Gyana Ranjan Rout
Anath Bandhu Das   *Editors*

Molecular Stress Physiology of Plants

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Foreword

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I am glad to foreword *Molecular Stress Physiology of Plants*, a book edited by Dr. G. R. Rout, Professor, and Dr. A. B. Das, Associate Professor, Department of Agricultural Biotechnology, College of Agriculture, Orissa University of Agriculture and Technology, Bhubaneswar, India. This book is divided in various chapters focusing on the effect of abiotic and biotic stresses exerted on plants' growth and its mechanism. *Molecular Stress Physiology of Plants* covers abiotic stresses like light, temperature, salinity, drought, heavy metals, osmotic, and submergence. The effect of growth regulators on plants' growth and molecular mechanisms including photosynthetic machinery has also been widely discussed. This is a much-needed book in this area that covers the topics that are essential to understand the molecular mechanisms that controls the stress physiology of plants. With the declining mangrove populations in the world, the aspects of salt stress genes in mangroves and molecular mechanism of salt tolerance and measurement of chlorophyll fluorescence are the essential topics to evaluate the declining plant populations. Biochemical and physiological adaptations in some halophytes are well documented in these chapters. Measurement of drought and high temperature stress signal in crop plants and its application is well discussed and can be correlated with the other aspects of stress physiology among angiosperm populations all over the world. The use of isothermal

calorimetry and Raman spectroscopy to study plant abiotic and biotic stress was unique and added a novel flavor in the reviewed chapter. Crop physiologists would be delighted to read the discussion on the physiology of reproductive stage and abiotic stress tolerance in cereals. Marker-assisted breeding for stress resistance in crops has depicted a novel strategy for crop improvement. The chapters also cover the approach with proteomics to understand the stress tolerance in plants and the role of calcium-mediated CBI-CIPK network in plants' mineral nutrition and abiotic stress that provides high impact on plant growth and metabolism. This book will help expand knowledge of stress physiology and improve understanding the mechanism involved. This book will be worth reading for students and researchers in plant physiology and plant biology.

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Preface

Progressive and sustainable rise in food grain production is essential for the country to maintain the level of nutrient supply. Growing population in both developing and developed countries already has alarmed to increased food grain production. Global climate changes exert multiple biotic and abiotic stresses which limit the crop production. The productivity of major staple food crops has reached to a plateau. There is very little scope to increase crop production area too. Abiotic stresses like drought, cold, salinity, and temperature are more emphasized with regard to crop productivity. Heavy metal stress creates the loss of crop production. Biotic stress also deals with the decrease in crop production. The soil reclamation is a costly affair, and it is temporary. Development of crop genotypes tolerant/resistant to the adverse conditions is the only alternative of such a problem. To develop tolerant/resistant genotypes, the plant breeder or plant biotechnologist should have keen knowledge regarding the injury and tolerance mechanisms in plant for specific stress and plant systems to identify the nature of abiotic stress, breeding methods, and modern biotechnological approaches. This book highlighted 17 invited chapters including various stresses like salt, drought, metal, osmotic, oxidative, submergence, temperature, chemical, hormonal, radiation, cold, and nutrient imbalance and its molecular mechanism, and stress mechanism in proteomic approaches. Emphases have been given to include latest development in the field of abiotic stresses with appropriate citations and application. Apart from this, the book also contains molecular mechanism of stress resistance of photosynthetic machinery, PS II fluorescence techniques for measurement of drought and high temperature stress signal in crop plants, isothermal calorimetry and Raman spectroscopy to study response of plants to abiotic and biotic stress, marker-assisted breeding for stress resistance in crop plants, physiology of reproductive abiotic stress tolerances in cereals, role of calcium-mediated CBL-CIPK network in plant mineral nutrition and abiotic stress, and DNA methylation-associated epigenetic changes in stress tolerance of plants. We hope that this book will help the students, researchers, teachers, and plant scientists in the field of basic and applied aspects of agriculture and botany.

We are extremely grateful to the contributors, specialist in the subject and also reviewers for their kind support in time. We are also thankful to all of our teachers for constant encouragement and support in promoting the

development of this book on *Molecular Stress Physiology of Plants*. We are thankful to Prof. Prasanna Mohanty, Eminent Plant Physiologist and ex-Dean, School of Life Sciences, JNU and INSA, Sr. Scientist, for the constant encouragement.

Bhubaneswar   Gyana Ranjan Rout
                                                                          Anath Bandhu Das

About the Editors

Professor Gyana Ranjan Rout is the Head of Department of Agricultural Biotechnology, Orissa University of Agriculture and Technology, Bhubaneswar, India. Professor Rout has worked in various aspects of plant sciences for over 25 years. He has made significant contributions in the field of plant improvement and propagation of various plant species, heavy metal toxicity and its tolerant mechanism, reclamation of mine through phytoremediation, and DNA fingerprinting. Professor Rout was elected as a Fellow of National Academy of Sciences, India (FNASc), in 1999 in the field of plant biotechnology and molecular biology. He was awarded Samanta Chandra Sekhar Award in 2005 honored by Orissa Bigyan Academy, Govt. of Odisha, for contributions to Life Sciences. Professor Rout was recipient of British Council fellowship, UK; BOYSCAST fellowship by DST, Govt. of India, FAO/IAEA/BADA fellowship, Belgium and DBT Overseas fellowship by Govt. of India. Professor Rout has 25 years of research and teaching experience in the field of plant biotechnology and heavy metal stress mechanism. He has published 175 research papers and 16 review chapters published in national and international peer-reviewed journals and 20 book chapters in contributory volumes. He has also been a principal investigator of 10 major research projects funded by ICAR, DBT, and NMPB.

Dr. Anath Bandhu is an Associate Professor in the Department of Agricultural Biotechnology, Orissa University of Agriculture & Technology, Bhubaneswar, Orissa, India. Dr. Das has worked assiduously in various aspects of plant sciences for over 26 years. He has made significant contributions in the field of cytotaxonomy, cytometry, DNA fingerprinting, and molecular physiology in various groups of medicinal plants, mangroves, cacti, orchids, and sweet gourd. He has reported for the first time chromosome number, karyotype, and genome size in ~350 species of angiosperms, especially on Indian mangroves that underpinned mining of de novo genomic diversity in diploids and polyploids. Molecular phylogeny of mangroves using various DNA markers resolved many discrepancies in taxonomic classifications. He is also working in molecular basis of high salt adaptation of secretor and nonsecretor mangroves to find out salt stress-resistant gene. His work on salt stress on mangroves has generated interest to study these fascinating processes of molecular physiology in other laboratories as evidenced by extensive citation of his work. He has published more than 150 research papers in international journals, 2 books, 10 book chapters, and 10 review articles. Dr. Das is honored with Hira Lal Chakravarty Award of ISCA and Samanta Chandra Sekhar Award by Orissa Bigyan Academy and recipient of DBT Overseas and National Associateships, Govt. of India; RI-LAT Fellowship (UK); and MIF Fellowship (Japan).

About the Book

Book Title: Molecular Stress Physiology of Plants

Crop growth and production is dependent on various climatic factors. Both abiotic and biotic stresses have become an integral part of plant growth and development. There are several factors involved in plant stress mechanism. The information in the area of plant growth and molecular mechanism against abiotic and biotic stresses is scattered. The up-to-date information with cited references is provided in this book in an organized way. More emphasis has been given to elaborate the injury and tolerance mechanisms and growth behavior in plants against abiotic and biotic stresses. This book also deals with abiotic and biotic stress tolerance in plants, molecular mechanism of stress resistance of the photosynthetic machinery, stress tolerance in plants (special reference to salt stress – a biochemical and physiological adaptation of some Indian halophytes), PSII fluorescence techniques for measurement of drought and high temperature stress signal in crop plants (protocols and applications), salicylic acid (role in plant physiology and stress tolerance), salinity induced genes and molecular basis of salt tolerance mechanism in mangroves, reproductive stage abiotic stress tolerance in cereals, calorimetry and Raman spectrometry to study response of plant to biotic and abiotic stresses, molecular physiology of osmotic stress in plants and mechanisms, functions and toxicity of heavy metals stress in plants, submergence stress tolerance in plants and adoptive mechanism, Brassinosteroid modulated stress responses under temperature stress, stress tolerance in plants (a proteomics approach), Marker-assisted breeding for stress resistance in crop plants, DNA methylation associated epigenetic changes in stress tolerance of plants and role of calcium-mediated CBL-CIPK network in plant mineral nutrition and abiotic stress. Each chapter has been laid out with an introduction, up-to-date literature, possible stress mechanism and applications. Under abiotic stress, plants produce a large quantity of free radicals, which have been elaborated. We hope that this book will be of greater use for post-graduate students, researchers, physiologists and biotechnologists to sustain plant growth and development.

Contents

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Abiotic and Biotic Stress Tolerance in Plants

1

Susana Redondo-Gómez

Abstract

Environmental stresses play crucial roles in the productivity, survival and reproductive biology of plants as well as crops. Plants are subjected to many forms of environmental stress, which can be included into two broad areas: abiotic (physical environment) and biotic (e.g. pathogen, herbivore). However, plants evolve different mechanisms of tolerance to cope with the stress effects. These mechanisms comprise physiological, biochemical, molecular and genetic changes. This chapter represents a general overview of the major mechanisms developed by plants to tolerate environmental stresses, both abiotic (drought, high temperature, chilling and freezing, UV-B radiation, salinity and heavy metals) and biotic (herbivory, pathogen and parasite and allelopathy). Since the length and complexity of the topic is so wide, the effects of the different stresses on plant physiology and biochemistry, as well as the synergies between types of stresses, are beyond the scope of this chapter.

Introduction

Tolerance of plant refers to its capacity to survive and reproduce under environmental stresses (Simms 2000). Plants are subjected to many forms of environmental stress. Some are abiotic physicochemical, such as drought, cold, heat and high salinity. Other sources of stress are biotic, such as herbivory, disease and allelopathy (Leavitt 1980). Plants alter their physiologies, metabolic mechanisms, gene expressions and developmental activities to cope with the stress effects. Therefore, plants possess unique and sophisticated mechanisms to tolerate stresses (Madhava Rao 2006). However, the degree of tolerance varies from plant to plant, from low to high (Smith et al. 2001). Anyway, the knowledge on the physiology and molecular biology of stress tolerance are certainly helpful to facilitate the biotechnological improvement of crop productivity in the near future.

The common theme of stress is the formation of reactive oxygen species (ROS, see Table 1.1) at cellular and molecular level, strong oxidants that can do significant damage to membrane

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Table 1.1 Formation of ROS by different types of stress

Stress	Reference
Drought	Smirnoff (1993)
Extremes of temperature	Rao and Dubey (1993)
UV radiation	Murphy and Huerta (1990)
Heavy metals	Cakmak and Marschner (1988)
Air pollutants	Mehlhorn (1990)
Mechanical and physical stresses	Legendre et al. (1993)
Pathogens	Sutherland (1991)

Table 1.2 Signal molecules in plants

Molecule	Reference
Nitric oxide	Shi et al. (2012)
Salicylic acid	Catinot et al. (2008)
Systemin	Rocha-Granados et al. (2005)
Jasmonic acid	Creelman and Mullet (1995)
Abscisic acid	Fujita et al. (2006)
Ethylene	Ludwig et al. (2005)

systems and DNA. ROS include superoxide, hydrogen peroxide, and superhydroxide (Scandalios 1993). Antioxidative systems, both enzymatic (superoxide dismutases, catalase, peroxidases, phenol oxidase, and ascorbic acid oxidase) and nonenzymatic systems (compounds that are strong reductants such as glutathione, phenols, flavonoids, and polyamines), play an important role in balancing and preventing oxidative damage (Foyer et al. 1994). Desikan et al. (2001) subjected *Arabidopsis* to oxidative stress and found an increase in genes involved in cell rescue and defence as well as other metabolic functions from the H_2O_2 treatment.

Additionally, ROS production, after exposure to biotic or abiotic stresses, has been described to be involved in signalling cascade. ROS are rapidly produced in plants as a defence response to pathogen attack (Bolwell et al. 2002). Furthermore, ROS signalling pathways are closely interwoven with hormone-signalling pathways in plant-insect interaction (Kerchev et al. 2012). Accumulation of the ROS, caused by salinity stress, seems to activate mitogen-activated protein kinase (MAPK) cascade (Kovtun et al. 2000), which is known as signalling module. Glutathione (GSH) and H_2O_2 act alone or in unison, in

intracellular and systemic signalling systems, to achieve tolerance to abiotic and biotic stresses. Other signal molecules produced in response to both biotic and abiotic stresses appear in Table 1.2.

Stress response and adaptation in plants is complex; there is a whole cascade of genes involved in stress tolerance, starting from stress perception followed by the formation of gene products that are involved in cellular protection and repair (Mantri et al. 2012). One central process of tolerance responses in plants is the activation of defence-related genes in response to biotic and abiotic stresses in their living environment (Xiang 1999). In this way, the signal transduction pathways that detect stress play a crucial role in the induction of stress tolerance in plants (Smalle and Vierstra 2004). Some genes involved in the stress signalling pathway appear in Table 1.3.

Moreover, many drought-inducible genes are also induced by salt stress and cold, which suggests the existence of similar mechanisms of stress responses. Interestingly, there are signalling pathways that are shared during abiotic and biotic stress responses. In a recent study, abscisic acid-induced myb1 (*SlAIM1*) gene from tomato (*Solanum lycopersicum*) encoding an R2R3MYB transcription factor was induced by pathogens, plant hormones, salinity and oxidative stress (Abuqamar et al. 2009). ROS and phytohormone signalling are also essential components of the inter-pathway crosstalk that allows plants to respond to multiple environmental inputs (Fujita et al. 2006). The main objective of this chapter is to present the major mechanisms developed by plants to tolerate environmental stresses, both abiotic and biotic.

Table 1.3 List of some genes in the stress signalling pathway

Gene	Function	Reference
ATHK1 (*Arabidopsis thaliana*)	Osmosensor	Urao et al. (1999)
etr1, ein2 (*Arabidopsis thaliana*)	Ethylene signalling	Larkindale et al. (2005)
AtCBF1 (*Populus* spp.)	Cold response	Benedict et al. (2006)
UVR8 (*Arabidopsis thaliana*)	UV-B response	Brown and Jenkins (2008)
OsCDPK7 (*Oryza sativa*)	Salinity response	Saijo et al. (2000)
OsMAPK2 (*Oryza sativa*)	Cu response	Yeh et al. (2003)
CYP79F1 (*Arabidopsis thaliana*)	Herbivory response	Mewis et al. (2006)

Abiotic Stressors

Drought

The major environmental factor that constrains the productivity and stability of plants is water stress (Araus et al. 2002). Water deficit affects plants on several levels. Cell expansion and growth are among the first processes to decline under water stress. With progressive water deficit, photosynthesis is adversely affected. On the cellular level, membranes and proteins can be damaged by a reduction in hydration and an increase ROS (Artlip and Wisniewski 2001). To overcome this, plants are equipped with various mechanisms to tolerate drought:

– *Reduction in water loss*: Plant leaves close their stomata immediately on sensing an increase in leaf-to-air vapour pressure difference, even if the roots have sufficient water, thereby reducing water loss through transpiration (Assmann et al. 2000). This response is induced by abscisic acid (ABA). The key role of ABA as a plant hormone regulating metabolism and stomatal behaviour under conditions of water stress is well established (Voesenek and Van der Veen 1994). The ABA is synthesized from carotenoid by ABA-synthesizing enzymes induced in root tip cells or parenchyma cells of vascular bundles by drought stress (Koiwai et al. 2004). ABA synthesized in the roots enters the xylem vessels in a free form or as a conjugate with glucose and is transported from here to the leaves (Sauter et al. 2002). Also, expression of the gene encoding abscisic aldehyde

oxidase has been revealed in the guard cells of dehydrated *Arabidopsis* leaves (Koiwai et al. 2004). Stomatal closure allows plants to preserve absorbed soil water, to improve water-use efficiency, to avoid damaging water deficits, or a combination of these. During long periods of water stress, stomatal closure is very important to maintain a favourable water balance and thus is an effective means of controlling cuticular water loss (Freitas 1997).

– *Protection of photosynthetic machinery*: As leaf water is lost, the turgor pressure of leaf tissues decreases and leaves begin to wilt. Wilting of the leaves works to protect photosynthetic machinery from direct rays of the sun (Larcher 1995). Moreover, stomatal closure under drought stress restricts the influx of CO_2 and hence photosynthesis, depriving plants of their largest consumer of solar energy. Different studies suggest that the cyclical electron flow around photosystem I (PSI) can lead to additional consumption of reducing equivalents and can thus act as an important electron sink for excessive excitation energy (Yokota et al. 2006), as well as an increase in thermal dissipation in the photosystem II (PSII) antennae (Teraza et al. 2003) and photorespiration (Parida and Das 2005). The thermal dissipation in the PSII is detected as non-photochemical quenching (NPQ) (Ma et al. 2003).

– *Osmotic adjustment*: Plant cells are required to maintain turgor pressure and synthesize and accumulate small molecule compounds and ions (fundamentally potassium). The ability of the cited organic molecules to balance ions sequestered in the vacuole and to

stabilize enzymes incubated with salt solutions has resulted in describing these compounds as compatible solutes. Compatible solutes include polyoles (e.g. sorbitol or mannitol), amino acids or amides (e.g. proline), quaternary ammonium compounds (e.g. betaine), and soluble carbohydrates (sugars) (Orcutt and Nilsen 2000). The compounds that accumulate most commonly are proline and glycine betaine (Hasegawa et al. 2000). High concentrations of compatible solutes can increase cellular osmotic pressure (Delauney and Verma 1993). Otherwise, their high hydrophilicity helps to maintain the turgor pressure and water content of cells which protect against water loss from leaves under drought (Yokota et al. 2006).

The plant response to drought is accompanied by the activation of genes involved in the perception of drought stress and in the transmission of the stress signal. There are genes that encode regulatory proteins that further regulate the transduction of the stress signal and modulate gene expression (Waseem et al. 2011). Other genes encode proteins that protect the cells from the effects of desiccation; these include those that govern the accumulation of compatible solutes, passive transport across membranes, energy-requiring water transport systems, and protection of cell structures from desiccation and damage by ROS. Low water status reduces the hydration of biomolecules such as proteins, which lead to denaturation and to disruption of membranes (Steponkus et al. 1993). Dehydrins have been proposed to ameliorate these effects by reducing hydrophobic aggregations or inappropriate interactions (Close 1996).

High Temperature

Heat stress due to high ambient temperatures is a serious threat to crop production worldwide (Hall 2001). High temperature stress occurs when plants experience temperature above that to which they are adapted and that adaptation depends on the makeup of the proteins and membranes of plants since both are strongly affected by temperature (Sharkey and Schrader 2006). Moreover, thermotolerance refers to the ability of an organism to cope with excessively high temperatures, and the term basal thermotolerance describes the plant response to high temperature in the absence of any period of acclimatization (Penfield 2008). Basal thermotolerance is highly dependent on salicylic acid (SA) action (Clarke et al. 2004). As little as 15 min after exposure to high temperatures, plants begin to acclimatize and the maximum tolerated temperature increases (Kaplan et al. 2004).

Plants exhibit a complex response to extreme high temperatures, including long-term evolutionary phenological and morphological adaptations and short-term avoidance or acclimation mechanisms such as changing leaf orientation, transpirational cooling, or alteration of membrane lipid compositions (Wahid et al. 2007). The sum total of metabolic changes elicited when living cells are subjected to a sudden and transient increase in temperature is referred to as heat shock (HS) response (Singla et al. 1997). Some major tolerance mechanisms, including ion transporters, osmoprotectants, free-radical scavengers, late embryogenesis abundant proteins and factors involved in signalling cascades and transcriptional control are significant to counteract the stress effects (Wang et al. 2004). Immediately after exposure to high temperatures and perception of signals, changes occur at the molecular level altering the expression of genes and accumulation of transcript that leads to the synthesis of stress-related proteins as a stress tolerance strategy (Iba 2002). Expression of heat shock proteins (HSPs) is known to be an important adaptive strategy in this regard (Feder and Hoffman 1999). The tolerance conferred by HSPs results in improved physiological processes such as photosynthesis, assimilate partitioning, water and nutrient use efficiency, and membrane stability (Wahid et al. 2007). HSPs also appear to protect plants against oxidative stress. Otherwise, production of ROS in the organelles is of great significance for signalling as well as production of antioxidants. Key role for ROS has been proposed in acquired thermotolerance (Penfield 2008).

Different mechanisms of plant tolerance to high temperatures are given below (Wahid et al. 2007):

– *Anatomical changes*: These are similar to those under drought stress (see the section "Drought"); there is a general tendency of reduced cell size, closure of stomata and curtailed water loss (Añon et al. 2004).
– *Physiological changes*: Plant water status is the most important variable under high temperatures. In general, plants tend to minimize water loss and synthesize and accumulate compatible solutes and ions (see the section "Drought"). For instance, glycine betaine (GB) plays an important role as a compatible solute in plants under various stresses, such as salinity or high temperature (Sakamoto and Murata 2002). Similarly, accumulation of soluble sugars under heat stress has been found in sugarcane (Wahid and Close 2007). On the other hand, photosynthesis is considered as the physiological process most sensitive to high temperatures. The rate of photosynthesis in most species declines above 35°C (Sage and Reid 1994). Overall, the rate of photosynthesis decreases while dark- and photorespiration increase considerably under high temperatures (Wahid et al. 2007). Both pathways can act as an important electron sink for excessive excitation energy.

 The integrity and functions of biological membranes are sensitive to high temperatures, as heat stress alters the tertiary and quaternary structures of membrane proteins. In the same way, carotenoids of the xanthophyll family and some other terpenoids, such as isoprene, are synthesized by plants in order to stabilize and photoprotect the lipid phase of the thylakoid membranes (Velikova et al. 2005). The resulting interaction of the xanthophyll molecules and the membrane lipids brings about a decreased fluidity (thermostability) of membrane and a lowered susceptibility to lipid peroxidation under high temperatures (Havaux 1998).

– *Molecular changes*: Increased production of HSPs occurs when plants experience either abrupt or gradual increase in temperature (Nakamoto and Hiyama 1999). Immunolocalization studies have determined that HSPs normally associate with particular cellular structures, such as cell wall, chloroplasts, ribosomes and mitochondria (Yang et al. 2006). However, in tomato plants, HSPs aggregate into a granular structure in the cytoplasm under high temperature stress that possibly protects the protein biosynthesis machinery (Miroshnichenko et al. 2005). Other proteins or mRNAs also increase in abundance during elevated temperature but are not considered HSPs. They include several glycolytic enzymes, protein kinases, and ubiquitin (Burke et al. 1988; Lindquist and Craig 1988; Moisyadi and Harrington 1990). Veirling (1991) suggested that glycolytic enzymes and protein kinases are involved in metabolic readjustment. Ubiquitin is probably required to remove aberrant proteins resulting from damage to translational machinery or thermally denatured proteins (Artlip and Wisniewski 2001). Dehydrins are also synthesized in response to heat stress (Wahid and Close 2007).

Chilling and Freezing

Plants experience chilling stress as a result of temperatures above 0°C and below some threshold temperature unique for each species. Freezing stress occurs at temperatures below 0°C or when radiative frosts occur with ice formation. Chill-sensitive plants comprise many major field crops, such as cotton, soybean, maize and rice. Plants face three major problems when exposed to low temperatures (Vézina et al. 1997) like (1) perturbation of membranes since a fall in temperature is accompanied by a decrease in membrane fluidity manifested by electrolyte leakage from tissues (Barták et al. 1998), (2) slow down of their chemical and biochemical reactions and (3) changes in water status and availability.

 Intracellular ice crystals are immediately lethal, as they can pierce the plasma membrane. Plants tolerate only extracellular freezing, which

is associated with cell dehydration and cell volume reduction. Osmotic potential of ice is lower than that of water; thus, cell water exits the cell towards the growing ice crystal in the apoplast (Rajashekar 2000). Desiccation via freezing is ameliorated by both biochemical and biophysical changes, particularly in woody plant species (Wisniewski and Arora 1993). Some freeze-tolerant plants can limit the growth of apoplastic ice crystals with proteins and polysaccharides, limiting the extent of protoplast dehydration from ice crystal growth (Breman 2006).

Tolerance to chilling is apparently a prerequisite for tolerance to freezing. Chilling tolerance is an inducible response, dependent on day length and temperature (Gray et al. 1997), and it is accompanied by an increase in the ABA content of cells. Low temperatures also induce numerous proteins or their mRNA, and evidence exists that some of these proteins are necessary for chilling tolerance (Artlip and Wisniewski 2001). For example, some of the heat shock proteins, or their transcripts, are cold inducible (Yacoob and Filion 1987; Guy and Li 1998). These proteins and others (including signalling molecules or transcription factors, metabolic enzymes and many hydrophobic or hydrophilic gene products) have shown the ability to inhibit ice propagation or recrystallization either in vitro or in vivo (Artlip and Wisniewski 2001).

Overall, the major mechanisms of plant tolerance to low temperatures are the following:

1. *Stabilization of membranes*: Perturbation of membranes could be due to phase transitions caused by the presence of minor lipid components in the membrane or, alternatively, failure to seal critical intrinsic membrane proteins into the cell membrane by non-bilayer-forming lipids (Williams 1990). Thus, changes in lipid composition have been proposed to augment the membrane stability against freezing stress (Rajashekar 2000). Yoshida and Uemura (1984) found that freezing tolerance was accompanied by an increase in phospholipids, especially phosphatidyl ethanolamine. Additionally, glutathione has been described to protect membrane protein, preventing protein denaturing. High levels of glutathione also have been found to be correlated with acclimated freeze hardiness in several plant species (Breman 2006).

2. *Cryoprotection*: Soluble sugars and other osmolytes have cryoprotective function. These compounds can protect cell membranes and organelles during freezing (Rajashekar 2000). The main sugars and sugar alcohols that increase are sucrose, glucose, fructose, sorbitol, mannitol, raffinose and stachyose. It is postulated that sugars replace water and decrease the degree of freeze-induced dehydration (Trischuk et al. 2006). Sugars also promote glass transitions that protect cells from desiccation injury (Wolkers et al. 1999). Furthermore, thylakoid membranes are protected from freezing inactivation by exogenous proline, argenine, threonine and lysine. Proline and glycine betaine are both postulated to act as cryoprotectants (Trischuk et al. 2006). Abscisic acid can activate the BADH gene, which encodes for one of the enzymes involved in the synthesis of glycine betaine (Ishitani et al. 1995).

UV-B Radiation

Increased solar UV-B radiation (280–320 nm), as a consequence of reductions in stratospheric O_3, has been shown to cause significant reduction in growth and other physiological responses in many sensitive crops (Kulandaivelu et al. 1997). However, plants have the capacity to develop various mechanisms of protection from the deleterious effects of UV-B radiation:

– *DNA repair*: One of the most important and sensitive targets of UV-B radiation is DNA. UV radiation induces various lesions in DNA, and the best studied are cyclobutane-type pyrimidine dimmers (Stapleton 1992). The dimmers can be repaired via photoreactivation (photolyase), excision repair, or recombinatorial repair (Smith 1989). The former type of repair is the best known, and it has been reported in several species (Artlip and Wisniewski 2001).

– *Accumulation of secondary metabolites*: Flavonoids and/or anthocyanins are induced by UV-B exposure (Beggs et al. 1986). Flavonoids and anthocyanins absorb UV radiation, and they generally accumulate in the epidermis, where they could keep UV radiation from reaching photosynthetic tissues. UV photoreceptors are responsible for the initial perception, and additional photoreceptors are required for anthocyanin or flavonoid biosynthesis in parsley (Stapleton 1992). Flavonoids also possess free-radical scavenging activity (Rice-Evans et al. 1997), which might offer additional protection to cell accumulating these compounds. Polyamines, waxes and specific alkaloids have all been suggested to contribute to UV tolerance. In soybean, a correlation was found between levels of polyamines and tolerance to UV-B radiation (Kramer et al. 1992). In *Dudleya*, accumulation of glaucescence, a powdery wax, increases reflection of UV-B to a larger extent than that of photosynthetically active radiation (PAR) (Mulroy 1979). Levels of UV-absorbing tetrahydrocannabinol increase linearly with UV-B dose in *Cannabis* (Lyddon et al. 1987).

– *Morphogenic changes*: Leaf curling is a photomorphogenic response observable at low fluences of UV-B that helps diminish the leaf area exposed to UV. A protective function has also been hypothesized for leaf or epidermal thickening (Jansen et al. 1998). In pea, leaf thickening is accompanied by a redistribution of chlorophyll away from the adaxial surface (Day and Vogelmann 1995).

– *Photosynthetic machinery repair*: D1 and D2 proteins form the core of PSII, and a very sensitive UV-B response is the rapid light-driven degradation of these two proteins. In this way, Jansen et al. (1998) suggested UV-B driven D1–D2 turnover is also part of a repair cycle, preventing accumulation of UV-inactivated PSII.

Salinity

Although salinity stress is related to water deficit by a decrease in water status, the presence of excess ions also appears to be detrimental to many plant processes. Thus, plants subjected to salinity stress appear to face two stresses at the same time. Based on general tolerance to salt stress, all plants can be roughly divided into two major groups: halophytes that can withstand even 20% of salts in the soil and non-halophytes or glycophytes that exhibit limited growth in the presence of sodium salts (usually higher than 0.01%). However, there are great differences in the level of salt tolerance within halophytes, which include eu-halophytes and facultative halophytes (Dajic 2006). Eu-halophytes show stimulation of productivity at moderate salinity (e.g. *Sarcocornia fruticosa*, *Arthrocnemum macrostachyum*; Redondo-Gómez et al. 2006; 2010a), while facultative halophytes show a slight growth enhancement at low salinity (e.g. *Plantago maritima*, *Aster tripolium*; Dajic 2006). Table 1.4 shows the salinity in which different halophytes have their optimal growth.

A few agricultural crops have moderate salt tolerance (e.g. barley, cotton, sugar beet, wheat, tomato, corn, rice, bean, beetle grass), and there can be a wide variation in salt tolerance among varieties or genetic lines of one crop species (Orcutt and Nilsen 2000).

Table 1.4 Optimal salinities for different halophytes

Species	Salinity (mM NaCl)	Reference
Arthrocnemum macrostachyum	171–510	Redondo-Gómez et al. (2010a)
Atriplex portulacoides	200	Redondo-Gómez et al. (2007)
Sarcocornia fruticosa	510	Redondo-Gómez et al. (2006)
Suaeda fruticosa	200–600	Khan et al. (2000)
Suaeda salsa	200	Lu et al. (2002)
Suaeda splendens	200–400	Redondo-Gómez et al. (2008)

Overall, mechanisms of salt tolerance are of two main types: those minimizing the entry of salt into the plant (or at least their accumulation in photosynthetic tissues) and those minimizing the concentration of salt in the cytoplasm (Munns 2002). This corresponds with two major adaptive strategies of plants to tolerate high salinity: *stress avoidance*, related to different physical, physiological and/or metabolic barriers with which the negative effects of stress are ameliorated, and *stress tolerance*, the linkage of adaptive mechanisms which enable successful survival despite the effects of stress internally (Dajic 2006).

Mechanisms of salt tolerance:

– *Salt exclusion*: Plants can limit salt accumulation in its tissues by inhibition of root uptake. However, in most plants such a mechanism is not efficient. Therefore, strategies have evolved to restrict salt transport into sensitive organs or tissues (Munns et al. 2002). Salt tolerance in beans (Awada et al. 1995), wheat or barley (Gorham 1993) is associated with Na^+ exclusion. Additionally, the presence of potassium (and calcium) ions has been shown to decrease Na^+ influx into plant cells. Salt tolerance in bread wheat and *Triticum turgidum* is associated with low rates of sodium transport to the shoots and high K^+/Na^+ discrimination (Gorham 1990; Munns et al. 2000). It has recently been reported that the genus *Triticum* expresses a range of genetic variation related to K^+/Na^+ discrimination (Munns et al. 2002). Salt tolerance in barley has been also associated with the ability to retain K^+ at elevated salinity (Chen et al. 2007).

  Sodium exclusion is accomplished by H^+-ATPase pumps and Na^+/H^+ antiporters. The H^+-ATPase complex creates the membrane electrical potential and provides the energy base for Na^+/H^+ antiporters. In fact, it has been suggested that the response of the H^+-ATPase genes to salinity may be a good indication of salinity tolerance in plants (Perez-Prat et al. 1994). According to Munns et al. (2002), the ability of plants to regulate the uptake and transport of salts is dependent on the following mechanisms: selectivity of uptake by root cells; preferential loading of K^+ rather than Na^+ into the xylem by the cells of the stele; removal of salts from the xylem in the upper parts of roots, the stem and leaf sheaths, based upon exchange of K^+ and Na^+; and loading of the phloem.

– *Salt excretion*: Halophytes frequently have anatomical structures designed for eliminating of excess salt ions from the plant into its environment. Salt glands and salt bladders are the main salt-excluding structures identified in plants. Both structures derive from epidermal tissue and have similar physiological function. Salt glands are embedded in the surface of leaves and salt bladders are specialized trichomes. The former are characteristics for, for example, *Avicennia* spp. mangroves (Griffiths et al. 2008) and the latter for members of the family Chenopodiaceae (Hagemeyer 1997; Orcutt and Nilsen 2000).

– *Intracellular ion compartmentation*: Sequestration of salts into leaf and/or shoot vacuoles is typical attribute of dicotyledonous halophytes. This accumulation is dependent on vacuolar H^+-translocating enzymes and tonoplast Na^+/H^+ antiporters, which are induced by saline environment (Barkla and Pantoja 1996). An immediate effect of salt stress is vacuolar alkalization, linked with Na^+/H^+ antiporters activity of tonoplast vesicles (Hasegawa et al. 2000). In this case, potassium ions and compatible solutes (see the section "Drought") should be accumulated in the cytoplasm in order to prevent dehydration and maintain the osmotic and ionic balance between these two compartments (Munns 2002). The effective capacity of halophytes to accumulate and utilize ions for osmotic adjustment to maintain turgor might explain their enhanced growth and control of their water regime in saline conditions (Dajic 2006). Succulence results from increased water uptake of the tissues, which may help to dilute absorbed salt ions (Munns et al. 1983). It is associated with the ability of intracellular compartmentation, to provide a larger capacity (volume of vacuoles) for salt storage.

Sarcocornia fruticosa (Chenopodiaceae) showed different photosynthetic areas with salinity treatments, which was a growth response (an increase in diameter of photosynthetic portions) mediated by an increase in turgor pressure (Redondo-Gómez et al. 2006). Otherwise, the *SOS1* (salt overly sensitive) locus has been described to be essential for Na$^+$ and K$^+$ homeostasis in *Arabidopsis*, as well as for the control of the long-distance Na$^+$ transport and loading Na$^+$ into the xylem under severe and mild salt stress, respectively (Shi et al. 2002). The *SOS2* gene is required for intracellular ion homeostasis (Liu et al. 2000).

Heavy Metals

Heavy metal pollution is gaining in importance day by day due to its obvious impact on human health through the food chain. Several vegetable, fruit and cereal crops are reported to accumulate heavy metals (Pieczonka and Rosopulo 1985; Mejuto-Marti et al. 1988; Prasad 1997). Heavy metal pollution is increasing in the environment due to industrial and agricultural activities such as mining and smelting of metalliferous ores, wastewater irrigation, and abuse of chemical fertilizers and pesticides (Redondo-Gómez et al. 2010a, b). Certain phosphate fertilizers have been found to contain high levels of cadmium and other trace metals (Roberts et al. 1994).

An excess of an essential or nonessential element can have detrimental effects on plant growth and development. The survival of plants growing on contaminated soils is considered to be the result of tolerance rather than avoidance since no plant has the ability to prevent metal uptake but can only restrict it (Baker 1981). However, plants differ among species with respect to the concentration of a specific metal they can tolerate. Plants growing on soils contaminated with high levels of metals are referred to as metallophytes, which have developed three basic strategies for growing in metalliferous soils (Baker 1981): (1) excluders, which prevent metal from entering their aerial parts over a

broad range of metal concentrations in the soil; (2) indicators, which take up metals at a linear rate relative to the concentration of metal in the soil; and (3) accumulators, which allow the uptake of very high levels of ions to the extent of exceeding the levels in the soil. Plants with enhanced tolerance versus pollutants are a promising tool in efficient bioremediation of areas contaminated with heavy metals.

Overall, metal tolerance mechanisms in plants include (Tomsett and Thurman 1988; Prasad 1997) the following:

– *Compartmentation*: There is some relationship between tolerance and accumulation characteristics in higher plants (Kuboi et al. 1987). The sequestering of metals in tissues (cell walls of roots and leaves) or cellular compartments (vacuoles), which are less sensitive to metals, away from metabolically active compartments (cytosol, mitochondria or chloroplast), has been described as a tolerance mechanism (Weis and Weis 2004). Manganese was accumulated in the cell walls of epidermis, collenchyma, bundle sheath cells and in a vacuolar compartment in the petioles of *Acanthopanax sciadophylloides* and in the leaves of tea plant (*Thea sinensis*) (Memon et al. 1980, 1981). Furthermore, Memon and Yatazawa (1984) explained that Mn was chelated with oxalic acid in a vacuolar compartment. With excess of Ni, vacuolization was observed in leaf mesophyll cells of *Brassica oleracea* (Molas 1997). Also, cereals are reported to accumulate trace metals (Piotrowska and Dudka 1994; Rivai et al. 1990). The mechanism involved in this preferential accumulation is not known.

– *Metal excretion*: Excretion is one of the important mechanisms of heavy metal tolerance. It has been shown that metals can be excreted in salt crystals released through salt glands of some halophytes (Krauss 1988). Redondo-Gómez et al. (2011) suggested that salinity could increase metal excretion and favour the tolerance and recovery of the photosynthetic apparatus of *Spartina densiflora* to the toxic action of zinc. Tobacco plants actively exclude Cd by forming and excreting

Cd/Ca-containing crystals through the head cells of trichomes (Choi et al. 2001).

– *Chelation*: A major factor governing the toxicity of a metal in soil is its bioavailability. Thus, to avoid undesirable metal penetration, plants are able to extrude material that can chelate free metallic cations in the extracellular space. Toxic metals can also be trapped once they are inside the cells (Bertrand et al. 2001).

– *Extracellular metal sequestration*: Differences in Al tolerance between several bean species have been attributed to the capacity of roots to exude citric acid, a strong Al chelator. Similar results were found for monocotyledons (barley, wheat, maize) for which better resistance to Al toxicity is associated with root exudation of citric acid, succinic acid and other organic acids (Bertrand et al. 2001). Root secretion includes organic ligands (e.g. carbohydrates, organic acids, nucleic acids) and inorganic ligands (e.g. Cl^-, SO_4^{2-}, NH_4^+, CO_3^{2-}). These substances functions as ligands to be chelated with heavy metals ions (Dong et al. 2007). Malate, citrate and oxalate are carboxylates exuded in the rhizosphere and implicated in the complexation of metals (Hinsinger 2001). Rice plant secretes phytosiderophores (amino acids) that can form much more stable complexes than carboxylates with Cd, Cu, Fe and Zn (Xu et al. 2005; Dong et al. 2007). Cambrollé et al. (2008) found that heavy metals combined as complex with oxides of Fe and Mn was accumulated in *Spartina densiflora* and *S. maritima* rhizospheres much more than in non-rhizosphere soil.

– *Intracellular metal sequestration*: Metallothioneins (MTs) and phytochelatins (PCs) are two protein families capable of sequestering metals. They are both cysteine-rich polypeptides having the ability to form metal-thiolate clusters (Cobbett and Goldsbrough 2002). MTs have characterized as gene-encoded proteins, whereas PCs are smaller enzymatically synthesized polypeptides (molecular weights of 5,000–20,000 and 500–2,300 Da, respectively). Although the precise physiological function of MTs has not yet been fully elucidated, expression and regulation of *Arabidopsis* MT genes have revealed that MTs have distinct functions in metal homeostasis, especially for Cu (Guo et al. 2003; Gasic and Korban 2006).

PCs are enzymatically synthesized directly from glutathione in the presence of metal ions by the enzyme PC synthase (EC.2.3.2.15) (Grill et al. 1989), and they are considered to have an important role in the cellular metal homeostasis (Steffens 1990). PCs also protect plant enzymes from heavy metal toxicity, and metal-requiring apoenzymes have been reactivated by PCs (Kneer and Zenk 1992). PC synthase genes in wheat (*TaPCS1*) (Clemens et al. 1999) and in *Arabidopsis* (*AtPCS1*) (Lee and Korban 2002) are regulated at the transcriptional level. However, transcriptional regulation of *AtPCS1* in *Arabidopsis* disappears as plants grow older (Lee and Korban 2002). Otherwise, under heavy metal stress, a high cysteine biosynthesis rate is required for the synthesis of GSH and PCs. *O*-acetylserine(thiol)-lyase (OASTL) is a key enzyme of a plant sulphur metabolism that catalyses the formation of Cys which serves as a precursor for GSH (Gasic and Korban 2006). Arabidopsis OASTL gene (*Atcys-3A*) has been described to be involved in cadmium tolerance (Domingues-Solis et al. 2001).

Biotic Stressors

Herbivory

Most of what we know about the mechanisms of herbivory tolerance in plants derives from studies of plant responses to mammalian (McNaughton 1983; Belsky et al. 1993; Lennartsson et al. 1998; Tiffin 2000). Empirical evidence indicates that relatively low levels of damage can be completely compensated by plants in terms of fitness. Further increments in the intensity of damage result in a decreasing ability to maintain

complete tolerance (Chapin and McNaughton 1989; Fornoni and Núñez-Farfán 2000; Fornoni et al. 2003).

Several plant traits that may buffer losses of fitness have been considered as components of tolerance (Strauss and Agrawal 1999; Fornoni et al. 2003):

– *Photosynthetic enhancement*: Partial defoliation may result in an increased supply of leaf cytokinins or root-derived cytokinins, which have been shown to increase net CO_2 fixation as a result of enhanced assimilate transport and nutrient uptake (Trumble et al. 1993). Furthermore, increases in production of the carboxylating enzyme ribulose bisphosphate carboxylase and chlorophyll contents in the remaining leaf tissue have been described, which might increase photosynthetic activity (Thorne and Koller 1974; Satoh et al. 1977).

– *High relative growth rates*: New leaves may become larger due to increase in cell size via mesophyll elongation or increased cell division (Satoh et al. 1977). Cell expansion may be influenced by chloroplast enlargement due to starch accumulation or via increased turgor pressure (Milthorpe and Moorby 1979).

– *Activation of dormant meristems*: When dominant apical meristems are removed, as the new growth tissue tends to be more succulent than older (lignified foliage), an increase in meristematic activity at nonapical locations can lead to increased branching (Trumble et al. 1993). Simulated herbivory in *Convolvulus chilensis* significantly affected plant architecture; there was an increase in number of stems/plant height (González-Teuber and Gianoli 2007).

– *Reallocation of stored resources*: Induced reallocation can occur rapidly while the herbivore is still present in order to safeguard resources, sequestering the primary metabolites from exposed tissues into storage organs (Orians et al. 2011). Also, resources can be mobilized from storage organs to growing tissues after the herbivory threat is over (Steinbrenner et al. 2011). Smith et al. (1990) found that amino acid concentrations in extrafloral nectaries increased following simulated herbivory on *Impatiens sultani*. Root feeding by the scarabaeid *Phyllopertha horticola* caused reallocation of resources to reproductive growth in an annual herb (Gange and Brown 1989).

– *Delay in senescence*: Increased supply of root-derived cytokinins as consequence of partial defoliation, which inhibit mRNA, suppress protein and enzyme degradation, increase stomatal opening, and maintain cell membrane integrity (Waring et al. 1968; Trumble et al. 1993). Studies suggest that plant architecture like branching ability and the activation of secondary meristems are the most important traits that allow plants to regrow and tolerate grazing (McNaughton 1983; Martínez Moreno et al. 1999). In contrast, plant usually respond biochemically and physiologically at the leaf or branch level when damaged by small herbivores or pathogens (Welter 1989; Marquis 1996). Traits that confer tolerance are controlled genetically and therefore are heritable traits under selection (Strauss and Agrawal 1999).

Pathogens and Parasites

Host plants have evolved defence mechanisms (i.e. resistance and/or tolerance) against pathogen and parasites attacks. Tolerance is defined as the ability to compensate in part for fitness decrements caused by pathogens or parasites, while resistance refers to traits that prevent infection or limit its extent (Boots and Bowers 1999). This allows infected host to live longer, which increases the infectious period and therefore increases rather than decreases pathogen prevalence, leading to a positive feedback (Best et al. 2008).

Clarke (1986) proposed the partitioning of tolerance into three types, namely, (1) tolerance to the parasite, the ability of a plant to endure the effects of levels of parasite infection; (2) tolerance to disease, the ability of a plant to endure the effects of levels of disease (i.e. host physiological damage); and (3) overall tolerance, the ability of a plant to endure the levels of parasite infection and disease.

Strategies that limit the extent of disease in an infected host (i.e. barriers to infection, immune response and rapid cell death in the immediate region around the wound) are sometimes interpreted as helping the host tolerate infection, but these are normally termed resistance strategies (Clarke 1986) because they combat the pathogen by limiting its spread (Roy and Kirchner 2000).

Tolerance often involves some degree of compensation for disease damage, for example (Roy and Kirchner 2000), namely, (1) *Photosynthetic enhancement*: Plants can tolerate infection by increasing the chlorophyll concentration in leaves, which might increase photosynthetic activity. (2) *Growth enhancement*: Plants can increase the size of new leaves or the number of new branches in response to a pathogen or parasite attack. (3) *Advancing the timing of bud break*: The advancement of bud break can increase the available photosynthetic area of the plant, and thus the net CO_2 fixation. (4) *Delaying the senescence of infected tissue*: The onset of senescence may be delayed due to increased levels of cytokinins (Trumble et al. 1993). (5) *Increasing nutrient uptake*: An enhancement of phosphorous uptake by a pathogen may increase plant development (Wehner et al. 2010).

These mechanisms of plant tolerance are similar to those developed against herbivory (Marquis 1992; Rosenthal and Welter 1995; Strauss and Agrawal 1999). However, in contrast to pathogen attack, herbivore attack is frequently associated with wounding, and the recognition of herbivore attack frequently involves modifications of a plant's wound response (Walling 2000). Blouin et al. (2005) demonstrated that the presence of belowground invertebrate activities improved tolerance of rice to parasitic nematodes, increasing plant biomass and photosynthetic activity. This response was mediated by the expression of three stress-responsive genes, coding for lipoxygenase, phospholipase D and cysteine protease. The host plant response to parasite attack is also associated with the formation of pathogenesis-related proteins (PRs) throughout the plant. The PRs occurring in the inter- and intracellular spaces are quite soluble, highly reactive and induced by signalling compounds such as salicylic acid, jasmonic acid, ethylene, xylanase and polypeptides (Orcutt and Nilsen 2000). Furthermore, the PR genes were found to be negatively modulated by an abscisic acid-inducible mitogen-activated protein kinase (MAPK) gene (*OsMAPK5*) in rice (Xiong and Yang 2003).

Allelopathy

Allelopathy refers to any direct or indirect harm of one plant (or microorganism) on the germination, growth or development of other plants through the production of chemicals (*allelochemicals*) deposited into the environment (Molisch 1937). It differs from competition wherein plants compete for a common resource. Allelopathic interactions between plants have been implicated in the pattering of vegetation and weed growth in agricultural systems and in inhibition of growth of several crops (Rice 1987; Liu and Lovette 1993; Devi et al. 1997).

The allelochemicals (see Table 1.5) concerned in higher plants interaction are typical secondary metabolites and appear to be mainly low-molecular-weight compounds of relatively simple structure. Most allelochemicals that have been positively identified are either volatile terpenes or phenolic compounds (Harborne 1993). The standard modes of release for allelochemicals are volatilization, residue decay, leaching or root exudation (Devi et al. 1997).

Tolerance of allelopathic compounds in plants could be due to a number of different processes like (1) *Exclusion*: The capacity to exclude allelochemicals at the root or leaf surface is due to morphological characteristics of the organ surface. Thus, the permeability of phenolic compounds into leaves varies depending on the lipid composition of the cuticle (Shafer and Schönherr 1985). (2) *Compartmentation*: Some tolerance to allelochemicals can be attributed to the ability to deposit these compounds in nonmetabolic compartments, such as vacuole or cell wall. Esculin and scopolin are accumulated in wheat vacuoles (Werner and Maitile 1985). (3) *Excretion*: The excretion of toxins that have been absorbed from