Floriculture, Ornamental and Plant Biotechnology

Advances and Topical Issues

Volume II

Edited by

Jaime A. Teixeira da Silva

CONTENTS: VOLUME II

Genetic Engineering and Transgenesis

Tissue Culture and the *In Vitro* Environment

Part 1 Somaclonal variation: cause, induction and control

Part 2 Somatic embryogenesis

Part 3 Techniques and tools for improved organogenesis and reduced somaclonal variation

Part 4 Scaling-up and mass production systems

Part 5 Applied case-studies

FOREWORDS TO VOLUME II

Andrea Allavena, Experimental Institute for Floriculture (Sanremo - Imperia), Italy

The era of plant engineering began with the breakthrough of *Agrobacterium*-mediated and direct transformation methods. In recent years, a rapid increase in the number of engineered species in combination with a plethora of transferred useful genes, demonstrate the potential of genetic transformation tools. By contrast the number of engineered species and cultivars approved for cultivation is still in a lag phase and the opinion that engineered plants may cause damages, either to human health or to the environment, is deep-rooted in public opinion. Scientists are often asked to adopt plant engineering technology that fits public opinion endorsement, and fulfil expected and going beyond current national laws. To breed a more acceptable generation of transgenic plants the following guidelines are proposed by the scientific community: 1) The correct sequence of the transgene only is integrated in the plant genome; 2) The transgene is integrated in the desired number of copies; 3) A desired level of expression is reached by selecting among transformation events and by using a tissue-specific or inducible promoter; 4) Transgenic plants do not contain unnecessary DNA sequence (selectable markers, ancillary sequences, backbone plasmid sequences, aborted copies of the transgene and of the marker, filling sequences, recombination sequences, T-DNA border, etc.); 5) Pathogen-derived genes may be inserted in strictly indispensable cases only; 6) Perturbation of plant genomic DNA is restricted to a minimum level; 7) Genetic strategies to limit the spread of transgenes by pollen (e.g. male sterility, transformation of chloroplasts) are adopted; 8) Accurate analysis of transgenic plants needed to select the wanted events of integration. Technical motivations, such as improving transgene and genome stability and allowing transgene pyramiding, also suggest the adoption of the herein indicated guidelines. Since the presence of selectable markers in transgenic plants is one of the major concerns, several methods that allow the recovery of transgenic plants free of marker gene were developed. These methods fall into three major categories: co-transformation and segregation, excision of the marker gene by recombination, and transformation with the useful gene only. Tools that assist the recovery of transgenic plants by these tiresome transformation methods were also adopted: virulent *Agrobacterium* strains and highly efficient direct transformation, plasmid vectors designed with minimum T-DNA and efficient borders; PCR-based methods for massive screening of putative transgenic plants, for genetic analysis of integration events and for verification of the occurrence of genome rearrangment; quantitative PCR and micro array technology to analyse the effect of transgene on endogenous genes. The articles of this Volume give a comprehensive review the of the scientific community's efforts in the field of ornamental plant genetic engineering and tissue culture. Most of the articles, in addition, report plant engineering technology that may contribute to limit the public opinion's concern about transgenic plants.

Andrea Allavena received the degree in Agriculture Science at the UCSC, Faculty of Agriculture (Piacenza) in 1975. He began his professional career in plant breeding and tissue culture of dry bean (*Phaseolus*) at the Experimental Institute for Vegetable Crops (Montanaso L.- Lodi). In 1991 moved to the Experimental Institute for Floriculture (Sanremo - Imperia) were he is still employed. The major fields of research are plant breeding, gene cloning and genetic transformation of ornamental species (*Anemone*, *Osteospermum* and *Kalanchoe*). He is member of International societies (ISHS, EUCARPIA, IAPTC&B) and of the SIGA (Italian Society of Agriculture Genetics).

Ervin Balázs, Agricultural Research Institute, Hungary

More than two decades have passed since the first transgenic plants were reported in 1983. Since than we have learnt about the creation of new organisms almost every day using transgenic strategies, and we could state that a new epoch has started. The first transgenic tobacco was quickly followed by many other crops bearing agriculturally important new traits. Corn, cotton, soybean and canola are already on the fields and have proved their superiority over their sister, non-transgenic cultivars. Genetic engineering became the most powerful technique for improving crops for environmentally friendly sustainable agriculture. Using transgenic plants significant reduction of pesticide treatments have been achieved and alternative solutions be envisaged for integrated pest management systems. From the beginning of the commercial cultivation of transgenic plants, since 1996 when only 1.6 million hectares were grown, the total in 2005 has topped 91 million hectares World wide. This large area is restricted almost exclusively for the four major crops, mentioned above. Other horticultural transgenic plants are also on the market, like carnations, crysanthemums, tomato, papaya, etc. The introduction of this new technology has been welcomed almost all over the Globe. But some concerns have been expressed because of past consequences of the Industrial Revolution in which environmental damage and occasional loss of life resulted. Biotechnology has been used for centuries during the selection of higher-yielding plants, microorganisms and animals by breeders in breeding programmes for particular phenotypic characteristics. This technology has been accepted and approved by different societies, and concerns over safety has never been a major problem.

This volume covers the highlighted topics of genetic engineering and transgenesis, including biosafety concerns related to GMOs. The section on selector and marker genes and vectors provides a comprehensive coverage of this area, describing the molecular techniques required for the removal of marker genes. It should be noted that today due to the development of PCR technology and highly efficient transformation, vectors and, in several cases marker genes, are not used. Safety concerns are also reflected in the experiments where new traits are specifically expressed in different tissues or organs of the plants. A section on novel transformation techniques extends our technology to other crops other than the major staple food crops to specific floricultural plants such as ornamentals and medicinal plants. The readers may also find a detailed survey on the use of transgenics in bioremediation and in stress tolerance.

This volume, especially for me – having graduated in horticultural sciences – has a unique flavor as most of the chapters cover research results on ornamental plants such as *Lilium* spp., carnations, chrysanthemums, pelargonium, Asteraceae, *Datura* spp., *Betula pendula*, *Malus prunifolia*, and others.

At the end of the volume there are chapters that cover the mode of actions of transgenes by describing the different mechanisms of gene silencing and also conditional gene expression in plants. Scientists working in the field of floriculture are well aware that these plants are usually excellent cash crops that give prosperity to growers, so it is obvious to finalize the volume with a chapter dealing with property rights.

In brief, enjoy the chapters written by respected experts.

Professor Ervin Balázs, founding scientific director of the Agricultural Biotechnology Center, Gödöllö, Hungary, has been serving as general director for a decade between 1990 and 2000. Early in his career he had been working in an International team in Strasbourg, France on the primary structure of cauliflower mosaic virus. He developed plant transformation vectors based on the promoters of that virus. Later, with his research team, he developed several transgenic virus resistant crops, such as tobacco, potato and pepper. He also participated in the regulatory work of gene technology at an International level. Currently he is establishing a DNA chip laboratory for plant breeders at the Agricultural Research Institute, Department of Applied Genomics, H-2462 Martonvásár Brunszvik u 2 Hungary.

Eva Casanova, University of Barcelona, Spain

The economic importance of ornamental flowers is increasing worldwide, which indicates a bright future for ornamental plant breeding. New, attractive varieties are continuously being created by breeders in response to consumer demand for novel products. Altered flower color and shape, improved fragrance, longer vase life in cut-flower species, as well as new plant morphology, are some of the desirable traits. Growers are also looking for plants with improved agronomic traits, such as increased production and resistance to pathogens.

New traits have traditionally been introduced into ornamental plants by classical breeding techniques. However, this is a slow and tedious process, which is based on crosses within the same species or between related species, and on the selection of offspring with desired features, which can take even several years. Moreover, in classical breeding the gene pool available for new traits is limited to the genetic background of the parents, and the sterility of many varieties of ornamental plants also restricts improvement by traditional breeding.

Nowadays, developments in genetic engineering, with new tools for transferring foreign genes into plants, combined with progress in the identification and isolation of genes, have allowed specific alterations of single traits in already successful varieties. Moreover, in addition to the overexpression or suppression of native genes, these techniques allow the available resources to be extended, since genes can be introduced into plants not only from other species but also from other kingdoms. Thus, in addition to the many genes suitable for ornamental plant breeding that have been isolated from plants, an ever-increasing number of genes from a wide range of living organisms are being and will be identified to improve plant traits.

Molecular breeding of ornamental plants is routine in only a few laboratories, due to the lack of efficient transformation systems. To date, monocotyledonous ornamental plants have been the most recalcitrant to transformation. Nevertheless, since 1987, when petunia became the first transgenic ornamental flower, both the efficiency of transformation procedures and the regeneration of transgenic plants have increased. This has allowed the successful transformation of more than 30 ornamental species, including commercially relevant plants, and the first genetically modified ornamentals are already on the market.

This book reviews recent information on basic and applied developments that have affected or are expected to affect ornamental breeding. Novel transformation techniques, such as *in planta* or floral dip transformation, are described. The book also covers new approaches and optimized methods to transfer genes into ornamentals, including both dicotyledoneous and monocotyledoneous species (liliaceous, grasses and turfgrasses). It also describes specific applications of molecular breeding, including delayed senescence in cut flowers and an increase in osmotic stress tolerance, and proposes new ways to transform plants for phytoremediation. The delivery of genes from other kingdoms, such as *rolC* from a bacterium or the gene of green fluorescent protein from a jellyfish, often with surprising results, opens new perspectives in the breeding of ornamentals, and these are also discussed.

Knowledge of the mechanisms of transgene expression and silencing in plants is essential to success in the production of transgenic plants. Recent discoveries in the examination of such mechanisms are reviewed. Moreover, the book deals with the use of suitable promoters, as well as with strategies to remove the marker gene, which would reduce environmental risks or consumer concerns. Although ornamental crops are ideal candidates for molecular breeding, since they may be more acceptable to the public than genetically modified food, the risks entailed by the application of genetic engineering are nevertheless examined in great detail. Finally, the legislation for the intellectual property of plant varieties is explained.

This book will undoubtedly contribute to the incorporation of molecular plant breeding as a complement to classical breeding to generate ornamental plants with new and improved traits.

Eva Casanova is a Researcher at the Department of Plant Physiology, Faculty of Biology, University of Barcelona, Spain, where she conducts research on the *in vitro* culture of carnation, specifically on adventitious organogenesis from tissues of this species. Furthermore, her specialization – of great importance to the global ornamental scientific community – are studies on the *in vitro* behaviour of *rolC*-transgenic carnations. She also closely examines the involvement of endogenous plant hormones on all of these processes.

Ming-Tsair Chan, Institute of BioAgricultural Sciences, Academia Sinica, Taiwan (ROC)

Since ancient times, Ornamentals have fascinated humanity and throughout the ages, people have capitalized on the diverse characteristics of the many species. Most Ornamentals are now systematically produced as a high value cash crop in the floriculture industry and as cut-flower and potted plants around the world. The creation of new hybrids with attractive or new flower patterns, color, shape, abiotic and biotic stress resistance has become an important factor in consumer demand and market direction for commercial production. Thus, the fascinating challenge of understanding the mechanisms of plant growth has gained a new urgency as we strive to exploit Ornamental crops for improved productivity. At no other time has this understanding been more relevant or the field more stimulating or provocative. Increasingly refined tools of biochemistry, molecular biology and plant transformation now allow us to approach these mechanisms at various levels of plant organization. The time is ripe to bring together new knowledge arising from this field, giving it a clear perspective and identifying research priorities for future improvements.

Plant growth regulators (PGRs) play a very important role in the regulation of plant growth and development. Floriculture has recently seen great strides in the effective use of this knowledge for chemical regulation of crop growth and productivity. In addition, gene gun or *Agrobacterium*-mediated genetic transformations have been reported for various ornaments involving regeneration by organogenesis from different explants. The integrated approaches of genetic engineering of Ornamentals have been of great value because of their commercial value. Henceforth research in the improvement of existing cultivars, or the establishment of new transformation protocols for new Ornamentals is essential. If floriculture researchers, hobbyists and commercial growers can be provided with convenient access to more recent research findings, clearly these would greatly enhance their efforts in meeting the challenge of improving the production of Ornamentals.

Like any other crop that has been found to be useful, Humanity has tried to improve the utility of Ornamentals through breeding. In recent years, introducing a new gene into an Ornamental plant gives it new “information” to synthesize a new protein that establishes a new trait. Furthermore, together with progresses in molecular biology, has allowed improvement through gene manipulation. Plant genetic engineering achieves in a very quick and precise way what breeders try to accomplish by years of crossing. Moreover, biotechnology makes it possible to isolate, characterize, and manipulate specific genes. This new technology provides a powerful tool to understand plant growth and development and offers a way to directly manipulate the processes leading to improved varieties with attractive flower qualities. In the future, the molecular science of genomics and proteomics will contribute substantially to Ornamentals’ improvement because genomics will help identify hundreds of plant genes that regulate all the processes of growth and development, adaptation to stress and defense against insects and pathogens. Molecular techniques will improve Ornamental crops by altering the regulation of those genes. A few chapters cover this important topic. Altogether, the material presented in this book combines and extends the cutting edge technologies for the sustainable productivity and improvement of Ornamentals.

Obviously, a book on “Floriculture, Ornamental and Plant Biotechnology**:** Advances and Tropical Issues (1st Edition)” will be an important and useful source of information for university students, professional professors and researchers. My hope is that this book will provide its readers with a strong awareness of current cutting edge research approaches being followed in the field and an appreciation of the potential of biotechnological tools to achieve improved Ornamental crops.

I congratulate the editor and authors for sharing their expertise.

Ming-Tsair Chan, Associate Research Fellow, Institute of BioAgricultural Sciences, Academia Sinica, Taiwan (ROC)

Jan Káš, Professor Emeritus, Czech Republic

After a detailed explanation of the basic terms and backgrounds of plant science in the 1st Volume the book logically continues with an excellent description of the hottest topics of modern Plant Biotechnology.

The first part of this Volume addresses the readers with up-to-date information about genetic engineering and transgenesis. The introduction defines the main tools and goals in genetic engineering of floriculture plants. Attention is then paid to the strategy of choosing proper selector and marker genes and suitable vectors. Tissue-specific promotors are not forgetten, nor are the major technology and research approaches applied for marker gene removal from transgenic plants. A few contributions deal with the quikly evolving field of transformation techniques and show their concrete applications both in ornamental plants and crops. Naturally, wide attention is paid to the problematics of high-level transgene expression and also to cosupression and silencing. The first part of the 2nd Volume is terminated by several actual GMO topics (e.g. abiotic and osmotic stress tolerance, bioremediation, ethylene production, tropane alkaloid production).

The second part of Volume II deals in detail with various aspects of tissue culture and the *in vitro* environment because the development of tissue culture techniques will affect remarkably the future success of plant genetic engineering. First of all attention is paid to somaclonal variation, somatic embryogenesis and also a detailed description of various techniques in relation to specific requirements. Showing the possibilities of scaling-up and mass production systems represents a culmination of the efforts of genetic engineers to utilize plant science achievements in favour of Humankind. The reader will find here the latest successes in planting cultivars of roses, *Pelargonium*, *Lilium*, *Gladiolus*, *Hyacinthus*, irises, tropical bamboos and medicinal plants for gaining secondary metabolites. The big advantage of this volume is that the reader will find here not only the survey of published data, but also many original findings. This approach makes possible to present a real, up-to-date review of this quickly developing field of science.

Jan Káš Born in 1934 (Žatec, Czechoslovakia). ***Education, degrees and positions:*** Engineer of Chemistry (1957, ICT Prague), PhD in Biochemistry (1970, ICT Prague), DSc.in Biochemistry (1987, Czechoslavak Academy of Sciences, Prague), Full Professor of Biochemistry (1990, ICT, Prague), Visiting Professor at the University of Luton, GB (1995-1997), Vice-Dean (1989-1991, 1992-1997), National Representative at IUPAC (1990-1996), IUPAC Titular Member and Secretary of the Commission on Biotechnology (1996-2001), National Representative in IUFoST (International Union of Food and Science Technology, Chairman of the Czech Biotechnology Society, Member of the Editorial Boards of the journals "Food and Agricultural Immunology", "Advances in Biotechnology", "Bioprospect" (Czech Biotechnology Society) and "Chemical Papers" (Czech Chemical Society). Head of the Department of Biochemistry and Microbiology (34 members of staff including technicians) 1994-2000. ***Long stays abroad*****:** Carlsberg Laboratory, Copenhagen, Denmark, 1974; University of Amsterdam, Dept. of Biochemistry, The Netherlands, 1975-1976; City College, New York University, USA, 1991-1992 (Fulbright scholar). ***Present position:*** Professor Emeritus. ***Teaching:*** General and Applied Biochemistry, Biotechnology. ***Research interests:*** enzymes, immunochemical methods, biosensors, separation techniques, genetic modifications. ***Publications:*** More than 250 original and review papers and 12 patents.

Awards:

1988 Honorary Acknowlegment of the Czech Union of Scientific and Technical Societies (for activities in biotechnology)
1988 Medal of the Research Institute of Food Industry, Prague
1994 Memorial Medal of the Faculty of Food and Biochemical Technology, Prague
1999 Balling Medal (Institute of Chemical Technology, Prague)
1999 Memorial Medal of the Palacky University in Olomouc, Olomouc
2004 Medal of Italian Agronomists, Italy

Nedyalka Zagorska, 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 markers 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.

Tomas Vanek, Academy of Sciences of the Czech Republic, Czech Republic

Currently, most commercial propagation *in vitro* is done by axillary bud propagation on gelled media. During growth of plants *in vitro*, new shoots are formed by activation of buds in the axils of leaves or branches. In this way, from every bud, a cluster of shoots is formed within a few weeks. Then, the newly formed shoots are excised and subcultured to allow outgrowth of their axillary buds in the next subculture cycle. This method closely resembles the way many plants are propagated *ex vitro*, by making cuttings.

Culture on gelled nutrient media requires frequent transfer of plantlets to fresh media which is exceptionally labour intensive. Two potentially faster methods are hardly being used commercially: axillary propagation in liquid medium and somatic embryogenesis. By using liquid medium instead of gelled medium, propagation is accelerated, culture transfer frequencies may be decreased and labour is less intensive. However, liquid media provide a substantially different environment for the plantlets and widespread use is hampered by several problems including hyperhydricity of the tissue, rapid spread of contaminants (e.g. bacteria and fungi), plantlet asphyxiation and changed direction of growth with respect to gravity. Even faster propagation can be obtained using somatic embryogenesis. In this method single cells or cell clusters, not buds, are propagated. When required, cells are stimulated to form embryos which then develop into new plants. Potential propagation rates are extremely high (millions of cells can be grown in a litre of medium) and labour input is very low. However, at the moment, large-scale somatic embryogenesis is feasible only in a few crops including conifers. Process bottlenecks are technical (bioreactor technology) or physiological (e.g. establishment of stable embryogenic cell suspensions, embryo maturation and conversion into whole plants).

In the case of secondary metabolite production, liquid systems are generally used. Unfortunatelly, some secondary metabolites are formed only in some stages of plant differentiation, so cultivation of plant organ cultures (instead of “single cell” cultures) is more usefull. For this purpose, equipment requirements for both micropropagation and secondary metabolite production are equal.

Labour input can be seriously reduced by automation of the cultivation system (robotisation); e.g. automation of bioreactor systems, automated media exchange according to the selected method, and selection of embryos from a suspension and transfer to maturation media.

The chapters within the section on **Scaling-up and mass production systems** highlight some of these fundamental approaches and give concrete examples of scaling-up cultivation of different plants both for micropropapagation purposes and secondary metabolite production. The latest techniques, and ways to counter production inefficiencies are dealt with. Novice scientists to the field will find this section facinating, while professionals may find some solutions to their mass production systems *in vitro*.

Tomas Vanek, **Education:** RNDr.: (equivalent to M.Sc.) Chemistry, Faculty of Natural Sciences, Charles University, Praha, CSc.: (equivalent to PhD), Czechoslovak Academy of Sciences, specialisation: organic chemistry. **Position:** Head of Department of Plant cell Cultures at the Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Praha, Czech Republic. Visiting professor of Jejijang University, Hangzhou, China. **Field of research:** isolation and identification biologically active compounds from plants and plant tissue cultures, production and biotransformation of biologically active compounds in plant tissue cultures (e.g. taxol, ginsenosides), utilization of plant cell cultures and plants for invironment protection (phytoremediation). **International cooperation:** Delegate and expert of the Czech Republic in the COST Technical Committee for Agriculture, Biotechnology and Food Sciences; Vice-chairman of COST 837 (1998-2003) and 859 (2004-2008). **Publication activity:** Author and co-author of 102 scientific papers, 14 patent applications, 24 invited and 61 of other lectures at international conferences and meetings.

CONTENTS: VOLUME II

Genetic Engineering and Transgenesis

Part 1 Introduction

There is considerable interest in the introduction of agronomically useful traits into ornamental species. Rapid progress in plant molecular and cellular biology has great potential to contribute to the breeding of novel floricultural plants for the market, utilizing recombinant DNA technology. Molecular genetic manipulation for applied purposes is only possible with the development of an efficient protocol for transformation. The genetic manipulation process is dependent on the ability of plant tissue to produce totipotent cells that can be regenerated into a completely viable plant. The use of genetic engineering to incorporate useful genes into elite breeding lines is dependent on the development of tissue culture techniques. The majority of transformation protocols used in ornamental species is based on gene transfer mediated by *Agrobacterium* and particle bombardment (biolistic process). In this chapter, we discuss the technologies developed to regenerate and genetically modify ornamental plants in order to introduce new traits, such as color, longevity, and scent in flowers and architecture, acclimatization efficiency, and tolerance to biotic and abiotic stress in vegetative organs.

Part 2 Selector and marker genes, and vectors

No single selectable marker can meet all research purposes, due to differences in transformation protocols, biological differences related to species and explant and the objective of the research. Various selectable markers have been developed for the generation of transgenic plants each with advantages and disadvantages. In additional to selectable markers, visible markers possess unique features and are an important companion to selectable markers for use in genetic transformation. The selectable and visible markers commonly used for plant transformation are described and assessed. Recently, new markers possessing novel features have been developed. Potential for wide usage of these markers in plant transformation is discussed. For selection effectiveness and efficiency it is important, and very often essential, to have appropriate levels of marker gene expression. Strategies to control marker gene expression are also reviewed.

Biotechnology contributes to the improvement of crops by introducing a single gene conferring a desired phenotype. To succeed in this process it is necessary to identify the gene able to confer the desired characteristic and a promoter region directing the expression of this gene in the adequate time and place. Each plant gene has its own promoter region, usually located upstream the coding sequences, presenting *cis*-acting elements responsible for tissue/organ specific expression as well as for induction/repression by external factors. Most recognition mechanisms are conserved in the whole plant kingdom, but significant differences have been observed between mono and dicot plants. Constitutive promoters are sequences that direct gene expression at a high level in the whole plant during all developmental stages. They have widely used for fundamental research as well as biotechnological tools, but their use leads to unnecessary metabolic expenses for the transformed plant. On the other hand, tissue/organ specific promoters have been described but expression directed by them is not enough strong to reach the desired acquired phenotype in the transformed plant. The use of artificial constructions combining specific *cis*-acting elements or entire weak promoters with enhancers is proposed. Several techniques developed to test a promoter's activity are presented. Finally, we discuss the possibility to obtain improved ornamental plants in the future by a combination of a suitable promoter with a coding sequence able to confer a desired phenotype. Changing petals color or obtaining a senescence delay became possible objectives.

Marker gene removal from transgenic plants is an important component of plant genetic engineering and biotechnology. Marker elimination will remove potential environmental risks, consumer concerns, and technical barriers for plant genetic engineering. Different methods for marker elimination have been described. This article discusses the major technologies and approaches which have been used for marker removal.

Plants present a rich source of biomass for the production of proteins in large quantities. Specifically, expression of foreign proteins by transient expression using plant viral expression vectors has several advantages over constitutive expression in transgenic plants. For example, proteins which are potentially toxic and cannot be expressed in transgenic plants can be expressed at high levels using plant virus-

based expression vectors. In general, higher levels of foreign protein can be expressed by plant viruses. Furthermore, plant virus-based expression avoids many of the biosafety concerns surrounding transgenic plants. In the past, most approaches for the expression of foreign genes have focussed on either expression of the foreign protein as a fusion to a viral protein or from a duplicated subgenomic mRNA promoter. In this chapter, we examine the most recent strategies that have been developed to utilize plant virus-based expression systems for a multitude of purposes. This chapter covers 'the deconstructed virus strategy' versus the 'full virus strategy' of plant virus-based expression vectors. We go over various means by which conventional plant virus vectors can be improved and applications for both commercial uses and as a research tool. Finally, we cover the development of application of plant viral vectors in terms of large scale field trials.

Part 3 Novel transformation techniques

This chapter describes simple and efficient *in planta* transformation methods using *Agrobacterium tumefaciens* for buckwheat (*Fagopyrum esculentum* M.), mulberry (*Morus alba* L.), kenaf (*Hibiscus cannabinus* L.) and rice (*Oryza sativa* L.). In the methods, meristems of either apical (buckwheat, kenaf and rice) or axillary bud (mulberry and kenaf) of young plants (buckwheat, kenaf, and mulberry) or soaked seeds (rice) were inoculated by *A. tumefaciens* after being pricked with a needle. The inoculated plants were grown to maturation in pots under nonsterile conditions. Transformation was demonstrated by several lines of evidence obtained by using mostly the progenies of the T_1 generation; phenotypic inheritance from T_0 plants to the plants of the following generation, detection of transgene and its transmission to the following generation, rescue of plasmids composed of T-DNA of the binary vector and flanking plant genomic DNA, detection of β-glucuronidase activity in the transformants and resistance of seed germination of transformants to antibiotics (geneticin or hygromycin).

The development of a robust *Agrobacterium*-mediated transformation protocol for a recalcitrant species like *Chenopodium rubrum* requires the identification and optimization of the factors affecting transformation efficiency. We have demonstrated that *C. rubrum* can be transformed in a stable and heritable way using the floral dip method and found that plant developmental stage and fitness after dipping were critical for successful transformation. The floral dip transformation system will increase the utility of the model plant *C. rubrum*. Further adaptation of this technique will provide a useful tool for the transfer of foreign genes into close relatives of *C. rubrum* such as spinach, sugarbeet and *Chenopodium quinoa,* all being agriculturally important crops.

The *rolC* gene is found on the RI plasmid of *Agrobacterium rhizogenes*. Plants expressing this gene have significant changes in phenotype, including reductions in height, apical dominance, internode length, and leaf size. These changes can be beneficial to plant breeders attempting to create novel, smaller versions of existing cultivars. The *rolC* gene has been introduced into several ornamental species, including *Antirrhinum majus*, *Begonia tuberhybrida*, *Betula pendula*, *Chrysanthemum morifolium*, *Datura arborea*, *Datura sanguinea*, *Dianthus caryophyllus*, *Eustoma grandiflorum*, *Lilium longiflorum*, *Limonium otolepis x Limonium latifolium*, *Malus prunifolia*, *Osteopermum ecklonis*, *Pelargonium x domesticum*, *Petunia axillaris x* (*P. axillaris x P. hybrida*) and *Populus tremula x P. tremuloides.* These species exhibited changes in morphological traits such as dwarfness, increased branching, increased flower number, and unique leaf morphology. These traits are heritable from one generation to another, and the degree of phenotypic change is affected by the amount of *rolC* produced under a specific promoter. The introduction of *rolC* into plant species is an effective way to produce dwarf plants and has potential for breeding unique ornamental cultivars.

Green Fluorescent Protein (GFP) from the jellyfish *Aequorea victoria* is a spontaneously fluorescent protein, that is widely used as a recombinant protein tag *in vivo*. A huge number of researchers have used GFP to label proteins, cells and organisms in a wide range of prokaryotic and eukaryotic species with the aim of investigating basic questions of cellular and developmental biology, neurobiology and ecology and of developing innovative biotech tools. A key reason for the invaluable usefulness of GFP as a biological marker is the fact that this protein does not require additional / external cofactors to generate fluorescence. The wild-type or modified GFP gene has been introduced with different tech-niques in many plant species (monocot and dicot). The introduction and expression of the *gfp* gene in ornamental genomes may allow to develop novel classes of flower colors. Furthermore, the ability of GFP to generate fluorescence in living material, and its detection in real time, would offer a novel method for the phenotypic monitoring of defined flower genotypes and to ascertain the diffusion and use of transgenic cultivars in the environment. However, the generation of fluorescent flowers remains by-and-large elusive. Major difficulty is the interference by background fluorescence and levels of GFP expression. These problems have been tackled and finally

This brief review focuses the most important stages of *Agrobacterium tumefaciens*-mediated cereal transformation. Simple and efficient approaches for improved transformation are suggested. For *Agrobacterium* activation, we recommend the use of an extract of sterile tobacco plants instead of acetosyringone. At the stage of *A. tumefaciens* co-culture with plant explants, a long-term contact of explants with bacteria evenly distributed above the agar-solidified nutrient medium (agrobacterial monolayer) is important. Furthermore, to stimulate plant regeneration from cereal calluses, we suggest the application of the antibiotic cefotaxime at 50-150 mg/l. Applicability of these approaches for transformation of ornamental grasses is demonstrated.

Part 4 Transgene expression, structure and silencing

The capacity of produce transgenic plants posed a revolution in modern plant biology. The ability to transform crops with genes from any other organism opens up the entire community of life as the source for new genes for a crop. Reporter genes and chemically induced genes are opening new ways to see and understand gene function. Although RNA silencing is a relatively recent topic of research, the mechanisms are now becoming clear and there is some indication of its biological roles. The discovery of RNA silencing has completely changed our view of RNA as a regulatory molecule in eukaryotic cells and it is likely that this view will continue to evolve as further discoveries emerge about the diversity of silencing mechanisms.

The precise timing and control of gene expression provides a mechanism by which plants have evolved developmentally and physiologically to cope with diverse environments and stressful conditions. The misexpression of endogenous or exogenous genes in plants leads to a better understanding of gene function and ultimately aids progress towards the development of new improved varieties. Advancement in inducible gene expression technologies facilitates more precise functional analyses, revealing additional roles for genes that act at multiple stages in the plant life cycle, and allowing the conditional complementation of genetic mutations at different stages in development. Conditional expression systems in agriculture may also increase public acceptance of genetically modified organisms and have already been used to generate marker-free transgenic plant lines. As more systems suitable for field application are developed, the conditional expression of genes in agriculture will allow traits to be modulated, or even reversed, providing flexibility in the production of future generations of crop plants.

Transgenic plant studies have lead to the breakthrough discovery of RNA silencing as a conserved mechanism for gene regulation across kingdoms. Recent molecular genetic studies have revealed a major role for RNA silencing in the formation of silent chromatin, characterized by histone modifications and dense DNA methylation. These epigenetic marks ensure stable, yet potentially reversible, transmission of the silent state of genetic elements such as transgenes and transposable elements through multiple cell divisions, and in some cases, through successive generations. It is now recognized that epigenetic control mechanisms play a fundamental role in preserving the integrity of the genome against invasive parasitic nucleic acid elements such as viruses and transposons. It is also becoming clear that epigenetic processes are of major evolutionary significance in plants by providing plasticity to the genome to adjust to environmental changes and by stabilizing the genome after polyploidization events. RNA silencing has become a powerful research tool to elucidate gene function in reverse genetics studies and has been applied in the production of virus resistant crops. An enhanced understanding of epigenetic processes is therefore not only of academic interest but will also provide new tools and techniques for plant scientists involved in conventional and biotechnology-based horticultural and agricultural crop improvement.

When we overexpress the transgene in plants, we encounter two completely different phenomena, namely, successful expression and silencing of the transgene. In the first half of this paper, we introduce several strategies for a high-level expression of the transgene. In the latter half we discuss another aspect of the transgene's fate, i.e., cosuppression. The RNA interference (RNAi) pathway seems to account for the molecular basis of cosuppression. The presence of siRNA, a key molecule of the RNAi pathway, is an indication of plants displaying cosuppression. However, its presence does not always corresponded to the incidence of RNA silencing, suggesting the presence of regulatory mechanisms involved in the action of siRNA, especially in cosuppression between transgenes and the corresponding plant

The global flower industry thrives on novelty. Genetic engineering is providing a valuable means of expanding the floriculture gene pool so promoting the generation of new commercial varieties. There has been extensive research on the genetic transformation of different flowering plant species, and many ornamental species have now been sucessfully transformed, including those which are most important commercially. To date, more than 30 ornamental species have been transformed, including anthurium, begonia, carnation, chrysanthemum, cyclamen, datura, daylily, gentian, gerbera, gladiolus, hyacinth, iris, lily, lisianthus, orchid, pelargonium, petunia, poinsettia, rose, snapdragon and torenia (Deroles *et al*. 2002) New ornamental plant varieties are being created by breeders in response to consumer demand for new products. In general terms engineered traits are valuable to either the consumer or the producer. At present only consumer traits appear able to provide a return capable of supporting what is still a relatively expensive molecular breeding tool. Commercialisation of genetically engineered flowers is currently confined to novel coloured carnations. The production of novel flower colour has been the first success story in floriculture genetic engineering. However, further products are expected given the level of activity in the field. Other traits that have received attention include floral scent, floral and plant morphology, senescence of flowers both on the plant and post-harvest and disease resistance. To date, there are only a few ornamental genetically modified (GM) products in development and only one, a carnation genetically modified for flower colour, in the marketplace. There are approximately 8 ha of transgenic carnation in production worldwide, largely in South America. The other breeding programs on colour modification or alteration of plant architecture and height remain focusses on rose, gerbera and various pot plant species.

Chrysanthemum will remain an important global floricultural crop leader due to its extensive range of plant and flower colours, shape and form. Recent studies have demonstrated genotype-independent phenotypic plasticity of this ornamental crop, making it a suitable candidate as a floricultural model plant for *in vitro* studies on development and transformation. Significant findings in *in vitro* regeneration from thin cell layers and biotechnological breeding protocols involving somatic embryogenesis, intergeneric somatic hybridization, mutation/classical breeding, and genetic transformation demonstrate to the reader significant research in understanding the dynamic processes underlying growth, development, and flower induction of this plant. Although there is now a trend to use ion beams and genetic transformation to primarily induce novel flower characters, classical mutation breeding (using physical and chemical mutagens) remains an important source of variation. Protoplast electrofusion has been used to generate intergeneric, rust-resistant hybrids with wormwood which, together with the successful introduction of several insect, virus/viroid, and fungal resistance genes in a wide range of cultivars, will serve as a model plant to overcome many pest-related problems plaguing ornamentals in the global floricultural industry. Preliminary studies also indicate that, due to the capacity of the plant to withstand great stresses *in* and *ex vitro*, chrysanthemums might very well be suitable candidates for ornamental phytoremediation. Well-studied, established regeneration and transformation protocols will undoubtedly allow for rapid advances in the biotechnology of this jewel plant.

Ethylene is a primary plant hormone involved in the senescence of carnation flowers. Inhibition of the synthesis or action of ethylene delays the onset of senescence symptoms and increases the vase life of the flowers. Leaves of cut chrysanthemum of some cultivars frequently become yellow, sometimes prior to the onset of flower senescence. The leaf yellowing is induced by the action of exogenous ethylene. Generation of transgenic plants with suppressed production or action of ethylene is an excellent way to lengthen the longevity of these ornamental plants. This chapter reviews our recent results on generation and characterization of carnations with reduced ethylene production and chrysanthemum with reduced sensitivity to ethylene.

Studies on the *Agrobacterium*-mediated production of transgenic plants in several Liliaceous ornamentals, *Lilium* spp., *Agapanthus* spp., *Muscari armeniacum* and *Tricyrtis hirta* are described. Different strains of *A*. *tumefaciens* were used, all of which harbored the binary vector carrying the neomycin phosphotransferase II (NPTII) gene, the intron-containing β-glucuronidase (GUS) gene, and the hygromycin phospho-transferase (HTP) gene in the T-DNA region. Utilization of organogenic or embryogenic calluses as a target material for transformation and acetosyringone (AS) treatment during inoculation and/or co-cultivation with *Agrobacterium* were found to be critical for successful production of transgenic plants in Liliaceous ornamentals. Following transfer of co-cultivated organogenic or embryogenic calluses onto hygromycin-containing media, several hygromycin-resistant (Hyg^r) tissues were obtained, and complete plants were subsequently developed from these tissues. Most of the plants were verified to be transgenic plants by GUS histochemical assay and PCR analysis. For *Lilium* ‘Acapulco’, *A*. *praecox* ssp. *orientalis* ‘Royal Purple Select’ and *M*. *armeniacum* ‘Blue Pearl’, Southern blot or inverse PCR analysis revealed the integration of 1-5 copies of the transgene into the genome of transgenic plants, but most of them had 1 or 2 copies. *Agrobacterium*-mediated

transformation systems thus established may be useful as a tool for molecular breeding as well as molecular biological studies.

23. Genetic transformation of two species of orchid.
Y Li, **X-R Nie**, **S-Z Men**, China, **JA Teixeira da Silva**, Japan, **C-H Wei**, China **184-192**

Orchids are distinctive flowering plants, which represent the largest family of ornamental plants among monocotyledons and occupy a wide range of ecological habitats and exhibit highly specialized morphological, structural, and physiological characteristics. The family *Orchidaceae* includes a number of commercially important species that are grown for cut flowers and potted plants. Vegetative propagation slows down the breeding process of orchids, and modern genetic engineering techniques provide an effective alternative. Genetic engineering technology has been used to create new traits and shorten breeding time to improve existing successful cultivars in other crops. However, our understanding of floral development in orchids at the molecular level is just beginning. Until recently, transgenic orchid plants have been reported for only a few orchid genera. The first transgenic orchid plant was reported in *Dendrobium* and was generated using biolistic bombardment. In all the above investigations, the transformation efficiency was relatively low. In this chapter the high efficiency of genetic transformation of two orchid species is introduced.

24. *Oncidium* tissue culture, transgenics and biotechnology. M-T Chan, **Sanjaya**, Taiwan, **JA Teixeira da Silva**, Japan …..... **193-198**

Oncidium is a commercially important orchid, and considerable research efforts of numerous biotechnologies have been deployed in the past few years to improve its mass propagation, gene transformation and disease resistance. The ability to regenerate plants from cultured cells, tissues or organs provides an efficient tool for plant transformation. Protocorm-like bodies (PLBs) were the most convenient target tissue in different gene transformation systems including *Agrobacterium*-mediated and microprojectile bombardment. A range of available molecular and biochemical tools to confirm transgene integration and expression made the outcome of such experiments more understandable. The integrated approaches of genetic engineering in *Oncidium* have been of great value because of its commercial value. Indeed, more recently *Oncidium* is the subject of new areas of research including functional genomics, proteomics, and metabolomics. For the successful application of these new approaches to improve traits in *Oncidium* a reliable and reproducible transformation technique is essential. This chapter attempts to highlight *Oncidium* tissue culture, with an emphasis on gene transformation and need for biotechnological applications, and the potential areas of research.

25. Optimization of parameters for delivery of transgenes to turfgrass cells. C Basu, **AP Kausch**, **JM Chandlee**, USA …..... **199-203**

Parameters for use of the PDS-1000/He (Bio-Rad, USA) particle delivery system were optimized for routine delivery of transgenes into turfgrass cells in an attempt to standardize methods for routine and efficient genetic transformation of turfgrass species (creeping bentgrass, cv. Penn A4). Among various callus types produced on callus induction medium, embryogenic callus was identified using electron microscopy. Suspension cultures of Penn A4 were bombarded using a ubiquitin rice promoter-GUS gene construct. Based on the number of 'GUS hits', the following conditions were determined to be optimal for transfer of foreign gene constructs into turfgrass cells: 1.0 μm gold, 7 μl DNA/ shot and 1100 psi rupture disc type. Transient GUS expression patterns in turfgrass suspension culture cells driven with three different promoters (PGEL1, ubiquitin rice-GUS and ubiquitin corn-GUS) were also evaluated. The ubiquitin rice promoter was determined to be an ideal promoter for driving high levels of foreign gene expression in turfgrass cells. This observed variation in reporter gene (GUS) expression provides important information regarding variability of promoter strengths and expression patterns that will be important when choosing a strong promoter for gene transfer experiments targeted for trait modification or enhancement in turfgrass.

26. Transgenic plants for bioremediation. S Eapen, **SF D'Souza**, India …..... **204-208**

Phytoremediation – the potential of plants to extract, sequester and/or detoxify toxic metals and xenobiotics has evoked considerable interest among plant biotechnologists and environmental biologists in the last few years due to its environmentally compatible and aesthetically pleasing qualities. Plants, although having the inherent ability to take up toxic metals and metabolize organic pollutants, the development of transgenic plants will give an opportunity for the transfer of genes across species barriers, resulting in enhanced efficiency of metal accumulation and detoxification of xenobiotics. Genes involved in uptake, translocation and sequestration of metals can be transferred from hyperaccumulator plants or from other sources into candidate plants for development of transgenic plants with improved metal accumulation. The detoxification of toxic organomercurial compounds and subsequent volatilization of elemental mercury by transgenic plants is an example of such a well conceived plant engineering strategy. Transfer of genes associated with xenobiotic metabolism from microbes/mammals to plants may also help in processing xenobiotics as in the case of transgenic plants with the mammalian cytochrome p4502E1 gene, which has resulted in plants metabolizing a number of xenobiotics such as trichloroethylene (TCE) and ethylene dibromide. As our understanding of the genetic basis of metal and xenobiotic metabolism improves, coupled with development of genome sequencing projects and analytical techniques, it will be possible to develop novel transgenic plants for environmental clean up.

27. Tropane alkaloids in plants and genetic engineering of their biosynthesis.

Tropane alkaloids occur mainly in the Solanaceae and include the anticholinergic drugs hyoscyamine and scopolamine, whose biosynthesis takes place in the plant root. Some of these Solanaceae plants have developed a wide diversity of morphological traits that make them suitable for ornamental purposes, but their main use is as a source of both hyoscyamine and scopolamine. Since scopolamine is more valuable, there has been a long-standing interest in raising the scopolamine content of producing plants and their hairy root cultures. At present, two flux-limiting steps in the biosynthetic pathway for scopolamine have been demonstrated and the corresponding genes cloned, making the metabolic engineering of the pathway possible. The first step is the conversion of putrescine into N-methylputrescine, the reaction being catalysed by putrescine:SAM N-methyltransferase (PMT). This enzyme removes putrescine from the polyamine pool and drives the methylated compound exclusively toward tropane alkaloid production. The second limiting step is at the level of the enzyme hyoscyamine-6β-hidroxylase (H6H), which biotransforms hyoscyamine into the epoxide scopolamine. The *pmt* gene of *Nicotiana tabacum* under the control of the CaMV 35S promoter was introduced into the genome of two scopolamine-rich plant species, *Datura metel* and a *Duboisia hybrid*, together with the T-DNA of the Ri plasmid from *Agrobacterium rhizogenes*. The results obtained showed a different pattern from hyoscyamine and scopolamine biosynthesis control in the hairy root cultures belonging to both species. In *Datura*, the ectopic expression of the transgene 35S-*pmt* increased the biosynthesis of hyoscyamine and scopolamine, whereas in *Duboisia*, the overexpression of this transgene increased the levels of N-methylputrescine but the alkaloid production remained similar to that of the control roots. In contrast, overexpression of the 35S-*h6h* gene of *Hyoscyamus niger* in hairy root cultures of *Duboisia hybrid* and *Hyoscyamus niger,* besides enhancing the bioconversion of hyoscyamine into scopolamine, also increased the hyoscyamine production. Enhancement of scopolamine production in hairy root cultures producing mainly hyoscyamine has also been achieved using metabolic engineering. An important conclusion from all these experiments is that the identification and cloning of regulatory genes might eventually be more successful for improving production, as such genes might control larger sections of the pathway, if not its entirety.

Helichrysum italicum and *H. stoechas* (family *Asteraceae*) are two spontaneous species of the Mediterranean region. The plants grow in arid soils, from the cliffs above the sea to the hills, where they flower from May to July. The bright yellow flower heads contain essential oils, tannins, caffeic acid and other useful substances. *H. italicum* and *H. stoechas* are used in folk medicine because of their antibacterial, antitoxic, diuretic and antiallergic properties; moreover they are appreciated as dried flowers. Tissue cultures from *H. italicum* and *H. stoechas in vivo* germinated seedlings were established. Micropropagation and rooting was achieved in basal Murashige and Skoog medium without any growth regulators. The organogenetic potential of leaf tissues was evaluated under light conditions, combined with different concentrations of growth regulators. Shoot induction from leaf tissue was obtained in *H. italicum* on a medium supplemented with zeatin (10 μM) and IAA (0.5 μM). Callus was induced from micropropagated leaf tissues on a medium enriched with 2,4-D (10 μM) and kinetin (1 μM). Fast growing *H. italicum* cell cultures were established in liquid medium. An efficient protocol for hairy root induction using wild type *Agrobacterium rhizogenes* has been established for both *H. italicum* and *H. stoechas*. Hairy root-regenerated plants were recovered in *H. stoechas*. *H. italicum* cell and hairy root liquid cultures could be used for secondary metabolite production: besides *H. stoechas* hairy root-regenerated plants could be studied to assess their ornamental and pharmaceutical value.

Part 6 Risks of GMOs and GMPs

The leading role of scientific and technological progress in the economy of developed countries has become a truism. Moreover, and informational and other high technologies are generally believed to play an exceptional role in the economy of postindustrial or information society, which is regarded as the next stage of the development of the world civilization. This point of intersection between society's expectations and actual possibilities of science is where a serious conflict is brewing. Often the impetuous development of technology and rapid adoption of latest scientific achievements is not accompanied by well-grounded estimation of their medical, ecological, and social consequences. To date, the economic outcome of the practical implementation of scientific developments is given priority over the principles of safety dictating the necessity of long-term, comprehensive tests of all new technologies and products. Nevertheless, taking into account the rate at which scientific knowledge and technology are developing and the global effect of technologies on the society and environment, mankind will have to solve this problem. In this chapter, I will estimate the risks entailed by the application of genetic engineering methods to floriculture and landscape horticulture.

Invasive species are a primary threat to biodiversity on the planet, second only to habitat destruction, and are one of the least reversible of all human impacts on the environment. An invasive plant is likely to spread to new areas or environments and develop self-sustaining populations, which then become disruptive to the ecosystem. Both native and non-native plants can be invasive, however this review focuses

on non-native invaders because of their association with the introduction and cultivation of ornamental plants. The enthusiasm of growers and consumers for novel ornamental plants drives plant breeders and others to develop cultivars from non-native species. Several characters contribute to invasive potential, however a major determinant for many invasive plants is seed production. Plants that are sterile or cannot set seed have a greatly reduced invasive potential. Using biotechnology to introduce sterility genes into plants with invasive potential has wide applicability. The development of sterility genes and their introduction into ornamental plants with invasive potential can be used to produce non-invasive cultivars. Sterility genes may alter expression of specific genes and disrupt the development of reproductive organs or may ablate tissue essential to reproduction. An essential factor for the application of either strategy is that the aesthetic quality of the flower is preserved.

The UPOV convention is the basis for legislation on plant breeders' rights (PBR) in currently 59 single countries and the European Union. The chapter explains - on the basis of the UPOV convention - who is entitled to plant breeders' rights and the scope of such rights including its conditions, restrictions, and exemptions. Furthermore, the document details keywords of the legislation to enable applicants for plant breeders' rights to understand the legal procedure of the granting process. The grant of PBR is independent from regulations on the production or commercialization of a given variety.

Tissue Culture and the *In Vitro* Environment

Part 1 Somaclonal variation: cause, induction and control

Tissue culture has been widely used for propagating disease-free floriculture crops. *In vitro* culture, however, has often been associated with somaclonal variation. Since the discovery of somaclonal variation in the 1970s, its potential as a source for cultivar development has been debated. Whatever the controversies are, the fact is that somaclonal variation has been an important source for cultivar development of floriculture crops. Floriculture crops are genetically heterozygous and propagated largely through vegetative means. Their value lies in the aesthetic appearance. *In vitro* culture of this group of crops generates somaclonal variants that could be novel and can be fixed via vegetative propagation. In this chapter, we document somaclonal variation in 58 genera across 33 families of floriculture crops. The occurrence of somaclonal variation may be a result of uncovering somatic mutations that have been accumulated during years of asexual propagation and/or induced mutations during *in vitro* culture. Changes at the chromosomal level, variation of DNA sequences including activation of transposable elements, and epigenetic effects have been shown to be the underlying mechanisms of somaclonal variation. This chapter also presents me-thods of somaclonal identification and characterization, and application of somaclone variation for new cultivar development of floriculture crops.

Plant cells have a unique capacity governing a strong efficiency for survival in a constantly changing environment. This capacity for nutrient accumulation should also govern our way of analysing and managing *in vitro* cultures. Knowing this, what can we then suggest or identify as potential causes for the high variability level observed in cell culture? And what can we do using which tools in order to reduce culture variability and reach the low levels usually observed for other biotechnological processes such as for bacteria, yeasts, fungi and animal cells? In addition to specific mechanical and hydrodynamic problems (mixing, shear), the capacity of plant cells to accumulate nutrients strongly complicates our capacity to make *in vitro* culture repeatable. There are increasing evidences that the cells nutritional state determines its potential towards growth and production. Therefore, to our point of view, the understanding and characterization of the role of a cell's nutritional state on cell behaviour is a key in reducing variability of *in vitro* culture. Then, this knowledge could be translated into mathematical models capable of describing and predicting cells growth and potential from the estimation of the cells physiological state along a culture (**Fig. 1**). Efficient tools are thus required for monitoring cell behaviour *in vitro*, feed the model development and then ultimately define actions to be done on the cells environment for manipulating the cell behaviour towards a desired potential. In this context, this contribution tries to offer some answers to the crucial problem of culture variability which is still limiting the emergence of *in vitro* plant biotechnology for the production of diverse products of interest such as somatic embryos, plantlets, secondary metabolites and recombinant proteins. More particularly, we will focus on major thoughts explaining plant cell culture variability and on potential solutions that have been identified, developed and validated. Emphasis is placed on suspension cell culture and comments are added for specific cases concerning plant tissues.

The term "Polysomaty" means polyploidization of somatic cells with various ploidy levels in developing plant tissues. In this chapter, we

Part 2 Somatic embryogenesis

In addition to the biomolecular, physiological and biochemical aspects of somatic embryogenesis, careful design of the lighting environment is necessary to ensure the successful induction and development of somatic embryos for different plant species. The effects of the lighting environment on somatic embryogenesis are certainly dependent on the particular plant species, cultivars and individual genotypes. Based, however, on the seminal studies that investigated the influence of the lighting environment on somatic embryogenesis, certain results exhibited enough consistencies to warrant forming the following two generalizations: (1) Red light generally has promotive effects on the induction of somatic embryos, but generally has neither promotive nor inhibitory effects on the development of somatic embryos; and (2) Blue light generally has inhibitory effects on the induction of somatic embryos, but generally has promotive effects on the development of somatic embryos. Indeed, these generalizations serve as important practical guidelines for the design of the lighting environment for all types of somatic embryogenic systems.

Tomatoes and cereals are becoming increasingly popular ornamentals in Japan, the former as fruiting pot plants, the latter as dry bouquet supplements. To obtain interspecific hybrids of tomato and its wild species, immature globular-stage embryos were taken out and cultured on suitable medium, on which somatic embryos were formed with globular, heart and torpedo stages. When the initially-obtained embryos were re-cultured on the same medium, secondary somatic embryos were formed and they grew to complete plants. Hereby a clonal propagation system of hybrid embryos was established. Cotyledons of tomato seedlings cultured in a controlled environment chamber were used as materials for protoplast isolation, and plant regeneration was obtained via somatic embryogenesis on TM medium (Shahin 1985). Subsequently, cotyledon protoplasts of tomato treated with iodoacetamide and suspension-culture-derived protoplasts of wild species were fused using polyethylene glycol, and somatic embryos were derived from greenish calli that formed from the fusion mixtures, developed progressively through the globular, heart, and torpedo stages, and finally formed complete plants. In parallel, to establish a simple and efficient culture system of vegetative tissues from monocots for gene transformation, guinea grass and bahia grass were used as starting materials. The leaflet tissues were taken from terminal tillers arising at the end of stolons, cut into 5-mm long pieces, washed, sterilized and then placed on MS medium (Murashige and Skoog 1962). Embryogenic calli were formed and they were observed by scanning electron microscopy, indicating somatic embryo structure with a scutellum and a coleoptile. Multiple shoot and plant regeneration were obtained from somatic embryos. A simple ovary culture system of both was also established via somatic embryogenesis.

For commercial floricultural crops, the promising system to perform massive *in vitro* propagation is nowadays represented by the possibility to obtain plantlets from somatic embryos and synthetic seeds. The complete structure of the somatic embryos have the possibility to be multiplied in liquid substrate with high multiplication factors that can reduce the manual labour cost and support the authomatization costs. In this paper we report some experiences on somatic embryogenesis and artificial seed production of three important ornamental species: *Lisianthus russellianus, Genista monosperma* and *Cyclamen persicum*. The culture protocols established for the three species are presented, and some peculiar aspects of the *in vitro* culture of each species are explained.

Hyacinthus orientalis cv. Jan Boss has great importance in cut-flower commerce. For *in vitro* production of bulbs of this cultivar, twin-scales were cultured on a modified Murashige and Skoog medium containing 3% sucrose and supplemented with 1 mg/l IBA + 0.1 mg/l BA or 1 mg/l IBA + 1 mg/l BA. Both media were appropriate for bulb induction, but the medium with equal amounts of both IBA and BA was the most suitable, as on this medium the number of responsive explants and the number of bulbs per explant was higher. Further growth of the bulbs and root production was achieved on a similar basic medium without growth regulators and with increased sucrose content (6%). Rooted bulbs with a mean diameter of 8 mm were adequately prepared for *ex vitro* culture and grown in a glasshouse with high success of transplantation rate.

An effective tissue culture system to regenerate *Melia azedarach* (Meliaceae), an important multipurpose – including ornamental value – tree, was established. The optimized protocol resulted in plant formation from cotyledon explants via organogenesis and somatic embryogenesis. Embryogenic callus induction occurred on full strength (salts and vitamins) MS medium containing 3 mg/L 1-naphthaleneacetic acid (NAA) and 1 mg/L 6-benzyladenine (BA) with 3% sucrose with or without 5 mg/L giberrellic acid (GA_3). Somatic embryos – induced at 447/g fresh

weight of callus – resulted in 100% conversion of somatic embryos to plants, all of which survived in the greenhouse. Organogenic callus was induced on full Murashige and Skoog (MS) medium supplemented with 0.5 mg/L NAA, 1 mg/L BAP, 1 g/L casein hydrolysate (CH) and 5% sucrose. Proliferation and elongation of adventitious shoots – derived from both embryogenic and organogenic callus – was achieved on full MS medium to which 1 mg/L BAP, 200 mg/L CH, 40 mg/L adenine, 80.5 mg/L putrescine and 2% sucrose, or 0.2 mg/l BAP, 0.01 mg/L GA_3, 161.1 mg/L putrescine and 3% sucrose were added. The addition of putrescine to the culture media had a positive effect on shoot proliferation. About 160 shoots/g (fresh weight) callus were produced. Rooted shoots were acclimatized and successfully transferred to soil with 100% survival and whole plants were obtained in ~4 months. Regenerated plants were phenotypically normal. In addition, we developed methods for the *in vitro* selection for salt tolerance. Organogenic callus cultures were screened for salt tolerance using direct selection methods and growth responses were examined. Despite the presence of salt in culture medium resulting in the loss of regeneration potential in organogenic callus, plantlets were obtained from culture media with 42.7 mm and 85.57 mm NaCl.

Part 3 Techniques and tools for improved organogenesis and reduced somaclonal variation

Novel methods and advanced culture systems concretely discussed in this chapter range from mutual relationship between microorganisms and plant root system, *in vitro* flowering, micropropagation for recalcitrant plants, synseed production, direct somatic embryogenesis and protocorm-like body formation, and several applied culture systems for plant regeneration, micropropagation, and materials for genetic transformation. All of the investigations have shown to be applicable for other cultivars/varieties and been considered to undergo further researches and to be up-scaled for commercial goals. Each investigation/study is presented with its materials and methods, the obtained results and suggestion for further research. Progress of plant biotechnology involved in these new methodologies has been reviewed, showing their advantages in modification of plant culture media, optimization of physical, chemical and physiological conditions for *in vitro* flowering, advanced micropropagation of recalcitrant plants, standardization of synseed production and preservation, applications and modification of advanced culture systems, and studies on shoot morphogenesis and regeneration, somatic embryogenesis and protocorm-like body formation. In addition to potential application and further research, a few problems that have arisen and disadvantages are also recorded with some solutions provided by presenting methodologies or other research ideas to be executed *a posteri* for improvement of these micropropagation methods and culture systems.

The commercial value of crop plants depends on the quantity and quality of the flowers it produces. Flowering is a complex phenomenon where a number of environmental and endogenous signals play a major role. These signals eventually lead to floral induction (Bernier *et al*. 1993, Levy and Dean 1998). The shoot apical meristem (SAM) integrates the inductive signals, which ultimately gives rise to the flower primordia. The SAM has been the focus of many physiological, biochemical and molecular studies directed toward the understanding of the processes involved in floral transition. Orchids, one of the largest flowering families in the world, are known for its unique flower shapes and attractive colour. Generally they have a long juvenile phase that delays the flowering period (Duan and Yazawa 1994, Kostenyuk *et al*. 1999). But flowering can be manipulated through photoperiod and plant growth regulators (Goh and Arditti 1985). Recent success of *in vitro* flowering in orchids, *Arabidopsis* and in many other plant species have opened up new avenues of research into the flowering mechanism. Such *in vitro* systems have enabled researchers to unleash the mechanisms involved in flower initiation and development. In this chapter we show how one such plant species, *Dendrobium sonia* was used as a model for *in vitro* flowering to explore the possibility of a potential substitute for potted plants. Thin cell layers (TCL) technology has proved to be a powerful tool in the *in vitro* manipulation of flowers in many species. It offers a solution to many of the issues hindering the efficient improvement of floriculture. TCL technology addresses the issue of plant breeding at the first stage of the problem i.e. regeneration. Since the regeneration of specific organs may be effectively manipulated through the use of TCL, *in vitro* flowering plant systems may also be potentially created. This review chapter is focused on flowering in general and *in vitro* flowering in particular to understand the facts and the factors that favor the flowering process. A study was made to describe the efficient nature of TCL technology towards *in vitro* flower production and briefly reviews the present state of control of *in vitro* flowering through case studies. Most importantly, an *in vitro* system for *Dendrobium sonia* has been standardized and provides the first step in a potential global movement towards a new fashion, “Test Tube Bouquets”.

Synthetic seed technology is today an important tool, available to breeders and scientists, for the *in vitro* propagation and conservation of ornamental plants. After an introduction on the main procedures for synseed preparation, this chapter provides information on the protocols that have been developed for the encapsulation of various explants (zygotic and somatic embryos, axillary buds, nodal segments, protocorms and bulblets) from ornamental species (trees, shrubs and cut flowers). Original results about the germinability and the average

application or high inoculation rates. Benefits of endophytes are manifold and not only include antagonism to pests and diseases, but also other benefits such as plant growth promotion. Endophytes employ several modes of action against pests and diseases: parasitism, competition, production of secondary metabolites, indirect effects through plant growth and induced plant resistance. Through induced plant resistance, endophytes trigger plant defence mechanisms prior to infection, resulting in reduced pest or pathogen damage. The role of induced resistance is only now beginning to be understood and offers new possibilities into targeting pests and diseases. Since endophytes are sometimes closely related to plant pathogens, the risks and fitness costs resulting from their potential use is discussed. Surprisingly, research into endophytes for the ornamental industry has been little explored. Lessons learnt from our work on banana endophytes are discussed, together with future applications such genetic modifications and application of endophyte combinations.

The vast majority of orchids are currently endangered due to extensive disturbance of their natural habitat and indiscriminate harvesting of the naturally growing plants. Therefore, it is urgently needed to establish reliable methods for conserving these orchids as valuable germplasms. Cryopreservation is found to be one of the most effective methods for long-term storage of genetic resources and significant progress has been made in the field of cryopreservation techniques in recent years. Three cryopreservation methods, namely desiccation (air-drying), vitrification, and encapsulation-dehydration methods, have already been applied for orchids, and various types of orchid genetic resources such as pollen, immature and mature seeds, protocorms, protocorm-like bodies, apical meristems and suspension cells have been successfully cryopreserved. Here these successful cryopreservation studies on orchids are summarized. Although these results are mostly encouraging with respect to the prospect for the long-term conservation of orchids, further studies are still required before a generalized protocol for orchid conservation is established. Therefore, problems encountered using these methods for orchid materials are also discussed.

After a concise outline of the recent advances in tissue culture, genetic engineering and *in vitro* conservation of the carnation (*Dianthus caryophyllus* L.), this chapter examines the potential of the cryogenic technology for the long-term preservation of valuable carnation germplasm. A comparison between various methods ("PVS2-droplet freezing", "DMSO-droplet freezing", and "encapsulation-dehydration") which allow the direct immersion of shoot tips in LN is reported, carrying out the study with 6 carnation cultivars ('Anthares', 'Coralie', 'Kinero', 'Pallas', 'Pink Candy' and 'Wanessa'). Maximum shoot formation after cryopreservation (from 53% for cv Coralie to 65% for cv Wanessa) was achieved after a PVS2-droplet freezing procedure was optimized. Lower percentages of shoot formation were obtained when the DMSO-droplet freezing and the encapsulation-dehydration methods were developed. After the plants from cryopreservation were potted and transferred to the greenhouse, a comparison with control plants did not evidence any sign of morphological alteration or abnormalities in growth.

This chapter deals with the cold storage of two types of cultures: callus and mainly shoot. The emphasis lies on the effect of cold storage on secondary metabolism in both types of cultures. Callus tissues survived cold storage at 4ºC for 1-8 months depending on the plant species. Production of secondary metabolites such as nicotine and betalain pigments was strongly inhibited by cold storage. However, anthocyanins and naphthoquinone derivatives were less affected following reculture of callus. *In vitro* shoot cultures of temperate plant species were able to tolerate cold storage at 0-5ºC for 1-2 years whilst those of tropical species at 10-15ºC for about 1 year without transfer. The plants regenerated from cold-preserved shoots had a normal biosynthetic capability, which indicated that cold storage of *in vitro* shoots is a dependable method for short- to medium-term germplasm conservation including secondary metabolism.

Culture *in vitro* has become an important technique in plant production. Substantial numbers of *in vitro* cultured plants do not survive transfer from *in vitro* conditions to the greenhouse or field environments. *In vitro* grown plants are very tender and delicate owing to high humidity in the culture vessel, controlled temperatures, low light intensities and hetero or mixotrophic mode of propagation under which they are normally grown. They lack protective mechanisms like waxy cuticles, stomatal regulation and leaf hairs, they show reduced differentiation of palisade cells and exhibit large inter-cellular spaces which make them vulnerable to desiccation once exposed to ambient temperatures. Understanding the physiological and morphological characteristics of *in vitro* cultured plants and the changes they undergo during the hardening process should facilitate the development of efficient transplantation protocols. Most species grown *in vitro* require an acclimatization process in order to insure that sufficient number of plants survive and grow vigorously when transfer to soil. Successful acclimatization procedure provides optimal conditions for minimal damage of plants in the micropropagation process and thus enhances the plant growth and establishment. The chapter discuss the anatomical, morphological and physiological factors that accounts for the fragility of

Part 4 Scaling-up and mass production systems

Conventional micropropagation technique is a typically labor-intensive means of producing elite clones. High production costs generally limit the commercial use of micropropagation. Bioreactor technology offers various advantages due to possibilities of automation, saving labor and reducing production costs by providing optimum growth conditions to achieve both maximum yield and high quality of propagules, or to keep the production costs as low as possible by integrating automated facilities and simple low cost devices. The use of bioreactor technology is starting to be commercialized and the results suggest the practical applicability of this technique in plant propagation. Here we attempted to focus on the development of cost-effective methods for commercial micropropagation of several important horticultural plants using a bioreactor system and also to identify the problems related to large-scale plant micropropagation.

Roots are much more than an organ that provides transport of water and solutes from the soil to the above ground parts of a plant. The recognition that roots possess a unique physiology and that they can contribute with biologically active chemicals to the environment expanded the field of root research. In this context, hairy roots became a unique experimental system, due to their high biomass growth allied to their biosynthetic capacity. In addition, hairy roots constitute a good system for the study of metabolic pathways, of root growth and primary and secondary metabolism, of their behaviour under stress-inducing environments, of the interrelationships of roots with biotic and abiotic factors, as well as the production of regenerants. The production of essential oils by hairy roots demonstrates a biosynthetic ability that can attain higher yields than in undifferentiated cultures and is sometimes equal to or higher than the parent plant. This capacity is strictly correlated with the differentiated state of the cultures, the level of production being severely impaired or lost when the hairy root phenotype is lost. Other factors, e.g. the type and/or age of the inoculums, the gap between subcultures, the combinations of nutritional and environmental stressing factors, including different medium composition, photoperiod conditions, and cultivation in a two-phase system and biotransformation, can also affect biomass growth and product performance. The possibility of growing and maintaining *in vitro* hairy root cultures of different species widens the indispensable basic knowledge needed to manipulate, in a controlled way, their molecular, biochemical and ecological potential.

Conventionally the culture medium is gelled to simulate a native soil-like environment for *in vitro* plant growth and differentiation. However, the use of gelling agent poses many problems such as, sensitivity of tissue to gelling agent type and concentration; alteration and/or inactivation of media components during heat sterilization; mechanical impedance of movement and availability of dissolved oxygen, water and ions; labor and time intensive cleaning of tissues and vessels before transplantation, transferring or sub-culturing. The use of liquid media circumvents these problems; allows even faster and more prolific tissue growth. Liquid systems are also amenable to automated large-scale micropropagation. However, direct and prolonged contact with liquid medium and lesser oxygen availability due to partial or complete submergence makes the tissue hyperhydric. Hyperhydric tissue exhibits recalcitrance and the affected plantlets strive to acclimatize upon *ex vitro* transfer. The use of inert support substrates abbreviates tissue hyperhydration, yet retaining the various advantages of liquid media. The present chapter is focused on various studies pertaining to the use of support systems like Coconut coir, Cotton fiber, Filter paper, Glass wool, Luffa sponge, Nylon cloth, Polystyrene foam, Polyester screen raft, Polypropylene membrane raft for micropropagation of floricultural crops. The chapter also highlights Poisson regression based analytical approach unlike customary ANOVA for critical assessment of *in vitro* shoot regeneration in liquid systems.

Isoprenoid biosynthesis occurs in plants via the plastidic and cytosolic pathways respectively named the methyl-erythritol phosphate (MEP) and mevalonate (MEV) pathways. For monoterpenoid indole alkaloids, the MEP pathway exclusively provides the terpenoid moiety. In this paper, we reviewed findings and observations that made *C. roseus* a model plant for monoterpenoid indole alkaloids biosynthesis and regulation studies. If the whole plant has been essentially used to establish the scheme of the spatial organization of the biosynthetic pathway between four different cell types, the cell suspension cultures have proven to be an excellent system for studying the regulation of the plastidial isoprenoid biosynthetic pathway. Due to the ability of cell suspensions both to take up chemical components and, with an apparent ease, to be genetically manipulated and transformed, a set of regulatory elements of monoterpenoid indole alkaloids biosynthesis starts to emerge. Technical approaches and results are discussed in the context of the recent literature.

Hypericum perforatum L. (St. John's wort) is a medicinal plant used for the treatment of neurological disorders and mild to moderate depression. *Hypericum perforatum* is also used as a garden ornamental plant in perennial hedges or in rock gardens. Recent studies have been conducted to investigate the productivity on a large-scale of *in vitro* growth systems such as undifferentiated calli, cell cultures, somatic embryos and regenerated shoots. Accumulation of bioactive molecules (hypericins, hyperforin, xanthones, flavonoids, procyanidins and anthocyanins) has been compared with that in wild and greenhouse-grown plants. *In vitro* studies demonstrated that shoot cultures accumulate greater amounts of different metabolites compared to calli and cell suspension cultures. Moreover shoot cultures demonstrated to be a good source for both hypericins and hyperforin production.

Part 5 Applied case-studies

The genus *Rosa* comprises hundreds of species and thousands of cultivars. Roses are, undoubtedly, one of the most economically important and favorite ornamental plants. Millions of rose bushes are planted in gardens or pots and billions of rose cut flowers are sold annually over the world. A number of species are also used for highly-prized essential oils (attar). Traditionally, rose species or cultivars are propagated by seeds, cuttings, budding or grafting. Propagation by seeds may not breed true-to-type and vegetative propagation is also very slow, time-consuming and may be a limitation in stock plants. Alternate systems of rose propagation are needed to eliminate or minimize these problems. *In vitro* culture of roses are one of the most exciting procedures of producing new cultivars, eliminating the use of rootstocks for different soil conditions, cultivars adaptable to different environmental conditions, providing rapid multiplication of superior cultivars and rootstocks, producing disease-free plants and speeding up breeding programs. In this review, with the emphasis on advances in the field of rose *in vitro* culture, we first briefly review early research, and then in separate sections, the studies related to media preparation (basic salts, sucrose, agar, pH and growth regulators) for callus culture, shoot regeneration and multiplication, rooting, cell suspension culture and embryogenesis. The stages of rose *in vitro* culture, including establishment, multiplication, *in vitro* rooting, acclimatization and genetic variation are also reviewed. Subsequently, investigations on methods of rose *in vitro* culture by callus, pollen, anther, cell suspension and protoplast culture are described. Finally, technological improvements for micropropagation, embryogenesis, biotechnology and plant breeding are discussed. Views and conclusive prospects of these techniques are also expressed.

Due to fact that geraniums (*Pelargonium* spp.) are of interest as ornamentals or as essential oils producers, *in vitro* tissue culture has been widely used for the multiplication and production of healthy plants. The majority of studies have been focused on commercially interesting species such as *Pelargonium* x *hortorum*, *P. peltatum, P.* x *domesticum* and *P. graveolens*. Even if the production of phenolic compounds is a big handicap to the *in vitro* establishment of *Pelargonium* explants in solid media, various authors have described the regeneration of geranium plants (*Pelargonium* spp.) using explants such as meristems, anthers, leaves, petioles, stem sections, roots, hypocotyls or cotyledons. In most cases, the regeneration of *Pelargonium* plants needed to pass through a callus state. Somatic embryogenesis can be used for large-scale vegetative propagation, but so far the production of Pelargonium somatic embryos has only been described for a small number of genotypes from seed-derived cultivars but not for vegetatively propagated cultivars. In of our investigations only root explants from vegetatively propagated geraniums were capable of embryogenesis. *Pelargonium* embryogenesis can be used not only for clonal multiplication, but for the breeding of new cultivars.

The patterns of clonal micropropagation *in vitro* of selected cultivars of *longiflorum*-Asiatic hybrid lily (*Lilium* L.) and gladiolus (*Gladiolus* L.) have been investigated. The optimal culture conditions maximizing regeneration capacity of plants at different stages of morphogenesis were determined. The effectiveness of these results, which are novel, are compared to others available in the literature for other cultivars.

The global focus on bamboo as a unique natural resource for the future has created an awareness of the importance of its cultivation at a plantation-scale. The existence of rare seeding and physiologically sterile species makes tissue culture an important technique for their propagule production and improvement. Axillary shoot proliferation and somatic embryogenesis have been used for propagule production in bamboo species. Culture initiation especially from adult field culms, difficulty of rooting of axillary shoots and the inconsistency in somatic embryogenesis are some of the difficulties encountered. Seeds are not dependable for culture initiation but could always be used whenever

available. The use of plant parts from field culms of known performance is more dependable. The determinate branching pattern of bamboo, bud dormancy and presence of microbial contaminants makes culture initiation from field culms difficult. However, when nodes with axillary buds are cultured at periods of physiological activity, they can readily sprout *in vitro.* Microbial contaminants are also eliminated relatively more easily than at other times. Reports show that rapid and continuously proliferating axillary shoots were developed from *in-vitro* sprouted shoots in the presence of a relatively high level of 6-benzlaminopurine (BA) in many species but the difficulty of rooting had made this technique commercially applicable for only a few species. Somatic embryogenesis was also possible but its application on a commercial-scale appears to be limited due to inconsistency in regeneration and germination of embryos. This chapter attempts to discuss the problems related to these techniques and identifies a protocol applicable for producing propagules by axillary shoot proliferation leading to a high degree of rooting and survival of plantlets.

In vitro plant regeneration of *Iris pumila* and *I. reichenbachii* from the culture of mature embryos and leaf bases of *in vitro* grown shoots as well as cell suspensions has been studied. During the regeneration process, two different regeneration pathways were observed: somatic embryogenesis and/or organogenesis. Embryogenic calli were developed on MS medium supplemented with 2,4-D alone or 2,4-D and kinetin in combination (1.0 mg l^{-1} each). Cell suspension cultures were established and maintained in MS liquid medium at the same concentration of 2,4-D and KIN as used for the induction and proliferation of embryogenic calli. These were initially composed of single cells, bi-, three and multicellular proembryos and cell aggregates. In prolonged cell suspension cultures (6-8 weeks) three types of embryogenic calli were observed: yellow, compact; yellow-green and white, friable. Friable (white, yellow green) suspension-derived calli had the greatest morphogenetic potential. Germination of normally developed somatic embryos (70%) was achieved on MS solid medium without hormones. Organogenic calli were cultured on MS medium supplemented with BAP and GA_3 (1.0, 0.1 mg l^{-1}, respectively) for ten years. Shoots can be easily rooted after one month on hormone free medium. Potted plants of investigated dwarf irises grew normally and flowered.

Micropropagation has been used in several ornamental species such as orchids, bromeliads, anthurium, African violet and heliconias for the large-scale commercial propagation of high quality plants. The *in vitro* establishment of a new species should be followed by field evaluation of its behavior to guarantee the maintenance of the agronomic characteristics. *Heliconia bihai* var. Lobster Claw I plants obtained from rhizomes and from *in vitro* multiplication were planted in the field. Two hundred plants of each were planted in alternate rows, 20 plants per row, with 10 replications. The experiment was evaluated by the number of suckers, time for flowering initiation and cut flower productivity. Flowering was initially observed after 7 months in the field in rhizome-derived plants. The number of suckers however was higher in micropropagation-derived plants, resulting in a higher productivity. In addition to a high cut flower production, the market price per plant derived from micropropagation is approximately one third of that for rhizome-derived plants, which makes establishment of a field crop of this species from *in vitro* propagated plants even more viable.

Hyacinthus orientalis cv. Jan Boss has great importance in cut-flower commerce. For *in vitro* production of bulbs of this cultivar, twin-scales were cultured on a modified Murashige and Skoog medium containing 3% sucrose and supplemented with 1 mg/l IBA + 0.1 mg/l BA or 1 mg/l IBA + 1 mg/l BA. Both media were appropriate for bulb induction, but the medium with equal amounts of both IBA and BA was the most suitable, as on this medium the number of responsive explants and the number of bulbs per explant was higher. Further growth of the bulbs and root production was achieved on a similar basic medium without growth regulators and with increased sucrose content (6%). Rooted bulbs with a mean diameter of 8 mm were adequately prepared for *ex vitro* culture and grown in a glasshouse with high success of transplantation rate.

Plant species that belong to the genus *Centaurium* are wide-spread in International traditional medicine. *Centaurium erythraea* Rafn. (European Centaury) is a biennial plant, with attractive pink flowers, and for potential as a medicinal-ornamental used in the treatment of gastrointestinal tract diseases, in the treatment of fever and anaemia, and for increasing appetite. The effects of some plant growth regulators, nutrient medium components and different light treatments on modification of the morphogenetic response of this plant were examined. The seeds collected in their natural habitat were surface sterilised, and germinated and establish *in vitro*. Seedlings were cultivated on MS basal medium. Root segments (average 15 mm long) isolated from seedlings were used as an initial material for establishing non-transformed root cultures. After ten days of growth on the basal medium, somatic embryos were observed to form on the

initial explants. Towards the end of the subculture period, a large number of adventitious buds also formed at the basal part of explants, beside somatic embryos. Both morphogenetic processes are asynchronous. Histological studies revealed that somatic embryos were formed directly from epidermal cells and adventitious buds from meristematic cells derived from root cortex tissues. *C. erythraea* seedlings were inoculated with *Agrobacterium rhizogenes* strain A4M70GUS so as to set up a transformed root culture. *In vitro* plant material, was analysed by PCR and results were compared to plants collected in nature. The results showed that *in vitro culture* of *C. erythraea* produced greater amount of secoiridoid glucosides and xanthones.