

Floriculture, Ornamental and Plant Biotechnology

Advances and Topical Issues

Volume I

Edited by

Jaime A. Teixeira da Silva

**Floriculture, Ornamental
and
Plant Biotechnology
Advances and Topical Issues**

First Edition

Volumes I-IV

Edited by

Jaime A. Teixeira da Silva

Department of Horticulture, Kagawa University, Japan

Floriculture, Ornamental and Plant Biotechnology: Advances and Topical Issues is a network of review articles, research and original papers, and a compendium of opinions and techniques that deals with the most important issues in the forefront of Floriculture and Ornamental Biology, Plant Science and Biotechnology. The book contains contributions from leaders at internationally reputed research institutions, universities and independent scientists who are at the forefront of research in issues and topics that affect and influence all plant scientists. The book also encompasses fields that are usually not associated with floriculture, and attempts to find, in the reader and in the book, potential links between inter-disciplinary topics of research, written by eminent scholars on various advanced aspects of floriculture and plant biotechnology. Flower-related topics include: research tools for floriculture biotechnology; flower colour and chimerism; manipulation of flower colour and shape; modification of plant and flower architecture and fragrance; molecular cloning, floral volatile analysis, scent-pollinator interactions; micro- and macro-arrays for floriculture; genomics, proteomics, and transcriptomics for ornamental and floricultural biotechnology; role of volatiles emitted from flowers and leaves; floral and leaf senescence and genetic engineering of ethylene-insensitivity; regulating growth of floriculture crops; light, lighting, photosynthesis and flowering; circadian clocks; domestication/introduction of novel ornamental crops; improvement of post-harvest shelf life of flowers, post-harvest techniques and quality control; standardization of cut foliage and dry flower production technology; germplasm collection and maintenance *in vitro* and *ex vitro*; *in vitro* thin cell layer technology, embryogenesis, synthetic seeds and cryopreservation; agrotechniques for improved flower crop production and analysis; nursery stock and ornamental foliage and pot-plant production; aeroponics, hydroponics, micro-irrigation and water optimization, environmentally-friendly and organic farming for ornamentals; *Agrobacterium* and viral expression vectors; classical and insertional mutagenesis; plastid, chloroplast and protoplast transgenics; commercialization of genetically modified plants; programmed cell death, transgenic expression mechanisms and silencing; genetic and metabolic engineering for drought-tolerance, pest resistance; pollen biology; analysis of ethylene signal transduction; plant stress, polyamines and signalling; integrated pest management (IPM) and control (IPC) strategies and effective quarantine measures; viruses and viroids, their resistance strategies and detection; weeds and ornamentals; plant-plant interactions and allelopathy, plant-microbe, plant-insect, plant-pest interactions; soil-water and soil-root/plant interactions and soil management strategies; greenhouse and protected structures and dynamics, management strategies, mechanization and robotics; ornamentals in landscaping and gardening; impact of the floriculture and ornamental industry and plant biotechnology in reducing poverty and hunger in developing and under-developed nations; cut-flower, ornamental plant, foliage and pot-plant economics and markets; certification, plant protection and patents; ornamental and floricultural ethics, and people-plant interactions; floriculture and its future in both science and society.

In addition, the book takes on a unique flavour by incorporating techniques commonly used in other plant species or other sectors of non-plant biotechnology, and examines its potential use in floriculture and plant biotechnology: space research, nanotechnology, allelopathy, mutagenesis, plants for revegetation and bioremediation, rare sugars, secondary metabolite production, ethnobotany, and herbs and medicinal plants as ornamentals and in cuisine.

This book series will be essential in the exposure of modern techniques and themes for undergraduate, postgraduate and research students of floriculture, plant science and plant biotechnology, as well as for scientists and policy makers involved with genetically-modified organisms, floriculture and the plant biotechnology industry.

This book series will take Flower and Ornamental Plant Biotechnology to a new frontier.

Jaime A. Teixeira da Silva



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FOREWORDS TO VOLUME I

Matyas Buzgo, Department of Botany, University of Florida, USA

The title of the book is almost misleading: it implies that most of the reviews deal with applied and commercial aspects. However, in **Volume I**, many reviews deal with fundamental science, involving developmental genetics, genomics, and physiology. This shows that, while commercial interests long for designed crops, ornamentals, or biosyntheses, many of the fundamentals still are not understood. However, in some cases amazing progress has occurred, and the flow of knowledge is by no means a one-way street.

Fundamental research in life sciences is strongly based on model organisms, most famous in plants, *Arabidopsis thaliana*, or as in one interesting example, green algae (*Chara* and *Cladophora*), which have a very restricted market. However, some fundamental research reviewed in this volume was conducted on important horticultural and crop plants, such as tulip, petunia, carnation, periwinkle, and grasses. For all life science, this broadens the availability of comparative data, allowing the assessment of the predictive power of fundamental concepts, and includes new aspects that previously could not be covered.

Further, the strong commercial interest behind applied research drives the development of new methods, which eventually will be available for fundamental research. For example, much of the study of odour described in this volume is backed by the perfume industry. Interestingly, the ability to collect and analyze odour compounds, and to understand their biosynthetic pathway is of high interest for ecological and evolutionary research as well, particularly in collaboration with population genetics (differentiation of strains and cultivars) and genetic regulation (transcription factors). Also, the connection of phytohormones to the administration of chemicals is quickly understood. However, the understanding of gene signaling pathways, involving genes responsible for cell fate and transcription factors, is closing in on phytohormones (namely ethylene and auxin), and the study of "new" or "unusual" forms helps to understand the evolution of land plants at large.

Therefore, nobody should be misled by the title. Applied researchers, be prepared to find articles that reach beyond cultivation, deep into the phenomenon of life on Earth, actually. Fundamental researchers, find what connections and methods have been found, and join the quest. Everybody, this compilation of review articles offers you the chance to read "what others do" in a context of your own research.

JS (Pat) Heslop-Harrison, University of Leicester, UK

Ornamental plants and cut flowers make a significant contribution to quality of life: something of natural beauty to be surrounded by, bringing biodiversity into the home or commercial environment. They have enormous economic importance, and, particularly when compared to the commodity crops, great potential for wealth creation and adding value by growers, whether smallholders selling to local markets or large companies exporting millions of plants or inflorescences.

These four volumes demonstrate the global impact of biotechnology (including genomic, molecular and physiological sciences) on plants selected and grown for this industry. Notably, they also cover the future directions, although I would loath to predict which of the chapters will impact the future of the industry the most, whether in the species that turns out to be most import, or in the biotechnology, or agronomy. The volumes are admirable in giving weight to discoveries in model species which can certainly be transferred and applied in floriculture. But we should also note that several significant breakthroughs in plant research in the last 20 years have come from species at the focus of this book - floral development, mutations, biodiversity exploitation and epigenetics, not to mention technology developments such as tissue culture, use of secondary products and other metabolites.

As the individual sections indicate, biotechnology impacts every part of floriculture. First, wild germplasm must be found and evaluated, before breeding, selection and perhaps modification or hybridization. Then propagation is required, often through tissue culture, before final growth of the product where characters of the item sold in terms of quality, shelf-life and consumer appeal become critical. Everyone will have noted the impacts of technological progress on every aspect of these, as well as the globalization of the horticulture market in the last decade. It is not unusual for a plant to visit three or four countries before final sale, with tissue culture in one country, growth in another, before wholesaling and transport to the final shop. The worldwide representation of authors in these volumes and the detailed discussion of the technological challenges presented here make this work a key reference for floriculture and ornamental plant research.

Denis J. Murphy, Professor of Biotechnology, University of Glamorgan, Wales, UK

The human manipulation of plants via agriculture and horticulture is the basis of our urban civilization. This process started when Neolithic hunter gatherers first began the process of crop domestication more than ten thousand years ago. For much of the ensuing millennia, plant manipulation has proceeded via a largely empirical process of selection and breeding that was uninformed by scientific knowledge or principles. The application of the scientific study of plant growth and development to crop improvement began gradually after the Renaissance, and was greatly accelerated by the rediscovery of Mendelian genetics in the early 20th century. As we move into the 21st century, new vistas of opportunity are opening up for the deployment of a host of new technologies for the improvement of an ever-widening list of crops. We are now moving beyond our previous focus on the major commodity staples, such as wheat, rice and maize, to being able to apply advanced breeding methods to a host of other useful plants, ranging from ornamental and medicinal species to plantation and forestry plants of

all shapes and sizes. In advancing this important project of applying the latest knowledge to practical use, it is important that the wider botanical community, both academic and commercially oriented, should have access to the latest scientific and technological developments. That, in a nutshell, is the ambitious task that has been set by the authors of this series of books on floriculture, and ornamental plant biotechnology.

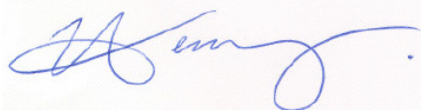
These volumes contain an impressive list of chapters from over 400 world authorities from 54 countries and the editor is to be commended in the doubtless challenging task of assembling and coordinating such a diverse array of talent. The subject matter of the various volumes covers a wide range of topics across the field of floriculture and allied areas. One innovative feature is the inclusion of focussed chapters with original data in specialised areas, as well as more conventional review chapters that give a more general overview of wider subject areas. The mixture of styles and content makes for a diverse and interesting potpourri of reading. The aim is to inform and update readers on the most recent developments that have the potential to influence and stimulate work on ornamental plants over the coming decades. The various chapters focus on new techniques and cutting-edge discoveries in plant biotechnology, as well as the refinement and improvement of existing tried and tested methodologies. These volumes should appeal to a broad audience ranging from undergraduate and graduate students to both academic and commercial research specialists at all levels. I wish this worthy project well and hope that it will not only inform and stimulate practical efforts to improve ornamental plant species and varieties, but will also stimulate a new generation of students and researchers to enter this dynamic and increasingly fast moving field of applied plant science.

Denis J Murphy

Denis J Murphy received a D Phil in plant biochemistry from the University of York, UK, in 1977. He spent four years as a Fulbright Scholar at the University of California, Davis, and Royal Society Postdoctoral Fellow at the Australian National University, Canberra. He has authored or edited more than 250 research articles and books, including the *Encyclopaedia of Applied Plant Science* (2003), *Designer Oil Crops* (1994), and most recently *Plant Breeding and Biotechnology, Societal Context and the Future of Agriculture* (due to be published by Cambridge University Press in late 2006). He has been involved in research and international consultancy in molecular biology and crop breeding since 1985, and was Head of the Brassica and Oilseeds Research Department at the John Innes Centre, Norwich from 1990-2000. He is currently Professor of Biotechnology and Head of the Biotechnology Unit at the University of Glamorgan in Wales, UK.

Leon A. Terry, Cranfield University, UK

The appearance, quality and longevity of cut flower species depend not only on genotype, but also on conditions of cultivation, correct harvest time and appropriate postharvest handling. Many of the postharvest challenges that face the cut-flower industry and the scientific community remain. However, since the seminal works by Halevy and Mayak, postharvest research of cut flowers and ornamentals has tended to be largely overshadowed by fresh produce research. This book, thus, represents an impressive set of chapters that helps to redress this balance and highlights the progress and future of cut-flower postharvest research. Specific chapters on ethylene, abscisic acid metabolism, senescence, floral scent biochemistry, development of vase-life solutions and general cut flower physiology and handling are of particular interest. The breadth of contributions from, for example, transgenic manipulation of vase-life to more applied issues centred on cut-flower longevity, will no doubt appeal to a broad audience ranging from academic to industrial research specialists alike. Accordingly, I wholly support this book and believe that it will act as a catalyst to encourage wider participation by the commercial and academic community in ameliorating and/or circumventing the problems that still afflict cut-flower quality after harvest.



Leon A. Terry received his PhD in postharvest technology from Cranfield University, UK. He is currently the Head of the Plant Science Laboratory at Cranfield University, which is the largest UK university-based group dedicated to research, consultancy and education in postharvest technology of fresh produce and cut-flowers. He is convenor of the Postharvest Biology Group within the Association of Applied Biologists and recipient of the Nuffield Foundation Award for New Lecturers in Science, Engineering and Mathematics 2005.

Radomíra Vaňková, Academy of Sciences of the Czech Republic, Czech Republic

Due to their sessile character, plants had to evolve some unique features. Apart from the ability to fix solar energy into the form of chemical bonds (usable also for other organisms); very important to the plant character is enormous plasticity and dynamicity of their metabolism. Plants produce a wide range of secondary metabolites, which enable them to interact with the environment. The function of these metabolites differs from repulsion of herbivora (phytoalexins) to attraction of potential pollinators (fragrances). Because of the lack of the possibility to move from the site of the stress, plants evolved different strategies to minimize the negative impacts of the stress. The elucidation of the function of "compatible solutes", important components of the plant defence system, may contribute to the understanding of the stress response mechanisms. This knowledge is necessary for the establishment of a suitable strategy for the increase of stress tolerance. A specific feature of plants is the existence of the cell wall. Regulation of its rigidity/loosening by galactoglucomannan oligosaccharides may have a significant impact in the control of cell growth. Recently a lot of attention has been paid to lipid biosynthesis and function. All these topical issues are covered in depth in the chapters within this section.

The vast variety in size and shape of plants requires an integration of shoot and root growth and development. The

important role of communication between these two organs is played by plant hormones, especially auxins and cytokinins. The primary sites of cytokinin biosynthesis are roots, while those of auxin are shoot meristems, especially the apical one. Both hormones are indispensable in the progression of cell division. Regulation of cytokinin signal during its way from roots to the site of action seems to involve cytokinin dehydrogenases. As hormones control plant behaviour and development, understanding of their mode of action could enable formation of an efficient strategy how to affect their performance in desired way. The recent advances in plant metabolism and hormone research are highlighted in this section of the book, with specific applications to ornamental plants.

Radomíra Vaňková: **Education:** RNDr.: (equivalent to M.Sc.) Biochemistry, Faculty of Natural Sciences, Charles University, Praha; CSc.: (equivalent to PhD), Czechoslovak Academy of Sciences, specialisation: plant physiology. **Position:** Senior scientist at the Institute of Experimental Botany, Academy of Sciences of the Czech Republic, Praha, Czech Republic. **Field of research:** Cooperation and mutual relationships between cytokinins, abscisic acid and auxins in regulation of plant development and stress response; cytokinin metabolism, especially glucosylation; methods of cytokinin and abscisic acid analysis. **Publication activity:** Author and co-author of 27 scientific papers.

Nedyalka Zagorska and N. Tyankova, Bulgarian Academy of Sciences, Bulgaria

This book series is very useful for scientists and persons related to the field of plant biology and biotechnology. It contains contributions from Internationally-reputed research institutions, universities and independent scientists who are highly qualified in all aspects of plant biotechnology. The book series provides a broad view of the subject, and although it covers fields that are usually not associated, an attempt to find potential links between interdisciplinary topics on various advanced aspects of floriculture and plant biotechnology has been made.

In addition, in the book series techniques and important results in other plant species and other sectors of biotechnology have been incorporated for potential use in floriculture and plant biotechnology: space research, nanotechnology, allelopathy, mutagenesis, and herbs and medicinal plants as ornamentals, among others topics.

In a number of chapters, special attention is paid to the most modern technologies and mainly to the cultivation of cell and tissue culture *in vitro* and molecular biology as their base.

Recently tissue culture or cultivation *in vitro* has found a growing application in many fields of biology. The great interest shown towards this method lies in the advantages of the simpler model systems of tissues and cells, which exist *ex planta*, and which make the study of current physiological, biochemical and genetic problems possible.

At present, not only are tissue cultures utilized for resolving some fundamental tasks of biology (such as differentiation of cells, regulation of morphogenesis and metabolism, mechanisms of tumor formation, and genetic transformation), but they are also used for solving practical genetic and plant breeding problems (for example overcoming incompatibility barriers and sterility limitations of interspecific hybrids, allowing pathogen-free plants in vegetable and ornamental species to be obtained, accelerating the processes for creating new cultivars: haploid induction from anther cultures following chromosome duplication, and the development of plants resistant to biotic and abiotic stresses, and other horticulturally and agronomically important traits.

Biotechnological methods, which supplement conventional breeding methods, have been developed, allowing new plant genotypes of greater quality and productivity to be obtained. These are extremely valuable today because of the high contamination levels in the environment caused by insecticides, herbicides and artificial fertilizers. Plant cell culture techniques also allow the isolation of mutant cell lines and plants, the generation of somatic hybrids by protoplast fusion, and the regeneration of genetically engineered plants from single transformed cells.

The progress of plant biotechnology and plant molecular biology is acceleration and reaching new areas. This offers unprecedented opportunities for the genetic manipulation of plants and for their improvement. The potential possibilities of these powerful biotechnologies contribute to the rapid expanse of the biotechnological industry.

New technologies bring new options, innovative openings and new potentials, but they can also lead to undesirable reactions, to restructuring of production and processing and to market dislocations. That is why they must be suitably targeted, adapted, tested, scaled up and accepted by the public. This book series, because of its wide scope, should help in achieving greater appreciation of the potentials of the biotechnologies and wider acceptance of their applications.

The book can also serve as a very good reference for one involved in the diverse areas of biology and plant biotechnology in general and in their use in floriculture, ornamental and plant biotechnology. The chapters are written by leading authorities in their fields. Although the book has a wide scope, it is well balanced and stresses the basic underlying principles and methodologies that are common to the diverse organisms covered. The book can also serve as a source of guided reading for advanced students in courses on biology and biotechnology. It can be highly recommended to research institutes, researchers, undergraduate, postgraduate and research students of floriculture, plant science and plant biotechnology, as well as to commercial biotechnological organizations, and to scientist and policy makers involved with genetically – modified organisms, floriculture and plant biotechnology industry.

This book will take floral and ornamental plant biotechnology to new frontiers.

Nedyalka Aleksandrova Zagorska (D.Sc.) is at the Institute of Genetics, Bulgarian Academy of Sciences (BAS), where she has held the position of Head of Department of Cell and Tissue Cultures, Sofia, Bulgaria since 1990. She obtained her PhD at the Institute of Genetics, Academy of Sciences, Moscow, former USSR, and held positions as a Research and Senior Research Scientist at the Institute of Genetics, BAS, until she obtained her DSc in 1995, and has been a full Professor since 1997. She is a member of several International Societies, has published over 200 articles and has managed 22 National and International collaborated research grants.



ABSTRACTS: VOLUME I

Structure, Metabolism, Development, Physiology and Genetics



Part 1 Flower growth, development, patterning, senescence and death

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1. **Genetics of floral development and patterning.** MP Running, USA 1-11

Flowers are valued both for their beauty and their economic importance, as flower parts form the major source of food for both humans and animals. Flowers also present an intriguing model for biological pattern formation: a small group of flower meristem cells gives rise to several different organ types in stereotypical positions in rapid succession. The use of molecular genetics approaches in the study of flower development has led to a series of insights into the biological mechanisms of generating flower patterns. This review focuses on the current knowledge of the genetic basis of flower patterning in *Arabidopsis thaliana*. Flower patterning requires several steps: the specification of lateral meristems as flowers instead of shoots, the establishment of proper floral organ identity, and the initiation of organs in correct number and positions. Floral meristem identity genes integrate environmental cues to establish a flower program, including the activation of flower homeotic genes, which specify sepal, petal, stamen, and carpel formation. Organ initiation occurs largely independently of organ identity and requires proper control of flower meristem size, proper regulation of cell division orientation, and spacing mechanisms that remain poorly defined. Future challenges include identifying additional genes involved in establishing the flower pattern, along with identifying more genes that act downstream of patterning genes to confer the final size and shape of flower organs across diverse species.

2. **The utility of snapdragon (*Antirrhinum majus*) to study flower development and coloration.**
N Efremova, R Castillo, C Navarro, Germany 12-24

The flower of the common ornamental snapdragon (*Antirrhinum majus* L.) and its mutant forms have been subject of study since the middle of the 19th century when its advantages as an experimental system were first recognised. In recent years, *Antirrhinum* has been one of the plant organisms contributing to the understanding of basic biological processes such as flower initiation and formation. *Antirrhinum* genetics combined with molecular approaches have enabled the cloning of numerous genes with a role in different aspects of flower development like the control of floral-organ identity, floral symmetry, inflorescence architecture and colouration. Newly developed research tools now allow studies of gene expression profiles during different stages of development and genes can be analysed in their genomic context. Comparison with *Arabidopsis* reveals conservation and divergence of gene functions leading to the formation of a basic floral structure. Uncovering peculiarities in these processes in different species demonstrates the usefulness of using different model systems to explain natural variability.

3. **Molecular mechanisms of hormone functions in flowering.** H Yu, T Dhavale, Singapore, S Yang, China 25-32

Flowering in plants involves several sequential developmental programs from floral transition to floral organ development. In most species, the shoot apical meristems initially generate primordia that develop into vegetative organs, and are reprogrammed to give rise to floral primordia during the transition to flowering. These floral primordia can differentiate into flowers with different whorls of floral organs during flower development. Although striking progress has been made in understanding of flowering mechanisms in recent years, there are still many unanswered fundamental questions. The function of plant hormones (phytohormones) in flowering process is one of these puzzles. The phytohormones, including abscisic acid, auxin, cytokinin, ethylene, gibberellin acid (GA), brassinosteroid, and jasmonic acid, are small molecules affecting a wide range of developmental programs in plants. Some of these phytohormones, such as GA, show significant impact on the control of flowering time and floral organ development. In the last several years, molecular genetic studies in the genetically facile model plant *Arabidopsis thaliana* have provided significant insights into the molecular basis of various roles of phytohormones in plant development. Here we summarize the recent advances in our understanding of molecular mechanisms of hormone functions in flowering, with the emphasis on the GA, cytokinin, and auxin pathways.

4. **Florigenesis in flower bulbs: classical and molecular approaches.** MA Flaishman, R Kamenetsky, Israel 33-43

Flower bulbs belong to numerous botanical taxa and show remarkable diversity with regard to morphology, developmental biology, genetic control and response to the environment. In this chapter we review the current data on morpho-physiological and biochemical aspects of the transition of bulbous plants from the vegetative to the generative phase, the development of reproductive organs from initiation to anthesis, and regulation of these processes by internal and external factors. We also discuss the factors involved in florigenesis with special emphasis on the prospects and future investigations of biochemical and molecular mechanisms of florigenesis in flower bulbs. Elucidating developmental mechanisms in these species may greatly contribute to the regulation of their florigenesis, as well as to the understanding of developmental processes in other higher plants. Molecular characterization of genes involved in flower morphology will help in developing novel floral architectures in ornamental bulbs by classical breeding or by genetic manipulations using transformation systems. In addition,

knowledge of bulb periodicity is essential for the control of flowering, while introducing modifications in this periodicity constitutes the basis of the techniques used to promote or delay flowering.

5. The differentiation of perianth morphologies in monocotyledonous plants. A Kanno, Japan 44-50

The morphological transition of the first whorl of flowers from petaloid tepals into sepals occurred frequently during the diversification of angiosperms. The *Arabidopsis* class B genes *APETALA3 (AP3)* and *PISTILLATA (PI)*, and the *Antirrhinum* class B genes *DEFICIENS (DEF)* and *GLOBOSA (GLO)*, are required for petal development in whorl 2, and their homologs have been isolated and characterized from various plants. A recent study of tulip flowers indicates that the morphology of petaloid tepals in whorls 1 and 2 is consistent with the expansion of class B gene expression. To clarify the evolutionary transition between sepals and petals in monocotyledonous plants, we isolated and characterized the class B genes from *Agapanthus*, *Muscari* and *Tricyrtis*, whose perianths consist of petaloid tepals in two whorls, and from *Tradescantia*, *Commelina* and *Habenaria*, whose perianths are differentiated into sepals and petals. Gene expression was examined by Northern hybridization and RT-PCR using dissected floral organs, or by *in situ* hybridization, and revealed the expansion of class B gene expression in whorl 1 in *Agapanthus*, *Muscari* and *Tricyrtis*, and a lack of *DEF/AP3*-like gene expression in whorl 1 in *Commelina*, *Tradescantia* and *Habenaria* species. These results suggest that the *DEF (AP3)*-like gene expression pattern may be correlated with a morphological transition from petaloid tepals into sepals in the first whorl in monocot flowers.

6. A proteomics approach to the study of distyly in *Turnera* species.

D Khosravi, Canada/USA, KWM Siu, JS Shore, Canada 51-60

Distylous species of *Turnera* are strongly self-incompatible, therefore they provide an excellent system for investigations of the proteins involved in distyly. Distyly is a genetic polymorphism in which there are two self-incompatible but cross-compatible morphs. The long- versus short-styled morphs have a reciprocal arrangement of reproductive organs. Little is known of the molecular basis of distyly in any species. Previous molecular investigations of *Turnera* species identified two proteins that are specific to the transmitting tissue of short styles: a polygalacturonase and an α -dioxygenase. Here we continue to search for proteins that might be involved in distyly, since we have not yet identified the genes that occur at the S-locus and no reliable candidate proteins from pollen have been identified to date. Comparative investigations were undertaken to detect and identify morph-specific proteins from styles and pollen using a proteomics approach. Using 2-D PAGE, and mass spectrometry, two new proteins were identified that are specific to short styles: a putative cysteine protease and a putative β -expansin. Neither the cysteine protease nor the β -expansin was detected in mutants or in self-compatible homostylous species of *Turnera*. They are absent in short styles sampled one day prior to anthesis. We speculate upon the function of these two proteins in the self-incompatibility system of short-styled plants. We also discovered a number of proteins unique to anthers of the long- or short-styled morph. We have not yet identified these proteins, but this remains an important aspect of our future work.

7. Mutations affecting corolla symmetry in sunflower. M Fambrini, D Bertini, G Cionini, V Michelotti, C Pugliesi, Italy 61-70

The inflorescence of sunflower (*Helianthus annuus* L.) is heterogamous with zygomorphic ray flowers located in the outermost whorl of the head and actinomorphic disk flowers arrayed in arcs radiating from the center of the head. The ray flowers are sterile with large flat ovaries with no ovules; disk flowers are hermaphrodite, carrying both male and female organs. Two mutants with altered corolla symmetry are known in sunflower: the first, named *Chrysanthemoides (Chry)* is characterized by a shift from the polysymmetric corolla of disk flowers into a monosymmetric-like corolla; the second, named *tubular ray flower (turf)*, shows a shift from the zygomorphic corolla of ray flowers into a nearly actinomorphic tubular-like corolla. We report in detail the morphological floral features of *Chry* and *turf*, demonstrating that both mutations also affect the development of stamens and carpels. Most disk flowers found in the peripheral whorls of *Chry* heads showed drastic reduction in stamen length, as well as the absence of ovules, and developed an unbranched style. In contrast, tubular-like ray flowers of *turf* achieved the ability to differentiate both fertile stamens and ovules. Homeotic transformations were also identified in the tubular-like ray flowers of *turf*, affecting both filaments and anthers that displayed petaloid-like traits. Notably, the *turf* mutation was not completely stable, occasionally reverting to nearly "wild-type" phenotype. Our results point to a primary role for *TURF* and *CHRY* in the programming of the corolla symmetry and suggest a key interaction of both genes with floral organ identity genes.

8. From the wild to the market: breeding of floral size and architecture in ornamentals.

J Weiss, E Cano-Vicente, M Egea-Cortines, Spain 71-75

The enormous number of ornamental plants present on the market today is the result of a continuous effort by private companies and individuals to obtain new plants that are attractive and have a long life. In spite of the obvious diversity caused both by choosing different plants and looking for new flowers, the fact is that floral development is a very well conserved developmental program. In this review we will give a perspective of three aspects that have been fundamental for the current status of ornamentals as commodities. First we will describe the general floral program, changes that have happened between some ornamentals and their wild relatives, and finally we will give a short description of some of the methods that are used to obtain new varieties.

9. Male gametophyte development and function. D Honys, D Reňák, Czech Republic, D Twell, UK 76-87

Male gametophyte development in higher plants is a complex process that requires the coordinated participation of various cell and tissue types and their associated specific gene expression patterns. The male gametophytic life cycle can be divided into a developmental phase leading to the formation of mature pollen grains, and a functional or progamic phase, beginning with the impact of the grains on the stigma surface and ending at double fertilisation. Pollen ontogeny is also an excellent model in which to dissect the cellular networks that control cell growth, polarity, cellular differentiation and cell signaling. Here we present an overview of important cellular processes in male gametophyte development and recent advances in genetics and genomic approaches that are advancing the field significantly. Genetic approaches have identified a growing number of gametophytic mutants affecting discrete steps during the developmental or progamic phases that are now beginning to uncover some of the key molecular processes involved. With recent technological advances pollen transcriptomic studies now provide the first genome-wide view of male reproductive cell development in *Arabidopsis*. These studies reveal at least two successive global gene expression programs and the identity of a large number of male gametophyte-specific genes and putative transcriptional regulators. Transcriptome analysis has revealed a striking overrepresentation of cell wall metabolism, cytoskeleton and signaling genes in preparation for the progamic phase. This quantum leap in gene-centered knowledge highlights the functional specialization of this pathway and offers many new opportunities for the dissection of cellular processes that control male reproductive success.

10. The role of polyamines in relation to flowering senescence. N Bagni, A Tassoni, Italy 88-95

Cut carnation (var. Reiko) and gerbera (var. Lisa) flowers were held under standard environmental conditions and treated with different polyamine (PA) concentrations, either by spraying directly on the petals or by supplying them in the vase water. In carnation, the greatest delay of senescence was evidenced with 10 mM spermidine (spd) in the watering solution (10 mM spd-V), while no significant effect was obtained by spraying the flowers. PA levels (free, PCA-soluble and PCA-insoluble conjugated) were determined in carnation control and 10 mM spd-V treated flowers. Starting from day 3, spd was absorbed from the watering solution and accumulated in the petals both in the free and PCA-soluble fractions. Putrescine (put) levels also increased with 10 mM spd-V treatment. Total anthocyanin, DNA, protein content and protease activity were determined in control and 10 mM spd-V supplied carnations. Spd was shown to stabilise and retard the degradation of DNA. In gerbera, the best results were obtained with 0.1 mM spd spray and with 10 mM spd supplied in the watering solution. Both these treatments prevented the bending of the stem. Free and PCA-soluble conjugated PAs were determined in control, 0.1 mM spd and 10 mM spd-V gerbera petals. In contrast to carnation, only 0.1 mM spd spray induced an increase in endogenous free and PCA-soluble spd and put content, while endogenous PA levels did not vary in 10 mM spd-V flowers. No evident difference in total DNA degradation was detected in control and treated gerberas.

11. Abscisic acid and cut flower senescence. A Ferrante, P Vernieri, Italy 96-100

The keeping quality of cut flowers, during the postharvest stages, is very important in the global market, where floricultural items must be extremely competitive. Postharvest physiological analyses of senescence point to plant hormones playing important roles in the promotion or inhibition of flower and leaf senescence. Among them, ethylene and abscisic acid (ABA) are the most important. Ethylene has been extensively studied and much information is available for modulating senescence in ethylene-sensitive flowers. In contrast, the role of ABA during cut flower senescence has been wrongly assigned secondary importance. In this chapter, the effect of ABA on leaf and flower senescence has been briefly reviewed in select species, with a brief discussion of ABA and gene expression.

12. Genetic control of floral abscission.

RB Aalen, MA Butenko, G-E Stenvik, NM Tandstad, Norway, SE Patterson, USA 101-108

Abscission is a process whereby plants shed their organs such as leaves, flowers or floral organs after having served their function. *Arabidopsis thaliana* is an ideal model for investigating the process of floral organ abscission since it is not obscured by other processes like senescence. Abscission is a program of cell separation that takes place between layers of small cells differentiated into an abscission zone at the base of the organ to be shed. Investigation of *Arabidopsis* mutants has shown that many of the growth regulators including ethylene, auxin, methyl jasmonate and gibberellic acid affect floral organ abscission. For example, mutations in ethylene receptor genes and other genes in the ethylene response signal transduction pathway lead to delayed floral organ abscission. However, observations on the *delayed in abscission (dab)* mutants and the *inflorescence deficient in abscission (ida)* mutant indicate that ethylene independent pathways regulate abscission, as these mutants have normal sensitivity to ethylene. Other mutants with delayed abscission have been identified; however, many have additional phenotypes not associated with abscission. Thus, we have selected to present an in depth discussion on *ida*. *IDA* encodes a small putative peptide ligand, and genes and transcripts encoding IDA-like (IDL) proteins have been identified in *Arabidopsis* and other dicot and monocot plant species. Promoters of *IDL* *Arabidopsis* genes can direct reporter gene expression in the floral organ abscission zone, at the base of the floral pedicel and in the columella cells of the root cap, suggesting involvement in abscission or abscission-like processes, like root cap sloughing. Overexpression of *IDA* leads to shedding of organs that normally are not abscised in *Arabidopsis*, e.g. the

pedicel, indicating that ectopic expression of *IDA* can activate preformed abscission cells. Preliminary experiments indicate that the *ida* phenotype can be recreated by RNA interference. Scientists' knowledge from studies on *IDA* and other genes regulating floral abscission may be utilized to modify floral crops, and we will present these applications. In summary, these recent insights leave the community poised to modify abscission in many ornamentals.

13. Molecular basis of ethylene signal transduction and control of abscission of flowers in *Delphinium*.

S Abe, S Kuroda, Y Hirose, E Davies, Japan 109-123

Florets of plants belonging to the genus *Delphinium* are known to undergo severe senescence a few days after harvesting the cut flowers, as well as in response to environmental cues such as rainfall and pollination. An ethylene sensing mechanism is thought to be involved. Recently, the molecular basis of the ethylene signal transduction pathway has begun to be uncovered. Ethylene Response Sensor (ERS)-type ethylene receptors are likely to be involved in the senescence of florets. *ERS1* for the ethylene sensor gene and *CTR1* for its downstream factor have been discovered in *Delphinium* and are likely to be involved in the abscission of florets. This chapter describes the structure, expression, and possible functions in abscission of florets in *Delphinium*. We postulate that floret abscission in *Delphinium* is caused by elevated levels of the ethylene receptor, *ERS1*, and that these will be influenced by endogenous ethylene generated after cutting the stem. This ethylene signal transduction pathway is crucial in the shelf life of cut flowers and in garden longevity of intact flowers against environmental cues, and poses a way to enhance them by either conventional methods or modern biotechnology such as transgenic plants.

14. Ornamental cut flowers: physiology in practice. JA Teixeira da Silva, Japan 124-140

Ornamental plants occupy an important part of society, culture, religion, and economy. Cut flowers from several ornamentals bear both a "butter and blade" symbolism, but independent of the viewpoint, there is no doubt that the quality of longevity is of great importance. In this review, the current status of postharvest technology applied to cut flowers and foliage is discussed. Basic considerations of the physical, biochemical and genetic mechanisms underlying some of the processes central to cut flower and foliage deterioration, such as abscission, senescence and programmed cell death, and some of their cross-talk mechanisms have been included. Moreover, through examples, solutions to increasing longevity through improvement of cultural practices and sanitation and through genetic engineering are covered, providing practical solutions to the global cut flower market. Long live the flower!

15. Programmed Cell Death in plants and flowers. GE Drury, P Gallois, UK 141-156

Programmed Cell Death is a process common to all multicellular organisms so far studied. Over the last thirty years it has been very well characterised in mammalian systems, as well as in organisms such as the nematode, insects and amphibians. Cell death in plants remained poorly understood in comparison until research in recent years has led to Programmed Cell Death (PCD) becoming a more eminent research area. Plant PCD occurs at many stages during development, and can occur in response to environmental conditions. Some of the better characterised occurrences of PCD include xylem formation, senescence and the hypersensitive response to pathogens and studying the occurrence of such processes will continue to bettering our understanding of a biologically imperative and prevalent process. Research in plant PCD has been heavily influenced by findings in mammalian PCD, but has yet to fully elucidate the events that culminate in PCD. The extent that PCD is conserved between the two systems is likely to be limited, and although evidence exists which implies there are similarities, the emergence of plant specific mediators has seen a shift in research focus.

16. Lace plant: a novel system for studying developmental programmed cell death.

AHLAN Gunawardena, C Navachandrabala, Canada, M Kane, USA, NG Dengler, Canada 157-162

The submerged aquatic plant, lace plant (*Aponogeton madagascariensis*, Aponogetonaceae) has a highly unusual leaf form, in which holes perforate the leaf blade in a regular lattice-like pattern. These perforations are formed by programmed cell death (PCD) shortly after leaf emergence from the apical region of the corm. Because PCD occurs in predictable locations in relation to the leaf vein pattern and at a known stage of leaf expansion, and because the thin, aquatic leaves are tractable to live-imaging microscopy, lace plant is an attractive model system for studying developmentally-regulated PCD in plants. One limitation of carrying out developmental and physiological analyses is the unknown effects of associated micro-organisms found in aquarium culture. Therefore, a primary objective of this study has been to establish and maintain axenic cultures of this species. We first characterized the formation of perforations through PCD in axenic cultures and found that the PCD process in culture did not differ significantly from that previously described. Since ethylene is an important component of PCD signaling pathways during many forms of plant PCD and also functions in growth regulation of other submerged aquatics, we also carried out experiments using 0.05 mM AgNO₃ as an inhibitor of ethylene perception and found a significant reduction in the number of perforations formed per leaf under treatment conditions compared to control plants. While preliminary, these experiments indicate that axenically cultured lace plant has considerable potential as a model system for the study of developmentally-regulated PCD in plants.

17. In vitro culture of the fern *Platyserium bifurcatum* as a tool for developmental and physiological studies.

The staghorn fern, *Platycerium bifurcatum* is an epiphytic homosporous fern. Different phases of its life cycle – spores, gametophytes and various parts of sporophytes – constitute useful systems for developmental and physiological studies. The morphological simplicity and simple culture requirements of the gametophyte enable various aspects of growth and development to be observed. Its great morphogenetic potential when cultured *in vitro* enables study of morphogenesis, including adventitious bud development and apospory. Direct adventitious bud development occurs on the whole surface of the excised juvenile leaf without use of any growth regulators. Morphogenesis was also obtained on excised bud scales. Apospory, the formation of gametophytes from sporophytic tissue without meiosis or sporulation, was induced on wounded juvenile leaves regularly and at a high frequency. In this article we provide an overview of the research on *P. bifurcatum* using *in vitro* techniques. Spore germination, gametophyte development and morphogenesis are discussed with respect to its potential use in plant development research, as well as to point out some similarities between ferns and seed plants.

18. *Zinnia elegans* is an excellent model for xylogenesis: *in vitro* and *in planta*.

E Pesquet, Sweden, A Jauneau, D Goffner, France 171-178

Vascular tissues play an essential role in plant growth, allowing for transport of raw sap via the xylem from roots to leaves and elaborated sap by the phloem from source to sink. The formation of xylem, or xylogenesis, is an integral part of the growth of each organ and allows vascular continuity in the organism, interconnecting organs and establishing irrigation of the entire plant. Xylem is a complex tissue comprised of several cell types (parenchyma, conducting cells and fibers), each formed by specific developmental processes and each with distinct morphological characteristics. One interesting cell type is the conducting cell, called the tracheary element (TE), which, in order to be functional, must undergo secondary cell wall formation and programmed cell death (PCD) processes. This results in the formation of an interconnected series of strengthened hollow tubes suitable for raw sap conduction. One of the major difficulties in studying TE formation *in planta* is that very few cells actually differentiate at a given moment and a given time. In order to study TE formation, an *in vitro* TE differentiation system has been established from isolated mesophyll cells of an ornamental asteraceae, *Zinnia elegans*. This system is characterized by a high rate of differentiation and the semi-synchronous nature of the morphological changes that occur. In order to compare TE formation *in vitro* and *in planta*, we provide herein i) a detailed anatomical description of the vascular system of *Zinnia elegans*, ii) a morphological comparison of TE differentiation *in vitro* vs. *in planta* and iii) gene expression localization both *in planta* and *in vitro*. The morphological and gene expression data provided in this chapter allow us to integrate *in vitro*-derived information in the context of the whole plant.

Part 2 Cellular mechanisms

19. The nuclear and chromosomal architecture of plant cells: development and dynamics.

S Fujimoto, S Matsunaga, K Fukui, Japan 179-184

In eukaryotic nuclei, genomic DNA is compacted as a result of interaction with various proteins. A hierarchical model is most commonly used to elucidate DNA organization in the nucleus. First, DNA is wound around core histones to form a nucleosome then interaction between linker DNA and histone H1 generates a heterogeneously folded 30-nm-long fiber structure. This fiber structure is organized into a 5-200 kb-long loop, which attaches to the nuclear matrix or scaffold, and during mitosis, forms the higher structure necessary for chromosome formation. This review is concerned with the physical properties of plant nuclei and features of proteins related to plant nuclear and chromosomal architecture. Some chromatin proteins are conserved among eukaryotes; however, many proteins related to nuclear structure are not. Therefore, identification of plant-specific proteins using visual screening such as random GFP fusion might contribute to our understanding of the specific characters of plant nuclei.

20. Mechanisms of cytokinesis in flowering plants: new pieces for an old puzzle.

JM Seguí-Simarro, Spain, LA Staehelin, USA 185-196

Cell division is the most distinctive stage of the cell cycle of eukaryotic cells due to the major changes in cell architecture that accompany the cell dividing process. Progress in plant cell division research during the past 130 years has been driven to a significant extent by improvements in light and electron microscope techniques. Most recently, improvements in cryofixation and freeze-substitution specimen preparation methods together with the use of dual-axis electron tomography techniques for visualizing the cryofixed samples has led to new structural and functional insights into the complex process of cell plate assembly as well of the associated changes in the phragmoplast cytoskeletal system. The main purpose of this chapter is to summarize the contribution of 3-D electron microscopy to the understanding of the mechanisms underlying cytokinesis in flowering plants. This information also provides an improved framework for interpreting light microscope observations of living cells, and for formulating new molecular hypotheses of plant cell cytokinesis.

21. Plant chimeras. J Wegner, France 197-202

This review chapter covers the origin of chimeras, explains the conditions for their existence and the reasons for their changes. The most common forms of chimeras and their features are described, and commonly used terms are explained. Furthermore, analytical methods are provided allowing the identification of a given plant as a chimera and to determine its structure. Finally, the impact chimeras may have on plant breeding is described, as are ways of making use of them when creating new varieties.

22. Plant peptide hormones, from defense to pollen self-incompatibility, cell fate and development: small peptides as signaling molecules in plants. DS Moura, MC Silva-Filho, Brazil 203-209

The first peptide hormone isolated from plants was an 18 amino acid peptide called systemin. Systemins are found in several Solanaceae species and are involved in the signaling transduction pathway responsible for the local and systemic production of defense proteins against herbivorous insects. Since the discovery of systemin, several other peptides involved in a diverse range of physiological processes have been identified in plants. Phytosulfokines (PSKs) are 5-amino-acid sulfated peptides initially isolated from suspension culture cells of asparagus and later from several other species. PSKs are mitogenic factors that have been implicated in cell proliferation and differentiation although their role *in vivo* is still unclear. The self-incompatibility (SI) system found in brassica is controlled by the S-locus that contains among others, a gene that codes for the S-locus cysteine rich protein (SP11/SCR). The SP11/SCRs are peptides of 47-60 amino acids in length that after interacting with their receptors on the stigmatic papilla cell membrane trigger the SI response through a signaling pathway that involves an S-locus glycoprotein, an arm-repeat protein and a recently identified membrane-anchored protein kinase (MLPK). The Arabidopsis mutant *clv3* is defective in a gene encoding a 79 extracellular signaling peptide named CLAVATA3 (CLV3). The CLV3 signaling pathway determines cell fate in the shoot apical meristem. RALF, a 5kDa secreted plant peptide, has been isolated from several plant species and cDNAs coding for the RALF precursor have been found in all plant tissues throughout the plant kingdom. RALF exogenously applied to germinating seeds of tomato and Arabidopsis was able to inhibit root growth and the peptide is thought to have a role in a basic cellular process. A review of the recent findings in this developing field in plant science highlighting the role and structure of the peptide ligands and the signaling pathways that are initialized by them will be presented herein.

23. Mitochondrial somatic genetics and homeotic effects on flower morphology. RJ Rose, JT Fitter, DW McCurdy, MB Sheahan, Australia 210-215

Homeotic floral structure, notably carpelloidy (stamen feminization), caused by alteration of the mitochondrial genome is a morphological manifestation of cytoplasmic male sterility (CMS). Mitochondria, which are usually maternally inherited are characterized by a recombinationally active genome and by being a dynamic organelle, undergoing fusion and fission. It is these latter phenomena that contribute to mitochondrial genomic changes that in turn lead to carpelloidy. There are three ways homeotic changes due to mitochondria can be induced: by mutation, by alloplasmic effects as a result of sexual hybridization and backcrossing, and by somatic hybridization or cybridization. All three ways are influenced by the multipartite nature of the mitochondrial genome, the recombination propensity of the mitochondrial genome and the fusion and fission of mitochondria to create novel, chimaeric genes and operons that produce "male sterilizing factors". All three ways are also influenced by nuclear-cytoplasmic relationships, both in affecting the mitochondrial genome through nuclear restorer genes and in responding to the consequences of the mitochondrial genomic effects which influence nuclear homeotic genes. This interplay within the mitochondrial population and between mitochondria and nucleus probably acts to prevent deleterious mutations, but with crosses between species or wider somatic hybridization the effect is to produce an adult that cannot produce pollen, thereby acting as a speciation barrier. As a consequence, however, fascinating floral morphologies are produced.

24. Genome structure and gene expression in polyploids. SC Pessino, LG Martelotto, Argentina 216-223

A raise in the number of total genomic complements in the nucleus or polyploidization is a common event in the evolutionary history of most angiosperms. New phenotypes often arise with polyploid formation and determine their success in nature or their further selection for use in agriculture. Enormous progress has been achieved in the last few years regarding the understanding of the molecular mechanisms concerned with polyploidization. It was shown that while in a number of species it appears to involve extensive alterations of the progenitor genomes, full additivity associated to genetic and epigenetic stasis is observed in others. Genomic changes are usually followed by a refined gene expression re-patterning which have most probably an adaptive significance. It could involve dosage-regulation, altered regulatory interactions and both rapid and long-term genetic and epigenetic changes. The objective of this chapter is to summarize the principal mechanisms leading to genome alterations and novel forms of gene expression in polyploid plants and briefly analyze their evolutionary significance.

25. Sex chromosomes in plants. R Hobza, B Vyskot, Czech Republic 224-235

A majority of plant species are cosexuals forming male (stamens) and female (pistils) sexual organs in each flower. About 5% of species are strictly dioecious and form unisexual flowers, either male or female, on different individuals. A high number of plant species represent

intermediate stages, i.e., different forms of flowers are present on one individual (e.g. monoecy) or sexually different individuals occur in populations of plants (e.g. gynoecey). It is well documented that these sexual forms also represent intermediate steps in the evolution of sexuality. Similarly as in animals, there are two basic mechanisms of sex determination in plants: genetic and environmental (hormonal). Among the dioecious species, only a few of them have evolved heteromorphic sex chromosomes, especially white campion (*Silene latifolia*) and common sorrel (*Rumex acetosa*). In these two classical species, different sex chromosome-based mechanisms have been described: white campion has the male dominant chromosome Y (the mammalian type of sex determination), while in sorrel the sexuality is controlled by a ratio between the number of X chromosomes and the number of autosomal sets (the drosophila system). Recent molecular analyses show that the plant sex chromosomes are evolutionarily much younger compared with the sex chromosomes in animal species. This fact makes them the optimum models to study early stages of sex chromosome evolution.

26. A wonder plant-microbe interaction between white campion and anther smut.

W Uchida, Y Kazama, S Matsunaga, S Kawano, Japan **236-242**

Infection with the smut fungus *Microbotryum violaceum* induces the development of male organs in female flowers of the dioecious plant *Silene latifolia*. The development of stamen primordia is prematurely arrested in healthy females, whereas in infected females it is derepressed, resulting in smutted anthers filled with fungal teliospores instead of pollen grains. Light-microscope analyses revealed that the derepressed stamens of infected females formed anther wall layers similar to those of healthy males. At this stage, the floral homeotic B-function gene, *SLM2*, was expressed both in the stamen primordia of healthy males and in the derepressed primordia of infected females, but not in the arrested primordia of healthy females. *SLM2* expression was induced in female *S. latifolia* by fungal infection, independently of the presence of the Y chromosome. To investigate the host-parasite interactions, electron-microscopic analysis was performed following fixation by a high-pressure freezing method. Parasitic fungal hyphae were observed throughout all organs in the young floral buds of infected plants. No morphological change in the fungus was found before or after the derepression of stamen primordia in infected females. The fungi were often observed adjacent to dying sporogenous cells of infected female anthers. We found an increasing number of dead and dying sporogenous cells, among which the fungus formed initial teliospores. The fungus accelerates cell death in the anther and utilizes constituents of dead host cells to form mature teliospores.

27. Integrating knowledge of transcription factors and cell engineering for modelling *Catharanthus roseus*: prospects and bottlenecks.

S Hedhili, France, **PK Pati**, India, **P Gantet**, France **243-252**

In *Catharanthus roseus*, studies on flavonoid as well as terpenoid indole alkaloids biosynthetic pathway holds immense potential looking at its ornamental and important medicinal properties. In general, studies on flavonoid pathway provide us the basis to realise that transcription factors are more promising and efficient molecular tools to engineer plant secondary metabolite pathways. Modelling plants with the knowledge of transcription factors not only helps in coordinated regulation of gene expression and cellular differentiation involved in the biosynthesis of a metabolite but also provides tremendous flexibility in bypassing the requirement to characterize different biosynthetic pathways at the biochemical or molecular level. The present chapter discusses the concept of transcription factor and regulation of key genes of terpenoid indole alkaloid (TIA) biosynthesis pathway in *C. roseus*. The need for functional interactions and determination of an efficient combination between transcription factors, genes and promoter sequences is also highlighted. For effective manipulation of *C. roseus* integration of the knowledge of transcription factors and efficient transformation/regeneration protocols is highly essential. The chapter highlights the various approaches, current status and major bottlenecks of genetic manipulation in *C. roseus*. Development of a new strategy to overcome the existing blockade in modelling *C. roseus* will greatly facilitate in improved production of TIAs and thus could be a boom to the pharmaceutical industry.

28. Glimpse into mechanisms of signal transduction in plant cells: role of non-receptor protein tyrosine kinases.

A Dash, India **253-255**

Two orders of green alga (*Cladophorales* and *Charales*) were investigated for the presence of protein tyrosine kinase activity. Proteins of 70 and 85 kD were found to be tyrosine phosphorylated in *Cladophora fracta*, with an additional phosphorylated band evident at the 120 kD region in *Chara vulgaris*, suggestive of the presence of putative tyrosine kinase activity in these algal species. A 70 kD protein was immunoprecipitated from both species using a polyclonal antibody against non-receptor protein tyrosine kinase Syk. These observations supported the presence of Syk-like kinase in the green algal species, which could have an important role in algal physiology. Parallels to signal transduction mechanisms in higher plants are drawn.

Part 3 Light, vernalization, clocks, rhythms and movements

29. Plant photoreceptors and the photoperiodic induction of flowering. **BL Montgomery**, USA **256-262**

Plant photoreceptors perceive light and transmit the information to downstream components that regulate the growth and development of

flowering plants throughout the plant life cycle. Three major classes of photoreceptors are active in flowering plants, including the red/far-red absorbing phytochromes, blue-light-absorbing cryptochromes and phototropins, and UV-B absorbing photoreceptors. The phytochromes and blue light photoreceptors have defined impacts on the photoperiodic induction of flowering in higher plants. This chapter will review the effects of phytochromes and blue light receptors, and the downstream components that they control, on the photoperiodic induction of flowering.

30. Thermoperiodic control of shoot elongation and involvement of gibberellins.

J-Anders Stavang, R Moe, JE Olsen, Norway 263-267

Stem elongation in many species is affected by the relationship between day (DT) and night temperature (NT). When plants are grown at the same average diurnal temperature, stem elongation increases with an increase in difference (DIF) between DT and NT, from a negative to a positive value. A negative DIF treatment (low DT and high NT) is a tool to produce compact flower plants and vegetable seedlings with short internodes without a delay in production time in commercial plant production in greenhouses. Also, in a number of flowering plant species, a short temperature drop of 2-4 hours in the beginning of the light period is efficient and commonly used to reduce stem elongation. Exogenous gibberellins (GA) stimulate stem elongation under negative DIF in a number of species. Also, negative DIF treatments and short diurnal temperature drops significantly reduce the level of active GAs as compared to a high DT/low NT regime or constant temperature. Using pea as a model system, we have shown that transcriptional control of a GA-deactivation gene, *GA2-oxidase2* (*PsGA2ox2*) is a key component in mediating thermoperiodic regulation of GA₁ levels and stem elongation. Inactivation of GA₁ by 2-oxidation apparently results in the reduced GA₁ levels and reduced stem elongation under negative DIF compared to zero and positive DIF. Also a temperature drop of 2 h in light increased the expression of *PsGA2ox2*, while a temperature drop in darkness had no effect on expression of GA-deactivation genes, but instead slightly stimulated expression of three GA-biosynthesis genes in pea, *NA*, *GA20-oxidase1* and *GA3-oxidase1*. The observed expression patterns contribute to explain why stem elongation is more restricted by temperature drops in light than in darkness and suggest that GA-metabolism is involved in plant acclimation to low temperature both in light and darkness.

31. The rhythms of circumnutation in higher plants. A Charzewska, Poland 268-275

Circumnutation is a rhythmic, rotary movement of elongating plant organs, such as stems, tendrils and roots. The tip of a plant organ describes ellipses, or circles, or pendulum-like movements that can alternate between the clockwise and counterclockwise direction. Circumnutation is caused by a turgor wave rotating around the stem that drives the helical, acidic growth of the stem. Although the basic rhythm of the movement is ultradian, circumnutation is also found to be modulated in a circadian and infradian way. Circadian modulation is brought about by the plant biological clock that drives circumnutation rhythms even in the absence of environmental cues, at least for several days. The role of the clock is also visible in the phenomenon of anticipation of light/dark cycles by rotating plants. Infradian modulation of circumnutation acquires a regular rhythm of the movement activity every several days. It is hypothesized to be correlated with stem modular growth. Circumnutation is a universal and unique oscillation in higher plants. The present chapter contains a review of recent studies executed on sunflower, *Arabidopsis*, rice and bean plants. The movement physiology based on recent studies is also discussed.

32. The use of photoperiodic lighting in floriculture in Mediterranean conditions: *Gypsophila paniculata*.

J Lopez, A Gonzalez, Spain 276-281

Gypsophila paniculata is used as a cut flower. Its beautiful inflorescence (panicle) is formed by many white or pink coloured, small flowers. This is a species native to the Mediterranean, which means that it is suited to intermediate environmental conditions for its natural growth, and although, being a long day plant, it needs a great number of light hours to bloom. These plants show excellent vegetative and floral development in non-natural growth condition cycles as long as their thermic and lighting needs are covered. It can be grown continuously with 2 to 3 cycles of flowering per year and can last 2 to 3 years. It needs sandy or porous soil in order to regulate its moisture uptake, while excess water can seriously harm it. These conditions can be provided by localised irrigation. Its rapid growth with resulting numerous, small secondary stems mean that it needs a guide, which is usually provided by several meshes at different heights. Vegetative development out of season is improved by the application of growth regulators e.g. gibberellins. Pests and diseases can be controlled. Stems are harvested when 30 or 40% of the flowers have started to open, although similar good quality results can be obtained by applying artificial opening techniques when only 5% of the flowers are open. Correct handling will ensure the longest possible vase life. Some varieties show different characteristics e.g. flower size, light needs, yield, duration of cultivation cycle and flowering phase, among others.

Part 4 Colour and scents

33. Flavonoid compounds in flowers: genetics and biochemistry. O Yu, M Matsuno, S Subramanian, USA 282-292

Flavonoids are one of the main determinants of flower colors. Flavonoid biosynthesis is one of the most extensively studied natural product metabolism. Flavonoid compounds are produced by the phenylpropanoid pathway, a major secondary pathway that exists in all higher plants.

Most enzymes involved in this pathway and their corresponding genes have been characterized; some proteins have been crystallized and structurally resolved. This chapter aims to provide a basic understanding of flavonoid biosynthesis and its regulation in flowers. The chapter has been organized into three sections: the first outlines key enzymatic reactions that lead to the production of anthocyanin and related co-pigments; the second summarizes major regulating factors that affect the biochemistry and genetics of the flavonoid compounds in flowers; and the last section describes examples of genetic engineering attempts that produced novel flower colors in many plant species by modifying the flavonoid biosynthesis pathway.

34. Model plants and functional genomic approaches applied to the characterization of genes involved in floral scent biosynthesis.

G Scalliet, Switzerland, **S Baudino**, **JM Cock**, **P Huguency**, France **293-299**

Flower scents have been subjected to extensive chemical characterization for many years, providing detailed analyses of the complex mixture of volatiles emitted by flowers. However, the past ten years have seen a rapid acceleration of progress in flower scent research, with the characterization of numerous genes involved in scent biosynthesis. This review focuses on the evolution of flower scent research, from the early biochemical to the present genomic approaches, illustrating how the use of different plant models, combined with functional genomic approaches, contributed to the present knowledge in this field.

35. Molecular breeding of flower color. K-Y To, C-K Wang, Taiwan **300-310**

Flower color contributes mainly to the market value of an ornamental plant, and coloration of flowers is determined by three classes of pigments: flavonoids, carotenoids and betalains. Flavonoids and carotenoids are widespread; however, betalains can be found only in plants of several genera in the order Caryophyllales, which belongs to one small group of angiosperms. Among these pigments, flavonoids (mainly anthocyanins) are the most common flower pigments contributing to a range of colors from yellow to orange to red to purple. During the past few decades, flavonoid biosynthetic pathway leading to anthocyanin production has been well established in various plant species, and genetic engineering of flavonoid/anthocyanin biosynthesis has been used to produce cultivars with novel pigmentation in flowers. Here we summarize the current status of molecular approaches in breeding flower coloration, and describe our study and prospective regarding flower color modification.

36. Causes of flower colour patterns with a focus on chimerical patterns. K Olbricht, S Plaschil, F Pohlheim, Germany **311-319**

Colour patterns in flowers are of ornamental value and are important for histological studies. Similar flower patterns may have different causes. Bicoloured flowers may result from genetically inherited patterns, instable alleles of floral genes, infections, or chimeral dispositions. A transgenic *Petunia* Juss. serves as an example of an epigenetic chimera. Bicoloured cultivars of *Angelonia* Humb. et Bonpl., *Pelargonium* L'Herit. ex Ait., *Petunia*, and *Saintpaulia* H. Wendl. and other species are examined in terms of their flower colour pattern. The investigation of the causes of flower patterns is based on inheritance analysis (self-pollination), *double marking* of the different apex layers, grafting and somatic segregation (spontaneous and by *in vitro* callus culture). In the case of chimerally determined flower patterns, conclusions about the participation of apex layers in the histogenesis of flower tissues can additionally be drawn by apex layer *double marking*.

37. Isolation and analysis methods of volatile compounds from flowers and leaves.

V Radulescu, **E Oprea**, **S Chiliment**, Romania **320-325**

The paper has an introductory part, which presents the basic principles for different methods of isolation and determination of volatile compounds from plants. In the experimental part, a comparison is made between the chemical composition of different plant products (essential oil, infusion and tincture), using as research material flowers and leaves of *Salvia officinalis*. The essential oil from leaves and flowers of *S. officinalis* was obtained through hydro-distillation in a Neo-Clevenger apparatus. The volatile compounds from infusion and tincture were extracted using solid phase extraction (C₁₈-SPE) and liquid-liquid extraction with hexane and dichloromethane. The volatile oil diluted with dichloromethane and the infusion and tincture extracts were analysed by gas chromatography coupled with mass spectrometry. The results were also compared with those obtained through headspace at 50°C and 80°C. The chemical composition of volatile oils extracted from leaves and flowers are very similar. The only significant difference is a higher contribution of monoterpenic hydrocarbons in flowers (8.59%) compared to leaves (4.54%). The analysis of volatile compounds from *S. officinalis* leaves shows that through infusion and maceration most of the monoterpenic hydrocarbons are lost. In headspace analysis, the amount of volatile compounds at an equilibrium temperature of 80°C is more than five times higher than at 50°C (the average ratio is 5.24).

Part 5 Metabolism, metabolites and hormones

38. Targeted transcriptomics to elucidate the regulation of benzenoid synthesis in *Petunia hybrida*.

JC Verdonk, **MA Haring**, **AJ van Tunen**, **RC Schuurink**, The Netherlands **326-338**

Flowers attract specific pollinators by a combination of color, shape and fragrance, which is referred to as a pollination syndrome. Generally, the fragrance emission by the flowers is at a maximal level when the pollinator is active, and consists of volatile molecules derived from different biochemical pathways. Studies regarding the fragrance of flowers have focused on scent composition rather than on the enzymes involved in its production and regulation. In *Petunia hybrida* cv. Mitchell, the fragrance of the flowers consists almost exclusively of benzenoids, which are mainly emitted during the evening and night. Microarrays were used to identify cDNAs whose expression increased with the production of floral benzenoids in the evening. We found genes from the shikimate pathway and the post-shikimate pathway that ultimately provides precursors for phenylpropanoid and benzenoid synthesis to be upregulated in the afternoon, prior to benzenoid production. Additionally, we identified an R2R3-like MYB transcription factor, *ODORANT1*, which regulates the expression of two genes in the shikimate pathway, and two post-shikimate pathway genes *chorismate mutase (CM)* and *phenylalanine ammonia lyase (PAL)*. Moreover, the expression of several genes that have a function in the biosynthesis of S-Adenosyl methionine (SAM) correlated with scent emission. SAM is the methyl donor in the reaction that forms methyl benzoate, the most abundant compound in the headspace of petunia Mitchell. These results illustrate the power of using targeted metabolomics and transcriptomics to unravel the biosynthesis of secondary metabolites.

39. **Plastid isoprenoids: biogenesis and molecular regulation.**

F Bouvier, JC Isner, Switzerland, **MS Alexis**, France, **O Dogbo**, Ivory Coast, **B Camara**, France 339-358

Plastid isoprenoids comprise a wide array of compounds (e.g. carotenoids, chlorophylls, tocopherols, phylloquinone, plastoquinone and diverse bioactive low molecular weight derivatives). Their biosynthesis starts either with isopentenyl diphosphate or dimethylallyl diphosphate and involves multiple enzymic transformations. Through a combination of biochemical and molecular approaches our knowledge about the genes and the enzymes governing their synthesis has significantly advanced. This review will discuss specific examples of this progress.

40. **Lipid biosynthesis in Spermatophyta.** **M Iriti, F Faoro**, Italy 359-372

Lipids are a heterogeneous group of compounds essential for all living organisms. Lipid bilayers provide a spatial separation between the extracellular medium and cytoplasm, as well as subcellular compartmentalization. Besides, lipids are a predominant form of chemical energy storage in seeds and some of them, namely octadecanoid (jasmonates) and eicosanoid (prostanoids) oxylipins act as mediators entailed in defence and immunity, via phospholipase/lipoxygenase (LOX)/cyclooxygenase (COX) pathway. Other classes of lipids, include, isoprenoids (consisting of pigments, hormones, vitamins and essential oils), phytosterols and polyunsaturated fatty acids (PUFA), such as omega-3, that enhance functional properties of foodstuffs, protecting against chronic disease. In this chapter, lipid biosynthesis will be examined, despite its complexity, emphasising on the parallelism/diversity between plants and animals, with the aim of offering a comprehensive description, beneficial for any biotechnological approach.

41. **The potential of carbohydrates in plant growth regulation.**

D Lišková, P Capek, K Kollárová, L Slováková, A Kákošová, Slovakia 373-378

Attention was devoted to enzymes assumed to be involved in the elongation/cessation of growth after treatment with galactoglucomannan oligosaccharides (GGMOs). Endoglycanases, endo-(1-4)- β -D-glucanase, endo-(1-3),(1-4)- β -D-glucanase, endo-(1-4)- β -D-mannanase, as well as XET, showed remarkable activity changes after GGMOs treatment indicating their role in cell wall remodeling and cell wall *milieu* stabilization during cessation of elongation growth. Studies on peroxidase – the enzyme participating in cell wall tightening – showed that elongation growth inhibited by GGMOs may be associated with the process of cell wall rigidification catalysed by peroxidase. Previously obtained results on glycosidases are also discussed.

42. **Cytokinins and plant phenolics: a cooperative metabolism.**

J Frébortová, O Novák, P Galuszka, I Frébort, Czech Republic 379-384

Cytokinin dehydrogenase (CKX) is a key enzyme in regulation of the levels of active cytokinins in plants. We have previously reported that CKX can use products of oxidation of plant phenolics by tyrosinase and peroxidase as acceptors of electrons from oxidized cytokinin and that the phloem sap of maize seedlings contains unknown phenolic precursor of this acceptor. In the present work we investigated a set of substituted phenolic acids for their ability to serve as precursors of electron acceptors in the CKX reaction and analyzed the phloem sap of maize seedlings for the presence of carboxylic phenolic acids using HPLC with a Q-TOF detector. Only *p*-coumaric, caffeic and chlorogenic acids were converted to effective electron acceptors in the presence of mushroom tyrosinase. Ferulic acid showed weak activity and analogous compounds derived from 4-hydroxybenzoic acid were not active. Compounds effective in *in vitro* CKX assay were identified in the phloem sap.

43. **Flower ovens and solar furnaces.**

I Lamprecht, Germany, **CM Romero, L Blanco**, Colombia, **JA Teixeira da Silva**, Japan 385-404

Struggle for life means struggle for energy, in animals and in plants. Because of their sessile existence plants depend strongly on photosynthesis, sometimes even on exploiting other plants. Metabolism is in general much lower in plants than in animals, but in special periods the demand may increase significantly. Two means of coping with such situations that are mainly connected with inflorescence, pollination and/or seed production are discussed in this chapter. The first deals with thermogenic plants, which use their own energy sources to increase the metabolic turnover rates and to heat up blossoms (“metabolic climax”, “flare-up”). Temperature increases of up to 35 K and for some hours, days or even weeks are observed. The mass specific rates are so high that they become equal to those of the most active animals like honeybee queens or hovering hummingbirds. A few thermogenic species even regulate the heat production to keep their elevated temperatures constant. Thus, there are a number of parallels to phenomena well known from homoeothermic animals and their brown fat tissue in hibernators or young mammals. The second means uses the direct sun light to achieve the necessary energy. Blossoms and leaves orient in a favourite angle with the incident beams to obtain a maximum quantum flux per area (phototropism). Specialists among them even follow the course of the sun by blossoms and/or leaves during the day (“solar tracking”, “heliotropism”). And some have bowl shaped flowers concentrating the radiation to their centres.

44. Osmoregulation versus osmoprotection: re-evaluating the role of compatible solutes.

S Shabala, TA Cuin, Australia 405-416

Growth and production of ornamentals is severely limited by dehydration stress imposed on plants by salinity, drought or temperature extremes. The ability of plants to accumulate so-called “compatible solutes” (small water-soluble molecules that may be accumulated in cells at high concentrations, without affecting metabolic reactions in the cytosol or major organelles), is frequently cited as a key mechanism for increasing dehydration tolerance. This review summarises recent progress in our understanding of the physiological roles of compatible solutes in plant adaptive responses to the environment and investigates prospects for using molecular tools to genetically engineer crop and ornamental species with greater dehydration stress tolerance. Despite the central dogma in stress physiology that the adaptive role of compatible solutes is in mediating osmotic adjustment, their concentrations in the cell are frequently far too low for conventional osmotic adjustment. Osmoprotective and/or regulatory roles are more likely. Such roles include PSII protection and repair, membrane and enzyme protection, ROS scavenging, energy sinks and redox potential buffering, the use of osmolytes as a source of N and C during the recovery from stress, signalling, and control over ion transport and partitioning. Major problems hindering the use of transgenic approach to improve plant dehydration tolerance are discussed, and prospects for future research are highlighted. It is concluded that combining transgenic approaches with *in planta* physiological measurements of plant adaptive responses at various levels of plant structural organization will help us to fully appreciate the complex role of osmolytes in cellular metabolism and make substantial progress in improving plant dehydration tolerance under field conditions.

Part 6 Seeds

45. Genetic control of plant embryogenesis and embryo dormancy in *Arabidopsis*. LO Baumbusch, Norway 417-428

Mature seeds in many higher plants undergo a period of dormancy prior to germination. The dormancy stage is characterised by the temporary failure to immediately germinate under favourable conditions until specific environmental stimuli release this inhibition of germination. Many different environmental factors influence dormancy and germination behaviour of a seed. Dormant *Arabidopsis* seed require stratification, as specific cold stimulation over a certain period of time, and light for germination. Various *Arabidopsis* ecotypes show differences in their degree of dormancy. The plant hormones gibberellic acid (GA) and abscisic acid (ABA) are implicated in the control of seed development, precocious germination and germination. Embryo development is divided into several discrete stages with unique gene expression patterns, such as the *LEA* genes, which are expressed late in embryogenesis, prior to the onset of dormancy. The transcription factors *LEC1*, *FUS3* and *ABI3* have been found to be general regulators for different aspects of seed development with an at least putative function in the process of dormancy. However, the temporal accumulation of the transcription factors *LEC1*, *FUS3* and *ABI3* and the *Em* genes are not or only marginally changed in different *Arabidopsis* ecotypes, which show variation in their dormancy behaviour, or in *INSOMNIAC (nsm)* mutants, which lack stratification-breakable seed dormancy. Therefore, it seems unlikely that these genes are the principal genetic controls for the establishment of dormancy in *Arabidopsis*. An alternative approach to identify embryogenesis and dormancy regulators in plants is to survey candidate genes. Expectations of potential dormancy genes, like genes involved in chromatin dynamics and interactions are discussed at the end of this chapter.

46. Ethylene in seed germination and early root development. E Cervantes, Spain 429-438

Seed germination is a model system for the analysis of plant development. Cell elongation and differentiation processes are under the control of hormones and submitted to the influence of environmental factors. Hormones (abscisic acid, auxin, gibberellic acid, ethylene and others) interact with each other as well as with diverse components of cell metabolism. *Arabidopsis* is the model system for the study of developmental processes in plants and research in this species is now helped by a multiplicity of resources (massive genome sequences

available, collections of mutants and lines expressing GFP, direct observation in confocal microscopy among others). The analysis of seed germination in *Arabidopsis* using these tools offers a unique opportunity to dissect the interaction of hormonal pathways during development and its relationship to cellular processes. Ethylene is one of the hormones whose mechanism of action has been most thoroughly investigated and may serve a pivotal role in this analysis. An important requirement is the accurate description of phenotypes for the known mutants with altered sensitivity or response to hormones. This chapter reviews our recent description of root apex curvature as a new phenotype associated with the ethylene signal-transduction pathway mutants. New *Arabidopsis* sequences induced during germination have been recently isolated. Their expression analysis may be a useful tool to investigate hormonal interactions during seed germination.

47. Endosperm development and regulation of starch biosynthesis. SE Lid, H Rudi, Norway 439-446

In angiosperms, double fertilization results in two fertilization products; the embryo and the endosperm. The endosperm represents a terminal organ limited to the seed stage during plant development, where its primary role is to nourish the growing embryo or germinating seedling. The endosperm of angiosperms has attracted much research attention primarily for two reasons. First, the endosperm represents our most important renewable source for food, feed and industrial raw material. Secondly, the relatively simple organization of the endosperm, with its limited number of cell types, also makes the endosperm an excellent system for basic studies of plant developmental biology. Therefore, progress in this field is not only expected to have an impact for developing plant varieties with improved characteristics for food and feed production, but for plant production in general. Due to the economic and agronomic importance of the monocots, much effort has been directed at understanding endosperm biology in cereal species. However, the use of *Arabidopsis thaliana* as a model species has in recent years contributed to the elucidation of several aspects of endosperm development. In this paper we will discuss both endosperm developmental biology as well as regulation of starch metabolism in the endosperm.

48. Expression of *Solanum americanum* genes encoding proteinase inhibitor II is essential for seed development. S-F Sin, China, EC Yeung, Canada, M-L Chye, China 447-453

Proteinase inhibitor II (PIN2) proteins not only confer protection against insects in transgenic plants by their action on exogenous proteinases, but can also regulate endogenous proteolysis. To investigate the endogenous roles of *Solanum americanum* (American black nightshade) genes encoding serine proteinase inhibitors SaPIN2a and SaPIN2b, we have examined their expression patterns and have shown that they are expressed in tissues destined to undergo programmed cell death, suggestive of their involvement in modulating endogenous proteolysis. Subsequently, we used RNA interference (RNAi)-induced post-transcriptional gene silencing (PTGS) to elucidate their functions. Examination of PIN2-RNAi transgenic plants revealed a reduction in seed set due to seed abortion. Aborted seeds had an abnormal endothelium that affected endosperm and embryo development, implicating that SaPIN2a and SaPIN2b are essential for seed development.

49. Physiological, biochemical and genetic aspects of GA influence on seed growth and development. LA Minasbekyan, Armenia, JA Teixeira da Silva, Japan 454-459

Macromolecule transport between the cytoplasm and the nucleus occurs through the nuclear pore complex (NPC) and is mediated by multiple families of soluble transport factors. The gibberellic acid (GA) receptor remains unidentified, and the molecular mechanisms by which plants respond to GA are still unknown. For this reason we studied phospholipid content in the composition of the nuclear membrane and matrix, which surround the NPC and play an important role in polarity, nuclear membrane surface charge and on the speed of diffusion through the NPC. While nuclear transport receptors promote NPC passage, cargo domains can drastically counteract the process through a hydrophobic permeability barrier (Ribbek and Görlich 2002). Differences in the content of particular phospholipids (PLs) in intact nuclei subfractions from 3-day-old wheat seedlings were found. The processing by GA resulted in the redistribution of PLs within each nuclear subfraction. A functional role caused by structural changes in the nuclear membrane during germination due to this PL redistribution is discussed. Evidence suggests differential sensitivity of nuclear subfractions to GA processing, and supposes that such rearrangements change nuclear membrane permeability and its surface charge. At the same time general architecture is shared by NPCs in all eukaryotes, and many of the NPC proteins are conserved across phyla (Rout and Aitchinson 2000, Hinshaw and Milligan 2003).

Genes, Genomes, Genomics, and Breeding

Part 1 Genetics and molecular techniques

50. Methodological advancement in molecular markers to delimit the gene(s) for crop improvement. P Azhaguvel, Germany, DV Saraswathi, Japan, A Sharma, USA, RK Varshney, India 460-469

Molecular markers, in recent years, have accelerated plant breeding methods significantly with an objective of crop improvement. At present a variety of molecular markers are available and the choice of using a particular type of marker depends on the user. With the advances in the area of genomics, new type and gene-derived markers as well as novel approaches such as genetical genomics, linkage disequilibrium

(LD)-based association mapping, etc. have been developed for identification of “perfect” markers for their use in breeding practices. The present article provides an overview on presently available but main type of molecular markers and their use in trait mapping, map-based cloning, estimation of diversity in germplasm collection to understand the population structure as well as in the area of comparative genomics. While dealing the above topics, major emphasis have been given on modern genomics tools and approaches such as functional molecular markers (EST-SSRs, EST-SNPs, SFPs), expression genetics or genetical genomics, high throughput approaches and automation technologies, public databases, etc. Utilization of modern genomics approaches such as functional genomics coupled with molecular marker technologies have a great potential to facilitate plant breeding practices and thus marker-assisted breeding seems to be evolved to genomics-assisted breeding in the near future.

51. Gene trapping and its applications in seed floricultural biotechnology.

AA Estrada-Luna, C Alvarez-Mejía, J-P Vielle-Calzada, México 470-475

A growing interest in the modification of floricultural traits through traditional breeding or genetic engineering is largely dependent on robust programs of seed genetic improvement necessary for their establishment and commercialization. Strikingly, little attention has been paid to the genetic basis and molecular mechanisms that regulate seed formation in floricultural species. Recent progress in large-scale insertional mutagenesis has opened new possibilities to systematically assess gene function in many plant species. Insertional mutagenesis relies on the introduction and activity of foreign DNA elements (mutagens) into the genome sequence of an organism. A powerful alternative to identify genes acting during specific developmental programs is the identification of expression patterns through enhancer detection or gene trapping. Both T-DNA and transposon insertions can be modified to function as either gene traps *sensu stricto* or enhancer detectors. In this chapter we briefly review our current knowledge of the genetic basis and molecular mechanisms that control female gametogenesis and early seed formation in a few model species, and describe some of the available enhancer detection and gene trapping tools that allow a systematic elucidation of gene expression and function. The developmental programs that control male and female gametogenesis, double fertilization, and early seed development appear to be tightly conserved among the Angiosperms. Therefore, the utilization of model species to investigate basic biological problems associated with floral organogenesis or seed formation promises to offer wide and unexpected possibilities for new biotechnological applications in floriculture.

52. Tilling in the botanical garden: a reverse genetic technique feasible for all plant species.

GW Haughn, EJ Gilchrist, Canada 476-482

Targeting Induced Local Lesions IN Genomes (TILLING) is a powerful technology that employs heteroduplex analysis to detect which organisms in a population carry single nucleotide mutations in specific genes. Genes are amplified by PCR using pooled genomic DNA from several individuals as a template. Following denaturation and renaturation of the amplified DNA, heteroduplexes form if organisms with wild type and mutant sequence are both present in the pool. The heteroduplexes can be detected by cleavage with an endonuclease and resolution of the resulting fragments on a sequencing gel. TILLING can be an effective reverse genetic technique if it is used to screen populations mutagenized with chemical mutagens such as ethyl methane sulfonate (EMS). Since such mutagens induce a diverse array of mutant alleles at a high frequency in any organism without the need of transgenic technology, TILLING is more versatile, universal and requires a smaller mutagenized population than other reverse genetic methods. TILLING can also be used to detect naturally occurring single nucleotide polymorphisms (SNP's) in genes among accessions, varieties, ecotypes or cultivars. These SNP's can serve as genetic markers in mapping, breeding and genotyping and can provide information concerning gene structure, linkage disequilibrium, population structure or adaptation.

53. Expressed Sequence Tags of genes involved in the flowering process of *Passiflora* spp.

MC Dornelas, SM Tsai, APM Rodriguez, Brazil 483-488

A major challenge of current plant biology is to understand the genetic basis and molecular mechanisms of all naturally occurring variation present among plant species, which have driven evolution by natural selection. Accordingly, developmental variation likely reflects adaptations to different natural environments and it is the origin of plant species differentiation. The genus *Passiflora* provides a remarkable example of floral complexity and diversity. The extreme variation of flower morphologies allowed a wide range of interactions with pollinators to evolve. The analysis of Expressed Sequence Tags (ESTs) as an approach for the detection of genes expressed during *Passiflora* reproductive development, allowed the identification of novel sequences from a relative small number of ESTs. Particularly interesting was the detection of a significant number of sequences related to the response to both abiotic and biotic stresses, as well as low abundance transcripts with high similarity to homeobox genes, transcription factors and signaling component genes that are likely to be determinant of the evolutionary meaningful novelty. The detection of these novel sequences can contribute to the development of EST-based markers for important agronomic traits as well as to the establishment of a genomic approach to understand the naturally occurring floral diversity among *Passiflora* species.

54. Gene profiling of plants with cDNA-AFLP. S Chaudhary, K Yu, Canada 489-497

In recent years gene profiling techniques have been increasingly used in plant research to study gene expression and to provide functional data for many genes simultaneously. Various gene profiling techniques are available to molecular biologists, including cDNA-Amplified Fragment Length Polymorphism (cDNA-AFLP), Differential Display (DD), Serial Analysis of Gene Expression (SAGE) and Microarray. This review outlines the role of cDNA-AFLP in plant genomic research. Applications of cDNA-AFLP to elucidate the molecular mechanisms involved in plants resistance to biotic stress, abiotic stress, and plant development are discussed. The advantages and disadvantages of this technique is compared to other gene profiling techniques used in plant research. cDNA-AFLP has a great potential in studying the expression profiling of floriculture and ornamental plant species since very little sequence information is available for these plants.

55. Molecular markers and their use in genetic studies in rose.

Z Yan, The Netherlands, **Y Bai**, China, **JA Teixeira da Silva**, Japan **498-503**

Molecular markers are becoming widely used in crops for varying purposes including genetic studies of traits, genotype fingerprinting and phylogenetic studies. Molecular genetic studies of roses have been performed on qualitative as well as quantitative traits in different mapping populations by using different marker types. Knowledge of the inheritance of the traits of interest have been gained, which is utilized to pave the roads towards marker-assisted breeding and detailed characterization of specific genes.

Part 2 Breeding and genetic resources

56. Modification of flower colour and plant form in selected ornamentals by molecular breeding.

M Boase, K Davies, New Zealand **504-511**

Flower colour and plant form are important traits that influence consumer selection of ornamental plant cultivars. Molecular breeding using recombinant DNA techniques offers opportunities to produce ornamental cultivars displaying novel flower colours and form beyond the range possible with traditional breeding. After giving some background information on the flavonoid pigments and the *ro/C* gene, we give examples from the literature on pigment profiling, development of transformation systems and the formulation and application of molecular breeding strategies to modify flavonoid biosynthesis in chrysanthemum, cyclamen, cymbidium, lisianthus, pelargonium and petunia. We also review research in which the *ro/C* transgene was introduced into chrysanthemum, pelargonium and petunia, to modify plant form. Despite the significant potential of genetic modification (GM) technology for ornamentals, the high costs of research, development and regulatory approval, the costs and difficulties of access to intellectual property (IP), and some market resistance present significant challenges for profitable applications of molecular breeding. Although consumer resistance may be less for genetically modified ornamentals than food products, the relatively small crop sizes exacerbate some of the other challenges. However, given the consumer appeal of novel traits that can be provided only by molecular breeding, the technology is still likely to have a major impact on the industry in the future.

57. Cytoplasmic, genomic and transgene induced male sterility. T Roitsch, T Engelke, Germany **512-522**

Cytoplasmic male sterility, or CMS has been most extensively studied in the past and various systems have been characterized in detail and successfully adopted for practical application in agriculture for hybrid seed production within various species. The key for the success in hybrid breeding is the selection of parental lines. Special attention is required for the maintainer lines, which not only need to carry the maintainer traits but also inherit as many as possible of the valuable agricultural traits. Combining both, male sterility on the one hand and agricultural traits on the other hand is a time-consuming process, since normally a number of backcrosses have to be done to reach this goal. This might be the reason why it took a long time from the first reports on CMS to reach the predicted potentials of hybrids like higher yields and greater uniformity compared to open-pollinated varieties. The development of molecular markers for a fast selection procedure of male-sterile plants and maintainer lines as well as haploid techniques to generate homozygous lines have opened new potentials and greatly facilitated hybrid breeding.

58. Cryopreservation in floricultural plants. Q Wang, China, **A Perl**, Israel **523-539**

Cryopreservation is considered an ideal means for the long-term conservation of plant genetic resources. This chapter provided comprehensive information on developments and advances of cryopreservation of shoot tips, cell cultures, somatic and zygotic embryos, pollen, anthers, seeds, immature seeds, and axes of zygotic embryos of floricultural crops. Although much progress has been made in the last several decades, existing data clearly showed that studies on cryopreservation in floricultural crops had been far lagging behind those on other crops such as fruit trees. Threats to genetic resources of floricultural crops are becoming much more serious than ever and, at the same time, the demand for new cultivars with novel traits by market is becoming much stronger than ever. Therefore, much more efforts should be invested in studies on cryopreservation, in order to maintain biodiversity and avoid genetic erosion of genetic resources, thus meeting the need by classical breeding and genetic engineering programs. Potential applications of cryopreservation to virus elimination and transformation should also be attempted.

59. Overcoming interspecific barriers in ornamental plant breeding.
T Eeckhaut, K Van Laere, J De Riek, J Van Huylbroeck, Belgium 540-551

In ornamentals, interspecific breeding is considered to be the most important source of genetic variation. The introgression of genes may be part of a breeding program towards disease resistance, better growth vigor, winter hardiness or morphological alteration in general. Both sexual and asexual hybridization, by pollination and protoplast fusion respectively, are fit to induce the desired introgression into the acceptor species. However, the number of possible barriers is very high. Obviously, apart from the actual "fusion barriers" also regeneration barriers are met; moreover, a thorough screening procedure of possible hybrids is needed. Whereas the hybrid status can be confirmed by molecular markers, visualization of introgression needs to be performed by cytogenetic techniques. In sexual crosses, both prezygotic and postzygotic barriers can inhibit the creation of a fertile interspecific hybrid; in asexual crosses regeneration of hybrid cells is often a bottleneck. For this reason *in vitro* techniques are indispensable for interspecific breeding in a large number of crops. Polyploidization protocols, whether driven by unreduced gametes or by mitosis arresting chemicals, can ease the fertilization or restore fertility of F₁-hybrids. In the latter case, unreduced gametes arise after FDR (first division restitution), SDR (second division restitution) and IMR (indeterminate meiotic restitution) still offer the possibility of direct gene introgression in the next generation, even if the hybrid produces no normal (haploid) gametes.

60. Molecular mechanisms of self-incompatibility in Brassicaceae.
M Watanabe, G Suzuki, H Shiba, S Takayama, Japan 552-555

Self-incompatibility (SI) is defined as the inability of a fertile hermaphrodite plant to produce zygotes after self-pollination. SI prevents self-fertilization by rejecting pollen from plants with the same genotype. The SI system in *Brassica* is controlled sporophytically by *S* multiple alleles at a single locus, and involves cell-cell communication between male and female. When the *S* phenotype of the pollen is the same as that of the stigma, the pollen germination and/or the pollen tube penetration are disturbed on the papilla cells. On the female side, SRK is a membrane-spanning receptor-like serine/threonine kinase. The SRK solely determines *S* haplotype-specificity of the stigma. On the male side, SP11 is a small cysteine-rich basic protein and is a male *S* determinant. SP11 works as a sole ligand to activate its cognate SRK specifically. Because the activity of the *S* allele is controlled sporophytically, dominance relationships influence the ultimate phenotype of both the stigma and pollen. Molecular analysis has demonstrated that the dominance relationships between the *S* allele in the stigma were determined by SRK itself, but not by virtue of its relative expression level. In contrast, in the pollen, the expression of *SP11* from the recessive *S* allele was specifically suppressed in the *S* heterozygote, suggesting that the dominance relationships in pollen were determined by the expression level of *SP11*. This understanding of SI in *Brassica* species will be applied to F₁ hybrid varieties in other species of Brassicaceae.

61. Advances in breeding of Japanese garden iris. T Yabuya, N Yoshihara, K Inoue, H Shimizu, Japan 556-563

Japanese garden iris, *Iris ensata* var. *ensata* is an important ornamental species which attracts many people because of the bright colors and beautiful patterns of the flowers during the rainy season from the end of May to June in Japan. This garden species which originated from the wild variety, *I. ensata* var. *spontanea*, has been extensively developed as a modern plant by ardent Japanese breeders. It is used as a cut and pot flower and also as a garden iris. One of the main objectives in the breeding of the garden species is the creation of novel flower colors such as blue, red and orange. In this chapter, breeding history, overcoming breeding barriers through tissue culture, embryo culture and cell fusion, characterization of anthocyanins, their biosynthetic enzymes and corresponding genes and breeding for flower colors in Japanese garden iris are discussed, and the breeding methods for blue, red and orange flowers of this species are proposed.

62. Apomixis: occurrence, applications and improvements. VTC Carneiro, DMA Dusi, Brazil, JPA Ortiz, Argentina 564-571

Apomixis is an asexual mode of plant reproduction through seeds. A common feature of all apomicts is the autonomous development of embryos and the generation of progenies that are exact genetic replicas of the mother plant. The aims of studying apomixis are to unlock the diversity of apomictic plants and to make it feasible to transfer apomixis to agriculturally important genotypes, therefore conferring them the ability of cloning through seeds. In this chapter we present a short review on the incidence of apomixis in plants of floricultural interest and the description of the recent advances in the molecular and genetic characterization of natural apomicts.

63. Advances in cloning and expression of apomixis-specific genes in flowering plants. LZ Chen, LM Guan, Japan 572-577

Up to megasporogenesis, there is no difference between sexual and apomictic plants of *Panicum maximum* and *Paspalum notatum*. The most important, notable difference in both is that while the formed megaspore does not divide, and becomes degenerated, aposporous initial cell (AIC) derived from nucellar tissue appears and divides into 4 nucleate embryo sacs containing one egg, two synergids and one polar nucleus in *P. maximum*, and one egg, one synergid and two polar nuclei in *P. notatum*. A novel classification method, based on ovary length, for sampling different developmental stages of embryo sac formation in obligate sexual and facultatively apomictic genotypes of *P. maximum*

has been employed to make a cDNA library and to isolate an AIC stage-specific cDNA clone, probably representing a full-length gene. The A2-134 cDNA, designed ASG-1 (apomixis-specific gene), was found to be expressed in flower buds of the apomictic but not of the sexual accession. The cDNA (1,177bp) codes for a protein of 305 amino acids with a molecular mass of 34.2 kDa. The *in situ* hybridization-analysis revealed that ASG-1 is not expressed in the ovule before and during megasporogenesis in both of *P. maximum* and *P. notatum*. With the appearance of AIC in the ovule of the apomictic type, ASG-1 expression is strong and specific to AIC and continues through different stages of the AIC-derived embryo sac development, but not in the sexual type. This indicates that the gene may play a role in this developmental process. In a heterologous system, using *P. notatum*, identical results were obtained.

- 64. Collection, conservation, evaluation and utilization of tea (*Camellia* spp.) genetic resources in China.**
L Chen, M-Z Yao, Y-J Yang, F-L Yu, China 578-582

Tea is the most popular non-alcoholic soft and healthy beverage across the globe and is an increasingly popular ornamental plant, easily found as a garden hedge in SE Asia. It has socio-economic and cultural importance and potential for some Asian and African countries, such as China, India, Japan, Sri Lanka and Kenya. Tea genetic resources are fundamental materials for production utilization, cultivar innovation and biotechnology research. In this chapter, the achievements and progress of tea genetic resource research in China in the last two decades are systematically reviewed from the following view-points: investigation and collection, conservation and cataloguing, appraisal and evaluation, as well as utilization. The authors also discuss the prospects of tea genetic resource research in China.

- 65. Genetic resources of Kazakhstan flora: Experience, basic targets and methods for conservation of flowering plants.**
AA Ivaschenko, LM Grudzinskaya, NG Gemedzhieva, Kazakhstan, JA Teixeira da Silva, Japan, NA Ryabushkina, Kazakhstan 583-588

The flora of Kazakhstan is virtually unknown to researchers around the world. This chapter aims to not only expose the wonders of the Kazakhstan flora, including several *Tulipa* and *Iris* spp., but also to encourage scientists who may be willing to assist in the recovery of unique endemic flora which faces extermination primarily caused by human activity. The plight of Kazakhstan botanists and biologists to engage in *ex situ* conservation efforts is highlighted, and new projects aimed at analyzing important secondary metabolites are emphasized.

Part 3 Mutations and mutagenesis

- 66. Biotechnology and mutagenesis in improving ornamental plants. SM Jain, Finland, MM Spencer, Austria 589-600**

Floriculture has benefited tremendously from biotechnology and induced mutations, and there is a great potential to further improvement of ornamental plants with commercial values. Floriculture industry is quite lucrative for exports in the international market, and has a great impact on enhancing the socio-economic aspects in the developing countries. The Joint FAO/IAEA Division initiated program of genetic improvement of ornamental plants by using biotechnology and radiation-induced mutations through Technical Cooperation projects in several countries such as Thailand, Malaysia, Indonesia, and Philippines. The Joint Division programme maintains a database of officially released mutant varieties world-wide (<http://www-mvd.iaea.org>). This review describes an overview on *in vitro* propagation techniques and mutagenesis in ornamental plants.

- 67. Mutations: the law of recurrent variation. W-E Lönnig, Germany 601-607**

In spite of an overwhelming acceptance of mutations as the ultimate source of evolution, the worldwide study of induced mutations – once hoped to include the potential to speed up evolution and *inter alia* revolutionize plant and animal breeding (Muller) – has led to an entirely different (overall) result for breeding research in particular and biology in general: the law of recurrent variation. It states that “treating homozygous lines with mutagenic agents generates large, but clearly finite, spectra of mutants. This consistently occurs when the experiments are carried out on a scale adequate to isolate the potential of alleles causing phenotypic and functional deviations (mutation saturation). However, due to almost invisible residual effects of changes in redundant sequences and/or of further chromosome rearrangements, the corresponding saturation curve is asymptotically approaching its limit for the micro-quantitative part of variation.”

- 68. Insertion mutagenesis for plant functional genomics. M Tadege, KS Mysore, USA 608-618**

Comprehensive understanding of systems biology requires understanding of gene function both as individual entities and as parts of a network. The last 25 years has seen a tremendous progress in gene cloning, sequencing, and transformation with the goal of identifying gene function for a better understanding of systems biology. Whole genomes have been sequenced from a variety of organisms including *Arabidopsis* and rice. Our understanding of gene function, however, is lagging behind because sequence information is not sufficient to accurately predict function. Perhaps, the most direct way to determine gene function is to introduce genetic mutations and analyze phenotypic repercussions. The four general methods that have been commonly used to generate mutants in plants are; post transcriptional

gene silencing, chemical mutagenesis, irradiation mutagenesis, and insertional mutagenesis. Insertional mutagenesis is a method of disrupting gene function by introducing a foreign DNA fragment (T-DNA, transposon, or retrotransposon) into the genome. This approach typically leads to a loss-of-function mutation or with modification it can be used for activation tagging which typically leads to a gain-of-function mutation by over-expressing the target gene instead of inactivation. In this chapter we discuss current advancements in insertional mutagenesis as applied to plants using T-DNA, DNA transposons or retrotransposons. Whenever possible, we have tried to describe these techniques from the genomics perspectives. Our evaluation of the relative advantages and disadvantages of the techniques as well as commentaries on regeneration efficiency and availability of *in planta* transformation strategies are thus reflecting this functional genomics approach taking into account the size of the entire genome of reference.

69. Advances of mutagenesis in flowers and their industrialization.

M Okamura, A Tanaka, M Momose, N Umemoto, JA Teixeira da Silva, T Toguri, Japan 619-628

The efficiency of mutation generation by ion beams, the characteristics of the mutants induced by the combined method of ion-beam irradiation with *in vitro* cell and tissue culture are investigated. Ion beam breeding can induce a wide variety of flower-color and -shape mutants, and that the combined method of ion beam irradiation with tissue culture is useful to obtain commercial varieties in a short time. The potential of ion beam breeding and its application to ornamentals is discussed. Transposable elements are considerably related to mutations because of their potential ability to transpose. Increasing knowledge on the relationship between mutated genes and transposable elements would improve mutation breeding.

70. Fast neutron bombardment (FNB) mutagenesis for forward and reverse genetic studies in plants.

H Wang, G Li, R Chen, USA 629-639

Genetic resources are indispensable in plant breeding and genetic studies. Their utility has been dramatically improved by the advancement in genome sequencing and availability of functional genomic tools in the past decade. Fast neutron bombardment (FNB) mutagenesis is effective in generating deletion mutants and has been successfully used in the generation of new crop varieties. Both forward and reverse genetic systems can be utilized in the cloning of genes and in high throughput screening of FNB deletion mutants. In this chapter, we highlight some of the key features of FNB mutagenesis and its applications in genetic studies of the model legume *Medicago truncatula*. FNB mutagenesis can be easily applied to crops and ornamental species.

71. Role of induced mutagenesis for development of new flower colour and type in ornamentals.

SK Datta, India, JA Teixeira da Silva, Japan 640-645

Induced mutagenesis and mutant induction through the application of physical and chemical mutagens remains, despite the advent of new technologies for novel variety production, an important part of ornamental and floricultural plant breeding. Through a relatively easy-to-apply technology, in both *ex vitro* and *in vitro* systems, novel characters in plant form, leaf and flower colour and shape can be induced without a negative, residual effect on human health (in the case of consumables). This is particularly relevant today where the consumer-market is much more safety-orientated, and where the floriculture industry is always seeking novel characters to surprise the market. This chapter looks at classical mutation breeding as one of the important hallmarks of novel colour, form, size and shape breeding in ornamentals.