Floriculture, Ornamental and Plant Biotechnology

Advances and Topical Issues

Volume III

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FOREWORDS TO VOLUME III

Frank G. Dennis, Jr., USA

This volume contains two sections, the first dealing with plant responses to the environment and the second with plant-organism interactions with regard to diseases and their control.

Section 1 The *Ex Vitro* Environment, Minerals, Water, Stress, Remediation

Part 1 in Section 1 covers effects of minerals on carbon and nitrogen metabolism in tulip bulbs, the effects of calcium ions in controlling responses to environmental conditions, the potential for seed treatment with impulse pressure to increase productivity by stimulating flowering and seed formation, and the effects of pH on nutrient balance, ionization of endogenous indoleacetic acid, and subsequent adventitious root formation.

Part 2 includes the effects of and mechanisms of resistance to various stresses, including drought, low temperature, mechanical stimulation, and lack of nutrients. Compounds that may be involved in affecting resistance to stress include reactive oxygen species, superoxide dismutase, enzymatic and non-enzymatic antioxidants, sugars and oligosaccharides, metalothioneins, salicylic acid and endogenous hormones.

Part 3 contains only three papers, all dealing with water management and/or characteristics of media. Emphasis is on the effects of medium density and fertilization on growth and flowering.

In Part 4 responses to both too little and too much water are discussed. Included are both genetic and physiological approaches to increasing tolerance, as well as reviews of the mechanisms by which flooding and anoxia induce stress.

Topics covered in **Part 5** are selection of species to be used for extracting heavy metals, such as cadmium, from soils, and the beneficial effects of compost in production of ornamentals.

Section 2 Plant-Organism Interactions, Disease and Control

Part 1 deals with molecular mechanisms, such as recognition of pathogens. Subjects include the roles of signal molecules, such as salicylic acid, jasmonic acid, and ethylene, and the possibility of isolating the genes involved in resistance and using them to induce resistance in susceptible genotypes. In addition, differences in susceptibility are related to both (a) genes that code for proteins that inhibit the action of polygalacturonases produced by the pathogen, and (b) secondary metabolites that inhibit growth of the pathogen. The mechanisms involved in the double immune response are also explained.

Part 2 The invasive characteristics of several rhododendron species, and use of surfactants with herbicides for control of weeds are covered in this brief section.

Subjects in **Part 3** include the development of fungal diseases of ornamentals as related to the importation of new species, the prospects for biotechnological methods of controlling fungal diseases in ornamentals, a review of the potential for inducing *Rhizobium*-induced nodulation in ornamentals, the role of phenolics and reactive oxygen species in defending against anthracnose, and the potential use of *Tricoderma* species for control of *Armillaria* root rot.

Part 4 focuses on plant-virus interactions. A method is described for large-scale systematic testing for viroids in ornamentals. The silencing of gene expression appears to play a role in viral pathogenicity. Recent research has led to several potential methods for controlling viruses, including the use of resistance genes and proteins with antiviral activity. The sequence of events during infection by tomato ringspot virus is described.

Part 5 covers plant-insect interactions. Molecular biology can play a role in protecting plants from insects, when used with standard methods of integrated pest management. For example, *Agrobacterium*-mediated transformation of bermudagrass has provided resistance to cutworm. The possibility of using botanical insecticides in place of synthetic ones is illustrated by successful use of azadirachtin A applied to tomato plants through the roots in hydroponic culture to reduce the population of whitefly.

Frank G. Dennis, Jr. was awarded a Ph.D. in Pomology by Cornell University in 1961, then spent a year in France on an NSF Post-Doctoral Fellowship. From 1962 to 1968 he conducted research on plant propagation and the effects of growth regulators on flowering and fruiting of tree fruits at the New York State Agricultural Experiment Station in Geneva, N.Y. In 1968 he moved to the Horticulture Department at Michigan State University to teach undergraduate and graduate courses and to continue research on both applied and endogenous hormones in relation to fruit development and dormancy of apple and cherry. He retired in 1996.

Anna Jäger, The Danish University of Pharmaceutical Sciences, Denmark

As is well-known, and can be seen from the many papers in this section dealing with various aspects of pharmacological activities of plants, medicinal and aromatic plants still play an important role in modern society. This chapter contains review papers on plants with antioxidant, antimicrobial, anti-inflammatory and antitussive activity. The paper on the potential use of *Salvia militiorrhiza* in the treatment of alcoholism deals with an effect which could be of tremendous importance to society, considering the grave problem of alcoholisms in most of the world today.

Back in the beginning of the 1980's, 'plant cell culture' was viewed with a lot of promise. It was thought that *in vitro* cultures of plant cells could be manipulated to produce interesting and valuable secondary metabolites in the same way bacterial and fungal cultures could. Towards the end of the decade, reality eventually sank in and it became clear that it wasn't going to be so easy. Many plant biotechnology research laboratories closed down. It is good to see that those who stayed in the field and made use of molecular biological methods today are able to do many of the things once hoped for. In this chapter we find interesting papers on a number of highly important medicinal plant species such as *Taxus, Catharanthus,* *Artemisia annua, Salvia, Curcuma* and food plants as tea and *Amaranthus* (red spinach). The authors have used ‘classical’ biotechnological methods or have used genetically transformed cultures to produce useful compounds. The paper on the regulation of the alkaloid biosynthesis in *Catharanthus roseus* and application of metabolic engineering deserves special mentioning as an example of how the latest molecular techniques can be applied to unravel the processes that take place inside plant cells and how we can manipulate them to our benefit.

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Phytoremediation has been defined as the use of green plants and their associated micro-organisms, soil amendments and agronomic techniques to remove, contain or render harmless environmental contaminants (Chappell 1997). Phytoremediation represents a typical *in situ* biological treatment, whose main advantage is that it allows the soil to be treated without being excavated and transported, resulting in potentially significant cost savings. However, *in situ* treatment generally requires longer time periods, and there is less certainty about the uniformity of treatment because of the variability in the soil and aquifer characteristics and because the efficacy of the process is more difficult to verify.

Phytoremediation is complementary to classical bioremediation techniques, which are based on the use of micro-organisms. It should be particularly useful for the extraction of toxic metals from contaminated sites and for the treatment of recalcitrant organic pollutants, like trinitrotoluene and nitroglycerin. Plant biomass could also be used efficiently for the removal of volatile organic pollutants or different priority pollutants, like pentachlorophenol, other polychlorophenols and anilines.

At present, phytoremediation is still a nascent technology that seeks to exploit the metabolic capabilities and growth habits of higher plants: delivering a cheap, soft and safe biological treatment that is applicable to specific contaminated sites and wastewaters is a relatively recent focus. In such a context, there is still a significant need to pursue both fundamental and applied research to provide low-cost, low-impact, visually benign and environmentally sound depollution strategies (Schwitzguebel and Vanek 2003).

One of the greatest forces driving increased emphasis on research in this area is the potential economic benefit of an agronomy-based technology. Growing a crop can be accomplished at a cost ranging from 2 to 4 orders of magnitude less than the current engineering cost of excavation and reburial. Expected applications will be in the decontamination of polluted soils and ground-water (phytoremediation) or in the clean-up of industrial effluents (plant cells, tissues or biomass immobilised in appropriate containers; whole plants cultivated in constructed wetlands or under hydroponic conditions).

Phytoremediation belongs to the fastest growing areas of research and application. From a literature survey from the Web of Science based on 1,500 research papers using the keyword “phytoremediation” this development is clearly visible - from 43 papers (2.8%) in 1996, the publication list grew to 151 (10.0%) in 2001 and doubled in 2004 to 291 (19.2%). Results from September 2005, 266 papers in total (17.6%) confirm this trend.

Concerning countries of origin, among the 1,500 papers published since 1990, 43.6% were published in the USA, 8.8% in China, 6.5% in the UK and Germany, followed by Spain (4.7%), Canada (4.3%), France (4.2%), India (4%) and Japan (3.3%). From a statistical point of view, the participation of Asian countries (mainly China) is growing very fast and reflects growing concern about environmental problems in these countries. Surprisingly, this research area is still not too popular in new EU member countries which have a seriously polluted environment – in the first position is the Czech Republic at 2.3% (14^{th} position among all countries) while others are below 1%.

Considering the above mentioned facts and global trends, the chapters in the **Phytoremediation and revegetation: techniques and applications** section of the book represent good and comprehensive examples of phytoremediation approach for solving environmental problems. Novel to the field of phytoremediation is a specific look at how Ornamentals can serve a dual purpose in Society: Decoration for Human satisfaction and well-being, and phytoremediation and revegetation for Environmental recovery and amelioration.

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CONTENTS: VOLUME III

The *Ex Vitro* Environment, Minerals, Water, Stress, Remediation

Part 1 Minerals

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Adventitious root formation is the primary regeneration process required for the successful propagation of cuttings. Among many physiological and cultural factors affecting rooting of cuttings, the role of pH in the medium is one of the least understood of variables. pH has a strong influence upon the availability of mineral nutrients needed for plant growth and developmental processes. Medium pH ranging between 5.5 and 6.5 is ideal for rooting of most horticultural crops, although cuttings of some species prefer lower or higher pH. The lowest recorded pH at which rooting occurred was 3.0 and the highest was 10.0. Typically, adventitious rooting is preceded by an initial wound-healing response in cells at the base of stem cuttings. This step is followed by various other morphological events: root primordia initiation (Stage IA) and root primordia development (Stage IB); and root emergence (Stage II). Each event is controlled by a complex balance of growth regulators, both promoter and inhibitor types, rather than by a single one. The presence of endogenously- and/or exogenously-applied auxin plays a pivotal role in triggering Stage IA, which is both auxin- and pH-dependent. At low pH (4.0-5.5) versus higher (6.5-7.0), auxin exists predominantly in the undissociated form. This form is more soluble in lipid environments, and passes more readily across the plasma membrane. Thus, a low pH medium allows greater accumulation and metabolism of auxin into plant cells in the base of cuttings, thereby increasing rooting performance. Stages IB and II are auxin-independent, and continuous supplies of auxin to cuttings in these stages are inhibitory to adventitious rooting. While it is clear that nutrients in the media are essential for root growth and development on or after root emergence, current research advances indicate that low pH-related disturbance or imbalances of nutrients such as P, Ca, Fe, Mn, Z, B, and Mo, singly or interactively, influence the development of roots, especially root hairs. Growth regulators are also involved, although the mechanisms of these complex and interactive processes require elucidation.

Tulip bulb scales contain a large amount of reserve carbohydrates, mainly starch and soluble sugars (sucrose and (2-1)-β-linked oligofructans). Starch accounts for about 60% of dry matter in scales just before planting, and over 60% of the starch decreases during the first three months after planting, coupled with a high accumulation of sucrose and oligofructans. The accumulation of sucrose and the related oligofructans may act as the temporary storage pool until they are converted to starch. Amylase in tulip bulb scales is an α-type. The optimum pH for α-amylase is about 5.6, and requires Ca^{2+} for its activity and stability. A cDNA of α-amylase was obtained by RT-PCR, and the deduced amino acid sequence has a high similarity (60% identity) to those of rice and maize. A major portion of nitrogen (N) in bulb scales at harvest is storage protein. Storage protein degradation is induced by cold temperature, and the accumulation of a soluble N fraction, especially glutamine, occurs. During the cold winter season, tulip roots absorb nitrate and ammonium and a high accumulation of N (glutamine) occurs in part of the root in direct contact with N. 4-methyleneglutamine may be a temporary N storage compound in leaves, stems and roots after sprouting. A large quantity of 2-oxo-4-methyl-3-pentene-1,5-dioic acid (OMPD) accumulation was discovered in leaves and stems of tulip, and OMPD may be a waste product by deamidation and deamination of 4-methyleneglutamine.

In the plants being exposed to environmental stresses, ion channels are likely activated to convert these external stimuli into intracellular signals. Among the ions taken up by the plant cells, Ca^{2+} plays an essential role as an intracellular secondary messenger in plants and thus the cytoplasmic free Ca^{2+} concentration ($[Ca^{2+}]_c$) is strictly regulated. Signal transduction pathways mediated by the changes in the $[Ca^{2+}]_c$ is termed Ca^{2+} signaling, and are mainly initiated by the activation of Ca^{2+}-permeable channels. When Ca^{2+} channels are activated in response to a variety stimuli, a drastic increase in the $[Ca^{2+}]_c$ is induced and the entered free Ca^{2+} binds to the sets of Ca^{2+}-regulated proteins such as calmodulin and calcium dependent protein kinases to modify the activities or affinities of these proteins in binding to specific targets. To date, a large body of electro-physiological and molecular biological studies has revealed that plants possess several Ca^{2+} channels belonging to distinct types with different gating mechanisms, and a variety of genes for Ca^{2+}-permeable channels have been isolated and functionally characterized. In this chapter, the topics covered include (1) the characteristics of molecularly cloned Ca^{2+}-permeable channels including voltage-dependent Ca^{2+}-permeable channels, cyclic nucleotide-gated cation channel, and ionotropic glutamate receptor; (2) the roles of Ca^{2+} at the key steps during environmental responses and regulations of growth and developments (eg. stomatal movements, tropisms, nutrient allocations, flowering, pollination, fertilization, and etc.); (3) the Ca^{2+}-dependent metabolic regulation of reactive oxygen species levels; and (4) the Ca^{2+}-mediated response during plant defense mechanism against pathogenic microorganisms.

Nitric oxide (NO) is a free radical gas formed endogenously in several biological systems, including plants, in which it performs a wide range of functions. Although many aspects of the physiological role of NO in plants remain to be elucidated, evidence is emerging that NO plays an important regulatory role in numerous processes, including stomatal closure, root development, stem elongation, seed germination, the host responses to infection, programmed cell death and senescence. In addition, it is now widely accepted that NO functions as a signal for hormonal responses in plants. Nonetheless, the mechanisms by which NO interacts with growth regulators is still far from clear, although considerable progress has been made during the last decade in our understanding of NO within the entire plant and in plant organelles. Herein, we specifically explore the role of NO and its cross talk with growth regulators in plants.

Impulse pressure – caused by explosion energy – was used for the treatment of seeds. It influenced the development of phloem and xylem in plants at different stages of ontogenesis and resulted in accelerated efflux of water to the leaves, and an increase in the transport of nutrients from leaves to flowers, fruits and roots. Seed treatment by impulse pressure of different strengths induces an increase in plant productivity by 25-70%. Even though this system was designed for and tested in buckwheat plants, the possibilities of its application to ornamentals, such as increased seed production or flowering, are tremendous.

Part 2 Stress in plant growth and development: mechanisms and pathways

Plants are exposed to a range of environmental stresses and have to adapt physiologically to these as the local environment changes. The recognition and signalling pathways regulating the responses to abiotic stresses (*e.g.*, drought, salinity, cold and heat) are similar to those used for responding to biotic stresses. The adaptation to one stress condition can therefore affect tolerance to other non-related stresses, a phenomenon referred to as cross-tolerance. The redundancy of some of the major signalling compounds, for example, salicylic acid, calcium, reactive oxygen might form the regulatory basis for developing such multiple tolerance mechanisms. Recent findings reveal a role of abscisic acid in biological defence and involvement of salicylic acid in abiotic stress, thereby indicating that these compounds have a broader importance than previously anticipated. Furthermore, cellular responses often depend on the intracellular concentrations and fluxes of some of these signalling molecules can constitute a secondary stress themselves. The ambiguous role of different stress messengers in biotic and abiotic stresses as signals and stress components is discussed.

Plants are exquisitely sensitive to the physical factors in nature, where exposure to precipitation, wind or touch results in shortened plants which are able to better tolerate stress conditions, and are capable of responding in terms of thigmomorphogenesis, alterations in the growth of plants to cope and compensate for the mechanical variables. An understanding of stress physiology in plant growth is important to many plant scientists, since physical stress has a major impact on biological diversity and agricultural productivity, as well as other environmental and ecological problems. In this chapter, a restricted definition of physical stress is presented as a complicated stress factor whose properties can be separated into several physical aspects, which would be induced by the exterior or internal physical environment and eliciting certain specific or non-specific responses in plant growth. Four interrelated phases that could qualify as primary cues for the differentiated courses induced by stress are restated here, and we are confirmed that there exists undoubtedly a bidirectional function of physical stress. In particular, the elegant experimental methodology designed to investigate the relationship between artificially delivered force and the resultant morphological, physiological, and genetic changes is reviewed. The experimental findings reveal that application of mechanical forces can elicit the response of plant in various aspects: growth and development of plant organism, biophysical characteristics (thermodynamic properties), biochemical metabolism, energy metabolism, signal transduction, resistance formation, and some genetic processes.

Abiotic stress is a complex environmental constraint limiting plant production. Bioengineering of stress-signaling pathways can contribute to the development of stress tolerant crops. Osmotic adjustment has been shown to be an effective component of such manipulations and accumulation of osmoprotectants (compatible solutes) is a common response observed in plant systems. Osmoprotectants include betaines certain amino acids like proline and ectoine, polyols and nonreducing sugars such as trehalose. Stress response is multigenic, as a number of processes involved in the tolerance mechanism are involved.

Development of crop cultivars tolerant to abiotic stresses is an important goal of national and international institutions engaged in plant research. Both traditional plant breeding methods and transgenic technology are being employed to achieve the above objective. Since conventional breeding approaches were not found sufficient, scientists are now attempting to explore advantages of the genetic engineering to develop transgenic crop plants tolerant to abiotic stresses *viz.* drought, salinity, cold and high temperature, etc. Although numerous studies have demonstrated the feasibility of developing such transgenics in an array of crop species, substantial data, barring in few cases, are, however, lacking on the response of these transgenics subjected to field stress conditions. In this chapter, we present the current status and future prospects of genetic modification for enhancing tolerance to abiotic stresses, with emphasis on drought, salt and cold stresses in crop plants in general, and in floricultural crops in particular.

Cold adversely affects the growth and productivity of many plants, while many others have the ability to respond and adapt to low temperatures. In some of these species, cold is an important prerequisite for flowering. The mechanism of a plant's response to cold is complex and varies among plant species. In this chapter, we summarize the progress on cold regulation on plant's growth and development. Plants have an in-built ability to adapt to low temperatures through processes such as cold acclimation and vernalization. The mechanisms underlying these processes are discussed in this chapter.

As an unfortunate consequence of aerobic life, reactive oxygen species (ROS) are formed by partial reduction of molecular oxygen. The imposition of biotic and abiotic stress conditions can give rise to excess concentrations of ROS, resulting in oxidative damage at the cellular level. Plants possess a complex battery of enzymatic and non-enzymatic antioxidants that can protect cells from oxidative damage by scavenging ROS. Glutathione *S*-transferases (GSTs) are involved in many stress responses in plants as well as in animals, for example, participating in the detoxification of xenobiotics and limiting oxidative damage. Manipulation of the expression of the enzymes involved in the ROS-scavenging systems by gene-transfer technology has provided a powerful tool for increasing the present understanding of the potential of the defense network against oxidative damage caused by environmental stresses. Transgenic plants overexpressing GST genes showed increased tolerance to environmental stresses, indicating that ROS-scavenging enzymes can play important role(s) in oxidative stress management. Such an understanding is fundamental for the production of ornamentals applied to phytoremediation, or for the application of transgenics for increased cut-flower production and postharvest physiology.

Activity of superoxide dismutase (SOD, EC 1.15.1.1) in the extracts from different parts of the stamen and pistil from healthy petunia (*Petunia hybrid* cv. "Hurrah") flowers was determined. The stamen had a higher level of SOD activity than the pistil. In the stamen, the anther had three times more SOD activity than the filament. In the pistil, the ovary had the highest level of SOD activity compared to the stigma and style. The levels of SOD activity in the different parts of the pistil were found to increase during flower development from immature flower bud (stage 1) to fully open flowers following anther dehiscence (stage 5). In contrast, those in the stamen, particularly in the anther, increased at the early stages of flower development but exhibited a marked decline at the later stages. Three isozymes of SOD were detected following native gel electrophoresis and the slowest migrating one seems to be a MnSOD. Overall, the isozyme analysis supports the observed developmental changes of SOD activity in the reproductive tissues of petunia. Taken together, these results are consistent with the idea that SOD activity is not necessarily only associated with stress responses but could also be involved in normal developmental processes under unstressed conditions.

Stress tolerance mechanisms are complex and involve both physical and biochemical changes. Salinity, drought, chilling, and freezing tolerance mechanisms often overlap and include such processes as osmoregulation, osmoprotection, protein accumulation, antioxidant protection, and reactive oxygen species signaling. Recent developments in stress tolerance are CBF (C-repeat binding factor) transcriptional activators, antifreeze proteins, and energy homeostasis. The raffinose family oligosaccharides or RFO are upregulated in response to several stresses. In *Petunia*, increased accumulation of RFO leads to freezing tolerance and increased growth under drought conditions. Variation seen among transgenic lines with confirmed genetic modification indicates a need to carefully screen whole plant growth response to stress conditions.

Metallothioneins (MTs) are low molecular weight cystein-rich proteins with the ability to sequester heavy metals such as copper (Cu), zinc (Zn) and cadmium (Cd). They are widespread throughout the animal and plant kingdoms and are currently classified into 15 families. In mammals it has been shown that MTs have an important function in heavy metal metabolism and detoxification and in the management of various forms of cellular stress. This chapter reviews the current knowledge on the classification, regulation and induction of plant MTs, with a particular emphasis on the MT genes that are expressed in flowers. It also considers the possible biological role of this protein in relation to heavy metals and oxyradicals scavenging capacity.

Mechanically-stressed Chrysanthemum (*Dendranthema* X *grandiflora* (Ramat.) Kitam.), induced by brushing daily for 30 days after planting produced more ethylene on the third day with a continuous high production. In contrast, control plants produced low levels of ethylene throughout experiments. On the other hand, gibberellic acid (GA)-like substances in the control plants were separated into 4 bands on silica gel TLC plates, at the same Rf as authentic GA_1, GA_3, and GA_7. GA_1- and GA_3-like substance contents decreased markedly in brushed plants as compared to control plants, suggesting that the growth of chrysanthemum dwarfed by mechanical stress is controlled not only by GA activity but also by ethylene production. This method provides an ecologically-sound and -friendly alternative to present chemical growth retardants.

Electrical impedance spectroscopy (EIS) is described as a method to study the properties of organic and inorganic materials. It is a diagnostic method based on the study of the passive electrical properties (determined by the observation of the tissue electrical response to the injection of external electrical energy) of a material. The impedance and phase angles of materials can be measured by a multiple frequency impedance analyser (impedance meter) that is able to scan each sample at different frequencies. The data obtained from EIS are commonly analyzed by fitting them to an equivalent circuit model; that is, the sample analyzed is characterized as an electrical circuit composed by resistors and capacitors. EIS is a combination of automatic measurement of impedance spectra and computer-assisted data analysis. Since impedance measurements are quick, repeatable and non-destructive, they can provide valuable insights into the behaviour of a large variety of substances, components and systems. The applications of electrical impedance measurement in plant tissues are numerous. In fact, this technique can be used to determine physiological conditions of plant tissues, particularly in relation to cold acclimation, freezing injury, dormancy induction, nutritional deficiency and heat injury. The fundamental studies and some applications of electrical impedance spectroscopy for the field of plant science are described.

Part 3 Soilless culture and hydroponic systems

Water, together with CO_2, nitrogen and light, is quantitatively the main essential nutrient. Water presents a double function: inorganic constituent and carrier of other nutrients. The hydric deficiencies generated by high osmotic potential values are essentially linked to the irrigation water quality, to the lixiviation levels or to disorders in the fertilization applied. Water uptake, transport and functions are analyzed in this chapter. The uptake capacity is related to potential gradients in the root zone and in the plant (root, xylem and leaf) and to the water climate demand. The knowledge of the different plant responses to the water climate demand can help to establish a specific irrigation management. Hydric status, defined by turgor pressure, water potential and relative water content (RWC), and drought stress physiology are also considered, focusing on the study of the complex response action mechanisms. Fertigation is the combined application of water and nutrients. Considering this criterion, irrigation scheduling can be defined as the process of determining how much nutritive solution to apply (irrigation volume) and the timing (when to apply). The function of an irrigation system is to ensure that the plant has the optimal amount of water, nutrients and oxygen in the root-zone. Different irrigation systems are discussed. Irrigation volume depends on the root-zone volume and the easily available water for the substrate. Irrigation frequency should be based on the amount of water lost since the last irrigation. The losses of the system crop are: plant transpiration, substrate evaporation and drainage. The adequate evaluation of these parameters permits an efficient water management. Nowadays, the irrigation of container-grown commercial ornamental crops is often very inefficient, because large quantities of water are wasted. In container crops, water application needs to be designed in order to establish a specific irrigation management that can improve the irrigation application efficiency, being compatible with a good quality production and friendly with the environment.

Three ornamental species, gloxinia (*Sinningia speciosa* Lood. Hiern.), lisianthus (*Eustoma grandiflorum* Shinn) and African violet (*Saintpaulia ionantha* Wendl.), were studied aimed at identifying and attending to the specific physical characteristics of the substrates to suit the growth and development needs of each species. When different substrate densities were evaluated for each culture, it was noticed that no general conclusions could be drawn regarding the recommendations for physical characteristics of substrates to suit all plant species, as each species shows specific needs. The best density for gloxinia cultivation was 0.55 gcm^{-3}, for lisianthus 0.75 gcm^{-3} and 0.95 gcm^{-3}, and for African violet, 0.75 gcm^{-3}. Substrates suitable for gloxinia growth should present 77% porosity, 26% air-filled porosity, 18% available water and 16% easily available water. These values are 72-75%, 12-19%, 19-26% and 17-23%, respectively for lisianthus, and 75%, 19%, 19% and 17% for African violet, respectively. Different mixtures of substrate materials, which had physical characteristics suitable for each culture, were analyzed in a second experiment. Gloxinia grew best in vermiculite : earthworm humus : perlite, lisianthus in cultivated peat : vermiculite; eucalyptus bark : conventional peat : sand substrates and in controls, while African violet grew best in eucalyptus bark : conventional peat : sand, earthworm humus : vermiculite : sand, pine bark : earthworm humus, cultivated peat : vermiculite, and the commercial substrates Eucatex® and Vida Verde®.

Anthurium is the main cultivated tropical cut flower all over the world. The yield elaboration in tropical environment and soilless cultivation is poorly known. The use of empirical cultivation systems can restrict production optimization and increase inputs costs and environmental risks. The plant morphogenesis and mineral and light requirements are essential elements of yield elaboration in sheltered cultivation under tropical conditions. Growth and development of tissue cultured plants have been followed with different nitrogen and calcium fertilization levels or under different shade levels. Plants, solutions and substrate mineral analyses, and photosynthesis measurements in our studies allow us to assess plant growth potential. Finally, recycling of the drainage solution was tested in order to seriously reduce pollution. Anthurium plantlets first have a monopodial growth without flower production; fertilization affects only this phase duration. Thereafter growth is sympodial. The plant produces successive articles, each including one leaf and one inflorescence. The leaves and flower size, the biomass, and plant composition depend on fertilization. The best results regarding flowers number and size were obtained for higher ammonium, total nitrogen and potassium concentrations, but not for high levels of calcium. Anthurium is a shade plant and its photosynthetic activity is enhanced by a lowered PAR level (3.6 mol photons $\times m^{-2} \times day^{-1}$). At 10.5 mol photons $\times m^{-2} \times day^{-1}$ PAR level, flower production increases but there is an obvious decrease in their size. In our tropical conditions, growing management requires a high shade level (85%), with the fertilization balance: 1-0.1-1.4-0.9-0.3 (N-P-K-Ca-Mg) during flower production.

Part 4 Water, hydric stress and salinity: control and manipulation

Under both natural and agricultural culture conditions, plants are frequently exposed to transient or permanent low O_2 levels in the soil atmosphere or soil solution as a consequence of flooding. Thus hypoxia and/or anoxia are environmental stresses commonly encountered by plant root systems. These O_2 restriction conditions will have drastic effects on plant growth, development and survival. Over the last decade, the introduction of large scale genomic and proteomic approaches coupled to physiological and morphological studies have allowed a great leap forward in our understanding of plant responses to flooding. As a result, the response and adaptation mechanisms involved in flooding tolerance are sufficiently understood allowing the selecting and breeding of stress tolerant plant varieties. However, it is crucial to understand the basic adaptive mechanisms that allow for the efficient selection of candidate species. This chapter reviews the metabolic, physiological and morphological responses and adaptation strategies of plants to flooding and assesses the prospect of improving plant tolerance to flooding through breeding, biotransformation and marker-aided selection.

Drought, salinity and freeze induced dehydration constitute direct osmotic stresses; chilling and hypoxia can indirectly cause osmotic stress via effects on water uptake and loss. Thus, improving crop resistance to osmotic stresses is a long standing goal of agricultural biotechnology. Upon exposure to osmotic stress, plants exhibit a wide range of responses at the whole plant, cellular and molecular levels. Morphological and developmental changes in life cycle, inhibition of shoot growth and enhancement of root growth constitute whole plant level responses. Molecular and cellular level responses include adjustment in ion transport (such as uptake, extrusion and sequestration of ions) and metabolic changes (e.g. carbon metabolism, the synthesis of compatible solutes) which are induced upon regulation of gene expression. The products of these stress-inducible genes have been classified in two groups: **1. Functional genes and products:** The first group includes proteins that directly protect against environmental stresses. They probably function by protecting cells from dehydration, such as the enzymes required for the biosynthesis of various osmoprotectants, late embryogenesis abundant proteins, antifreeze proteins, chaperones, and detoxification enzymes. **2. Regulatory genes and products:** The second group of gene products regulates gene expression and signal transduction in the stress response. They include transcription factors, protein kinases, and enzymes involved in phosphoinositide metabolism. Stress-inducible genes have been used to improve the stress tolerance of plants by gene transfer. It is important to analyze the functions of stress-inducible genes not only to understand the molecular mechanisms of stress tolerance and the responses of higher plants, but also to improve the stress tolerance of crops by genetic manipulation. In this chapter, we highlight recent studies on transgenic plant technology for improving environmental stress tolerance by metabolic engineering and manipulation of signalling pathways.

Over the last few years there have been major advances in applying the use of plant tissue culture to the study of poikilohydric or resurrection plants. The three main species (*Craterostigma plantagineum*, *Haberlea rhodopensis* and *Ramonda myconi*) which have been studied as model resurrection plants have now been introduced into tissue culture and two of them (*C. plantagineum* and *R. myconi*) were successfully transformed with marker genes (Toldi *et al*. 2002, Tóth *et al*. 2005). A large number of genes have been identified which may play a role in underpinning the ability of the plant tissues to survive severe tissue water loss. There have been a number of elegant experiments which have used tissue culture to study metabolism in resurrection plants. The time is now ripe for the large scale molecular analysis of the poikilohydric ability of these plants using tissue culture as a major tool to drive research forward. Large-scale isolation of drought stress associated genes with unknown biological roles requires functional analysis. Here we show that conventional genetic transformation techniques, via *in vitro* plant regeneration systems, still represent an unavoidable part of the high-throughput functional genetics analyses. Similarities and differences in tissue culturing and genetic transformation of these resurrection plants, as a consequence of their common physiological specialisation, are summarized, and future prospects of advances in this area of research are discussed.

The nutritional status of a plant is disrupted by environmental extremes and stresses which results in a marked reduction of plant performance. The traditional method of whole-tissue analysis of plant material hides a multitude of heterogeneity of physiological responses within the plant, thus severely restricting our understanding of the responses of plants to environmental stresses. The development and use of techniques to investigate plant processes and responses to stress events at the level of a single plant cell is enabling us to investigate and understand plant responses to stress at a much higher resolution. This review covers a number of techniques which can be used to describe and investigate the effects of various environmental stresses on the cellular composition, transport of ions and metabolites into and out of cells, intracellular metabolic processes and gene expression at the resolution of the individual plant cell. The applications of such techniques are described, as are their potential pitfalls and drawbacks. Knowledge gained about metabolic and nutritional processes from single-cell techniques and how these processes are perturbed by stress events will enable substantial progress in the understanding of physiological responses to stress in plants, leading to the development of plant species with greater stress tolerance.

Changes in oxygen concentration are a frequent phenomenon when plants are subjected to flooding. During flooding, when roots are submerged in water, low oxygen concentration limits respiratory activity and induces fermentation processes. When water levels recede, increased oxygen concentration results in high respiratory activity and oxidative stress symptoms. Both anaerobiosis and post-anaerobiosis can severely damage plant tissues due to structural and activity changes in mitochondria. Inhibition of respiration during anaerobiosis increases reduction of respiratory chain components leading to increased reactive oxygen species (ROS) production during post-anaerobic period. We discuss in this chapter the mitochondrial respiratory chain as the main source of ROS during post-anaerobiosis and induction of antioxidative plant defense systems in root tissue.

This study examines the water stress response of *Impatiens wallerana*, a highly popular flowering ornamental plant which is very susceptible to wilting. No intercultivar differences in transpirational water loss rates were observed. In accord with this, the abaxial stomatal index for leaves from each cultivar was virtually identical. Under drought stress, the water content of roots, stems, and buds remained high (88-92%) even when leaves were wilted and had lost 26% of their water. Analysis of the "succulent" stem anatomy revealed prolific cortical and pith tissue parenchyma cells, facilitating water retention. Leaf photosynthetic rates were severely decreased during water stress, and at wilting point, O_2 evolution was only 15% of initial rates in watered plants. Re-watering did not fully reverse the loss of photosynthetic capacity. The decline in photosynthesis was not explained by a decrease in extractable activity of the rate-limiting enzyme, Rubisco, which was not appreciably affected by stress conditions. It is possible that inward CO_2 diffusion was impeded in wilted leaves. In addition, it is likely that other Calvin cycle enzymes and photosynthetic electron transport reactions are decreased under water deprivation. The physiological response of *Impatiens wallerana* to water stress is of particular interest because of its unusually high sensitivity to drought, and its early lethal wilting point. In addition, its significant commercial value warrants a solution to this problem either using cultural techniques, or via breeding programs identifying sources of drought resistance in existing germplasm collections.

Part 5 Phytoremediation and revegetation: techniques and applications

More and more attention has been paid to phytoremediation of contaminated soils by heavy metals with the continuous increase in soil contamination by heavy metals. This chapter begins from emphasizing the importance of phytoremediation and the significance of selecting plants with the hyperaccumulative ability, and generalizes the basic characteristics of hyperaccumulators and their evaluating standards including critical content, accumulative coefficient, transfer, endurance, and basic accumulative ratio. In view of the fact that more and more ornamental plants have been applied to our daily life and environmental protection with the improvement of people's living levels, the potential of ornamental plants applied to remediation of contaminated soils by heavy metals was systematically analyzed by describing ornamental plant resources and their application to remediation of contaminated soils by heavy metals, particularizing the identification of ornamental hyperaccumulators and their advantages compared with other hyperaccumulators which were documented, discussing not only their endurance and accumulating traits to heavy metals but also types of contaminated soil remediation using ornamental hyperaccumulators. It can be thus anticipated that the identification of hyperaccumulators from ornamental plants and their application to remediation of contaminated soils by heavy metals will provide an entirely new research area in environmental sciences and engineering.

The use of green plants to contain, inactivate or degrade harmful environmental contaminants is an emerging technology. In this chapter an overview is given of existing information concerning the use of revegetation for the remediation of metal and organic contaminated soils. Revegetation techniques in use at both laboratory and field scale for mine, metallurgical and organic polluted soil are described. In addition to the plant itself, the use of soil amendments for mobilization and immobilization is discussed. Also the metabolic pathways and translocation and tolerance mechanisms occurring during revegetation are treated. Also the revegetation strategies adopted in semiarid zones and for salt affected landscapes are illustrated. More fundamental research is needed for the selection of appropriate tolerant plants, cropping schemes, and soil amendments and to better exploit the routes to increase the levels of metallothioneins (MT) or phytochelatins (PC) concentrations in plant cells with the hope to improve resistance. Further, demonstration experiment are needed to exploit the advantage of engineered transgenic plants which contain large amounts of recombinant proteins with possible roles in chelation, assimilation, or membrane transport of trace elements.

This chapter provides a broad overview of the variety of potential mechanisms present in higher plants that may be involved in the detoxification and tolerance to metals at the cellular level for their potential application in remediation technology of metal toxicity. The naturally tolerant plants which hyperaccumulate metals form the basis for investigations on the improvement of metal tolerance. Tolerance to metal stress relies on plant's capacity to detoxify metals having entered cells. The major mechanisms for metal tolerance discussed are metal ion efflux, organic ligand exudation, compartmentalization in certain cell compartments, metal sequestration by organic compounds, and induction of antioxidative enzymes. Application of molecular-genetic technologies led to the better understanding of mechanisms of metal tolerance in plants and subsequent development of transgenic plants with increased metal uptake and tolerance.

Organic wastes make up a large part – up to 40% – of the municipal waste stream. Therefore, organic wastes should be recycled from an ecological as well as from an economical point of view. Compost utilization in agriculture and horticulture yields many beneficial effects, mostly associated with organic matter. The benefits of compost use, such as improved soil structure, porosity and water holding capacity, the fertilizer value of compost concerning nitrogen, phosphorus, potassium and lime, the increased microbial biomass and activity and the suppression of plant diseases are discussed as well as potential risks of compost use, like the accumulation of heavy metals in soils and crop plants and the leaching of nitrogen to the ground water. Numerous examples of compost use in different fields of agriculture, in vegetable production, in field production of ornamental shrubs, and in container media show the feasibility and the positive effects of compost utilization.

This article links two terms wastelands and fly ash, which sound distinct but have several common implications. In their present forms both are waste and a rather problematic one that could spread further if poorly managed. Their management itself is difficult and economically unfavorable. At the same time both could have great potential if utilized prudently. The present article emanates from the need to realize the veiled potential and to find out how fly ash and wasteland can complement each other for the benefit of Humanity and our environment. Consequently this article introduces and deals individually with wastelands and fly ash in the first two sections followed by a synergy (concept of joint or symbiotic management of wastelands and fly ash) in the subsequent section. Lab/field scale applications of fly ash in wasteland reclamation are summarized with special emphasis on the benefits of organic/ microbial culture-amended fly ash. Prospects of floriculture on abandoned fly ash dumps are also discussed.

Water hyacinth (*Eichhornia crassipes*) is a troublesome weed with an inherent ability to survive in polluted environments and wide ranging temperature, pH and saline conditions despite its ornamentally attractive value. These attributes equip water hyacinth with exceptional ability and versatility to treat different kinds of waste waters. The present article briefly deals with the phytoremediation potential of this plant by highlighting the recent developments in utilization of water hyacinth for treatment of domestic, industrial and farm house effluents. The recent interest in the role of plant-microbial interactions for improving the efficacy of biological remediation has also been stressed. Subsequently the article narrows down to the phytoremediation of selenium and the last section describes the experimental investigations on the factors enhancing selenium removal capacity of water hyacinth. Selenium removal from selenium enriched (0.1-10 mg/L) sterilized half strength Hoagland nutrient medium by water hyacinth was studied. A maximum of 84% Se removal was obtained in 6-day period at 5 mg/L selenium concentration. The Se concentration in the plant root (2.67 g/kg dry wt.) was about 1.5 to 2 times higher than the shoot concentration (1.88 g/kg dry wt.). Detopping of plants to remove shoots further enhanced the Se uptake capacity of roots to 3.35 g/kg dry wt, subsequently resulting in 92% Se removal. Bactericide treatment of the roots decreased Se removal by 36% of the control (no treatment), indicating an important role of the root zone bacteria in Se removal from contaminated solutions. Various nutrient supplementations (5.0 g/L acetate, 5.0 g/L D-glucose, 2.0 g/L albumin) increased the total number of root zone bacteria. However, acetate supplementation resulted in maximum (98.6%) of Se removal. Mulching of chopped leaves (4 g/L) instead of nutrient supplementations also enhanced the bacterial number as well as Se removal to 94%. These results call for the optimization of conditions favoring plant-bacterial interaction during phytoremediation.

The ubiquitous metalloid arsenic is a toxic environmental pollutant. Arsenic pollution of groundwater is a major health concern worldwide. Remediating arsenic contaminated soil and groundwater using currently available engineering methods is costly and difficult. The discovery of the Chinese brake fern (*Pteris vittata* L) and related species to hyperaccumulate arsenic in their fronds has led to the development of environmentally-friendly and cost-effective phytoremediation technology for remediation of arsenic contaminated sites. An understanding of how *P. vittata* hyperaccumulates arsenic will be helpful to further improve and fully utilize the phytoremediation potential of this fern. This review summarizes recent research on the mechanisms of arsenic hyperaccumulation in *P. vittata* and its relevance to application in phytoremediation.

In the present study, we analysed the differences in endodermal barrier development, plant age- and timing of cadmium (Cd) treatment-dependent dynamics, uptake and translocation, and bioaccumulation coefficient as a response to Cd treatment of three different populations of *Silene dioica* L. The populations differ in the degree of soil contamination with toxic metals. *S. dioica* plants can accumulate Cd to a very high concentration in their bodies. The results show specific adaptation of plants from highly polluted areas. These plants, following treatment with a low concentration of Cd (10^{-5} M $Cd(NO_3)_2.4H_2O$) develop apoplastic barriers close to the root tip thus reducing the transport of metals to the above-ground plant parts. The endodermal apoplastic barriers develop closer to the root apex in older plants than in the younger ones after Cd treatment. The bioaccumulation coefficient of Cd after low Cd treatment differs between the examined populations. It is much higher in the plant population originating from piles of old copper mines (1,800 in the shoots, 17,200 in the roots) than in those from the vicinity of old mines (60, and 2,200, respectively).

Plant-Organism Interactions, Disease, and Control

Part 1 Disease and resistance: general mechanisms, techniques and control

Plants have evolved sophisticated innate immunity system to resist constant attacks from microbes. This system consists of multi-layered defense programs. Milestone advances have been achieved in the last fifteen years towards an understanding of the molecular mechanisms by which plants recognize the invading microbes and activate appropriate defense programs. Over 60 plant disease resistance (*R*) genes have been cloned from model and crop species. The majority of these genes encode proteins containing domains (e.g. "TIR", "NBS" and "LRR") that appear to be conserved in the plant and animal kingdoms for innate immunity function. The molecular basis for the long-standing enigma of the so called "gene-for-gene" interaction between a plant R protein and a cognate pathogen-encoded effector is being elucidated with increasing evidence for a recent model – the "guard hypothesis": Plant R proteins are part of a protein complex that functions in the plant surveillance system to detect the activities of pathogen effectors inside the host cell, rather than receptors for the pathogen-derived ligands. Many components of the complex defense signaling pathways have been identified and characterized. Moreover, biochemical cascades involved in some of the genetically defined signaling steps are being revealed. Finally, with more *R* genes and regulatory components of the innate immunity system are being characterized, greater efforts are to be made in exploiting plant natural disease resistance through genetic engineering of plant *R* genes and their signaling pathways.

To protect themselves from disease, plants have evolved sophisticated inducible defense mechanisms in which the signal molecules salicylic acid, jasmonic acid and ethylene often play crucial roles. Elucidation of signaling pathways controlling induced disease resistance is a major objective in research on plant-pathogen interactions. The capacity of a plant to develop a broad-spectrum, systemic acquired resistance (SAR) after primary infection with a necrotizing pathogen is well known and its signal transduction pathway extensively studied. Plants of which the roots have been colonized by specific strains of non-pathogenic fluorescent *Pseudomonas* spp. develop a phenotypically similar form of protection that is called rhizobacteria-mediated induced systemic resistance (ISR). In contrast to pathogen-induced SAR, which is regulated by salicylic acid, rhizobacteria-mediated ISR is controlled by a signaling pathway in which the phytohormones jasmonic acid and ethylene play key roles. In the past decade, the model plant species *Arabidopsis thaliana* has been extensively explored to study the molecular basis of systemically induced resistance. Here we review the current knowledge on induced disease resistance signaling in plants.

Accumulation of defensive secondary metabolites is a well-studied strategy of plants against various adversary environmental stresses, and it especially occurs in plants exposed to pathogens or elicitors. Pathogens or elicitors stimulate biosynthesis of defensive secondary metabolites through various signal transduction pathways. Understanding these elicitor signal transduction networks is one of the most important areas for revealing mechanisms of plant-environment interactions. This chapter summarizes the progress made on aspects of elicitor signal transduction leading to production of defensive secondary metabolites, including: elicitor perception by plant receptors; GTP binding proteins; ion fluxes and Ca^{2+} signaling; medium alkalinization and cytoplasmic acidification, oxidative burst and reactive oxygen species; inositol trisphosphates and cyclic nucleotides (cAMP and cGMP); salicylic acid and nitric oxide; jasmonate and ethylene signaling; others oxylipins and lipid signals, as well as cross talk among these elicitor signaling pathways. The review also highlights the linkage of above signaling components in elicitor signal transduction network by protein phosphorylation and dephosphorylation, as well as the integration of elicitor signaling pathways into various transcription factors, which eventually regulate multiple-step biosynthesis of plant secondary metabolites. Perspectives were also presented at transcriptome and metabolome points of view for production of useful secondary metabolites and improvement of agronomic defenses of crops.

Effective management of diseases in plant and flower production requires integration of physical and chemical approaches and utilization of plants' own disease resistance. Resistance to diseases in many cases is controlled by plant disease resistance genes (*R* genes). Availability of effective tools to identify, track, dissect and isolate *R* genes is of critical value to plant breeders for genetic improvement of disease resistance. Recent advances in molecular genetics and biology hold great promises to realize this hope. Molecular characterization of cloned plant *R* genes has shown that plants may use just a few classes of *R* genes and their gene products contain well conserved domains and motifs for pathogen recognition and defense activation. This conservation has allowed development of strategies to isolate and identify resistance gene analogs (RGAs) from plants of interest. RGA sequences are highly similar to and contain motifs typical of cloned plant *R* genes. It has been shown that many of them are tightly linked, genetically and physically, to known *R* gene loci, and that some of them are or hybridize to members of known *R* genes. They have found wide applications as molecular markers for tagging, mapping, cloning, and characterization of plant *R* genes. Isolation, identification and application of RGAs in floriculture and ornamental plants can allow tagging and mapping major *R* genes, thus facilitating selection, introgression and pyramiding of multiple disease resistance genes based on marker genotypes and expediting cloning of *R* genes for developing transgenic disease resistance.

Calcium-dependent protein kinases (CDPKs) are a family of plant specific proteins that link calcium-signal perception and protein phosphorylation. Little is known about their biological role but it was proposed, based on biochemical and transcriptional activation data, that some CDPKs function in multiple signal transduction pathways triggered by environmental and developmental stress cues. We have identified NtCDPK2 from tobacco as a crucial regulatory component in biotic and abiotic stress signal transduction pathways and have studied the enzyme's biochemical activation mechanism *in vivo*. Our data suggest that a modification of specific sites mimicking the enzyme's activated state may be exploited to engineer plants with altered stress behaviour.

Polygalacturonases (PGs) are the first cell wall degrading enzymes produced by plant pathogens and are virulence factors. Plants have evolved polygalacturonase-inhibiting proteins (PGIPs), which are anchored on the cell wall and interact with PGs on the same surface. PGIP encoding genes are up regulated by pathogen infection and stress. The *pgip* are regulated differentially in resistant and susceptible varieties/cultivars, incompatible and compatible reactions and by different signal molecules. PGIPs have a characteristic leucine-rich repeat region in their β-strand/β-turn, involved in protein recognition. This is the first protein-ligand interaction elucidated with direct evidence among the LRR defense proteins. The PGIPs with different specificities have coevolved along with PGs to counteract them.

The N-methylated compounds as inducers act within two concentration ranges characterized by low infection rates. In the case of a short induction time (2 days) these concentration ranges are around 10^{-5}-10^{-6} and 10^{-12}-10^{-13} mol/l, respectively, of the applied inducer. Between these active ranges there is always an inactive range (5-7 decimal dilutions) characterized in general by a high rate of infection. This unique phenomenon is called the double immune response, which is a non-linear effect. If the time interval between pre-treatment and inoculation increases the active concentration values for double immune response shift towards original higher dose values because of the continuous metabolism of methylated inducer in the host plant. The inactive range between the two active dose values remains the same. The multiple pre-treatment of plants with inducer gives contrasting results: the active dose ranges shifted towards the original lower values, however, the inactive range also shifted but remained unchanged. It seems that the two active concentrations of the inducer are always the same, and about equal to values observed after short induction. Methylated inducers are potential formaldehyde (HCHO) generators, therefore, it is especially interesting that HCHO (in diluted formalin solutions) generates a time- and dose-dependent immunostimulating activity on the host-parasite relationship. HCHO (mainly in bound form) and H_2O_2 can be formed intercellularly and extracellularly by almost all plant cells. These two small reactive molecules can interact (also endogenously) and the very reactive singlet oxygen and excited HCHO can form. The singlet oxygen may participate in the oxidation of water and in biological systems previously unknown oxidants including dihydrogen trioxide and ozone, are generated. It seems that these small very reactive molecules can not be avoided in the cell proliferation, apoptosis and disease resistance. These reactive molecules - from HCHO to O_3- form the biochemical basis of the double immune response of plants to pathogens and determine the common bridge between the innate (natural) and adaptive (induced) resistance in plants and animals, alike.

Vascular wilt caused by the fungus *Fusarium oxysporum* (*Fo*) affects over 100 different plant species, including economically important crops and ornamental plants. In this review, we propose that gene technology, based on the manipulation of plant defence signalling pathways, can be used as a novel method for controlling *Fusarium* wilt disease in genetically modified (GM) plants. First, the pathogenesis and biology of the *Fo* pathogen are briefly described. Then, particular aspects of the disease and the potential of GM resistance are illustrated for banana, cotton and ornamental plants. This is followed by an overview of the transgenic strategies that can be used to engineer *Fusarium* wilt resistance, with particular focus on the manipulation of defence signalling components. Finally, the potential of transferring the knowledge gained from studies in model plants species to crops is discussed.

Part 2 Plant-plant interactions and allelopathy

Invasive plants are one of the most devastating ecological problems in the 21st century, causing a $35 billion loss per year to the economy in the United States alone. More than 50% of all invasive plant species and 85% of invasive woody plants were introduced originally for ornamental and landscape use. Because many non-native plants are commercially important and widely utilized for various purposes, completely banning their use and prohibiting their imports are unpractical solutions. On the other hand, currently used methods to control the spread of non-native plants are ineffective, expensive, or environmentally problematic. Recent advances in plant molecular biology and plant genetic transformation may enable us to create sterile cultivars of these non-native ornamental crops of commercial value. The use of sterile cultivars should reduce or eliminate the undesirable spread of some non-native invasive plants into natural areas.

A dilemma surrounding *Rhododendron* confers upon this genus unique characters among plants. Great ornamental values (spectacular flowers, species-richness, ease of hybridization and broad geographical range) will continue to make *Rhododendrons* highly celebrated horticultural plants around the world. Their highly invasive ecophysiology (e.g. shade- and cold-tolerance, great resource use efficiency, allelopathy), life strategy (e.g. plastic physiological, morphological, and behavioral response to varying environments and shifting between generative and vegetative reproduction as primary source of colonization), and difficulty to control also will continue to make this shrub a notorious woody weed world-wide. This chapter focuses on the two most highly problematic *Rhododendron* species (*R. ponticum* L. and *R. maximum* L.) that exert profound impacts on natural ecosystems on both sides of the Atlantic. The broad geographical range and noteworthy similarities in climate, topography, and ecology of *Rhododendron* species can provide an opportunity to understand the ecology and control of invasive *Rhododendron* species. Information transfer on the ecology and effective, cost-efficient control of *Rhododendron* may significantly improve management practices not only for the current *Rhododendron*-invaded ecosystems but also for other parts of the world where this shrub is becoming an ecological threat.

Great rhododendron has become an abundant woody weed in the understories of eastern U.S. forests, reducing tree regeneration and growth significantly. Imazapyr and triclopyr ester can effectively control similar rhododendron species; however, the waxy leaf cuticle poses a major obstacle to chemical uptake and translocation to the roots. Vegetable oils, organosilicones, and nonionic surfactants enhance cuticle penetration of and enhance uptake and efficacy of herbicides in many herbaceous and woody weeds. This research was designed to assess uptake and describe translocation patterns of two rates of a conventional nonionic surfactant (Mixture B®), an organosilicone (Silwet 408®), and a methylated sunflower seed oil (Sun-It II®) on the uptake and translocation of two rates of radiolabeled triclopyr ester and imazapyr in 15-month-old great rhododendron. On average, foliar uptake of the lipophilic triclopyr ester was substantially greater (>60%) than uptake of imazapyr; translocation to roots, however, was lower (almost 50%). Surfactants had no effect on triclopyr ester uptake and its translocation out of the treated leaf. Although imazapyr uptake was much less than that of triclopyr ester, its cuticular diffusion, foliar uptake, and translocation to roots were enhanced significantly by the addition of Sun-It II and Mixture B. Increased herbicide rates and surfactant concentrations did not increase translocation. Combinations of low-rate imazapyr with low-rate Sun-It II or Mixture B may provide enhanced herbicidal efficacy and cost-effectiveness of controlling great rhododendron.

Part 3 Plant-fungus and plant-bacteria interactions

Legumes have the unique capacity to interact symbiotically with rhizobia, with the formation of root nodules as a result, in which nitrogen fixation takes place. The microsymbionts offer the plant an unlimited nitrogen source by fixing atmospheric nitrogen in the newly developed organs. Because of their symbiotic nitrogen fixation capacity and the accumulation of high protein levels in their seeds, legume crops are of major importance in a sustainable ecological agriculture. *Medicago truncatula* and *Lotus japonicus* are the model legume systems for the scientific community to study the biological features of indeterminate and determinate root nodulation, respectively, but also to investigate the improvement of grain legumes. Comparative genome and syntenic studies are currently performed, resulting in a potential new tool to extend the knowledge of the model legumes to agriculturally important crops, such as soybean, alfalfa and pea. The biological and molecular researches are mainly accomplished thanks to recent advances in biotechnological techniques with newly developed tools, such as genome analysis, bioinformatics, transcriptomics, proteomics and metabolomics. The knowledge of the signaling pathways leading to nodulation could eventually result in the nodulation of non-legume crops. In this chapter, major findings are discussed with the aim at providing the reader with a clear overview of the latest insights into the legume-*Rhizobium* nodulation field.

Plants have evolved a number of inducible defense mechanisms against pathogen attacks. Recognition of certain non-pathogenic rhizobacteria can trigger a systemic resistance reaction that renders the host less susceptible to subsequent infection by a virulent agent. Since this induced systemic resistance (ISR) is long-lasting and not conducive for development of pathogen resistance, disease control strategies based on this phenomenon are promising both for greenhouse cultures and under field conditions. The list of beneficial rhizobacteria reported to induce ISR is growing rapidly. Data compiled here also show that ISR may occur in various dicotyledonous and monocotyledonous plants and can be effective against a wide range of pathogens. This review emphasizes the molecular aspects of this three-step process involving sequentially i) the perception by plant cells of elicitors produced by the inducing agents that initiates the phenomenon, ii) signal transduction that is needed to propagate the induced state systemically through the plant and iii) expression of defense mechanisms *sensu stricto* that limit or inhibit pathogen penetration into the host tissues. The current state of knowledge about rhizobacteria-stimulated ISR is discussed in parallel with the more well-characterized systemic acquired resistance induced by incompatible pathogens.

The production of ornamental plants is a thriving and expanding industry, economically important in the USA, Canada, South America, Australia, and Europe as well as in many developing countries. During the last few decades significant changes occurred, with many new crops being introduced, new products such as pot plants replacing cut flowers, improved techniques for growing, treating and handling plants being implemented. Such changes had a profound influence on disease development and management. This chapter focuses on the evolution of the most important fungal diseases of ornamental plants during the past few decades as well as on developments in their management.

Fungal pathogens cause severe yield losses which are of great economic significant in a wide range of ornamental plants. Strategies for combating fungal diseases include traditional technologies such as plant breeding and chemical applications. Biotechnology now provides sustainable solutions to the problem of plant disease resistance. Several economically important ornamental plants have been already benefited by techniques of plant biotechnology. However, efforts to create new varieties have been mainly concentrated on morphological traits, such as flower colour, shape and fragrance, or plant architecture. This review summarizes successful attempts to engineer fungal resistance, and highlights promising approaches for generating broad protection, in transgenic ornamental plants. Strategies that are based on the expression of genes of plant origin, genes whose protein products either have antifungal activity or are controlling the synthesis of products with such activity, are presented. The expression of antifungal genes of non-plant origin is an alternative approach that has been successfully applied to obtain fungal resistance in transgenic plants. Progress in gene identification together with improvements in the efficiency of transformation and tissue culture technologies of ornamental plants provide powerful tools for the development of new varieties improved in their resistance to fungal pathogens. This review also discusses specific issues related to the regulation and exploitation of genetically modified ornamental plants.

To elucidate the defence mechanisms of *Hypericum perforatum* L. against *Colletotrichum gloeosporioides*, we utilized cell suspension cultures. When primed with methyl jasmonate, *H. perforatum* cells showed a double oxidative burst upon *Colletotrichum gloeosporioides* elicitation typical of a hypersensitive response. Phenolic profile of the cells was modified upon various stimuli such as salicylic acid, MeJ and *C. gloeosporioides*. The possible importance of reactive oxygen species production and phenolics (xanthones) as components of defence mechanism of *H. perforatum* against biotic stress is discussed.

Phytophthora species are oomycetes that can infect many plant species including vegetables, fruit and forest trees, field crops, and ornamentals. They cause a large array of diseases including damping off, root, crown, stem, tuber and corm rots, as well as leaf blights. The ability of *Phytophthora* sp. to infect ornamentals is favored by wet, saturated conditions in warm soils. Receiving high levels of moisture by ornamentals during the summer is highly conducive to infection of these plants by *Phytophthora* sp. This chapter will describe a few examples of *Phytophthora* sp. causing diseases on ornamentals, and provide an update of disease control and plant-pathogen interactions so far investigated in model plant species.

Armillaria occurs worldwide either as facultative pathogens or obligate saprophytes wherever there are suitable hosts or substrates. *Armillaria mellea* is associated with root rot and death of trees and shrubs, but it also attacks some herbaceous plants. Control by physical and chemical methods alone is inadequate. Phenolic fungicides can be detoxified. Prophylactic biocontrol of *Armillaria* by *Trichoderma* species is feasible. Growth of the antagonist on a carrier substrate, the time of application and several environmental conditions, influence efficacy. Other antagonists include some cord-forming fungi. The integration of biological, chemical and other control methods requires further study.

Part 4 Plant-virus interactions

RNA silencing is an evolutionarily-conserved process in eukaryotes that suppresses gene expression through small RNA-guided RNA cleavage or translational inhibition. In plants, RNA silencing functions as an antiviral defense mechanism; viruses are both an inducer and a target of RNA silencing, and plants encode multiple silencing factors to cope with viral infections. To evade RNA silencing, viruses have evolved various counter-defense strategies. One of these is to encode RNA silencing suppressors, which function to interfere with the assembly of silencing complexes or to prevent the spread of silencing signals. RNA silencing is therefore likely to be an important selective force for the evolution of viral and subviral genomes. Evidence is emerging that RNA silencing plays a key role in the pathology of viruses and subviral agents; induction or suppression of endogenous gene silencing by viral infections are two possible causes of viral pathogenicity. The demonstrated effect of silencing suppressors on endogenous silencing pathways suggests that viral infections play a positive role in plant genome evolution. Viral infections may interfere with the silencing of transposable elements and therefore increase their transposition frequency, leading to host genomic changes. Recent understanding of RNA silencing has led to the development of several transgene-mediated antiviral strategies. The most successful of these is to express inverted-repeat transgenes encoding hairpin RNA targeting viral genomes. Although several issues, such as viral escape, remain to be addressed, the silencing-based technologies hold tremendous potential for the control of viral pathogens in plants.

Viral diseases have a considerable impact on global agronomic production. While conventional breeding procedures aiming at the selection of virus resistant crop varieties are sometimes too slow to meet the challenges of fast-evolving pathogenic viruses, transgenic approaches might be a promising alternative. Recent advances in understanding of the molecular basis of viral infections and plant defence mechanisms facilitated the development of a variety of different concepts and strategies for engineering virus resistant plants. These include on the one hand the utilization of endogenous defence mechanisms either by transgenic expression of resistance genes or by triggering the RNA-silencing machinery, and, on the other hand, the expression of proteins or peptides exhibiting dominant antiviral activities *in vivo*.

Ornamental plant nurseries play a significant role in the aesthetic landscape of urban and rural centres. Flowers and foliage plants, shrubs, plants for hanging baskets, and landscapes from all regions of the world are available at ornamental plant nurseries. A variety of plant species, lack of isolation between plant species, rapid vegetative multiplication (mainly by cuttings), and an absence of disease-symptoms in propagating material, render nurseries as an ideal environment for viroid evolution through recombination and mutation. Species-jumping of *Columnea latent viroid* from an ornamental plant to tomato and its transfer to potato has demonstrated its high infection rate in the tomato crop and a high percentage of potato tuber yield reduction under field conditions. Similarly, the potential of developing multiple viroid infections through recombination in vegetatively propagated plants and experimental demonstration of 'inverse' chimeric viroid appearance are other examples of the role played by ornamental plants in viroid evolution. Although only scattered reports of viroids from ornamental plants have been made, it has been shown that viroids remaining symptomless in ornamental plants can cause severe economic losses upon transfer to crop plants. To facilitate large-scale systematic surveys of viroids in ornamental plants, a *Pospiviroid* specific primer pair and a simplified nucleic acid preparation protocol for RT-PCR detection of viroids using nitrocellulose membrane binding for storage and long-distance transportation are discussed.

Tomato ringspot nepovirus (ToRSV) is an economically important pathogen of small fruits and fruit trees in North America. It also causes serious diseases in ornamentals. In this review, we discuss recent advances towards the elucidation of the ToRSV replication cycle at the molecular level. In particular, we describe our current understanding of how (1) viral proteins are produced through regulated proteolytic processing of large polyprotein precursors, (2) large viral replication complexes are assembled in association with intracellular membranes from the host and (3) virus particles move through the plant. At each step of the replication cycle, we point out the role of essential host factors which are recruited by viral proteins. We also briefly discuss the interaction between the virus and the plant post-transcriptional gene silencing antiviral pathway. Finally, the impact of these advances for the design of new antiviral strategies is considered.

Part 5 Plant-insect interactions

Floricultural crops are damaged by a variety of insect pests. Insect damage drastically decreases the value of floricultural products in the market. Host plant resistance to insects is one of the most important components of integrated pest management. However, introgression of host plant resistance is a long and tedious process and has many limitations. Protection of crops using pesticides, although very effective, has many deleterious effects. In recent years, it has become possible to incorporate novel traits such as insect resistance in plant species by using molecular biology techniques. Biotechnological strategies will be useful to manage the pests in floriculture. Insecticidal proteins of *Bacillus thuringiensis* will be an important component of insect pest management in transgenic ornamental crops cultivation. Other insecticidal proteins such as proteinase inhibitors and lectins have also been tested. Aphids are very important insect pests of floriculture crops especially in protected cultivation. Novel molecules such as linalool for efficient management of aphids in transgenic plants are being discovered. One of the important considerations of introduction of insect resistant transgenic plants into the environment is to prevent the development of resistance in insects towards insecticidal proteins such as Bt toxins. Various resistance management strategies have been suggested to prevent or delay the development of resistance to Bt. Another important consideration in the large-scale cultivation of transgenic plants is to rationally deploy integrated pest management (IPM) strategies. Various measures of IPM include crop sanitation, biological control methods, botanical pesticides, entomopathogenic fungi and viruses and minimal application of chemical pesticides.

To perform efficient genetic transformation of turfgrass species, it is necessary to identify the ideal explant type and optimize the *in vitro* conditions, mainly using different concentrations of various growth regulators for each genotype. Among turfgrass genera, common bermudagrass [*Cynodon dactylon* (L.) Pers.] is a warm-season perennial species widely used for turf, forage, and soil stabilization. The black cutworm (*Agrotis ipsilon* Hufnagel) is the most commonly encountered pest of bermudagrass, especially on golf course greens. Developing insect-resistant cultivars is a very desirable substitute, both environmentally and economically, to using current synthetic pesticides. Here we report, for the first time, *Agrobacterium*-mediated transformation of common bermudagrass 'Arizona Common' with the *Bacillus thuringiensis* Berliner *cry1Ac* gene encoding an endotoxin active against black cutworm. Mature seeds were used for producing embryogenic callus, and calli were transformed with a plasmid containing a synthetic *cry1Ac* and the kanamycin resistance (*nptII*) genes. Putative transgenic calli and plantlets were selected on media containing 100 and 50 $mg \cdot L^{-1}$ G418, respectively. RNA-blot analysis of PCR-positive lines revealed the expression of the *cry1Ac* transgene in three out of five putative transgenic lines. The larvae fed on transgenic plant leaves experienced highly significant (over 80%) mortality.

The effect of low concentrations of azadirachtin A (AzaA) applied systemically through root tissues of tomato plants (*Lycopersicon lycopersicum* L.) cv. Vilma on the development and population size of greenhouse whitefly (*Trialeurodes vaporariorum* Westwood (Hemiptera.: Aleyrodidae) was studied. The concentrations of AzaA tested were: 5 and 0.5 ppm. The effectiveness increased, dependent on dosage and the number of applications. If at least two applications at 5 ppm or 3 applications at 0.5 ppm were made, the number of each stage was reduced from 70-80%. Such a significant decrease in the *T. vaporariorum* population can be sufficient for pest regulation in tomato plants grown in hydroponics and may serve as a model basis for other crops and ornamentals growing hydroponically.