittacorum Red Opol; H. psittacorum Red Gold; Heliconia x nickeriensis Maas & de Rooij; H. latishoptha Bentham (orange); H. latishoptha Bentham cv Yellow Gyro; H. rauliniana; H. latishoptha Bentham cv. Distans; H. rostrata R. & P. (10 days); H. rostrata R. & P. (3 days); H. wagneriana Peters; H. bihai (L.) cv. Kamehameha; H. psittacorum x H. spathocircinata Aristegueta cv. Golden Torch; H. bihai) were evaluated during 18 months for these traits, since December of 2003. The inflorescences were harvested when they presented between 2 and 4 open bracts. It was observed significant (5%) differences for all the traits among genotypes. The DIH ranged from 14,85 to 29,53 days and the plants have 4,69 to 6,29 leaves. This information is important to help the growers on planning the harvesting program according to the species cultivated. The FWS varied from 0,36 kg to 0,04 kg and the DI 27,34 to 5,18 mm. The SL ranged from 56,58 to 125,47 cm, and the IL from 15,6 to 32,87 cm. It is important to notice that large and heavy inflorescences are not suitable for transportation. These results give indications of genotype selections for further studies and commercial use.
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P30. SCREENING FOR RESISTANCE OF LILY LEAVES BY BOTRYTIS SPP.
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The purpose of this study was to conduct for screening of resistance to Botrytis spp. through a “leaf tip test.” Disease severity was evaluated on an abaxial and adaxial foliar surface, ranging from 1 resistant (no lesions) to 6 (a high degree of necrosis with mycelium or even spores). After 7 days of inoculation of Botrytis elliptica on adaxial foliar surface, the DSS (Disease Severity Score) was showed Asiatic hybrids 3.8, LA (L. longiflorum x Asiatic) hybrids 3.2, Oriental hybrids 2.3, FH (L. formolongi x L. henry) hybrid 1.6, OH (Oriental x L. henry) hybrids 1.5, FO (L. formolongi x Oriental) hybrids 1.4, L. longiflorum hybrids 1.4 and OT (Oriental x Trumpet) hybrids 1.2. After 7 days of that on abaxial foliar surface, the DSS was showed from Asiatic hybrid 6.0, LA hybrids 5.84, FH hybrid 5.77, FO hybrid 5.38, L. longiflorum hybrids 5.35 and FO hybrids 5.0. After 7 days of inoculatin of Botrytis cinerea on the adaxial and abaxial foliar surface, DSS was showed below 2. Therefore, strong pathogen of botrytis spp. among lily cultivars was showed botrytis elliptica.

P31. A STUDY OF TRANSMISSION OF CHRYSANTHEMUM STUNT VIROID IN THE MARGUERITE DAISY (ARGYRANTHEMUM FRUTESCENS)
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Chrysanthemum stunt viroid (CSV) is a highly contagious and potentially damaging disease of Chrysanthemum sp. Currently, there is no data are available on CSV natural transmission through seed, pollen and insect vectors. Seed transmission in marguerite daisy is unknown. Heterologous encapsidation between viruses and in mixed infections has been reported to facilitate aphid transmission of viroids (Querci et al., 1997). In some globally available commercial cultivars of marguerite daisy is unknown. CSV transmission was approximately 25-92% for CSV and CSV+CBV. This study demonstrated that CSV is seed transmissible in the marguerite daisy, CSV is not able to transmit by aphids from mixed infection, and CSV is easily graft transmitted with or without CBV infection. This is the first report of seed transmission of CSV in the marguerite daisy.

P32. EFFECT OF CHrysanthemum B CARLAVIRUS AND CHRYSANTHEMUM STUNT VIROID IN THE MARGUERITE DAISY (ARGYRANTHEMUM FRUTESCENS)
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Frequent disease infection of ornamental plants can significantly affect aesthetic quality and reductions in yield. Virus diseases have an adverse effect on plant health, an increase of growth inhibiting substances and reduction of growth regulatory substances (Agrios, 1997). The marguerite daisy (Argyranthemum frutescens) is an important ornamental plant belonging to the chrysanthemum complex (Fam. Asteraceae). Chrysanthemum B Carlavirus (CBV) and Chrysanthemum stunt viroid (CSV) were detected in marguerite daisy in a preliminary studies, and both cause economically important diseases in Chrysanthemum sp. This study was undertaken to determine the effect of CBV and CSV in the marguerite daisy. Each of three uninfected cultivars (Comet Pink, Sultan’s Pride and Bright Carmine) were approach grafted with three naturally infected commercial cultivars (Frosty and Butterfly) with double infection (CBV+CSV), one cultivar (Sugar baby) with CSV alone and one Australian bred line (2000-101) with CSV alone. After 4 weeks the grafted plants were tested to confirm transmission by DAS-ELISA for CBV and by One-step RT-PCR for CSV. Cuttings were taken from the infected positive plants and after two weeks they were transferred to a commercial nursery to evaluate. The infected plants were 11-25% shorter (stunted), had increase bud formation, flowered 1-2 weeks later, reduced the number of flower 30-80% and deformation of flower growth, colour bleaching and colour breaking. On Comet Pink the effect on flowering was generally more severe than other two cultivated. Double infection has more severe effects on plants than single infection. This study demonstrated that both double and single infection has deleterious effects on the three cultivars. The difference between effects on different cultivars may be due to the uneven distribution of infective agents in plants or due to difference in plant resistance.

P33. FIRST RESULTS OF AN ITALIAN ORNAMENTAL CITRUS BREEDING PROGRAMM
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Italian producers are the main suppliers to the European ornamental Citrus market. Around 90% of the current nursery production is made up of four species (lemon, orange, kumquat, calamondin and chinotto). To differentiate the produce and keep the market alive, it is important that Italian nursery have new ornamental genotypes. At this purpose we started a breeding
program finalized to obtain valuable ornamental hybrids. Different crossing pollinations were therefore carried out, using monoembryonic female parents, such as Meyer lemon and bergamot, and several ornamental male parents, such as calamondin, 'Doppio sanguigno' orange, Othaeite rangpur lime and small leaf chinotto. About four hundred ornamental Citrus hybrids were obtained. Two replications of each one of the seventy-eight hybrids showing poor thorns were grafted, between 2003 and 2004, on sour orange and grown up in 6.5 litre plastic pots, placed 40 cm apart, along two lines of a drip system. Some hybrids showed, in 2005, the first fruits. In particular those obtained from Meyer lemon x Othaeite rangpur lime crossing, showed interesting fruit traits, such as small size, oval shape and different rind colour, while some others, obtained from lemon Meyer x small leaf chinotto, showed other original traits, such as leaves larger than chinotto's and smaller than Meyer lemon's, joint to very dense canopy and yellow or red fruits.

P34. 'Marins' and 'Olimpo' – two new Hippeastrum hybrids from IAC
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The amaryllis bulb is an important product for the international flower market. The IAC develops, since 1982, a program of improvement with the Hippeastrum based in the exploration of the genetic variability of the Brazilian native species, objectifying the creation of new products. The genus Hippeastrum (Amaryllidaceae) is represented by endemic bulbous species of the great Amazonian Basin and possess around 55 to 75 species, which many natives of Brazil. The cultivated varieties are normally tetraploids, results of crossings that involved diverse botanical species, carried through for the first time by Johnson, in 1799 in England, through the crossing of H. reginae and H. vittatum. Bulbs of native species have been systematically collected in diverse regions of the country, and used in controlled pollinations with botanical species, hybrids and tetraploid commercial varieties, as 'Red Lion' and 'Apple Blossom'. As a result of this research program, two new varieties must briefly be registered in the SNPC - National Service of Plant Protection: 'Marins' (IAC 313) and 'Olimpo' (IAC 244). These new varieties are vigorous and well adapted to the ecological conditions of the State of São Paulo plateau. The multiplication can be made in field by lateral bulblets, which normally blossom after two cycles of plantation. Description of the two new releases: 'Marins' (IAC 313) - Green and narrow leaf, also green floral escape and long of narrow thickness, with 4 simple flowers, green pedicel and of average length, flower of star format and medium size, wide perianth, with small overlapping of tepals; external tepals of straight-elliptical format, light choral coloration with greenish center, stamens and pistil also of light choral coloration and pink anthers, stigma of small size. 'Olimpo' (IAC 241) – Green leaf of average width, also green floral escape and long of average length and thickness, with 4 simple flowers, pedicel green and short, flower of star format and small, wide perianth, with small overlapping of tepals; external tepals of elliptical format, choral coloration with greenish center, pinkish stamens and pistil, blue anthers, small stigma.

P35. Anthurium hybrids for pot plant
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Eight anthurium cultivars: Astral, Krahô, Sonata, Kinã, Prelúdio, Serenata, Aikanã and Cantata, selected by IAC, cultivated in a plastic greenhouse in Sto. Antonio de Posse (SP). The experimental unit was one micropropagated plant per pot, filled with coconut fiber and fed by hydroponic system; 15 pots per treatment (cultivar). Plants were 8 months old at the experiment beginning. The characteristics observed, every 2 months, were: plant height, length and width of the largest leaf and number of leaves, offsprings and of flowers and blossoms per pot. After 10 months of observations, the average data showed that the highest plants were found in the cultivar Prelúdio (55.5 cm ) and the lowest for Astral (31.5 cm) and Serenata (31.4 cm). The largest leaves were found in the cultivar Kinã (35.8 cm long and 21.0 cm large). Krahô also produced the long leaves (36.7 cm) and Prelúdio large leaves (20.4 cm). The shortest leaves were observed for Aikanã (21.0 cm long and 11.0 cm large). The highest number of leaves were found in the cultivars Astral (19.7) and Sonata (19.0), and the smallest in Krahô (8.5). The highest number of plants were produced by Serenata (4.7) and Aikanã (3.6), and the smallest by Krahô (1.1). The very initial flowering of plants was observed by the fourth month of the experiment start, but general cultivars’ flowering was observed only after other four months. The best flowering cultivars were Astral and Serenata. It also must be considered that these hybrids were primarily selected for cut flower, and it is very interesting to notice that some of them are indeed suitable for pot plant production. The final results were compared to a common pot plant variety Red Queen at the commercialization standard, and it was noticed that Red Queen has smaller leaves in larger quantity, and also larger number of offsprings. Krahô produced very large flowers in a very striking red color, and can be produced as a large pot plant. Serenata and Astral were the most recommended for pot plant production because of their compact plant development of large number of offsprings, and high number of flowers. Flower color is also a characteristic, which influences the costumer, at this point of view Aikanã has light green spathes, which is not a very common color for a pot plant variety, and also presents a short height. Prelúdio, for its very vigorous development, red tulip type spathe and redish color of the new large leaves, can be suitable as a large garden plant. It is worth to remember that most of the IAC cultivars are A. andraeanum type, from a different origin of Red Queen and most of the compact cultivars. It was also the first experience of hydroponic cultivation with these cultivars, which were selected under an organic basis, and their behaviour was surprisingly good.

XXII EUCARPIA Symposium (Section Ornamentals) "BREEDING FOR BEAUTY": 11-15 September 2006, Sanremo (Italy)
**HEMEROCALLIS BREEDING AT INSTITUTO AGRONÔMICO (IAC), BRAZIL**

**P37.**

**BREEDING WITH HIPPEASTRUM PAPILIO**

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The IAC carries on a breeding program on *Hippeastrum* since the early 80's, and has released at least five new selections, which looks much more like the native species than the traditional “amaryllis” (tetraploid Dutch hybrids), for this reason they have been called “açucena” (ah-soo-sa-nah), a Brazilian popular name for these plants. Among the hundreds of interspecific crosses made during these two decades, the *H. papilio* hybrids showed particular interesting characteristics and, for this reason, it was created separated breeding lines. An important point of this research is that the *H. papilio* mother plants employed in the crosses were not the same one clone commonly spread worldwide, but plants collected in the nature, in Southern Brazil, and for this reason they show larger variability in their characteristics, as for example, four-flowered plants, more opened tepals, besides the variation on flowers’colors and shapes. The different clones of *H. papilio* were crossed with *H. puniceum*, *H. damazioanum*, *H. psittacinum* and ‘Apple Blossom’, the Dutch hybrid cultivar. The so interesting characteristics founded in these *H. papilio* crosses, like plant vigor, leaf color, color and design of tepals, inspired then the creation of separated breeding lines, and a new program is now created and from this time it will be carried on at the Apta Regional Sudoeste, in Capão Bonito(SP).

**P36.**

**BREEDING WITH HIPPEASTRUM PAPILIO**


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Daylilies become more and more important as a garden plant in the country. Since old times the introductions like *H. flava*, the double flowered cv. Flore Pleno, and other unnamed cultivars of *H. fulva* showed as very successful crop as ornamental for landscape. Further on, the introductions of new cultivars by Roberto Burle-marx and his new approaches on their uses in his world famous projects made the daylily even more popular. The program of improvement of *Hemerocallis* in development at the IAC, since 1990, introduces new foreign varieties for the evaluation of their behavior in the country, creates hybrids for the creation of new varieties adapted to the São Paulo State climate and develops micropropagation techniques aiming at the massal multiplication of the materials of higher interest for the floriculture and the national landscape. The main partner is the Agricola da Ilha Ltda., an agricultural company paradoxically from Sta. Catarina State, a Southern state. This enterprise’s 2005 catalogue shows the thirty IAC cultivars, being the most of them tetraploid. Their names and main colors are: Daniela Esther Nass (light yellow), Carmem Bovée (light greenish yellow), Ilha Formosa (golden yellow and red throat), Ilha Mirtesosa (light yellow), Gabriel Matheus Nass (mauve), Campinas (golden yellow with red throat), Amanda (coral), Cora Coralina (coral and yellow), Primavera (rose and red throat), Alessandra (salmon), Santa Catarina (mauve), Barbara (red), Maria Bonita (striped light rose), Olga Ullmann (pink), São Paulo (cream), Canário (yellow), Carolina (light orange), Longhi (dark red), Rainha Silvia (illic), Dona Francisca (yellow), Graziela Barroso (yellow and brown throat), Joinville (orange), Lígia Fagundes Telles (yellow and brown throat), Margaret Mee (maroon and brown throat), Alvorada (red), Castanho (maroon), Guaratiba (pink), Jundiai (red) and Santa Eliza (yellow). Besides these there are also the introduced varieties from USA: Allegretto (pink and red throat) and Sebastian (mauve) and Sirocco (salmon). This large choice of cultivar promises to expand even more the daylily in the public and private gardens. The adaptation of these selection is very good and they flower during at least six months (from October until March) in the southeast part of Brazil, because the Northern region lack of cool season, important for flower induction. Nowadays, daylilies seems to become a new hobby for the garden lovers, and the enterprise Agricola da Ilha Ltda. estimates this new demande and offer to the customers the opportunity to get new hybrids especially selected for collectors. All these hybrids are still on the way to be registered at the SNPC – National Service of Plant Protection, an office of the Brazilian Ministry of Agriculture.

**P38.**

**STUDIES ON BEHAVIOUR OF SOME ROSE CULTIVARS TO THE SPECIFIC PATHOGENS IN SOUTH-EASTERN ROMANIA**

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In the South-Eastern Romania as in all country the rose culture is highly praised for its ornamental value both in parks and domestic garden. In this zone of our country the rose culture is more important because the Black Seaside provide a better environment (82 km). Beside the growing of forign varieties from Europe Companies, Romania has done a breeding work (Wagner, 1999; Roman, 2001; Argatu, 1997) to develop autochtonous cultivars (Rusticana, Ambassador, Bordura de nea, Rosagold, Simina, etc.) better adapted to our local conditions. In rose breeding besides the ornamental value of these flowers (nice leaves, colours and shapes) the disease resistance has been taken into account. Among the specific pathogens very harmful for the rose culture, one can mention: *Sphaerotheca pannosa* var. *rosae* Lev. (powdery mildew), *Diplocarpon rosae* (black spotting), *Phragmidium tuberculatum* (rust) and *Botrytis cinerea* (grey mould). One of the most effective methods to prevent these pathogens attack is breeding new cultivar and genetically resistant genitors (Wagner and Raureanu, 1996). These papers present the behaviour of 150 genotypes from collection of Fruit Research Station Constanța and their response to the natural infections of such pathogens expressed by attack frequency (%I) and intensity (I). The genetnically resistant varieties are: Queen Elisabeth, Foc de tabara, Rubín, Parfum, Emerald d’or, Bel Ange, Algold.
In lilies, commercial tetraploid varieties have been distributed to market because they have superior agronomic traits such as large flowers and resistance to physiological disorder (Okazaki and Hane 2005). Okazaki et al (2005) developed the method of direct 2n pollen induction in tulips by applying N2O to bulbs undergoing meiosis. This method can reduce the time necessary to obtain 2n pollen, compared to use of 2n pollen through tetraploidization of diploids by colchicine treatment. In the present study, we attempted to induce 2n pollen of Asiatic hybrid lilies by arresting meiotic process with nitrous oxide gas. To determine which meiotic stage is optimal for induction of 2n pollen, plants having buds undergoing different meiotic stage were treated with N2O for 24 h. The plants were treated in a pressure-tolerant cylinder (20cm inner diameter, 100 cm long). No diploid pollen was induced using plants including anthers of prophase I, while mixed pollen grains of differing size were produced. No diploid pollen grains were induced using plants including anthers of prophase I, while mixed pollen grains of differing size were produced using plants undergoing meiotic metaphase. Giant pollen whose shape is circular (normal pollen is elliptical) were induced. Flow cytometry analysis showed that giant pollen grains were diploid. Besides when mixed pollen including normal and giant pollen was crossed to tetraploid cultivars, the resulting seedlings were tetraploid and aneuploid, indicating that giant pollen grains are diploid and can generate tetraploid seedlings through the fusion of diploid eggs supplied from tetraploid female parent. Treatment with N2O is useful for the production of 2n pollen of Asiatic hybrid lilies and may provide a new approach of lily breeding at the tetraploid level.
the numbers of the petals were measured. The SERK gene was chosen among the genes involved in the somatic embryogenesis pathway, with the aim to select a marker of the embryogenic potential. Set of degenerated primers were designed on nucleic acid or protein alignment of all the SERK genes cloned in the different plant species and used in a PCR strategy on cDNA material of a high embryogenic lines of Cyclamen. It was possible to clone a fragment (1300bp) of Cyclamen SERK cDNA (Somatic Embryogenesis Receptor Kinase). It showed a high homology with the sequences of other species: 96% with Citrus unshiu, 95% with Medicago truncatula, 93% with Daucus carota and 93% Arabidopsis thaliana.

P43. **IN VITRO REGENERATION OF VACCARIA PYRAMIDATA**

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Vaccaria pyramidata belonging to Caryophyllaceae family is generally known in Turkiye as a weed and host plant for chickpea green larva. However, it has been used in medicine and cut flower sector in the world because of its valuable proteins and esthetic flowers. Vaccaria pyramidata resembles to Gypsophylla due to its natural plant habitus. But, it has larger, striking, pink flowers and seems feasible to improve better varieties through breeding. Also, it is thought that it may be used in Gypsophylla breeding. In the preliminary studies made with the seeds of wild germplasm which was found at high elevations with cool climate conditions, it was realized that Vaccaria pyramidata adapted to low elevations with hot climate and has a very high regeneration capacity in vitro in regard to callus induction, multiplication and rooting. The objective of this study was to establish in vitro regeneration system of Vaccaria pyramidata. For this aim, initially immature seeds were used as explant. The most striking two improvements in the seedlings as soon as germination began were a dense hairy root development and in vitro flowering in the aging period. Additionally, a dense proliferation and rooting were observed when the shoot tips and single noder of these in vitro seedlings were cultured.

P44. **BREEDING OF LISIANTHUS (EUSTOMA GRANDIFLORUM RAF. SHINNERS) BY MEANS OF MUTATION INDUCTION AND CROSS POLLINATION**

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Lisianthus is known botanically as Eustoma grandiflorum (Raf.) Shinners with common names of prairie gentian or Texas Bluebell. Lisianthus is mainly cultivated for cut flower production, with 115 million stems produced in 1995 (pot culture is less significant, with only an estimated 600,000 pots production). Although more than 150 cultivars are available, no one cultivar type accounts for more than 5% of the total production. This is because they are all essentially similar, none showing a decided superiority at any one stage of production, distribution or use (Ohkawa and Sasaki, 1999). The botanical, technical and commercial features of lisianthus make this species very interesting for cultivation in the Italian pedoclimatic conditions, mainly in the South. Even if this species naturally flowers in summer, it also responds positively to programmed flowering techniques. In fact, in the southern environments, it is possible to produce flowers for almost ten months per year. A breeding activity on lisianthus by means of mutation induction and cross pollination was started in 2002 in order to obtain new varieties characterised by new flower color combinations, by novel forms of the flower and architecture of the plant and by a longer vase life. This last trait is very important for the development of new varieties because this plant, evolved in very hard environmental conditions (Texas prairies), has naturally a short reproductive cycle and, after pollination, the flowers rapidly senesce. Seeds of six ecotypes of lisianthus (‘ECHO’ Pink Picotee n.1 and n.2, ‘ECHO’ Blu Picotee n.1 and n.2, ‘ECHO’ Champagne and ‘ECHO’ Yellow) were treated with different γ-rays doses (50 to 700 Gy) in summer 2002. Seed germination and plantlet survival resulted very low with the higher doses (almost 100%) and only two progenies were obtained with the 600 and 700 Gy treatments, whereas increasing survival percentages were obtained decreasing γ-rays doses. From 2003 to 2005 the plants showing interesting features of the flowers (new forms and color combinations) were selected and free- or self-pollinated. In 2006, the most promising M3 progenies were evaluated for aesthetic value, agronomical traits and flower longevity. The most interesting genotypes are shown in this paper.

P45. **THE GENETIC STABILITY OF PLANTS OF PELARGONIUM X HORTORUM REGENERATED FROM SOMATIC EMBRYOS AND ADVENTITIOUS SHOOTS**

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It has long been known that microplant formation in Pelargonium may occur from both adventitious shoots and somatic embryos (Cassells, 1979). Subsequently, it was shown that somatic embryogenesis in Pelargonium was increased by thidiazuron TDZ (Gill et al., 1994) and by TDZ and dark induction (Croke and Cassells, 1997). Studies on the genetic stability of the progeny plants showed the presence of somaclonal variation (Cassells et al., 1997), however, it was not determined unequivocally whether somaclonal variation was the same, or higher or lower in the adventitious shoot-derived progeny versus the somatic embryo-derived progeny. Here, using a microscope, somatic embryos were removed from explants, plated and observed for bipolar growth, the progeny of these structures where compared with progeny from unselected regenerants (that is, regenerants from both somatic embryogenesis and adventitious regeneration), and from F1 hybrid seed of the variety 'Rio' and analysed for genetic stability using flow cytometry, RAPDs and image analysis, as described previously (Cassells et al., 1997). The results showed a lower incidence of somaclonal variation in the progeny from somatic embryogenesis compared with those from the mixed pathways of regeneration. The results are discussed in the context of transformation and cloning of Pelargonium.
P46. **In vitro multiplication and conservation of some horticultural-important taxons of Achillea, Dianthus and Paradisea genera**

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The biotechnology of *in vitro* cultures represents an efficient and largely utilized method for the multiplication and conservation of some horticultural, agronomical, pharmacetical, etc. important species. Using this method, our goals are to start the *in vitro* culture for taxons belonging to some horticultural important genera, to micropropagate them and to preserve them *ex situ*, both *in vitro* as well as in the botanical gardens. The genera on which we focused our experiments were: *Achillea, Dianthus, and Paradisea*. We mention just some of the studied taxons which have both horticultural and phytogeographic importance (due to endemic status or because they are considered endangered at different intensity levels in Europe): *Achillea pyrenaica* Sibth. *ex Godr.* – endemic for the Pirinei Mts., *Dianthus anatolicus* Boiss. – characteristic for Turkey, *Dianthus lusitanus* Brot. – endemic for the Iberic Peninsula, *Dianthus pyrenaicus* Pour. – endemic for the Pirinei Mts., *Dianthus ferrugineus* Mill. subsp. *liburnicus* (Bartl.) Tutin – characteristic for Northern and Western Italy and former Yugoslavia, *Dianthus glacialis* Harenke ssp. *geidus* (Schott., Nyman & Kotschy) Tutin – an endemic alpine species from S and E of the Carpathians; *Dianthus spiculifolius* Schur – an endemic species of the Carpathians; *Dianthus petraeus* Waldst. & Kit. ssp. *simonkaianus* (Péterfi) Tutin – found only in the Carpathians and the Balkan Mts.; *Dianthus giganteus* D’Urv. – an important Balkan species; *Dianthus alpinus* L. – an endemic species for the N-E of Alps; *Dianthus gratianopolitanus* Vill. – considered as rare plant in the Alps; *Dianthus ferrugineus* Mill. – Mediterranean element spreading from SE France to Albania; *Paradisea liliastrum* (L.) Bertol. – characteristic for the central and Southern European mountains. For the initiation of the cultures, seeds originating from various alpine parks, European botanical gardens or “Alexandru Borza” University Botanical Garden (Cluj-Napoca) were mainly used. The influence of different hormonal balances designed for the multiplication and rhizogenesis processes was studied. A photoautotrophic step preceded the transplantation of the vitroplants *ex vitro*, in order to facilitate their acclimatization. The acclimatization had a good efficiency for most of the taxons. Some of the taxons are preserved both *in vitro* as well as outdoors, in the “Alexandru Borza” University Botanical Garden (Cluj-Napoca).

P47. **Chromosome doubling of Ranunculus asiaticus**

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Three mitotic inhibitors, i.e. oryzalin, trifluralin and colchicine were used as doubling agents for *Ranunculus asiaticus* L. The chromosome number of the diploid plants was 16 and that of tetraploid plants was 32. In *vitro* plants of *Ranunculus asiaticus* were incubated on fresh stock medium enriched with the antimitotic agents oryzalin (0.5, 1, 3 or 10µM) or trifluralin (1, 2, 3 or 10µM). After this incubation period plants were transferred to stock medium; at the end plant material was harvested for flow cytometrical measurements. The colchicine was applied in a different way: plants were kept on a stock medium for different time intervals before transfer to a liquid medium, supplemented with colchicine (100 or 200µM). Plants were kept in the liquid medium for 16 or 24 hours and then transferred to stock medium. The effect of antimitotic agents on genome doubling and development of *Ranunculus asiaticus* revealed promising results with trifluralin and colchicine. Application of colchicine resulted in the production of mixoploids. No plants with a doubled chromosome number in all their cells were detected. A treatment of trifluralin resulted also in the induction of mixoploids and resulted in tetraploid plants. Oryzalin had a negative effect on the *in vitro* regeneration of the plants when supplemented with the highest concentrations (3 or 10 µM). Furthermore a high mortality before the end of the experiment was observed due to the high concentration of this mitotic inhibitor. Therefore experiments were repeated with lower concentrations (0.5 or 1µM).

P48. **Development of a plant regeneration protocol for Spathiphyllum and Anthurium protoplasts**

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Interspecies crosses within the Arecaceae are impossible due to prezygotic and postzygotic incongruity that prohibit fertilization or later development of a zygote. Protoplast fusion is an alternative tool to get round these barriers. The aim of the presented research is to develop an efficient regeneration protocol for non-fused protoplasts. Different cultivars of *Spathiphyllum wallisii* and *Anthurium scherzerianum* (Hamidah, 1997) were evaluated in our studies. Viable protoplasts could be isolated from young leaves of *in vitro* plants in an isolation mixture containing 1 % cellulase, 0.5 % macerozyme R-10, 0.5 % driselase and 0.5 M mannitol. Preincubation of leaf tissue in 0.5 M mannitol before isolation increased the release of protoplasts. A yield between 1 x 10^3 and 50 x 10^3 per g fresh weight of tissue was obtained using this protocol. Purified protoplasts were cultured at densities of 1 x 10^4 per ml using three different culture methods, particularly liquid modified Kao & Michayluk (KM) medium, agarose beads and sodium alginate beads. After solidification of both agarose beads and sodium alginate beads, a liquid overlay modified KM-medium containing 30 g/l sucrose, 4,5 % mannitol, 0,1 mg/l 2,4-dichlorophenoxyacetic acid, 0,5 mg/l 6-benzylaminopurine and 0,5 mg/l o-naphthalene acetic acid was added. After 1 week, the liquid overlay medium was removed and replaced with the same medium containing 2,5 % mannitol; after 2 weeks the liquid medium was replaced with medium without mannitol. The protoplasts of the three culture methods were incubated in darkness at 21°C and constant agitation of 30 rpm. Protoplasts in liquid culture and sodium alginate beads were unable to divide, whereas protoplasts embedded in agarose beads could divide. Both *Spathiphyllum* and *Anthurium* protoplasts developed a cell wall after 1-2 days. After 6 days the first
divisions were visible of some Spathiphyllum protoplasts, after 4 weeks tetrads were observed. Anthurium protoplasts showed first divisions after 12 days.

P49. **Production of hybrids for restoration of seed fertility in Cosmos atrosanguineus**
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Interspecific hybrids between Cosmos atrosanguineus (2n=48) and C. sulphureus (2n=24) were successfully produced by using ovary culture. The hybrids had a chromosome number of 2n=36 and intermediate characteristics between the parents. Moreover, they showed strong tolerances to diseases and hot humid climate as compared with C. atrosanguineus. Although they exhibited pollen and seed sterility, a progeny plant was obtained when one of the hybrids was back-crossed as maternal parent with C. sulphureus. However, this BC1 plant had no pollen fertility and showed diploid level of chromosome number (2n=24). Amphidiploid of the BC1 plant was successfully produced by the treatments of in vitro shoots with 100 mg/l colchicines for 9 hours. Expectedly, this amphidiploid restored pollen fertility and successfully produced seeds by self-pollination although the efficiency was relatively low. Hybridization between the amphidiploid of BC1 and C. atrosanguineus are now under investigation.

P50. **Assessment of ornamental value and antibacterial activity in Helichrysum stoechas plants regenerated from wild type hairy roots**
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Helichrysum stoechas is a Mediterranean species that is grown as ornamental plant. The aim of the present study was to assess the ornamental value of the plant and to test its antibacterial activity against three Gram negative and five Gram positive bacteria and against three mycetes by disc diffusion test and agar dilution method. Micropropagation was achieved from hairy roots derived from wild type plants by the direct organogenesis from leaf petiole explants. The induction medium containing myo-inositol (100 mg l–1), thiamine (M), and BAP (1.0 mg/l) plus NAA (0.54% M) alone or with BAP (4.0–44.0 µM) plus active chlorine. After sterilization, the petioles were sliced into 0.5–0.7 cm segments and the explants were cultured in the dark for 4 hours. Expectedly, this amphidiploid restored pollen fertility and successfully produced seeds by self-pollination although the efficiency was relatively low. Hybridization between the amphidiploid of BC1 and C. atrosanguineus are now under investigation.

P51. **Chromosome doubling of creeping gloxinia in vitro**
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Creeping gloxinia (Lophospermum erubescens D. Don, Asarina erubescens (D. Don) Pennell, Maurandya erubescens (D. Don) A. Gray) belongs to the cosmopolitan family Scrophulariaceae and comes from the Mexican mountains. Many members of this family are highly ornamental and are commonly planted in gardens. Several representatives of this family (such as Mimusulus, Bacopa, Nemisia and Diascia) are now widely grown as ornamental plants. Plants of this genus have recently been used for planting and other purposes, and could potentially be used for breeding, polyploidisation in vitro and so on. One-year-old maternal plants that were grown from seeds were stored during the winter at a temperature of 10°C. The following spring, they were sprouted and grown in a greenhouse at 15–25°C solar irradiance. Leaves were cut from plants when they reached 60 cm in height, avoiding the upper and lower 10 cm sections of the shoot. Freshly harvested leaf petioles were surface sterilized in 70% EtOH for 20 sec, followed by soaking for 20 min with occasional agitation in a 20% v/v commercial bleach ‘Jewel’ containing 2.6% active chlorine. After sterilization, the petioles were sliced into 0.5–0.7 cm segments and the explants were placed onto Murashige and Skoog (MS) agar medium. The induction medium containing myo-inositol (100 mg/l), thiamine (0.1 mg/l), nicotinic acid (0.05 mg/l) and sucrose (5% w/v) was supplemented with BAP (4.0–44.0 µM) alone or with BAP (4.0–44.0 µM) plus NAA (0.54 µM). Regeneration was achieved via direct organogenesis from leaf petiole explants. Regenerated plantlets were exposed to different colchicine concentrations at various exposure times. The treatment applied 0.05% colchicine for 48h was the most effective. Significant differences were observed for the size of flowers and etc. This research has demonstrated that ploidy of creeping gloxinia can be doubled using colchicine.
hybridization in Lilium is very difficult because of incompatibility problems. The genus Lilium generally includes allogame species that present a distinct self-incompatibility with the consequent inability to produce seeds when they are self-pollinated. The intraspecific crosses produce a large quantity of seeds, whereas crosses between different species create many problems. A breeding program on Lilium was carried out at the Experimental Institute for Floriculture in Pescia since 2003 in order to overcome barriers of interspecific incompatibility and to originate hybrids presenting agronomical and commercial characters from various species. Several crosses between Asiatic hybrids (‘Golf’, ‘Polyanna’, and ‘Girondine’), Oriental hybrids (‘Lombardia’), and Longiflorum hybrids (‘White Haeven’, and ‘White Magic’) were made using just breaking flower buds and cut-style-method. In order to obtain Lilium hybrids a second test was carried out using pollen-free clones selected at the Institute for several characters like flower colour and shape. Several wild species like L. pumilum, L. regale, L. aurilian, L. leucanthum, and L. New Zealand were included. In order to overcome the physiologic and metabolic barriers that inhibit interspecific hybrids germination a direct method for embryo rescue has been used for a long time but in these experiments an alternative method using the whole ovary and the ovules has been tested. Seven and fourteen days after pollination ovaries were cut and cultivated in vitro using three different substrates with (IAA, BA + NAA) and without growth regulators for 50 and 60 days; two different concentrations of sucrose were also tested. Subsequently the well developed ovules were taken out from ovary and subcultivated onto two substrates containing IBA 0.5 or NAA 0.1 for 180 days. The embryo germination was highly affected by the genotype and by the cross type. The developed plants were cloned in vitro and transferred to soil. The genetic evaluation of the germinated plants is in course in order to determine if the obtained material represents hybrids, containing parental characters.

The present research offers the opportunity to obtain interspecific hybrids to evaluate with the aim to increase the quality of the most important bulbous plant cultivated in Italy.

P52. **Interspecific Hybrids of Lily spp.**

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Lily genus includes more than one hundred species from a large territory, including temperate zone of Europe, Asia, and North America. The main goal of Lilium breeders is to combine in one genotype several positive traits of different species such as biotic stress resistance (fungi, bacteria, viruses) and abiotic stress resistance (cold, water stress, salinity), but interspecific hybridization in Lilium is very difficult because of incompatibility problems. The genus Lilium generally includes allogame species that present a distinct self-incompatibility with the consequent inability to produce seeds when they are self-pollinated. The interspecific crosses produce a large quantity of seeds, whereas crosses between different species create many problems. A breeding program on Lilium was carried out at the Experimental Institute for Floriculture in Pescia since 2003 in order to overcome barriers of interspecific incompatibility and to originate hybrids presenting agronomical and commercial characters from various species. Several crosses between Asiatic hybrids (‘Golf’, ‘Polyanna’, and ‘Girondine’), Oriental hybrids (‘Lombardia’), and Longiflorum hybrids (‘White Haeven’, and ‘White Magic’) were made using just breaking flower buds and cut-style-method. In order to obtain Lilium hybrids a second test was carried out using pollen-free clones selected at the Institute for several characters like flower colour and shape. Several wild species like L. pumilum, L. regale, L. aurilian, L. leucanthum, and L. New Zealand were included. In order to overcome the physiologic and metabolic barriers that inhibit interspecific hybrids germination a direct method for embryo rescue has been used for a long time but in these experiments an alternative method using the whole ovary and the ovules has been tested. Seven and fourteen days after pollination ovaries were cut and cultivated in vitro using three different substrates with (IAA, BA + NAA) and without growth regulators for 50 and 60 days; two different concentrations of sucrose were also tested. Subsequently the well developed ovules were taken out from ovary and subcultivated onto two substrates containing IBA 0.5 or NAA 0.1 for 180 days. The embryo germination was highly affected by the genotype and by the cross type. The developed plants were cloned in vitro and transferred to soil. The genetic evaluation of the germinated plants is in course in order to determine if the obtained material represents hybrids, containing parental characters.

The present research offers the opportunity to obtain interspecific hybrids to evaluate with the aim to increase the quality of the most important bulbous plant cultivated in Italy.

P53. **Comparison of Caffeine, Oryzalin, and Colchicine Treatment for In Vitro Chromosome Doubling in Lilium spp.**

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The purpose of this study was to obtain tetraploidy lilies comparing effectively among colchicine, oryzalin and caffeine in vitro chromosome doubling of 8 lily cultivars. In colchicine treatment, tetraploids were obtained five explants after treatment with 0.005% and 0.1% colchicines in the Asiatic lily ‘Corrida’. Four tetraploids were obtained by treating with colchicines of 0.01%, 0.05% and 1% concentration in the Oriental lily ‘Siberia’. Three tetraploids were obtained by treating with 0.1 and 0.5% colchicines concentration in the FA (Fomolongi x Asiatic) hybrid ‘Supia’. In ‘Rodrigo’, ‘Rosato’, ‘Raizan’ and ‘Migreen’, it was no effect of colchicines in vitro chromosome doubling. In caffeine treatment, treating with 0.1 to 3% caffeine concentration in the Asiatic lily ‘Corrida’ and Oriental lily ‘Siberia’ obtained thirteen tetraploids. Other lily cultivars have no effect in caffeine treatment. In oryzalin treatment, except for FA hybrid ‘Migreen’ and Fomolongi hybrid ‘F1 Augusta’, six lily cultivars were obtained thirty-nine tetraploids from 0.001% to 0.005% (low) oryzalin concentration. From these results, oryzalin was proved to be the most effective lily chromosome-doubling agent as compared to colchicine and caffeine. The effect of oryzalin was possible low concentration (0.001% to 0.005%), that of colchicines was high concentration (0.1% to 0.05%) and that of caffeine was very high concentration (0.1% to 3%).

P54. **Isolation of Female and Male Gamete Cell from Flower of Lilium Oriental Hybrid ‘Casa Blanca’**

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Oriental hybrid lilies are one of the important groups of Lilium commercial cultivars, because the cultivars of the group have showy, large flowers and good sweet fragrance. However, the group displays minor variation in flower colors, compared with that of Asiatic hybrid lilies. So it is one of the assignment that the Oriental hybrid lilies will hybridize with other groups for the expansion of flower color variation. Although several techniques, such as cut-style pollination, ovule culture for embryo rescue and so on, had been developed for overcoming the cross-incompatibility, there are several combinations that cannot obtain crossbred embryos, up to now. Recently, some techniques had been developed for the isolation and culture of female and male gamete cell, for direct in vitro fertilization in maize (Kranz et al. 1996). So we tried to apply the technique for Lilium breeding.

In this report, we showed the preliminary results, that is, isolation processes of female and male gamete cells from flower of Lilium ‘Casa Blanca’. Embryo sac was located in deep inside hemitropous ovule entirely covered with thick nucellar tissue. For isolation of the embryo sac, ovules treated with an enzyme mixture solution for 4.5 hr were dissected using a hand made glass needle under a binocular microscope to release the embryo sac from ovule. The isolated embryo sacs were treated with another enzyme mixture solution. After 3 hr treatment, each of the embryo sacs was dissected using a micromanipulator system to release female gamete cell. Pollen was enzymatically treated for isolation of pollen protoplasts. For isolation of generative cells, the pollen protoplasts were ruptured by a hypodermic needle in a suspended solution. The suspension solution was filtrated by 50μm nylon mesh filter to collect generative cells. The procedures mentioned above, caused to get considerable number of female and male gamete cells repeatedly, for direct in vitro fertilization.
P55. **Efficient in vitro regeneration and polyploidization in Pelargonium x hortorum Bailey**

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The objective of this research was to find essential factors affecting in vitro regeneration from somatic tissues and conditions of colchicine-induced polyploidy in zonal geranium plants. Polyploidization is a very useful tool employed in plant breeding. Physical and chemical factors of an efficient in vitro regeneration were explored in Pelargonium x hortorum cultivars 'Black Velvet Scarlet' and 'Gizela'. Initial explants were taken from segments of petals and intact blades from in vitro seedlings subcultured using microcuttings on half-strength MS (Murashige and Skoog, 1962) medium with growth regulators benzyladenine (BA), zeatin or IBA (indolebutyric acid). Explant incubation in darkness for 4–5 weeks and consequent cultivation at day/night 22/18 °C under a photoperiod of 55 µmol m\(^{-2}\) s\(^{-1}\) in a controlled room proved to be a stimulating factor to induce shoot regeneration and cytokinin TDZ (thidiazuron) or the combination of cytokinin mT (meta-topolin) with auxin IBA showed the best results. Indirect organogenesis was obtained on full-strength MS medium supplemented with 50 mg l\(^{-1}\) polyvinylpyrrolidone (PVP), 50 mg l\(^{-1}\) casein enzymatic hydrolysate, 2 mg l\(^{-1}\) mT, 0.5 mg l\(^{-1}\) IBA or TDZ at concentration 2 mg l\(^{-1}\).

A difference was determined in regeneration ability of leaf explants in Petri dishes and Erlenmeyer flasks. Methods of colchicine application were tested under in vitro conditions. In colchicine-treated greenhouse seedlings, tetraploidy was found using flow cytometry analysis.

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P56. **Somaclonal variability in chrysanthemum cultures**

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The aim of the studies was to determine somaclonal variability in 'Ja Dank' cultivar of chrysanthemum propagated for 10 years in vitro. Phenotypic and genotype differences were determined with relation to the control plants of mentioned cultivar propagated in vitro for 6 months. Phenotype variability of the plants was determined at the plantlets stage in vitro culture and next after planting them in a pot experiment. Genotypes of tested plants were compared on basis of analysis of DNA using ISSR-PCR method. The studies indicated that the age of the culture affected the observed during in vitro propagation and after plant regeneration at the stage of mature plants. At the stage of culture the changes in the number of leaves, the plant height, their weight and the number of internodes were observed in comparison with control plants. The plants from 10-year culture had thin, partially lignified shoots, pale green colour, physiological diseases such as glassiness, and lighter colouring than the control group. As it had been mentioned above, the culture age had a significant influence on condition of chrysanthemum plants at the stage of mature plant. In the plants from 10-year cultures flowering was delayed. The changes in flower morphology, the shape of leaf blade and its edges were also observed. 7% of the plants under study had chlorophyll changes in leaves and 42% - changed shape of leaves. The observed phenotype changes were confirmed at DNA level by ISSR-PCR. It was found that on the average there was 96.2% similarity. Between the plants from 10-year cultures and those from 6-month culture the short plants and the plants with chlorophyll changes from 10-year cultures were similar to the bulk sample made up of 10-culture and to the plants from 6-month culture, 85.5% and 84.6% similarity respectively. The short plants without chlorophyll changes from 10-year cultures were similar to the bulk sample of 10-year culture and to the plants from 6-month culture, 95.6% and 91.9% similarity respectively. Among the 50 microsatellite primers applied, 16 gave products visible on agarose gel. There were the following primers: 808, 812, 815, 816, 817, 818, 820, 821, 827, 828, 830, 833, 834, 835, 837, 843. The differences between the plant under study, at DNA level were determined by means of ISSR primers: 808, 812, 815, 816, 818, 820, 821, 827, 828, 833, 834, 835, 843.

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P57. **Strategies for Anemone coronaria breeding**

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Most of the Anemone coronaria cultivars ( cvs), grown for cut flower production, are multiplied by seed and sold for cultivation as one year old rhizomes. The cvs are population of hybrid plants obtained by crosses between heterozygous parents; therefore they lack of uniformity. The extent of genetic divergence between and within 5 widely commercialised cultivars (Cristrina, Monalisa, Tetraelite, Wicabri and Mistral) has been evaluated by means of AFLP markers. Due to the large dimension of A. coronaria genome, an AFLP protocol based on restriction with Smal (an eight bp enzyme) and Msel (a four bp enzyme) was developed. A total of 402 bands were yielded of which 152 were polymorphic. Genetic similarities among accessions were calculated according to Simple Matching Coefficient. A dendrogram based on the unweighted pair group (UPGMA) method was constructed using arithmetic averages. The dendrogram resolved the entries into three major branches: A, which included sub-cultivars of Cristrina; B, which included two clusters grouping the sub-cultivars Monalisa and Mistral; C which included two clusters grouping the sub-cultivars Tetraelite and Wicabri. The majority of the plants within each sub-cultivar clustered with one another, but in some cases no clear genetic differentiation between sub-cultivars was detectable. The hierarchical analysis of variance (AMOVA) demonstrated significant degree of differentiation within sub-cultivars (approximately 42%) and comparable levels of differentiation among sub-cultivars within cultivars (approximately 27%) and among cultivars (approximately 30%). These data support the opinion that the synthesis of F1 cvs is the elite breeding strategies needed to maintain a high level of heterozygosity, in order to avoid inbreeding depression, and stabilize cultivars for agronomic and commercial characters. To accomplish this purpose, two approaches were attempted to yield pure lines from the cvs Cristrina, Monalisa, Tetraelite, Wicabri: selfing and anther cultures. Not more than one cycle of selfing can be achieved per year in anemone. Low fertility was observed from 52 generations. In repeated experiments of anther cultures, embryo-like structures and plantlets were regenerated from all the cvs tested. Up to 16,9 regenerations per 100 anthers were yielded. Time elapsing from another
culture to plant in vitro acclimatisation was of fifteen months on average. Cytological analysis on root tips of regenerated plants showed that eleven out of nineteen plants had a 2n or n plus 2n karyotype, compatible with F1 hybrid synthesis. RAPD analysis demonstrated that all tested plants differed from the anther donor plants, definitely confirming their androgenetic origin. Shortening considerably the time required for homozygous line production through androgenesis, may convince seed companies to breed F1 hybrid.

P58. **Induction of morphogenesis in Phlox paniculata L.**

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**Phlox paniculata** cultivars ‘Fuji’ and ‘Rijnstroom’ belong to important border perennials. We compared morphogenesis of leaf explants from *in vitro* plants and from 6- month-old greenhouse plants. The explants were cultured on induction full-strength MS salts (Murashige and Skoog, 1962) medium containing 0.5–1.5 mg/l TDZ (thidiazuron), IAA (indoleacetic acid) or 2,4-D (2,4-dichlorophenoxyacetic acid) for 6–10 weeks and subcultured onto medium without growth regulators. MS medium was supplemented with 30 g l⁻¹ sucrose, B5 vitamins and 7.5 g l⁻¹ agar Sigma. Leaf explants were incubated at day/night 22/18 °C under a 16 h photoperiod at 55 µmol m⁻² s⁻¹ in a controlled room A difference was found in regeneration ability of *in vitro* and *ex situ* leaves. In both types of explants, organogenesis rate depended on the cultivar and concentration of TDZ. Correlation coefficients between regeneration percentage and the number of shoots per leaf explant were significantly positive. Fixed specimens were dehydrated in an EOH/n-butyl alcohol series, and infiltrated and embedded in paraffin wax. Microtome sections were deparaffined and stained with safranin/fast green. Histological observations demonstrated organogenic and embryogenic capacity of calli. We will exploit this efficient protocol of *in vitro* regeneration in a subsequent study of colchicine-induced triploidy.

**P59. Gametophytes formation and establishment of cell suspension cultures of the fern Asplenium nidus**

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A reproducible system for gametophytes formation and establishment of cell suspension cultures of *Asplenium nidus* has been developed. Gametophytes could be developed when spores collected from frond bearing mature sporangia were cultured on Murashige and Skoog, (MS) medium supplemented with 1mg/l BAP + 0.1mg/l NAA. Prothallus was formed after 10 days of culture and further developed into gametophytes 4 weeks later. The gametophyte-derived callus cultures were initiated when the young and fresh gametophytes were cultured on MS medium supplemented with 4 mg/l 2,4-D. Within 3 weeks, the soft and green callus was produced. After 2 weeks, suspension culture was successfully established when callus produced was transferred into liquid MS medium supplemented with 4 mg/l 2,4-D.

**P60. Results in in vitro propagation of Sorbus redliana ‘Burokvölgy’, a Hungarian breed ornamental tree**

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The Hungarian cultivar *Sorbus redliana* ‘Burokvölgy’ is a decorative small tree, suitable for planting in gardens and streets. Similarly to other Hungarian endemic *Sorbus*, its propagation by conventional methods is slow and it is difficult to find the suitable rootstock. Data on the *in vitro* propagation of endemic Hungarian sorbs are rare. The main aim of our study was to find the optimal growth regulator and its optimum concentration. Beside, to determine the chlorophyll content of plants, growing on different media. During the proliferation, Murashige and Skoog (MS) medium supplemented with 1mg/l BAP + 0.1mg/l NAA. The cultures were incubated at day/night 20-24 °C in 8/16 hours dark/light photoperiod for 50-52 days. At the end of the proliferation period the number of shoots was counted, the length of shoots, the length and width of leaves was measured. The leaves’ chlorophyll content was determined too. Data were evaluated by two-sample analysis (t- and F-test). The plants developed only 1.13-1.33 shoots on the MS media containing KIN. The widest (11.2 mm) and longest (17.8 mm) leaves were found in case of using 1.0 mg/l KIN + 0.05 mg/l IBA. The shortest (24.27 mm) plants was obtained on the medium with 0.75 mg/l KIN + 0.05 mg/l IBA. On the other hand, significantly more shoots were found on the media containing mT and the highest number of shoots (3.2) were observed in case of using 0.75 mg/l mT + 0.05 mg/l IBA. Every concentration of mT effected significantly taller plants, and the highest plants (35.46 mm) were obtained on medium with 1.0 mg/l mT + 0.05 mg/l IBA. The highest number of shoots and the smallest leaves were achieved on media with BA. Furthermore, on the medium containing 0.75 mg/l BA + 0.05 mg/l IBA the longest leaves were only 10.73 mm. The highest chlorophyll content was found in case of using KIN and the best results was obtained on the medium with 1.0 mg/l KIN + 0.05 mg/l IBA (1.569 mg/l total chlorophyll). Lower chlorophyll contents were determined in the case of using BA and mT. The lest chlorophyll content (0.271 mg/l total chlorophyll) was observed in case of using 0.5 mg/l mT + 0.05 mg/l IBA. This work was supported by OTKA (Project Number: T049642).
P61. In vitro culture of several endemic species Dianthus in Balkan peninsula
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The aim of this study is the endemic plants diversity protection and conservation of endemic Dianthus species, with special focus on them as resources important for horticultural use. Dianthus giganteus subsp. croaticus is subendemic species distributed in the western part of the Balkan peninsula (from Slovenia eastward to Serbia). It is a perennial herb. D. ciliatus subsp. dalmaticus is endemic species growing in Mediterranean and submediterranean zone of the Adriatic coast in Dalmatia (Croatia) and Montenegro. It is the semi woody shrublet. D. petreus subsp. noeans is subendemic species distributed in the Carpathians and the Balkan peninsula. It is perennial herb forming loose cushions. Multiplication of these species was achieved through micropropagation from meristem and/or stem segments culture. Plant regeneration of D. petreus was obtained from meristem culture (clone "DP") and from adventitious buds (AB) formed from organogenic calli (OC) in stem segments culture (clone "DPS"). Meristems consisting of two leaf primordia formed numerous leaf rosettes on MS1= MS + IBA (0.02 mgL⁻¹) + NAA (0.2 mgL⁻¹) + Kin (1.0 mgL⁻¹). Multiplication of shoots of clone "DP" and "DPS" were achieved on media MS and/or MS= MS + IBA (0.02 mgL⁻¹) + NAA (0.2 mgL⁻¹) + BAP (1.0 mgL⁻¹). Stem segments were cultivated on MS= MS + 2,4-D + Kin (1.0 mgL⁻¹), each ) + I-proline (250 mgL⁻¹) on which they subsequently formed the OC. After transfer of the OC on MS, medium, the AB were observed. Shoots of both clone were rooted on media MS and MS=MS + IBA (0.5-1.0 mgL⁻¹) + Kin (0.05 mgL⁻¹). Rooting for clone "DP" (7and 27%) and "DPS" (70 and 91%) depended on the concentration of IBA (0.5-1.0 mgL⁻¹, respectively). Multiplication of D. ciliatus and D. croaticus was achieved through micropropagation from stem segments culture on medium MS= MS + NAA (1.0 mgL⁻¹) + BAP (1.0 mgL⁻¹) + IBA (0.5 mgL⁻¹). The same rooting protocol used for these species. Carantion plantlets were grown in the greenhouse until flowering.

P62. Effect of ethylene and 1-methylcyclopropene (1-MCP) on flower senescence of Oncidium and Odontoglossum
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Ethylene is a natural gaseous hormone and is responsible for a number of processes in the plant, amongst them the senescence and abscission of organs like flowers and fruits. Thus, the exposure of plants to ethylene may result in a reduction of the plant quality, particularly in faster wilting of flowers and the abscission of florets and petals. Objectives of the present study were to examine the effects of ethylene and the ethylene perception inhibitor 1-methylcyclopropene (1-MCP) on the senescence of flowers of the orchid genera Oncidium and Odontoglossum. Our experiments involved cut inflorescences and on the other hand entire potted plants. First, cut inflorescences of one Oncidium and three Odontoglossum genotypes were treated with 1 ppm ethylene continuously and compared to controls. In control, one half of the flower stalks was pre-treated with 200 nL/1-MCP for 6 hours at 20 °C. After two to four days, depending on the genotypes, the inflorescences treated with 1 ppm ethylene showed symptoms of senescence, like wilting of the florets and petals. Other remarkable effects of ethylene were the yellowing of the pedicels as well as bud dropping. Open flowers, however, were not abscised, but turned wilted and in red-coloured genotypes changed their petal colour. The untreated control flowers showed these symptoms three to seven days later. On the other hand, the plants pre-treated with 1-MCP expressed an increase in shelf life of two up to seven days in both variants, the control and the ethylene treatments. To compare and confirm these results and to monitor further effects of ethylene, the second experiment with the same treatments is currently carried out with entire potted plants. Preliminary results showed that also on the whole plants similar symptoms of the inflorescences could be observed. In conclusion, the two genera Oncidium and Odontoglossum have to be considered as sensitive to ethylene, and 1-MCP can improve their post-harvest quality by far.

P63. Factors affecting in vitro germination and seedling growth of terrestrial orchids
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The substantial factors affecting seed germination and seedling growth under in vitro asymbiotic conditions were determined in three critically endangered species of terrestrial orchids (Dactylorhiza incarnata subsp. serotina, Dactylorhiza maculata subsp. maculata and Liparis loeselii). The effect of two sterilization substances, calcium and sodium hypochlorites, on the germination rate and the influence of nitrogen and growth regulators on the growth and development of seedlings was studied. The surface sterilization of mature seeds using 7.2% calcium hypochlorite (until decolourization of brown to ivory colour) stimulated the germination rate. Addition of peptone in the concentration 1 g l⁻¹ or auxins 3-indoleacetic acid (IAA; 1.43 µM) and 1-naphthylacetic acid (NAA; 1.34 µM) into cultivation medium significantly increased the growth parameters of seedlings after 12 months. Owing to the present results, we have been able to start a pilot study of in vitro germination under symbiotic conditions with two mycorrhizal isolates from roots of D. incarnata subsp. serotina and D. maculata subsp. maculata.

P64. In vitro propagation using adventitious buds technique as a source of new variability in chrysanthemums
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Eleven cultivars of chrysanthemums (Chrysanthemum x grandiflorum/ Ramat./Kitam.): 'Richmond' and its ten radionutants representing the Lady group, were propagated in vitro with two kinds of explants: shoot tips and leaves. The aim of this study...
was to investigate if the micropropagation method affects the genotype and phenotype of chrysanthemums. Plants regenerated from shoot tips and adventitious buds formed on leaves were rooted in vitro, acclimatized and cultivated in glasshouse up to full-flowering. The colour and shape of inflorescences of plants obtained from two different explant types were compared within the cultivars. All plants derived from shoot-tip explants showed inflorescences colour and shape typical for the cultivars. Inflorescence features of plants derived from adventitious buds formed on leaves were true-to-type in four cultivars: 'Richmond', 'Lady Amber', 'Lady White' and 'Lady Yellow'. 'Lady Apricot' (originally: golden beet) and 'Lady Salmon' (salmon) propagated with adventitious buds technique showed altered inflorescence colour in 100% (respectively: purple gold; white and pink). 'Lady Bronze' (originally: reddish brown), 'Lady Orange' (orange brown) and 'Lady Rosy' (purple gold) propagated with adventitious buds technique had both typical and changed inflorescences colours (respectively: yellow; yellow and red; reddish pink). 'Lady Vitroflora' showed altered inflorescence shape while propagated with adventitious buds technique. Those changes might be an effect of either somaclonal variation or chimeral structure of the plants investigated. The variation appears only if non-meristemical explants are used. Adventitious buds technique might be useful in chrysanthemum breeding as a source of variability.

P65. PRODUCTION OF TRANSGENIC PHALAENOPSIS PLANTS BY INTRODUCING GLUTATHIONE S-TRANSFERSASE GENE INTO PROTOCORMS AT AN EARLY STAGE AFTER GERMINATION

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Phalaenopsis orchids, one of the most popular orchids, are grown for cut flowers and pot plants around the world. In recent years, wide variations of Phalaenopsis cultivars have been produced through inter-specific and inter-generic hybridization. However, it is expected that improvement of this orchid crop by using genetic transformation method, to produce cultivars with novel traits such as disease resistance, flower color and tolerance to temperature stress. We have previously established a transformation system, by targeting protocorms at an early stage after germination via Agrobacterium. By using this system, Glutathione S-transferase (GST) gene isolated from rice was introduced into Phalaenopsis genome to confer the resistance to various biotic and abiotic stresses. Protocorms obtained 21 days after sowing on liquid New Dogashima medium were inoculated with Agrobacterium strain EHA101 (pEKH35S163P) harboring GST and hygromycin resistant genes. A total of 68 transgenic plants derived from independent protocorm were obtained from 6325 mature seeds 6 months after infection with Agrobacterium. Regenerated plants were grown in greenhouse and all plants bloomed within 2 years. Since the aim of this study is to breed the plants with desirable traits and the transgene among the seedling population, we selected 6 plants showing suitable characters for commercially production such as big flower size, flower shape and flower stem length among 68 transgenic plants. A total of 13 pods of seeds were obtained after self-pollination or cross combinations among the 6 transgenic clones. Analysis of GST in T1 plants are in progress.

P66. DEVELOPMENT OF EST MARKERS AND EVALUATION OF THEIR USE IN EVERGREEN AZALEA ANALYSIS

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Starting from a cDNA library made from flowers of the Rhododendron simsii hybrid ‘Flamenco’, 200 cDNA fragments were randomly picked and sequenced. The putative functions of the cDNA fragments were determined by comparison of the sequences with EMBL accessions. Reliable homologies were found for 30 % of the fragments. Primers were developed on 87 cDNA fragments and used for PCR amplification on 6 different azalea cultivars and species. Polyacrylamide gel electrophoresis was used to separate the fragments and after UV staining, the presence of polymorphic bands was evaluated. In those cases that no polymorphisms could be detected, new primers were developed and the procedure was repeated once. In the end, this resulted in 32 polymorphic EST markers. Ten of them were selected and the application of these EST markers to study genetic diversity and relationships in and evergreen azalea gene pool was investigated. Forty species and varieties that most concurred in the origin of the known cultivars and 44 cultivars chosen among distinguishable horticultural groups were genotyped using 3 AFLP primer combinations, 6 microsatellite loci and 10 ESTs (Scariot et al., submitted). EST markers revealed a higher genetic distance detection capacity than AFLPs. Similarity matrices produced for each marker technique showed weak, yet significant, correlations when Mantel’s test was applied. Performing the analysis of molecular variance, for all marker techniques used most of the genetic diversity was attributable to differences among cultivars within horticultural groups. However, EST markers outperformed AFLP and STMS markers concerning Fst values indicating a low but significant differentiation among horticultural groups. Although ESTs and STMSs appear to be the most appropriate markers for paternity analysis and assessment of narrow genetic relationships, AFLP still remains the best technique for phylogenetic studies. EST markers could be particularly useful for QTL mapping using a candidate gene approach because they target expressed genes and for comparative mapping studies because they are derived from gene coding regions, which are more likely to be conserved across populations and species than non-coding regions.
Azalea flower colour is mainly determined by two groups of pigments, anthocyanins and flavonols. Genes coding for two key enzymes in the biosynthesis of these pigments, chalcon synthase (chs) and dihydroflavonol-4-reductase (dfr) were isolated from an azalea cDNA library and fully characterised. The expression of these two genes in the petals of 10 azalea flower colour sports of the ‘Hellmut Vogel’ sporting series will be used as a model for the development of a real-time RT-PCR protocol for gene expression analysis in azalea. Real-time RT-PCR is currently the most sensitive method, especially suitable because very little amounts of RNA are sufficient. Optimisation was needed at all crucial steps from RNA isolation up to the final quantification. Since no intron-spanning primers could be developed, DNase treatment of mRNA samples appeared to be a good alternative for preventing the co-amplification of contaminating DNA, as shown by the low or non-existing amplification in the noRT-samples. There exist many different quantification strategies, but, although most labour-intensive, the use of standard curves remains the most reliable method. However, reproducibility and stability of these dilution series was a major problem. This problem could be circumvented by linearisation of the used plasmids and by diluting them in a yeast tRNA solution. The most critical step for the whole quantification process was the selection of the right housekeeping genes. In previous analysis, only GAPDH was used for this purpose, but the use of at least two housekeeping genes is recommended. Starting from the ‘Flamenco’ cDNA library, 200 cDNA fragments were randomly picked and sequenced. The putative functions of these fragments were determined by comparison of the sequences with EMBL accessions, 60 potential azalea genes could be identified. Twelve potential housekeeping genes (coding for enzymes active in photosynthesis, cell cycle, ...) were selected and primers suited for real-time PCR were developed. One primer pair gave no amplification; another one resulted in aspecific signal. The remaining ten genes were tested together with GAPDH in real-time PCR and by using the geNorm software (Vandesompele et al., 2002) we could state that the use of two housekeeping genes, coding for histone H3 and pyruvate dehydrogenase kinase, will be sufficient for expression analysis in the ‘Hellmut Vogel’ sporting series. In the end the expression of CHS and DFR was determined and different quantification strategies will be compared to find a relation between gene expression and azalea flower colour.

P68. EVIDENCE FOR A FORMA SPECIALIS SPECIFIC EFFECT OF ANTIFUNGAL FLAVONOLS IN FUSARIUM OXYSPORUM

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Previous experiments evidenced that three natural flavonols, extracted from carnation (Dianthus caryophyllus), are provided with an antifungal activity towards the 'forma specialis' "dianthi" of the pathogenic fungal species Fusarium oxysporum. In the present research, the same three molecules have been assayed against further four 'formae specialiae' of Fusarium oxysporum affecting ornamental plants: f. sp. asparagi, f. sp. ranunculi, f. sp. cyclaminis and f. sp. lilii. The compound effect was evaluated as ability of inhibiting mycelial growth and spore germination. Among the assayed flavonol aglycones, the 4'-methoxylated flavonol kaempferide was particularly effective, and generally speaking the glycosylated forms were less active than the free aglycones. The inhibiting effect of a same flavonol proved to vary depending on the different fungal 'forma specialis', therefore indicating that fungal tolerance towards fungitoxic flavonoids could be genotype-dependent. This also means that flavonols fungitoxic to Fusarium oxysporum exert an effect which is strictly specific for each forma specialis. Further studies should be aimed to assess if this tolerance mechanism is nondegradative or relies instead on the antifungal compound metabolism.

P69. VARIOUS PROFILES OF FLORAL SCENT IN PETUNIA AXILLARIS AND THEIR GENERATING PROCESSES

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Petunia axillaris, which has white nocturnally fragrant flowers, is classified into 3 subspecies based on their flower organ morphologies: axillaris, parodii and subandina. From these subspecies lines, we chose 13 specimens whose scents are different each other. By comparing their emitted and endogenous scent compounds, we investigated factors to generate the variation of fragrances among P. axillaris lines. Floral scents were composed of aromatic compounds in all specimens. Methylenbenzoate is commonly occurred as a dominantly emitted component with quantitative variation, that corresponded to the strengths of fragrance. Different from emitted components, endogenous components showed both quantitative and qualitative variations. Emission of floral scents seemed to be basically physical phenomenon, so that lower boiling point compounds were more emphatically expressed in proportion of emitted compounds. We isolated a novel aromatic compound of scent-less and high boiling point and identified it to be dihydroconiferyl acetate. The total amounts of benzenoids including dihydroconiferyl acetate showed good correlations with the amounts of phenylalanine. Now we could understand that various floral scent profiles in P. axillaris generate through following three processes: 1) determination of endogenous amounts of "total" aromatic compounds depending on biosynthetic activities of common precursors, 2) determination of the endogenous composition of 'scent' compound depending on their own metabolism ratios and 3) evaporation of each 'scent' compound depending on its vaporing pressure related to its boiling points.
P70. **ANALYSES OF GENOME CONSTITUTION OF DARWIN HYBRID TULIPS**

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Darwin Hybrid tulips have been developed by interspecific crosses of *Tulipa gesneriana* cultivars and *T. fosteriana* Hoog ex W. Irving (Lefeber, 1960). Most of the Darwin Hybrid tulips are triploid, whereas one is a diploid and a few are tetraploids. Despite the high popularity of triploid Hybrid tulips cultivars for cut flower and in gardens, both their genome constitution and how triploidy occurred through the interspecific hybridization are unknown. To understand the process of the formation of triploid Darwin hybrid tulips we analyzed diploid cultivars of *Tulipa fosteriana* and *T. gesneriana* (2n = 2x = 24) and triploid Darwin Hybrids (2n = 3x = 36) by karyotype analyses, genomic in situ hybridization and flow cytometry. To arrange chromosomes in karyograms, the total length, relative length, centromeric index and arm ratio were compared. Based on the karyotypical analysis, median chromosomes were proposed as a marker for diploid genotypes. Discriminant analysis with respect to total chromosome length and short arm length showed the significant difference in the larger median chromosomes of *T. gesneriana* and *T. fosteriana*. Comparison of the median chromosomes length in Darwin Hybrid tulips showed that two larger chromosomes and one smaller chromosome were derived from *T. gesneriana* and *T. fosteriana*, respectively. This founding was unambiguously confirmed by simultaneous hybridization of differently labelled genomic probes of *T. fosteriana* and *T. gesneriana* to metaphase chromosomes of triploid cultivar ‘Yellow Door’ where twelve chromosomes of *T. fosteriana* ‘Red Emperor’ were uniformly painted red, whereas the remaining chromosomes showed hybridization pattern similar to that observed on *T. gesneriana* cultivar ‘Queen of Night’. In addition, verification of hybridity of Darwin hybrid tulips was readily accomplished by flow cytometry. Providing that triploid Darwin hybrid tulips have two copies of the *T. gesneriana* genome and one copy of the *T. fosteriana* genome, their theoretical relative DNA content can be calculated by adding the 2C value (148) of *T. gesneriana* and the 1C value (59) of *T. fosteriana*, resulting in a value of 207. This value is very close to the 2C value of Darwin hybrid tulips obtained in our study (208.7 ± 4.2). To sum up, on the basis of the karyological analysis, GISH and flow cytometry analysis, triploid Darwin Hybrid tulips combined the diploid genome of *T. gesneriana* and the single genome of *T. fosteriana*. Our results enhance our understanding of the process of *Tulipa* cultivar formation and will be useful for interspecific hybridization breeding.

P71. **DETERMINATION OF THE ANTHOCYANIDIN PATTERN IN FLOWERS OF *CALLISTEPHUS CHINENSIS***

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*Callistephus chinensis* (China aster) is a popular ornamental plant which is worldwide used as cut flower. The broad flower colour spectrum is determined by various mixtures of derivatives of the anthocyanidins pelargonidin, cyanidin and delphinidin. In order to elucidate the determination of the anthocyanidin pattern in *Callistephus*, we investigated three chemogenetically defined lines: line “01” (genotype RR; predominant accumulation of delphinidin derivatives), line “02” (genotype rr; predominant accumulation of cyanidin derivatives) and line “03” (genotype rr; pelargonidin and cyanidin derivatives in a ratio of about 60 : 40, no delphinidin derivatives). Enzyme assays with petal extracts showed that chalcone synthase, the key enzyme of anthocyanin biosynthesis, mainly catalyses the formation of the precursors of pelargonidin derivatives in all lines. Therefore, the determination of the anthocyanidin pattern is supposed to be due to the presence and activity of flavonoid 3’-hydroxylase (*F3’H*; necessary for the synthesis of cyanidin derivatives) and flavonoid 3’5’-hydroxylase (*F3’5’H*; necessary for the synthesis of delphinidin derivatives). This is supported by the treatment of petals with tetacyclacis, an inhibitor of *F3’H* and *F3’5’H*, leading to a predominant accumulation of pelargonidin derivatives in all lines. We performed enzymatic analysis of *F3’H* and *F3’5’H* activity in microsomal preparations and furthermore analysed the expression of the respective genes by RT-PCR. In accordance with the accumulation of cyanidin derivatives in all lines investigated, *F3’H* expression and activity of *F3’5’H* was detected in all lines. In contrast, *F3’5’H* activity was measured only in line “01”. Since delphinidin derivatives accumulate in low amounts in line “02”, *F3’5’H* activity may be to weak to be detected. The lack of delphinidin derivatives in line “03” corresponds to the absence of *F3’5’H* gene activity in-vivo. Using gene-specific primers for the amplification of the complete coding region by RT-PCR, expression of the *F3’5’H* gene was found for line “01” and line “02” but not for line “03”. However, by using an unspecific reverse primer, two amplificates were obtained for line “03”, both being substantially shorter than compared to those of lines “01” and “02”. Sequencing of these fragments revealed that both *F3’5’H* transcripts are prematurely terminated and polyadenylated. Therefore, no functional *F3’5’H* protein can be synthesised in line “03”. The premature termination and polyadenylation may be due to altered splicing and/or due to polyadenylation signals within introns. Isolation and sequencing of the genomic *F3’5’H* will deliver an explanation for the described phenomena in future studies.

P72. **ESTABLISHMENT OF A MANNOSE-BASED SELECTION SYSTEM FOR THE TRANSFORMATION OF TORENSIA HYBRIDS**

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Traditional selection markers for the production of transgenic plants such as genes for antibiotic resistance and herbicide tolerance are discussed controversially in the public. A promising alternative system consists in the use of mannose as selective agent. Mannose can not be metabolized by many plant species and accumulates as mannose-6-phosphate resulting in growth inhibition. By the use of a gene encoding phosphomannose isomerase (*PMI*) as a selection marker, transgenic cells are enabled to use mannose-6-phosphate as carbon source, since *PMI* catalyses its conversion to fructose-6-phosphate. A mannose-based selection system has already been successfully established for the production of transgenic crops such as sugar beet, rice, maize or potato. We intend to use this “positive” selection system for the transformation of ornamental plants and chose *Torenia* as a model plant. *Torenia* leaf explants were inoculated with the *A. tumefaciens* strain LBA4404 harbouring a binary vector with the...
P73. **Superoxide dismutase (SOD) gene transferred into cultivars and breeding lines of Petunia hybrida**

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To develop new petunia cultivars resistant to environmental stresses, we have conducted genetic transformation for introduction of superoxide dismutase (SOD) gene. Four cultivars and 15 breeding lines of Petunia hybrida were cultured on MS medium supplemented with 1.0 mg/L BA and 2 mg/L IAA after co-culturing with Agrobacterium tumefaciense including SOD gene isolated from Escherichia coli. The host plants were 4 cultivars (Millenium White, Glory Red, Glory Blue, and Glory Purple) and 15 breeding lines. Kanamycin was used as a selective agent. Leaf segments were inoculated with the disarmed Agrobacterium tumefaciense strain GV3101 containing pBl121 vector in which SOD gene was introduced as sense in the direction. The shoots survived at the first selection medium containing 50 mg/L kanamycin and 400 mg/L cefotaxime for 4 - 6 weeks were transferred to the second selection medium containing 100 mg/L kanamycin and 400 mg/L cefotaxime for 3 - 4 weeks. To confirm the putative transgenic plants, PCR analysis was conducted using the neomycin phosphotransferase II (npt II) and SOD gene specific primers. Transgenic plants, which were confirmed by PCR analysis, were transferred to MS medium containing 200 mg/L cefotaxime for rooting for 3 weeks. The number of plants, which were survived on the second selection medium, was as many as 75. From PCR analysis, 58 plants derived from 4 cultivars and 2 breeding lines were found to contain both npt II and SOD genes.

P74. **Green Fluorescent Protein (GFP) as a tool for selection of transgenic Petunia hybrida plants**

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Selectable marker genes are needed for efficient transformation of plants. In most cases, selection is based on antibiotic or herbicide resistance. Due mainly to consumer concerns, strategies (site-specific recombination, homologous recombination, transposition and co-transformation) have been developed to eliminate the marker gene after selection. The present study focused on testing the applicability of the Green Fluorescent Protein (GFP) for selecting transgenic Petunia hybrida plants. The Green Fluorescent Protein (GFP) from the jellyfish Aequorea victoria has proven to be a convenient and powerful vital marker in transgenic studies. Its expression can be detected non-destructively, in real time, simply by UV-light excitation. This property of GFP holds promise in monitoring the presence and expression of transgenes in higher plants. In our experiments, we first studied in a transient assay the detectability of two different versions of GFP genes (mGFP-4, smRS-GFP). Two days after infiltration of Agrobacterium tumefaciens into leaf epidermal cells was recorded, whereas the intensity of smRS-GFP fluorescence was higher and better seen than that of mGFP in Petunia. The first experiments aiming at stable transformations involved vectors containing the bar gene and the smRS-GFP gene (pGreenII0229+smRS-GFP). Leaf explants were co-cultured with different A. tumefaciens strains (LBA4404, GV3101, GV2260, EHA105) carrying this vector for three days. Thereafter the explants were cultured on shoot regeneration medium containing phosphinothricin. GFP expression was monitored 3, 7, 10, 14 and 21 days after transformation. GFP detection was very clear during the first days of culture in putatively transformed dividing cells at the explant margins and also in young shoot buds. However, its intensity decreased with growth of the shoots. The next studies will employ observations on localization of GFP activity without selection applying phosphinothricin. Possible implications for further studies in Petunia transformation are discussed.

P75. **Trying to control floral scent of Gypsophylla using genetic transformation**

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Besides beauty of flowers, floral scent is one of the most characteristic features of flower crops for ornamental use. Flowers of two species of genus Gypsophylla emit unpleasant odor after opening the florets. We tried to reveal the cause of this bad order and to control the emission by molecular breeding technique, using two cultivars of G. elegans (‘Covent Garden Market’ [CM] and ‘Crimson’ [CL]) and two cultivars of G. paniculata (‘Bristol Fairy’ [BF] and ‘Golan’ [GO]). Among these cultivars, CM emits less order. Investigation by headspace adsorption/gas chromatography indicated that major volatile compounds from florets were ocimene, 3-methylbutyric acid and 2-methylbutyric acid. 3-Methylbutyric acids were the highest in BF and GO. They
were undetectable in non-scent CM. Methylbutyric acids are produced by degradation of leucine or isoleucine. It was suggested that α-ketoisocaproate was decarboxylated oxidatively to isovaleryl CoA, then decarboxylated to 3-methylbutyric acid. In non-scent plants, α-ketoisocaproate may be decarboxylate to form isovaleraldehyde by pyruvate decarboxylase (PDC). From this point of view, PDC activity was measured from crude enzyme extract of florets of GO. The enzyme activity was high in swollen flower buds and low in open flowers. These results indicated that low PDC activity leaded to synthesis of methylbutyric acids. In order to estimate PDC gene expression in flower buds, total RNA was extracted from flower buds (tight, swollen, full open) of CM, BF and GO. Primer pair was designated from Arabidopsis thaliana PDC gene to amplify 230 bp DNA fragment from cDNA synthesized from total RNA. The results of RT-PCR using these primers indicated that transcription level of PDC gene was continuously high in non-scent cultivar CM and low in full open florets of BF and GO. Agrobacterium-mediated transformation system of G. elegans cv. Cl was also established. Leaf segments were pre-cultured for two to three weeks on regeneration medium then co-cultivated with Agrobacterium tumefaciens harboring hpt and GUS reporter genes. All plants regenerated from leaf explants after selection on hygromycin-containing regeneration medium for 4 weeks showed strong expression of GUS gene. We are now planning to obtain transgenic G. elegans plants over expressing PDC gene to know the possibility to control the methylbutyric acids biosynthesis.

P76. **Agrobacterium–mediated transformation of protocorm-like bodies of Vanda**

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A transformation procedure using protocorm-like bodies (PLBs) of Vanda was established by using Agrobacterium tumefaciens strain EHA 101(pG121Hm) harboring both β-glucoronidase (GUS) and hygromycin resistant genes. PLBs cultured in liquid New Dogashima medium (NDM) supplemented with 0.1 mg/l NAA, 1 mg/l BA and 30 g/l maltose under continuous light or complete darkness were used for transformation. PLBs were sub-cultured in liquid NDM containing 100 μM acetosyringone (AS) 2 days before inoculation. After 3 days of co-cultivation, the PLBs were transferred to a medium containing 10 mg/l meropenem for 2 weeks after which they were transferred into a selection medium containing 10 mg/l meropenem and 10 mg/l hygromycin for 2 months. Surviving PLBs were cut transversely and placed on recovery medium without hygromycin for 2 months. The proliferated PLBs were further transferred onto selection medium and the hygromycin-resistant PLBs regenerated showed histochemical blue staining for GUS. Transformation of these PLBs were confirmed by PCR analysis. PLBs cultured in complete darkness with the addition of 100 μM acetosyringone and subjected to longer period of inoculation (4h) with Agrobacterium gave the highest transformation efficiency.

P77. **Transformation in Curcuma**

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A condition for regeneration and genetic transformation was investigated for Curcuma. A high frequency of regenerate shoots from multiple shoots was achieved with a modified Murashige and Skoog's medium (MS), with 0.1 mg l⁻¹ IAA, 4 mg l⁻¹ IMA and 0.5 mg l⁻¹ TDZ. Agrobacterium tumefaciens harbouring the binary vector, pBI121, pCAMBIA 1303, pCAMBIA 1304 and pSCV1.6 were used. The explants were co-cultivated in difference time such as 1, 2 and 3 days with the bacteria. After co-cultivation the explants were transferred onto regenerate medium, containing 50 mg l⁻¹ kanamycin and 500 mg l⁻¹ vancomycin. Within 8 weeks, the shoots were separated onto the elongation medium and detected transgenic. Transformation events were confirmed by PCR analysis, histochemical GUS assay and southern blotting of the transgenic plants.

P78. **AFLP analysis of Dendrobium Sonia white mutant lines**

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Dendrobium Sonia white mutant lines were developed from purple flower D. Sonia. Amplified Fragment Length Polymorphism (AFLP) technique was used to compare genomic variations in these mutant lines with the control. Our objectives were to isolate and characterize polymorphic fragments from these mutants to provide useful information on genes involving in flower colour expression and develop these fragments as DNA markers to identify the mutants. In this method, preamplification products were used as templates for selective amplification using two AFLP primers (EcoR I and Mse I primer) with each containing three selective nucleotides. A total number of 30 primer combinations have been tested and five produced clear fingerprint patterns. Of these, 13 polymorphic bands have been successfully isolated and sequenced. The sequences generated were analysed using NCBI GenBank Database. The BLASTx search revealed that half of the fragments showed high homology to Class III Chitinase gene.

P79. **DNA fingerprinting of carnation varieties through AFLP markers**

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The use of molecular markers in ornamental plant breeding is very considerable for applications related to genotype fingerprinting including variety identification and phylogenetic analyses, but still limited in application to gene or QTL tagging.
P80. **CHARACTERIZATION OF Fusarium spp. AND F. Oxyosporum USING RAPD AND PCR MARKERS**

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The selection of resistant forms in breeding material of Callistephus chinensis NEES again Fusarium sp. was researched for specific active forms (formae speciales) of this. Gene pool of Fusarium was collected, purified and tested with plant material. The genomic methods were used to determination of differences between Fusarium spp. and its formae speciales (f.sp.). The random amplified polymorphic DNA (RAPD) and PCR technique were used to analyse genomic DNA of 13 Fusarium spp. included F. oxyosporum and its f.sp. callistephi, cyclaminis, dianthi, lycopersici, radicis-lycopersici, narcissi, pisi, tracheiphilum and 40 isolates of Fusarium sp. collected from different hosts plants, mainly Callistephus chinensis. These isolates are deposited in University fungal collection. 10 deca-hexamers random primers and 3 pairs of specific primers were used for amplification in this study. New PCR markers based on conservative sequences in mitochondrial Fusarium oxyosporum genome were tested. Products of amplification were always detected. Obtained spectra were evaluated similarly as RAPD and added to RAPD data for genetic similarity estimation. A dendrograms of genetic relationships and methodology of identification of each isolates were created.

P81. **CHANGES IN Petunia hybrida INDUCED BY ETHYLENETHANESULPHONATE (EMS) AND METHYL METHANESULPHONATE (MMS).**

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The studies aimed at determining phenotypic and genotype changes occurring in Petunia hybrida grandiflora "Flash", with red flowers, after exposure to chemical mutagens EMS and MMS. The seeds, first soaked for 24 hours in water, were next for 2 hours soaked in 0.5, 1.0, 1.5 and 2.0 mM solutions of EMS and MMS (pH – 4.0) in the presence of a buffer (orthophosphoric acid). Next, the seeds were rinsed in distilled water and placed in cellulose pots filled with a mixture of pit and perlite in a 3:1 ratio. Initially, the plants grew in a growth chamber (51 days) and after transplanting into larger pots – in a glasshouse. The experiment was arranged in random blocks, each in three repeats, saving 150 seeds from each experimental variant. During vegetation observations were made of the phenotypic differences between plants. DNA was isolated from plants differing in phenotype from the control and analysed using the ISSR-PCR technique. The results obtained demonstrated that the chemical mutagens, used in the present work, affected the germinating ability of seeds, height of plants, number of leaves and their colour, the length of roots, number of buds and the number, diameter and colour of flowers (darker veined, lighter or white and wavy margins of corolla petals, mottled petals and leaves, etc.). The frequency of the changes observed in the case of EMS depended on its concentration in the solution (the higher the concentration the higher the frequency). In all mutants specific ISSR-PCR products were observed, differentiating them from control plants. This made it possible to elaborate a fingerprinting, specific for each genotype examined. It was observed that the Petunia genotypes, selected for testing, were similar in 22.2- 71.6%. The greatest phylogenetic similarity to the remaining forms of petunia was demonstrated by plants with a changed wavy margins of corolla petals, obtained from seeds soaked in a 2.0 mM solution of MMS.

P82. **GENETIC SIMILARITY OF CHosen Styringa genotypes determined by the ISSR-PCR technique**

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The present work aimed at determining by the ISSR-PCR technique the genetic similarity between fourteen genotypes of lilacs from Przelewice Dendrological Garden (S. vulgaris Krasavitsa Moskvy’, S. vulgaris ‘Niebo Moskwy’, S. vulgaris, S. × prestoniae ‘Jaga’, S. meyeri ‘Palibín’, S. vulgaris ‘Mme Lemoine’, S. vulgaris ‘Jules Simon’, S. vulgaris ‘Mirabeau’, S. × chinensis, S. × prestoniae ‘Oktavia’, S. vulgaris ‘Katherine Havermeyer’, S. × prestoniae ‘Gopala’ and S. vulgaris ‘Mrs. Ellen Wilmott’). Only thirty primers homologous to microsatellite repeats based on various di-, tetra- and penta- SSR motifs with 3’-selective nucleotides for anchoring, were screened against the onion genome by 2 % agarose gel electrophoresis. PCR conditions were optimised to obtain high quality patterns. Out of the 30 primers 13 were chosen for final study. These amplified a total of 723 bands of which 698 (96.5%) were polymorphic. Clearly detectable amplified ISSR ranged from 215 to 3731 bp in size. The average number of bands generated per primer was four. Species-specific ISSR fragments (25) was detected for nine lilacs genotypes. The highest number (5) of specific fragments were obtained for S. × chinensis, whereas the lowest species-specific number (1) was detected for S. vulgaris ‘Krasavitsa Moskvy’, S. meyeri ‘Palibín’, S. vulgaris ‘Katherine Havermeyer’ and S. vulgaris ‘Mirabeau’. Genetic similarity between genotypes was estimated using Jaccard’s coefficient of similarity. UPGMA cluster analysis was used to construct a dendrogram and to estimate the genetic distances between the lilacs. Their similarity ranged from 30.4 to 72.4 %. The analysed genotypes of lilacs were divided into four groups. In the first group, there were genotypes: S. prestoniae ‘Jaga’ and S. vulgaris ‘Mme Lemoine’ (similarity – 58.9 %), in the second one - S. meyeri ‘Palibín’, S. × prestoniae, S. vulgaris, S. vulgaris Krasavitsa Moskvy’ and S. vulgaris ‘Niebo Moskwy’ (similarity from 49 to 69.1 %), in the third one – S. vulgaris ‘Katherine Havermeyer’, S. vulgaris ‘Mirabeau’ and S. vulgaris ‘Jules Simon’ (similarity from 69.2 to 72.4 %).
and in the fourth one = S. vulgaris ‘Mrs. Ellen Wilmutt’, S. × chinensis, S. prestoniae ‘Oktavia’ and S. × prestoniae ‘Goploana’ (similarity from 49 to 52.6 %). These results suggest that the ISSR-PCR method is potentially useful for genetic fingerprinting and molecular characterization of the lilacs genotypes.

P83. CHEMICAL MUTAGENESIS - A TOOL TO ANALYSE GENETIC RELATIONSHIP AMONG CARNATION VARIETIES?
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Mutation a major tool in flower breeding program is used to create variability and to create novel types of carnation. In order to create variability, selected carnation (Dianthus caryophyllus) genotype IIHRS-1 was subjected to mutagenesis under in vitro condition using Ethyl Methane Sulphonate (EMS) at various concentrations (0.01% to 1%). Survival, growth and abnormality response was found to decrease with the increase in concentration of EMS incorporated into media. Abnormal response of the explant under in vitro condition after two months of culturing was found to be dependent on both direct and interaction effect of concentration and incubation duration. Comparison of results considering survival, growth and abnormal response observed in both the experiments gave a clear indication of selecting the right method of treatment as well as mutagen concentration that can be used for mutagenesis. Established plants were evaluated for their morphological characters. Variations were noticed for flower colour. Mainly four colour groups were identified in mutated population. Interestingly, these colors were found to be similar to some of the genotypes that were already available in our collection. Morphological evaluations of all the mutants were taken up utilizing twenty morphological parameters. Based on the morphological characterisation, genetic distance was worked out to understand the relationship between the mutants. The entire study throws light on possibility of utilizing mutation as a tool for understanding the genetic relationship among varieties and as one of the supportive tools for sorting out the issues related to Plant Breeders rights.

P84. COMPARATIVE STUDY UTILIZING MORPHOLOGICAL, MOLECULAR AND BIOCHEMICAL ANALYSES FOR VARIETAL DISTINCTNESS IN MUTANTS OF CARNATION
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Breeding of flower crops like carnation (Dianthus caryophyllus) concentrates on creating new colour types and to come out with novelty. Often with an attempt to create variation, breeder ends up in realizing the genotypes with similar colours already existing in the gene pool of the crop. To work out the distinctness of a variety, evaluation can be made utilising morphological, molecular and biochemical analyses. In the present study we have attempted to establish the uniqueness of a stable mutant of carnation (IIHRP-1) in comparison with an unstable mutant (IIHRS-1), and its wild parent (CG-109). The minimum genetic similarity value derived between IIHRP-1 and IIHRS-1 was 0.656, while the maximum GS value derived between CG109 and IIHRP-1 was 0.832. Twenty five morphological characters, 50 molecular markers and estimation of anthocyanin was utilized to establish the uniqueness of the mutant. Morphological evaluation grouped the IIHRP-1 closer to IIHRS-1 on the contrary molecular markers grouped the IIHRP-1 closer to CG-109. A comparative analyses of various estimations is presented for establishing the uniqueness of variety.

P85. COOPERATION BETWEEN PUBLIC RESEARCH AND INDUSTRY IN ORNAMENTAL BREEDING
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In recent years, private and public breeding research in ornamentals focuses more and more on the major (mainly cut flower) crops. On one hand, application of advanced technologies in minor ornamental species is too expensive for individual small sized companies. On the other hand introducing new cultivars successfully is a difficult task for public research institutes, due to their limited access to markets and to promotion. Therefore the Department of Plant Genetics and Breeding (CLO-DVP) set up long term partnerships with growers for different ornamental crops. "BEST-select cvba" and "Het Azaleainnovatiefonds" are examples of Flemish grower associations that were founded during the last five years under impulse of CLO-DVP. The major aim of such alliances is the financial support of innovative breeding in respectively woody ornamentals and pot azalea.

Participating growers pay therefore a yearly contribution, which is invested in breeding activities and research. Especially for promising projects, the input from the growers functions as a kind of "seed money" to acquire subsidies from innovation investment funds. In Flanders, e.g. IWT, a governmental organisation stimulating and supporting innovation, favours high subsiding rates only if a collective of private partners is involved and a purpose of general interest for a sector is aimed for. At this moment, research projects of this kind are carried out on interspecific hybridisation, embryo rescue, polyploïdisation and disease resistance breeding. Moreover, we run a technological advice service to gather and transfer scientific and technological expertise to participating companies. In return, the grower associations have their voice in the breeding research topics and are involved at an early stage in evaluating new candidate cultivars. Finally, they get the exclusive rights on the new products from the breeding work. New releases are protected by plant breeding rights and marketed by the growers association under own label and trademark. By this program, scheduling propagation and market introduction can be coordinated. At the same time, an adequate publicity and promotion campaign for the new introduction can be made. Marketing funds are gathered by collecting a royalty on each sold plant. These partnerships between public research and private growers associations made it possible for small companies to get access to new cultivars with valuable qualities.
APPLICATION OF COMPUTER IMAGE ANALYSIS FOR EVALUATION OF GENETIC VARIABILITY OF FLORAL MORPHOLOGY

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Floral morphology, i.e., shape and size, is important for many ornamental crops because of the commercial value of their flowers, and thus target characteristics for breeding. Although the variation of floral morphology in many plant species is generally continuous, until now it has been evaluated qualitatively. Therefore, an objective and quantitative evaluation method is vital for evaluation of genetic variability of floral morphology. Recent improvements in computer performance and reductions in the cost of digital imaging hardware and software, have contributed to the widespread use of digital image processing in biological and agricultural research. We report here an application of such computer image analyses to quantitatively evaluate genetic variability of floral morphology. Elliptic Fourier descriptors and principal component analysis (EF-PCA) were applied to evaluate floral morphology of lisianthus (Eustoma grandiflorum). This method describes an overall shape mathematically by transforming contour coordinates into Fourier coefficients, and summarises these coefficients by principal component analysis. EF-PCA has two major advantages: first, it can detect small variations in shape, and second, it can evaluate the shapes of objects independently of their size. EF-PCA could describe several shape elements (principal components) such as aspect ratio and the curvature of the proximal and distal part. These new quantitative variables revealed wide variation in the floral morphology of lisianthus. Results of ANOVA indicated highly significant varietal effects in these shape elements. Although not designed to estimate heritability, this results indicated that the major source of variation in the floral morphology is genotypic. Digital images can provide information on shape and size of objects; this information composed of locations and colour depths of pixels. Thus, image analyses are considered to be effective ways of evaluating these characteristics of biological organs, and giving us quantitative measures which could not be obtained by human visual assessments. In fact, the results of our researches indicated the image analyses are effective to evaluate genetic variability of floral morphology, and perform breeding researches of biological organs for breeding.