PHYSIOLOGY AND MOLECULAR BIOLOGY OF STRESS TOLERANCE IN PLANTS

Physiology and Molecular Biology of Stress Tolerance in Plants

Edited by

K.V. MADHAVA RAO
Andhra University, India

A.S. RAGHAVENDRA
University of Hyderabad, India

and

K. JANARDHAN REDDY
Osmania University, India

Springer

A C.I.P. Catalogue record for this book is available from the Library of Congress.

ISBN-10 1-4020-4224-8 (HB)
ISBN-13 978-1-4020-4224-9 (HB)
ISBN-10 1-4020-4225-6 (e-book)
ISBN-13 978-1-4020-4225-6 (e-book)

Published by Springer,
P.O. Box 17, 3300 AA Dordrecht, The Netherlands.

www.springer.com

Cover Illustration:

Statice (*Limonium latifolium*) plants are utilized as a model to understand metabolic adaptations to environmental stress. Synthetic pathway to the osmoprotectant beta-alanine betaine was discovered in this species and cDNA for beta-alanine N-methyltransferase involved in this pathway is utilized for metabolic engineering of crops for enhanced tolerance to salinity and drought (See Chapter 9).

Printed on acid-free paper

Printed in the Netherlands.

WE THANK

Mrs Syamala Madhava Rao
Mrs Rama Raghavendra
Mrs Sunanda Janardhan Reddy

About the Editors

K.V. Madhava Rao obtained Ph.D. from Sri Venkateswara University, Tirupati, India. He held different teaching positions as Lecturer in Botany at Sri Venkateswara University, as Reader in Botany and as Professor of Botany at Andhra University, Visakhapatnam. His research areas include stress physiology and molecular biology, seed ageing mechanisms and nutritional physiology. He has published 75 research papers and edited few books. He has retired recently and is associated with Biotechnology Division, Andhra University.

A.S. Raghavendra obtained Ph.D. from Sri Venkateswara University, Tirupati, India. He is currently the Dean, School of Life Sciences at University of Hyderabad, Hyderabad. His research interests include photosynthesis, particularly of C_4 plants and stomatal guard cell signal transduction. He has published more than 160 research papers and edited 4 books. He is in the editorial board of several journals including Photosynthesis Research.

K. Janardhan Reddy obtained Ph.D. from Osmania University, Hyderabad, India. He is on the faculty for the last 25 years as Lecturer, Reader and Professor. His fields of research include plant nutirtional physiology and biotechnology of medicinal plants. He published several research papers and authored / edited few books.

CONTENTS

Chapters

LIST OF CONTRIBUTORS

K. AKASHI
Graduate School of Biological Sciences
Nara Institute of Science and Technology
Nara 630-0101
Japan

ZORA DAJIC
Faculty of Agriculture
University of Belgrade
Nemanjina 6
11080 Belgrade
Serbia and Monte Negro

KSENIJA GASIC
Department of Natural Resources and Environmental Sciences
University of Illinois
Urbana
Illinois 61801
USA

LAWRENCE V. GUSTA
Department of Plant Sciences
University of Saskatchewan
Saskatoon
Saskatchewan S7N 5A8
Canada

RAMANDEEP KAUR
Horticultural Sciences Department
Plant Molecular and Cellular Biology Program
University of Florida
Gainesville FL 32611-0690
USA

SCHUYLER S. KORBAN
Department of Natural resources and Environmental Sciences
University of Illinois
Urbana
Illinois 61801
USA

AGEPATI S. RAGHAVENDRA
Department of Plant Sciences, School of Life Sciences,
University of Hyderabad, Hyderabad 500 046
India

K.V. MADHAVA RAO
Biotechnology Divission
Andhra University
Visakhapatnam 530003
India

BALA RATHINASABAPATHI
Horticultural Sciences Department
Plant Molecular and Cellular Biology Program
University of Florida
Gainesville FL 32611-0690
USA

K. JANARDHAN REDDY
Department of Botany
Osmania University
Hyderabad 500 007
India

ATTIPALLI R. REDDY
Department of Plant Sciences, School of Life Sciences,
University of Hyderabad, Hyderabad 500 046
India

NAVINDER SAINI
Department of Plant Molecular Biology
University of Delhi South Campus
New Delhi-110021
India

THOMAS D. SHARKEY
Department of Botany
University of Wisconsin-Madison
430 Lincoln Dr
Madison
Wisconsin 53706
USA

BRIAN S. SCHILLING
Arvesta Canada Inc
Saskatoon
Saskatchewan S7L 0K4
Canada

STEPHEN M. SCHRADER
Department of Botany
University of Wisconsin-Madison,
430 Lincoln Dr
Madison
Wisconsin 53706
USA

K. TAKAHARA
Graduate School of Biological Sciences
Nara Institute of Science and Technology
Nara 630-0101
Japan

RUSSELL G. TRISCHUK
Department of Plant Sciences
University of Saskatchewan,
Saskatoon
Saskatchewan S7N 5A8
Canada

AKHILESH K. TYAGI
Department of Plant Molecular Biology
University of Delhi South Campus
New Delhi-110021
India

SHUBHA VIJ
Department of Plant Molecular Biology
University of Delhi South Campus
New Delhi-110021
India

M. WISNIEWSKI
USDA-ARS
2217 Wiltshire Road
Kearneysville WV 25430
USA

A. YOKOTA
Graduate School of Biological Sciences
Nara Institute of Science and Technology
Nara 630-0101
Japan

PREFACE

Increasing agricultural productivity to meet the demands of growing population is a challenging task. Abiotic stresses are among the major limiting factors on agriculture. The knowledge and research programmes on the physiology and molecular biology of stress tolerance are certainly helpful to counter act this negative effect to a great extent. The present literature deals in detail mostly with plant responses to different abiotic stresses. There have been extensive studies, in the past few decades, on the physiology and biochemistry of plant responses to abiotic stress conditions, in the laboratory as well as in the field. However, the interest has shifted to molecular biology of stress tolerance, modes of installing tolerance mechanisms in crop plants. Microarray technology, functional genomics, development of high throughput proteomics would benefit and guide the physiologists, molecular biologists and biotechnologists to enhance stress tolerance in plants. We therefore, felt very strongly that there is an immediate and urgent need for a textbook on this important topic.

This book would be an ideal source of scientific information to the postgraduate students, research workers, faculty and scientists involved in agriculture, plant sciences, molecular biology, biochemistry, biotechnology and related areas.

We would like to thank the authors for their interest and cooperation in this exciting venture. We are grateful to Jacco Flipsen and Noeline Gibson of Springer for their continuous support and technical advice in bringing out the book.

K.V. Madhava Rao
A.S. Raghavendra
September 2005. K. Janardhan Reddy

CHAPTER 1

INTRODUCTION

K.V. MADHAVA RAO

Biotechnology Divission, Andhra University, Visakhapatnam 530003, INDIA
(email: kvmadhavarao@yahoo.co.in)

Keywords: *Abiotic stresses, functional genomics, genetic engineering, gene products, gene transfer, signal transduction*

Higher plants are sessile and therefore cannot escape from abiotic stress factors. They are continuously exposed to different abiotic stress factors without any protection. On the other hand animals are mobile and can escape the direct harsh conditions. The immobile nature of plants needs more protection. This enabled them to develop unique molecular mechanisms to cope with different stress factors. However, variations do exist in tolerance mechanisms among plants. Certain morphological features of some plants however, make them avoid stress factors. But it may not be the case in all plants. The only option for plants is to 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 abiotic stresses. Those plants that have better tolerant, resistant, protective and acclimation mechanisms alone can survive while others cannot. Gene products play a key role in the molecular mechanisms of stress tolerance in plants.

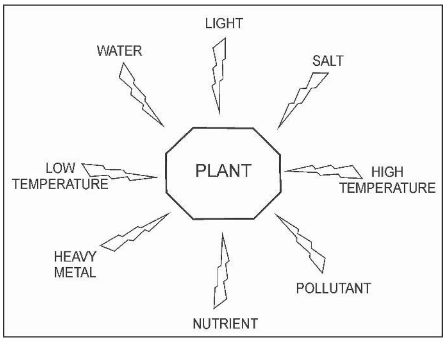

Figure 1. *Some common abiotic stress factors that affect plants*

K.V. Madhava Rao, A.S. Raghavendra and K. Janardhan Reddy (eds.),
Physiology and Molecular Biology of Stress Tolerance in Plants, 1–14.

Abiotic stresses are commonly caused by drought, salinity, high or low temperatures, light, deficient or excess nutrients, heavy metals, pollutants etc either individually or in combinations (Figure 1). The stress caused by abiotic factors alter plant metabolism leading to negative effects on growth, development and productivity of plants (Figure 2). If the stress become harsh and/or continues for longer period it may lead to unbearable metabolic burden on cells leading to reduced growth and in extreme cases results in plant death. However, plant stress may vary from zero to severe through mild and moderate levels. In nature, plants may not be totally free from stresses. Plants are expected to experience some degree of stress of any factor or factors. To combat these stresses, plants exhibit several mechanisms which make them withstand the stress with the formation of new molecules and molecular mechanisms of stress tolerance.

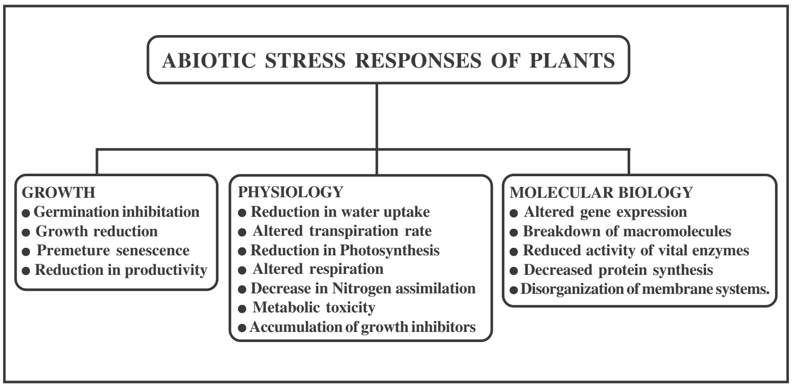

***Figure 2**. Some of the common plant responces to abiotic stresses*

Avoidance mechanisms though considered to be advanced, which by modification of morpholgy and anatomy prevents plants from various stress factors, they may not be of much importantce in immediate crop improvement. Therefore, the immediate emphasis is on the development of tolerance mechanisms in plants, since plants exhibit great variations in their tolerance mechanisms, within species, between species and among the plants of different groups. These variations are highly significant in developing stress tolarence in plants.

1. THE MOLECULAR “CROSS ROADS”

Most of the stress factors produce certain common effects on plants although each stress factor has got its own specific effects. The common targets of most of abiotic stress factors are the membrane systems, which under normal conditions perfom several life maintenace processes (Figure 3.). Therefore, all membrane involving processes will be affected by abiotic stresses. Active oxygen species (AOS) are always associated with aerobic life (Vranova et al., 2002). Abiotic stresses such as water stress, salt stresss, temperature stress, light stress, nutrient stress, heavy metal stress and pollution stress are known to accelerate the production of AOS in plants that cause damage to membrane systems and other cellular processes (Dat et al., 2000; Mittler, 2002; Mittler et al., 2004). Antioxidative systems, both enzymatic and nonenzymatic systems, play an important role in balancing and preventing oxidative damage (Bowler et al., 1994; Foyer et al., 1994). However, the prodction and efficiency of the antioxidative systems depend on plant type and genetic make up of the plant. In spite of the close association of AOS with aerobic life, their production, role, stress involvement, importance in signaling phenomena and their scavenging are not clearly elucidated. In addition, abiotic stresses

- ⋆ **Boundary Layer of cells and organelles**
- ⋆ **Metabolic compartmentations**
- ⋆ **Electron transport**
- ⋆ **Signal perception**
- ⋆ **ATP generation**
- ⋆ **Solute transport**
- ⋆ **Ion pumps**
- ⋆ **Ion channels**
- ⋆ **Carrier proteins**
- ⋆ **Electrical excitability**
- ⋆ **Anchoring points for proteins**

Figure 3. *Certain functions of plant membrane systems*

SOME OF THE PROMINENT ABIOTIC STRESS TOLERANCE MECHANISMS

- Activation of signaling factors
- Altered gene expression
- Accumulation of compatible solutes
- Synthesis of stress proteins
- Enhanced antioxidative metabolism
- Ion homeostasis and compartmentation
- Facilitated membrane transport
- Accumulation of polyamines
- Adjustment of hormonal balance

Figure 4. *Some of the prominent abiotic stress tolerance mechanisms*

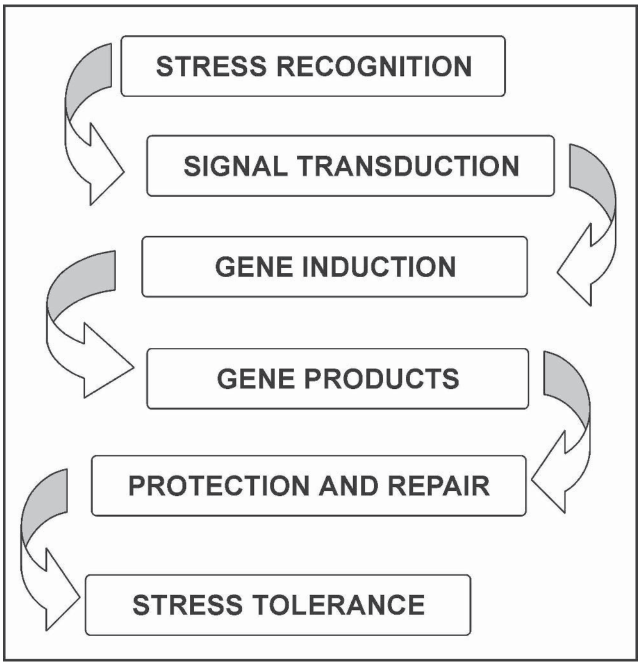

Figure 5. *The path of stress tolerance in plants*

affect photosynthesis, respiration, nitrogen assimilation, protein synthesis and several other processes (Figure 2). To combat stress effects plants develop some common tolerance mechanisms as well as stressor specific mechanisms to cope up with stress (Figure 4). However, the degree of tolerance varies from plant to plant, from low to high. Stress tolerance mechanisms start with stress perception followed by the formation of gene products that are involved in cellular protection and repair (Figure 5). The signal transduction pathways that detect stress play a crucial role in the induction of stress tolerance in plants (Smalle and Vierstra, 2004). One of the important ways to develop stress tolerance is by gene transfer (Figure 6).

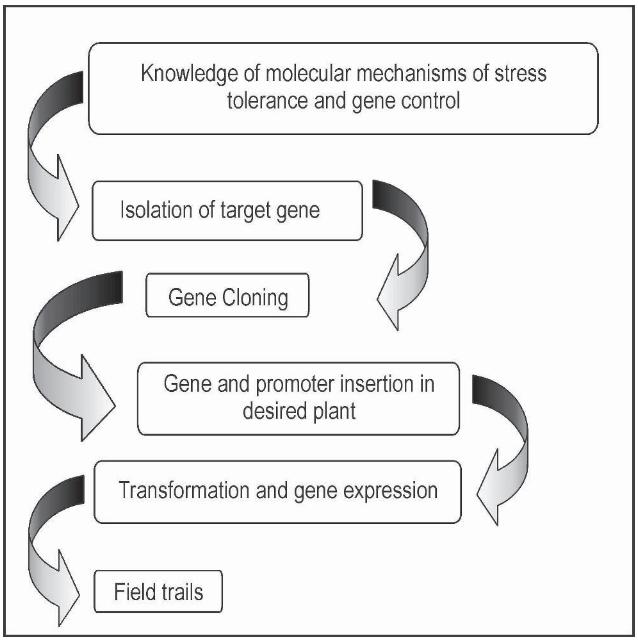

Figure 6. *Strategies of gene transfer in plants*

This book attempts to present an overview on the physiology and molecular biology of plant tolerance mechanisms in response to most important abiotic stress factors. The present chapter describes the scope of the articles included in this book. There have been some books published earlier on this topic (Jones et al., 1989; Fowden et al., 1993; McKersie and Leshem, 1994; Basra, 1994; Basra and Basra, 1997; Pessarakli, 1999; Cherry et al., 2000; Hirt and Shinozaki, 2002; Di Toppi and Pawlik-Skowronska, 2003; Ashraf and Harris, 2005; Jenks and Hasegawa, 2005; Chakraborty and Chakraborthy, 2005). Some of these books may either deal with physiology or molecular biology, but none on physiology and molecular biology together.

2. WATER STRESS

Drought leading to water stress in plants is a major problem in reducing agricultural productivity especially in tropical, semi-arid and arid regions of the world. Water deficits result from low and eratic rain fall, poor soil water storage and when the rate of transpiration exceeds water uptake by plants. The cellular water deficits results in the concentration of solutes, loss of turgor, change in cell volume, disruption of water potential gradients, change in membrane integrity, denaturation of proteins and several physiological and molecular components (Grifth and Parry, 2002; Lawlor 2002; Lawlor and Cornic, 2002; Raymond and Smirnoff, 2002: Parry et al., 2002; Bartels and Souer, 2003). The stress effects depend on the degree and duration of the stress, developmental stage of the plant, genotypic capacity of species and environmental interactions. Several attempts were made to understand the water stress recognition and the subsequent signal transduction (Bohnert et al., 1995; Leung and Giraudat, 1998). The gene induction leads to the formation of gene products such as proline, glycinebetaine, and other products, which may act to maintain cellular function through protection of cellular processes by protection of cellular structures and osmotic adjustments (Bray, 1993; 1997; 2002). Abscisic acid concentration increases under water stress as well as under some other abiotic stresses (Christmann et al., 2005). In fact abscisic acid is considered as a 'stress hormone' (Zeevaart and Creelman, 1988) although it may serve several other functions in the absence of stress. Understanding the functions of the various gene products formed, which are usually involved in osmotic adjustment, protection and repair of cellular structures, are of great value in evaluating water stress tolerance mechanisms and to develop water stress tolerant plants. A large number of genes with a potential role in water stress tolerance have been identified and characterized (Ingram and Bartels, 1996). In spite of the considerable progress made in understanding plant molecular responses to water deficits and its impact on whole-plant physiology, the details of water stress perception; signal transduction and molecular biology of water stress tolerance are yet to be evaluated (Chapter 2, Yakota, Takahara and Akashi).

3. SALT STRESS

Salinity affects agricultural production and its quality in arid and semiarid regions, where rainfall is limited and is not sufficient to transport salts from the plant root zone (Quesada et al., 2000; Tester and Davenport, 2003). Poor water management also results in salinity. The basis for salinity is evaporation in which water evaporates in a pure state leaving salts and other substances behind (Carter, 1975). Salinity arises due to increase in the concentration of salts like sodium chloride, sodium carbonate, sodium sulphate or salts of magnesium. The dominant salts are either sodium chloride or sodium sulphate or mixtures of the two. The saline soil management includes crop selection, crop stand establishment, leaching requirement, drainage and other reclamation practices. It is also anticipated that the importance of salinity as a breeding objective will increase in the future (Flowers and Yeo, 1995). The effect of salinity on plants is complex and its adverse effects include ion toxicity, water deficits and nutrient imbalance and deficiencies. Much information is available on morphological and anatomical adaptations in response to salinity (Poljakof-Mayber, 1975). Considerable information on physiological and molecular responses of plants to salinity stress is also available (Adams et al., 1992; Moons et al., 1995; Hasegawa et al., 2000; Munn, 2002; Zörb et al., 2005). Salt tolerance and resistance mechanisms are highly complex since the effects are diverse and are controlled by a number of genes or groups of genes (Flowers and Yeo, 1995). Salt tolerance is generally associated with regulated ion uptake, compartmentation of ions and gene products including stress proteins (Flowers and Yeo, 1986; Cheeseman, 1988; Winicov, 1998; Zhu, 2001). Ion homeostasis is an important component of salt tolerance (Zhu, 2002). However, based upon its complexicity, the mechanisms underlying salt tolerance are to be investigated in detail. Dajic in Chapter 3 deals in detail the molecular basis of salt tolerance in addition to related physiological, genetical and biotechnological aspects.

4. HIGH TEMPERATURE STRESS

High temperature stress in plants arises in response to many factors such as the exposure of plants to high ambient temperatures, exposure of germinating seeds to the soil which is warmed by absorbed infrared radiation from the sun, more plant transpiration followed by less water absorption, reduced transpiration capacity in certain plant organs, forest fires, natural gas blowouts, etc. Though, much work has been carried out at ultrastructural, molecular and gene expression level under different temperature extremes, the temperature perception and the molecules involved in the perception are not known clearly (Burke and Usda-Ars, 1988; Iba, 2002; Rao et al., 2002; Camejo et al., 2005). All the cells of an organism respond to high temperature stress. All organisms when exposed to rapid increases in external temperatures, generally 5 to 10 ^{0}C above normal growth temperatures for a period of few minutes to a few hours exhibit synthesis of an elite set of proteins called heat shock proteins (HSPs) which are not present, or are present in small quantities in unstressed organisms (Sridevi et al., 1999). These HSPs are involved in cellular repair, rescue, cleanup and/or protection during the stress and from its recovery. Understanding the mechanisms and development of thermotolerant plants is of great significance in tropical, semi-arid and arid regions of the world. Sharkey and Schrader (Chapter 4) emphasizes the effect of high temperature stress on various physiological and molecular biological processes and discusses several strategies for improving heat tolerance in plants.

5. FREEZING STRESS

Plants growing in temperate and frigid areas are exposed to freezing temperatures. It is well known that membrane systems are the primary sites of freezing injury to plants (Rudolf and Crowe, 1985; Hughes and Dunn, 1996; Thomashow, 1999). In addition, cell damage in response to freezing stress is also caused by protein denaturation. Freezing tolerance is characterised by changes in metabolite levels and enzyme activities (Levitt, 1980; Mazur, 1968; Steponkus, 1984; Guy, 1990). Freezing tolerance is associated with the accumulation of sugars, several types of proteins including heat shock proteins, lipids, abscisic acid and other products of altered metabolism (Siminovitch et al., 1968; Nagao et al., 2005). They are expected to depress the freezing point of the tissue, may act as nutrient and energy source and play a key role in rectifying the cellular damage caused by freezing stress. Freezing tolerance increases with decreasing water content. Abscisic acid accumulation also increases freezing tolerance. However, much information is not available regarding the freezing injury and tolerance mechanisms against freezing stress. Recently much interest has been shown towards the identification, characterization and functioning of genes with roles in freezing tolerance and the mechanisms involved in low temperature gene regulation and signal transduction (Thomashow, 1999). In this connection, a systems biology approach to study cold acclimation of plants possesses great significance (Chapter 5: Trischuk, Schilling, Wisniewski and Gusta).

6. PHOTOOXIDATIVE STRESS

Among the abiotic stress factors, light stress is one of the important environmental constraints that limit the efficiency of photosynthesis and plant productivity (Foyer and Noctor, 2000; Das, 2004; Reddy et al., 2004). When absorbed light energy exceeds the capacity for light energy utilization in plant photosynthesis, then the photosynthetic efficiency will be reduced due to the formation of AOS, which can damage photosynthetic apparatus and chloroplast components. In order to mitigate the photooxidative stress, plants have developed certain strategies of tolerance mechanisms (Mittler, 2002; Mittler et al., 2004). Understanding how plants respond to light stress has a high priority in several plant biotechnological programmes. Foyer et al., (1994) and Apel and Hirt, (2004) reviewed the mechanism of photooxidative stress tolerance in higher plants. Chapter 6 (Reddy and Raghavendra) covers the recent advances in elucidating the pivotal role of AOS metabolism in response to photooxidative stress, in addition to variuos physiological and molecular strategies of plants to develop tolerance mechanisms under photoinhibitory conditions.

7. NUTRIENT STRESS

Plant growth, development and yield are contributed by 17 essntial elements (Hopkins and Hüner, 2004). Plants may be subjected to nutrient stress due to several factors such as negligence of the farmer leading to nutrient deficiency or excess supply of nutrients because of the farmer's over enthusiasm to obtain more yield, natural deposits or mining processes etc. Nutrient stress and associated metabolic disorders decrease plant growth and yield (Lynch and Brown, 2001). Plant growth and metabolism is also affected by heavy metal and salinity stress. Developing nutrient stress tolerance in crop plants may help to extend agriculture to unexplored harsh and nutrient poor soils (Cobbet, 2000; Clemens, 2001). Plant growth response to low or excess nutrient stress and related remedial measures to improve crop yields are discussed by Reddy (Chapter 7).

8. HEAVY METAL STRESS

Supra-optimal concentrations of heavy metals such as Cd, Pb, Hg, Cu, Zn and Ni affect growth, development and yield of plants (Pahlsson, 1989; Sresty and Rao, 1999). However, Cu, Zn and Ni are essential micronutrients at low concentrations. Heavy metals affect several physiological (Barceló and Poschenrieder, 1990) and metabolic processes (Van Assche and Clijsters, 1990; Hall, 2002; Schützendübel and Polle, 2002). Plants have developed several mechanisms that control and respond to the uptake and accumulation of both essential and nonessential heavy metals (Cobbet and Goldsbrough, 2002). These tolerance mechanisms in plants vary from species to species and their genetic background. The important heavy metal tolerance mechanisms include, metal binding to wall, reduced transport across the cell membrane, active efflux of metals, compartmentalization, chelation and sequestration of heavy metals by particular ligands such as phytochelatins and metallothioneins (Tomsett and Thurman, 1988; Cobbet and Goldsbrough, 2002). Antioxidative systems are also involved in heavy metal tolerance (Rao and Sresty, 2000). Certain plants specially many brassicaceae family members including numerous Thalspi species have relatively high tolerance for heavy metals such as Ni and Zn and act as hyperaccumulators which can be used for phytoremediation (Clemens, 2001; Freeman et al., 2005). Gasic and Korban (Chapter 8) explore different heavy metal tolerance mechanisms and discuss the importance of hyperaccumulators in phytoremidiation.

9. METABOLIC ENGINEERING

To cope up with different abiotic stresses plants alter their metabolic pathways to adjust to changed environments (Rathinasabapathi, 2000). The metabolic pathways such as proline, glycinebetaine, polyols, antioxidant components become more active to keep the plant survive under stress conditions. However, the initiation and efficiency of these pathways differ from species to species or genotype to genotype to a great extent. Installing these stress tolerating pathways utilise recombinant DNA technology (Stephanopoulos, 1999). Stitt, (1995) has given an interesting account of production of transgenic plants for metabolic design. The use of novel approaches combining the techniques of genetic, genetic engineering and molecular biology are expected to provide exciting avenues for future research (Madlung and Comai, 2004). Understanding the mechanisms by which plants perceive and transduce stress signals to initiate adaptive response is essential for engineering stress-tolerant crop plants (Xiong and Zhu, 2001). In this direction, various metabolic engineering strategies for stress tolerance in plants is presented by Rathinasabapathi and Kaur in Chapter 9.

10. FUNCTIONAL GENOMICS

Development of techniques such as cDNA libraries, molecular markers, PCR amplifications and microarray technologies made it possible to determine transcript patterns and to identify differentially expressed genes in plants. Comparision of transcript patterns with proteome data may provide information whether the intracellular concentration of specific proteins is preferentially regulated at the level of transcription or by post-transcriptional mechanisms. These techniques help to record the genome wide expression patterns very rapidly and with high accuracy (Kuhn, 2001; Derra, 2004). The information so obtained can be integrated with functional genomic information that contributes to our understanding of the correlation between genes and phenotype of a plant. Based on these techniques, Tyagi, Vij and Saini (Chapter 10) describe the genome-wide approach to develop stress tolerance in plants.

11. PROPELLING FORWARD

Various physiological and molecular mechanisms in association with the applications of plant breeding and genetic engineering can improve the scope for stress tolerance in plants (Figure 7). The present literature on molecular biology deals in detail mostly with abiotic stress tolerance and modes of installing tolerance mechanisms in plants with a view to have desired yields even under harsh environments. The importance given to this line of research is quite evident from the large number of publications appearing on this topic every year. This trend will continue in future. New molecules, their new roles, new concepts and new molecular mechanisms, more attention on products related to stress inducible genes, importance of signal transduction pathways, microarray analyses and functional genomics pervade the field of abiotic stress tolerance. We there-

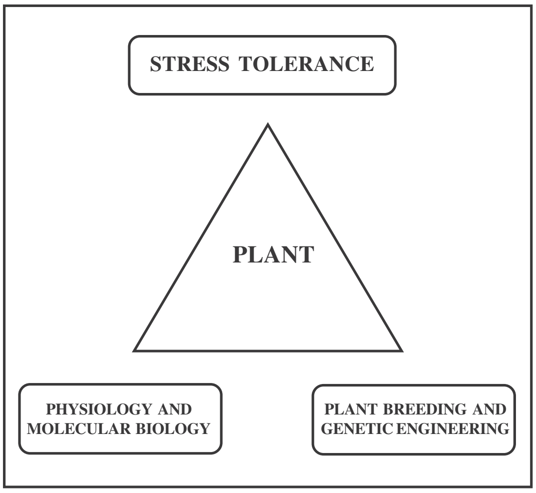

Figure 7. *Knowledge of physiology and molecular biology combined with plant breeding and genetic engineering techniques are expected to enhance stress tolerance in plants*

fore felt very strongly that there is an immediate and urgent need for an advanced level textbook on this important topic. This book would not only review the present status but also trigger further research on this exciting field.

12. REFERENCES

Adams, P., Thomas, J.C., Vernon, D.M., Bohnert, H.J. and Jensen, R.G. (1992) Dstinct cellular and organismic responses to salt stress. *Plant and Cell Physiol.* **33,** 1215-1223.

Apel, K, and Hirt, H. (2004) Reactive oxygen species: metabolism, oxidative stress and signal transduction. *Annu. Rev. Plant Biol.* **55,** 373-379

Arora, R. (2004). *Adaptations and Responses of Woody Plants to Environmental Stresses.* Haworth Press Inc., New York.

Ashraf, M. and Harries, P.J.C. (eds) (2005). *Abiotic Stresses: Plant Resistance through Breeding and Molecular Approaches.* Haworth Press Inc., New York.

Barceló, J. and Poschenrieder, Ch. (1990) Plant water relations as affected by metal stress: A review. *Plant Nut.* **13,** 1-37.

Bartels, D. and Souer, E. (2003). Molecular responses of higher plants to dehydration. In: *Plant responses to abiotic stress. Vol. 4. Topics in Current Genetics.* Berlin; Springer; p.9-38.

Basra, A.S. (1994). *Srtess-induced Gene Expression in Plants.* Taylor and Francis, London.

Basra, A.S. and Basra, R.K. (eds) (1997). *Mechanism of Environmental Stress Resistance in Plants.* Harwood Academic Publishers. The Netherlands.

Bohnert, H.J., Nelson, D.E., Jensen, R.G. (1995) Adaptations to environmental stresses. *Plant Cell* **7,** 1099-1111.

Bowler, C., Van Camp, W., Van Montagu, M. and Inze, D. (1994) Superoxide dismutase in plants. *Crit. Rev.Plant Sci.* **13:** 199-218.

Bray, E.A. (1993). Molecular responses to water deficit. *Plant Physiol*. **103,** 1035-1040.

Bray, E.A. (1997). Plant responses to water deficit. *Trends Plant Sc.* **2,** 48-54.

Bray, E.A. (2002). Classification of genes differentially expressed during water-deficit stress in *Arabidopsis thaliana*: an analysis using microarray and differential expression data. *Ann. Bot.* **89,** 803-811.

Burke, J.J. and Usda-Ars, K.A.O. (1988). The heat-shock response in higher plants: a biochemical model. *Plant Cell Environ*. **11,** 441-444.

Camejo, D., Rodriguez, P., Morales, M.A., Dell'Amico, J.M., Torrecillas, A. and Alarcon, J.J. (2005). High temperature effects on photosynthetic activity of two tomato cultivars with different heat susceptibility. *J. Plant Physiol.* **162,** 281-289.

Carter, D.I. (1975). Problems of salinity in agrculture. In: Poljakoff-Mayber A and Gale J eds. *Plants in Saline Environments.* Springer-Verlag Berlin. pp. 25-35

Chakraborty, U. and Chakraborty, B. (eds) (2005). *Stress Biology*, Narosa Publishing House, New Delhi.

Cheeseman, J.M. (1988). Mechanisms of salinity tolerance in plants. *Plant Physiology.* 87: 547-550.

Cherry, J.H., Robert, D.L. and Rychter, A. (eds) (2000). *Plant tolerance to Abiotic Stresses in Agriculture: Role of Genetic Engineering.* Kluwer Academic Publishers, The Netherlands.

Christmann, A., Hoffmann, T., Teplova, I., Grill, E. and Müller, A. (2005) Generation of active pools of abscisic acid revealed by in vivo imaging of water stressed Arabidopsis. *Plant Physiol*. **137,** 209-219.

Clemens, S. (2001). Molecular mechanisms of plant metal tolerance and homeostasis. *Planta.* **212,** 475-486.

Cobbet, C. and Goldsbrough, P. (2002). Phytochelatins and metallothioneins: roles in heavy metal detoxification and homeostasis. *Annu. Rev. Plant Biol.* **53,** 159-182.

Cobett, C.S. (2000). Phytochelitins and their roles in heavy metal detoxification. *Plant Physiol*. **123,** 825-832.

Das, V.S.R. (2004). *Photosynthesis: Regulation Under Varying Light Regimes*. Science Publishers, Inc., UK.

Dat, J., Van Denabeele, S., Varanova, E., Van Montagou, M., Inze, D. and Van Breusegem, F. (2000). Dual action of the active oxygen species during plant stress responses. *CMLS Cellular Mol Life Sci* **57,** 779-795.

Derra, S. (2004). How to make the most use of your microarray data. *Drug Disc. Dev.* 39-44.

Di Toppi, L.S. and Pawlik-Skowronska, B. (eds) (2003). *Abiotic Stresses in Plants.* Kluwer Academic Publishers, The Netherlands.

Flowers, T.J. and Yeo, A.R. (1986). Ion relations of plants under drought and salinity. *Aust. J. Plant Physiol.* **13,** 75-91.

Flowers, T.J. and Yeo, A.R. (1995). Breeding for salinity resistance in crop plants: where next? *Aust. J. Plant Physiol.* **22,** 875-884.

Fowdern, L., Mansfield, T. and Stoddart, J. (1993). Plant adaptation to environmental stress. *Chapman and Hall, London.*

Foyer, C.H., Descourvieres, P. and Kunert, K.J. (1994). Protection against oxygen radicals: an important defense mechanism studied in transgenic plants. *Plant Cell Environ.* **17,** 507-523.

Foyer, C.H., Lelandais, M. and Kunert, K.J. (1994). Photooxidative stress in plants. *Physiologia Plantarum.* **92,** 696-717.

Foyer, C.H. and Noctor, G. (2000). Oxidant and antioxidant signaling in plants: a reevaluation of the concept of oxidative stress in a physiological context. *Plant Cell Environ*. **28,** 1056-1071.

Freeman, J.L., Garcia, D., Kim, D., Hopf, A. and Salt, D.E. (2005). Constitutively elevated salicylic acid signals glutathione-mediated nickel tolerance in Thalspi nickel hyperaccumulators. *Plant Physiol.* **137,** 1082-1091.

Griffiths, H. and Parry, M.A.J. (2002). Plant responses to water stress. *Ann. Bot.* **89,** 801-802.

Guy, C.L. (1990). Cold acclimation and freezing stress: role of protein metabolism. *Annu.Rev. Plant Physiol. Plant Mol. Biol.* **41,** 187-223.

Hall, J.L. (2002). Cellular mechanisms for heavy metal detoxification and tolerance. *J. Exp. Bot.* **53,** 1-11.

Hasegawa, P.M., Bressan, R.A., Zhu, J-K, and Bohnert, H.J. (2000). Plant cellular and molecular responses to high salinity. *Annu.Rev. Plant Physiol. Plant Mol. Biol.* **51,** 463-499.

Hirt, H. and Shinozaki, K. (2002). *Plant Responses to Abiotic Stress.* Springer-Verlag, New York Llc.

Hopkins, W. and Hüner, N.P.A. (2004) Introduction to Plant Physiology.Third edition. p.245. John Wiley & sons Inc.

Hughes, M.A. and Dunn, M.A. (1996). The molecular biology of plant acclimation to low temperature. *J.Exp. Bot.* **47,** 291-305.

Iba, K. (2002). Acclimative response to temperature stress in higher plants: approaches of gene engineering for temperature tolerance. *Annu. Rev. Plant Biol***. 53,** 225-245.

Ingram, J. and Bartels, D. (1996). The molecular basis of dehydration tolerance in plants. *Annu. Rev. Plant Physiol. Plant. Mol. Biol* **47,** 377-403.

Jenks, M. and Hasegawa, P. (eds) (2005). *Plant Abiotic Stress.* Blackwell Publishers, U.K.

Jones, H.G., Flowers, T.J. and Jones, M.B. (eds) (1989). *Plants under Stress.* Cambridge University Press, Cambridge.

Kuhn, E. (2001). From library screening to microarray technology: strategies to determine gene expression profiles and to identify differentially regulated genes in plants. *Ann. Bot*. **87,** 139-155.

Lawlor, D.W. (2002). Limitation to photosynthesis in water stressed leaves: stomata vs. metabolism and the role of ATP. *Ann. Bot*. **89,** 1-15.

Lawlor, D.W. and Cornic, G. (2002). Photosynthetic carbon assimilation and associated metabolism in relation to water deficits in higher plants. *Plant Cell Environ.* **25,** 275-294.

Leung, J. and Giraudat, J. (1998). Abscisic acid signal transduction. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **49,** 199-222.

Levitt, J. 1980 *Responses of plants to environmental stress.* Vol. 1. 2nd ed. Academic Press, New York.

Lynch, J. and Brown, K.M. (2001) Topsoil foraging – an architechtural adaptationof plants to low phosphorus availability. *Plant Soil* **237,** 225-237.

Madlung, A. and Comai, L. (2005). The effect of stress on genome regulation and structure. *Ann. Bot.* **94,** 481-495.

Mazur, P. (1968). Freezing injury in plants. *Annu. Rev. Plant Physiol.* **20,** 419-448.

McKersie, B.D. and Leshem, Y.Y. (1994). *Stress and stress coping in cultivated plants.* Kluwer Academic Publishers, The Netherlands.

Mittler, R. (2002). Oxidative stress, antioxidants and stress tolerance. *Trends Plant Sci.* **7,** 405-410.

Mittler R., Vanderauwera S., Gollery, M. and Van Breusegem, F. (2004). *Trends Plant Sci*. **9,** 490-498.

Moons, A., Bauw, G., Prinsen, E., Van Montagu, M. and Van Der Straeten, D. (1995) *Plant Physiol.* **107,** 177-186.

Munn, R. (2002). Comparative physiology of salt and water stress. *Plant Cell Environ.* **25,** 239-250.

Nagao, M., Minami, A., Arakawa, K., Fujikawa, S. and Takezawa, D. (2005). Rapid degradation of starch in chloroplasts and concomitant accumulation of soluble sugars associated with ABA-induced freezing tolerance in the moss *Physcomitrella patens*. *J. Plant Physiol*. **162,** 169-180.

Pahlsson, A.M.B. (1989) Toxicity of heavy metals (Zn, Cu, Cd, Pb) to vascular plants: a literature review. *Water Air Soil Pollut.* **47,** 287-319.

Parry, M.A.J., Andralojc, P.J. Khan, S., Lea, P.J. and Keys, A.J. (2002) Rubisco activity: effects of drought stress. *Ann. Bot*. **89** : 833-839

Pessarakli, M. (Ed) (1999) *Handbook of Plant and Crop Stress.* Marcel Dekker, USA.

Poljakoff-Mayber, A. (1975) Morphological and anatomical changes in plants as a response to salinity. In: Poljakoff-Mayber A and Gale J eds. *Plants in Saline Environments.* Springer-Verlag, Berlin. pp. 97-117.

Quesada, V., Ponce, M.R. and Micol, J.L. (2000). Genetic analysis of salt–tolerant mutants in *Arabidopsis thaliana*. *Genetics* **54,** 421-436.

Rao, K.V. Madhava, Sridevi, V and Satyanarayana N.V. 2002 Heat shock induced lipid changes and solute leakage in germinating seeds of pigeonpea. *Biol. Plant.* **45,** 71-76.

Rao, K.V. Madhava and Sresty, T.V.S. (2000) Antioxidative parameters in the seedlings of pigeonpea (*Cajanus cajan* (L.) Millspaugh) in response to Zn and Ni stresses. *Plant Sci.* **157,** 113-128.

Rathinasabapathi, B. (2000). Metabolic engineering for stress tolerance: installing osmoprotectant synthesis pathways. *Ann. Bot.* **86,** 709-716.

Raymond, M.J. and Smirnoff, N. (2002). Proline metabolism and transport in maize seedlings at low water potential. *Ann. Bot.* **89,** 813-823.

Reddy, Ramachandra Attipalli., Chaitanya, K.V. and Vivekanandan, M. (2004). Drought-induced responses of photosynthesis and antioxidant metabolism in higher plants. *J Plant Physiol.* **161,** 1189-1202.

Rudolf, A.S. and Crowe, J.H. (1985) Membrane stabilization during freezing: the role of two natural cryoprotectants, trehalose and pralines. *Cryobiology* **22,** 367-377.

Schützendübel, A. and Polle, A. (2002). Plant responses to abiotic stresses: heavy metal-induced oxidative stress and protection by mycorrhization. *J. Exp. Bot.* **53,** 1351-1365.

Smalle, J. and Vierstra. (2004). The ubiquitin 26S proteosome proteolytic pathway. *Annu. Rev. Plant Biol.* **55,** 555-590.

Siminovitch, D., Rheaume, K., Pomeroy, K. and Lepage, M. (1968). Phospholipid, protein, and nucleic acid increases in protoplasm and membrane structure associated with development of extreme freezing resistance in black locust tree cells. *Cryobiology* **5,** 202-225.

Sresty, T.V.S. and Rao, K.V. Madhava . (1999). Ultrstuctural alterations in response to zinc and nickel stress in the root cells of pigeonpea. *Environ. Exp. Bot.* **41:** 3-13.

Sridevi, V., Satyanarayana, N.V. and Madhava Rao, K.V. (1999). Induction of heat shock proteins and acquisition of thermotolerance in germinating pigeonpea seeds. *Biol. Plant.* **42,** 589-597.

Stephanopoulos, G. (1999). Metabolic fluxes and metabolic engineering. *Metabol. Engn.* **1,** 1-11.

Steponkus, P.L. (1984). Role of the plasma membrane in freezing injury and cold acclimation. *Annu. Rev. Plant Physiol.* **35,** 543-584.

Stitt, M. (1995) Regulation of metabolism in transgenic plants. *Annu. Rev. Plant physiol. Plant Mol. Biol.* **46,** 341-368.

Tester, M. and Davenport, R. (2003) Na^+ tolerance and Na^+ transport in higher plants. *Ann. Bot.* **91,** 503-527.

Thomashow, M.F. (1999). Plant cold acclimation: freezing tolerance genes and regulatory mechanisms. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **50,** 571-599.

Tomsett, A.B. and Thurman, D.A. (1988) Molecular biology of metal tolerance of plants. *Plant Cell Environ.* **11,** 383-394.

Van Assche, I. and Clijsters, H. (1990) Effect of metals on enzyme activity in plants. *Plant Cell Environ.* **13,** 195- 206.

Vranova, E., Inze, D. and Van Breusegem, F. (2002) Signal transduction during oxidative stress. *J. Exp Bot.* **53,** 1227-1236.

Winicov, I. (1998). New molecular approaches to improving salt tolerance in crop plants. *Ann. Bot.* **82,** 703-710.

Xiong, L. and Zhu, J-K. (2001) Abiotic stress signal transduction in plants: molecular and genetic perspective. *Physiol. Plant.* **112,** 152-166.

Zeevaart, J.A.D. and Creelman, R.A. (1988) Metabolism and physiology of abscisic acid. *Annu. Rev.Plant Physiol. Plant Mol. Biol.* **39,** 439-473.

Zhu, J-K. (2001) Plant salt tolerance. *Trends Plant Sci.* **6,** 66-71.

Zhu, J-K. (2002). Salt and drought stress signal transduction in plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **53,** 247-273.

Zörb, C., Noll, A., Karl, S., Leib, K. and Yan, F. (2005). Molecular characterisation of N^+/H^+ antiporters (ZmNHX) of maize (*Zea mays* L.) and their expression under salt stress. *J. Plant Physiol.* **162,** 55-66.

CHAPTER 2

WATER STRESS

A. YOKOTA, K. TAKAHARA AND K. AKASHI

Graduate School of Biological Sciences, Nara Institute of Science and Technology,
Nara 630-0101, Japan
(Email: yokota@bs.naist.jp)

Keywords: *Abscisic acid, compatible solutes, desiccation, drought, photosynthesis, rubisco*

1. INTRODUCTION

Plants use ribulose 1,5-bisphosphate carboxylase/oxygenase (RuBisCO) to fix CO_2 during photosynthesis. RuBisCO also reacts with O_2, lowering productivity through inevitable photorespiration and increasing the CO_2 compensation point of C_3 plants to 50 to 70 bar. The Km value for CO_2 of RuBisCO is 10 to 15 µM and CO_2 activation is necessary for activity (Roy and Andrews, 2000). Diffusion barriers to CO_2 in the stomata, plasma membranes, cytosolic fluid and chloroplast envelopes lower CO_2 concentrations around RuBisCO to approximately 7 µM during active photosynthesis (von Caemmerer and Evans, 1991; Noctor et al., 2002), even with the aid of aquaporins for quick diffusion at plasma membranes (Terashima and Ono, 2002; Uehlein et al., 2003). Consequently, less than 20% of all RuBisCO catalytic sites actually participate in photosynthetic CO_2 fixation in chloroplasts (McCurry et al., 1981). Nevertheless, plants fix a total of 200 Gtons of CO_2 every year by investing a large amount of nitrogen in RuBisCO synthesis and by maximizing the density of stomata per leaf unit area and the size of stomatal apertures (Terashima et al., 2005).

These properties of RuBisCO are the most critical factors influencing the physiology of plants under water-stressed conditions (Whitney and Andrews, 2001). The amount of water transpired from leaves through stomata is 500 to 1000 times more than the amount of CO_2 absorbed on a molar basis (Larcher, 1995). Consequently, plants need an enormous amount of water for growth. The water use efficiency of C_3 plants is 1.3 to 2 g of dry matter production per kg of water used and this is 2-fold higher in C_4 plants. This indicates the importance of water as a determinant of plant productivity in the field; for example, in the US drought is the most serious environmental stress affecting agricultural production (Table 1) (Boyer, 1982).

K.V. Madhava Rao, A.S. Raghavendra and K. Janardhan Reddy (eds.),
Physiology and Molecular Biology of Stress Tolerance in Plants, 15–39.

Table 1. *Distribution of insurance indemnities for crop losses in the United States during the last 40 years (Boyer, 1982)*

Cause of crop loss	*Proportion of payment (%)*
Drought	40.8
Excess water	16.4
Cold	13.8
Hail	11.3
Wind	7.0
Insects	4.5
Disease	2.7
Flood	2.1
Others	1.5

To maximize productivity, plants optimize the morphology, physiology and metabolism of their organs and cells; however, this strategy causes vulnerability to water deficits. To overcome this, plants are equipped with various mechanisms of adaptation to water-limiting environments. The following chapter describes recent advancements in physiological, biochemical and molecular and cellular research related to water deficiency in plants. Although flooding and excess water are other extreme water stresses encountered by plants, and although the inhibitory effect of heavy rainfall on leaf photosynthesis has also been reported (Ishibashi et al., 1996), these topics are not dealt with here.

2. PHYSIOLOGICAL RESPONSES TO DRYING ENVIRONMENT

2.1. Sensing Drying Environments

The absence of precipitation in natural environments causes dryness of the atmosphere and soil, the latter mostly due to evaporation of water from the soil surface in the daytime. In general, drying of soil is slow (Larcher, 1995), but decrease atmospheric humidity can sometimes be quick. Accordingly, plants need suitable systems both in their roots and leaves that sense environmental dryness.

Plant leaves close their stomata immediately on sensing an increase in leaf-air vapor pressure difference, even if the roots have sufficient water (Mott and Parthurst, 1991; Assmann et al., 2000); this response is completed in several minutes (Assmann et al., 2000). Whether this stomatal closure system is abscisic acid (ABA)-dependent or independent is unknown. Expression of the gene encoding abscisic aldehyde oxidase has been revealed in the guard cells of dehydrated Arabidopsis leaves (Koiwai et al., 2004). Moreover, four other enzymes involved in the ABA-synthetic pathway are known to be expressed in leaves (Iuchi et al., 2001; Tan et al., 2003), but their functional localization remains to be determined. Since exposure of leaves to dry air causes decreases in the turgor of epidermal cells and transpiration rate without any significant effect on the leaf water potential (Shackel and Brinckmann, 1985), the sites of perception of signals of atmospheric dryness and ABA synthesis are thought to be close to or in the guard cells. Although ABA genes are known to be up-regulated under drought conditions, rapid closure of stomata has also been observed in *abi1* and *aba2 Arabidopsis* mutants (Assmann et al., 2000). This is possibly the result of a low basal level of ABA in these mutants, sufficient enough to transmit the leaf-air vapor pressure difference, or indicative of guard cells as the sensor and transducer of humidity signals (Maier-Maercker, 1983).

Evaporation of water lowers the water potential and increases the salt concentration of soil. In general, other stresses such as osmotic and high salt concentration stresses also affect roots in combination with water deficits, while heat stress is a further stress in leaves. This is thought to be reflected by the activation of numerous common factors in inter-/intracellular signal transduction pathways with different environmental stimuli (Yamaguchi-Shinozaki and Shinozaki, 2005). Deficits in the water content of the soil environment might be sensed as an increase in the salt concentration around root surfaces and/or an increase in the osmotic pressure of root cells. However, no water sensor or potential low water sensor has so far been identified in plants. An *Arabidopsis* mutant showing no hydrotropism or directed growth of roots to gradients in moisture has been isolated (Eapen et al., 2005), but the mutated gene(s) remains to be determined.

The ABA is synthesized from carotenoid by ABA-synthesizing enzymes (zeaxanthin epoxidase, 9-*cis*-epoxycarotenoid dioxygenase and aldehyde oxidase) induced in root tip cells or parenchyma cells of vascular bundles by drought and salt stresses (Koiwai et al., 2004). ABA synthesized in the roots enters the xylem vessels in a free form or as a conjugate with glucose, and from here is transported to the leaves (Sauter et al., 2002). How the conjugates are formed in the cytosol of the cortex remains to be determined. The conjugated form is thought to be suitable for long-distance delivery from roots to leaves, since the free form might possibly escape from the acidic xylem sap to surrounding tissues. The ratio of free to conjugated forms of ABA in xylem sap varies from plant to plant, but in all species, the total amount of ABA increases significantly under drought and salt stresses (Sauter et al., 2002).

The ABA conjugates are hydrolyzed into a free form by β-D-glucosidase in the apoplastic space (Dietz et al., 2000), inducing stomatal closure aided by a signaling system in the guard cells (discussed below). The guard cells in the leaves of plants grown under well-irrigated conditions are large in size, while inversely, the stomata of plants grown with limited water are smaller but more dense (more stomata per unit area)

(Elias, 1995). Smaller stomata are advantageous in that the stomatal aperture can be reduced within a short period after guard cells sense ABA. Stomatal closure in many plants is incomplete even after application of high concentrations of ABA (Mustilli et al., 2002). However, field-grown plants, woody plants and wild watermelon plants show almost complete stomatal closure and transpiration rates of almost zero during severe drought stress (Davies et al., 1994; Loewenstein and Pallardy, 1998; Yokota et al., 2002). Since complete stomatal closure cannot be accomplished by application of 300 μM ABA in wild watermelon plants, a possible alternative drought signal from the roots to leaves has been suggested (Yokota et al., 2002).

2.2. Responses of Leaf Photosynthetic Systems to Drying Environments

During progressing drought, plants attempt to protect against evaporation by closing their stomata. However, many plants lose water through stomata that remain open as well as through their cuticles. The water conductance of the cuticle varies greatly from species to species (Kerstiens, 1996); however, the reason for this large variation remains unknown. The lowest conductance value so far reported was with the cuticle of Vanilla plants; this value was much lower than those of artificial food-storage films such as polyvinylchloride and liquid crystal polymer (Kerstiens, 1996; Riederer and Schreiber, 2001). Despite the lack of evidence suggesting a close correlation between cuticle conductance and drought resistance in crops (Kerstiens, 1996), water filled pores of molecular dimension are thought to contribute to cuticular transpiration (Riederer and Schreiber, 2001).

As leaf water is lost, the turgor pressure of leaf tissues decreases and leaves begin to wilt. Wilting or curling of the leaves functions to protect photosynthetic machinery from direct rays of the sun (Larcher, 1995). Since the leaves of some plants such as wild watermelon do not wilt after stomatal closure, they are thought to possess specialized systems able to endure full sunlight virtually in the absence of CO_2 fixation (Kawasaki et al., 2000; Yokota et al., 2002). The morphology of the plant body as well as the molecular and biochemical characteristics of photosynthetic organs has evolved to maximize photon capture and use of these photons in CO_2 fixation. Accordingly, stomatal closure under drought stress deprives plants of their largest consumer of solar energy.

Under non-stressful conditions, half the electrons in plastoquinone enter the Q cycle enabling transportation of more protons to the lumenal side of thylakoids in order to meet the ATP/NADPH ratio required by the photosynthetic carbon reduction (PCR) cycle (Shikanai et al., 2002; Cramer et al., 2004); these electrons are therefore not passed to cytochrome f and consequently photosystem I (PSI). With progressing stomatal closure, the rate of utilization of electrons in PCR and the photorespiratory carbon oxidation (PCO) cycle decreases. Although the rate of oxygen fixation by RuBisCO (photorespiration) increases under these conditions (Cornic and Fresneau, 2002; Noctor et al., 2002), considering the relative specificity of plant RuBisCO and CO_2

and O_2 concentrations in chloroplasts during photosynthesis (Noctor et al., 2002), the rate of energy utilization of photorespiration does not exceed that under non-drought conditions. Electrons in PSI are directed to electron transport chains (Cornic et al., 2000; Golding and Johnson, 2003; Golding et al., 2004) causing oxygen reduction (Asada, 1999; Biehler and Fock, 1996) when utilization of NADPH slows down. Under such conditions, the ATP/ADP ratio increases and the lumenal side of the thylakoids is acidified (Kramer et al., 2004).

In PSII, two carboxyl groups of photosystem II subunit S (PsbS) are protonated and synthesis of zeaxanthin from violaxanthin is promoted at a low luminal pH (Li et al., 2004). Zeaxanthin blocks energy transfer from light-harvesting chlorophylls to the reaction center chlorophyll P680 in PSII (Holt et al., 2004). The energy in light-harvesting chlorophylls is dissipated mainly as heat and partly as fluorescent light, and blocking of energy transfer to P680 or conversion of this energy to heat is detected as non-photochemical quenching (Ma et al., 2003). An increase in non-photochemical quenching is detected in leaves where the supply of photon energy exceeds the demand of RuBisCO-related reactions under drought, strong light and salt stresses (Golding and Johnson, 2003; Teraza et al., 2003). The PSII D1 protein is continuously degraded and replenished during photosynthesis under moderate conditions when no phenotypic damage occurs. The turnover of D1 starts with damaging of grana thylakoids and is completed with the return of the PSII complex replenished with newly synthesized D1 in appressed and stromal thylakoids. Experiments with a cyanobacterium *Synechocystis* sp. PCC6803 have shown inhibition of a translation step in protein synthesis to be the main cause of photoinhibition of PSII at high light intensities (Nishiyama et al., 2004).

Three different routes for the cyclic electron flow around PSI have been suggested. One is through stromal NADPH and plastoquinone (Shikanai et al., 1998a) while another is through ferredoxin and plastoquinone (Munekage et al., 2002). The protein entities of these two routes remain unclear; however, since an Arabidopsis mutant in which the single genes involved in both routes are mutated shows severely suppressed growth (Munekage et al., 2004), both are thought to be essential for normal photosynthesis and likely to function under stresses. The cytochrome b_6f complex isolated from spinach leaves contains a ferredoxin: NADP reductase ratio of 0.9 reductase/1 cytochrome *f* (Zhang and Cramer, 2004), suggesting the existence of the third path in which electrons of NADPH are thought to return to PSI without involvement of plastoquinone.

It is possible that PSI cyclic electron transport increases in momentum under drought stress when solar energy trapped by the thylakoids greatly exceeds the demand from carbon metabolism. PSII is severely down-regulated preventing release of electrons from P680 through acidification of the thylakoid lumen (Golding and Johnson, 2003; Teraza et al., 2003). Since PSI receives photons at a similar frequency to PSII, energy dissipation in PSI should not be neglected. The flux of electrons in PSI cyclic electron transport plays only a minor role relative to the total PSI flux under moderate conditions. In barley leaves suffering CO_2 limitation under drought, the quantum effi-