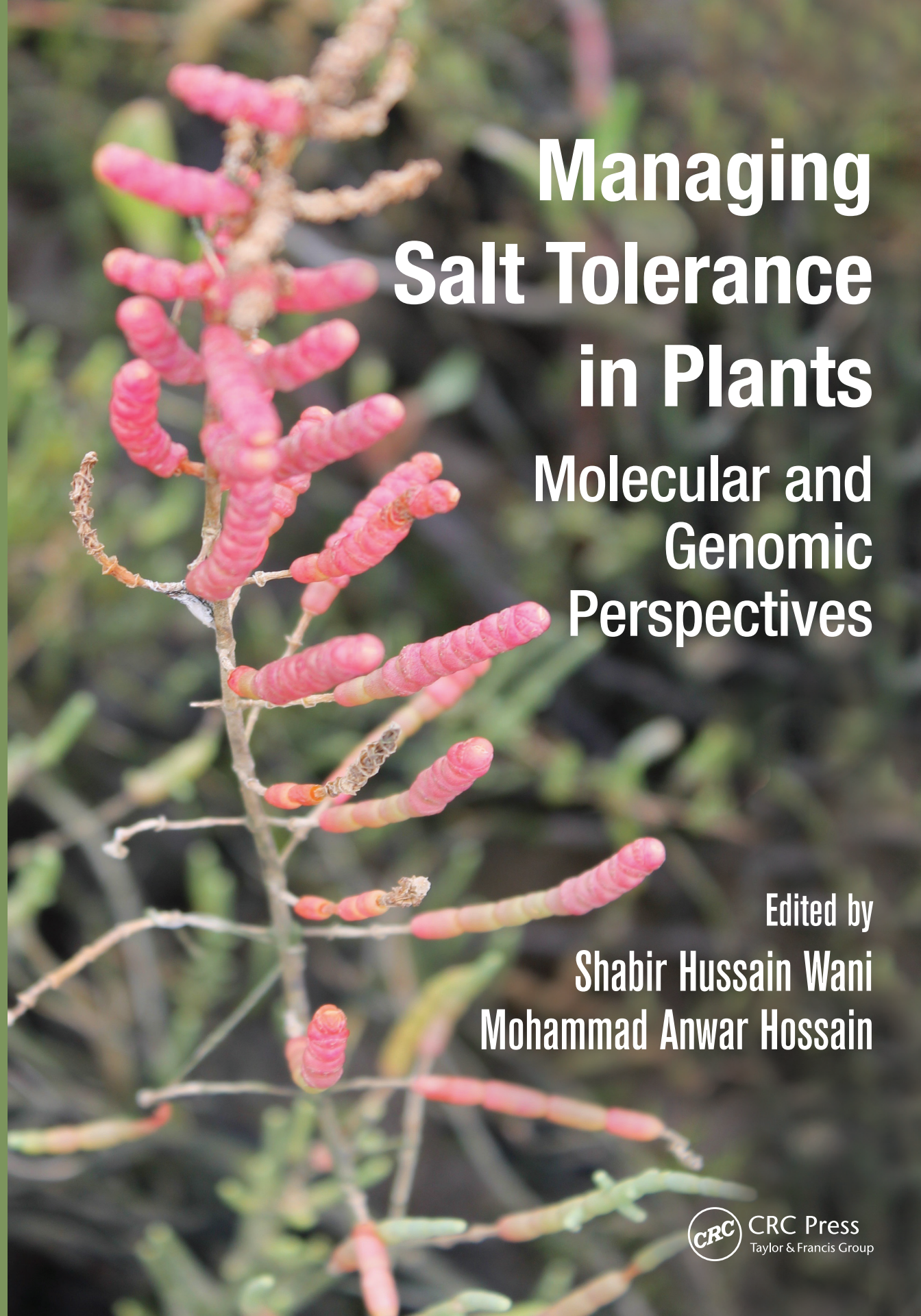

Managing Salt Tolerance in Plants

Molecular and Genomic Perspectives

Edited by
Shabir Hussain Wani
Mohammad Anwar Hossain

CRC Press
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PREFACE

Abiotic stress has become one of the decisive factors dwindling crop productivity worldwide. Salinity stress is one of the major abiotic stresses affecting agriculture, with more than 80 million hectares of irrigated land affected worldwide. This book describes salinity stress in plants and its effects on plant growth and productivity. It also addresses the management aspect of salinity stress in crops through molecular and genomic approaches. This edited book attempts to bring together all the biochemical, physiological, and molecular techniques exploited to develop crop plants with increased salinity tolerance. Through this book, an attempt has been made to integrate the most recent findings about the key biological determinants of salinity stress tolerance with contemporary crop improvement approaches. Twenty-two chapters written by leading scientists involved in plant salinity stress research worldwide provide an ample coverage of the key factors impacting world crop production.

Chapter 1 discusses the understanding of salt stress response from the gene to the whole plant level. Chapter 2 explains the mechanisms of salt stress tolerance in halophytes. Chapter 3 is concerned with vacuolar sodium sequestration in plant breeding for salinity tolerance. Chapter 4 discusses salt stress signaling pathways. Chapter 5 discusses the physiological and biochemical approaches for salinity tolerance. Chapter 6 describes the plant cell organelle proteomics and salinity tolerance. Chapter 7 depicts the function of heat shock proteins in salt tolerance. Chapter 8 deals with the transcription factors involved in salt tolerance. Chapter 9 discusses the role of the glyoxalase system and salinity stress tolerance, and Chapter 10 depicts ROS metabolism and salt stress tolerance in plants. Chapter 11 describes the insights of hydrogen peroxide–induced salt stress tolerance, Chapter 12 deals with the roles of ascorbate-glutathione cycle in salt stress tolerance of plants, and Chapter 13 discusses polyamine metabolism and salinity stress tolerance. In Chapter 14, metabolomics and salt stress tolerance are reviewed. Chapter 15 discusses plant–microbe interaction and salt stress tolerance, Chapter 16 summarizes molecular breeding for salt stress tolerance, and Chapter 17 is about mutation breeding for salt stress tolerance. Chapter 18 is concerned with the present status and future prospects of transgenic approaches for salinity tolerance, Chapter 19 discusses proline engineering for enhanced salt stress tolerance, Chapter 20 describes salt stress tolerance in plants in relation to ion transporters and genetic engineering, and Chapter 21 reviews transgenic plants for higher antioxidant contents and salt stress tolerance. The final Chapter 22 summarizes the insights of salinity tolerance–based transcriptomic studies.

The facts presented in this book call attention to primary genetic, physiological, and biochemical acquaintance of plant salinity stress, which may lead to both conventional and biotechnological applications that finally lead to enhanced

crop productivity in stressful environments. We hope that the book will be very helpful for plant abiotic stress researchers, graduate students, and university teachers. It will also be of interest to environmental scientists, biochemists, and policy makers.

We give special thanks to all the authors for their stupendous and sensible work in producing such fine chapters. We also thank Dr. C. R. Crumly (senior acquisitions editor, CRC Press), Stephanie Morkert (project coordinator, CRC Press), and other members of CRC Press, Taylor & Francis Group, for their guidance and support during the progress of this important book. Thanks also to all the well-wishers, teachers, colleagues, research students, and family members. Without their unending support, motivation, and encouragement, the grueling task of writing this book would not have been possible.

Finally, we bow in reverence to Allah, who blessed us with the favor of plentiful academic work.

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Dr. Mohammad Anwar Hossain received his PhD in agriculture, with specialization in plant abiotic stress physiology and molecular biology from Ehime University, Japan, in 2011. He received his bachelor's and master's degrees in agriculture and genetics and plant breeding from Bangladesh Agricultural University, Bangladesh. He also earned his master of science in agriculture from Kagawa University, Japan. He has been an associate professor at the Department of Genetics and Plant Breeding, Bangladesh Agricultural University since September 2011. Dr. Hossain teaches plant breeding, advanced plant breeding, molecular plant breeding, and stress breeding. He has published 17 research articles, 6 book chapters, and 4 review articles on the important aspects of plant physiology and breeding, plant stress responses, environmental problems in relation to plant species, and the role of micronutrients on plant defense system. Presently, he is working on elucidating the molecular mechanisms of

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CHAPTER ONE

Understanding Plant Stress Response and Tolerance to Salinity from Gene to Whole Plant

Kaouthar Feki, Walid Saibi, and Faiçal Brini

CONTENTS

Abstract. Salinity is a major environmental stress that limits agriculture production. Hence, it is essential to produce salt-tolerant crops for sustaining food production. Understanding the molecular basis of salt-stress signaling and tolerance mechanisms is essential for breeding and genetic engineering of salt tolerance in crop plants. Plant adaptation or tolerance to salinity stress involves complex physiological traits, metabolic pathways, and molecular or gene networks. In many plants, the salt tolerance is associated with the ability to exclude sodium from the shoot, to prevent its accumulation in photosynthetic tissues. Salinity stress involves changes in various physiological and metabolic processes, depending on severity and duration of the stress, and ultimately inhibits crop production. In this chapter, we mainly discuss about the effect of salinity on plants and tolerance mechanisms that permit the plants to withstand stress.

Keywords: Functional genomics, Ion homeostasis, Salt stress, Salinity tolerance

1.1 INTRODUCTION

Salinity is one of the most serious factors limiting food production, because it limits crop yield with adverse effects on germination and restricts use of land previously uncultivated (Munns and Tester 2008). High salinity causes water stress, ion toxicity, nutritional disorders, oxidative stress, alteration of metabolic processes, membrane disorganization, reduction of cell division, and genotoxicity (Zhu 2002, 2007; Munns 2002). The complex "plant response to abiotic stress" involves many genes and biochemical molecular mechanisms. The analysis of the functions of stress-inducible genes is an important tool not only to understand the molecular mechanisms of stress tolerance and the responses of higher plants but also to improve the stress tolerance of plants by genomic strategies. The susceptibility or tolerance to high salinity stress in plants is a coordinated action of multiple stress-responsive genes, which also cross talk with other components of stress signal transduction pathways. Several types of gene belonging to different metabolic functions have been identified and used for over-expression into glycophytic plants to enhance salinity stress tolerance. The stress-related genes are generally classified into two major groups. The first one is involved in signaling cascades, transcriptional control, and the degradation of transcripts or proteins. The member of the second group functions in membrane protection and osmoprotection as antioxidants and as reactive oxygen species (ROS) scavengers (Pardo 2010). Plant responses to salinity and mechanisms conferring plant salinity tolerance have been studied for a long time. Using modern genetic approaches like genome sequencing, reverse genetics methods, and identification and characterization of key genes involved in salt-stress signaling, the understanding of salt tolerance mechanisms is substantially in progress especially salt ion signaling and transport (Hasegawa et al. 2000; Flowers 2004; Kosova et al. 2013). This chapter provides an overview of our current understanding of the mechanisms contributing to salt-stress tolerance in plants and the contribution of the genomic, transcriptomic, proteomic, and metabolic investigations to understand plant salinity tolerance.

1.2 EFFECTS OF SALINITY ON PLANTS

Salt stress imposes a major environmental threat to agriculture, and its adverse impacts are serious problems in regions where saline water is used for irrigation. It is estimated that about 7% of world agricultural land is affected by salinity and that this number could increase up to 20% in the future due to land salinization as a consequence of artificial irrigation and unsuitable land management. Regarding irrigated soils that contribute to roughly one-third of the global food production, it is estimated that nearly one-half of the total area of irrigated land could be adversely affected by salinization (Munns 2002; Munns and Tester 2008). High salinity causes hyperosmotic stress and ion disequilibrium that produce secondary effects (Hasegawa et al. 2000; Zhu 2001). Indeed, salts dissolved in the soil solution reduce the water potential (i.e., diminish water availability to the plant), and water uptake by roots is thermodynamically hampered. Thus, plants have to cope with osmotic effect by the mechanisms of osmotic adjustment. The stomatal closure often observed in salt-treated plants ameliorates tissue dehydration by limiting water losses (Fricke et al. 2004). Due to a reduced stomatal conductance, the rate of photosynthetic CO_2 assimilation is generally reduced by salinity. Salinity may cause nutrient deficiencies or imbalances, due to the competition of Na^+ and Cl^- with nutrients such as K^+ and Ca^{2+}. Plants cope with increased ion concentrations either via salt ion exclusion from the cells or salt ion compartmentation in the intracellular compartments. Plants are generally classified as glycophytes or halophytes referring to their capacity to grow on highly saline environments (Flowers et al. 1977). Under salt stress, halophytes accumulate salts and have a capacity to growth on salinized soils in coastal and arid regions due to specific mechanisms of salt tolerance developed during their phylogenetic adaptation. However, glycophytes, including most crop plants, tend to exclude salt, and they are severely inhibited or even killed by 100–200 mM NaCl (Zhu 2007). Halophytes represent an ideal target for understanding the genetic and molecular basis for their adaptation in saline conditions (Subudhi and Baisakh 2011). Some halophytes have evolved unique adaptations such as salt glands and bladders, succulence, life cycle avoidance, and salt-induced facultative metabolism to cope with salinity (Flowers et al. 1977, 1986, 2010; Shabala and MacKay 2011).

Salt stress has various effects on plant physiological processes such as increased respiration rate and ion toxicity, changes in plant growth, mineral distribution, membrane permeability (Gupta et al. 2002), and decreased efficiency of photosynthesis (Boyer 1976; Downton 1977; Hasegawa et al. 2000;

Munns 2002; Kao et al. 2003). The chlorophyll content decreases in many salt-susceptible plants such as tomato (Lapina and Popov 1970), potato (Abdullah and Ahmed 1990), and pea (Hamada and El-Enany 1994). The derived reduction in the photosynthetic rate of salt-sensitive plants can increase the production of ROS. Salinity is well established to induce oxidative stress, which occurs due to the production of ROS such as superoxide radical (O^-), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH^-). These species of oxygen are highly cytotoxic and can seriously react with vital biomolecules such as lipids, proteins, and nucleic acid, causing lipid peroxidation, protein denaturation, and DNA mutation, respectively (Scandalios 1993; McCord 2000; Breusegem et al. 2001; Mittler 2002). ROS are rapidly removed by antioxidative mechanisms, which include specific ROS-scavenging antioxidative enzymes and small nonenzymatic molecules that act as ROS scavenger such as ascorbate, glutathione (GSH), α-tocopherol, flavonoids, anthocyanines, polyphenolic compound, and carotenoids.

1.3 SALT TOLERANCE MECHANISM

The plant response to salinity consists of numerous processes that must function in coordination to alleviate both cellular hyperosmolarity and ion disequilibrium.

1.3.1 Ion Homeostasis: Transport Determinants and Their Regulation

Elevated salts lead to a passive salt ion penetration via plasma membrane and to an accumulation of salt ions in cell cytoplasm, which can lead to inhibition of intracellular enzyme activity (Munns and Tester 2008). Some halophytes are able to reduce shoot Na^+ accumulation through an intracellular sensing mechanism that indirectly regulates inward K^+ conductance (Robinson et al. 1997; Véry et al. 1998). Many salt-tolerant halophytes accumulate higher shoot Na^+ concentrations than less salt-tolerant halophytes or glycophytes, which is indicative of greater Na^+ homeostasis capacity (Rus et al. 2006; Flowers and Colmer 2008; Munns and Tester 2008; Baxter et al. 2010). In general, to prevent the accumulation of Na^+ in the cytoplasm, plants have developed three mechanisms that function in a cooperative manner, which are (1) reduction of Na^+ entry into the cell, (2) activation of Na^+ extrusion at the root–soil interface, and (3) compartmentalization of Na^+ in the vacuole (Tester and Davenport 2003).

1.3.1.1 Na^+ Influx

Under salinity, sodium enters into root cell through cation channels or selective or nonselective transporters or into the root xylem stream via an apoplastic pathway (Chinnusamy et al. 2005). One of the key responses to salt stress is to maintain cellular ion homeostasis by restricting the accumulation of toxic sodium (Clarkson and Hanson 1980; Tester and Davenport 2003). The major pathway for passive Na^+ entry into roots at high soil NaCl concentrations is the voltage-dependent nonselective cation channels (NSCCs) (Tester and Davenport 2003; Demidchik and Maathuis 2007). Many studies have demonstrated that NSCCs are directly involved in a multitude of stress responses, growth and development, uptake of nutrients, and calcium signaling. NSCCs can also function in the perception of external stimuli and as signal transducers for ROS, pathogen elicitors, cyclic nucleotides, membrane stretch, amino acids, and purines (Demidchik and Maathuis 2007). Due to the similarity between Na^+ and K^+, voltage-dependent K^+ inward rectifiers or outward rectifiers appear to be one path for Na^+ entry into root cells (Blumwald et al. 2000). The members of the HKT gene family are Na^+-specific transporters, although they are initially described as high-affinity K^+ transporters. Generally, the HKT members of subfamily 1, which has a highly conserved serine in the first pore loop of the protein, have a relatively higher Na^+ to K^+ selectivity than subfamily 2 HKT transporters (Horie et al. 2009; Pardo 2010; Yao et al. 2010). Whereas the HKT family is comprised of a single gene in *Arabidopsis thaliana*, encoding a Na^+-selective transporter (Uozumi et al. 2000; Mäser et al. 2001), it is much larger in cereals: nine *HKT* genes are present in rice (*Oryza sativa*), and 6–24 *HKT* are expected to be present in barley (*Hordeum vulgare*) and wheat species (Garciadeblás et al. 2003; Huang et al. 2008; Ben Amar et al. 2013). In *Arabidopsis*, AtHKT1 has been shown to control Na^+ accumulation in the shoots in salt-stress conditions by mediating Na^+ retrieval from the ascending xylem sap in the roots (Sunarpi et al. 2005; Davenport et al. 2007) and Na^+ recirculation from shoots to roots via phloem sap loading (Berthomieu et al. 2003). Many studies in cereals have shown that natural variation in the activity or expression of HKT transporters may be a genetic resource for enhanced NaCl tolerance. Indeed, the inactivation or suppression

of low affinity Na^+ transporter can improve plant salt tolerance. For example, the *hkt1* mutation suppresses the salt hypersensitivity and K^+-deficient phenotype of the *Arabidopsis sos3* mutant (Rus et al. 2001). In addition, transgenic wheat expressing antisense *HKT1* showed less Na^+ uptake and significant growth under salinity compared with control plants (Laurie et al. 2002).

1.3.1.2 Na^+ Exclusion

A critical factor of salinity tolerance in plants is the ability to exclude Na^+ from the shoot, and the modification of specific Na^+ transport processes has yielded enhanced salinity tolerance. In shoots, high concentrations of Na^+ can cause a range of osmotic and metabolic problems for plants. Sodium exclusion is one of the major mechanisms conferring salt tolerance in cereal crops including rice, wheat, and barley (Gorham et al. 1990; Munns et al. 2006). Bread wheat (*Triticum aestivum*, AABBDD) is, in general, a better Na^+ "excluder" than durum wheat (*Triticum turgidum* ssp. *durum*, AABB), a trait controlled by the *Kna1* locus on chromosome 4D, which corresponds to an *HKT1*;5-like gene (Dvorák et al. 1994; Dubcovsky et al. 1996; Byrt et al. 2007). However, an unusual durum wheat named Line 149 has a salt-tolerant phenotype similar to the bread wheat. This is due to the presence of two major genes for Na^+ exclusion, named *Nax1* and *Nax2* (Munns et al. 2003). The *Nax1* locus is associated with the exclusion of Na^+ from leaf blades only upon salt stress, both by retrieval of Na^+ from the xylem in roots and leaf sheaths and by recirculation of Na^+ from the shoots to the roots via the phloem. Concerning the *Nax2* gene, it also reduces the Na^+ transport from root to shoot and has a higher rate of K^+ transport, resulting in enhanced K^+ versus Na^+ discrimination in the leaf (James et al. 2006). High-resolution mapping and sequencing analyses of known Na^+ transporter genes have suggested that the *Nax1* and *Nax2* loci are attributable to polymorphisms in wheat *HKT* genes encoding protein of the subfamily 1 with preferred Na^+ transport (Huang et al. 2006; Byrt et al. 2007). Nax2 was shown to be homologous to Kna1 in *T. aestivum*, namely, TaHKT8 (Byrt et al. 2007).

In *Arabidopsis*, the plasma membrane Na^+/H^+ antiporter SOS1 plays a crucial role in sodium extrusion from root epidermal cells at the root-soil interface under salinity (Shi et al. 2000, 2002). In salt-stress conditions, SOS1 protein controls long-distance Na^+ transport since this ion is transported from the root to the shoot via the xylem (Shi et al. 2002). This critical function was demonstrated also in the halophytic *Arabidopsis* relative *Thellungiella salsuginea* (Oh et al. 2009) and in tomato (Olías et al. 2009). SOS1, SOS2, and SOS3 proteins are three essential components of SOS signaling pathway, which mediate cellular signaling under salt stress, to maintain ion homoeostasis (Figure 1.1). SOS1 protein is the direct target of SOS signaling pathway, and it is regulated through protein phosphorylation by the alternative SOS2/SOS3 and SOS2/CBL10 protein kinase complexes (Qiu et al. 2002; Quintero et al. 2002, 2011; Quan et al. 2007). ABI2 is the negative regulatory of this pathway, through the inhibition of SOS2 kinase activity or the activity of SOS2 targets, suggesting a cross talk between the ABA pathway and SOS pathway (Ohta et al. 2003).

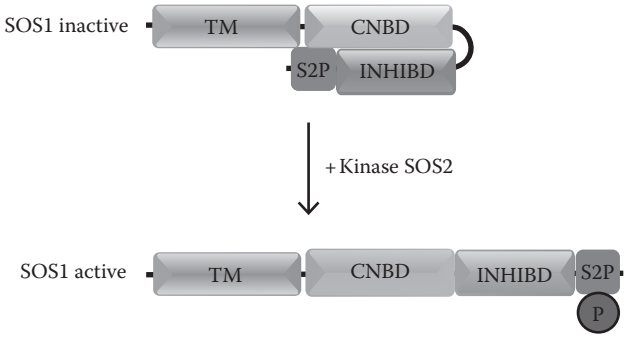

Figure 1.1. The different steps of SOS signaling pathway for salt-stress adaptation. Salt stress induces an accumulation of sodium in the cytosol, producing Ca^{2+} signal that activates the SOS3/SOS2 protein kinase complex. SOS2 activates the antiporter SOS1 by phosphorylation, the tonoplast Na^+/H^+ antiporter NHX and CAX1 (H^+/Ca^+ antiporter), restoring the ionic homeostasis.

Many other SOS1 proteins are also regulated by *Arabidopsis* SOS2/SOS3 complex, such as rice OsSOS1 (Atienza et al. 2007), tomato SlSOS1 (Olías et al. 2009), bread wheat (Xu et al. 2008), and durum wheat TdSOS1 (Feki et al. 2011). The activation mechanism of *Arabidopsis* and durum wheat SOS1 proteins involves the phosphorylation by the kinase SOS2 and inactivation of an autoinhibitory domain located at the C-terminal end of these transporters. Indeed, SOS1 is maintained in a resting state by the autoinhibitory domain, which is the target of SOS2/SOS3 complex and interacts intramolecularly with an adjacent domain that is essential for SOS1 activity. Upon salinity stress, the Ca^{2+}-dependent SOS2/SOS3 protein kinase complex phosphorylates SOS1 at the phosphorylation sites and relieves SOS1 from autoinhibition, presumably by displacing the autoinhibitory domain (Figure 1.2) (Quintero et al. 2011; Feki et al. 2011). SOS3 is a calcium-binding protein capable of sensing calcium transients elicited by salt stress (Liu and Zhu 1998; Ishitani et al. 2000). SOS2 is a serine/threonine protein kinase whose catalytic domain is evolutionarily related to that of the yeast protein SNF1 and animal AMP-activated kinases (Liu et al. 2000). It was shown that SOS3 recruits SOS2 to the plasma membrane to achieve efficient interaction with SOS1 (Quintero et al. 2002). In addition to the direct target SOS1 protein, CAX1 (H^+/Ca^{2+} antiporter) and NHX proteins are an additional target of SOS2 activity (Quintero et al. 2002; Cheng et al. 2004).

It has been reported that SOS proteins may have novel roles in addition to their functions in sodium homeostasis. For example, these proteins play a role in the dynamics of cytoskeleton under stress. Indeed, SOS3 plays a key role in mediating Ca^{2+}-dependent reorganization of actin filaments during salt stress (Ye et al. 2013). In addition, SOS1 is phosphorylated by MPK6, which is implicated in the organization and dynamics of mitotic and cortical microtubules (Müller et al. 2010; Yu et al. 2010).

1.3.1.3 Na^+ Sequestration

Under salt stress, plants have evolved an osmotic adjustment mechanism that maintains water uptake and turgor under osmotic stress conditions. For osmotic adjustment, plants use some organic compatible solutes such as proline, betaine, and soluble sugars and also inorganic ions like Na^+ and K^+. Proteins of the NHX family function in the sequestration of Na^+ in the vacuole, endosomal transporter, luminal pH control, and vesicle trafficking (Pardo et al. 2006). Plant NHX exchangers have 10–12 transmembrane domains

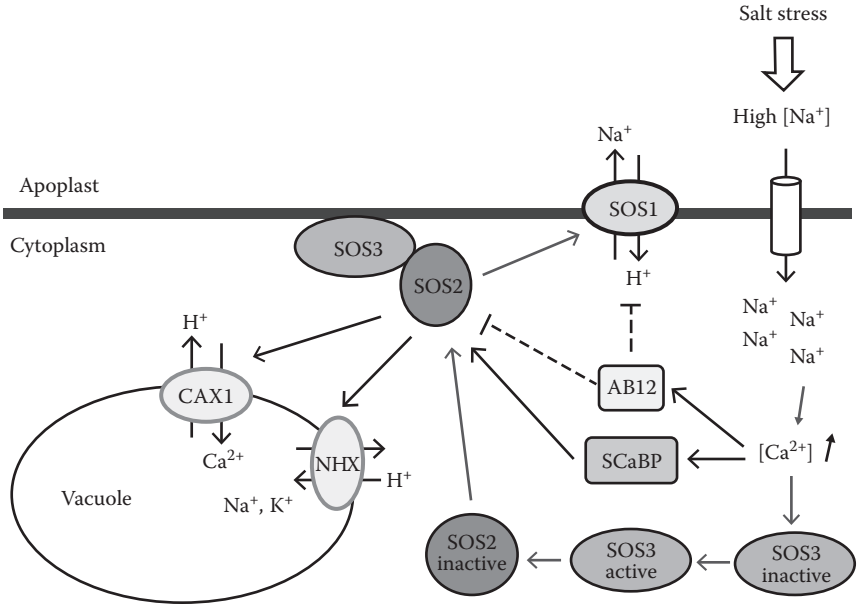

Figure 1.2. Model illustrating the activation mechanism of AtSOS1. (Adapted from Quintero, F.J. et al., *Proc. Natl. Acad. Sci. USA*, 108, 2611, 2011.)

with a hydrophilic C-terminal tail, which has vacuolar localization (Yamaguchi et al. 2003). The Na^+/H^+ exchange was electroneutral and driven by the vacuolar proton gradient established by the activity of the proton pumps V-ATPase and V-PPase (Blumwald 1987). The first Na^+/H^+ antiporter exchanger identified in plant was AtNHX in *Arabidopsis* (Gaxiola et al. 1999). The NHX proteins belong to the NHX/NHE subfamily of the cation proton antiporter CPA1 family (Saier 2000). The NHX proteins were subdivided into two classes. In plants, the class-I NHX proteins are localized in the tonoplast and function as $(Na^+, K^+)/H^+$ exchanger that accumulate Na^+ and K^+ into vacuoles, thereby contributing to osmotic regulation and the generation of turgor essential for cell expansion. However, the localization of class-II NHX proteins is in the endosomal compartments, and they function as K^+/H^+ exchange to prevent the accumulation of potentially toxic Na^+ into the endosomal lumen (Pardo et al. 2006). The microarray analysis showed that class-I AtNHX antiporters are expressed in leaves (AtNHX1, 2 and 4) or roots (AtNHX3) under the application of salt or osmotic stress. AtNHX1, 2, and 5 are expressed especially in guard cells compared to surrounding mesophyll cells (Shi and Zhu 2002; Rodriguez-Rosales et al. 2009). Although the first suggestion that AtNHX1 is specific to Na^+ transport, later studies have shown that AtNHX1 expressed in plants also catalyzes K^+/H^+ antiporter, albeit with lower affinity (Apse et al. 1999, 2003; Yokoi et al. 2002). Tomato LeNHX2 protein was purified and reconstituted into liposome showing that this protein catalyses relatively specific K^+/H^+ antiport (Venema et al. 2003). In addition to ionic homeostasis regulation, plant NHX proteins are implicated in endosomal and vacuolar pH regulation. Involvement of plant *NHX* genes in vacuolar pH regulation was most clearly demonstrated analyzing the dependence of flower color on vacuolar pH (Yoshida et al. 2005; Fukada-Tanaka et al. 2000). The involvement of ScNHX1 in pH regulation was demonstrated in yeast by the elimination of this protein, producing an acidification of the vacuolar and cytoplasmic pH (Brett el al. 2005). Both class-I and class-II plant NHX isoforms complement NaCl, KCl, and hygromycin sensitivity of the yeast ScNHX1 disruption mutant (Quintero et al. 2000; Yokoi et al. 2002; Venema et al. 2003). It is thus tempting to suggest a role for plant NHX proteins in endosomal pH regulation and protein trafficking as well.

The overexpression of NHX antiporters isolated from different plant species induced tolerance not only to salt but also to drought stress (Apse et al. 1999; Zhang and Blumwald 2001; Xue et al. 2004; Liu et al. 2010; Brini et al. 2007). Both class-I and class-II NHX antiporter from glycophytes or halophytes seem to have a similar effect on salt tolerance (Rodriguez-Rosaled et al. 2008; Shi et al. 2008).

1.3.1.4 Sodium Transport in the Whole Plant

Sodium is transported from soil solution through symplastic, apoplastic, or transcellular pathways up to the endodermis where a hydrophobic barrier that includes the Casparian strip restricts apoplastic movement (Schreiber et al. 2005; Plett and Moller 2010). The movement of Na^+ from root to shoot in xylem vessels occurs by bulk flow, driven primarily by xylem vessel tension caused by the vapor pressure gradient (Epstein and Bloom 2005).

NSCCs, and possibly other cation transporters like HKT and KUP/HAK transporters, are thought to mediate Na^+ influx in root cells. HKT mediates Na^+ entry in roots under low external concentrations of Na^+ and K^+, yet they are not likely to play a significant role in the salinity tolerance of plants (Horie et al. 2009). In *Arabidopsis*, SOS1 protein mediates sodium exclusion at the root–soil interface thereby reducing the net uptake of Na^+. Under moderate stress, SOS1 functions to load Na^+ into the xylem in roots for its transfer and storage in leaf mesophyll vacuoles. Whereas under severe salt stress, SOS1 is proposed to function in unloading Na^+ from the root xylem to reduce Na^+ damage of leaves that might be caused by exceeding the capacity of Na^+ sequestration in leaf cell vacuoles (Shi et al. 2002). SOS1 protein and AtHKT1;1 protein work in concert to regulate the Na^+ distribution between roots and shoots (Figure 1.3). Indeed, AtHKT1;1 is preferentially expressed in the vasculature, and it unloads Na^+ from xylem vessels to xylem parenchyma cells (Sunarpi et al. 2005). To translocate Na^+ back to roots, Na^+ is loaded into shoot phloem cells via symplastic diffusion (Sunarpi et al. 2005) or facilitated by HKT1-like proteins, preventing Na^+ overaccumulation in shoots (Berthomieu et al. 2003). However, the retranslocation of Na+ from leaf via phloem is little compared to the amount imported in the transpiration stream via the xylem (Tester and Davenport 2003; James et al. 2006).

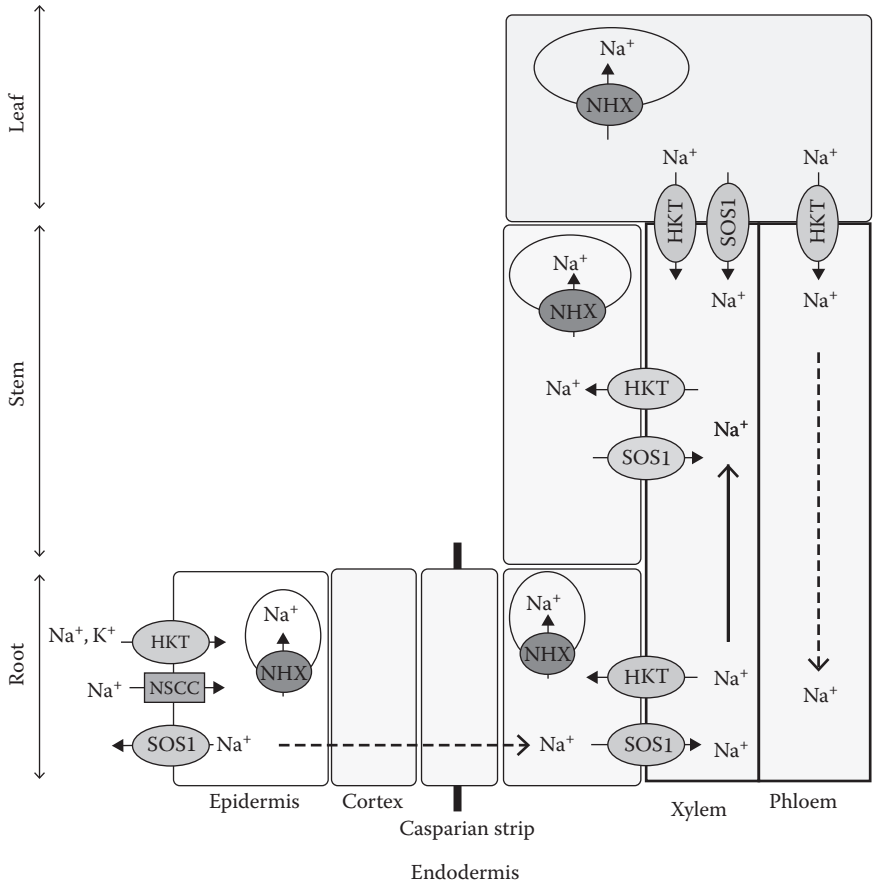

Figure 1.3. Sodium transport in the whole plant subjected to salinity stress. Thick arrows in the xylem and phloem indicate the flow of Na^+ in the sap. The dashed arrow symbolizes the reduced basipetal flow of Na^+ via phloem compared to the acropetal flux via xylem. (Adapted from Pardo, J.M., *Curr. Opin. Biotechnol.*, 21, 185, 2010.)

1.3.2 Osmotic Tolerance

Under salinity conditions, salt ions induce a decrease of osmotic potential with a passive salt ion penetration into plant cells. Osmotic stress decreases water availability for the cells, and it leads to a decreased water uptake resulting in cellular dehydration. The decrease in cellular turgor is sensed by the osmosensor histidine kinase at the cell plasma membrane (Urao et al. 1999). The osmotic stress is then transduced via a series of phosphorylation and calcium signaling to nucleus producing changes in gene expression. Plasma membrane phospholipids like phospholipases C and D are also implicated in osmotic stress signaling, and they lead to formation of small signaling molecules such as inositol-1, 4, 5-triphosphate (IP_3), diacylglycerol, and phosphatidic acid. These molecules induce Ca^{2+} signaling events leading to signal transduction to nucleus.

To regulate the osmotic balance within the cells, plants produce compatible osmolytes known as osmoprotectants. They are organic compounds of uncharged polarity and do not interfere with the cellular metabolism even at high concentrations (Wyn Jones et al. 1977). They mainly include proline, glycine betaine, sugar, and polyols, and they are implicated in the protection of the structure and in the regulation of osmotic balance within the cell via continuous water influx (Hasegawa et al. 2000). Contrary to the other amino acids, proline concentration rises in salt-stress condition (El-Shintinawy and El-Shourbagy 2001).

Proline is synthesized either from glutamic acid or ornithine, and the biosynthetic pathway comprises two major enzymes, which are the pyrroline carboxylic acid synthetase and pyrroline carboxylic acid reductase. It has been demonstrated that the addition of proline enhanced salt tolerance in many plants like olive (*Olea europaea*), tobacco (*Nicotiana tabacum*), and *A. thaliana*, by increasing the activity of some antioxidative enzyme activities (Abraham et al. 2003; Hoque et al. 2008; Ben Ahmed et al. 2010).

Glycine betaine is an amphoteric quaternary ammonium compound, and it stabilizes protein and protects the cell by osmotic adjustment and the photosynthetic apparatus from stress damages and reduction of ROS (Gadallah 1999; Makela et al. 2000; Ashraf and Foolad 2007; Cha-Um and Kirdmanee 2010). It has been showed that the treatment with glycine betaine ameliorates the ultrastructure of *O. sativa* seedlings when exposed to salt stress (Rahman et al. 2002). In addition, the application of glycine betaine to stressed plants increases the photosynthetic rate and growth level (Cha-Um and Kirdmanee 2010; Ahmad et al. 2013).

Under salt stress, some carbohydrates like sugars are accumulated within the cell in a number of plants belonging to different species to assure osmoprotection, carbon storage, and scavenging of ROS (Gupta and Huang 2014). ABA may regulate osmolyte biosynthesis in plants under salt stress. For example, Xiong et al. (2001) demonstrated that the production of ABA under osmotic stress regulates the *P5CS* gene involved in proline biosynthesis.

1.3.3 Antioxidant Regulation of Salinity Tolerance

Salt stress induces an accumulation of ROS that are detrimental to cells at high concentrations because they cause oxidative damage to membrane lipids, proteins, and nucleic acids (Gómez et al. 1999; McCord 2000; Breusegem et al. 2001; Mittler 2002). Despite the potential of ROS for causing harmful oxidations, it is now well established that they are also implicated in the control of plant growth and development as well as priming acclimatory responses to stress stimuli (Foyer and Noctor 2005, 2009). To cope with ROS, living organisms evolved antioxidant defense systems, comprised of enzymatic and nonenzymatic components. Several enzymes are involved in the detoxification of the activated oxygen species like superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT), glutathione reductase (GR), and glutathione peroxidase (GPX). Transgenic plants overexpressing ROS-scavenging enzymes showed increased tolerance to oxidative stress (Wang et al. 1999; Roxas et al. 1997, 2000; Foyer et al. 1995). SOD is one of the most important enzymes used against oxidative stress in the plant defense system, and it occurs ubiquitously in every cell of all types of plants (Ashraf 2009). With a few exceptions, Cu/Zn-SODs are generally found in the cytosol of eukaryotic cells and chloroplasts; the Mn-SODs are found in the matrix of mitochondria and in prokaryotes; the Fe-SODs are generally found in prokaryotes and have been reported to exist in some plants (Duke and Salin 1985). A membrane-associated Mn-SOD has been reported in chloroplasts of some plants (Hayakawa et al. 1984). CATs are one of the H_2O_2-metabolizing proteins in plants, and they are highly active enzymes that do not require cellular reductants as they primarily catalyze a dismutase reaction (Mhamdi et al. 2010). CATs have a very fast turnover rate, but a much lower affinity for H_2O_2 than APX and PRX (peroxiredoxins), which have KM values below 100 μM (Mittler and Zilinskas 1991; Konig et al. 2002). Three CAT genes are present in the genome of *Arabidopsis*, in which two are located on chromosome 1 (*CAT1* and *CAT3*) and one on chromosome 4 (*CAT2*) (Frugoli et al. 1996). The *CAT1* gene is mainly expressed in pollen and seeds, *CAT2* in photosynthetic tissues but also in roots and seeds, while *CAT3* is associated with vascular tissues but also leaves (Mhamdi et al. 2010). APXs are thought to be the most important H_2O_2 scavengers operating both in the cytosol and chloroplasts. They use ascorbic acid (AsA) as a reducing substrate and form part of a cycle, known as the ascorbate–GSH or Halliwell–Asada cycle.

The nonenzymatic antioxidant system includes AsA, GSH, α-tocopherols (vitamin E), flavonoids, anthocyanines, polyphenolic, and carotenoids compounds (Noctor and Foyer 1998; Schafer et al. 2002). AsA and GSH, the most abundant soluble antioxidants in plants, play a key role in plant defense against oxidative stress (Foyer and Noctor 2011). It has been demonstrated that salinity tolerance is positively

correlated with the activity of antioxidant enzymes, such as SOD, CAT, GPX, APX, and GR, and with the accumulation of non-enzymatic antioxidant compounds (Sairam et al. 2002, 2005; Mandhania et al. 2006; Koca et al. 2007; Khosravinejad et al. 2008; Gapinska et al. 2008; Turhan et al. 2008).

1.4 PLANT ADAPTATION TO SALINITY: GENOMIC, TRANSCRIPTOMIC, PROTEOMIC, AND METABOLIC REGULATIONS

In general, four major factors could determine the tolerance of plants to abiotic stress at the molecular level, which are genomic, transcriptomic, proteomic, and metabolic levels.

1.4.1 Genomic Regulation

Various mechanisms of gene regulation have been identified from transcriptional initiation, to RNA processing, and to the posttranslational modification of a protein. Great advances in the comparison of genomes and the transcriptomes of different organisms have contributed to the development of comparative genomics as one of the most promising fields in the area (Caicedo and Purugganan 2005). Despite the genomes of *A. thaliana* and *Thellungiella parvula* having very similar size and gene number, there are significant differences in gene copy number in certain functional categories important for stress tolerance. In fact, the *T. parvula* genome reveals a higher gene copy number of several genes involved in transport like *AVP1*, *HKT1*, and *NHX8* than *A. thaliana* genome. In contrast, the *T. parvula* genome contains lower gene copy numbers of several genes involved in signal transduction with respect to *A. thaliana* (Dassanayake et al. 2011). Among monocotyledonous plant species, rice has a high degree of synteny with genomes of others cereals plants like wheat, barley, and maize (Caicedo and Purugganan 2005; Paterson et al. 2005).

1.4.2 Transcriptomic Regulation

Transcriptomic analysis provides detailed knowledge about gene expression at the mRNA level, which is widely used to screen candidate genes involved in stress responses. The availability of the complete genome sequence of some model plants like *A. thaliana* and *O. sativa* has allowed the development of whole genome tiling microarrays. This constitutes a new powerful technology that has already made possible the identification of several unannotated transcripts responsive to abiotic stress (Gregory et al. 2008; Matsui et al. 2008). Many genes induced by salinity have been identified by the analysis of gene expression profile (Kreps et al. 2002; Oono et al. 2006; Jianping and Suleiman 2007). It has been reported that in *A. thaliana* and rice, the transcript profile changes under different abiotic stresses like high salinity, cold, and drought (Rabbani et al. 2003; Gong et al. 2005).

Salinity produces an upregulation of some genes and transcription factor in different plant species. These genes can be classified into the following functional categories: ion transport (*SOS*, *AtNHX*, *H+-ATPase* genes); ROS-scavenging; molecular chaperones; and dehydration-related transcription factors (Table 1.1). SOS1 is a component of the SOS signaling pathway, and it plays an important role in ion homoeostasis. *SOS1* gene is upregulated by salinity in monocotyledonous and dicotyledonous species like in *A. thaliana* and in bread wheat (Shi et al. 2002; Xu et al. 2008). In ROS-scavenging genes, we cited the case of *Apx* gene, in which its expression is rapidly induced by various stress conditions, such as paraquat, abscisic acid, ethylene, drought, and heat shock, suggesting an important role in stress tolerance (Mittler and Zilinskas 1991). Molecular chaperones play an important role in salt-stress response, like heat-shock proteins (HSP). In rice, the application of high salt stress induced an upregulation of *OsHsp17.0 and OsHsp23.7* genes (Zou et al. 2009). Genes involved in osmoprotectant biosynthesis are also upregulated under salt stress (Zhu 2002). In response to salinity, many transcription factors have been identified, and they are capable of controlling the expression of a broad range of target genes by binding to the specific *cis*-acting element. The expression of the transcription factor bZIP genes was upregulated in the salt-sensitive wheat cultivar, compared to the salt-tolerant cultivar (Johnson et al. 2002). Thus, in response to salinity, the expression profile is different between the salt-tolerant and salt-sensitive cultivars. This suggestion was observed in other plants like in *Arabidopsis* and *Arabidopsis*-related halophyte (*Thellungiella halophyla*). Indeed, contrary to

TABLE 1.1
Examples of upregulated genes in response to salinity stress.

Gene functions	Gene name	Species	References
Ion transport	*SOS1*	*Arabidopsis thaliana*	Shi et al. (2002)
		Triticum aestivum	Xu et al. (2008)
		Oryza sativa	Atienza et al. (2007)
		Solanum lycopersicum	Olías et al. (2009); Wang et al. (2010)
		Puccinellia tenuiflora	
		Populus trichocarpa	Tang et al. (2010)
ROS scavenging	*Fe-SOD*	*Thellungiella halophyla*	Taji et al. (2004)
	Apx	*Spinacia oleracea*	Yoshimura et al. (2000)
		Pisum sativum	Mittler and Zilinskas (1992)
Heat-shock proteins	*HSP*	*Oryza sativa*	Zou et al. (2009)
		Daucus carota	Song and Ahn et al. (2011)
Transcription factor	*DREB*	*Arabidopsis thaliana*	Tang et al. (2011)
	AlSAP	*Aeluropus littoralis*	Ben Saad et al. (2010)

Arabidopsis, a large number of known abiotic and biotic stress-inducible genes, such as *Fe-SOD*, *P5CS*, *PDF1.2*, *AtNCED*, P-protein, β-glucosidase, and *SOS1*, were expressed in salt cress at high levels even in the absence of stress. Thus, it is possible that the salt tolerance of salt cress is due to constitutive overexpression of many genes that function in stress tolerance and that are stress inducible in *Arabidopsis* (Taji et al. 2004). Comparative analysis of salt-sensitive rice (line IR64) and salt-tolerant rice (Pokkali) has led to identification of some salinity-responsive genes, displaying a higher expression in Pokkali than in IR64 (Kumari et al. 2009). These two examples showed that salt-tolerant plants are able to cope with stress due to the expression induction of some genes implicated in salt-stress response and as a consequence the production of more proteins and response efficiently to stress.

In addition to the upregulation of some genes, downregulated genes are emerging now as essential components of the response to salinity. For example, downregulation of β-carotene hydroxylase increases β-carotene and total carotenoids enhancing salt-stress tolerance in transgenic cultured cells of sweet potato (Kim et al. 2012). Therefore, it is possible that in plants there is a mutual regulation mechanism between different genes and proteins and signals underlying different processes of plant adaptation to abiotic stress.

1.4.3 Proteomic and Metabolic Regulations

Several common stress-responsive proteins are expressed in response to various abiotic stresses in different plants species, which are either upregulated or downregulated by salinity stress (Zhang et al. 2012). Several functional groups of proteins affected by salt stress include proteins involved in ion transport, signaling, energy metabolism (photosynthesis, respiration, ATP production), protein and lipid metabolism, metabolism of osmolytes and phytohormones, and stress-related proteins. Comparative proteome responses to salt stress have been analyzed in some related plant species with contrasting salinity tolerance like *A. thaliana* and *T. salsuginea* (Pang et al. 2010), rice and salt-tolerant wild rice *Porteresia coarctata* (Sengupta and Majumder 2009), and common wheat (*T. aestivum*) cv. Jinan 177 and *T. aestivum*/*Thinopyrum ponticum* (Wang et al. 2008). These studies give information about differential protein abundance but not about protein function under salinity, and therefore, validation of comparative proteomics should be done by protein functional analysis. Other approaches like posttranslation modifications, protein–protein interactions, tissue and subcellular localization, and phenotype influence by silencing or overexpressing the gene coding for a protein interest have to be employed to unravel the role of the proteins in acquisition

and development of salinity tolerance in plants. Consequently, large-scale high-throughput proteome analyses must be integrated with transcriptomic and metabolomic analyses to improve our understanding of the stress response.

Another significant research approach in plant system biology is metabolomics, which involves the study of metabolome. Plant metabolites implicated in salinity tolerance include polyols like mannitol; dimethylsulfonium compounds; glycine betaine; sugars such as sucrose, trehalose, and fructans; or amino acids like proline. The concentration of these osmolytes increases in plant subjected to salt stress, suggesting their importance in salt tolerance.

1.5 CONCLUSIONS AND FUTURE RESEARCH PERSPECTIVES

Salinity tolerance involves a complex of responses at molecular, cellular, and whole plant levels. An understanding of how single-cell responses to salt are coordinated with an organism and whole-plant responses to maintain an optimal balance between salt uptake and compartmentation is fundamental to our knowledge of how plants successfully adapted to salt stress. Despite the significant advancements in the fields of genomic, transcriptomic, proteomic, and metabolomic techniques, there is lack of the integration of information among these four regulation levels. Therefore, the combined approach is essential for the determination of the key pathways or processes controlling salinity tolerance.

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CHAPTER TWO

Mechanisms of Salt Stress Tolerance in Halophytes

BIOPHYSICAL AND BIOCHEMICAL ADAPTATIONS

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CONTENTS

Abstract. The salinization of soils is one of the most important factors impacting plant productivity. About 3.6 billion of the world's 5.2 billion ha of agricultural dryland have already suffered erosion, degradation, and salinization. This arises the need to arrange solutions to overcome the stress imposed by salinity to the typical glycophytic crops, such as the improvement of these crops or the use of halophytes in their substitution. Halophytes typically are considered to be plants able to complete their life cycle in environments where the salt concentration is around 200 mM NaCl or higher, representing 1% of the world flora. Different strategies are identified to overcome salt stress as adaptation mechanism from these type of plants. The adjustment to salinity is a complex phenomenon characterized by a high degree of ecological complexity, structural changes, and physiological adjustments both at the biochemical and biophysical levels. These adaptations have naturally evolved in halophytes as responses to their colonization of saline ecosystems, and therefore making halophytes good model plants to study the tolerance mechanisms underlying these salinity constrains. As photosynthesis is a prerequisite for biomass production, halophytes adapted their electronic transduction pathways and the entire energetic metabolism to overcome the stress imposed by the excessive ionic concentration in their cells. The maintenance of the homeostasis between the Na+, K+, and Ca+ concentrations is in the basis of all cellular stress in particular in terms of redox potential and energy transduction. A salt-stressed cell is unable to process the electronic energy fluxes leading to the accumulation of lethal excessive energy. In the present work, the biophysical mechanisms underlying energy capture and transduction in halophytes are discussed and their relation to the biochemical mechanisms (osmocompatible solute production, pigment profile alteration, antioxidant enzymatic and nonenzymatic defenses), integrating data from the photosystem light harvesting complexes (LHC), passing through electronic transport chains (ETCs) to the quinone pools and the carbon harvest and energy dissipation metabolism and the inevitable antioxidant processes, in order to draw a map of some of the diversity of metabolic mechanisms of salt stress tolerance.

2.1 INTRODUCTION

Earth is in fact a salt planet. Seventy percent of its surface is covered by salt water, the oceans, with concentrations of Na+ around 500 mM in contrast with the low K+ concentrations of 9 mM (Flowers, 2004). Also, the remaining 30% of Earth's surface is being severely affected by an increased salinization phenomenon, mostly due to the increased soil use to agriculture and its irrigation procedures (Zhang and Shi, 2014). Aggravating this situation are the ongoing climate changes increasing drought, air temperature, and salt water intrusion in coastal soils (Duarte et al., 2013a). This will have severe impacts in the planet's terrestrial primary production with a special emphasis on the crop production. Salinity-induced damage in plants include reduction of leaf expansion, stomata closure, reduced primary production, biomass losses due to water deficit, and deficiency in essential nutrients like K+ (Mahajan and Tuteja, 2005; Rahnama et al., 2011; James et al., 2011). Although this is true for most of Earth's flora, halophytes are the exception, being highly productive under saline conditions.

Halophytes are defined as plant species that can survive and reproduce under growth conditions with more than 200 mM NaCl, comprising only 1% of world flora (Flowers and Colmer, 2008). Some of these species are what may be called "obligate halophytes," like *Suaeda maritima* and *Mesembryanthemum crystallinum*, requiring saline environments for optimal growth, while other species like *Puccinellia maritima* and *Thellungiella halophila* are "facultative halophytes" with optimal growth without salt in their substrate but tolerating high NaCl concentrations (Flowers, 1972; Gong et al., 2005; Gao et al., 2006, 2012; Agarie et al., 2007; Wang et al., 2007, 2009). Salt tolerance results from a complex network of mechanisms involving multiple biochemical and physiological traits. Over the last decades, this issue attracted several investigation groups as the global soil salinization problem intensified, and the need to understand these mechanisms increased with the main objective of applying this knowledge to economically important crops. On the other hand, another source of interest arises as some halophytes were identified as potential food sources with high nutritional value and with possibilities to be cultured in arid environments of the poorer regions of the planet, such as the African desert countries. Several halophytes like *Aster tripolium* (Ventura et al., 2013), *Chenopodium quinoa* (Eisa et al., 2012), and *Salicornia sp.* (Ventura and Sagi, 2013) are already identified and commercially used as food sources in some countries.

2.2 MORPHOLOGICAL ADAPTATIONS

Some of the more evident adaptations to arid salt environments are immediately detected while observing halophyte morphology. There are typically two mechanisms that halophytes undergo in order to overcome high salinities: secretion and exclusion. The secretion-based strategy implies the existence of specialized salt glands, normally located at the leaf surface, which excrete the excess salt and thus avoiding its potential negative effects on cell metabolism (Figure 2.1). This tolerance mechanism is probably one of the most well studied in halophytes (Rozema et al., 1981; Waisel et al., 1986). The accumulated salt crystals on the leaf surface are then washed by rain or, since most halophytes inhabit coastal areas, are washed away by tidal waters (Balsamo et al., 1995).

On the other hand, *Suaeda fruticosa* (Amaranthaceae), for example, is a typical excluder, retaining higher amounts of K^+ and Ca^{2+} inside its cells, thus avoiding the entrance of Na^+ (Figure 2.2). This exclusion strategy is often accompanied by a dilution strategy implying that the halophyte increases its intracellular water content in order to decrease

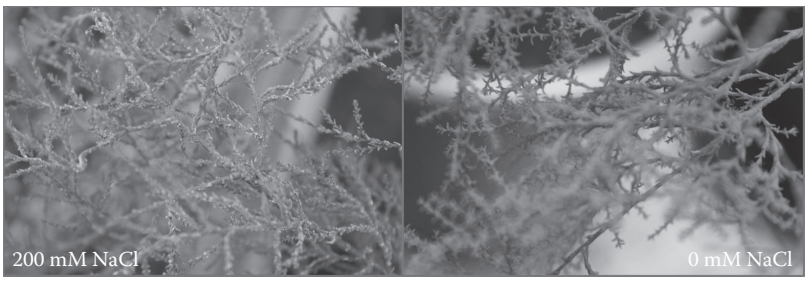

Figure 2.1. *Tamarix gallica* leaves of individuals subjected to 200 and 0 mM NaCl. (Photo by B. Duarte.)

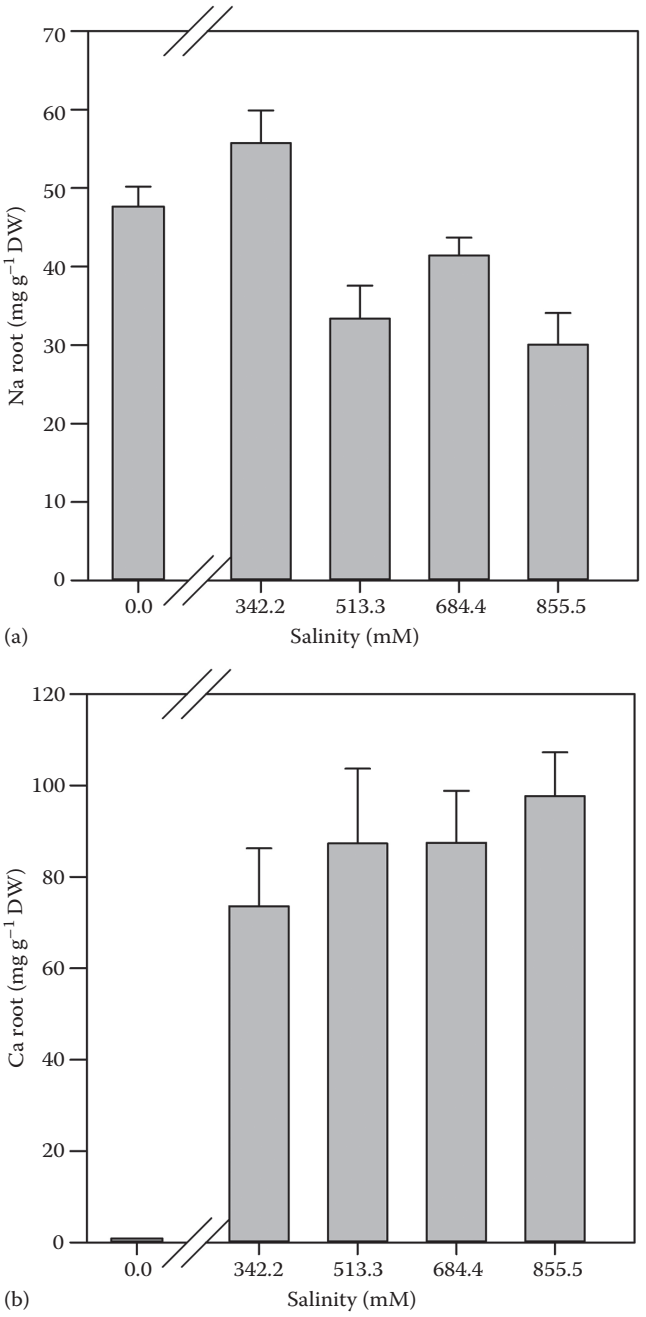

Figure 2.2. Na (a) and Ca (b) ionome in the roots of *Suaeda fruticosa* exposed to increased salinity levels.

the ionic concentration inside its cells (Figure 2.3). Nevertheless, all these morphological adaptations have implications at the biophysical and biochemical levels. This was also observed for *T. halophila* retaining higher potassium and accumulating less sodium, while increasing its transpiration rate resulting in a high water uptake (Volkov and Amtmann, 2006). On the basis of this differential ionic absorption are specific protein-like ionic channels. A total of 32 salt-induced differentially expressed proteins were identified in *T. halophila* (Pang et al., 2010). In stress situations, K^+ transporter proteins are preferentially expressed counterbalancing the extracellular Na^+ concentration.

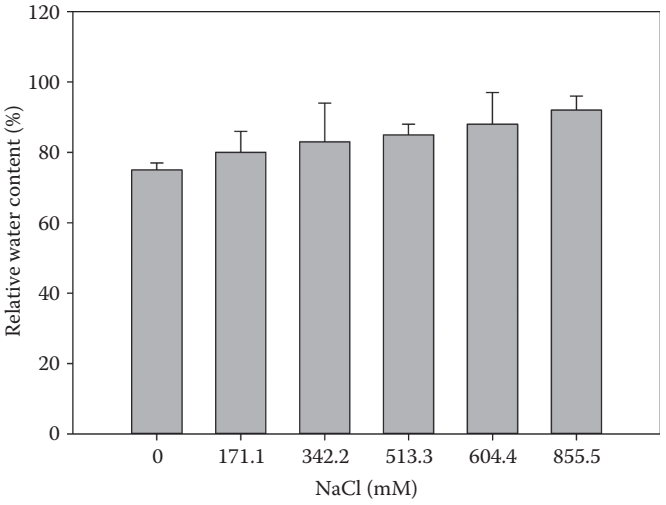

Figure 2.3. *Suaeda fruticosa* photosynthetic stems relative water content exposed to increased salinity levels.

2.3 BIOPHYSICAL CONSTRAINS

As all other excessive ionic accumulation, excessive salinity also has its redox implications at the cellular level, unbalancing the electronic fluxes inside the cell. A decrease in photosynthesis capacity is very common in salt-stressed plants (Munns and Termaat, 1986; Munns, 1993; Qiu et al., 2003; Jaleel et al., 2007), mostly due to a low osmotic potential of the soil solution (osmotic stress), specific ion effects (salt stress), nutritional imbalances, or, more usually, a combination of these factors (Zhu, 2003). One of the consequences of salinity-induced limitation of photosynthetic capacity is the exposure of plants to excess energy with inevitable consequences on the photosystem II (PSII), if the dissipation mechanisms are not efficient enough (Demming-Adams and Adams, 1992; Qiu et al., 2003), since plants under salt stress use less photon energy for photosynthesis (Megdiche et al., 2008). The effects of salinity on photosynthesis include several other consequences besides the damage on PSII. Also the photosynthetic carbon harvesting is affected by disturbances on leaf water relations and osmotic potential (Munns, 2002; Zhao et al., 2007) on the chloroplast membrane systems and on the pigment composition (carotenoids and chlorophyll). To avoid damages to the PSII, plants have developed several strategies to dissipate excessive energy, protecting the photosynthetic apparatus. Comparing a glycophyte species with a halophyte one, the differences in a global examination of the PSII activity are evident (Figure 2.4). Both real (operational) and maximum activities of PSII suffer drastic decreases in activity due to salt stress in glycophyte species. On the other hand, a halophytic species very well adapted to salt environments shows almost no differences along a salinity gradient even at oceanic salt concentrations.

PSII quantum yield gives rapid and valuable informations on the overall ongoing processes in the PSII, but in order to understand the causes of these changes as well as the mechanisms that allow halophytes to overcome salt stress, one has to delve deeper into the biophysics and energetics of the chloroplast. PSII efficiency relies essentially on two major processes: (1) photon harvesting, entrapment, and transport throughout the transport chain and (2) excessive electronic energy dissipation. Examining the first one and specially the electronic transport depending on the tolerance and defense mechanism, two behaviors can be observed (Figure 2.5). Observing the rapid light curves obtained for both species at different stress levels, it is possible to observe that there are evident differences either between species or, in particular for *Halimione portulacoides*, between stress levels. In *S. fruticosa*, the maximum electron transport rate (ETR), photosynthetic efficiency, and the onset of light saturation are very similar among healthy and stressed individuals, only with small differences also regarding the rETR at different light exposures. On the other hand,

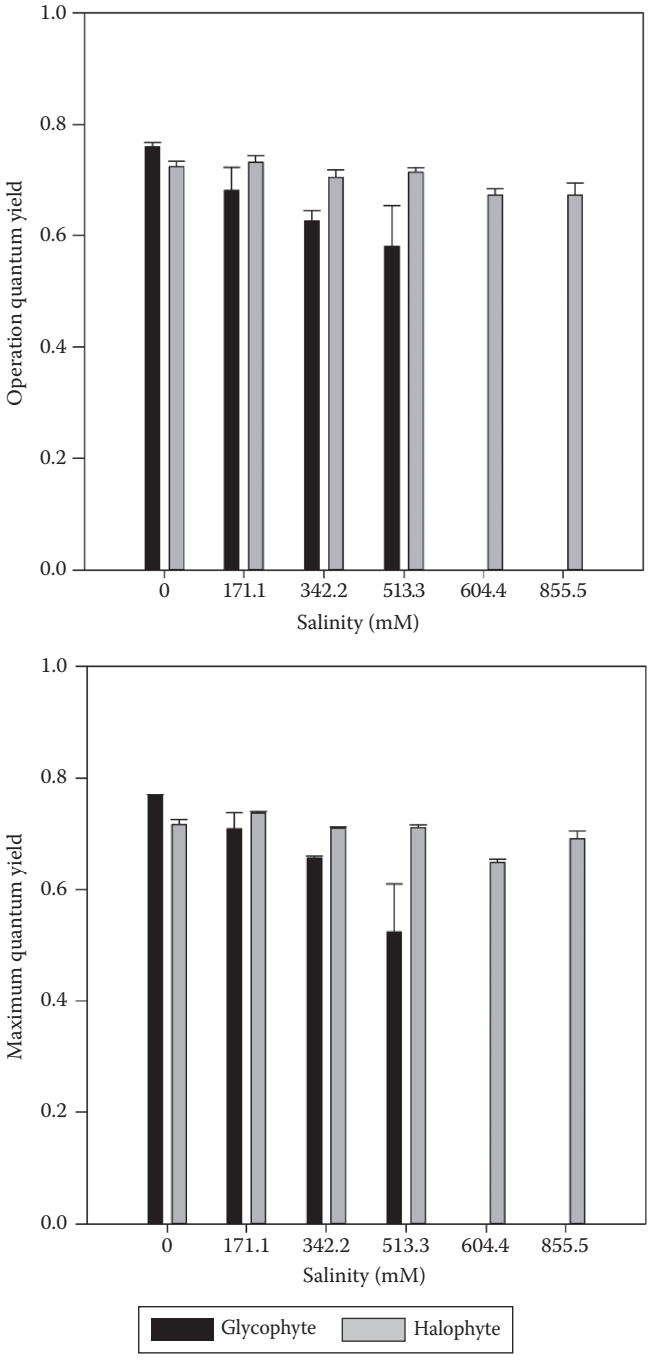

Figure 2.4. Operational and maximum photosystem II efficiency in a glycophyte and in a halophyte along a salinity gradient.

H. portulacoides stressed and healthy individuals exhibited very distinct photosynthetic parameters. Not only the photosynthetic efficiency and the onset of light saturation were reduced to zero, but also the maximum ETR was lower in stressed individuals. Observing *S. fruticosa* healthy and stressed individuals, there are no major differences neither between the ETR nor in the onset of light saturation, indicating a normal functioning in the ETC. As for *H. portulacoides*, not only the

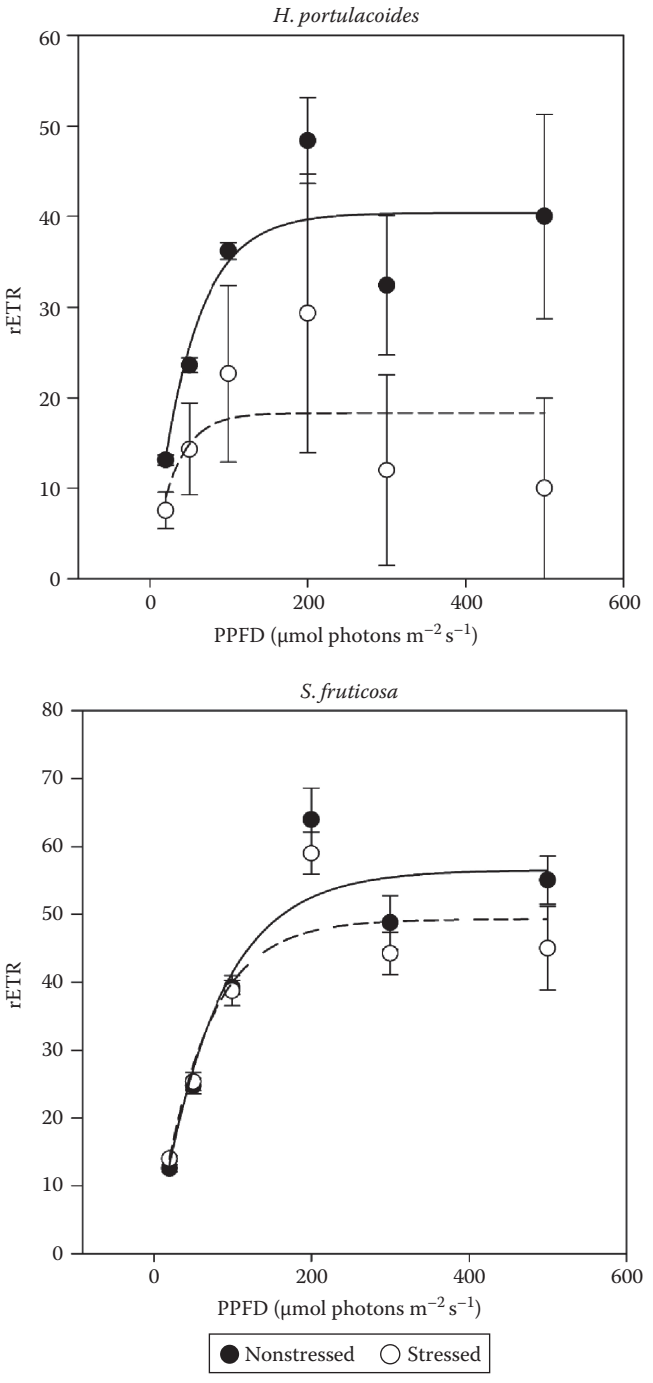

Figure 2.5. Electron transport rate at different light intensities in *Halimione portulacoides* and *Suaeda fruticosa* stressed and nonstressed individuals. (From Duarte, B. et al., *Plant Physiol. Biochem.*, 67, 178, 2013b.)

ETR is rather decreased in stressed individuals, but also these individuals have a smaller onset for light saturation, indicating incapacity to use the absorbed photons into primary photochemistry. This inevitably leads to an accumulation of large amounts of lethal energy that, as stated before, can destroy the D1 protein, impairing the photochemical apparatus. Again two tolerance mechanisms are evidenced between these two Amaranthaceae species. *S. fruticosa* has salinity tolerance mechanisms that allow the photosystems to absorb light even at high Na concentrations, while in *H. portulacoides* these mechanisms appear to be absent or inactivated, leading to lower light and carbon harvesting efficiencies. In fact *S. fruticosa* exhibits a common feature among halophytes: elevated salt concentrations improve some energetic mechanisms. Delving even deeper into the electronic processes, one can distinguish how the energy fluxes that, in sum result in the PSII activity, are affected by salt stress.

Looking deeper into the photochemical mechanisms (Figure 2.6), it can be observed that in *S. fruticosa* the major factor responsible for the decrease in the photosynthetic rate is due to salinity adverse effects in the quinone pools. Both the electron flow from the ETC to the quinone pool (Sm) and the quinone reduction turnover rate were rather decreased, leading to an excess of energy accumulated at this level (Kalaji et al., 2011). In *H. portulacoides*, the negative effects driven by salt stress leads to higher amounts of energy dissipated rather than trapped in the photochemical reactions, ultimately having as a consequence the destruction of the D1 protein (Rintamäki et al., 1995). In these individuals, there is a small probability that an incident photon can move an electron throughout the ETC and also a reduced efficiency of a trapped electron to move further than the oxidized quinone, reducing this way the maximum yield of primary photochemistry (Kalaji et al., 2011). Although excessive salt acts at different levels in the two different analyzed species (in the photon reception in *H. portulacoides* and in the reduction of the quinone pool in *S. fruticosa*), all these effects are well summarized overlooking the reduced performance index in stressed individuals, due to its dependency on the efficiency, yield of energy transfer, and primary photochemistry (Figure 2.7). The behavior exhibited by *S. fruticosa* is very similar to the one found in *T. gallica* when supplied with 200 mM NaCl and can be easily detected using a rapid induction Kautsky curve (Figure 2.8). This type of analysis is very quick and allows a rapid interpretation of the overall energetic fluxes underlying the PSII activity. In this assessment, two phases can be distinguished: O-J phase or photochemical phase and the J-I-P phase or thermal phase. The first one is considered to be a good proxy of the photochemical energy production work ongoing inside the chloroplasts, while the second one reflects the ability to dissipate excessive amounts of energy throughout thermal dissipation. It is possible to observe that *T. gallica* individuals have similar photochemical activity both with and without salt, but the individuals supplemented with 200 mM NaCl have a higher ability to dissipate excessive energy throughout heat formation. This is one of the mechanisms that halophytes exhibit to overcome excessive energy absorbed to the photosystems while under stress, avoiding this way the photodestruction of the photosynthetic apparatus (Duarte et al., 2013b).

2.4 BIOCHEMICAL IMPLICATIONS

Beyond the biophysical processes, halophytes have also a battery of biochemical adjustments to counteract, at the molecular level, the cellular stress imposed by excessive ionic concentrations, namely, Na. Still in the chapter of the photosynthetic light harvesting mechanisms, also the pigment profiles are often affected by elevated salt concentrations. On the other hand, under favorable conditions, the increase in efficiency of the photosystems, consequence, for example, of optimal salt concentrations, a frequently observed strategy is the decrease of the antenna size since there is no need to have large LHC as it would be in stressful conditions for maximization of light harvesting (Rabhi et al., 2012), as it can be evaluated by its pigmentar proxy, the chl a/b ratio (Figure 2.9). The increase of the chl a/b ratio is directly related to an increase in the number of active light harvesting reaction centers and is commonly used as indicator of plant photochemical capacity enhancement, leading to an increase in processing the absorbed light, even at normal light conditions. On the other hand, when the halophyte is away from its optimum salt

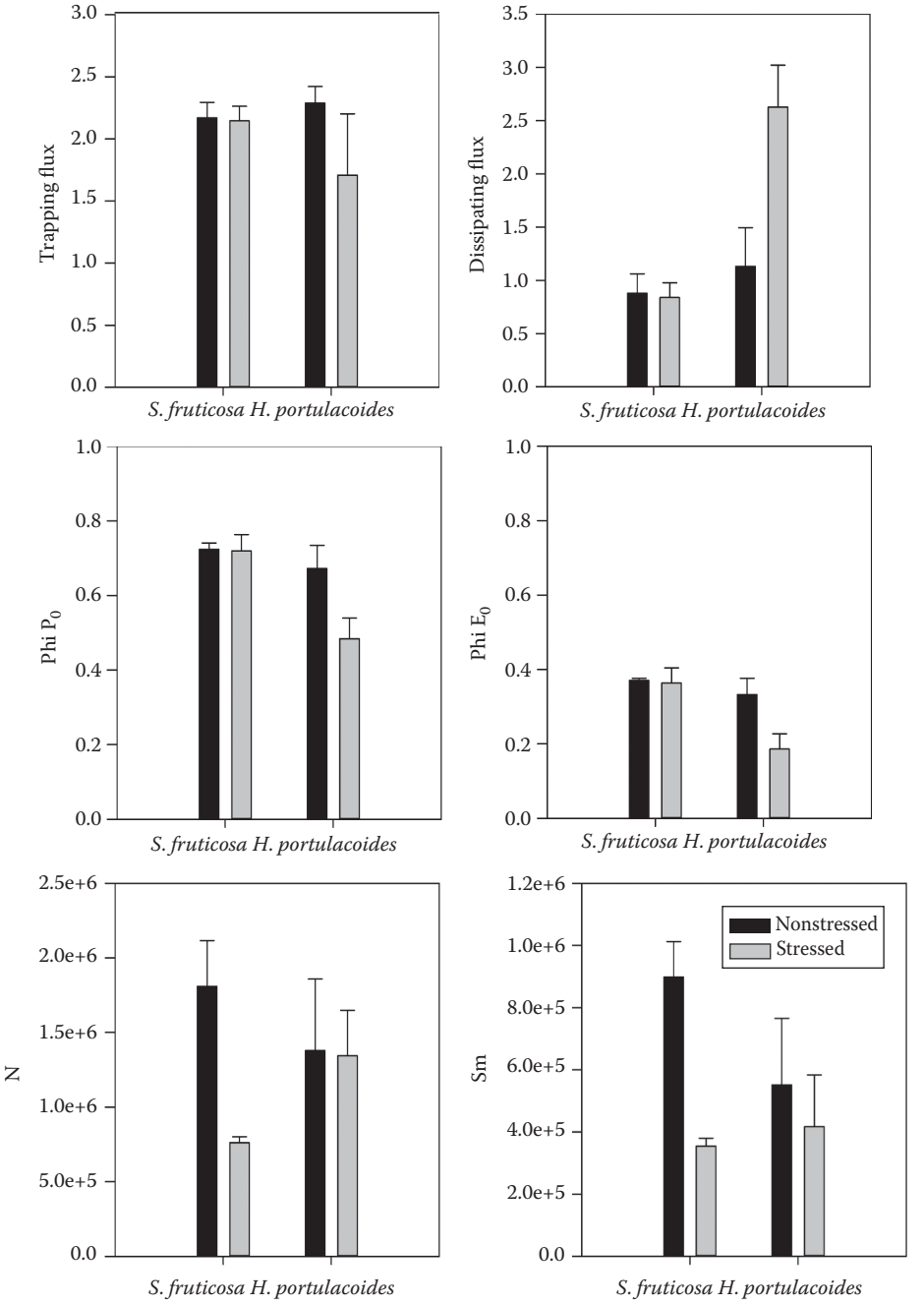

Figure 2.6. Rapid transient O-J-I-P curve calculated parameters in stressed and nonstressed individuals of *Suaeda fruticosa* and *Halimione portulacoides* species. (From Duarte, B. et al., *Plant Physiol. Biochem.*, 67, 178, 2013b.)

conditions, the excessive energy reaching the photosystems must be dissipated (Duarte et al., 2013b). *H. portulacoides* appears to have a physiological optimum at median NaCl concentrations (513.3 mM) similar to those observed in its natural habitat at the estuaries.

On the other hand, when this increase in LHC is not enough to process all the incoming solar radiation, the plant needs to dissipate its energy, either by fluorescence quenching or by a pigment cycle involving a class of carotenoids called xanthophylls (Demming-Adams and Adams, 1992).

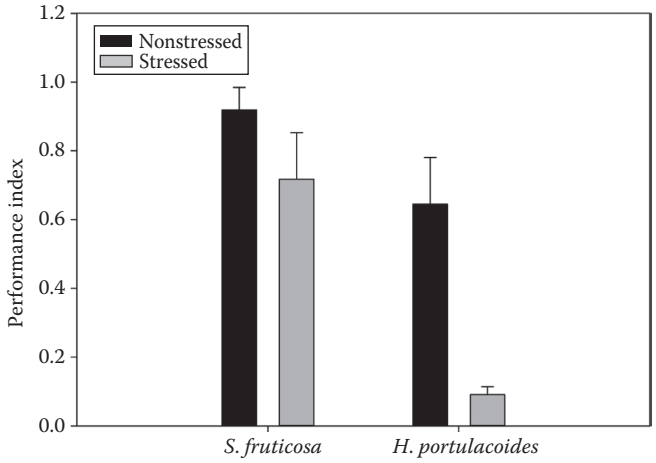

Figure 2.7. Performance index derived from the J-I-P test, in stressed and nonstressed individuals of *Suaeda fruticosa* and *Halimione portulacoides* species. (From Duarte, B. et al., *Plant Physiol. Biochem.*, 67, 178, 2013b.)

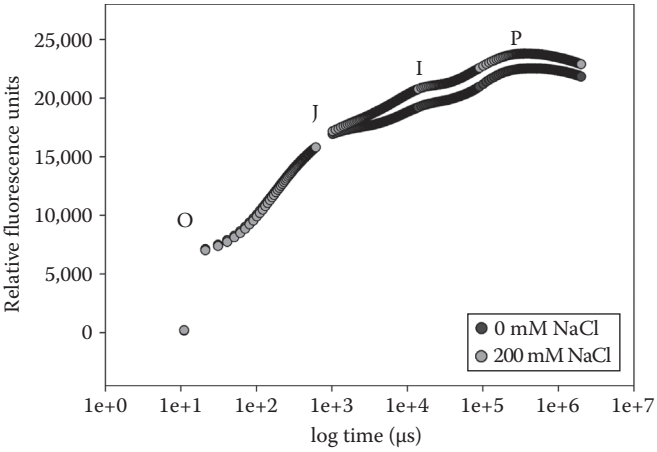

Figure 2.8. Kautsky curve from *Tamarix gallica* individuals exposed to 0 and 200 mM NaCl.

Additionally in extreme stress conditions, an unhealthy plant cannot withstand what in normal cases would be considered a normal dose of light, and thus even at low solar radiances, it undergoes photoinhibition and thus needs to dissipate energy. Another evident signal of environmental stress is the xanthophyll cycle malfunctioning, as revealed by the increase in the de-epoxidation state index (Figure 2.10). When the absorbed light exceeds the plant photochemical capacity (as revealed previously by the decrease in the chi a/b ratio), even in normal light conditions, this excess energy may be transferred to the ever-present oxygen. In this context, the conversion of violaxanthin to zeaxanthin throughout the xanthophyll cycle is considered to be one of the most effective energy dissipation mechanisms (Demmig-Adams and Adams, 1992). Also the total-chlorophyll-to-total-carotenoid ratio points in the same direction. An increase in this ratio occurred in stressed individuals of both species, indicating that, although all pigments suffer a drastic decrease under stress, chlorophylls decrease in a smaller proportion than carotenoids, enhancing the light harvesting efficiency and counteracting stress (Figure 2.10).

Although this turnover toward the carotenoid production is not evident to the naked eye, sometimes another phenomenon can be seen overlooking halophytic extensions, especially during summer. During warm seasons, sediment water evaporates, greatly increasing the salinity in the

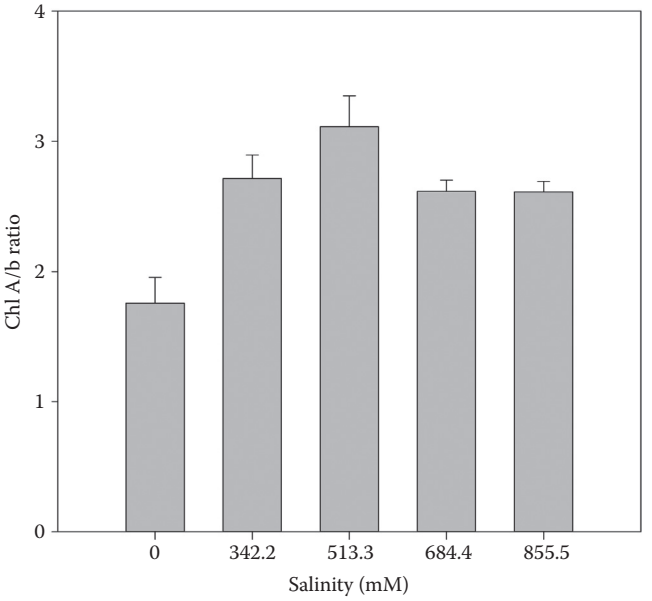

Figure 2.9. Chlorophyll a/b ratio in *Halimione portulacoides* leaves from individuals exposed to a salinity gradient.

sediments sometimes to values twice those observed in seawater. In these conditions, it is often observable in Amaranthaceae salt marshes large extensions of plants exhibiting a strong red coloration (Figure 2.11). This coloration is due to a presence of water-soluble pigments from the betacyanin class, normally produced as a response to salinity, anoxia, or thermal stresses (Wang et al., 2006). Betacyanins play an important role in scavenging reactive oxygen species (ROS) (Stintzing and Carle, 2004), generated under environmental stress conditions. Wang et al. (2006) found similar results for other Amaranthaceae species (*Suaeda salsa*), suggesting that this betacyanin production is part of a common defense mechanism against environmental stresses, namely, salinity. Commonly, these pigments can also be indicators of a high betaine production, a quaternary ammonium compound, mainly accumulated in the chloroplast in order to counteract high Na concentrations in this compartment (Rhodes and Hanson, 1993; McNeil et al., 1999). As for the cytosol, the plant tends to accumulate proline, an amino acid but also a quaternary ammonium compound, in this compartment as an effective osmoregulator of the ionic pressure exerted by excessive salt concentrations. Comparing a glycophyte with a halophyte species, the differences are evident (Figure 2.12), with the halophyte species highly adapted to salinity, with an enormous production of betain in order to balance and regulate the osmotic potential inside its photosynthetic compartments.

Halophytes are often classified as extremophyte species inhabiting extremely arid environments under extreme abiotic conditions adverse to life support, particularly high salinity levels. Another interesting adaptation developed by this group of plants was the acquisition and development of a highly efficient battery of antioxidant enzymes. As any other excessive ionic concentration, high Na concentrations generate ROS due to its reactions with the cellular biological elements (Duarte et al., 2013c). Halophytes evidence a highly efficient enzymatic system of rapid response to salinity changes that are rapidly activated when the medium conditions are shifted aside from the halophyte optimum (Figure 2.13).

This battery has its higher expression while overlooking its first line of defense, superoxide dismutase. This enzyme catalyses the conversion of the highly toxic superoxide anions to hydrogen peroxide. As a second line of defense, intrinsically connected to the first one are the peroxidase class of enzymes such as catalase, ascorbate peroxidase, and guaiacol peroxidase. All three enzymes have as major function in the hydrogen peroxide detoxification and thus in the reduction of ROS to nondamaging concentrations.

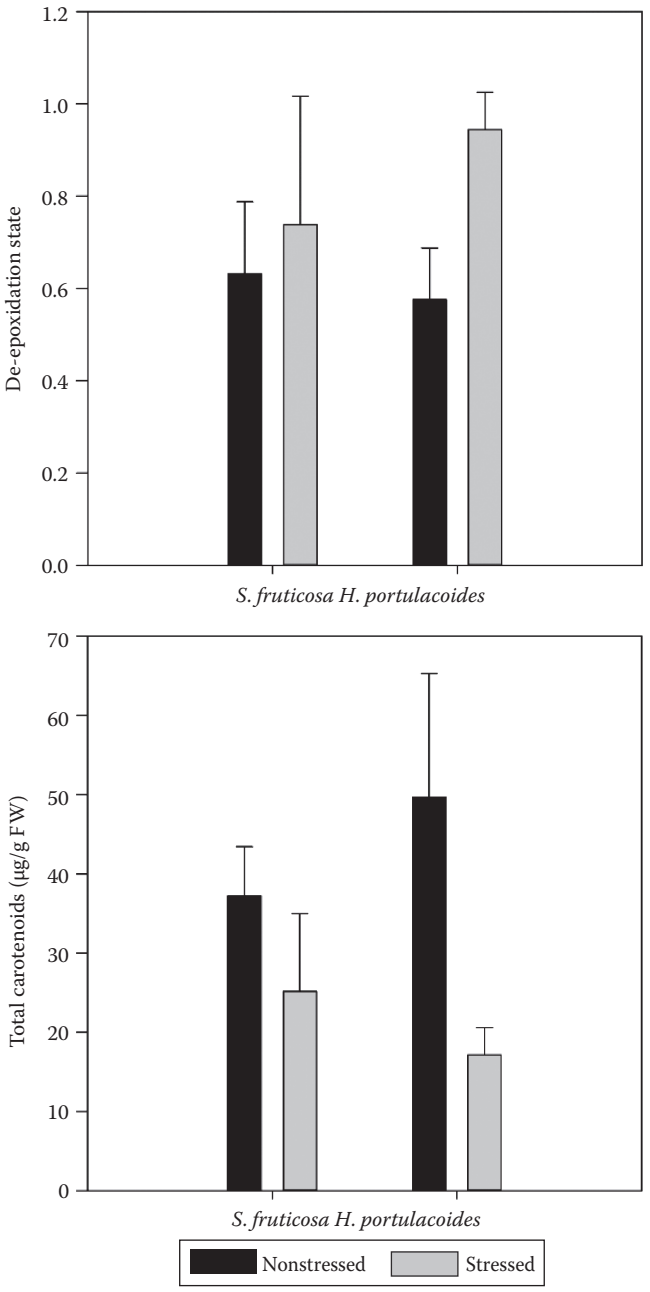

Figure 2.10. De-epoxidation state and total carotenoids in *Suaeda fruticosa* and *Halimione portulacoides* stressed and nonstressed individuals. (From Duarte, B. et al., *Plant Physiol. Biochem.*, 67, 178, 2013b.)

It is possible to observe that these enzymes are activated both at very low concentrations of Na (below the physiological optimum) and at seawater Na concentrations (considered excessive), pointing out to a physiological Na dependence in certain halophytes, like *H. portulacoides* (Figure 2.13).

2.5 FINAL REMARKS

Halophytes are extremely plastic species with a high degree of adaptation to saline habitats being therefore excellent models to study salt resistance and tolerance mechanisms. Nevertheless, some halophytes have recently been pointed