Floriculture, Ornamental and Plant Biotechnology

Advances and Topical Issues

Volume IV

Edited by

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FOREWORDS TO VOLUME IV

Lynn Frewer, Marketing and Consumer Behaviour Group, The Netherlands

The research described in **Volume IV** provides a fascinating insight into potential developments of flowering plants arising as scientific knowledge and understanding progresses. Novel applications of biotechnology which have been developed in order to produce plants with advantageous traits has the potential to improve quality of life in society, and various applications have been developed which confer benefits in terms of human nutrition and micronutrient delivery (see section 3 on health), biosecurity and development of varieties which grow in hostile environmental conditions or are resistant to pests or pesticides (chapter on agricultural biotechnology and developing countries, Acqaah *et al*.), or which have other desirable qualities such as improved aesthetic presentation (for example, the aesthetic history of the daffodil is described by Rivera et al), or improve quality of life in other ways (for example, the potential use of flowering plants in the reduction of indoor pollution has been described in the chapter by Weidner and Teixeira da Silva). Ultimately, however, the successful implementation of any emerging technology, and the commercialisation of its products, is dependent on consumer responses to both the technology in an abstract sense, and specific applications. In the area of plant biotechnology, there is a considerable literature focused on understanding consumer reactions to food and medical applications, but much less research conducted into, for example, consumer reactions to the genetic modification of ornamental plants. However, it is possible to extrapolate from the existing research evidence to identify those factors which may be utilised as part of an effective commercialisation strategy and governance policy.

In a democratic society where choice exists, people will not buy novel products of emerging technologies that they associate with some negative attribute. Various factors may contribute to concerns. These include, for example, consumer beliefs that there is potential for negative environmental impact associated with production processes or agricultural practices, or perceptions that potentially negative effects to human health or other risks are not being disclosed by producers or regulators to serve their own vested interests. Ethical concerns are also important (for example, that a particular technology is in some way “tampering with nature”, or that unintended effects are unpredictable and thus unknown to science. However, consumers are much more positive towards applications which they perceive to be associated with some desirable and tangible consumer benefit. Some technologies may also be described as “transformative” as they have potential consequences for the way in which society is organised, which may be viewed positively or negatively by its members. In other words, societal responses to the application of technology innovations may be driven by concerns about the impact that the technology will have on societal and social structures and relationships, for example, employment practices in the agri-food sector, such as the requirement for workers with a different skill base, or the reduction the agricultural labour force, necessitating population shifts to urban areas. Finally, the public is not homogenous in terms of acceptance of the novel products of the biosciences. Differences in preferences and concerns can be identified according to demographic, psychological and cultural variation within a given population. Thus, for example, we might predict that some consumers will be positive towards genetically modified ornamental plants only if there is a tangible benefit to consumers (for example, increased or more intense colour ranges, bigger blooms or longer cut-flower life), or positive results for the environment (reduced use of pesticides). The issue of consumer choice is also very important, necessitating the implementation of effective traceability and labelling systems for genetically modified products. The failure to provide consumer choice was one of the most influential factors driving the negative reaction to genetically modified foods in Europe, for example, and similar mistakes should not be made in the context of commercialisation of other novel applications of the biosciences to plants.

The issue of consumer acceptance of the products of emerging technologies should not been taken lightly. A transparent system of risk analysis, coupled with the implementation of effective systems to ensure consumer choice, is an essential part of consumer acceptance. It is also important to develop novel products which consumers want and need, whether in terms of consumer health, environmental advantages, or aesthetic values. Clearly an understanding of consumer preferences through systematic investigation would make the process of technology innovation and application more effective.

Volume 4 provides an informative and inclusive overview of novel developments in the area of flowering plants. The potential of existing and emerging technologies to deliver novel varieties with profound consumer benefits is thoroughly encapsulated in this volume. An effective commercialisation strategy is, of course, contingent on understanding consumer perceptions and desires, and it is in this area that there is also potential for future research. I am very happy to recommend this volume to those academics and students of flowering plants, and to those researchers interested understanding how society may respond to different application of technological innovation in this area.

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The future is photosynthesizing

Why is it often quite difficult, and sometimes a challenge, to convince the brighter students to embark on a botanical career? Is it because plants are devoid of cuteness? Surely not, because a Valentine gift consists classically of red roses instead of, say, a salmon with a ribbon around its neck. Is it because botanists are still considered to be freakish people who spend their time strolling around the fields, picking something green now and then, to dry it and store it for ever and ever? I certainly don't do that. Do you? Or do students associate 'botany' with what they perceive as an endless series of leaf margin types?

Or are students less interested in botany because animal scientists have just always been better at linking their subject to what lives in the heart of young people, talking about toxic effects, cures against cancer, exotic endangered species, great technological progress? Well, dear fellow botanists, our striving to inspire enthusiasm for botany in the minds of our students has just been made easier. Whereas the first three volumes of the book series on "Floriculture, Ornamental and Plant Biotechnology: Advances and Topical Issues" will become their topical study material, this fourth volume has the potential to linger in their heads, because of the thrilling nature of the topics.

Students in botany with a general interest in engineering feats will be perusing the chapters on the use of microchips (by Peng *et al*.), the electronic nose (by Lozano Rogado), nanotechnology in botanical research (by Minunni) and silicon deposition (by Coradin *et al*.). Those who are drawn towards biomedical sciences, may be fascinated by the chapters on fighting vitamin deficiencies (Navarrete *et al*.) or on phytopharmaceutical production (Li and Jiang; Fennell *et al*.; Do and Bernard). Socio-economic aspects of botany are discussed as well (see the chapters by Acquaah *et al*. or Xia *et al*.), and for those who are easily swayed by the more luxurious sides of life, there are insights to be gained on perfume therapy, steviosides and etheric oils (treated, respectively, by Klála *et al*., Meireles *et al*. and Singh *et al*.). These, and many more of the chapters in this volume, show a glimpse of the future of plant sciences. And that future is founded, of course, on the knowledge of leaf margin types and primary root structures, but extends way beyond.

In short – this volume is like the flower on the whole series – adding to the beauty of the whole, but sure enough hoping to attract cross-pollinators.

Have a fertile journey through its petals.

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Mankind has been utilizing plant products for ages. From 30,000 known natural substances approximately 80% are of plant origin and are used as traditional medicines, fragrances, spices, and colourants.

Even most of the used pharmaceuticals are of plant origin. They are either directly isolated from plants (e.g. digitoxin), or for economic reasons now chemically synthesized (e.g. acetylsalicylic acid). Other pharmaceuticals are analogues or derivatives of natural substances. Also intact plants or their parts are still being utilized as medicaments or promotive agents, e.g. in teas or infusions. At present, the research of plants as potential sources of medicaments is growing. Examples include taxol, found in the bark of *Taxus brevifolia*, or campothecin from *Camptotheca acuminata*, the invention of which led to the synthesis of number of analogues.

In some cases the "active principle" of medicinal plants has not been discovered (e.g. in *Panax ginseng*), and their effect is probably based on a synergic action of several substances. Despite the fact that the mechanism of their effect has not been elucidated yet, these plants have been successfully and very intensively used for several thousands of years, especially in East Asian traditional medicine. Published tests proved the improvement of both physical and psychical conditions (especially physical persistance and capability of long-term psychical concentration).

The chemical potential of plants is however, still largely unexplored. Chemical diversity has only been analysed in about 10% of all land plants, and even here only the most abundant compounds have been well characterized. There are unprecedented possibilities for the discovery of novel chemicals, for diverse uses from pharmaceuticals through to fine chemicals. Their discovery and use will be accelerated by genomic and evolutionary analysis of the large gene families that encode the rich array of enzymes that contribute to their synthesis, by rapid advances in the analytical methods to define structures, and by the development of high throughput platforms to characterize their chemical and biological properties (Oksman-Caldentey and Inzé 2004).

The chapters in the section on **Health, important secondary metabolites, herbs, medicinal, and aromatic ornamentals** give many examples from this field of research, starting from general contributions summarizing the future of phytopharmaceutical discovery through to contributions about plant metabolism to the utilization of single compounds, e.g. stevioside. Moreover, a unique flavour to this section is the specific emphasis of plants that are BOTH ornamental and medicinal. The health of Humans lies not only in the physical, conferred by the secondary metabolites, but also by the visual and aesthetic beauty of the flowers; this section shows emphasizes the double importance of such ornamental plants, for example *Catharanthus roseus*, and shows their importance as novel model plants to study physico-chemical and genetic mechanisms in plants.

Oksman-Caldentey KM, Inzé D (2004) Plant cell factories in the post-genomic era: new ways to produce designer secondary metabolites. *Trends in Plant Science* **9**, 433-440

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Flowering Plants: the Future

Part 1 Emerging and hot topics **Page**

and informal; and medicinal plant biodiversity is threatened by irresponsible harvesting practices. The advantages of cultivating medicinal plants are obvious, but few are produced on a large scale even in westernized industries. This paper outlines the benefits that can potentially be derived from integrating biotechnology – represented by rapid, simple and low budget methods to highly sophisticated methods – in an industry that is dominated by simple technologies. Such solutions, including testing medicinal extracts for efficacy, establishing pharmacopeias (where none previously exist), *in vitro* and/or *ex vitro* medicinal plant cultivation and the drafting and implementation of appropriate policies and legislation, are particularly relevant to the Developing World where the medicinal plant industry remains largely unregulated. Case studies illustrate the successes of the ethnopharmacological-biotechnological approach, while future challenges and opportunities for such a strategy in the medicinal plant industry are considered.

The detoxification potential for air pollutants, predominantly formaldehyde and nicotine, of indoor ornamental plants, here represented by *Epipremnum aureum* and *Ficus benjamina,* was investigated. The evaluation relies mostly on ^{14}C-tracer experiments and, to a lesser extent, on *in vitro* measurements of relevant enzyme activities. Both, the air path and the soil path of pollution abatement were studied. Noxes were applied in gaseous (e.g. formaldehyde) or in aerosolic form (nicotine): direct pollutant uptake and metabolization in the leaves was observed. Alternatively, the substances were applied in solution to the roots. ^{14}C-formaldehyde is not taken up by the roots but rather assimilated by the leaves and transformed into photosynthates after transient oxidation to $^{14}CO_2$. In contrast, nicotine is only adsorbed onto the leaf surface and does not enter the mesophyll. However, it is taken up by the roots of *E. aureum*, but not of *F. benjamina.* In *E. aureum*, the xenobiotic (with respect to this species) is tranlocated to the leaves - driven exclusively by root pressure - where it accumulates at high concentrations without being metabolized. The growth of *Pseudomonas putida* and *Arthrobacter nicotinovorans* strains, bacteria which degrade formaldehyde and nicotine, respectively, is strongly stimulated by root exudates, provided as organic C (mostly sugars), or as co-substates. *E. aureum,* and supposedly other plants as well, enhance bacterial detoxification rates 5-10-fold. Moreover, bacterial degradation products and microbial wastes are absorbed by the roots and subsequently transformed into plant biomass. Perspectives for the development of a self-regenerating biofilter system for indoor air purification are outlined.

Lignin is an essential component of terrestrial plants, accounting for 15-36% of woody plants. To utilize the plants effectively, in particular woody plants, including woody ornamentals, an understanding of the lignin structure is required, as well as the carbohydrate structures co-existing with lignin in wood cell walls. However, lignin is unfortunately still far from being structurally described in detail. The main problem in its structural elucidation is the difficulty of isolating intact lignin from other wood components. Furthermore, many diverse linkages that occur between the monomeric units and the chemical resistance of certain linkages limit the extent to which analytical and degradation procedures can be used to elucidate the lignin structure. Analytical pyrolysis combined with gas chromatography (GC) and/or mass spectrometry (MS) has revealed to be a very powerful technique in the structural elucidation of lignin due to its speed and sensitivity, in combination with wet chemical and spectroscopic methods available in the study of lignin. The recent development of a new pyrolysis technique in the presence of tetramethylammonium hydroxide (TMAH) has provided new possibilities to analyse the lignin polymer. This article examines several recent advances in pyrolysis-GC/MS in the presence of TMAH. Selected aspects in the field are exemplified using original results.

Part 2 Futuristic techniques, novelties and challenges

Gene targeting has long been a major goal for researchers interested in the production of transgenic plants with stable and predictable patterns of gene expression. Unfortunately gene targeting in plants has been extremely difficult. We review the current status of gene targeting in plants along with recent breakthroughs in novel approaches to the use of site-specific recombinases, mutant host plants, and homologous recombination techniques. While the new gene targeting technologies are presently being used in model plants such as Arabidopsis, rice and tobacco, the new findings bode well for gene targeting technologies becoming available for economically important crop and horticultural plants.

Higher plants exhibit a complex organisation which can be described anatomically as well as physiologically, e.g. roots mediate nutrient uptake and in leaves, photosynthesis takes place. In most conducted experiments on plants, the organ level is the spatial limit in the analysis. This totally ignores the fact that the high degree of organisation also becomes apparent in the microscopical scale, which are tissues and single cells. For instance in a leaf, photosynthesis is restricted to mesophyll cells, whereas gas exchange is controlled by guard cells in the

epidermis. However, specific physiological contributions of particular cells cannot be assigned properly due to the mixture of cell types in the staring material. Therefore, a number of methods for sampling and analysis of tissues and individual cells have been developed in the last years. In this chapter I will give an overview about developments, applications, and capabilities of these methods. To avoid describing well established and commonly-used protocols, I focus this chapter on recently published methods which exhibit high potentials and will undoubtedly be frequently used in the future. For reasons of clarity, I give no actual protocols in the text. Instead, key references for the discussed methods are summarised in **Table 1** and **Fig. 1**.

10. **Phytoanalysis: challenges and solutions.** **G Stecher**, **R Bakry**, **I Feuerstein**, **CW Huck**, **GK Bonn**, Austria **109-130**

This review is concerned with analytical methods for the qualitative and quantitative determination of plant ingredients and phytopharmaceutical products. For this purpose a subdivision in three main chapters was performed to differentiate between the classical analytical approach, i.e. extraction and separation (subchapter A) and the general approach in direction of system biology (subchapter B). Finally in subchapter C applications for ornamental research are described, focusing on volatiles, plant hormones, pigments and phytoremediation.

11. **Novel lighting technologies and strategies for plant production on Earth and in space.** **JL Cuello**, USA **131-136**

Three novel lighting technologies or strategies that could significantly reduce the high electric-power demand and/or the high thermal load associated with the use of electric lighting in controlled-environment plant growing structures for both Earth and space applications include: (1) hybrid solar and electric lighting (HYSEL) systems; (2) water-cooled high-pressure sodium (HPS) lamps; and (3) light-emitting diode (LED) arrays. Using a fiberoptic-based Solar Irradiance Collection, Transmission and Distribution System (SICTDS) for the HYSEL system, and with the SICTDS having an overall efficiency of 40.5%, the SICTDS was found to achieve significant potential electric-energy savings by harnessing the available solar irradiance. Using solar irradiance data for Tucson, Arizona, the average energy savings for a whole year would be 0.9 MW-hr m^{-2} when replacing an HPS lamp and 1.7 MW-hr m^{-2} when replacing a cool-white fluorescent (CWF) lamp. Meanwhile, tests of water-cooled high-pressure sodium (HPS) lamps revealed that 72% of a total thermal load of 197 W was transported from a water-jacketed lamp with water thickness of 4 mm, while 98% of a total thermal load of 198 W was transported from a water-jacketed lamp with water thickness of 9 mm. Also, the electrical conversion efficiency of the LED array tested was 27.3% based on total radiation output or photosynthetically active radiation (PAR) output, and was practically the same as that of the control HPS lamp of 27.3% based on PAR output.

12. **3-D flow control for single-cell experiments: potentials and applications for plant science on a microchip.**
XYL Peng, China, **PCH Li**, Canada, **JA Teixeira da Silva**, Japan **137-151**

The conditions of on-chip single-cell experiments have been discussed. A key issue is how to safely retain a cell for microscopic observation and to deliver liquid medium to it for control-feed experiments. The three-dimensional flow control concept considers an experiment on a fragile cell in the microchip. This kind of chip provides on-chip cell selection from a group of suspension cells, for the study of on-chip single cell culture, chemical introduction and pure cellular fluorescent signaling. As an example of such single-cell experiments, the dynamic fluorescent cellular signals in single yeast cells caused by the metabolism of fluorescein diacetate (FDA) and intercellular Ca^{2+} stimulated by pH and glucose have been studied. Data shows a great deal of valuable, but complex, information. Complex data from single cells derived from the multiple stimulation of one cell reveals detailed cellular biochemical kinetics. Mathematical models can be established based on only one cell's data. In addition, these on-chip experiments are of man-cell conversation style, which means we can derive much information repeatedly for only one live cell. The potential applications in plant biotechnology of 3-D flow control on a microchip are numerous: dynamic observation of cell suspension and protoplast cultures, development, tissue formation and hybridization using fluorescent techniques such as FISH and GISH, protoplast fusion, study of chimerism, or even screening of transformed cells. This technique may prove to be one of the vital links that was missing between the cellular, tissue and molecular levels of analysis in Plant Science.

13. **New technology in sensing odours: from human to artificial noses.** **J Lozano Rogado**, Spain **152-161**

The Human nose is much more complicated than other human senses like the ear and the eye. It is still the primary 'instrument' to assess the smell of various products. Sensory evaluation using the human sense of smell is subjective; careful design and rigorous training of assessors allows it to become a more objective, but still expensive option. Instrumental methods, such as gas chromatography/ mass spectrometry (GC/MS), are also expensive and require trained personnel. The concept of the electronic nose has attracted attention in many branches of industry for its potential in routine odour analysis. Being first reported in 1982 by Persaud and Dodds (Persaud 1982), the research in the field rapidly increased, and a number of companies have now been established to commercialize the concept. Basically, an electronic nose has the mammalian olfaction as a model and consists of a sensor array with partially overlapping selectivities and a pattern recognition system. It can be trained to detect and discriminate a large number of both simple and complex odours. The electronic nose concept is widely used as an analytical tool in industry today. The commercialization of the electronic nose began in 1993 as the concept

Visual information is very important for cytological studies. For example, the visual characteristics and quantity of plant chromosomes can be useful indicators for parental selection in a hybrid breeding program. An unbiased observation of chromosomes can best be achieved by the quantification of chromosomal visual information contained in digital images. Image analysis is an effective tool for plant chromosome analysis and other cytogenetic / genomic aspects of plant breeding. Faster, cheaper, desktop computers, has enabled more wide spread use of this technology in recent years. Though there are numerous image analysis packages that have been designed to be “user-friendly”, the understanding of the basic principles behind these algorithms is necessary to fully understand the potential use and limitations of this technology. Here, we describe the basics of image analysis, and present practical examples for chromosome research and other areas applications applied to plant research.

Allometry, ontogeny and plasticity are three fundamental biological phenomena of developmental, evolutionary and ecological significance. They can now be integrated with a new systems approach constructed by a unified genomic mapping model. We review a general framework for understanding the genetic regulation of ontogenetic growth, allometric scaling and phenotypic plasticity. We can characterize the dynamic patterns of genetic effects of quantitative traits loci (QTL) governing growth curves and estimate the global effects of the underlying QTL during the course of growth and development. The framework model can decipher the genetic architecture of trait expression adjusted to different biotic and abiotic environments and genetic relationships for growth rates and the timing of life history events for any organism.

Accurate and rapid cultivar identification is especially important in vegetatively propagated plant species such as most fruit trees, and ornamental plants both for practical breeding purposes and for proprietary rights protection. Unfortunately, the traditional methods for characterization and assessment of genetic variability, based on morphological, physiological and biochemical studies are time consuming and are often affected by the environment. On the other hand, molecular methods have proven to be a powerful tool to identify and characterize these species, but these methods, though effective, are resource and labour-intensive, and require a skilled and experienced technical staff to be exploited effectively. Therefore, the use of artificial neural networks (ANN) as a possible alternative for genetic distinction and identification in plant material seems to be a powerful new method. An ANN is configured for a specific application, such as pattern recognition or data classification, through a learning process. Artificial neural networks are an attempt to emulate (very roughly) the basic functions of the mammalian brain to perform complex functions that computer systems are incapable of doing. ANNs can be effectively used to differentiate cultivars and accessions through phyllometric and fractal parameters. Though one of the acknowledged advantages of the neural networks is the capacity to overcome the need for a sample statistically representative of a population, they also have the capability for generalization beyond the training data, to produce approximately correct results for new cases that were not used in training.

Rapid hot air thermocycling of small samples in glass capillaries is a well-known technique for performing highly specific PCR reactions in 10-30 minutes. Compared to conventional slow cycling, rapid cycling considerably increases the quality of reactions for sequencing and allele-specific PCR. However, capillary-based cyclers are awkward when large numbers of samples have to be manipulated. Therefore, so far little has been done to apply rapid cycling to the various fingerprinting techniques. In this chapter we describe a novel rapid cycling technology based on ultrathin-walled multiwell plates combined with a rapid Peltier-driven heat-block thermal cycler. The technique combines the advantages of rapid cycling with the advantages of conventional cyclers. We tested several single-primer and two-primer DNA fingerprinting techniques in an attempt to reduce reaction times as much as possible.

Enhanced protection afforded by induction of resistance through exposure to a weak strain of a microorganism or chemical compound on plants, the term ‘induced resistance’ (IR), is an alternative approach for plant protection. Since plants, unlike animals, neither possess a circulatory system, nor immune surveillance system, the mechanisms of defense must be entirely different. Indeed, immunization of an animal leads to the production of antibodies that are highly specific for the antigen encountered, whereas IR occurs non-specifically in plants. Induction of disease resistance appears to constitute another layer of interaction between pathogen and plant, requiring its induction along with the defenses triggered upon primary infection, but expressed only when challenge actually occurs. There are many ways to trigger

disease resistance in plants through systemic acquired resistance (SAR) and induced systemic resistance (ISR), which have found widespread acceptance in describing the state of enhanced defensive responsiveness throughout a plant, resulting from local infection with a pathogen inducing necrotic lesions, such as in hypersensitive response. IR is the additional capacity for defensive activities, resulting from the primary infection, and dependent on the concomitant triggering of resistance responses. Once a plant has been stimulated in this way, it can express the enhanced defensive capacity irrespective of whether the challenging pathogen gives rise to an incompatible or to a compatible interaction. The purpose of this short review is to provide the reader with a closer understanding of the advantages and disadvantages of SAR and ISR inducers that may show possible alternative control methods of plant pathogens in floriculture and ornamental plants. We will discuss how they work, and most importantly their advantages and disadvantages, compared to traditional chemical fungicides.

Silicon (Si), the second most abundant element on Earth, has long been considered as a non-essential (trace) element. However, most plant muticellular taxa and some marine unicellular groups readily accumulate Si and for some species it is even essential for growth. Recent renewal of interest about this trace element results from the increasing body of evidence of its implication in various abiotic and biotic stresses. Recent researches have even started to examine the genetic and molecular basis of Si absorption and deposition pathways from both marine and terrestrial organisms. It was shown that Si is taken up as an uncharged molecule, silicic acid, and transporters have now been characterized in different plant species and cloned in some unicellular algae, *i.e*. the diatoms. Moreover, the Si-biomineralization process is also under investigation through the isolation and the characterization of several bio-organic compounds that may be directly involved in biogenic silica (or Si dioxide) formation. In parallel, the great potentiality of using Si biomineralization processes has recently been realized, for both plant biotechnology purposes and for the development of new biomimetic or “eco-friendly” approaches in Si chemistry and new materials design.

Chromosome analysis techniques for maize and its relatives are described. These approaches should be universally applicable to plant species. Procedures are described that result in improved signal strength for Fluorescence *in situ* hybridization (FISH) procedures. These improvements allow localization of individual genes, including transgenes, to specific chromosomal locations. To identify each chromosome in the karyotype, a set of tandemly arranged repetitive sequences was assembled. The copy number of the elements in the individual arrays varied from line to line. Also, the method of Retroelement Genome Painting is described that uses specific retrotransposons, which have amplified differentially in the genomes of related species to permit the distinction of diverged chromosomes in interspecific hybrids or allopolyploids. Cytological visualization of chromosomes using this technique can reveal evolutionary relationships between species and guide attempts to introgress portions of one genome into another.

Part 3 Plants in space

Flowering plants have coevolved with their pollinator animals. Gravity has been one of selection pressure for the evolution of flowers. Gravity dominates morphology and other features of flowers in many ways. Interaction between flowers and their pollinator is one of factors that determine the fitness of both organisms. Evolution of flower morphology and its molecular basis are reviewed briefly. Anemophilous flowers are also under the influence of gravity in a different manner. Shape of flowers has been highly diverged. Tropic response of those flowers and its mechanism are summarized with recent findings on gravitropism and phototropism of pistils and stamens.

Fundamental studies were conducted to develop a facility having an adequate environmental control system for growing healthy plants over a long-term under microgravity conditions in space farming. To clarify the effects of gravity on heat and gas exchanges between plant leaves and the ambient air, surface temperatures and net photosynthetic rates of sweet potato and barley leaves were evaluated at gravity levels of 0.01, 1.0 and 2.0 g for 20 seconds each during parabolic airplane flights. Thermal images were captured using infrared thermography. The net photosynthetic rates were determined using a chamber method with an infrared gas analyzer. Mean leaf temperatures increased by 0.9 - 1.0°C and decreased by 0.6°C over 20 seconds as gravity decreased from 1.0 to 0.01 g and increased from 1.0 to 2.0 g, respectively. The increase in leaf temperatures was greater at the regions closer to the tip of the barley leaf and at most 2.5°C over 20 seconds as gravity decreased from 1.0 to 0.01 g. The net photosynthetic rate decreased by 20% with decreasing gravity levels from 1.0 to 0.01 g and increased by 10% with increasing gravity levels from 1.0 to 2.0 g at a PPFD of 500 µmol m^{-2} s^{-1}. The heat and gas exchanges between leaves and the

ambient air were suppressed more at the lower gravity levels. The retardation would be caused by heat and gas transfers with less heat convection. Restricted free air convection under microgravity conditions in space would limit plant growth by retarding heat and gas exchanges between leaves and the ambient air.

Compared to the plant growing conditions on Earth, a totally unusual space environment, such as high-energy ion radiation, microgravity, space magnetic field, ultra vacuum, may have either direct or indirect effects on plant growth and metabolic activities. Plants in space must response those different conditions in order to complete a full life cycle. Genetic mutagenesis of seeds or seedlings may thus take place when being flown with retrievable satellites and spacecrafts. This opens potential opportunities for screening new plant varieties by ground-based observation and selection. This paper summaries recent progress in earth-grown plants returned from China's retrievable satellites (No.9 to No. 20) and spacecrafts (Shenzhou 1 to Shenzhou 6) to screen new floricultural, vegetable and medicinal varieties from 1987 to 2005 in China. A total of +140 ground-grown plant species (+1000 varieties) have been tested, including 14 field crops, 59 floricultural plants, 27 vegetable and fruits, 27 medicinal plants and 13 forest trees. Of them, more than 50 selected new varieties have been successfully planted in large scales, and more than 200 varieties are promising. Space mutations lead to a great potential approach in screening diverse valuable plants in the future.

Ornamental Plants and Flowers in Art and Society

Part 1 Plant and floral development and form: evolutionary and palaeobotanical perspectives

Extensive research has been carried out, especially in model dicotyledonous species such as *Arabidopsis*, on the characterization of the genetically controlled successive conversions of meristem identities that lead to the concentric arrangement of floral organs in specific whorls. Using data from the Sugarcane Expressed Sequence Tags (ESTs) Project (SUCEST) database, we have identified sugarcane genes putatively involved in reproductive meristem and flower development. Sequence comparisons of flower-related genes among sugarcane and other plants have uncovered conserved evolutionary pathways of flower development and flower pattern formation between dicots and monocots. We have paid special attention to the analysis of the pathways that promote or repress flowering and to the role of the MADS-box multigene family of transcription factors during early flower development.

Environmental sex determination is defined in plants and animals when environmental factors influence the sexual development of individuals. Under evolutionary theory, we expect to find a 50:50 sex ratio in offspring of organisms when males and females are equally abundant in the environment, increase in fitness at equal rates with increasing size or age, have equal fitness in all environmental patches, and cost the same to produce. When there are deviations from these conditions, there is a selective advantage to biasing the sex ratio of offspring or individuals. Accordingly, biased ratios of unisexual flowers or individual plants are found in nature. The developmental processes that produce unisexual flowers vary among species. However, species with unisexual flowers can be categorized in two independent ways: those that abort or arrest developing reproductive organs vs. those that selectively initiate reproductive organs and those that coordinate the signals for unisexual development by chromosomal linkage vs. those that control the signals by hormonal controls. We propose that when biased sex ratios can be selectively advantageous, sexual lability will evolve in those species that control unisexual development by hormonal controls.

In addition to the many anti-herbivore defense mechanisms, plants have probably also adopted various types of defensive coloration: (1) undermining herbivorous insect camouflage, (2) aposematic (warning) coloration of thorny and poisonous plants, (3) camouflage, (4) insect and dead leaf mimicry, (5) various unexplained types of leaf variegation, (6) delayed leaf greening, and (7) signaling about trees' defensive quality by red and yellow autumn leaves. At this initial stage of study of defensive plant coloration, many additional types and aspects of defensive plant coloration very likely remain experimentally unexplored, and even not recognized and described. Visually oriented animals were the selective agent for such types, by attacking defended and non-defended genotypes differentially. Many of the defensive types of coloration certainly perform various physiological functions concomitantly.

The purpose of this chapter is to analyze the biogeographical characteristics of *Narcissus* L. subgenus *Ajax* in which a wide range of floral morphological variability is shown. This variability has been interpreted in terms of genetic drift leading towards the emergence of species confined to small areas (micro endemics) in SW Europe. In this chapter, biogeographical comparison of the species and taxonomic assemblages, as well as the models of evolution is presented. The distribution is discussed. The more relevant habitat types colonized by species of subgenus *Ajax* are deciduous forests, thickets and wet grasslands. Most taxa of this group grow in supra-Mediterranean and montane bioclimatic belts, at altitudes between 600 and 1,800 m above sea level and under subhumid and humid precipitation regimes. Almost 90% of taxa are diploid, but triploids, tetraploids and hexaploids also occur. The center of diversity of this subgenus is the region lying between the Sierra Nevada in Southern Spain and the Northern Slopes of the Cantabric and Pyrenean mountains.

Part 2 Society and lifestyle

Agbiotech has the potential to be used to address hunger and food security needs of developing countries. To achieve this, several challenges will have to be addressed chief of which are inadequate scientific capacity for biotechnology, unfavorable government policies, lack of biosafety regulations, capacity to effectively manage intellectual property, and lack of financial resources. Developing countries vary in the degree to which the challenges exist in the nation. Some countries such as China, India, South Africa, and Brazil have well established agbiotech infrastructure and are already commercializing certain products. Contrary to the fact that agbiotech is not as advanced in most developing countries, some of those countries are among the leaders in the adoption of GM crops for commercial production, the leaders being Argentina, Brazil, South Africa and China. Most developing countries use second generation biotechnology, that is, primarily tissue culture. A relatively few number of countries have high scientific capacity to conduct research and development using third generation biotechnology involving transgenesis. The decision to adopt agbiotech should be made by each nation according to its needs, and not imposed or obstructed by external interests.

In Hungary, where they blossom twice a year, *Salvia officinalis* and *Salvia sclarea* are popular ornamental plants in rocky gardens. In addition to their aesthetic effect they also serve as a melliferous, and as medicinal plants. During the flowering period the scent released enhances the aesthetic effect in the limbic system. They are important basic materials in the perfume indusry because of their volatile oil content and are also applied in therapeutics, mainly externally, for their antiseptic effect. Owing to its ornamental effect, coriander is often used as a bordering plant in vegetable gardens. In Northern Europe coriander seeds are used for marinating meat and the seeds of coriander is applied for flavouring of meat and in Southern Europe (e.g. Spain) the dried leaves are applied as spice. Biologically active agents of *Salvia officinalis*, *Salvia sclarea* and *Coriandrum sativum* were studied. A qualitative comparative study was performed for terpenes obtained from volatile oils by a traditional- and supercritical fluid extraction. Detailed examination was performed for oil extraction from coriander seed with CO_2, CO_2-propane and propane as solvents, under sub- and supercritical conditions. The composition of essential oils extracted greatly depends on the solvent and parameters of the extraction applied.

With the continuous development of greenhouse technology, and advances in plant biotechnology, transportation conditions, and marketing strategies, floriculture has reached a historical maximum hub of activity and competitiveness. The Netherlands, United States and Japan are the three most important global producers and consumers of floricultural products. As a result, three essential floriculture centers have formed: Europe-Africa, America, and Asia-Pacific. To give an overview of the current global floriculture industry, we describe the production, consumption, and trade in each of these three centers. The most important production and consumption countries have been highlighted. In the market chain, auctions and tele/Internet flower auctions as marketing strategies play an increasing important role in the floriculture industry. Moreover, rare insights into some of the rationale behind consumer-driven floriculture markets, especially in Japan, which, together with China, may easily be the new global market floricultural trend-setters, are discussed. Most importantly and to create a holistic, balanced view of the floricultural industry, the “not-so-rosy” side of the global floricultural industry is also depicted.

Ornamental area remains very modest in North African countries and varies from 74.5 ha in Algeria to 210 ha in Morocco. Cultivated plants are cut flowers, indoor and outdoor plants. Main species of cut flowers are rose, carnation, Bird of Paradise and *Gladiolus*. A wide range of important trees, shrubs and bedding plants are grown for landscape use and indoor ornamentals, mainly foliage plants. Flower and ornamental products are sold solely in local markets as in Algeria and Libya or exported such as in the case of Tunisia (318.3 tons) and Morocco (2230 tons). Export of cut flowers concerns particularly rose, carnation, buds of jasmine and dry flowers. To expand local markets and to produce competitive export products, technical and marketing constraints must be solved. The climatic conditions in summer (heat and drought) and the quality of irrigation water impose a choice of tolerant species for landscape use. Many floral crops are adapted to these conditions and their use in arid and semi-arid regions is recommended.

Floriculture in general and trade of cut-flowers is an increasingly globalised business. Production of cut-flowers is of growing importance in newly industrialized countries and third-world-countries. To guarantee implementation and acceptance of workers' rights, of ecological and social responsibility as well as transfer of knowledge and guidance certification-systems and labelling were introduced. Not only to ensure consumers on the final markets, i.e. place of use but also to minimize economic risk for the players in the flower-supply-chains. About a potentially strong new exporting country, Brazil, detailed information about dispersal and use of eco-socially based labelling were not available. In a field study Brazilian institutions of importance in floriculture (Universities and research stations, marketing institutions, growers) were visited and representatives interviewed. No floricultural nursery could be found, knowledge about certification and labelling was not wide-spread. Interest in eco-social labelling was expressed.

This chapter introduces some of Chile's new and exciting endemic flora, which have shown promise as cut flowers, especially species from the genus *Leucocoryne* (Alliaceae) and *Chlorea* (Orquidaceae). The chapter deals with steps taken at domestication of these species for the purposes of genetic improvement.

The "Ajax Group" (i.e. *Pseudonarcissus*) is one of the most important ancestors of modern daffodils cultivars. The manner in which these plants were introduced into the English, French and Dutch gardens appears relatively obscure since most are endemic to the Iberian Peninsula. This chapter compares data from Arab texts of agriculture, European Renaissance, and Prelinnaean texts and illustrations, with the morphological characteristics of primitive cultivars of trumpet daffodils and the related wild taxa from of Spain and Portugal. The relationships among wild plants, domesticated plants, and primitive cultivars were investigated through a cluster analysis of the characters available from figures or botanical illustrations. The tree resulting from the Complete linkage analysis and UPGMA analysis distinguished 26 different groups including wild; cultivated and wild; and cultivated daffodils. A comparison of early descriptions, localities, and illustrations with currently wild species confirmed that several Iberian Peninsula endemics were cultivated in Central European gardens between the 16th and 18th centuries. Examples are: *Narcissus abscissus* Pugsley*, N. jacetanus* Fernández Casas*, N. asturiensis* Hénon*, N. hispanicus* Gouan*, N. nobilis* (Haw.) Schult. var. *leonensis* (Pugsley) A. Fernandes*, N. pallidiflorus* Pugsley and *N. pseudonarcissus* L. The characters involved in flower pigmentation evolved independently from other morphological characters. It obviously occurred in different places and at different times. Thus any colour flower group, even whites, is polyphyletic.

Part 3 Health, important secondary metabolites, herbs, medicinal, and aromatic ornamentals

Plants have provided strong medicines, or at least the starting materials for powerful drugs, for ages. Furthermore plants make up a significant percentage of available therapeutics even today. In the foreseeable future, they will continue to be an important source for new compounds and novel structures with biologic activities, due to the chemical diversity found across this biologic kingdom. However, the discovery process for new medicines or bioactive molecules from plant sources is often an involved, time-consuming, or expensive endeavor. This forward reviews historic and current discovery methodologies based on ethnobotany or laboratory high-throughput screening (HTS), and discusses the advantages and problems associated with each. Emerging techniques including data mining for new leads, and speculation on future trends as they apply to this discovery process are also considered.

The present resume reports on the naturally occurring antioxidants isolated from flowering plants, including several ornamentals, covering the literature from 2000 to early 2005. Chemical classifications of the compounds as well as their natural distribution and antioxidant property are discussed in detail. Applications of flowering plants in the traditional system of medicine are also mentioned.

Now-a-days, search for antioxidant and antimicrobial agents through natural sources such as essential oils and extracts from aromatic plants, many of which are also ornamentals, are gaining much more interest. Since, the products obtained from natural resources are not toxic in comparison with synthetic materials, there is need to find out substitutes for synthetic ones. Briefly, this review article covers the applications of essential oils and oleoresins as antioxidant, antimicrobial and insecticidal agents. It also includes the methods adopted for the evaluation of antioxidant activity in edible oils, and further describes the various methods such as DPPH, β-carotene bleaching and static headspace chromatography assays.

In the flowers of many species of several genera of Clusiaceae (Guttiferae) belonging to both subfamilies recognized today, oily or resinous exudates have been observed. Most of these are secreted by stamens or staminodes, but in species of *Clusia* (sect. *Cordylandra*) the stigma also secretes lipids in considerable amounts. The androecial exudates are involved in basically two major functions in the pollination process: as a reward (fragrant oils and resins) and as a vehicle to improve pollen transport. The stigma exudate in *Clusia* sect. *Cordylandra* enhances the chances of a successful germination of the pollen grains that are deposited by pollinators on the stigma within resin droplets. We studied the chemical composition of various floral exudates and found that they are either pure isoprenoids or poliketides possessing isoprenoid radicals, some of them with considerable biological activity.

Promising results are reported for several herbal extracts and remedies in a variety of diseases including virus infections, autoimmune syndromes, and malignant, cardiovascular or neurodegenerative disorders. In the same clinical conditions, inflammation and immune system activation are deeply involved in the pathogenesis, and immune activation cascades appear to contribute to disease progression. Enzymes GTP-cyclohydrolase I and indoleamine (2,3)-dioxygenase are specifically induced by Th1-type cytokine interferon-γ: Likewise, cellular immune activation can sensitively be monitored by determination of neopterin concentrations and tryptophan degradation in patients. This is also feasible *in vitro* by using peripheral blood mononuclear cells (PBMC) stimulated with, e.g., mitogens phytohaemagglutinin or concanavalin A. The increased neopterin production and tryptophan degradation in stimulated PBMC was found to be significantly suppressed by several plant extracts, e.g., from *Uncaria tomentosa* and *Hypericum perforatum*, green and black tea from *Camellia sinensis* or by the Tibetan herbal remedy Padma 28. Also beverages rich in antioxidants like green and black tea prepared from *Camellia sinensis*, beer and wine and also pure antioxidants, like vitamins C and E and resveratrol, achieved similar effects in this *in vitro* system. Data show that plant compounds are able to down-regulate Th1-type immune response by reducing the expression of the cytokine interferon-γ. Antioxidant compounds seem to be important for this suppressive activity. The assay described allows in a simple manner to quantify anti-inflammatory properties of plants, plant extracts and plant compounds.

Local anaesthetics (LAs) are drugs that block conduction of nerve impulse in animals resulting in both sensory and motor paralysis. Studies on the influence of anaesthetics on plants has largely escaped the attention of researchers, probably due to an apparent lack of a parallel neural system. Having both lipophilic aromatic and hydrophilic amine moieties in their constitution, renders these anaesthetics interactive with biological membranes, which formed the basis of numerous investigations involving synthetic as well as natural membranes, including those of chloroplasts. Relatively recently, studies have been initiated to understand their influence on growth and morphogenetic processes of higher plants as well, taking LAs as elicitors. The observations, quite intriguingly show significant modulations of seedling growth and developmental responses. These LA-induced influences are suggestive of irreversible long-term modulations of plant systems, obviously involving altered gene expression. It is unlike short-term reversible blockade of nerve conduction in animals or even the motor mechanism of leaflet movements in the sensitive plant. Weather the mechanisms of action for the two types of responses are differently sited or executed is not known as yet. Nevertheless, many striking similarities between the mechanism of anaesthetic-caused human anaesthesia on one hand

and cyanobacterial and yeast growth as well as leaflet movements on the other have been exhibited.

This review summarizes several characteristics of *Stevia rebaudiana* (Bertoni). The plant, whose extract is commonly used as a natural sweetener, does not as yet have any ornamental application, despite its showy leaves and petite, attractive flowers. But through this chapter we hope to expose the importance of Stevia, not only to human health, but also its potential for ornamental greenhouse or field cultivation as a foliar spray for pest control and in stress studies. This possibility lends itself from the particularly wide-ranging characteristics of its major sweetening compound, stevioside. Aspects including the tissue culture, bioreactors, chemistry and genetics will reveal that Stevia may be more than just a greener view to healthy living, but may actually become an important model plant, along with the ranks of Arabidopsis, tobacco and tomato. We demonstrate, too how this importance may often be masked by the numbers of patents which heavily outweighs the numbers of original publications. An open exposure of the current status of this plant through this chapter may reverse this trend, allowing R&D to find solutions to the presently major challenge facing Stevia today: how to increase the endogenous levels of both stevioside and rebaudioside-A.

Tea is the most popular non-alcoholic soft and healthy beverage across the world. It contributes greatly to wealth and job opportunities in several Asian and African countries and has great value as a source of secondary metabolic products. Molecular biology and biotechnology of tea plant are two of the most active and kinetic research fields of tea science in the last decade. In this paper, the recent 10 years', especially the latest 5 years' progress in the research of 1) molecular markers, 2) functional gene isolation, cloning and expression, 3) functional genome, 4) gene transformation of tea plant have been reviewed. And brief prospects of tea plant molecular biology are given, great attentions and efforts should be focused on functional genomics and proteomics and, gene transformation used currently and in the near future.

The genus *Fritillaria* L. belongs to the Liliaceae, which is an important steroidal alkaloid-bearing plant family. Bulbs of this genus, commonly known as "Bei-mu" or "Pei-mu" in Chinese and "Bai-mo" in Japanese, have long been known as one of the principal Chinese crude drugs. The dried bulbs or a decoction of *Fritillaria* species be prescribed to treat cough, asthma, bronchitis and some other disorders. The phytochemical and biological activity properties of *Fritillaria* L. species are reviewed along with recent studies in this chapter.

The callus culture line 95 initiated from young stem segments of *Taxus baccata* L. established on Gamborg B5 agar medium produced paclitaxel from the 21st day of cultivation. The highest content was determined on the 49th day. In suspension cultures derived from this callus culture the elevation of taxanes content in cells and their excretion into medium were observed. The highest amount of total taxanes (in cells and excreted into the medium), as well as the high content of paclitaxel produced by suspension cultures in modified Gamborg B5 medium with addition of sucrose (2%) in combination with cellobiose (3%) was determined.

The present chapter describes the lines of experimental evidence that feature the extracts of the roots of *Salvia miltiorrhiza*, a Chinese medicinal plant, as a potential pharmacotherapy for alcoholism. Indeed, it has been reported that the acute or repeated administration of extracts of *S. miltiorrhiza* decreased alcohol intake and different alcohol-motivated behaviors in rats of the Sardinian alcohol-preferring (sP) line, an animal model of excessive alcohol intake. Clinical studies are now needed to assess whether the reducing effect of *S. miltiorrhiza* extracts on alcohol intake observed in sP rats may be generalized to human alcoholics.

Aloe species are used for medicinal and cosmetic purposes since ancient times. There are 300 species but only a few have purported curative properties. This work reports on *Aloe* classification of species as well as their reported medicinal uses; the information also

considers the chemical structure and bioactive compounds identified in several species. The crassulacean acid metabolism of species is reviewed for photosynthesis and transpiration processes. Finally, *Aloe vera* is discussed in detail for botanical classification, cropping and alternatives for bioprocessing including the results obtained by our research group.

Plant polysaccharides from various parts of medicinal plants *Althaea officinalis, Malva mauritiana*, *Salvia officinalis*, *Verbascum thapsiforme*, *Mahonia aquifolium* and *Rudbeckia fulgida*, and a wild plant *Arctium lappa*, in form of a mucilage, a crude mixture or isolated and characterized carbohydrates, were tested for cough suppressive ability. Their antitussive activities evaluated on the basis of the cough reflex parameters were found lower than that of narcotic antitusives but higher than that of non-narcotic cough suppressive drugs. High antitussive activities, minimal influence on the reduction of expectoration and rare side effects make herbal polysaccharides prospective drugs for suppression of the cough reflex.

The genus *Salvia* (*Lamiaceae*) includes ca 1,000 species distributed throughout the world. Sage plants are widely employed as spices, as well as in the food and fragrance industry. With an increase in popularity, sage plants also play an important role as herb garden ornamentals. Medicinal properties of some of the species are also known since long time. The present chapter describes salient morphological, histological and chemical features of sage species. Biotechnological methods to reproduce high quality plant material able to synthesise the bioactive metabolites are discussed and results compared with *in planta* molecular phenotype.

Sage (*Salvia officinalis* L.) is a reputed plant species with respect to its multiple ethnobotanic uses. In recent years, it has been intensively studied for its bioactivities and potentialities as a source of phytomedicines and chemicals for several industrial purposes. In this chapter, results from analysis of essential oils, phenolics and lipid-like extracts are reported and discussed. A new method for simultaneous extraction of non-polar and semi-polar compounds including essential oils, phenolic diterpenes and lipid constituents of sage and their analysis by GC and GC-MS, as trimethylsilylated derivatives of their hydroxyl and carboxyl functionalized compounds was assayed and the respective results are presented and discussed in this chapter. In this method ninety compounds were detected, and mostly identified, in sage *n*-hexane extracts which contained the essential oil constituents, free fatty acids, sterols, triterpenols, including ursolic and oleanolic acids, and some phenolics. Phenolic extracts obtained, as a polar fraction of the *n*-hexane extracts and analysed by HPLC-DAD, were composed of four to five phenolic compounds not reflecting the composition of phenolic extracts obtained from sage by maceration with acetone as is usually done. This method was applied to sage plants growing in Nature and to *in vitro* cultures including shoots, calli and cell suspensions grown in MS medium supplemented with 0.05 mg/L 2,4-dichlorophenoxyacetic acid (2,4-D) and a cytokinin, kinetin (KIN) at 2,0 mg/L (shoots); benzyladenine (BA) at 1.5 mg/L (calli) and KIN at 0.5 mg/L (cell suspensions). Two different lines of sage cell suspensions were induced in the same MS medium from calli originated from different types of aseptic sage shoot explants (leaves and inter-nodal segments). Sage *in vitro* shoots showed a high capacity for producing essential oils and phenolic compounds while cell suspensions produced high levels of phenolic acids, especially rosmarinic acid.

Amaranthus spp., locally known as spinach or "bayam", is one of the most popular green vegetables consumed in Malaysia. Five types of "bayam" namely 'bayam putih' (*Amaranthus spp*), 'bayam merah' (*Amaranthus gangeticus*), 'bayam itik' (*Amaranthus blitum*), 'bayam duri' (*Amaranthus spinosus*), 'bayam ekor kucing' (*Amaranthus caudatus*) can be found in Malaysia. However, urban and rural community most commonly consumes 'bayam putih 'and' bayam merah. *Amaranthus* spp. have high antioxidant capacity among other vegetables. Besides other traditional antioxidant components, 'bayam' is also a good source of phenolic compounds. These compounds are able to act as a potent antioxidant component in *Amaranthus* spp. This green leafy vegetable is cooked before being consumed. Cooking practices may affect the antioxidant capacity and phenolic content in vegetables. Major losses of antioxidant capacity and phenolic content of the fresh 'bayam' were observed after cooking processes (boiling) for more than 10 min. However, this depended on the species of 'bayam'. The consumption of certain fresh vegetables is important to promote a high intake of antioxidant components. Minimal heat treatment in cooking practices should be recommended to prevent the major loss of antioxidant properties and also to maintain safety and quality.

The genus *Hypericum* has recently received attention due to the andidepressant activity of *Hypericum perforatum* L. Several species of this genus are used in traditional medicine and some of them have ornamental value. In *Hypericum* species phenolic compounds such as polycyclic quinones, xanthones, flavonoids and phloroglucinol derivatives are commonly found. Of the 20 Brazilian species of *Hypericum* a few have been analyzed. Xanthones and phloroglucinol derivatives were isolated from the leaves and flowers of *H. brasiliense* Choisy, and extracts containing these substances inhibited the monoamine oxidases (MAO). The research with native species native to south Brazil resulted in the isolation of benzopyrans from the aerial parts of *H. polyanthem*um Klotzsch ex Reichardt, phloroglucinol derivatives from *H. myrianthum* Cham. & Schlecht., *H. carinatum* Griseb., *H. polyanthemum*, *H. caprifoliatum* Cham. & Schlecht. and *H. connatum* Lam., and benzophenone derivatives from *H. carinatum*. All species presented flavonoids and tannins, and essential oil in small amounts but none of them presented hypericins or hypericin derivatives. Pharmacological studies demonstrated that some of these species present antidepressant, antinociceptive, MAOI, antiviral, antibacterial, antifungal and antiproliferative activities. Aiming the conservation of the native species, the establishment of protocols for propagation of *H. carinatum* Griseb., *H. caprifoliatum* Cham. & Schlecht., *H. myrianthum* Cham. & Schlecht. and *H. ternum* A. St. Hil. are currently under investigation.

Biotechnology is defined as a collection of technologies that use living cells and/or biological molecules to solve problems and make useful products. Medicinal curcuma plants have been widely implicated in a number of ailments and studied extensively for their biological activities, predominantly anti-inflammation, tumor-resist, antioxidant, anticarcinogenic, antimutagenic, anticoagulant, antifertility, antidiabetic, antibacterial, antifungal, antiprotozoal, antiviral, antifibrotic, antivenom, antiulcer, hypotensive and hypocholesteremic activities. We have integrated published papers on Curcuma plant research: the chemical components, biology activities, chemical technology and biotechnology used. Biotechnology as a powerful tool used in Curcuma research, such as transgenics, molecular authentication, amplification-refractory mutation system (ARMS) analysis, Polymerase chain reaction (PCR), Reverse transcriptase PCR (RT-PCR), Western blotting and Northern blotting, single-nucleotide polymorphism (SNP) analysis, molecular markers (randomly amplified polymorphic DNA (RAPD), isoenzymes), molecular genetic identification, molecular cloning and tissue culture, have provided a novel way for the identification of Curcuma plants and the standardization of medicinal Curcuma plants and their products. Further research using biotechnology to study Curcuma taxonomy, pharmacy, quality control and clinic trials is merited.

The most relevant aspects of the enzymology and genetics involved in the synthesis of monoterpene indolic alkaloids in *Catharanthus roseus*, an important medicinal and ornamental plant, are reviewed. The regulation of this pathway in different tissues is also covered, mainly focused on the production of the dimeric cytotoxic alkaloids, vinblastine and vincristine, which cannot be formed in *in vitro* cell cultures. Particular emphasis is given to specific cell type gene expression, which is absent in undifferentiated cell cultures, as well as to the role of regulatory genes participating in the induction of alkaloid biosynthesis in response to stress conditions. The use of alternative strategies for the production of valuable pharmaceutical compounds through *in vitro* cell culture technologies is also discussed, covering from the most traditional approaches to the recent application of metabolic engineering, based on the overexpression of enzymes representing potential rate-limiting steps, as well as the feeding of intermediaries.

Artemisia annua L. is an aromatic herb, which is the only source of artemisinin (Qinghaosu), an endoperoxide sesquiterpene lactone. Artemisinin and its derivatives are the most potentially important and clinically useful agents in the treatment of malaria. In this paper, advances in regulation of artemisinin biosynthesis have been reviewed, which include the artemisinin biosynthesis pathway, the establishment of a transgenic system for *A. annua* L. via *Agrobacterium tumefaciens*, overexpression of the FPS gene in *A. annua* and its effects on artemisinin production, effects of the *ipt* gene expression on the physiological and biochemical characteristics of *A. annua*, as well as effects of the *fpf1* gene on *A. annua* flowering time and the link between flowering and artemisinin biosynthesis.

Two cultivars of bananas (*Musa troglodytarum*) with local names ‘Kulasr’ and ‘Kulundol’ are known to have high contents of carotene, the precursor of vitamin A. These cultivars of banana are endangered species and probably endemic to the Federated States of Micronesia. Vitamin A deficiency is a major nutritional disorder among the population of Oceania. Micropropagation and field planting with community participation saved these cultivars from extinction. Half strength MS medium supplemented with 250 mg l^{-1} KH_2PO_4, 1 mg l^{-1} thiamine HCl and 1 mg l^{-1} 6-benzyalamino purine proved to be excellent for establishment of the explants in culture. Incubation of these cultures on

modified MS medium produced six to eight well developed shoots. Micro-shoots profusely rooted on half-strength MS basal medium. Acclimatisation of transplanted plantlets in a controlled-environment glasshouse resulted in the establishment of 90-96% of the plants. Micropropagated plants exhibited better field establishment, vigour, growth, reduced pre-bearing period and improved yield than conventional suckers. As banana is a staple food for the population of Oceania, availability of these nutritious bananas at an affordable price may alleviate the vitamin A deficiency among these people. This work also demonstrated the successful introduction of biotechnology to a tiny, remote island with a population of just 7,500 people.

Novel Ornamental Gems and Floricultural Assets

Part 1 Orchids

In contrast to most tropical orchids the European temperate orchids have proven to be very difficult to grow in soil and in tissue culture. The plants can be grown in tissue culture and then can be transferred to soil but require up to 5 years to attain a flowering size. In addition there are no propagation methods for many species of these orchids. This long time investment has meant commercial growing of these plants is very rare. In this work we report the tissue culturing of the green winged orchid and the transfer to soil. Once in soil experiments have been performed on the plants whereby the nutrients and growth regulators used in tissue culture have been applied in the soil. Using the optimized methods quoted in this work it has been possible to accelerate growth in the plants such that they flower within 2 years of sowing seeds and in addition can be forced to propagate. This work could provide a powerful tool for orchid conservation and for the commercialization of these rare and unusual plants.

Currently many methods are available for conferring useful traits to orchids besides breeding. Elaboration of methods for gene transfer to orchid cell has been possible due to the development of reproducible *in vitro* propagation techniques. Moreover nutritional requirements (minerals), organic compounds (carbohydrates, vitamins and amino acids), environmental factors and treatment with growth regulators have helped orchids in achieving a high proliferation rates to allow commercially viable micropropagation. In this article an overview of *in vitro* seed germination process, organogenesis and embryogenesis from different explants and synthetic seed production of *Cymbidium* orchid is presented. In addition future prospects of different biotechnological approaches to enrich *Cymbidium* genome are discussed.

This article emphasises the importance of an innovative fungal bioassay approach in modern plant biotechnology addressing the question of orchid seed viability, within the new concept of “mycovitalism”. According to this approach, seed viability evaluation using a *Fusarium* compatible strain leads to viable seed coloration (before dormancy arrest) and germination. *Fusarium* was isolated on MBA selective medium and subsequently identified. Then, the phylogenetical status was defined based on sequences of the EF-1alpha gene. The culture-independent PCR-DGGE fingerprinting method was optimised to asses *Fusarium* diversity *in situ* and select promising strains for orchid seed viability testing in orchid production biotechnology. Finally, this study proposed the *Orchidaceae* as a model to study seed viability in plants with minute seeds.

An overview of the recent discoveries concerning the ecology and chemistry of pollination in Brazilian orchids is presented, preceded by a brief summary of orchid systematics, morphology and breeding systems. Floral features promoting cross-pollination are described in detail. Recent discoveries in Brazilian orchids pollinated by Euglossini and oil-gathering bees are discussed and illustrated. Pollination in Brazilian Maxillariinae orchids is described in detail. Fragrance main components in some rewardless species are provided and illustrated. In addition, floral secretions in some reward-offering species are discussed and illustrated in detail. Study cases concerning sexual mimicry, or “pseudo-copulation”, in which morphological and chemical flower features elicit the attraction of sexually-excited insects that promote pollination during copulation attempts with the flowers, are emphasized. We expect that the study of the chemistry and ecology of pollination in Orchidaceae will not only shed light on character evolution, but also be helpful for systematic purposes, by providing non-molecular characters to support or diagnose clades.

According to International Union for Protection of New Varieties of Plants (UPOV) requirements, a new plant variety should be distinct, uniform and stable (DUS) to qualify for Plant Breeder Rights. On the other hand, genetic profiling techniques like amplified fragment length polymorphism (AFLP) and simple sequence repeats (SSR) are highly polymorphic and able to distinguish even minor differences in genetic make up. This paper was to evaluate the utility of fluorescent AFLP in identification of *Dendrobium* hybrids in the spirit of UPOV requirements. For tropical orchids like those of the popular genus *Dendrobium*, intensive breeding activities have given rise to many commercial cut flower hybrids with smaller genetic distances. Clear identification of similar hybrids is difficult. We have subjected 39 *Dendrobium* hybrids to fluorescent AFLP analysis with inclusion of siblings and tissue culture mutants. The AFLP profile for one hybrid was also evaluated for its stability in different parts of a single mother plant and among random samples from its vegetatively propagated populations. It was found that each hybrid tested had a distinct AFLP fingerprint profile except for the tissue cultured mutants. Sibling hybrids were closely clustered (with genetic distance <0.09) followed by those sharing one parent. These results suggest that AFLP profiling gives accurate and objective molecular identities to *Dendrobium* hybrids tested. It was also found that the AFLP profile of one mother plant for *Dendrobium* Lucian Pink remained stable in different parts of the plant and was faithfully inherited in vegetatively propagated populations at different developmental stages. We conclude that AFLP fingerprinting profiling has the potential to be an integral part of current new plant varieties protection system. Limitations and other issues are also discussed.

An efficient and rapid *in vitro* method was developed for high frequency regeneration of *Cymbidium* using nodal segments of mature nursery plants. Nodal segments cultured on half strength Murashige and Skoog (MS) medium supplemented with N^6-benzyladenine (BA; 11.1 μM) and 10% coconut water (CW) produced an average of 12 protocorm-like bodies (PLBs) within 4 months. PLBs were multiplied by subculturing on the same nutrient medium, where a PLB multiplied into 12-15 PLBs within the following 2 months. Plantlet development from PLBs, was achieved by subculturing on half strength MS medium supplemented with banana pulp (50 gl^{-1}) + 1 gl^{-1} activated charcoal + 10% CW with or without BA (11.1 μM) within 2 months, by which time new PLBs were induced from the base of developing shoots. By repeated subculture of PLBs on plantlet regeneration medium, plantlets as well as PLBs could be continuously produced. About 8 months were required from culture initiation to the production of plantlets for transplantation.

An efficient continuous high frequency *in vitro* regeneration method was developed for four cultivars of *Phalaenopsis amabilis* from leaf segments of emerging young leaves of mature plants. Leaf segments of four cultivars of *Phalaenopsis* hybrids cultured on half strength Murashige and Skoog (MS) medium supplemented with N^6-benzyladenine (BA; 8.88 μM), α-naphthaleneaceetic acid (NAA; 2.7 μM), 2% (w/v) sucrose, 10% (v/v) coconut water (CW), 2 gl^{-l} peptone and 1 gl^{-l} activated charcoal produced an average of 16-26 protocorm-like bodies (PLBs) after 12 weeks. PLB clumps were cut into four pieces and subcultured on agar-gelled ½ MS medium with 2% (w/v) sucrose + 10% (v/v) CW + 2 gl^{-l} peptone + 150 mgl^{-l} L-glutamine + 1 gl^{-l} activated charcoal, where each clump of PLBs produced 198-268 PLBs, within 8 weeks. After a further four weeks of subculture the PLBs were found to be enlarged with leafy shoots. Plantlet development from leafy shoots was achieved on ½MS medium supplemented with 2 gl^{-l} peptone, 2% (w/v) sucrose, 10% (v/v) CW and 1 gl^{-l} activated charcoal, upon which 100% explants developed into plantlets with stout roots within 8 weeks. By repeated subculture of PLB clumps on proliferation medium and culturing leafy shoots on the plantlet regeneration medium, a continuous high frequency of plantlets could be produced.

Part 2 Other gems

Introducing a new flowering crop requires investigations into aspects such as seed germination, propagation practices, growth characteristics, flowering control, disease and pest resistance, yield potential and post-production issues. The genus *Watsonia* has been little researched compared to the closely related genus *Gladiolus* and shows much potential for development as both a cut flower and a container plant. Our work indicates that *Watsonia borbonica* and *W. tabularis* exhibit a facultative vernalisation requirement for flowering while *W. pillansii* exhibits an obligate vernalisation response. Long-day treatment of plants increased flowering percentage, suggesting that photoperiod also acts to regulate flower induction. *In vitro* seed germination showed that species from the winter rainfall area germinate best at 10°C while those from the summer rainfall area germinate best at 25°C. Micropropagation of four species (*W. gladioloides*, *W. laccata*, *W. lepida*, and *W. vanderspuyiae*) demonstrated that good multiplication rates could be achieved and that scaling up in liquid cultures by meristemoid induction offers the potential for rapid and mass production of propagules. *W. vanderspuyiae* was the only species in which corms could be induced to form. Plant height of tall species could be reduced by inhibiting leaf and inflorescence stem elongation using paclobutrazol as a post-emergent soil drench without compromising marketability. Combined, these findings could open the door for the successful

commercialisation of species and hybrids from this genus.

As a large and diverse tropical genus, *Begonia* has been highlighted as a research subject at the Royal Botanic Gardens Edinburgh and has been the subject of several projects over the past few years. We have been conducting some preliminary studies of evolutionary development in the genus and in this chapter discuss the potential of *Begonia* for research of this kind. Advances in methods for working with *Begonia* are introduced, including DNA and RNA extraction protocols. Preliminary results are described which further demonstrate the particular interest of the genus for studies at the level of gene, population and species.

Cut-flowers and cut-greens usually undergo stresses during the post harvest period, e. g. dehydration, grading, sorting, cooling, transportation, modified storage chamber-atmosphere, ethylene impact, micro-organisms, post-harvest pests and diseases. Final stress-effects occur during vase-life at the consumption stage. Application of vase-water-additives like commercial fresh-flower-refreshments (fresh flower food, floral preservatives) or non-commercial chemicals is possible throughout handling in the post-harvest-chain. Main targets of application are: control of microbial growth, support relationships (uptake, flow, transpiration), provide energy (e.g. by carbohydrates) and regulate senescence (carbohydrates, synthetic growth regulators, anti-ethylene-compounds). To study the effects and efficiency of post-harvest management strategies experiments were conducted with different cut-flower species and applied both additive groups: 1) commercial flower-refreshments; 2) several chemical compounds as post-harvest-effectors. For standardization we followed the suggestion of van Meeteren *et al*. (1999) and introduced his standard-vase-solution (SVS). Flowers were re-cut and prepared for treatments (stem end treatment of sunflowers, impact of commercial preservatives on vase life: e.g. *CHRYSAL clear*®, *FLORA 2000*, *BIOVIN*®, *OASIS*®, *FLOWER-FRESH* and effects of sucrose, ethanol and other chemicals were studied. Efficiency of treatments was evaluated by monitoring physiological parameters correlated with components of quality as the impact on vase life at ambient room conditions: Chlorophyll fluorescence parameters were measured at ambient conditions (F_0, F_v, F_m; Mini PAM, Walz, Germany), colour (CIE *L*a*b**; CR-200 Minolta, Germany), Brix-value of petal press-sap (digital Brix-meter, Atago, Japan), osmotic potential of petals (Wescor Osmometer, USA) development of individual fresh-weight and dry mass content at final stage of senescence (electronic balance, Sartorius, Germany).

Since the 16th century passionflowers are considered the most fascinating plants in the tropics. Their astonishingly beautiful flowers are reminiscent of exotic ambiances. The genus *Passiflor*a, which includes about 500 species, is mostly distributed throughout the warm temperate and tropical regions of the New World. Several species are grown in the tropics for their edible fruits, the most widely grown being *Passiflora edulis* (the yellow or purple passion fruit, also named granadilla). Nevertheless many other passionflower species are grown outdoors in the warmer parts of the world or in the glasshouses for their exotic flowers. In this chapter we mostly focus on Brazilian species of passionflowers and we attempt to address the potential ornamental use or their leaves, flowers and fruits. We also point out aspects of their vegetative propagation, including *in vitro* techniques.

Protea cynaroides, with its attractive blooms, is a well known cut flower in many parts of the world. Its commercial production has been relatively limited due to difficulties encountered during propagation and cultivation. Its lengthy growth period from plant establishment to production of commercially acceptable flowers takes years, which makes it an expensive flower to produce. Blanching of cuttings has improved both the rooting rate and rooting percentage of the cuttings. *In vitro* propagation is widely used for rapid mass propagation of disease-free plants. *In vitro* embryo culture, micro-grafting and somatic embryogenesis of *P. cynaroides* reported in this chapter, is showing great potential for future commercial mass propagation.

Bromeliaceae comprise three sub-families of exotic plants of neotropical origin with ecological, agronomical, ornamental and pharmacological importance. Their form and ability to capture water and nutrients from rainfall and other environmental fluids and debris, the microclimate created by the rosettes in many species and mainly the stunning beauty of their inflorescences has called the attention of researchers, environmentalists and ornamental plant producers. Of agronomical importance, the pineapple is the most important example in the Bromeliaceae family. In this chapter we mostly focus in biotechnological aspects of bromeliad propagation, from *in vitro* germination and

vegetative *in vitro* propagation through general aspects of greenhouse cultivation. The chapter is illustrated with selected ornamental species from a few genera and aspects of the micropropagation process.

Trees contribute significantly to the livelihood of Humans in many ways: sources of food, ornamentals, timber, poles and medicines. In Southern Africa, Jacket plum (*Pappea capensis*), Mobola plum (P*arinari curatellifolia*) and large sour plum (*Ximenia caffra*) are important wild tree species indigenous to Africa. They have multiple values and hence have been identified as future tree crops with commercial value. They are utilized mainly as a source of delicious fruit, edible oil, as ornamentals, or medicine and their woody stems are used in making handles for various tools from which many rural communities in Southern Africa generate a substantial income and hence these tree species have an economic value. Their existence in the wild is threatened by a high rate of deforestation. It is conceivable that integrating these tropical tree species into landscape or agriculture will benefit many communities through income generation, improved nutrition and health. They also add beauty to the landscape. However, incorporation of these future tree crop species onto farmland is hampered by the lack of sound planting materials, inadequate information on propagation methods and farmland management. Moreover they are recalcitrant to conventional propagation methods. Currently, there is no research dedicated to overcome propagation bottlenecks. Polyphenolic compound production and poor rooting of cuttings and seed dormancy are some of the major problems encountered in the propagation of these three wild tree crop species.

Ebenus cretica is a native herbaceous perennial evergreen subshrub growing on the island of Crete, Greece. The aerial plant parts are densely covered with whitish non-glandular hairs. The leaves are compound and amphistomatic. The flowers are zygomorphic with a pink coloration and organized in racemes, 5-15 cm long. Flowering begins in early May and lasts about 6 weeks. A great variability exists in the major morphological characteristics of the plants; however, color variants are very rare. In the field, flowers become pollinated by insects, while self-incompatibility is not observed. Glutelins constitute the major fraction of the totally extracted seed proteins. Four ecotypes of *E. cretica* were distinguished by SDS-PAGE on the basis of the totally extracted seed proteins. Morphological and anatomical features of *E. cretica* revealed the xeromorphic character of the plant. Plant regeneration is feasible by seeds. Rooting of shoot cuttings is possible by using growth regulators, though the various genotypes show different rooting ability. Peroxidases can be used as biochemical markers for both germination and rooting processes. Regeneration *in vitro* can be achieved by culturing shoot-tip explants from young seedlings on MS medium. Rooting occurs on an auxin-free MS medium. Lethal temperatures are below -5°C. Plants of *E. cretica* have low temperature requirements for flower bud initiation. Thus, plants which are maintained outdoors during the winter and then are moved to the greenhouse, produce new growth and abundant flowers. High light irradiance also promotes flowering. Paclobutrazol, GA_3 and daminozide applied to outdoor-grown plants, promote plant shape and flowering. Selection and breeding are however, necessary prerequisites before the plant can be marketed.

The taxonomical description of native species is of utmost importance in terms of biodiversity survey and for checking the maintenance conditions of ecosystems. The fact that many species have ornamental features add value to their study because they will consist of important materials for production and trade, helping to reduce the predatory collection and to foster sustainable development. On the other hand, studies on mitotic and meiotic chromosomes – be they through conventional techniques, banding techniques or through techniques of molecular cytogenetics – and also studies on the pollinic morphology through optic or electron microscopy will be of great help for the taxonomical delimitation of genera which, very often, are poorly defined and comprehended.

Aster is interesting because it can be easily cultivated in the Mediterranean region due to its adaptability to a wide range of habitats. The genus *Aster* includes about 500 species that differ greatly in plant and inflorescence morphology, and day-length response. A number of species and interspecific hybrids are grown as cut-flowers or pot plants. This chapter shows the techniques needed to control flowering; uniform flowering in cultivars with different growth rhythms by a photoperiod treatment, flowering at periods which are commercially significant, and accelerating the flowering cycle. The effect of photoperiod on vegetative development and floral induction of *Aster* is studied, short day conditions are important for the production of basal buds, whereas long day conditions are needed in order to prevent their elongation until new rosette shoots are required. *In vitro* culture is used in *Aster* to micropropagate the most interesting species for

commercial use.

Light is probably the most complex and variable ambient factor that acts on plants. It has a very important role to give energy for photosynthesis and to act as a stimulus for growth and development. A good lighting system will place the proper quantity and quality of light where it is needed in a cost-effective way. The amount of light needed is highly dependent on the task(s) that need to be performed. Depending on the culture, the climate and other factors, artificial light or alternatively, shading materials, are needed to reach the optimal light level. Artificial light can be applied in horticulture for two main objectives: to increase the irradiance level for photosynthesis and to increase the effective daylength (photoperiodism). It may also serve for two other purposes: as a substitute for daylight in growing rooms where plants are grown for commercial purposes under tightly controlled environmental conditions and as a substitute for daylight in laboratories (phytotrons) where plants are grown for research purposes. By using supplementary light, growers' aims are to: increase production, improve product quality, and create possibilities for earlier or year-round production and a more regular labour requirement, these being especially important in floriculture production. On the other hand, in Mediterranean areas, the intensity of light during the summer period is very high and shade cloths must be used. As a result, there are some studies about the use of coloured shade cloth to control some light responses. Light sources available for agriculture lighting applications include incandescent, fluorescent, low pressure sodium and high intensity discharge (HID). HID sources include mercury, metal halide and high-pressure sodium lamps. There are some aspects to consider about lighting: the cost of the equipment, the energy efficiency, the ease of management and the light quality. The initial quality of light in a work area must be considered in addition to the quantity. An installation's light quality is influenced by the spectrum of the light source, light uniformity, horizontal and vertical illuminance. Conventional artificial lighting, where the lamps are mounted above the canopy, provides unequal irradiation distribution. Mobile assimilation light has often been claimed to be more effective in terms of plant growth than static illumination. Compared with those traditional lamps, the improved features of the light-emitting diode (LED) include smaller mass and volume, a longer life, and a single wavelength. In recent years LED's have become more powerful and more multicoloured. Although present LED-based illumination is cost-efficient only for niche applications, a constant decrease in price of LEDs and an increase in power per chip within the last decade harbinger an optimistic perspective for wider applications, such as greenhouse lighting. For the commercial grower, the decision whether or not to install a plant irradiation system will largely depend on the outcome of a profit *versus* cost analysis.