Volume 6, Special Issue 1, 2012

ISSN 1749-0294

ISBN 978-4-907060-03-9

FLORICULTURE & ORNAMENTAL BIOTECHNOLOGY

Bulbous Ornamentals I

Guest Editors: Jaap M. Van Tuyl, Paul Arens

GLOBAL SCIENCE BOOKS

Floriculture and Ornamental Biotechnology

Abbreviation: Floriculture Ornamental Biotech. (FOB)
Print: ISSN 1749-0294
Frequency and Peer status: Biannual, Peer reviewed

Scope and target readership: *Floriculture and Ornamental Biotechnology* is dedicated to significant advances in ornamental plant science and biotechnology.

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2) *In vitro* propagation (micropropagation, somatic embryogenesis, tissue culture);
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4) Physiology, molecular biology, structural botany (integrated, pure and applied);
5) Phytopathology;
6) Post-harvest technology as applies to cut flowers and foliage (deterioration, preservation, shipping, and marketing);
7) Production of secondary metabolites, organic and inorganic biochemistry, and phytochemistry;
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For publication in *Floriculture and Ornamental Biotechnology* the research must provide a highly significant new contribution to our understanding of floricultural or ornamental plants and must generally be supported by a combination of either physiological, biochemical, genetic or molecular analyses. All areas of study are welcome and the experimental approaches used can be wide-ranging.

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Head Office and Editorial Office
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Miki cho, Ikenobe 3011-2, P.O. Box 7
761-0799, Japan

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Guest Editors

Prof. Jaap M. van Tuyl, Dr. Paul Arens

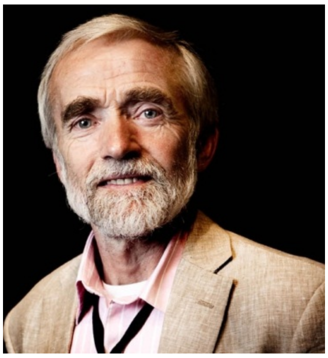
Wageningen University and Research Centre, The Netherlands

Cover photos: Top row (from left to right): Somatic embryogenesis induction in tissue culture of *Fritillaria meleagris* (Petrić *et al*., pp 78-89). 'Freida Hemple' caladium (Zhanao Deng, pp 53-61). *Zephyranthes latissimifolia* from Mexico (Tapia-Campos *et al*., pp 129-139). Center row (from left to right): *Lachenalia* 'Rainbow Bells' (Kleynhans *et al*., pp 98-115). Cytotype of *Echeandia mexicana* 236 (Palomino *et al*., pp 140-152). *Sprekelia formossisima* (Tapia-Campos *et al*., pp 129-139). *Polianthes geminiflora* var. clivicola (Barba-Gonzalez *et al*., pp 122-128). Bottom row (from left to right): *Hymenocallis jaliscensis* (Tapia-Campos *et al*., pp 129-139). *Echeandia breedlovii* (Palomino *et al*., pp 140-152). Tulip 'All Seasons' (William B. Miller, pp 35-44).

Printed in Japan on acid-free paper.
Published: December, 2012.

The Guest Editors

Prof. Jaap M. van Tuyl



Bulbous ornamental plants also called flower bulbs or ornamental geophytes are an important group of ornamental plants which play worldwide an important role in the flower industry. Although native in all continents, are Asia and South Africa the most important gene centres. The main crops are tulip, lily and narcissus which are as bulb crops predominantly grown and traded in The Netherlands. Bulbs are exported worldwide and grown as cut flower, pot plant and garden plant. The breeding history of ornamental bulbous crops goes back centuries ago but well described for especially for tulip only 3-4 centuries. Nowadays for the main crops consist an broad assortment of thousands of cultivars mainly originated from interspecific hybridization.

After a career of more than 35 years in breeding research of mainly lily and tulip at Wageningen University and Research Centre, it was a pleasure for me to invite leading scientists from all over the world to write contributions on bulbous crops in areas like taxonomy, plant breeding, tissue culture and physiology. This special issue contains 38 reviews and original research papers written by 90 authors from 18 different countries. With many of the authors I had a long cooperation as student, guest researcher and colleague. I am proud on my former PhD-students which graduated on bulbous subjects in my flower bulb group. They returned to their country of origin and continued research in this field. Most of the authors I know personally quite well from international meetings from ISHS (International Symposia on Flower Bulbs and herbaceous perennials, International Symposia on the genus Lilium) and EUCARPIA (International Symposia on Breeding of Ornamentals) and these contacts go back more than 30 years. I am very happy that so many of them were able to contribute to this book. The many reviews represent the current state of knowledge in these areas.

The taxonomy and phylogeny of *Lilium* is reviewed by Pelkonen and Pirttilä**,** while Wu *et al.* focussed on the *Lilium* species native to China. Okubo and co-workers describe "New insights on *Lilium brownii"*, while Hiramatsu *et al.* report on "The evolution of early flowering ability in *Lilium formosanum"* from its progenitor *L. longiflorum.* Eisuke Matsuo worked as guest researcher in 1983 with me, he is retired several years ago and jumped into name of *Lilium longiflorum* **"**Historical survey of Easter Lily name in association with *Lilium longiflorum."* Fred Bos, a retired vegetation specialist, discovered wild habitats of *Lilium bulbiferum* spp *croceum*, the only Lilium species native in The Netherlands. This orange lily was discovered around 1850 growing wild in rye fields in the northern part of the Netherlands and North-western Germany. He describes interesting cultural aspects of this lily and shows us that it can be found in 17th century Dutch and Flemish flower paintings as is an important symbol.

Kleynhans *et al*. contributes with the "Cytogenetic and Phylogenetic Review of the Genus *Lachenalia".* Agnieszka Marasek-Ciolakowska was during 5 years a guest researcher in my group and specialized in tulip. She evaluated the **"**Breeding and cytogenetics in the genus *Tulipa".* Nadeem Khan was my PhD-student from 2006-2009 and contributes with *"*A molecular cytogenetic analysis of introgression in backcross progenies of intersectional *Lilium* hybrids". Ramanna, although retired, was for more than 10 years our resource with his in depth cytogenetic expertise. He contributed to many papers and in this issue he presents "The significance of polyploidy for bulbous ornamentals: A molecular cytogenetic assessment" in which he compares *Crocus*, *Narcissus*, *Tulipa*, *Alstroemeria* and *Lilium.* Also Songlin Xie, another former PhD-student is co-author in this paper. Under supervision of Ki-Byung Lim (also graduated in our group) Hwang *et al.* reports on "Genome analysis of *Lilium tigrinum* by chromosome microdissection and molecular cytogenetic techniques. Two papers of Neil Anderson and co-workers focus on the discovery of novel traits in seed-propagated *Lilium xformolongi*. Proscevičius *et al*. show promising results with the mixed pollen method to overcome interspecific barriers in lilies:

"Application of mixed incongruous pollen for interspecific crosses of lilies". Okazaki *et al*. show the important implications of mitotic and meiotic polyploidization by using laughing gas in lily and tulip: **"**Application of nitrous oxide gas as a polyploidizing agent in tulip and lily breeding." Takejiro Takamura was in 1999 guest researcher in our research group and specialist in genetics of *Cyclamen.* He shows results of DNA-content differences in Cyclamen: "Specific differences in nuclear DNA content in the Genus *Cyclamen."* Emmy Dhooge *et al*. investigated intergeneric hybridisation between *Anemone and Ranunculus* and present this work entitled "Cytological and Molecular Characterization of Intertribal Hybrids Between the Geophytes *Anemone coronaria* L. and *Ranunculus asiaticus* L. (Ranunculaceae)". Kashihara *et al.* examined the possibilities of intergeneric hybridization between *Alstroemeria* and *Bomarae*: "Towards intergeneric hybridization between *Alstroemeria* L. and *Bomarea* Mirb". Recent advances of *Caladium* breeding and genetics is reviewed by Zhanao Deng. The genetics, physiology and cut flower production of the herbaceous peony (*Paeonia)* is reviewed by Rina Kamenetsky and John Dole. A review on Genetic transformation in the breeding of flower bulbs is written by Avner Cohen and Frans Krens. Former PhD-student Rodrigo Barba and co-workers give us an insight in some native genera of Mexico. They contributed three chapters on Mexican geophytes genera: *Polianthes, Hymenocallis, Sprekelia, Zephyranthes* and *Echeandia*.

In the field of tissue culture Geert-Jan de Klerk reviewed the technology and present state of micropropagation of bulbous crops. Ruffoni *et al*. describe their results using advanced techniques in micropropagation of *Gladiolus* "Biotechnological support for the development of new *Gladiolus h*ybrids". Petrić et al. are reporting the successful micropropagation of a number of *Fritillaria* species "Morphogenesis *in Vitro* of *Fritillaria* spp." Małgorzata Podwyszyńska is an expert in the field of micropropagation of bulbous crops. For this issue she contributed with "The mechanisms of *in vitro* storage organ formation in ornamental geophytes." Dariusz Sochacki and Małgorzata Podwyszyńska investigated the use of *in vitro* chemotherapy for obtaining virus-free stocks "Virus eradication in *Narcissus* and *Tulipa* by chemotherapy. Faouzi Haouala and Emna Chaïeb present their work in *Gladiolus* **"**Effects of Explant Position and Polarity on Callus Induction and Shoot Regeneration of *Gladiolus* (*Gladiolus hybridus* Hort.)". Cathy Kamo and Bong Hee Han optimized the callus regeneration protocol in lily: **"**Optimized growth and plant regeneration for callus of *Lilium longiflorum* 'Nellie White'." Paladines *et al.* present their results on microspore culture and haploid production in *Anemone coronaria:* "Prospects of isolated microspore culture for haploid production in *Anemone coronaria* L."

Saniewski *et al.* studied the role of auxin in the stem elongation of dark-grown tulips **"**Evidence for a role of auxin in the stem elongation of dark-grown tulips". William B. Miller of Cornell University shows the importance of growth regulators for the pot culture in the US: "Current status of growth regulator usage in flower bulb forcing in North America." Chad Miller *et al.* describe the possible cause of chlorotic symtoms in *Oxalis*: "Iron deficiency may result in interveinal chlorosis of Shamrock plant (*Oxalis regnellii*)". Juodkaitė *et al.* of the Botanical Garden of Vilnius studied in 299 tulip cultivars the vegetative reproduction capacity: "Assessment of the Vegetative Reproduction Potential of Tulips (*Tulipa* L.)". Peter Knippels investigated some specific propagation techniques like scoring, chipping, scaling and leaf cuttings in *Eucomis* and *Hymenocallis*: "Advanced *in vivo* propagation techniques for specialty bulbs."

The last four years my life's work with ornamental plants is taken over step by step by Paul Arens and I want to thank him for the pleasant cooperation in our research as well as in the editing of this book. This project was initiated four years ago by Jaime A. Teixeira da Silva, Editor-in-Chief of Global Science Books, who invited me as guest editor for these special issues of Floriculture and Ornamental Biotechnology. I am grateful to Jaime and all contributors for their willingness to share their knowledge.

December, 2012

Dr. Paul Arens

From the moment I got involved into the breeding research of bulbs, I have been impressed with the enormous variety of shapes, appearances and colours of these ornamentals. In many crops interspecific hybrids have been used in breeding to create the stunning wealth of cultivars as we know today whereas in other crops already a huge variation was accomplished within a single species. Nevertheless, for both situations still many new possibilities to extend breeding exist and those can add to further extend this large genetic variation. A number of the chapters go into more recently developed breeding possibilities for specific crops whereas others give a nice overview of more general interest.

As breeding of most bulbous crops requires a considerable amount of patience due to the relative long life cycle and slow vegetative reproduction, breeders and researchers in these crops are not only skilled professionals but are also very passionate for bulb flowers from a personal view. This makes the breeding research in bulbous ornamentals besides very interesting also very enjoyable. During the preparation of this book Jaap van Tuyl and I have been working with many researchers all over the world who made contributions to the book and this has been a very stimulating experience for me. Therefore I would like to thank all contributors, Jaime A. Teixeira da Silva, Editor-in-Chief of Global Science Books, who initiated the project to write this book and especially Jaap van Tuyl from who I have learned a lot in these past years and who has been the perfect person to work with as guest editors of this book. I hope this book will be as inspiring for people interested in bulbous crops as it was for me to work on this special issue of Floriculture and Ornamental Biotechnology.

December, 2012

Invited Foreword

Evert Jacobsen

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Floriculture and breeding of bulbous ornamentals is worldwide an important activity. The beauty of these ornamentals is shown in many exhibitions, parks and gardens. To enjoy human life is in this sense an important task of ornamental breeders. Breeding of this group of bulbous ornamentals, which are mostly vegetative propagated, always starts with domestication of certain phenotypes which afterwards were used as intraspecific breeding parent in order to combine such phenotypic traits with other ones. Important aspects of ornamental breeding are besides domestication, genetic variation, selection methods, biotechnological techniques and healthy vegetative propagation. In this special issue these different aspects are updated for the individual ornamentals like *Lily*, *Tulip*, *Gladiolus*, *Caladium*, *Peony*, *Fritillaria*, *Lachenalia*, *Crocus*, *Narcissus*, *Alstroemeria*, *Polianthus*, *Amaryllis*, *Sprekelia*, *Echeandia*, *Oxalis*, *Anemone* and *Ranunculus*. New developments have been described in (disease-free) micropropagation, seed propagated Lily, genetic transformation, introgression by interspecific hybridization and back crossing, improved techniques for wide crosses, further domestication of less developed bulbous species, (natural) polyploidisation, new interspecific hybrids and even intergeneric hybrids, cytogenetics using GISH and FISH, molecular markers but also chromosome micro-dissection, using “chemotherapy” for virus eradication and microspore culture for (doubled) haploid production.

It is clear that bulbous ornamental breeding is following modern genomic research like whole genome sequencing and many other molecular techniques at a distance. This is mainly due to the large genome size and the smaller amounts of money available in these ornamental crops. However, micro-chromosome dissection in lily is a surprising and interesting development. The use of genetic modification at variety level is still absent in this group of ornamentals despite the interesting possibilities for a number of specific traits.

I expect that this updating made by many different authors’ will stimulate breeding research but also variety breeding in this group of ornamentals. So, that we can enjoy the emergence, not only of new improved varieties within existing species but also of an increasing number of new, artificial, species obtained by interspecific hybridization with interesting combined phenotypic traits.

December, 2012

SPECIAL ISSUE: CONTENTS

Geert-Jan de Klerk (The Netherlands) Micropropagation of Bulbous Crops: Technology and Present State (pp 1-8)

ABSTRACT

Invited Review: Conventional propagation of bulbous crops must be supplemented with micropropagation to satisfy the requirements of present-day horticulture with respect to fast production of disease-free, superior starting material. Adequate micropropagation protocols for bulbous crops are therefore a *sine qua non*. The successive steps in micropropagation of bulbous crops are reviewed: initiation, multiplication, bulb formation, dormancy breaking and planting. In the first two steps, new shoots or bulblets are generated by axillary bud outgrowth or adventitious regeneration. During initiation, endogenous contamination may be a severe problem since bulbs grow subterraneously and have often been propagated vegetatively in the field for many years. Other drawbacks are insufficient axillary branching, poor adventitious regeneration and inferior growth. The latter, inferior growth, is likely the most significant problem and is caused by poor translocation of medium ingredients to the growing regions within the explant. In micropropagation of bulbous crops, bulblets should be produced because of, among others, easy handling and acclimatization. For optimal performance after planting in soil, preparatory treatments are required in particular a dormancy breaking treatment. A phase-change from juvenile to adult and protective pretreatments are also profitable. It is concluded that when major problems like that of inferior growth have been solved, commercial micropropagation of bulbous crops will experience a second heyday.

Małgorzata Podwyszyńska (Poland) The Mechanisms of *in Vitro* Storage Organ Formation in Ornamental Geophytes (pp 9-23)

ABSTRACT

Invited Review: In most ornamental geophytes, the rate of conventional vegetative propagation is low. Ten or even twenty years can pass until the commercial release of a new genotype. Therefore, numerous micropropagation methods have been developed to enhance the propagation rate, but also to obtain healthy elite stock material, speed up the breeding process, provide new genotypes on the market and restore endangered geophytes. In a number of geophyte species, formation of bulbs, corms or tubers is an essential step in the micropropagation process because only a storage organ shows high rooting ability and good field performance. In nature, abundance of photosynthesis-derived sugars together with some inducing environmental factors (low temperature, short or long day) trigger a sequence of biochemical, physiological, and finally, morphological events leading to storage organ formation. In *in vitro* conditions, however, due to insufficient light intensity, sugars have to be provided exogenously. *In vivo*, sugars exist as the multifunctional internal factors, while *in vitro*, they act both as internal and external factors. This can lead to certain disturbances in the course of the *in vitro* tuberization process. Therefore, success in storage organ formation often requires administration of the proper growth regulators whose endogenous production is insufficient (cytokinins, abscisic acid, jasmonates, auxins and polyamines), and sometimes other specific compounds such as inhibitors of gibberellin biosynthesis. This review focuses on the recent findings about the tuberization process *in vitro* of ornamental geophytes in relation to the newest knowledge concerning tuber formation in potato as model plant. Also some aspects of storage organ formation *in vitro*, the stages and the factors regulating this process on the morphological, physiological and biochemical levels are discussed in relation to storage organ formation occurring in nature (*in vivo*).

Avner Cohen (Israel), Frans A. Krens (The Netherlands) Genetic Transformation in the Breeding of Flower Bulbs (pp 24-34)

ABSTRACT

Invited Review: Ornamental geophytes are used for the production of cut-flowers, potted flowering plants or in gardening (collectively known as flower bulbs). Most flower-bulb cultivars have been produced by cross hybridization and mutation breeding and are propagated vegetatively. Biotechnological techniques have been used to breed and propagate these plants. Plant breeders use *in vitro* techniques, such as cut-style in vitro fertilization, embryo rescue, ovary-slice culture and ovule culture, to overcome pre- or post fertilization compatibility barriers and generate interspecific hybrids. Recently, biotechnological tools such as molecular markers and genetic engineering have also been introduced. Genetic transformation may be defined as the utilization of isolated recombinant DNA based technology to aid the effective incorporation of a limited number of valuable traits (that are not available in the original plant genome or in closely related species) into improved cultivars lacking such traits. Transformation techniques supplement the other methods available to plant breeders and are especially valuable for clonally propagated crops, such as flower bulbs. Flower bulbs have been transformed using both *Agrobacterium*-mediated and microprojectile-acceleration methods. In both systems, the success of the transformation depends upon the successful

assembly of several key components and the calibration of the entire system. One component is the availability of a genetic construct carrying target genes under the control of appropriate promoters. A second component is the target organ or tissue, which must be competent for genetic transformation. That is, it must be capable of accepting the foreign DNA, into the genome of its own cells, expressing the genes and maintaining the ability to regenerate into plants. Introduced genes in many agricultural crops include those that confer resistance to biotic or abiotic stresses, as well as genes that alter plant phenotype (e.g., flower color). Although transformation systems for many flower-bulb crops are available, few attempts to produce genetically engineered flower bulbs with commercially valuable traits have been successful and, to date, none have resulted in a registered cultivar. In order to be commercially viable, any genetically engineered flower bulb cultivar would contain mostly proprietary technology covered by freedom-to-operate agreements. Marker-free technology is needed to ease the risk-assessment process and to address public concerns.

William B. Miller (USA) Current Status of Growth Regulator Usage in Flower Bulb Forcing in North America (pp 35-44)

ABSTRACT

Invited Review: North American greenhouse companies are mainly focused on potted crops, and due to lower geographical concentration, individual greenhouse firms, tend to have a very diversified product offering. As such, individual crops are often grown under less than optimum conditions. Plant growth regulators offer a set of tools that allow growers to better tailor bulb crops to containers, and the more liberal market and regulatory environment in North America continues to allow a high degree of specialization of PGR use. It is hoped the specific PGR and crop information presented herein and available online will stimulate additional research and interest in these products and crop uses worldwide.

Barbara Ruffoni, Marco Savona, Sara Barberini (Italy) Biotechnological Support for the Development of New *Gladiolus* Hybrids (pp 45-52)

ABSTRACT

Invited Review: The genus *Gladiolus* L. (*Iridaceae*) includes important ornamental species and hybrids which are successfully treated on the market since the last century. Recently breeders have selected new genotypes with the aim to increase the production in winter and late spring especially in countries bordering the Mediterranean Sea. In order to speed up the volume of the new hybrids and to produce virus-free stocks of mother plants, it is possible for *Gladiolus* to apply biotechnological tools of *in vitro* propagation through liquid culture or simple bioreactors such as temporary immersion system to reduce production costs and to enhance multiplication rate and cormel quality. *Gladiolus* micropropagation was reported first by Ziv *et al.* in 1970 and subsequently by several other authors exploring the performances of different species and varieties. Bulbs and corms of several species are commercially propagated in liquid culture in semi-automatic systems as temporary immersion; in *Gladiolus*, Ruffoni *et al*. presented in 2008 data about high efficiency micropropagation using temporary immersion compared with the culture on agar-solidified medium suggesting an efficient semi automatized protocol. The present paper takes into consideration the different ways for *in vitro* culture initiation and the efficiency of the meristem excision for the establishment of pathogen-free cultures including data coming from direct experiences and bibliography search. Moreover it will compare the different growth strategies (solid vs liquid micropropagation) evaluating finally the performances of the temporary immersion system.

Zhanao Deng (USA) Caladium Genetics and Breeding: Recent Advances (pp 53-61)

ABSTRACT

Invited Review: Caladiums are important ornamental aroids; they are valued for their colourful and variably-shaped leaves. Numerous advances have been made in recent decades in caladium breeding and genetic studies. Techniques have been developed to increase flower production, store pollen, and maintain seed viability. Sources of genetic resistance have been identified for important diseases and pests (such as Fusarium tuber rot, Pythium root rot, bacterial blight, and root-knot nematodes) and abiotic stress factors including chilling injury. Mode of inheritance for important foliar traits has been elucidated through analysis of trait segregation in progeny populations. Caladiums have evolved three alleles at one locus that control colour of leaf main veins (red, white or green) and two co-dominant alleles at an independent locus that determine leaf shapes (fancy, lance, or strap). Gene loci for leaf spotting and blotching are both simply inherited but tightly linked to green veins. *In vitro* culture and plant regeneration were successful with several types of tissues/organs through somatic embryogenesis and/or organogenesis. Shoot-tip culture has been used to eliminate viral and fungal pathogens and invigorate planting stock; protoplasts isolated from leaf callus regenerated into whole plants; foreign genes from maize or humans have been introduced

into caladium through *Agrobacterium* co-cultivation. Molecular markers, including highly specific and informative SSRs, have been developed and applied to caladium to distinguish cultivars, assess genetic diversity, and analyze genetic relationships. The availability of these improved techniques, sources of desirable traits, and cellular or molecular tools will be very valuable for enhancing caladium breeding efficiency, achieving specific breeding objectives, and developing valuable new cultivars.

Rina Kamenetsky (Israel), John Dole (USA) Herbaceous Peony (*Paeonia*): Genetics, Physiology and Cut Flower Production 62-77)

ABSTRACT

Invited Review: Peony (*Paeonia* spp.) is one of the most popular garden plants in temperate regions. They were introduced into cultivation in China hundreds of years ago, and have since been spread widely to many countries. According to morphological traits and life form, the genus is divided into tree and herbaceous peonies. Numerous cultivars of herbaceous peonies have been developed to satisfy demand for colors, fragrance, flowering time, and disease resistance. In the last two decades, the popularity of peonies as cut flowers has resurged, and has resulted in additional requirement for new research, production methods and postharvest technology. Today, more than 25 countries produce cut peony flowers, with the primary markets being in Europe and the USA. Despite the popularity of herbaceous peonies, their production and use are restricted due to a lack of reliable systems for mass propagation, a long juvenile period, complicated flowering physiology and ineffective postharvest handling procedures. In this review, highlights of the recent scientific research in herbaceous peony are presented, along with up-to-date information on peony propagation, postharvest handling and cut flower marketing.

Marija Petrić, Angelina Subotić, Milana Trifunović, Slađana Jevremović (Serbia) Morphogenesis *in Vitro* of *Fritillaria* spp. (pp 78-89)

ABSTRACT

Invited Review: The genus *Fritillaria* includes 100 species of bulbous plants and is found throughout the temperate region of the Northern Hemisphere. *Fritillaria* species are often used as ornamental plants, but various species have also been used in traditional Chinese, Japanese and Turkish medicine. Many species from the genus *Fritillaria* are endangered, rarely found in the wild and protected by law. Micropropagation techniques have great importance for germplasm conservation and commercial multiplication of fritillaries. Successful propagation methods have been developed for the following *Fritillaria* species: *F. anhuiensis*, *F. alburyana*, *F. camtschatcensis*, *F. cirrhosa*, *F. hupehensis*, *F. imperialis*, *F. meleagris*, *F. pallidiflora*, *F. przewalskii*, *F. roylei Hook*, *F. sinica*, *F. sichuanica*, *F. thunbergii*, *F. taipaiensis*, *F. unibracteata*, *F. ussuriensis* and *F. whitallii.* This paper summarises the various techniques of *in vitro* morphogenesis induction and rapid propagation of fritillaries, as well as successful acclimatisation. The most potent explant types for the induction of morphogenesis *in vitro* are bulbs, bulb scales, inflorescence parts and immature or mature zygotic embryos. Whole plant regeneration of fritillaries has been achieved by bulblet production, as well as by direct or indirect somatic embryogenesis. The influence of different media compositions, hormone concentrations and temperature requirements for the induction of morphogenesis and overcoming of dormancy are discussed. This review also describes major secondary metabolites in *Fritillaria* (alkaloids and non-alkaloid constituents), their nature and perspective for production by methods of *in vitro* culture which can be used in the pharmaceutical industry.

Agnieszka Marasek-Ciolakowska (The Netherlands/Poland), M.S. Ramanna, Paul Arens, Jaap M. Van Tuyl (The Netherlands) Breeding and Cytogenetics in the Genus *Tulipa* (pp 90-97)

ABSTRACT

Invited Review: Tulip (*Tulipa*) is one of the most important ornamental bulbous plants, which has been cultivated for cut flower, potted plant, garden plant and for landscaping. Species from the different sections display complementary agronomic characteristics and breeding techniques are used to combine desired features. The main goals of modern tulip breeding are the introgression of resistance against Tulip Breaking Virus (TBV), *Botrytis tulipae* and *Fusarium oxysporum* (bulb-rot), and also characteristics such as a short forcing period, good flower longevity and new flower colours and flower shapes into the commercial assortment of *T. gesneriana*. *T. gesneriana* has been crossed successfully with only 12 out of the approximately 55 tulip species by using conventional breeding methods. Many successful crosses have been made between *T. gesneriana* cultivars and TBV resistant *T. fosteriana* cultivars resulting in highly resistant Darwin hybrids tulips. The majority of tulip cultivars are diploid ($2n = 2x = 24$) however, there have been many attempts to obtain polyploid tulips. The production of tetraploids was described in the late sixties when young ovaries were treated, under pressure, with laughing gas (N_2O). In breeding of polyploid tulip laughing gas has also been used to induce 2*n* gametes. Several new tetraploids were also obtained by making crosses between tetraploid lines. Polyploids have been derived from interploidy crosses between diploid, triploid, and tetraploid cultivars. Several other polyploids have resulted from 2*n* gametes, spontaneously produced by diploid F1 hybrids. Molecular cytogenetic tools such as FISH and GISH permitted detailed studies of genome composition and chromosome recombination in the progenies of interspecific hybrids. In this context, tulip breeding and the use of cytogenetic techniques for genome analysis of hybrids are discussed.

Riana Kleynhans, Paula Spies, Johan J. Spies (South Africa) Cytogenetic and Phylogenetic Review of the Genus *Lachenalia* (pp 98-115)

ABSTRACT

Invited Review: The genus *Lachenalia* (family Asparagaceae), endemic to southern Africa, is a horticultural diverse genus, with many species featuring in the red data list of southern Africa. The extensive morphological variation within some species complicates species delimitation and has led to taxonomic confusion. The genus is utilised in a breeding programme where cytogenetic and phylogenetic information is important for the development of breeding strategies. Chromosome numbers of 89 species have been recorded in literature, with $2n$ = 10 to 56 and n = 5 to 28. B-chromosomes have been described in some species. Basic chromosome numbers include x = 5, 6, 7, 8, 9, (probably 10), 11, (probably 12), 13 and (probably 15). Polyploidy was reported in 19 taxa (23%), and is most common in the x = 7 group. Molecular cytogenetic studies using 5S rDNA, 18S rDNA probes and DAPI staining, as well as molecular systematic studies using *trnL-F* and *ITS1-2* were used to assess the phylogeny of the genus. All these studies indicated that species with the same basic chromosome number are closely related. The one deviation is that it appears as if there are two separate groups within the x = 7 group. The cytogenetic and molecular studies are further supported by breeding studies, where improved results are generally obtained from crosses within a phylogenetic group or between closely related groups. This review of the literature reveals how different studies obtain similar results regarding the phylogenetic relationships within the genus and how these results can be utilized to improve breeding strategies. It also accentuates that further multidisciplinary studies are needed to solve the evolutionary history of the complex genus *Lachenalia*.

M. S. Ramanna (The Netherlands), Agnieszka Marasek-Ciolakowska (Poland), Songlin Xie (The Netherlands), Nadeem Khan (Sweden), Paul Arens, Jaap M. Van Tuyl (The Netherlands) The Significance of Polyploidy for Bulbous Ornamentals: A Molecular Cytogenetic Assessment (pp 116-121)

ABSTRACT

Invited Mini-Review: Most of the bulbous crops, viz., *Crocus*, *Narcissus*, *Tulipa*, *Alstroemeria* and *Lilium* that are commercially important, share certain common characteristics. The present day cultivars are all derived from hybrids between distantly related species, and in almost all cases spontaneous polyploidization has played a prominent role and there is a tendency to replace diploids by polyploid cultivars. Molecular cytogenetic techniques such as genomic in situ hybridization (GISH) and fluorescence in situ hybridization (FISH), along with other techniques, have greatly facilitated our understanding of the modes of origins of polyploids. Because the bulbous crops generally have large chromosomes, the parental genomes, individual chromosomes, as well as intergenomic recombinant chromosomes, can be accurately identified in the interspecific hybrids and their backcross progenies. This enables an assessment of the potential genetic variation that might occur in the progenies as well as the extent of introgression. Although the superiority of polyploids as compared to their diploid parents is beyond doubt, the actual explanation for their superiority is still elusive. Of the several explanations, chromosome dosage, optimal amounts of 4C DNA values of the complements, heterozygosity and favourable gene interactions transmitted by the $2n$ gametes to polyploid progenies are some of the factors that might be considered at present. Undoubtedly, more studies on the bulbous ornamental crops using molecular techniques might be rewarding.

Rodrigo Barba-Gonzalez, José Manuel Rodríguez-Domínguez, Ma. Claudia Castañeda-Saucedo, Aaron Rodríguez (Mexico), Jaap M. Van Tuyl (The Netherlands), Ernesto Tapia-Campos (Mexico) Mexican Geophytes I. The Genus *Polianthes* (pp 122-128)

ABSTRACT

Invited Mini-Review: Members of the genus *Polianthes* L. are bulbous ornamentals in the Agavaceae, includes 15 species three varieties and a two cultivars native to Mexico. *Polianthes tuberosa* L. (Tuberose) is the only species cultivated as an

ornamental cut flower in tropical and subtropical areas. The cultivation of tuberose occupies a prime position in the floriculture industry in countries such as Mexico, China, India, New Zealand and Taiwan. The Flower colour of all known cultivars of *P. tuberosa* is white; however, attempts have been made to introduce colors from related species. Besides its use as an ornamental, it is cultivated for use in manufacturing: as a source of fragrant essences in perfumery, to extract polysaccharides and glycosides; in addition *P. geminiflora* (Llave & Lexarza) Rose is utilized as a source of saponins for soap. The main diseases in this crop are caused by virus and it is affected by a coleoptera (*Scyphophorus acupunctatus*) whose larva feeds on the bulbs. In this review we will cover the uses, distribution, species, of the genus and the current state of tuberose breeding as a further reference for tuberose breeding programmes.

Ernesto Tapia-Campos, Jose Manuel Rodriguez-Dominguez, María de los Milagros Revuelta-Arreola (Mexico), Jaap M. Van Tuyl (The Netherlands), Rodrigo Barba-Gonzalez (Mexico) Mexican Geophytes II. The Genera *Hymenocallis*, *Sprekelia* and *Zephyranthes* (pp 129-139)

ABSTRACT

Invited Review: Among hundreds of bulbous ornamental plants native to tropical and subtropical America, different genera from the Amaryllidaceae family such as *Hymenocallis*, *Sprekelia and Zephyranthes* present an enormous potential as ornamental crops. The genus *Hymenocallis* comprises over 60 species distributed from the north of Brazil to the south east of the United States; many of them are endemic to Mexico. The flowers are star shaped and white. The different species grow in a wide and contrasting diversity of habitats, near rivers and streams, on occasion completely submerged under water and sometimes in dry areas. The genus presents a complicated phylogeny, where in some cases it is difficult to distinguish species from hybrids. The genus *Sprekelia* is a monotypic genus native to Mexico; *Sprekelia formosissima* is cultivated as an ornamental pot plant in many countries. It presents solitary red flowers, their stems reaches up to 80 to 90 cm. The genus *Zephyranthes* comprises over 70 species distributed in tropical and subtropical America; different species are cultivated all over the world as an ornamental crop. The different species have beautiful flowers from white to yellow with various tints from lemon to sulphur and pink. In this review we will cover taxonomical, chromosomal and phenological aspects of these genera, with the aim of providing a reference of useful traits for breeding programs.

Guadalupe Palomino, Javier Martínez, Rodrigo Barba-González, Ignacio Méndez, Benjamín Rodríguez-Garay (Mexico) Mexican Geophytes III. Cytotypes and Meiotic Behavior in Mexican Populations of Species of *Echeandia* (Anthericaceae) (pp 140-152)

ABSTRACT

Original Research Paper: *Echeandia* Ortega includes about 85 perennial herbaceous species. The subgenus *Echeandia* is distributed from USA, to Argentina and Chile. Mexico is considered to be the genus center of origin and diversity. *Echeandia* is considered as a monobasic genus with x = 8. Diploid plants (2n = 16, n = 8, x = 8) have been reported for 35 species of *Echeandia*. Chromosome numbers for 22 polyploid species for the genus have been the reported (4x, 5x, 6x, 8x, 10x and 11x-4). These reports detail karyotype, meiotic chromosome behavior and, pollen fertility of 23 populations of eight species: *Echeandia echeandioides*, *E. hintonii*, *E. mexicana*, *E. montalbanensis*, *E. nana*, *E. pubescens*, *E. reflexa* and *E. tenuis.* All species of *Echeandia* were diploid (2n = 16, n = 8, x = 8). Each species had a distinctive karyotype that varied among populations of the same species. Spontaneous heterozygotic exchanges in species and cytotypes of *Echeandia* have a common behavior pattern in karyotype variation. The exchanges were observed in heteromorphic pairs of chromosomes with satellites, and, in metacentric, submetacentric and subtelocentric chromosomes. The origin of these rearrangements was evident in heteromorphic bivalents (IIs) and quadrivalents (IVs) observed in MI. Additional evidence for translocations and chromatid exchange comes from the low level of meiotic irregularities observed in anaphase I (AI), including U-type bridges, side arm bridges and lagging chromosomes. Populations of *E. nana*, display only two cytotypes. Based on these results, the translocations and chromatid exchange follow a behavior pattern common to species and cytotypes of *Echeandia*, and these chromosome aberrations have played a major role in evolution of the genus, providing a larger potential of colonization and distribution in new habitats.

Volume 6, Special Issue 2, 2012

ISSN 1749-0294

ISBN 978-4-907060-04-6

FLORICULTURE & ORNAMENTAL BIOTECHNOLOGY

Bulbous Ornamentals II

Guest Editors: Jaap M. Van Tuyl, Paul Arens

GLOBAL SCIENCE BOOKS

Floriculture and Ornamental Biotechnology

Abbreviation: Floriculture Ornamental Biotech. (FOB)
Print: ISSN 1749-0294
Frequency and Peer status: Biannual, Peer reviewed

Scope and target readership: *Floriculture and Ornamental Biotechnology* is dedicated to significant advances in ornamental plant science and biotechnology.

Floriculture and Ornamental Biotechnology aims to examine:
1) Breeding biotechnology (genetic modification, somatic hybridisation and embryo rescue);
2) *In vitro* propagation (micropropagation, somatic embryogenesis, tissue culture);
3) Mycorrhizal symbioses (and effects on plant physiology, productivity, reproduction and disease resistance);
4) Physiology, molecular biology, structural botany (integrated, pure and applied);
5) Phytopathology;
6) Post-harvest technology as applies to cut flowers and foliage (deterioration, preservation, shipping, and marketing);
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For publication in *Floriculture and Ornamental Biotechnology* the research must provide a highly significant new contribution to our understanding of floricultural or ornamental plants and must generally be supported by a combination of either physiological, biochemical, genetic or molecular analyses. All areas of study are welcome and the experimental approaches used can be wide-ranging.

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Guest Editors

Prof. Jaap M. van Tuyl, Dr. Paul Arens

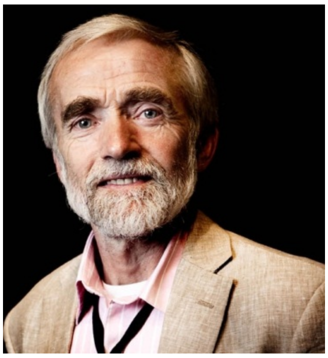
Wageningen University and Research Centre, The Netherlands

Cover photos: Top row (from left to right): *Lilium davidii* (Wu *et al*., pp 28-38). Crossing product of *Ranunculus asiaticus* 'Krisma' as maternal plant and *Anemone coronaria* 'Mistral Wine' as paternal plant (Dhooghe *et al*., pp 104-107). *Nomocharis aperta* (Wu *et al*., pp 28-38). Interspecific hybrids AC (Asiatic hybrid × *L. candidum*) (Proscevičius *et al*., pp 89-93). Bottom row (from left to right): Chlorotic ringspot symptoms associated with a potyvirus in oxalis (*Oxalis regnellii*) (Miller *et al*., pp 99-103). *Lilium bulbiferum* subsp. *croceum*. (Bessler 1613) Courtesy of Teylers Museum Haarlem. (Fred Bos, pp 53-62). Pollen tube entry into the micropylar region of *Alstroemeria aurea* ×*Bomarea coccinea*, observed by fluorescence microscopy (Kashihara *et al*., pp 146-149).

Printed in Japan on acid-free paper.
Published: December, 2012.

The Guest Editors

Prof. Jaap M. van Tuyl



Bulbous ornamental plants also called flower bulbs or ornamental geophytes are an important group of ornamental plants which play worldwide an important role in the flower industry. Although native in all continents, are Asia and South Africa the most important gene centres. The main crops are tulip, lily and narcissus which are as bulb crops predominantly grown and traded in The Netherlands. Bulbs are exported worldwide and grown as cut flower, pot plant and garden plant. The breeding history of ornamental bulbous crops goes back centuries ago but well described for especially for tulip only 3-4 centuries. Nowadays for the main crops consist an broad assortment of thousands of cultivars mainly originated from interspecific hybridization.

After a career of more than 35 years in breeding research of mainly lily and tulip at Wageningen University and Research Centre, it was a pleasure for me to invite leading scientists from all over the world to write contributions on bulbous crops in areas like taxonomy, plant breeding, tissue culture and physiology. This special issue contains 38 reviews and original research papers written by 90 authors from 18 different countries. With many of the authors I had a long cooperation as student, guest researcher and colleague. I am proud on my former PhD-students which graduated on bulbous subjects in my flower bulb group. They returned to their country of origin and continued research in this field. Most of the authors I know personally quite well from international meetings from ISHS (International Symposia on Flower Bulbs and herbaceous perennials, International Symposia on the genus Lilium) and EUCARPIA (International Symposia on Breeding of Ornamentals) and these contacts go back more than 30 years. I am very happy that so many of them were able to contribute to this book. The many reviews represent the current state of knowledge in these areas.

The taxonomy and phylogeny of *Lilium* is reviewed by Pelkonen and Pirttilä**,** while Wu *et al.* focussed on the *Lilium* species native to China. Okubo and co-workers describe "New insights on *Lilium brownii"*, while Hiramatsu *et al.* report on "The evolution of early flowering ability in *Lilium formosanum"* from its progenitor *L. longiflorum.* Eisuke Matsuo worked as guest researcher in 1983 with me, he is retired several years ago and jumped into name of *Lilium longiflorum* **"**Historical survey of Easter Lily name in association with *Lilium longiflorum."* Fred Bos, a retired vegetation specialist, discovered wild habitats of *Lilium bulbiferum* spp *croceum*, the only Lilium species native in The Netherlands. This orange lily was discovered around 1850 growing wild in rye fields in the northern part of the Netherlands and North-western Germany. He describes interesting cultural aspects of this lily and shows us that it can be found in 17th century Dutch and Flemish flower paintings as is an important symbol.

Kleynhans *et al*. contributes with the "Cytogenetic and Phylogenetic Review of the Genus *Lachenalia".* Agnieszka Marasek-Ciolakowska was during 5 years a guest researcher in my group and specialized in tulip. She evaluated the **"**Breeding and cytogenetics in the genus *Tulipa".* Nadeem Khan was my PhD-student from 2006-2009 and contributes with *"*A molecular cytogenetic analysis of introgression in backcross progenies of intersectional *Lilium* hybrids". Ramanna, although retired, was for more than 10 years our resource with his in depth cytogenetic expertise. He contributed to many papers and in this issue he presents "The significance of polyploidy for bulbous ornamentals: A molecular cytogenetic assessment" in which he compares *Crocus*, *Narcissus*, *Tulipa*, *Alstroemeria* and *Lilium.* Also Songlin Xie, another former PhD-student is co-author in this paper. Under supervision of Ki-Byung Lim (also graduated in our group) Hwang *et al.* reports on "Genome analysis of *Lilium tigrinum* by chromosome microdissection and molecular cytogenetic techniques. Two papers of Neil Anderson and co-workers focus on the discovery of novel traits in seed-propagated *Lilium xformolongi*. Proscevičius *et al*. show promising results with the mixed pollen method to overcome interspecific barriers in lilies:

"Application of mixed incongruous pollen for interspecific crosses of lilies". Okazaki *et al*. show the important implications of mitotic and meiotic polyploidization by using laughing gas in lily and tulip: **"**Application of nitrous oxide gas as a polyploidizing agent in tulip and lily breeding." Takejiro Takamura was in 1999 guest researcher in our research group and specialist in genetics of *Cyclamen.* He shows results of DNA-content differences in Cyclamen: "Specific differences in nuclear DNA content in the Genus *Cyclamen."* Emmy Dhooge *et al*. investigated intergeneric hybridisation between *Anemone and Ranunculus* and present this work entitled "Cytological and Molecular Characterization of Intertribal Hybrids Between the Geophytes *Anemone coronaria* L. and *Ranunculus asiaticus* L. (Ranunculaceae)". Kashihara *et al.* examined the possibilities of intergeneric hybridization between *Alstroemeria* and *Bomarae*: "Towards intergeneric hybridization between *Alstroemeria* L. and *Bomarea* Mirb". Recent advances of *Caladium* breeding and genetics is reviewed by Zhanao Deng. The genetics, physiology and cut flower production of the herbaceous peony (*Paeonia)* is reviewed by Rina Kamenetsky and John Dole. A review on Genetic transformation in the breeding of flower bulbs is written by Avner Cohen and Frans Krens. Former PhD-student Rodrigo Barba and co-workers give us an insight in some native genera of Mexico. They contributed three chapters on Mexican geophytes genera: *Polianthes, Hymenocallis, Sprekelia, Zephyranthes* and *Echeandia*.

In the field of tissue culture Geert-Jan de Klerk reviewed the technology and present state of micropropagation of bulbous crops. Ruffoni *et al*. describe their results using advanced techniques in micropropagation of *Gladiolus* "Biotechnological support for the development of new *Gladiolus h*ybrids". Petrić et al. are reporting the successful micropropagation of a number of *Fritillaria* species "Morphogenesis *in Vitro* of *Fritillaria* spp." Małgorzata Podwyszyńska is an expert in the field of micropropagation of bulbous crops. For this issue she contributed with "The mechanisms of *in vitro* storage organ formation in ornamental geophytes." Dariusz Sochacki and Małgorzata Podwyszyńska investigated the use of *in vitro* chemotherapy for obtaining virus-free stocks "Virus eradication in *Narcissus* and *Tulipa* by chemotherapy. Faouzi Haouala and Emna Chaïeb present their work in *Gladiolus* **"**Effects of Explant Position and Polarity on Callus Induction and Shoot Regeneration of *Gladiolus* (*Gladiolus hybridus* Hort.)". Cathy Kamo and Bong Hee Han optimized the callus regeneration protocol in lily: **"**Optimized growth and plant regeneration for callus of *Lilium longiflorum* 'Nellie White'." Paladines *et al.* present their results on microspore culture and haploid production in *Anemone coronaria:* "Prospects of isolated microspore culture for haploid production in *Anemone coronaria* L."

Saniewski *et al.* studied the role of auxin in the stem elongation of dark-grown tulips **"**Evidence for a role of auxin in the stem elongation of dark-grown tulips". William B. Miller of Cornell University shows the importance of growth regulators for the pot culture in the US: "Current status of growth regulator usage in flower bulb forcing in North America." Chad Miller *et al.* describe the possible cause of chlorotic symtoms in *Oxalis*: "Iron deficiency may result in interveinal chlorosis of Shamrock plant (*Oxalis regnellii*)". Juodkaitė *et al.* of the Botanical Garden of Vilnius studied in 299 tulip cultivars the vegetative reproduction capacity: "Assessment of the Vegetative Reproduction Potential of Tulips (*Tulipa* L.)". Peter Knippels investigated some specific propagation techniques like scoring, chipping, scaling and leaf cuttings in *Eucomis* and *Hymenocallis*: "Advanced *in vivo* propagation techniques for specialty bulbs."

The last four years my life's work with ornamental plants is taken over step by step by Paul Arens and I want to thank him for the pleasant cooperation in our research as well as in the editing of this book. This project was initiated four years ago by Jaime A. Teixeira da Silva, Editor-in-Chief of Global Science Books, who invited me as guest editor for these special issues of Floriculture and Ornamental Biotechnology. I am grateful to Jaime and all contributors for their willingness to share their knowledge.

December, 2012

Dr. Paul Arens

From the moment I got involved into the breeding research of bulbs, I have been impressed with the enormous variety of shapes, appearances and colours of these ornamentals. In many crops interspecific hybrids have been used in breeding to create the stunning wealth of cultivars as we know today whereas in other crops already a huge variation was accomplished within a single species. Nevertheless, for both situations still many new possibilities to extend breeding exist and those can add to further extend this large genetic variation. A number of the chapters go into more recently developed breeding possibilities for specific crops whereas others give a nice overview of more general interest.

As breeding of most bulbous crops requires a considerable amount of patience due to the relative long life cycle and slow vegetative reproduction, breeders and researchers in these crops are not only skilled professionals but are also very passionate for bulb flowers from a personal view. This makes the breeding research in bulbous ornamentals besides very interesting also very enjoyable. During the preparation of this book Jaap van Tuyl and I have been working with many researchers all over the world who made contributions to the book and this has been a very stimulating experience for me. Therefore I would like to thank all contributors, Jaime A. Teixeira da Silva, Editor-in-Chief of Global Science Books, who initiated the project to write this book and especially Jaap van Tuyl from who I have learned a lot in these past years and who has been the perfect person to work with as guest editors of this book. I hope this book will be as inspiring for people interested in bulbous crops as it was for me to work on this special issue of Floriculture and Ornamental Biotechnology.

December, 2012

Invited Foreword

Evert Jacobsen

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Floriculture and breeding of bulbous ornamentals is worldwide an important activity. The beauty of these ornamentals is shown in many exhibitions, parks and gardens. To enjoy human life is in this sense an important task of ornamental breeders. Breeding of this group of bulbous ornamentals, which are mostly vegetative propagated, always starts with domestication of certain phenotypes which afterwards were used as intraspecific breeding parent in order to combine such phenotypic traits with other ones. Important aspects of ornamental breeding are besides domestication, genetic variation, selection methods, biotechnological techniques and healthy vegetative propagation. In this special issue these different aspects are updated for the individual ornamentals like *Lily*, *Tulip*, *Gladiolus*, *Caladium*, *Peony*, *Fritillaria*, *Lachenalia*, *Crocus*, *Narcissus*, *Alstroemeria*, *Polianthus*, *Amaryllis*, *Sprekelia*, *Echeandia*, *Oxalis*, *Anemone* and *Ranunculus*. New developments have been described in (disease-free) micropropagation, seed propagated Lily, genetic transformation, introgression by interspecific hybridization and back crossing, improved techniques for wide crosses, further domestication of less developed bulbous species, (natural) polyploidisation, new interspecific hybrids and even intergeneric hybrids, cytogenetics using GISH and FISH, molecular markers but also chromosome micro-dissection, using "chemotherapy" for virus eradication and microspore culture for (doubled) haploid production.

It is clear that bulbous ornamental breeding is following modern genomic research like whole genome sequencing and many other molecular techniques at a distance. This is mainly due to the large genome size and the smaller amounts of money available in these ornamental crops. However, micro-chromosome dissection in lily is a surprising and interesting development. The use of genetic modification at variety level is still absent in this group of ornamentals despite the interesting possibilities for a number of specific traits.

I expect that this updating made by many different authors' will stimulate breeding research but also variety breeding in this group of ornamentals. So, that we can enjoy the emergence, not only of new improved varieties within existing species but also of an increasing number of new, artificial, species obtained by interspecific hybridization with interesting combined phenotypic traits.

December, 2012

SPECIAL ISSUE: CONTENTS

Veli-Pekka Pelkonen, Anna-Maria Pirttilä (Finland) Taxonomy and Phylogeny of the Genus *Lilium* (pp 1-8)

ABSTRACT

Invited Review: Lilies have a long history as ornamental plants. Today, there is an ever increasing variety of new lily cultivars due to the significant progress in the propagation and development of new methods in breeding. The domesticated native species have retained their place along with new hybrids in commercialized horticultural industry, and they have sustained their invaluable potential for the breeding of new cultivars for garden use as well as for greenhouse culture. Systematics has always played an important role in plant breeding, giving guidelines for hybridization, although biotechnology has introduced new solutions for many problems that were evolutionary obstacles especially in inter-specific crossings before. The genus *Lilium* has been a subject of variable suggestions for classification systems, and the process still continues. The currently accepted concept for the phylogenetic and taxonomic system for all species is based on geographical, structural and genetic information. In our review, we give an insight into the latest progress in revealing the taxonomical relationships within the genus. According to the existing GenBank sequence data, we have constructed a phylogenetic tree consisting of the main species and sections of the genus. Provided with species photos, the tree gives a brief overview of phylogeny- and morphology-based classifications, which are not always congruent. In the tree mainly all species grouped into sections defined within the genus, but *L. bulbiferum* and *L. dauricum* grouped equally with the species in *Sinomartagon* and not with each other. Even though these two species share many morphological features, the phylogenetic tree questions the existence of the section *Daurolirion* and potentially gives a blueprint for classification in the future.

Eisuke Matsuo (Japan) Historical Survey of Easter Lily Name in Association with *Lilium longiflorum* (pp 9-12)

ABSTRACT

Invited Mini-Review: Based on the English dictionaries from around 1900, the author traces back how *Lilium longiflorum* came to be referred to as the Easter lily. The term “Easter lily” was given to any lily-like flowering species that bloomed around the Easter day, being called Easter flowers. Among them, *L. candidum* became the most famous one known as “Easter lily”. The evidence suggests that when more easily forcible bulb production began in the Bermuda Islands, the superiority of *L. longiflorum* over *L. candidum* in forcing ability and price, might have led to an explosive promotion of the usage of *L. longiflorum* as the Easter flower instead of *L. candidum*. Thus, the name "Easter lily" came to be transferred from *L. candidum* to *L. longiflorum*.

Nadeem Khan (Sweden), Agnieszka Marasek-Ciolakowska (Poland/The Netherlands), Songlin Xie, Munikote S. Ramanna, Paul Arens, Jaap M. Van Tuyl (The Netherlands) A Molecular Cytogenetic Analysis of Introgression in Backcross Progenies of Intersectional *Lilium* Hybrids (pp 13-20)

ABSTRACT

Invited Review: The genus *Lilium* is comprised of about 100 species and has been divided into seven taxonomic sections. The abundance and diversity of species within the genus *Lilium* offers numerous and rewarding possibilities to lily breeders. Species within the same section can be crossed by conventional hybridization and this has led to different hybrid groups of great commercial importance such as Longiflorum, Asiatic and Oriental lilies. On the other hand, the divergence of species between various taxonomic sections causes considerable difficulties for intersectional crosses. Such difficulties include crossing incompatibility barriers, embryo abortion, sterility and reduced fertility in intersectional hybrids. For these reasons, various pollination techniques followed by *in vitro* embryo (sac) rescue and ovary culture, chromosome doubling, and 2*n* gametes are used frequently to obtain progeny between parents from different sections. Being the largest genome in the plant kingdom, lily is used as a model plant for cytogenetic analysis. The genome composition of the hybrids and backcross progenies were monitored through genomic *in situ* hybridization (GISH). The progress in molecular cytogenetic studies has been associated with the analysis of introgression of chromosomal segments in backcross progenies of various interspecific hybrids, contribution of individual genome in the resultant progenies, and the mechanism of 2*n* gamete formation. Based on the cytological analysis of progenies derived from the use of both haploid and 2*n* gametes, cytological maps of three different genomes have been constructed and the relevance of these analyses for introgression in *Lilium* is demonstrated.

Michikazu Hiramatsu, Jun-ichiro Masuda, Satomi Sakazono, Hiroshi Okubo (Japan) Evolution of Early Flowering Ability in *Lilium formosanum* from its Progenitor *L. longiflorum* (pp 21-27)

ABSTRACT

Invited Mini-Review: Attention to the evolutionary background of 'early flowering ability', i.e. extraordinary shorter period from seed germination to the first flowering in *Lilium formosanum*, could have great potential for reducing costs in commercial production of lilies. We clarified the phylogenic relationship and geographic divergence of the early flowering ability and its related life history traits using seedling populations from natural populations of *L. formosanum* and its progenitor species, *L. longiflorum.* Based on the data obtained, we propose a hypothesis for evolution of the early flowering ability in *L. formosanum*.

Xue Wei Wu, Li Hua Wang, Li Fang Wu, Su Ping Qu (China), Jeung Keun Suh (South Korea), Ji Hua Wang (China) Native Species of the Genus *Lilium* and the Closely Related *Nomocharis* in Yunnan, China (pp 28-38)

ABSTRACT

Invited Review: The lily is an important ornamental flower that has been cultivated for over 3,000 years, and with the development of breeding technologies, has been widely used in the garden, as potted flower, and for cut flower, floral designing. Lily belongs to the genus *Lilium* of family *Liliaceae*, which comprises over 180 species. China is the major center of *Lilium* distribution in the world; and almost one-third of the species found in China are distributed in Yunnan Province, China. 34 species of the genus *Lilium* are reported: *L. brownii*, *L. rownii* var. *viridulum*, *L. wenshanense*, *L. sulphureum*, *L. sargentiae*, *L. lophophorum*, *L. lophophorum* var. *linearifolium*, *L. nanum*, *L. souliei*, *L. henrici*, *L. henrici* var. *maculatum*, *L. bakerianum* var. *bakerianum*, *L. bakerianum* var. *aureum*, *L. bakerianum* var. *delavayi*, *L. bakerianum* var. *yunnanen*, *L. bakerianum* var. *rubrum*, *L. sempervivoideum*, *L. amoenum*, *L. pinifolium*, *L. nepalense*, *L. nepalense* var. *burmanicum*, *L. nepalense* var. *ochraceum*, *L. wardii*, *L. taliense*, *L. duchartrei*, *L. lijiangense*, *L. papilliferum*, *L. davidii*, *L. fargesii*, *L. stewartianum*, *L. habaense*, *L. lankongense*, *L. primulinum*, and *L. lancifolium*; Seven *Nomocharis: N. aperta*, *N. saluenensis*, *N. forrestii*, *N. basilissa*, *N. farreri*, *N. meleagrina*, and *N. pardanthina.* Sampling quantity, environmental survey of wild lily introduction, domestication note and crossing history have been previously investigated. Present and the proposed utilization classification characteristics of 41 native species are discussed in this paper.

Keiichi Okazaki, Shotarou Nukui, Hideaki Ootuka (Japan) Application of Nitrous Oxide Gas as a Polyploidizing Agent in Tulip and Lily Breeding (pp 39-43)

ABSTRACT

Invited Mini-Review: Nitrous oxide has been successfully applied to zygotes as a polyploidizing agent in various crops. More recently nitrous oxide treatments have been applied at male gamete formation resulting in production of diploid gametes in tulips and lilies. Additionally this treatment can be used to overcome pollen sterility of interspecific hybrids via polyploidization of archesporial cells in developing anthers. This paper provides a review of some of the literature and results of our experiments using nitrous oxide for chromosome doubling of gametes and zygotes, as well as pollen mother cells to overcome pollen sterility of the interspecific hybrids. These methods have important implications for lily and tulip breeding.

Hiroshi Okubo, Michikazu Hiramatsu, Jun-ichiro Masuda, Satomi Sakazono (Japan) New Insight into *Lilium brownii* var. *colchesteri* (pp 44-52)

ABSTRACT

Invited Review: *Lilium brownii* var. *colchesteri* has been widely cultivated for long time by its perfect flower shape with its colour arrangement and fragrance. However, it did not receive enough attention in recent lily research programs, and information on the history and culture is lacking. An overall research project on this species including breeding, flowering control, propagation, virus-free bulb production, flower pigment and scent along with the surveys of old literature and arts to clarify the introduction history of the species into Europe and Japan, has been conducted. The major results are: 1) *L. brownii* var. *colchesterii* was probably introduced in about 1600 from Korea to Fukuoka, 2) there was confusion of the species nomenclature of this species in Europe at the time of introduction, 3) all individuals of our present collection in Japan and Korea are clones, 4) F_1 hybrids of *L. formosanum* × *L. brownii* var. *colchesteri* obtained through cut-style pollination and ovary slice culture methods showed the early flowering traits of *L. formosanum*, but the flower shape and colour were similar to those of the pollen parent, 5) F_2 seedlings were obtained from self-pollination of F_1 through ovary-slice culture, 6) control of flowering was successful by temperature treatments, 7) an *in vitro* propagation procedure was established, 8) virus-free bulblets were obtained by a combination of meristem tip culture and chemotherapy, and 9) pigments that characterize the flower colour were identified.

Fred Bos (The Netherlands) *Lilium bulbiferum* L. Subsp. *croceum* (Chaix) Arcang., The Orange Lily, A Special Plant of Lowland NW Europe (pp 53-62)

ABSTRACT

Invited Review: The life history of the *Lilium bulbiferum* subsp. *croceum* has been described and analyzed since the orange lily was discovered around 1850 growing wild in rye fields in the northern part of the Netherlands and northwestern Germany. In this article the status of the orange lily has been discussed. The lily does not seem to be a garden escape as was thought in the 19th century, but should be treated as a native species. The orange lily belonged to the agricultural weed plant community, the *Scleranthо annui-Arnoseridetum*, of the so called “eternal” rye fields on the poor sandy soils in the old-morainic landscape. Due to changes in agricultural use both the orange lily and the other weeds became very rare. Recent research shows that the lily has mainly survived in gardens. In Govelin in Lower Saxony (Germany) the very last rich plant community of the *Scleranthо annui-Arnoseridetum* with thousands of *Lilium bulbiferum* subsp. *croceum* is still present. Because of its beauty the lily has and has had important cultural aspects. In flower symbolism, in medieval paintings and in 17th century Dutch and Flemish flower paintings it is an important symbol. For its orange colour several links to the Dutch royal family, the House of Oranje (= Orange) Nassau, were discovered.

Neil O. Anderson, Erika Berghauer, David Harris, Kari Johnson, Jenni Lonnroos, Mary Morey (USA) Discovery of Novel Traits in Seed-Propagated *Lilium*: Non-vernalization-requiring, Day-neutral, Reflowering, Frost-tolerant, Winter-hardy *L.* x*formolongi*. I. Characterization (pp 63-72)

ABSTRACT

Original Research Paper: *Lilium* are important floricultural crops worldwide. The objectives of this research were to examine *L.* x*formolongi* hybrids and a parental species (*L. longiflorum*) in photoperiods (Exp. 1) and environments (greenhouses, Exp. 1; field, Exp. 2) for flowering without vernalization, post-emergence photoperiod, potted plant/field performance, frost tolerance, and winter hardiness. Seed germination (4-15%) and yield potential (3.5-12.5%) varied between genotypes. In Exp. 1, short/long days had a significant effect on visible bud date only, but not on leaf unfolding rates, plant height, leaf number, or flowering dates; cultivar differences were highly significant. Cultivar × photoperiod interactions were nonsignificant except for flowering date (P = 0.04). ‘Nellie White’ (case-cooled bulbs) flowered in 213 d, while *L.* x*formolongi* cultivars took 247 d (‘Sakigake Raizan’) to 306 d (‘Raizan No. 3’) from sowing. Both VBD (h^2 = 0.93) and flowering date (h^2 = 0.91) were highly heritable (Exp. 1) and correlated. Regardless of photoperiod and environment, *L.* x*formolongi* flowered in <1 yr from sowing without cold. Leaf number (h^2 = 0.81, Exp. 1) and unfolding rates (h^2 = 0.93) were not as tightly linked in *L.* x*formolongi* as ‘Nellie White’. No seed-propagated hybrids (98-164 cm) were as short as ‘Nellie White’ (62 cm). ‘Augusta F_1’ had the highest flower bud counts (6.9 / plant). Shoot numbers ranged from n=1 (‘Nellie White’) to n = 3.8 (‘Sakigake Raizan’). *L.* x*formolongi* reflowered continuously in the field, but varied for frost-tolerance (25-75%; Exp. 2). Winter survival ranged from 0 to 87.5% in *L.* x*formolongi* over two years (Exp. 2). Flowering and reflowering of seed-propagated *L.* x*formolongi* in <1 yr. without vernalization, frost-tolerance, day neutrality, and winter hardiness are novel trait combinations for *Lilium*.

Neil O. Anderson (USA) Discovery of Novel Traits in Seed-Propagated *Lilium*: Non-vernalization-requiring, Day-neutral, Reflowering, Frost-tolerant, Winter-hardy *L.* x*formolongi*. II. Photoperiodism in Parents and Hybrids (pp 73-83)

ABSTRACT

Original Research Paper: The discovery of seed-propagated lily hybrids which flower in <1 year from sowing in any photoperiod presents unique opportunities for transforming lilies. Recent research documented such hybrids to possess additional traits such as reflowering capabilities, frost tolerance, and winter hardiness. Objectives of this research were to examine *Lilium* x*formolongi* hybrids, backcrosses, and parental species (*L. formosanum*, *L. longiflorum*) in photoperiods (SD/LD) to clarify flowering in more stringent environments (growth chambers, greenhouses) than previously. Case cooled (CC) and non-CC bulbs (*L. formosanum*, *L. longiflorum* ‘Nellie White’); non-vernalized *L. xformolongi* seed-propagated cultivars (5 cultivars, 9 seed lots), *L. longiflorum* ‘Snow Trumpet’, and *L. xformolongi* backcrosses (5 BC_1F_1) were tested. Seed germination ranged from 4% to 83.3%; yield potential was similar. Flowering *L. xformolongi* (growth chamber) did not differ from *L. longiflorum* ‘Nellie White’ for VBD. Cultivar x photoperiod interactions were not significant except flowering date (P = 0.04). ‘Nellie White’ (CC) flowered in 213 d, while *L. xformolongi* cultivars flowered in 247 d (‘Sakigake Raizan’) to 306 d (‘Raizan No. 3’) from sowing. Non-vernalized *L. formosanum, L. longiflorum* bulbs never flowered in either photoperiod or environment. Both VBD and flowering date were highly heritable and correlated. Regardless of photoperiod and environment, seed-propagated *L.*

xformolongi flowered in <1 year. One backcross was day-neutral for flowering. Two *L. xformolongi* BC_1F_1 and *L. longiflorum* 'Snow Trumpet' produced significantly less leaves than 'Nellie White'. Leaf number ($h^2 = 0.83$) was not as tightly linked in *L. xformolongi* as 'Nellie White'. Plant height in *L. formosanum* (CC bulbs, several backcross *L. xformolongi* hybrids) did not differ from 'Nellie White'. In contrast, only 'Sakigake Raizan' was taller than 'Nellie White' in growth chambers.

Yoon-Jung Hwang (South Korea), Paul Arens, Jaap M. Van Tuyl (The Netherlands), Tae-Jin Yang, Ki-Byung Lim (South Korea) Genome Analysis of *Lilium tigrinum* by Chromosome Microdissection and Molecular Cytogenetic Techniques (pp 84-88)

ABSTRACT

Original Research Paper: Chromosome microdissection and microcloning are powerful tools for plant genome research. Here we describe the isolation of chromosome #1 derived sequences from *L. tigrinum* with these techniques and their characterization. Detailed chromosome analysis was performed by FISH and then chromosome #1 was isolated from metaphase chromosomes of *L. tigrinum* by microbeam dissection. DOP-PCR and LA-PCR were used to amplify a DNA of chromosome #1 segments. PCR products from the microdissected chromosome were cloned into a plasmid vector to construct a chromosome #1 specific library and sequenced. BLAST-nr revealed that 28% of the sequences were matched with known genes and transposons, and the rest of 72% did not match with known sequences from NCBI database of plant taxa. The unknown sequences were putatively divided into five classes and we called them lily unique unknown repeats. FISH confirmation with some clones confirmed that the products from both methods were indeed amplified from the chromosome #1 of *L. tigrinum* genome. These results provide important information for not only the composition of the *Lilium* genome but also for detailed sequence information of huge genome sized plants.

Juozas Proscevičius, Vida Rančelienė, Violeta Kleizaitė (Lithuania) Application of Mixed Incongruous Pollen for Interspecific Crosses of Lilies (pp 89-93)

ABSTRACT

Original Research Paper: Creating interspecific hybrids between distantly related species is the first critical step in breeding of lilies. Different methods are used to overcome pre-fertilization and post-fertilization barriers in incongruous interspecific crosses of lilies. Compatibility between female pistil and pollen play an important role on fertility of crosses. Results are presented showing that pollen incongruous to female pistil can perform fertilization if they are used in mixtures with other incompatible pollen. To overcome pre-fertilization barriers cut-style pollination and pollination of stigma by mixed incompatible pollen were performed. In some cases recalcitrant crosses were more efficient when stigma was pollinated by mixed pollen of incongruous species than when cut-style was pollination by separated pollen of one incongruous male. However, cut-style pollination was efficient in cases when long style possessing females were pollinated by short style possessing male species. Though it is known that crosses of Asiatic hybrid (Asiatic hybrids (A) with *Lilium longiflorum* or Oriental hybrids (O) possible perform only in one direction where Asiatic hybrid participate as male, the pollen of *L. longiflorum* and O stimulated fertilization of Asiatic hybrid when they were added in mixtures with pollen of *L. candidum*, *L. henryi*, *L. monadelphum* or Trumpet and Aurelian hybrids (T). However, any stimulation of fertilization was observed when pollen of *L. longiflorum* and O were added in mixtures with pollen of *L. concolor* or *L. pumilum* to pollinate native pistil of Asiatic hybrid. Pollination of Asiatic hybrid by mixed pollen of incongruous species resulted progeny among which hybrids Asiatic hybrid x *L. regale* and Asiatic hybrid x *L. candidum* were screened by inheritance of molecular markers characteristic to male species.

Kathryn Kamo (USA), Bong Hee Han (South Korea) Optimized Growth and Plant Regeneration for Callus of *Lilium longiflorum* 'Nellie White' (pp 94-98)

ABSTRACT

Original Research Paper: Rates of growth and regeneration were compared for compact callus, friable callus, and suspension cells of *Lilium longiflorum* 'Nellie White' to determine the optimal culture conditions. The highest frequencies of embryogenic callus induction (60-90%) occurred from compact callus cultured on either picloram (0.5, 1, or 2 mg/L) or dicamba (2 mg/L). Fresh weight (FW) was higher for compact callus induced from bulb scales cultured on MS medium supplemented with picloram (0.5, 1, or 2 mg/L) compared to scales cultured on MS medium supplemented with dicamba (2, 4, or 8 mg/L). Compact callus cultured on picloram (0.5, 1, or 2 mg/L) or dicamba (2, 4, or 8 mg/L) grew slowly with a 1.2X increase in FW/month compared with suspension cells grown in 0.5 mg/L picloram that increased 1.7X in FW/month. Regeneration rates were similar (23-35 plantlets/g FW callus) for compact callus cultured on either dicamba (2 or 4 mg/L) or picloram (0.5 or 1 mg/L), but 3% of

the plantlets regenerated from dicamba were phenotypically abnormal while none were abnormal with picloram. Suspension cells showed a lower regeneration rate than compact callus with a maximum of only 12 plantlets regenerated from one g fresh weight suspensions cells grown in 0.5 mg/L picloram. A fast-growing, friable callus was induced and selected from compact callus cultured on MS medium with 2 mg/L dicamba and 9% sucrose but not from 3, 6, or 12% sucrose. Friable callus grew 5X faster than compact callus and formed numerous somatic embryo-like structures when cultured on MS medium with 1% activated charcoal, but only a few embryo-like structures germinated to form plants with roots.

Chad T. Miller, Benham Lockhart, Margery Daughtrey, William B. Miller (USA) Iron Deficiency May Result in Interveinal Chlorosis of Shamrock Plant (*Oxalis regnellii*) (pp 99-103)

ABSTRACT

Original Research Paper: *Oxalis regnellii* is a geophytic ornamental pot plant grown primarily for its clover-like leaves. During greenhouse production, the leaves often become chlorotic for unknown reasons, possibly including virus infection, iron (Fe) and/or manganese (Mn) deficiencies, and improper greenhouse forcing temperatures. We conducted a series of experiments to address these hypotheses. *Shamrock chlorotic ringspot virus* (SCRV) has been reported before in *Oxalis regnellii*. Oxalis plants exhibiting virus-like symptoms were analyzed and a potyvirus was detected, although this virus was not further confirmed to be SCRV. To confidently test other hypotheses, any suspected viral infected material was discarded. Plants grown at 13°C exhibited slowed growth and development; however, the incidence of leaf chlorosis did not increase compared with plants grown at warmer temperatures of 21/16°C (day/night); 22°C constant; or 22 to 16°C (plants were moved to 16°C when 50% of the plants were in first flower). To assess the ability to correct an iron (Fe) deficiency, a media drench of ferric ethylenediaminedi (*o*-hydroxyphenylacetic) acid (Fe-EDDHA) was applied to chlorotic, Fe-deficient oxalis plants and plants successfully re-greened within 5 days.

Emmy Dhooghe, Dirk Reheul, Marie-Christine Van Labeke (Belgium) Cytological and Molecular Characterization of Intertribal Hybrids Between the Geophytes *Anemone coronaria* L. and *Ranunculus asiaticus* L. (Ranunculaceae) (pp 104-107)

ABSTRACT

Original Research Paper: *Anemone coronaria* L. and *Ranunculus asiaticus* L. both belong to the Ranunculaceae, a large plant family with many ornamentals of horticultural importance. There are considerable differences between these species leaves, flower morphology and flower colour therefore intergeneric crosses between the species might result in new interesting hybrids. Crosses between *Anemone coronaria* L. and *Ranunculus asiaticus* L. were performed and the F1 progeny was examined. In this study, the F1 hybrid generation was investigated at morphological, molecular and cytogenetic levels. More than 85% of the F1 plants had very similar flowers to the maternal plants and although seeming to have a limited paternal contribution, AFLP analyses confirmed the partial hybrid character of the F1 plants. GISH experiments revealed that the F1 plants were mixoploids (plants composed of cells with different chromosome numbers) or showed many chromosome rearrangements.

Marian Saniewski (Poland), Hiroshi Okubo, Kensuke Miyamoto, Junichi Ueda (Japan) Evidence for a Role of Auxin in the Stem Elongation of Dark-grown Tulips (pp 108-113)

ABSTRACT

Original Research Paper: Shoot growth of fully cooled tulip bulbs cvs. 'Gudoshnik' and 'Apeldoorn' grown in continuous dark conditions was investigated in relation to the role of exogenously applied auxin. Continuous darkness caused much more stem elongation than natural light conditions in the greenhouse. In both cultivars, all internodes were longer in the dark than those in the light. Auxin (indole-3-acetic acid, IAA, applied in the place of a removed flower bud on a stem with no leaves) greatly stimulated the growth of all internodes in the dark in comparison to that in the light whereas almost no growth in all internodes was observed in the absence of exogenously applied auxin both in the dark and in the light. These results confirm that auxin is a major factor responsible for growth of all internodes in etiolated tulip stems. The hormonal control and its metabolic significance during the etiolation of tulips are discussed.

Dariusz Sochacki, Małgorzata Podwyszyńska (Poland) Virus Eradication in Narcissus and Tulip by Chemotherapy (pp 114-121)

ABSTRACT

Original Research Paper: The aim of the research was to obtain virus-free stock plant material of several cultivars and breeding clones of narcissus (*Narcissus* L.) and tulip (*Tulipa* L.) by *in vitro* culture using chemotherapy with ribavirin. Virus indexing by ELISA was done several times to detect the most important viruses infecting tulips and narcissus. Genotypes of both crops, totally or heavy infected by viruses, were subjected to chemotherapy in a few experiments. The results of virus eradication showed that none of the three concentrations of ribavirin (12.5, 25 and 50 mg l^{-1}) did result in death. However, shoot regeneration and growth on the medium with the highest concentration of ribavirin was significantly retarded, both for tulips and narcissus. Chemotherapy joined with further consecutive virus indexing and roguing the virus-suspected plantlets of tulip S2 and S3 enabled selection of virus-negative plants. The results of chemotherapy obtained for the new tulip cultivars (S2, S6 and S7) in the next experiment were very promising. The ribavirin treatment resulted in virus eradication from the newly forming shoots. In turn, in the old cultivars (E and F) and one breeding line (P7), whose shoots were totally infected, chemotherapy appeared to be ineffective. Virus eradication was unsuccessful for all treated plantlets of narcissus 'Lajkonik' infected by *Narcissus mosaic virus* and potyviruses in two experiments. Virus eradication for breeding clone 0.985T infected by *Narcissus latent virus* and potyviruses was successful in 27 among the 80 plantlets which started the experiment. The results leads to conclusion that the effect of ribavirin depends on the concentration of this antiviral agent, kind of virus and their concentration in plant tissue and on the genotype of plant.

Regina Juodkaitė, Angelė Meilutė Baliūnienė, Zenonas Jančys (Lithuania) Assessment of the Vegetative Reproduction Potential of Tulips (*Tulipa* L.) (pp 122-132)

ABSTRACT

Original Research Paper: The principal aim of this research was the assessment of the vegetative reproduction potential of different size tulip bulbs. Bulbs were arranged by size into 7 fractions. Vegetative reproduction capacity of different size tulip bulbs of 299 cultivars was calculated using a number of specific reproduction coefficients: total reproduction coefficient (TRC), generative bulb reproduction coefficient (GRC) and forcible bulb reproduction coefficient (FRC). Reproduction coefficients were calculated individually for each different bulb size class of the investigated tulip cultivars. TRC is a quantitative indicator specifying the mean number of all daughter bulbs per clone. GRC is a qualitative indicator specifying the mean number of bulbs per clone that is capable to blossom next year. FRC is a qualitative indicator specifying the mean number of forcible tulip bulbs per clone. By modulating the data on TRC, GRC and FRC of all cultivars of different size bulbs, indexed reproduction coefficient (IRC) was deduced. IRC indicates a comparative reproduction value. Empirical tulip cultivar dispersion analysis demonstrated that this coefficient most objectively reflects reproduction capacity of all bulbs of the studied tulip cultivars. Based on IRC, the investigated tulip cultivars were grouped into 5 classes of reproduction capacity. Most tulip cultivars were ascribed to 2nd–4th classes (correspondingly 24, 30 and 30%), whereas a small number of the studied cultivars were attributed to one of the outer classes 1st and 5th (8%).

Faouzi Haouala, Emna Chaïeb (Tunisia) Effects of Explant Position and Polarity on Callus Induction and Shoot Regeneration of *Gladiolus* (*Gladiolus hybridus* Hort.) (pp 133-139)

ABSTRACT

Original Research Paper: Different types of explants were used for callus induction in *Gladiolus* tissue culture of cultivars 'ChaCha' and 'Priscilla'. Different concentrations of 2,4-dichlorophenoxyacetic acid (2,4-D) or α-naphthaleneacetic acid (NAA) were tested. Explant polarity was studied for leaves by placing fragments of these organs horizontally and vertically on the culture medium. Also, explants were taken from the apical, middle and basal parts of leaves and petals. Apical buds, leaves and flower stalks showed excellent callus formation (100%). However, petals were characterized by a low callus formation ability (10%) while floral stems, bracts and floral spikes showed no callus formation. The rate of callus development was significantly higher for horizontally cultured leaves and decreased from basal explants to middle and apical ones. The highest rate of callus formation was obtained on media containing either 2,4-D (1 or 3 mg l^{-1}) or NAA (2 or 5 mg l^{-1}). Callus budding was significantly higher on medium supplemented with 1 mg l^{-1} BA. The budding rate of callus obtained from apical buds was 93.3 and 100%, respectively, for cultivars 'Priscilla' and 'ChaCha'. For callus derived from leaf fragments, the rate of budding was 100% for both cultivars.

Rosalia Paladines, Diandra Jurado (Ecuador), Tjitske Riksen-Bruinsma (The Netherlands), Ana Maria Quiñones (Ecuador) Prospects of Isolated Microspore Culture for Haploid Production in *Anemone coronaria* L. (pp 140-145)

ABSTRACT

Original Research Paper: The aim of this study was to establish a procedure to obtain haploid plants from microspore cultures of *Anemone coronaria* L., an important ornamental crop known worldwide due to its commercial value in the cut flower industry. Microspores were isolated from two genotypes of *A. coronaria*: 'Blue' (plants obtained through one cycle of selfing) and 'Lilac'. The effect of different treatments to interrupt the gametophytic development of microspores and promote sporophytic development was evaluated. High temperature, culture media composition and developmental stage of microspores at the moment of isolation were the assessed factors. Achieved microspore-derived embryo formation was 0.53% for 'Blue' and 0.06% for 'Lilac'. Different treatments were tested for microspore-derived embryo germination. Organic supplements had a positive effect on triggering germination, while growth regulators were needed to complete the development of the plantlets. Germination percentage was 2.13 and 2.41 for 'Blue' and 'Lilac', respectively. The ploidy analysis revealed the existence of haploid and doubled haploid plants of both genotypes. We identified 18 haploid plants and 9 doubled haploid plants of 'Blue', and 4 haploid plants and 3 doubled haploid plants of 'Lilac'.

Yukiko Kashihara, Koichi Shinoda, Hajime Araki, Yoichiro Hoshino (Japan) Towards Intergeneric Hybridization between *Alstroemeria* L. and *Bomarea* Mirb. (pp 146-149)

ABSTRACT

Original Research Paper: There are many interspecific hybrids of *Alstroemeria*. In this study, the possibility of intergeneric hybridization between *Alstroemeria* and *Bomarea* Mirb. was examined through the development of pollination procedures and ovule culture based on the histological observation of embryo and endosperm development after intergeneric pollination. Three methods of pollination (stigmatic, cut-style, and non-style) were combined with four different pollen types (fresh, frozen, non-germinated, and pre-germinated). We observed that the pollen tubes of *Bomarea coccinea* (Ruiz & Pav.) Baker could reach to the ovules of *Alstroemeria aurea* Graham 48 hours after stigmatic pollination with frozen pollen. Histological observations revealed that a primary embryo was formed, but subsequently aborted during development. This study demonstrates the possibility of intergeneric hybridization between *Alstroemeria* and *Bomarea*, but showed that there are post-fertilization barriers between *A. pelegrina* and *B. coccinea*. Further study is needed to investigate the optimum conditions for obtaining hybrid progeny.

Takejiro Takamura, Hideki Yamashita (Japan) Specific Differences in Nuclear DNA Content in the Genus *Cyclamen* (pp 150-153)

ABSTRACT

Original Research Paper: Relative nuclear DNA contents in *Cyclamen* species were estimated by flow cytometry with 4', 6-diamidino-2-phenylindole (DAPI) staining and propidium iodide (PI) staining. The relative fluorescence intensity (RFI) values in each species ranged from 1.521 to 8.071 and from 1.485 to 7.941 in flow cytometry with DAPI and PI staining, respectively. The ratio of RFI with PI staining to that with DAPI staining ranged from 0.93 to 1.15. Species belonged to the same subgenus indicated almost the same ratio of RFI with PI staining to that with DAPI staining. Subgeneric difference of the ratio was also observed. Flow cytometry seemed to be useful for the identification for interspecific hybrids in *Cyclamen* species, because the RFIs of interspecific hybrids were intermediate between that of their maternal and paternal species. The results of the present study should indicate that flow cytometry might be one of the effective tools for classification and identification of interspecific hybrids in the genus *Cyclamen*.

Peter J.M. Knippels (The Netherlands) Advanced *in Vivo* Propagation Techniques for Specialty Bulbs (pp 154-157)

ABSTRACT

Original Research Paper: Bulbs can be propagated by natural propagation techniques like by seeds and off setts, as well as by techniques like scoring, chipping, scaling and leaf cuttings. These techniques are mostly used for *in vivo* propagation, less for *in vitro* propagation. This article concerns the first case. Specialty bulbs can successfully be propagated by scoring and chipping, either starting at the beginning of the dormant period or at the end of this period. Of various species of the genera *Eucomis*, *Lachenalia* and *Ornithogalum*, it is known that they can successfully be propagated by leaf cuttings. In the presented experiments the techniques scoring, chipping and leaf cuttings were applied on *Eucomis autumnalis* and *Hymenocallis festalis*. Scoring and chipping resulted in new newly formed bulblets with *E. autumnalis*. Propagation by leaf cuttings resulted only with *E. autumnalis* to the formation of adventitious bulblets. Crucial is the timing of propagation, which will count for all genera and

species. In previous research with *Lachenalia* species it has been proved that the regeneration potential is highest when leaf cuttings are taken at visible bud stage.. A preliminary conclusion is that with *Eucomis* the regeneration potential is the highest before flowering. It is not possible to draw conclusions for *Hymenocallis*, because this genus belongs to another family than *Eucomis* and *Lachenalia* and the experiment with leaf cuttings failed with this genus. Due to the low number of used bulbs a statistical evaluation was not possible.

Antra Balode (Latvia) Effect of Microbiological Products on Bulblet Development of *Lilium* spp. in Scale Culture (pp 158-160)

ABSTRACT

Short Communication: The aim of the present study was to investigate the effects of two locally produced microbiological products *Trihodermins* B-J and *Vitmins* on scale promotion and bulblet development in *Lilium*. Lily scales were given three treatments: untreated controls; *Vitmins* solution (10 ml L^{-1}) and *Vitmins* solution (10 ml L^{-1}) + *Trihodermins* B-J (dry powder form, at 10 g kg^{-1} in the substrate). After treatment, scales were placed in a polyethylene bag in peat moss. Bulbs of three cultivars from different groups were used: Asiatic hybrid ‘Gardenja’, *Longiflorum* × Asiatic (LA) hybrid ‘Sonora’ and Trumpet hybrid ‘Elegija’. The scales were incubated in a plastic greenhouse at 15°C for 12 weeks; at 5°C for 10 weeks; and 18°C for 4 weeks. The effectiveness of microbiological products was evaluated by the number of bulblets per scale, the diameter of bulblets, shoot height, root length, and the percentages of dead plants. Significant difference ($P < 0.05$) between shoots and roots among the three cultivars and variants were found. The mean values obtained for shoots by treatment with *Vitmins* + *Trihodermins* B-J were: ‘Gardenja’ - 138.5 ± 9.8 mm, ‘Sonora’ – 102.0 ± 4.7 mm and ‘Elegija’ – 7.0 ± 3.0 mm. The coefficient of variation for number of bulblets per scale was recorded in the range from 19.7 to 39.2%. Treatment with *Vitmins* + *Trihodermins* B-J resulted in a significantly higher number of bulblets per scale and greater shoot height ($P < 0.05$ vs. control).