Floriculture, Ornamental and Plant Biotechnology

Advances and Topical Issues

Volume V

Edited by

Jaime A. Teixeira da Silva

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Advances and Topical Issues

First Edition

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FOREWORDS TO VOLUME V

Arie Altman, The Robert H. Smith Institute of Plant Sciences and Genetics in Agriculture, The Hebrew University of Jerusalem, Rehovot, Israel

The comprehensive book series "Floriculture, Ornamental and Plant Biotechnology", so thoughtfully, meticulously and skillfully edited by Jaime A. Teixeira da Silva, contained previously four volumes. Here, the 5th volume ("Advances and Topical Issues") is presented. As all other volumes in this series, this one is also characterized by its wide scope of subjects, presenting up-to-date and informative reviews, divided into seven major sections (Markets, policy, industry, plant invasion and conservation; Cut flowers and flower colour; Breeding and domestication; *In vitro* culture, tissue culture, micropropagation; Functional biology, development and cellular biology; Orchids; Greenhouse environments, soil and pathology). This wide scope of disciplines and subjects, ranging from practical agronomical issues and specific *in vitro* procedures to basic biology, molecular biology and biotechnology, characterizes all former volumes which deal with floriculture and ornamental plants (but also with other plants of interest). The wide scope of the subjects, contributed by over 400 authors from more than 50 countries, is an important asset to all plant scientists and students who are interested in the academic and commercial facets of plant and specifically ornamental and flower biotechnology. The editor is to be commended in his successful achievement of an immense challenge of putting together such a valuable source of information.

It is noteworthy that flowers and ornamental plants (e.g., *Gerbera*, carnations, *Pelargonium*, orchids, *Ficus benjamina* and Boston fern) were the first to be commercially clonally propagated *in vitro*, thus proving the potential and realization of plant biotechnology – long before the era of plant genetic engineering. Thus, the title of this series of volumes pays tribute to the true pioneers of plant and agriculture biotechnology.

Plant biotechnology – especially *in vitro* regeneration and cell biology, DNA manipulation and biochemical engineering – is already changing the agricultural scene in three major areas: control of plant growth, protecting plants against biotic stress, and production of specialty foods, metabolites and pharmaceuticals. Plant biotechnology faces several major challenges in the coming decades: alleviating the hazards of abiotic stress (especially salinity, drought, and extreme temperatures), improving pest control, maintenance and improvement of the environment, improvement of food quality and design of 'specialty food' using biochemical engineering, and production of biomaterials. Two parallel research approaches will most likely exist simultaneously in the near future: the transgenic approach (expression of unique genes and specific promoters and transcription factors), and the non-transgenic approach (genomics-assisted gene discovery, marker-assisted selection, efficient mutations, and clonal agriculture). Molecular breeding has now become routine in agriculture, and floriculture and ornamental plants – to which this series of volumes is devoted – are an integral part of it. Achievements today in plant biotechnology have already surpassed all previous expectations. The full realization and impact of the new developments depend not only on continued successful and innovative research and development activities, but also on a favorable regulatory climate and public acceptance. Plant scientists now have a central role in society.

Arie Altman, Professor of Horticulture at the Hebrew University of Jerusalem, Israel, and former President of the International Association of Plant Tissue Culture and Biotechnology (IAPTC&B), received his PhD from the Hebrew University of Jerusalem. Following a post-doctoral period at the University College of Wales, Aberystwyth, he was a Visiting Professor and Visiting Scientist at Yale University, Hoffman La Roche and Scripps Institutes of Molecular Biology, Universite Paris VI, and Amsterdam Free University. His main research interests include general plant biotechnology, in vitro propagation and molecular control of plant response to abiotic stress. He has authored more than 180 research articles and edited several books, including "Agricultural Biotechnology" (Marcel Dekker, 1998), and is an Editor or member of the Editorial Board of several major plant biotechnology journals. He served in many international and national organizations and committees, was Director of the Otto Warburg Center of Biotechnology in Agriculture, and is the Founding Head (1999-2003) of the Robert H. Smith Institute of Plant Sciences and Genetics in Agriculture.

Jana Pospíšilová, Institute of Experimental Botany, Academy of Sciences of the Czech Republic, the Czech Republic

Floriculture attracts attention not only of thousands of researchers and growers but also of millions of amateur gardeners. Biotechnological methods can significantly help them in their hard effort to improve quality, widen the assortment, decrease the price of cultivated flowering plants, as well as to preserve endangered naturally grown species. The comprehensive (more than 2000 pages) book series "Floriculture, Ornamental and Plant Biotechnology: Advances and Topical Issues" is very useful source of up-to-date information presented by well-known experts from all over the world. With the aim to put in order contributions from this extremely broad field, the already appeared volumes I to IV are divided into sections and every section is further divided into several specialized parts. The first volume comprises two sections "Structure, metabolism, development, physiology and genetics" and "Genes, genomes, genomics, and breeding". The second volume consists of sections "Genetic engineering and transgenesis" and "Tissue culture and the *in vitro* environment". The third volume includes sections "The *ex vitro* environment, minerals, water stress, remediation" and "Plant-organism interactions, disease, and control". The fourth volume contains three sections "Flowering plants: the future", "Ornamental plants and flowers in art and society" and "Novel ornamental gems and floricultural assets".

Extremely rapid progress in plant biotechnology asked for continuation of this series. Topics discussed in the new volume V include improved protocols for growing of different species of ornamental plants *in vitro* as well as *ex vitro*, improvement of flowering and retardation of flower senescence, interspecific hybridization, breeding for resistance, or production of economically important secondary metabolites. On one hand, there are contributions concerning conservation of endangered plant species and, on the other hand, those showing the influence of horticulture on the risk of plant invasions. Attention is also paid to importance of mycorrhizal symbiosis, functional biology of carnivorous plants, large-scale production of anti-bodies and cryopreservation. As in previous volumes, some fundamental research is reviewed in this volume, e.g. crosstalk between ethylene, abscisic acid and sugar signalling pathways, dynamics of cytoskeleton during stomatal movement, biosynthesis and degradation of carotenoids, molecular mechanism and impact of cytokinins, proteomics of whole tissue and subcellular compartments, a role of *KNOX* genes in reproductive biology. Commercial biotechnological organizations appreci-ate contributions dealing with advances in technology for improvement greenhouse environments or a role of micropropa-gation in the development of the foliage plant industry.

I am convinced that researchers, teachers, students and growers certainly welcome the appearance of this new volume and I wish it great success.

Jana Pospíšilová: *Education*: RNDr. (equivalent to M.Sc.), Faculty of Natural Sciences, Charles University, Prague; CSc. (equivalent to PhD) Czechoslovak Academy of Sciences, specialization plant physiology, 1968. *Position*: lead scientist at the Institute of Experimental Botany, Academy of Sciences of the Czech Republic, Prague; lecturer and thesis supervisor at Faculty of Natural Sciences, Charles University. *Fields of research*: characteristics of plant water relations, effects of stress deficit on photosynthetic parameters, stomatal and nonstomatal limitations to photosynthesis, adaptations and acclimations of plants to environmental conditions, acclimations during micropropagation, hormonal control of plant responses to abiotic stresses. *Publication activity*: Author and co-author of more than 130 scientific papers and 17 books. *Executive editor* of the international journal Biologia Plantarum.

Jan KÁŚ, Professor Emeritus, Institute of Chemical Technology, the Czech Republic

Flowers and other ornamental plants accompany people since their birth to death and they co-exist with almost all happy events in life such birthday celebrations, weddings, carrier progress, etc. In addition, they form our best partners in our everyday life in our flats, offices, different public spaces, parks, gardens and elsewhere. They are an inseparable part of the culture of all nations and nationalities. This is the reason why people since time immemorial have tried to improve or change flowers and other ornamental plants according to their imagination, dreams and practical aspects of planting. The original fondness for flower beauty has changed within the last centuries in well organized breeding attempts utilizing continuously increasing scientific progress. This effort was terminated with successful large-scale production of nice flowers and other ornamental plants available almost to everybody in developed countries and often regardless of the season and geographical location in the world.

Nevertheless, people always want to have new plant varieties with new colors and fragrances, plant shape, resistant against all types of possible obstacles, including stress factors (dryness, moisture, etc.), all kinds of diseases and adaptation to various environmental conditions. Flowers and other plants, however, are not here only for our pleasure. They may be simultaneously prosperous in production of various pharmaceutically important substances, in phytoremediation, improvement of soil quality, nature protection against weather or even climate changes, etc. The previous four volumes of "Floriculture, Ornamental and Plant Biotechnology" represent outstanding collection of 280 interesting contributions showing new perspectives in all indicated aspects leading to the further improvement of quality and general utility of plants. The description of modern genetic and biotechnological approaches plays a dominate role here.

The fifth volume terminates the effort by the editor to present a coherent image of the contemporary status of science in this area and also to remember some practical aspects of plant use in society. The additional 53 contributions completed the total number of the contributions in this five volume compendium to an admirable "nice number" of 333. Besides continuing the description of new technologies, attention is also paid to highly discussed problem of regulation and public acceptance of genetically modified plants. The reader will also find here a detailed description of legislative regulation of handling with genetically modified organisms (GMOs) in different countries, as well as a discussion on the policy of reducing the risk of the introduction of invasive plants into nature via the horticulture trade.

Jan KÁŚ: Professor Emeritus of the ICT in Prague, was born in 1934 (Žatec, Czechoslovakia). He received PhD in Biochemistry (1970, ICT Prague), DSc. in Biochemistry (1987, Czechoslavak Academy of Sciences, Prague), and he was appointed Professor of Biochemistry (1990, ICT, Prague), Visiting Professor at the University of Luton, GB (1995-1997), Vice-dean (1989-91, 92-97). He served in several university positions and in several international bodies (e.g. IUPAC, IUFoST), national scientific organizations and editorial boards. His teaching and research activities included general and applied biochemistry, biotechnology, applied enzymology, immobilized biological systems and immunochemistry. His publication activity includes more than 250 original papers, 75 review papers, 12 patents and several monograph chapters.

Sergio J. Ochatt, Physiologie Cellulaire, Morphogenèse & Validation (PCMV), UMR LEG, INRA, France

Ornamental and flower crops are produced mainly for their aesthetic value, thus the propagation and improvement of quality attributes such as leaf types, flower colour and fragrance, longevity and form, plant shape and architecture, and the creation of novel variation are important economic goals for the ornamental industry. Aside from their ever-increasing economic value, ornamental plants and particularly the wild ones serve for an improved knowledge of the evolution of species and contribute highly also to the understanding of some of the fundamental mechanisms underlying plant growth, population genetics and the study and validation of gene function. The latter is notably true as a large amount of research on gene signalling is mainly conducted on model species and requires its extension to cultivated crops for an unequivocal validation of gene activity.

This series of books is probably the most comprehensive available in the literature to date devoted to ornamental and floricultural crops. The fifth volume herein continues the high quality standard of the previous four volumes and extends the range of species for which the development and application of biotechnological approaches is reviewed all along its more than fifty chapters. Importantly too, contributing authors come from all over the world and thus introduce their own, enriching vision on the various aspects, challenges and contributions of biotechnological tools as applied to the ornamental and flower crops. The summation of these facts will certainly render this book a key reference not only for floriculturists and commercial companies, but also for researchers interested in various fundamental aspects of plant science, biotechnologists, and students.

Sergio Ochatt: agronomist, PhD, HDR. Heads the PCMV team at UMRLEG, INRA Dijon (France) since 1998. Chairman of COST Action 843 (E.U.) and FAO expert on development of biotechnological plant breeding. Over 100 publications plus several book chapters. Associate Editor of Plant Cell Tissue Organ Culture, Referee of several journals, has co-edited three books. Having worked on fruit tree biotechnology until 1995 and on protein legumes since then, some landmark results include: plant regeneration from protoplasts, somatic hybridisation, demonstration of role of electricity on DNA synthesis and regeneration competence, production of drought/salt resistant genotypes, haplo-diploidisation, acceleration of generation cycles *in vitro*, elucidation of genetic basis of hyperhydricity, flow cytometry characterization, gene mapping, identification of markers of somatic embryogenesis.

Andy V. Roberts, School of Biosciences, University of East London, United Kingdom

The first four volumes of ‘Floriculture, Ornamental and Plant Biotechnology’ include a substantial collection of competently edited, interesting and attractively presented articles. The broad range of topics enables readers to gain new perspectives on their own specialist areas and explore others with which they are less familiar. Volume 5, which is subtitled ‘Advances and Topical Issues’, continues in the same style. The areas covered include disease prevention and the promotion of plant health, propagation (*in vitro* and *in planta*), floral biology, genetics and plant breeding, biochemistry and physiology, biotechnology and environment issues. But the authors have not been constrained by a rigidly prescribed general theme. Interest is stimulated by a diversity of approaches and the international flavour brought by contributors from 26 countries. From my perspective, articles on interploid hybrids in lily breeding, various aspects of *in vitro* culture and perspectives of industry on barriers to plant biotechnology are essential reading. But articles such as those dealing with the molecular biology of the digestion of animals by insectivorous plants, and antibody production in plants for medicine, phytoremediation and plant improvement are irresistible. I welcome this series of books which I believe make an important contribution to the literature of pure and applied plant science.

Andrew V. Roberts was born and educated in the UK, obtaining a BSc (Hons) degree in Botany at Cardiff University and a PhD in Agricultural Botany at Reading University. His first appointment was in Lesotho, as a lecturer in Botany at the University of Botswana, Lesotho and Swaziland. After returning to the UK, he was appointed as a lecturer in genetics and then as a professor at the University of East London, where he is now an emeritus professor. His main publications are in the taxonomy, cytogenetics, tissue culture, pathology and physiology of roses, and in the tissue culture of several other plant genera. He was editor-in-chief of the Encyclopedia of Rose Science, which was published by Elsevier in 2003.

ABSTRACTS: VOLUME V

Pandora's Box

Part 1 Markets, policy, industry, plant invasion and conservation   Page

The development and commercialization of Genetically Engineered (GE) ornamentals faces significant challenges. These include overcoming product development costs, obtaining freedom to operate, and obtaining regulatory approval. This chapter examines each of these challenges in detail and points to possible solutions that may encourage further development and commercialization of this technology.

Ornamental horticulture is considered to be the main pathway for the introduction of non-native plants into a country and a small percentage of these species are serious threats to native biodiversity. In recent years, this pathway and its mechanism has been more closely analysed by ecologists. It has been possible to link the frequency of species in the horticultural trade with their subsequent success in escaping from cultivation and spread into unintended habitats. Policies are being developed to address the problem, such as voluntary codes of conduct and lists banning certain species from sale. This chapter will review the influence of ornamental horticulture on the risk of plant invasions as an introductory pathway, through its effect on propagule pressure and through cultivation. It will also analyse the existing legal framework and future research needs in this area.

Invasive alien plant species are known to cause both economic losses and ecological damages. However, the introduction of a non-native species can be simultaneously classified as destructive by one segment of society and desirable by another. Indeed, many potentially invasive plant species are deliberately introduced for economic benefit despite the risks associated with them. Notably, the horticulture industry is the most significant pathway for intentional introductions of invasive alien plants. Although it is increasingly recognized that the nursery trade and the invasive alien plant problem are inextricably intertwined, existing regulations do little to tackle deliberate introductions of potentially invasive plant species. Indeed, prevailing policies largely ignore the horticultural dimension of the problem. Therefore, novel policy options to address the problem have been developed and could be implemented to reduce the risk of invasive plant introductions via horticultural trade. Here, we describe the current regulatory framework, and its shortcomings, for dealing with the introduction and sale of potentially invasive plant species in Canada and the United States. The chapter also outlines new policy options for limiting the risk of invasive alien plant introductions by the green industry, including the use of economic instruments and risk assessment procedures. We discuss the merits of these options as compared to the approaches that are applied at present. Finally, preliminary results from a survey of professional horticulturists are presented.

This study outlines a pioneer initiative concerning the native plant conservation that is considered as one of the most important plant refuges in Europe. The Balkan Botanic Garden of Kroussia has designed a system built on people, infrastructure and a strategy of seven hierarchical and complementary policies. To formulate and implement this strategy, we have created a flexible research team with scientists from different disciplines that collaborate interdisciplinary. In this team, information flows horizontally, experience and expertise are joined and used collectively and know-how is delivered vertically to all interested or involved parties. In the frame of this strategy, target plants are being initially explored, located and collected from the wild and Important Plant Species are maintained, evaluated and studied. Explicitly documented living plant collections are maintained with classical and innovative methods and species-specific propagation protocols for wild plants are being developed. Environmental awareness is promoted and several educational activities on the native biodiversity are organized. Numerous plant conservation actions are undertaken; all attempt to integrate the *ex-situ* with the *in-situ* plant conservation and contribute to the implementation of the targets of the Global Strategy for Plant Conservation at local, regional and (inter-) national levels. Furthermore, evaluation of the medicinal, cosmetic, flavouring, floricultural and ornamental value of selected native plants is performed, aiming at their sustainable exploitation in collaboration with the state, the stakeholders and the market. This approach has the ultimate goal to deliver promising and unique new crops that are carefully selected and designed, sustainably produced and managed, successfully launched and fair traded internationally.

Part 2 Cut flowers and flower colour

The genus *Gentiana* consists of 400 species and is mainly distributed in the mountain area of temperate zones. Some gentian species are utilized as floricultural crops. The blue flower color of *Gentiana triflora* is derived from a unique diacylated anthocyanin, gentiodelphin. We have characterized the biosynthetic pathway leading to gentiodelphin by isolating of the structural genes in the pathway and conducting a biochemical study. Some of the genes, such as flavonoid 3′,5′-hydroxylase and flavone synthase, are shown to be useful molecular tools to modify the flavonoid composition and, thus, the flower color of heterologous plants. We also isolated carotenoid and disease-related genes that are expected to be useful to yield novel transgenic plants. Color- and morphology-modified transgenic gentian plants have been obtained via an *Agrobacterium tumefaciens*-mediated transformation system. Gentian infected with *A. rhizogenes* yielded dwarf phenotypes that can be utilized as pot plants. Molecular breeding will introduce novel characters into gentians to obtain more commercially competent varieties. It will be necessary to overcome some problems, such as gene silencing of an introduced promoter and the negative public sentiment toward genetically modified organisms. Our studies in gentians will provide constructive models and lessons to those who adopt molecular breeding to improve floricultural crops.

Flower senescence is of particular economic importance in horticulture. Ethylene, sugar and abscisic acid (ABA) have all been implicated in the regulation of this process. Ethylene plays an important role in the senescence of climacteric (ethylene-producing) flowers. ABA addition has been observed to accelerate, and sugars to delay, flower senescence. Evidence for crosstalk between ethylene, ABA and sugar signalling has emerged for *Arabidopsis* vegetative tissues or isolated cells. Ethylene-sugar interactions are best characterized for the transcription factor EIN3, a component of the ethylene signalling pathway, which is continuously degraded by proteasomes, a process that for isolated cells is inhibited by ethylene and stimulated by glucose. In addition, glucose represses transcription of the gene for EIN3. These effects of glucose are probably exerted via its detection by the sensor hexokinase 1 (HXK1). A lower EIN3 level decreases ethylene sensitivity. Sugar induces, also via HXK1, leaf senescence. The transcription factor ABI5, involved in ABA signalling, is required for this sugar-induced senescence. Exogenous sugars improve longevity for many cut flowers. This observation is in contrast to the accelerating effect of sugar on leaf senescence, and could possibly be due to sugar-induced EIN3 degradation and decreased *EIN3* transcription, as observed for *Arabidopsis* cells. Furthermore, the observation that ABA promotes senescence in some climacteric flowers may be due to a decreased level of CTR1, an ethylene signal protein, since ABA decreases *CTR1* transcription in *Arabidopsis* seedlings. However, the crosstalk between sugar, ABA and ethylene in flower senescence is still not totally understood, and clarification will have to await further investigation of the signalling pathways and their interactions in flowers.

Eustoma grandiflorum, native to North America, was introduced into Japan about 70 years ago. *Eustoma* flowers are sensitive to ethylene and ethylene production from flowers increases during senescence. Pollination is known to accelerate flower senescence in many plants such as orchid, petunia and carnation. In cut *Eustoma* flowers, senescence is also accelerated by pollination, and ethylene production by the pistil increases rapidly after pollination. The distance from stigma to anther varied among 13 cultivars studied, and this distance was negatively correlated with rate of natural self-pollination. This result indicates that flowers are more apt to be self-pollinated if this distance is short. Pollination levels on the stigma affect flower senescence, that is, flower senescence accelerates as the area of pollination increased. Ethylene production of flowers increases earlier as the area of pollination increases. Our results indicate that the distance from the stigma to the anther and the pollinated area of the stigmatic surface are involved in pollination-induced senescence in *Eustoma* flowers.

The petal color of yellow-flowered chrysanthemums originates mainly from carotenoids. The yellow coloration is recessive trait against a white one. Although a single dominant gene inhibiting carotenoid formation was postulated (Hattori 1991), the gene has not been identified. To find a factor that inhibits carotenoid biosynthesis or accumulation in white petals, we compared the expressions of genes coding for carotenoid biosynthesis and degradation between yellow and white petals of chrysanthemum. In white petals, all genes for carotenoid biosynthesis were expressed at the late stage of development, when carotenoids were nearly undetectable. A gene encoding carotenoid cleavage dioxygenase (*CmCCD4a*) was specifically expressed in white petals. Petals of a white-flowered cultivar turned yellow after an RNAi construct of *CmCCD4a* was introduced. These results indicate that in white petals of chrysanthemum, carotenoids are synthesized but are subsequently degraded into colorless compounds.

Part 3 Breeding and domestication

The value of haploids in genetic analysis and plant breeding has been known for a long time. Natural haploid embryos and plants, derived from gametophytic cells, have been described in about one hundred species of angiosperms. However, haploids occur only rarely in nature. To be useful for breeding programmes, they must be produced in large numbers. Haploid plants can be obtained by: a) selective elimination of chromosomes in a hybrid embryo, b) by using male sterile plants, c) by induced androgenesis, d) by induced gynogenesis. The proposed chapter will preferentially focus on induced androgenesis and gynogenesis, like effect of stress, gene regulation, proteomics, etc. Besides, to provide reader with the most up-to-date topical view on gametic embryogenesis, the main aim of the chapter will be to highlight the recent rapid progress in the field of gametic embryogenesis as well as economic and biotechnological impact of haploid and/or dihaploid plant production.

Interspecific hybridization is one of the most important strategies for creating variations in ornamental plants since it has the potential to combine useful traits, i.e. favorable morphology, disease resistance and some environmental tolerances, of both parents, that could not be achieved by the cross within a single species. Carnation (*Dianthus caryophyllus* L.) is native to Mediterranean areas and most of the carnation cultivars have been bred in Europe and the U.S.A. with a relatively cool climate. Consequently, most cultivars do not have tolerance to hot and humid climate and tend to grow unfavorably in the hot, humid summer of Japan, which results in various disease problems and production of deteriorated cut flowers. To increase the yield and quality of cut flowers, carnation should be cultivated to produce flowers before the hot summer arrives. Therefore, for stable and quality carnation production, it is important to breed cultivars with both heat tolerance and early flowering. In this study, two indigenous *Dianthus* species to Japan, *D*. *japonicus* Thunb. and *D*. × *isensis* Hirahata et Kitam. were cross-hybridized with carnation to introduce useful traits of these two species such as heat tolerance and early flowering into carnation. On reciprocal interspecific hybridization between carnation and *D*. *japonicus*, hybrid plants were obtained only when carnation was used as the seed parent. Although hybrid plants rarely obtained were sterile they successfully restored fertility by producing amphidiploids through artificial chromosome-doubling treatment. When reciprocal interspecific crosses were carried out between carnation and *D*. × *isensis*, fertile hybrid plants were obtained in both cross-directions. Since these two kinds of interspecific hybrids exhibited useful traits of the donor species, they will be efficiently used as valuable germplasm for further carnation breeding.

Bacterial wilt (*Burkholderia caryophylli*) is one of the most important and damaging diseases of carnations (*Dianthus caryophyllus*) in Japan. Interspecific hybridization between carnation and *D*. *capitatus* ssp. *andrzejowskianus*, a wild species that is highly resistant to bacterial wilt, was carried out to introduce its resistance into carnation cultivars. A new resistant line (‘Carnation Nou No. 1’) was selected from the F_1 progeny. Using the random amplified polymorphic DNA (RAPD) technique, a RAPD marker tightly linked to a bacterial wilt resistance gene was identified. This marker was successfully converted into a sequence-tagged site (STS) marker suitable for use in marker-assisted selection. This marker is now being used in practical breeding programs to improve resistance to bacterial wilt. To improve the vase life of carnations, conventional cross-breeding techniques were used to develop many carnation lines with a long vase life. Two new cultivars, ‘Miracle Rouge’ and ‘Miracle Symphony’, with a genetically determined long vase life, were developed. They had vase lives of 17.7 to 20.7 days (3.2 to 3.6 times the vase length of the ‘White Sim’ cultivar) under standard conditions (23°C, 12-h photoperiod, 70% RH). The ethylene biosynthesis pathway in these cultivars was almost completely blocked during natural senescence, which was responsible for the long vase life. Differences in ethylene sensitivity among cultivars were evaluated using a time-lapse video recording system, which provides a simple and accurate way of evaluating ethylene sensitivity. The video system revealed that the ethylene sensitivity of carnation flowers after anthesis decreased with increasing age.

In recent years the cultivation of ornamental sunflower has become widespread and at present this species takes a significant portion of the cut flower market. Cultivated sunflower (*Helianthus annuus* L.), regardless of purpose for which it is grown (as oil crop, for food, or ornamental) has a very narrow genetic base which makes development of commercial cultivars resistant to prevalent diseases, such as white rot, very difficult. Therefore, an increase of genetic variability by hybridization with wild *Helianthus* species has great potential in sunflower breeding for resistance, since these species are very often sources of genes for resistance. Since crossing via conventional methods had limited success due to poor crossability and sterility of interspecific hybrids, somatic hybridization presented itself as an alternative for incorporation of genetic material from wild species to cultivated sunflower. Electrofusion is the most frequently used technique for generation of somatic hybrid plants between different species because of better maintenance of protoplast viability and reduction in membrane damage, protoplast distortion and disruption. In this chapter we present a protocol for the production of somatic hybrid plants by using asymmetric electrofusion of sunflower with *H. maximiliani* and *H. mollis* protoplasts. Protoplasts of white rot-resistant clones of *H. maximiliani* and *H. mollis* were electrically fused with protoplasts of the cultivated sunflower inbred lines. Fusion products were embedded in agarose droplets. Developed microcalluses were transferred into solid media and subjected to different regeneration protocols. After shoot regeneration and development, a morphological and RAPD analysis confirmed a hybrid nature of the regenerated plants.

With the main goal of generating new groups of lilies, that combine agronomic characteristics of major importance, a large number of crosses were performed among the three most important groups of lilies (*Lilium*), viz., Asiatic, Longiflorum and Oriental; and to some species, to produce F_1 inter-specific hybrids. These hybrids, as well as many other F_1 hybrids, were sterile and two approaches were used to overcome sterility: i) chromosome doubling through chemicals and ii) the use of naturally occurring or induced unreduced (*2n*) gametes. The first approach produced many fertile hybrids, however, due to autosyndetic pairing in the allotetraploids, recombination was not detected through DNA *in situ* hybridization techniques (GISH), and introgression was not achieved. On the other hand, through extensive meiotic analyses and pollen germination tests, we selected hybrids that were able to produce *2n* gametes, and in some cases, hybrids in which *2n* gametes could be induced. Many plants were obtained in reciprocal backcrosses and recombination as well as introgression was detected through GISH and FISH analyses. In this chapter we present the success in breeding lilies from different taxonomical sections through the use of naturally occurring and induced *2n* gametes. The mechanisms of *2n* gamete formation are described and the genetic considerations of their use to achieve introgression and to generate variability are examined.

Lily comprises more than 80 species belonging to 7 sections. Within the sections cultivars bred from Sinomartagon, Archelirion, and Leucolirion are the most important in the commercial market. At this moment, the most promising breakthrough in lily breeding is the raising of new cultivar through interspecific hybridization with introgression of useful genetic traits from species or breeding materials belonging to the wild species which are not commonly used so far for commercial breeding. We have been crossing almost all different cross combinations and have succeeded in more than 28 cross combinations since 1980. The F_1 hybrids between the species have shown the intermediate phenotypic characteristics. Making interspecific or intergeneric hybrid is laborious but finding the clues, affecting to the most successful embryo formation, and growth is even more difficult and time consuming. Therefore research on the successful interspecific hybridization between distantly related species was carried out at several laboratories in the world. For the successful interspecific hybridization breeding, not only production of F_1 interspecific hybrids but also successful production of subsequent generations using interspecific hybrids to introgress valuable trait(s) is important. One of the promising crosses is of course between Orientals and several species such as *L. henryi*, Asiatics, and trumpet lilies. In this context, we demonstrate the possible methods and some valuable instances of the interspecific hybridization in lilies.

Despite a long history of cultivation, a large number of lily (*Lilium*) cultivars were still diploid (2n=2x=24) till recently. But polyploid cultivars are rapidly increasing. In addition to having robust stems, large flowers, thicker and larger leaves, polyploids can also serve to combine desirable characters from species of different taxonomic sections; not only from the cultivated groups, viz., Sinomartagon, Archelirion and Leucolirion, but also from sections that include non-cultivated species. Because the F_1 hybrids between the species of different taxonomic sections are highly sterile, it is imperative that breeding has to be carried out at the polyploidy level. This chapter is based on our results on using intersectional interspecific hybrids of Longiflorum x Asiatic (LA) and Oriental x Asiatic (OA) groups of lilies and their polyploid backcross progenies BC1, BC2 and BC3). In order to cross genotypes of different ploidy levels, i.e., interploidy crosses, a knowledge of embryo and endosperm ploidy levels is valuable because, unlike in most other plant species, lily has very large chromosomes and the embryo sac formation is of tetrasporic 8-nucleate type. In order to highlight the differences, a comparison is made with the most commonly occurring, monosporic 8-nucleate type of diploid potato, with 24 chromosomes (same as lily). Some of the limitations and constraints associated with interploidy crossing are considered.

Antirrhinum majus, commonly known as snapdragon, is a well known plant as it is widely used as an ornamental all over the world, as well as a model plant in biotechnological research. The species, which belong to the botanical genus *Antirrhinum*, are distributed around the

Mediterranean Sea, being the Iberian Peninsula considered its genetic centre as it concentrates most of the diversity of the genus. Most of the species are narrow range endemics and several of them are under threat. Wild species of *Antirrhinum* are perennial diploids. Flowers are hermaphrodite and ecalcarate with a gibbous corolla; fruits are capsules in which dozens of tiny seeds are produced. With the exception of *A. siculum*, which is self-compatible, wild species of *Antirrhinum* are self-incompatible and cross-pollinated, with pollinators able to discriminate differences in shape, colour and scent. This chapter aims to show current information on biological traits such as reproductive system, pollination and between species hybridization ability, species range and ecology as well as data on genetic diversity and its partition within and among populations. All these data allow a better understanding of the threat to species and are valuable tools to evaluate the consequences of habitat fragmentation and genetic erosion and can help species conservation managers to make decisions to preserve natural populations.

Many plant genera native to South America such as *Petunia*, *Glandularia*, *Begonia* and *Alstroemeria* have contributed to the development of commercial varieties. However, the countries of this continent had not exploited the use of genetic resources with ornamental value. Since 1999, in an attempt to revert this situation, the Floriculture Institute of INTA-Castelar, Argentina began research activities to develop ornamental varieties from native plants. Since then, more than 2,300 accessions have been collected, and many of them characterized. Such breeding activities have been initiated in *Tabebuia*, *Nierembergia*, *Jacaranda*, *Calibrachoa*, *Passiflora*, *Glandularia*, and *Gloxinia*. Here we present two successful breeding programs set up by the Floriculture Institute of INTA-Castelar, in *Tabebuia* and *Nierembergia*. In both genera, we carried out interspecific hybridizations, segregated progeny in some cross combinations, and obtained new ornamental genotypes. We also studied the pollen tube growth in pistils in order to characterize different kinds of interspecific incompatibility.

Part 4 *In vitro* culture, tissue culture, micropropagation

Gels and mechanical supports have been used in the culture of microorganisms and plants for nearly 200 years. The early studies to develop methods of growing and examining organisms on solid media, initially bacteria and fungi then latterly plant tissues, are traced to the point where agar came into common use. Each of the major gel types in current use – agar and agarose, gellan gum, carrageenan and alginate – is described. Details of their history, discovery and development, the chemical structures and gelation mechanisms leading to functional use(s) in plant tissue culture, and their advantages and deficiencies are given. A wide variety of mechanical supports and their evolution from the earliest use of filter paper with liquid media to more contemporary specialized applications of porous foam and microcarrier beads are also described. Finally, a survey of the newer gels appearing in the literature either alone and/or in novel combinations is presented.

Cytokinins are plant hormones that are often implicated in the source-sink relations among plant organs. They are thought to be integral in growth regulation through their promotion of cell cycle or sugar metabolism enzymes. These mechanisms strongly influence traits important to floriculture, like the control of plant architecture and flowering effort. With this in mind, the state of knowledge is reviewed about the role of cytokinins in processes that directly impact floriculture such as flower initiation, sex determination and fruit set and development. Implications are discussed for trade-offs between such reproductive traits and competing vegetative growth processes like branch initiation, whole plant architecture, phyllotaxis and senescence. Physiological data, emerging functional genomics and transgenic technology is assessed to help pinpoint how manipulation of CK synthesis or target tissue sensitivity may lead to improvements in horticultural species and the floricultural industry.

The foliage plant industry is one of the fastest growing sectors in U.S. agriculture, posting a wholesale value of $710 million in 2005, which is an almost 54-fold increase compared to 1949. The rapid increase in wholesale value can be attributed to technological advances in production and to new cultivar releases that meet consumers' demand for novel plants. As one of the major technological advances, micropropagation has steadily and significantly changed the spectrum of foliage propagation and production. The foliage plant industry was the first to successfully demonstrate the commercial profitability of micropropagation. Annually more than 507 million foliage plantlets including 150 million orchids are produced worldwide. Micropropagation helps to eliminate systemic diseases in starting materials, dramatically reduces greenhouse space required for maintaining stock plants, and provides growers with healthy and uniform liners on a year-round schedule. Micropropagation has also become a prosperous avenue for obtaining new cultivars through the selection of somaclonal variants; more than 80 commercial cultivars have been selected from somaclonal variants. Furthermore, micropropagation speeds the introduction of hybrid cultivars. New cultivars reach sufficient numbers to become commercially available in 2 to 3 years through micropropagation compared to the 5 to 10 years needed via traditional propagation methods. This article is intended to document the history of foliage plant micropropagation and the contributions this technology has made to the rapid development of the foliage plant industry.

The micropropagation of cactus has been carried out over three decades of research, where the application of cytokinins and auxins to media culture as well as the use of phylloclades as explants are the two main keys to promote areole activation, growth and development. Most procedures are highly specific and poorly competent. In this chapter we present a brief review on the propagation of cactus plants – mainly of horticulture interest or needed for conservation – by tissue culture. We also attempt to identify innovation in technology and novelties in techniques that would allow for the methodologies to be widely applied to all members of the Cactaceae.

The genus *Iris* is the largest and most complex genus of the family Iridaceae. Several Iris species occur naturally in Jordan. Of these, *Iris atrofusca*, *Iris petrana* and *Iris vartanii* are threatened species. Callus from the former two species was successfully propagated using juvenile flower bases. Irone-type essential oil was first determined by TLC and confirmed using GC-MS.

In vitro-grown apical shoot tips of *Limonium* (hybrid statice; *L. altaica* Mill. x *L. caspium* Mill. cv. 'Blue Symphonet') were cryopreserved in liquid nitrogen (LN) by following three cryogenic procedures; vitrification, vitrification with encapsulation (encapsulation/vitrification), and encapsulation/dehydration technique. When dehydration tolerance was well developed by preconditioning and cryogenic procedures were well optimized, these three procedures produced nearly the same levels of growth recovery (70 to 75%). Bulbous structures consisting of meristematic clumps (designated "shoot primordia") were induced from a meristematic culture of statice. Cold-hardened, precultured small segments of shoot primordia were successfully cryopreserved in LN by vitrification. Shoot primordia appear promising for large-scale production and cryopreservation for statice.

The genus *Phyllostachys*, a major bamboo found in Japan, provides useful resources for local economics. Recently it has been considered as a renewable bio-resource. We have established an efficient cell suspension culture system for bamboos, genus *Phyllostachys*. Uptake of carbohydrates by bamboo cells was characterized using this cell suspension culture system. In order to demonstrate the effect of cell densities on proliferation of bamboo suspension cells, a portion of maintained liquid suspension cells was re-suspended in fresh 50 ml of modified ½MS medium in 200-ml conical flasks at cell densities of 1, 10, 50 and 100 ml sedimented cell volume (SCV) per liter of the medium, respectively. Ten ml SCV l^{-1} was the most effective for proliferation of the cells (35.6-fold) while high-cell-density culture strongly suppressed their growth (2.6-fold). Osmolality in each liquid medium, which reflected consumption patterns of nutrients by the cells in a culture period, was measured and found that the value dramatically decreased in high-cell-density conditions (50-100 ml SCV l^{-1}). In practice, no or less fructose, glucose, and sucrose contents in the media of high-cell-density conditions were detected by high performance liquid chromatography analysis suggesting that sucrose catabolism might be highly activated in bamboo suspension cells. A micro-plate cell culture method was employed for detailed analysis of growth and sugar uptake of bamboo plants. The unique characteristics of bamboo cells were discussed in comparison with that of rice Os cells and tobacco BY2 cells.

Plant regeneration and a histological study of somatic embryogenesis in mature zygotic embryos culture of Persian onion (*Allium aflatunense*), an ornamental onion, is presented. Induction of morphogenesis *in vitro* was achieved on media supplemented with BDS mineral solution according Dunstan and Short (1977) with 2,4-dichlorphenoxyacetic acid (2,4-D, 2.0 mg L^{-1}) or thidiazuron (TDZ, 2.0 mg L^{-1}). Multiplication of somatic embryos and bulblet formation were achieved on the same media. After removing plant growth regulators, multiplication of somatic embryos continued and lasted for more than 2 years. Somatic embryos formed indirectly on embryogenic callus derived from BDS medium supplemented with 2,4-D. A histological study of the explants at various developmental stages of somatic embryogenesis revealed that somatic embryos developed from the cotyledonary cell layers of mature zygotic embryos and were multicellular in origin. Somatic embryos passed through the globular stage, and developed into somatic embryos with a cotyledon, shoot and root meristem. Persian onion, due to its ability to regenerate plants through somatic embryos can be considered as a useful model system for further studies of *in vitro* morphogenesis.

In vitro plant regeneration by organogenesis of *Iris pseudopallida* Trinajstic, an endemic species of the Balkan Peninsula has been studied and reported for the first time ever. Zygotic embryos were cultured four weeks on MS (Murashige and Skoog 1962) solid media supplemented with several concentrations of thidiazuron (TDZ; 0.0-2.2 mgL^{-1}) where organogenic calli, as well as, adventitious shoots are induced. The average number of shoots per zygotic embryo varied from 2.2-4.2. The best multiplication index (12.3) was obtained after subseqent removal of TDZ from the medium. Long-term multiplication could be maintained on MS media supplemented with 6-benzylaminopurine and gibberellic acid (1.0 and 0.1 mgL^{-1}, respectively). The best rooting rate (77%) was achieved on MS medium without plant growth hormones. *In vitro* plants were successfully acclimatized to a greenhouse and garden conditions. Potted iris plantlets flowered in the next season and produced seeds normally. The protocol we described can be used for rapid, large-scale micropropagation of this endemic iris species and may also serve as a good platform for the development of protocols for other *Iris* spp.

A simple and efficient protocol has been developed for regeneration and conservation of *Gloriosa superba* through microtuber induction from *in vitro* culture of apical and axillary buds. Ninety two per cent of the cultures of apical and axillary buds of young sprouts from naturally grown *Gloriosa superba* plants regenerated four shoots per culture in MS basal medium fortified with 1.5 $mg.l^{-1}$ BA + 0.5 $mg.l^{-1}$ NAA. Repeated subcultures in the same medium resulted in rapid shoot multiplication with an average of eight shoots per culture. The addition of 15% (v/v) coconut water (CW) and 2 $g.l^{-1}$ activated charcoal increased the number of shoots up to an average of 15 per culture. *In vitro* raised shoots rooted on half strength MS with 1.0 $mg.l^{-1}$ IBA + 0.5 $mg.l^{-1}$ IAA added as supplement. When rooting medium was enriched with 8% sucrose or rooted shoots were subcultured in MS medium with 8% sucrose an average of eight microtubers were induced per culture. Microtubers were harvested and could be stored at 25°C for one year. About 80% of microtubers germinated *in vivo* and morphological parameters of regenerated plantlets were found to be normal. This protocol is feasible for mass propagation as well as for conservation of *G. superba*.

Senno is a traditional ornamental plant and presently only thirteen strains have been confirmed to be under cultivation in ten localities in Chugoku and Kyushu districts of West Japan. Analyses on chromosome number and nuclear DNA contents using flow cytometry revealed that all of the strains are triploid (2n=36). The average of meiotic chromosome configuration (10.1III+1.9II+1.9I) observed in strain MS indicated that Senno is an autotriploid. Chromosome bridges, lagging chromosomes and micronuclei were observed in microsporogenesis. Although about 70% of pollen showed stainability with cotton blue solution, Senno plants conserved in Japan did not produce any viable seeds by self-pollination. A protocol for the micropropagation of triploid Senno was established by the axillary bud method. MS (Murashige and Skoog 1962) medium supplemented with 10 mg/l BA induced an average of eight shoots per node. Ploidy stability of these regenerants was confirmed by flow cytometric (FCM) analysis. The regenerants showed uniform flower characters such as color, shape and size, which were also the same as those of the original plant. However, plants were taller and flowered earlier than original plant. Node segments of *in vitro* plantlets of triploid Senno were irradiated with ^{12}C ion beams in a dose range of 2.5 to 50 Gy. After acclimatization, several mutations were observed in plants grown in a glasshouse. The expression of the hairless mutation was stable, but flower shape and variegated leaves were not evaluated after two years of cultivation.

Part 5 Functional biology, development and cellular biology

Carnivorous plants trap and utilise tiny animals by means of highly specialised trap leaves. The inorganic ions of their prey enable them to colonise habitats extremely poor in nutrients. Plant carnivory is restricted to a very specific combination of environmental factors including high light, poor soils, sufficient humidity and low competition. Here we summarise the features of carnivory including insights into the costs and benefits of the carnivorous strategy. Carnivorous plants exhibit a wide range of specific physiological features that are closely related to the trapping process and therefore cannot be studied on model organisms. We discuss the main properties including mechanical sensitivity, rapid movements mediated by action potentials like in animal neurons, protein secreting glands, foliar absorption of nutrients and drastic cytomorphological changes upon stimulation. Our knowledge on the functional genetics of carnivorous plants is still limited but recent research already enlighted the molecular background of prey utilisation. Physiological and molecular studies on carnivorous plants require availability of plant material grown under controlled conditions. The specific needs of carnivorous plants on the one hand and the particular requirements for molecular investigation on the other hand make tissue culture the method of choice. We present protocols for the establishment and maintenance of sterile cultures for most carnivorous genera and discuss the main results gained with this technique. Besides basic research, sterile culture of carnivorous plants is used for the production of pharmaceutical metabolites as well as for the preservation of endangered species.

Carnivorous plants have developed special mechanisms for trapping and digesting insects to provide essential nutrients when photosynthesis is limited. The mode of carnivory varies and includes adhesive traps (*Drosera* and *Pinguicula*), suction traps (*Utricular*ia), snap traps (*Dionea*) and pitchers (*Sarracenia*, *Nepenthes*). The latter (pitfall traps) have been shown to secrete hydrolases including proteinases, nucleases, phosphatases and chitinases into the pitcher liquid resulting in efficient digestion of the prey. Several genes encoding aspartic proteinases, nucleases, chitinases as well as transporters of amino acids, peptides and ammonium have been cloned from various *Nepenthes* species. At the initial stage of prey capture, chitin degradation products, resulting from the activity of constitutively expressed plant chitinases, induce the synthesis and secretion of additional chitinases and a β-1,3-glucanase. Ammonium, as the major absorbed nitrogen source, activates a plasma membrane H^+-ATPase whose activity as a proton pump acidifies the trap liquid thereby enhancing plant hydrolase activities and prey digestion. In addition to nutrition, carnivory also relies on the synthesis of secondary metabolites as a tool for self-defense against perturbing organisms. In this chapter we review the current knowledge about pitcher plants, their genes and secondary metabolites.

Calla lilies (*Zantedeschia* spp.), aroid plant species native to moist places of southern Africa, comprise commercially significant cut flowers. At present, these ornamental species belonging to the Araceae family consist of a multitude of cultivars that make significant contributions to floriculture industries. Each plant has an underground tuberous rhizome and bears a typical inflorescence consisting of a funnel-shaped spathe surrounding a central spadix. This leaf-like organ is responsible for the wide range of colours displayed by *Zantedeschia* cultivars. Economic interests in the production of hybrid cultivars have imposed improvement programs, not only for increasing resistance to the significant soft rot disease, but also for improving flowering and inflorescence development. Floral spathe development of the only one evergreen species – *Zantedeschia aethiopica* – has been well studied. The floral bud spathe undergoes a whitening process, which leads to its death when fructification does not occur. Contrarily, spathe whitening is inhibited and regreening is observed. It was shown that in spathe cells that undergo regreening, dramatic changes occur, namely the chloroplasts and peroxisomes restructuration, resulting in the re-acquisition of photosynthetic and photorespiratory capacities. Although less importance has been given to spathe whitening, recent reports considered this process as being a natural senescence-like process. The use of *Z. aethiopica* spathe development as a natural model for studying senescence may be valuable because the same organ undergoes senescence and self-inhibition of senescence after fructification, allowing the understanding of regulatory aspects of this developmental process.

KNOTTED1-like homeobox (*KNOX*) genes act in the shoot apical meristem (SAM) to provide an uncommitted population of cells for organogenic programs. In addition to playing a role in SAM function and leaf morphology, there is also evidence of the involvement of *KNOX* genes in some features of flower development. The expression pattern of *HtKNOT1*, a class I *KNOX* gene, in inflorescence meristems, floral meristems and floral organs of two related species, *Helianthus tuberosus* and *H. annuus* strengthens this view. *HtKNOT1* mRNAs have been detected in the inflorescence and floral meristems as well within lateral organ primordia (i.e. floral bracts, petals, stamens and carpels). In more differentiated flowers, the expression of *HtKNOT1* was restricted to developing ovules and pollen. Class I *KNOX* genes may play a dual role being required to maintain the meristem initials as well as initiating differentiation and/or conferring new cell identity. In the genus *Helianthus*, some *KNOX* genes could cooperate at the floral level with additional factors that more specifically control floral organs and pollen development.

The expression of antibodies in plants has several promising applications that are currently being developed. Plants are being considered for the large-scale production of antibodies needed for medical purposes. The benefit of using plants is that they are able to perform post-translational modifications of proteins that bacteria cannot, and antibody production in plants is less expensive than in mammalian cells. There is potential for antibodies expressed in plants to be used for phytoremediation by accumulating and concentrating a targeted pollutant within the plant or by binding and neutralizing a targeted molecule in the rhizosphere if they are secreted by the roots. Plants are also being transformed with antibody genes for protection against various plant pathogens. Effective virus resistance has been engineered in transgenic plants using antibodies that bind to the coat protein, nucleoprotein or RNA-dependent RNA polymerase of the targeted virus. A high degree of resistance to *Fusarium* has been achieved by combining a fungus-specific antibody with an antifungal protein. Because many of the plant pathogens affect numerous species of plants including many ornamental plant species, antibodies may be an effective approach for controlling pathogens of ornamental plants in the future. In addition to using antibodies in ornamental plants for disease resistance, there is the potential of using antibodies directed to plant hormones and enzymes for altering characteristics of ornamental plants. The ornamental plant industry is always interested in new plant forms such as flower color, increased flower scent, and changes in the plant's morphology. For example, plants with decreased height can be grown as container plants or in populated urban areas where there is less land available for the large shrubs and trees.

Posttranslational modifications are critical to the functions of many eukaryotic proteins. One key modification, the attachment of lipid moieties to proteins, is commonly used to facilitate membrane association and promote protein-protein interactions. This review covers three types of lipid modifications found in plants: prenylation, N-myristoylation, and S-acylation. While lipid modifications of plant proteins have been known for quite some time, in recent years much progress has been made in the identification of lipid modifying enzymes in model plants and in the characterization of mutants in these enzymes. Such studies have shown roles for lipid modification in a number of plant processes of broad interest, including meristem function, flower development and hormone responses. The number of proteins known to be lipid-modified has greatly increased, and with it our knowledge of the functional role of lipid modification in plants. While basic lipid posttranslational modification mechanisms appear to be conserved among eukaryotes, plants show important differences in the target sequences recognized, the types of target proteins present, and the interplay of different lipid modification enzymes.

A stoma is an epidermal pore, formed by a surrounding pair of guard cells, that by opening and closure regulates gaseous exchange and transpiration in response to environmental stimuli. Such stomatal movement is regulated by changes in guard cell shape and volume. As plant cell morphology is largely dependant on cytoskeletons and vacuoles, we describe in this chapter the cytoskeletal and vacuolar dynamics of guard cells during stomatal movement. Microtubules and actin microfilaments showed radial arrays in open stomata but these structures appeared to have positive and negative roles, respectively, during stomatal movement. Vacuolar structures became complicated in closed stomata and demonstrated luminal membrane structures that were continuous with the vacuolar membrane. The possible roles of these structures in stomatal movement are discussed, and critical points in the handling of plant materials for observation of stomatal movement are also described.

The first decade of plant proteomics research made great strike to the plant sciences. The ability to quantify and identify hundreads of protein in the analyzed sample opened wide doors to functional biology. The localization and correlation with quantitative information proposed function for theoretical proteins. Additionally, well-known proteins were identified in unexpected tissues and/or subcelular compartments that sugested new function for these proteins. Furthermore many unknown proteins has been identified suggesting missing pieces of biological puzzles. This review chapter sumarises proteomics efforts on model plant *Arabidopsis thaliana* up to 2008. Proteomics of whole tissue and subcellular compartments are the main area of interest. The effort to obtain complete proteome of various organelles confirmed old and proposed new functions. The whole tissue proteomics foccussed mainly on reproductive tissues such as pollen and seeds, besides investigation of mutants and ecotypes. Proteomics of plant stress responses attracted big attention due to ability of proteomics to generate protein maps based on two-dimensional electrophoresis. As conclusion, many proteins are still waiting for confirmation or discovery. Proteomics discoveries of new proteins might reveal new biological functions, which will help in genome annotation, and discovery of new biotechnological targets.

Recent advances in molecular biology have opened up unlimited possibilities for the construction of molecular linkage maps and for gene transfer across sexual barriers. A basic requisite in such work is a precise understanding of chromosome numbers and morphology. Cacti are a very important group of ornamental plants that require little attention to grow and have ample scope in genetic improvement through crossing for development of new cultivars or varieties with a lucrative market value. As a prerequisite for any breeding work, I provide a current update of meiotic and mitotic chromosome numbers in ~385 species belonging to 57 genera of cacti of the family Cactaceae. Chromosome numbers of n = 11 with 2x, 3x, 4x, 5x, 6x, 8x and greater polyploidy *Opunctia* showed a high level of ploidy despite the small chromosomes. Genome size estimates of 145 species of cacti through cytophotometry and flow cytometry (FC) are reported with a calculated value of nucleotide base pairs. 4C DNA content of *Astrophytum*, *Ferocactus*, *Echinopsis*, *Gymnocalycium*, *Mammillaria*, *Melocactus*, *Parodia* and *Rebutia* were determined with fulgen cytophotometry while *Opunctia* 2C DNA estimates were determined by FC using fluorescent dyes. To establish molecular phylogenetic relationships among different tribes and subtribes of cacti genetic markers like, isozymes, proteins, RAPD, cpDNA markers, *rbcL* gene were used to suplement the taxonomic classifications in large groups (*Mammillaria*, *Astrophytum*, *Echinopsis*, *Ferocactus*, *Molocactus*, *Parodia* and *Rebutia*). To established introgession of genetic materials of colour inheritance genomic *in situ* hybridization (GISH) was used to interpret intergeneric hybrids of cacti. This chapter provides vital information on cactus cytology, isozyme markers, DNA markers and molecular cytogenetic markers which can be used for a cactus breeding programme for intergeneric and interspecific hybrid development, particularly for the introduction of novel colours and other traits that would enrich its horticultural value.

Part 6 Orchids

38. Techniques and applications of *in vitro* orchid seed germination.
PJ Kauth, **D Dutra**, **TR Johnson**, **SL Stewart**, **ME Kane**, **W Vendrame**, USA **375-391**

In nature orchid seeds germinate only following infection by mycorrhizal fungi that provide the developing embryo with water, carbohydrates, minerals, and vitamins. Orchid seeds were first germinated at the base of wild-collected potted orchids, but germination was unreliable and seedling mortality rates were high. *In vitro* germination techniques, which were developed in the early 1900s, have resulted in more reliable germination and propagation of many orchid taxa. The earliest *in vitro* orchid seed germination techniques utilized mycorrhizal fungi found in nature to stimulate germination and seedling development. In 1922 Lewis Knudson germinated orchid seeds *in vitro* by sowing seeds on sterile nutrient medium amended with sucrose. This technique is known as asymbiotic seed germination since no fungal mycobiont is used to promote germination. For both symbiotic and asymbiotic orchid seed germination to be effective, many conditions must be addressed such as photoperiod, temperature, and mineral nutrition. In the case of symbiotic germination, another important factor is fungal compatibility. In recent years, the limitations that seed dormancy poses to the germination of orchid seeds have also been examined. In this chapter techniques and applications of asymbiotic and symbiotic orchid seed germination will be discussed in relation to photoperiod, temperature, nutrition, seed dormancy, and fungal mycobionts.

39. The anatomical basis of floral, food-reward production in Orchidaceae.
KL Davies UK, **Malgorzata Stpiczyńska**, Poland **392-407**

The publication of *On the Various Contrivances by which British and Foreign Orchids are Fertilized by Insects and on the Good Effects of Intercrossing* in 1862 generated great interest. In his seminal work, Charles Darwin sought to relate differences in the morphology of orchid flowers to the ways in which they are pollinated and fertilized. Since then, almost a century and a half have passed and enormous strides have been made in the field of orchidology. However, with the exception of comparative taxonomical investigations, micromorphological and anatomical studies of the orchid flower have been largely neglected. This is ironic given the importance of the flower in reproduction and ultimately, evolution. Recently, the dangers of using information based solely on floral morphology to elucidate the reproductive biology of orchids have been highlighted. However, a comprehensive, multidisciplinary approach involving reliable field records and, where possible, biochemical data still has the capacity to yield much useful information. Floral food-rewards occur in many orchid species and clearly play an important role in their pollination. To date, however, little is known about their production and evolution. Where information is available, it mostly concerns nectar formation and our knowledge of the ultrastructural changes that accompany the production of other floral food-rewards such as food-hairs or pseudopollen, resin-like secretions and floral oils is scant. The present chapter outlines what is currently known of the anatomy and histology of food-reward-producing tissues and explains how these contribute towards the evolutionary and reproductive success of arguably the largest family of flowering plants, the Orchidaceae.

40. Floral scent in *Phalaenopsis*. **Y-Y Hsiao**, **W-C Tsai**, **W-H Chen**, **H-H Chen**, Taiwan **408-420**

Floral scent is a key modulating factor in plant-insect interactions and thus plays a central role in successful pollination. Closely related plant species that rely on different insects for pollination produce different odors. *Orchidaceae*, containing more than 25,000 species, is one of the largest monocotyledon families. In orchids, the floral scents serve as attractants for species-specific pollinators that have played a major role in orchid evolution. The range of odors produced by orchids is enormous, providing an inexhaustible basis for specificity. Such diversity is advantageous in the evolution of an obviously successful family. Although volatile components have been identified in hundreds of orchid species, the biochemical and molecular biological knowledge of the biosynthesis of scent compounds is still limited. We have combined genomics and bioinformatics approaches to investigate candidate biosynthesis pathway and enzymes involved in the floral scent formation of orchid. These approaches demonstrate the opportunity to study the non-model plant such as floral scent in orchids and will accelerate molecular breeding in orchid industry.

41. *In vitro* precocious orchid flowering: a strategy for basic research and commercial approaches.
APA Vaz, **GB Kerbauy**, Brazil **421-426**

Despite the extensive literature on orchid plants, flowering studies are so far rather rare, mainly due to the relatively long time from sowing to flowering. An *in vitro* system became a convenient tool to study the switch-on of the flowering mechanism, as it allows for better control than *ex vitro* conditions and also can provide more obvious landmark events during development. Although *in vitro* flower formation has been observed in some plant species, its occurrence is relatively uncommon and poorly understood. In orchids, recent successes on *in vitro* flowering have shown that it is possible to shorten, to some degree, the relatively long juvenile phase, providing further insights into the flowering process of these plants, including environmental, physiological, genetic and molecular aspects. However, most of the published papers on this matter are just concerned with the description of flowering, while the events that trigger the process are poorly studied. The main goal of this chapter is to synthesize and discuss the available knowledge in the field of *in vitro* flowering of orchid plants during the last decade.

In the present study, the effects of medium composition (nutrients, carbohydrates and hormones), photoperiodism and temperature on *in vitro* flower formation of *Psygmorchis pusilla* were investigated. Low nitrogen concentration and high concentrations of potassium and calcium in the culture medium favoured floral development, with a preferential requirement for ammonium. A positive correlation between flowering and sucrose, glucose or fructose at different concentrations was also detected. The presence of BA in the culture medium stimulated flowering, but floral buds developed abnormally. Endogenous levels of Z, ZR and iP increased during the initial development of the floral spike. Long photoperiods did not affect plant growth and leaf number, but enhanced floral spike development. However, flower anthesis did not occur under photoperiods longer than 20 hours. The most favourable temperature for *Psygmorchis pusilla* growth and flower formation *in vitro* was 27°C. The events of resumption of floral buds, growth of floral spikes and floral bud anthesis were associated with different optimal media composition, temperatures and photoperiods.

The potential role of endophytes and their biologically active metabolites in its association with their host has been briefly studied in order to explore an increase in the efficiency of *Cremastra appendiculata* (D.Don.) Makino (Orchidaceae), an ornamental orchid, micropropagation. We established an effective method for endophyte isolation, identification, cultivation and preparation of endophyte extract and subsequent component analysis from the orchid explants. We found that endophyte extracts had a significant positive effect in the improvement of *in vitro* tissue culture and micropropagation of this orchid.

Part 7 Greenhouse environments, soil and pathology

Greenhouse production is becoming increasingly dependent on technology. The total area of greenhouses, including ornamentals, is increasing and consumers are demanding higher quality produce. The greenhouse industry is responding to these challenges through the adoption of new greenhouse structures, advanced environmental modification technologies and innovative greenhouse management practices. The role of key greenhouse climate parameters of light, temperature, relative humidity and carbon dioxide are now well appreciated. The manipulation of the greenhouse environment to achieve optimum growing conditions that will produce sustainable outcomes is a significant challenge. Plastic coverings have continued to be developed in the key areas of strength, durability and light transmission. Spectral modification has the potential to provide enhanced production environments. The supplementary heating of greenhouses to achieve out of season production is a strategy commonly employed in ornamental crops. There are numerous technologies available to produce heat and deliver it into the greenhouse. The greenhouse industry is investigating alternative heat sources including solar, phase change materials (PCM), cogeneration and geothermal heat sources. Energy conservation strategies and techniques are continuing to be developed. The improved ventilation of naturally ventilated greenhouses has been an important technological development. Achieving effective ventilation in greenhouses fitted with insect screens is continuing to be addressed and is still a challenge in warm climates. Temperature reduction through evaporative cooling is now widely used. Techniques include sprinkling, fan and pad systems and fogging. The hot dry conditions of some greenhouse regions are well suited to the combined use of fogging systems in conjunction with naturally ventilated greenhouse structures. The management of the greenhouse environment is becoming more important in producing quality ornamental crops. Optimization of all of the greenhouse crop production inputs, including technology, is fundamental to sustainable ornamental greenhouse production.

Mycorrhizal symbiosis is an important natural phenomenon of plant and soil fungi interaction. The beneficial biofertilizing and biocontrolling effects of this ubiquitous symbiotic relationship between soil-borne mycorrhizal fungi and most vascular plant species have valuable potential to be used as an economical, sustainable and environmentally friendly technology for the production of numerous horticultural and floricultural crops. Theoretical aspects of the functions and mechanisms of the most common types of mycorrhizal symbioses associated with horticultural and floricultural crops are described. In horticulture and floriculture the greatest potential of mycorrhizal inoculation is, among others, its use in the treatment of micropropagated plants, thus increasing their performance after transplanting. Other potentials include the use of mycorrhiza in vegetable production or in the production of plant stock transplanted into harsh field environments. Mycorrhiza plays an important role in sustainable cropping systems and is able to increase growth, yield and uniformity of horticultural crops as well as to decrease agrochemical inputs during production. Examples of various nutritional and non-nutritional effects of symbioses on various horticultural crops are given. The potential of implementing mycorrhizal technology (targeted inoculation of cultivation substrates) and aspects of industrial production and commercial application of mycorrhizal inoculants and the benefits of introducing mycorrhizal biotechnology are discussed.

46. *Rhodococcus fascians*, an emerging threat for ornamental crops.
S Depuydt Belgium, **M Putnam** USA, **M Holsters**, **D Vereecke**, Belgium ……………………………………………………………… **480-489**

The actinomycete *Rhodococcus fascians* is a biotrophic pathogen that is capable of deregulating plant development and provoking the formation of multiple shoots. Naturally occurring infections have been reported for 43 families of mostly herbaceous plants. Because of its broad host range and its capacity to disfigure plants, *R*. *fascians* causes important local losses to the ornamentals industry. As global movement of plants is expanding and no efficient eradication measures are available, *R*. *fascians* infection is becoming an emerging threat to herbaceous nurseries worldwide. To facilitate detection and identification of possible targets for control procedures, several fundamental aspects of the interaction of *R*. *fascians* with model plants have been studied. Elucidation of the colonization strategy and the early steps of the interaction might shed new light on the epidemiology of the disease, whereas elaborate knowledge on the virulence determinants of the bacterium might allow new diagnostic tools to be developed. On the other hand, evaluation of the plant response to *R*. *fascians* infection might yield fundamental insights into plant growth and meristem formation. Here, we present an overview of the current in-the-field and primary knowledge available on this plant-pathogen interaction.

47. Assessment of fertility of virus-tolerant transgenic chrysanthemum and survey on viruses in wild chrysanthemum populations in Western Japan. **T Toguri**, **N Umemoto**, **M Yoshioka**, **O Nawata**, **M Okamura**, **K Taniguchi**, Japan ……………………………… **490-495**

We have produced transgenic chrysanthemum (*Chrysanthemum morifolium* Ramat.) expressing double-stranded RNA-specific ribonuclease, and found that this enzyme conferred dual-tolerance against both *Chrysanthemum stunt viroid* and *Tomato spotted wilt virus*. In order to cultivate these transgenic plants in the field in Japan, however, we need to pre-evaluate safety aspects of these plants, especially concerning the effect on the surrounding environment caused by pollen spread from transgenic plants to wild relatives. There is an argument that virus-tolerant transgenic plants might retain a selective advantage compared with wild plant species during the course of competition under pathogenic selective condition, and that this might eventually change the natural habitat of the wild species. To know whether this kind of selection could operate under natural conditions, we assessed the fertility of transgenic plants with wild relatives under greenhouse condition, and the pollen spread in the simulated field. Next, we surveyed the distribution of plant viruses and viroid in the populations of wild chrysanthemum species, *C*. *indicum*, and the formation of hybrid plants between cultivated and wild species as a model case in western Japan. Surprisingly, we found a lot of wild populations were infected by *Chrysanthemum virus B* in several prefectures facing the Sea of Japan. Virus infection did not seem to cause any phenotypic change in infected plants.

48. Ornamental *Limonium* grown in Mediterranean conditions. **J López, A González**, Spain ……………………………………………… **496-503**

Several species of the genus *Limonium* form an important part of the group of plants used for complimenting flower arrangements. An important aspect of these plants is dense multi-flowering and great inflorescences. Their tolerance to salinity and types of soil means that they are favoured for introduction into areas with a Mediterranean environmental profile. This environment supplies nearly all the thermal needs of the plant. The great demand for these different species especially for *L. sinuatum* and *L. latifolia*) has meant that large scale production has been vegetative, originating from meristems. Consequently *L. sinuatum* which was traditionally reproduced from seeds is now generally produced by cloning carried out in *in vitro* cultures. The development of new hybrids which improve their agronomic and ornamental qualities has also been a factor in using this form of reproduction. The shortening of the growth cycle due to winter cultivation demands additional husbandry practices such as training on mesh; this enables the plant to be kept erect, being gradually strengthened by the addition of adequate top fertilisation. Phytosanitary problems such as rot caused by botrytis affects the quality of production; therefore ventilation factors should be taken into account. The yellow varieties and to a lesser extent the white need to be improved in order to increase their productivity.

49. Introduction of ornamental *Asparagus* spp. to semiarid climates. **J López, A González**, Spain …………………………………… **504-509**

In recent years there has been a gradual increase in the importance given in ornamental floriculture to so-called cut foliage which is used to improve the appearance of cut flower arrangements. These complementary plants can have very different edaphoclimatic needs and consequently need different cultivation infrastructures. Many areas of the Mediterranean coast have a climatic profile particularly suitable for the production of some of these complementary flowers such as asparagus fern. Any deficiencies in the environment can be resolved by means of auxiliary systems using existing technology. This type of plant needs special thermal and luminous needs to achieve continual production throughout the year. The first need is satisfied by the environment of the cultivations themselves. The plants reduced need for light can be achieved using passive systems of shade. Sandy soils produce optimum growth but corrections can be made in areas where clay soils are a limiting factor. Experiments have been carried out on adapting different species with different morphology of plumes and with greater resistance to the shedding of leaves with the intention of providing a greater diversification of the plants on offer for the market. Other aims of this chapter are to examine technologies of cultivation to: shorten the period until beginning of production; to adapt to integrated production; and to prolong the useful life of the plants.

Part 8 Other ornamental biotechnological advances

Having a great genetic diversity, shape and color, *Narcissus* is a bulbous ornamental geophyte scattered from Europe to East Asia. Beside their ornamental value, *Narcissus* species produce alkaloids which can be used for the treatment of neurological diseases such as Alzheimer's by biotechnological approaches. The main issue in commercializing this plant is its very slow natural vegetative propagation rate. Manipulated vegetative propagation approaches such as chipping and twin scaling are time consuming and usually altered with degeneration and virus and disease infection. Therefore producers are led to micropropagation techniques which are more productive and efficient. In this chapter many biotechnological applications for *Narcissus* such as *in vitro* culture, somatic embryogenesis, gene transfer and molecular markers besides mutation induction, virus-free stock production and cytogenetics of *Narcissus* are discussed.

The genus *Alstroemeria* belongs to the family Alstroemeriaceae and comprises many ornamental species. This genus, including more than 60 species, is indigenous to South America. Thus far, numerous cultivars, which are used as cut flowers and potted plants worldwide, have been produced by interspecific hybridization and mutation breeding. Recently, biotechnological approaches are being applied in order to improve *Alstroemeria* strains. Interspecific hybrid plants have been produced by ovule cultures. By improving certain culture techniques, sexual incompatibility was overcome in some cross combinations using ovule cultures. Plant regeneration systems that involved the use of explants, immature ovules, leaves, etc., through callus cultures have been reported. Isolation of protoplasts and cultures resulting in plant regeneration were achieved by using the embryogenic callus. Particle bombardment and *Agrobacterium*-mediated procedures were applied for genetic transformation, and some transformed plants with marker genes were produced. The procedure of *in vitro* fertilization using single isolated gametes has been developed to study fertilization and early zygotic embryogenesis in higher plants. This technique will also be utilized as a novel strategy in plant breeding for inducing the fusion of gametes obtained from distantly-related incompatible species and for achieving direct gene transfer into isolated zygotes. In the case of *Alstroemeria*, isolation of egg cells and zygotes from ovules has been attempted in order to develop an *in vitro* fertilization technique.

The Amaryllidaceae (daffodil family) of subclass monocotyledonae includes perennial herbs from a bulb with contractile roots, comprising 86 genera and 1300 species. Consisting mostly of bulbous plants, this family occurs naturally throughout the tropics and warm temperate regions of the world. Many species have extravagantly lovely flowers and are highly prized garden plants. A large number of species in several genera are widely cultivated for their attractive flowers. These include species of *Boophone* Herbert, *Crinum* L., *Haemanthus* L., *Galanthus* L., and *Pancratium* L. The genus *Narcissus* L., which includes the popular daffodils, narcissi, and jonquils, is very extensively cultivated. Besides, some members of this family are also used for obtaining natural compounds, including alkaloids and lectins in particular, with desired biological activities important for human health. Because of the medical magnitude of their secondary metabolites, some plant biotechnology methods such as tissue culture, micropropagation, and molecular cloning have been employed for production of bioactive compounds from the species of Amaryllidaceae. The intent of this chapter is to summarize this type of studies performed on a number of genera belonging to this family.

In North European countries floricultural production in greenhouses is highly controlled and intensive. However, instruments for adequate planning and quality control are still insufficient and need to be improved to face market demands. To make full use of the available technologies for providing the desired environment for a target product quality and delivery date, crop models are essential. Although modelling product quality is of special importance in floricultural production, including quality attributes in crop modelling, has not been given deserved importance. This chapter provides an overview of the models that have been developed for predicting visual quality attributes (e.g. stem length, number of flowers per plant, flower size) and time to flowering of cut flowers and pot plants. The importance of controlling and predicting visual quality is emphasized. Different types of crop growth models and their limitations are presented and several examples of existing visual quality models for ornamental plants are given. Stem length and time to flowering are the attributes that have received most attention from modellers. We conclude that in the last decade more effort has been put in modelling visual product quality. Recent developments and future trends include functional-structural plant models (FSPM; combining process-based models with architectural models) and incorporation of genetic information in crop models. Industry is showing interest in the application of quality models but the large diversity in product attributes and the need for species-specific parameterisation have been a major constrain for their practical implementation.