Mohd Sayeed Akhtar *Editor*

Salt Stress, Microbes, and Plant Interactions: Mechanisms and Molecular Approaches

Volume 2

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This book is dedicated to my grandfather,
Nana

Muhammad Aziz-ur-Rahman (1907–1985)

A great scholar, statesman, social reformer,
and famous hakim of the twentieth century

Foreword

Global warming and other potential climatic abnormalities associated with it cause different types of biotic and abiotic stress conditions that lead to affect plant growth and yield severely. Though not visible to our naked eyes, plant-microbe interactions occur in several ways. In fact, plant interacts with microbes at a certain phase of their life, and this communication is not inevitably harmful for the plant. Indeed, there are copious examples of plant-microbe associations that effectively benefit the plants by enhancing their growth, survivability, and productivity. The plant-microbe interaction is very essential for combating any stressful conditions. The microbes associated with plants secrete volatile compounds that encourage plant growth and/or provide resistance against abiotic and/or biotic stresses. Microorganisms, occupying in different environments, have variable biological and metabolic mechanisms to overcome ecological stresses. As the microbial diversity in the environment is very high, it is required to characterize and clarify microbial associations with plants in relation to protect them against several ecological encounters. The microbiota of different soils and their beneficial effect on plants need to be studied in detail, so that they can be used as a consortium to improve crop productivity under different agro-climatic conditions. Moreover, understanding the physiological and molecular mechanisms involved in plant-microbe interactions will be very useful for improving the agronomic applications of rhizospheric microbes. The recent advancements in modern techniques have helped in identifying and quantifying the microbial diversity linked with plants. This has allowed us to understand the immense interactions of microbes with the plants. The knowledge of these research accomplishments provides a comprehensive understanding on multidimensional communications between microbes and plants. Thus, it is required to have a collective database on the existence of varied stress combinations in agronomically important areas, and this will allow one to make use of the beneficial microbes to provide stress tolerance.

The present volume to be published by “Springer,” *Salt Stress, Microbes and Plant Interactions: Mechanisms and Molecular Approaches* (Volume 2), includes 13 chapters contributed by the authors from different parts of the world. Chapter 1 by the Indian authors provides information on the role of microbes in the management of crop salinity. Also, it discusses on the mechanisms and microbial applications in overcoming salinity stresses. Chapter 2 contributed by the Malaysian authors describes in detail about the adaptive mechanisms of plant responses to salts. In Chaps. 3 and 4, the Indian authors have summarized the molecular approaches and salt tolerance mechanisms in leguminous plants and crop improvement through microbial biotechnology. In Chapter 5, the Indian authors discuss the role of phytohormones in recuperating salt stress. Similarly, Chaps. 6 and 7 by the Malaysian and Indian authors, respectively, describe the genetic responses of plants to salt stresses and the use of microbial biotechnology in improving salt stress and crop productivity. Chapter 8 discusses the consequences of bioinoculants and intercropping approach to alleviate salinity stresses. However, Chap. 9 entails the pathways of signaling molecules in improving salt stress in plants. Chapter 10 by the Indian authors describes the mechanisms and molecular approaches for salt tolerance enhancement. Similarly, Chaps. 11 and 12 by Pakistani contributors describe the methods used to reduce and mitigate the adverse impact of soil salinity and to overcome salt stresses through bacterial inoculation. Lastly, Chap. 13 by the Indian authors describes the diverse roles of proline and its mechanism of tolerance and adaptation under salinity stress. In this edited volume, an attempt has been made to highlight both the hypothetical and practical aspects of salt stress tolerance in plants. Furthermore, it highlights on the physiological, ecological, biochemical, and molecular mechanisms involved in various plant-microbe interactions to overcome salt stresses. I personally congratulate Dr. Mohd Sayeed Akhtar for his noble academic efforts in bringing out this book volume.

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Mallappa Kumara Swamy

Preface

Salt stress has pessimistic crash on the yields of various agricultural crops, and it may directly affect the livelihood of farmers worldwide. The excessive salts in soil lower the availability of water, inhibit metabolic processes, and affect nutrient composition, osmotic balance, and hydraulic conductivity that result to stunted growth and low productivity of cultivated crop plants. Plants have developed number of processes involved in the tolerance mechanism, such as various compatible solutes, polyamines, reactive oxygen species and antioxidant defense mechanism, ion transport, and compartmentalization of injurious ions. To overcome this problem, the exploitation of genetic variation; the use of plant hormones, mineral nutrients, and soil microbes; and other mechanical practices are of prime importance. It is a fascinating subject, which is multidisciplinary in nature, and concerns scientists involved in plant heath. There have been marked advances in this field during the past few decades.

Salt Stress, Microbes, and Plant Interactions: Mechanisms and Molecular Approaches (Volume 2) is a very timely effort in this direction. This book volume describes both the theoretical and practical aspects of salt stress tolerance and the physiological, ecological, biochemical, and molecular mechanisms involved in understanding these multi-tropic interactions. I hope that the book will be helpful to graduate students, teachers, researchers, and industry persons who are interested in agronomy, ecology, stress physiology, environmental science, crop science, and molecular biology.

I am highly grateful to all our contributors for readily accepting our invitation, for not only sharing their knowledge and research but also venerably integrating their expertise in dispersed information from diverse fields in composing the chapters, and for enduring the editorial suggestions to finally produce this venture. I greatly appreciate their commitments. I am also thankful to Professor Mallappa Kumara Swamy for his suggestion and for writing the foreword of this volume. Moreover, I am thankful to my beloved wife, Mrs. Shagufta Bano, and lovely sons, Mohd Rafaan and Mohd Almaan, for their unconditional encouragement, support, and moral boost up throughout the compilation of this book volume. I also thank the team of Springer Nature, especially Dr. Kapila Mamta, Raman Shukla, and Raagaipriya Chandrasekaran, for their generous cooperation at every stage of the publication.

Shahjahanpur, Uttar Pradesh, India Mohd Sayeed Akhtar

About the Book

The interaction between plant, soil, and microbes is fairly intricate and is essential for combating any stressful condition. The presence of excessive salt in soil lowers the availability of water, inhibits metabolic processes, and affects nutrient composition, osmotic balance, and hydraulic conductivity that resulted in the stunted growth and lower productivity of crop plants. *Salt Stress, Microbes and Plant Interactions: Mechanisms and Molecular Approaches* (Volume 2), along with the recently published *Salt Stress, Microbes and Plant Interactions: Causes and Solution* (Volume 1), provides a detail account on the physiological, ecological, biochemical, environmental, and molecular levels for this multi-tropic interaction. Specifically, these two titles are focus on both the theoretical and practical aspects and also provide a solid foundation for students, teachers, researchers, and industry persons interested in agronomy, ecology, stress physiology, environmental science, crop science, and molecular physiology.

Contents

About the Editor

Dr. Mohd Sayeed Akhtar (PhD) is working as an assistant professor in Gandhi Faiz-e-Aam College, Shahjahanpur, UP, India. He has received his PhD degree from Aligarh Muslim University (AMU), India, in 2008, prior to conducting postdoctoral research at the Botanical Institute, University of Basel (BIB), Switzerland, in 2008–2010, and Chonbuk National University (CBNU), Republic of Korea, in 2011. He was an assistant professor at Jimma University, Ethiopia (2011–2014), and a fellow researcher at the International Institute of Tropical Agriculture, Universiti Putra Malaysia (UPM) (2014–2015). He has more than 15 years of research and 10 years of teaching experience in soil microbiology, applied microbiology, environmental microbiology, molecular biology, plant pathology, and plant nanobiotechnology. He is author and coauthor of more than hundred articles in peer-reviewed journals, conference proceedings, and book chapters and has edited 12 books with international publishers. He is serving the scientific community as editorial board member and reviewer of several high-impact international journals. His current research is focused on the rhizospheric plant-microbe interactions and their molecular biotechnology, bioremediation, biomineralization, nano-fertilizers, and nanobiotechnology.

Microbial Management of Crop Salinity Stress: Mechanisms, Applications, and Prospects

1

Hillol Chakdar, Dnyaneshwar Namdev Borse,
Shaloo Verma, Prassan Choudhary, and Sudipta Das

Abstract

Salinity stress is a major deterrent to crop growth worldwide. A high concentration of salt in soil and irrigation water reduces water uptake by plants, which triggers a range of cellular and metabolic processes ultimately resulting in lowered crop yield. Genetics of salinity tolerance in plants is well understood and has been successfully utilized to breed improved crop varieties; however, such strategies are long drawn and cost intensive. Microorganisms due to their immense metabolic diversity can be very useful to devise low-cost strategies to ameliorate salinity stress in crop plants. An intricate and highly complex interaction between plant and microbe results in alleviation of salt stress. Although the cross talk between the plant and microbe is not clearly understood, regulation of osmotic balance and ion homeostasis by microorganisms are mostly mediated through modulation of phytohormone production, alteration in gene expression, protein function, and metabolite synthesis in plants. Consequently, improved antioxidant activity, accumulation of compatible solutes, proton extrusion mechanism, salt compartmentalization, and improved nutrient status in plants can reduce the osmotic shock and ionic toxicity. Microbial management of salt stress offers an eco-friendly, cost-effective approach which however requires a rigorous selection, testing, and validation of the microbial strain(s) besides understanding the cross talk between the plant and microbe. Therefore, the aim of this chapter is to discuss the salinity response in plants and also to understand the mechanisms of microbe-mediated salinity stress alleviation on the molecular basis.

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Keywords
Salinity · Plant-microbe interaction · Osmolytes · Antioxidants · Gene expression · Phytohormone · ACC deaminase

1.1 Introduction

Soil salinity has become a major constraint in agriculture as high salt content in soils restricts crop growth and reduces yield. The saline soils are characterized with high concentration of soluble salts with an electrical conductivity (EC) >4 dS m^{-1} and exchangeable sodium percentage <15. Salt concentration of saline soils is more than 40 mM NaCl equivalent which generates an osmotic pressure of more than 0.2 MPa (Sharma et al. 2016). Major causes of natural soil salinity have been attributed to salt water intrusion and deposition of salts through wind. However, poor soil drainage, irrigation with highly saline water, improper irrigation, and excessive use of manures/composts have also been shown to increase salinity of soils (Kotuby-Amacher et al. 2000). Worldwide saline soils cover approximately 260 Mha with most extensive soil salinity reported in the arid and semi-arid regions of North Africa, the Near East, the former Soviet Union, and Central Asia (Cherlet et al. 2018). Osmotic pressure of soil solution increases due to high salt concentration which in turn adversely affects plant growth by reducing water uptake and balanced absorption of essential nutrients (Tester and Davenport 2003). Reduced uptake of water and nutrients hampers major metabolic functions of plant cells which ultimately affect germination, plant vigor, and crop yield (Munns and Tester 2008). Salinization in root zone may cause yield reduction to the tune of 30% (Cherlet et al. 2018). About 30 species of crop plants provide ~90% of plant-based human food which under moderate salinity reduce average yields by 50–80% (Panta et al. 2014). Annually the loss in crop production due to salt-induced land degradation globally costs approximately US$ 27.3 billion (Cherlet et al. 2018).

Globally researchers are making huge efforts to develop salinity-tolerant varieties to combat the ill effects of salinity on crop production, but only very little success has been achieved (Munns and Tester 2008). Sustainable, eco-friendly, and low-cost management practices for successful reclamation of saline soils for crop production have become a challenge for the researchers and policy makers. Microorganisms due to their multiple plant beneficial functions and ability to adapt to a wide range of environmental conditions have long been used in agriculture. Many of such plant beneficial microorganisms can be useful to crop plants for their better growth and sustenance under saline conditions. Microbes have been reported to alleviate salinity stress to plants through production of phytohormones, repressing production of stress hormones, enhancing plant stress defense mechanisms, improving plant nutritions, etc. Soil microflora and endophytes have an intricate interaction with agricultural plants to maintain productivity under stressed conditions. Microorganisms from hypersaline environments, halophytes represent a unique biological resource for salinity alleviation. Microbiologists and environmental scientists are putting huge efforts to bring out low-cost microbial technologies for salinity amelioration in crop plants. Published reports show the potential of such technologies, but still their large-scale application in agriculture is yet to be materialized globally. Therefore, the aim of this chapter is to discuss the salinity response in plants and also to understand the mechanisms of microbe-mediated salinity stress alleviation on the molecular basis.

1.2 Effect of Soil Salinity on Plant Growth and Productivity

High salt concentration in soils limits agricultural productivity by exerting negative effects on germination, seedling growth, development of root, flowering, and fruit setting (Fig. 1.1a, b) (Alam et al. 2004; Munns and Tester 2008). When concentration of solutes in root zone increases, the water potential of soil decreases. Reduced soil water potential hinders plant in maintaining turgor and thereby limits the water uptake. Broadly, salinity affects growth of plants in the following ways, viz., (a) imposing osmotic stress through reduced soil water potential; (b) inducing ionic imbalance in cells, specifically reducing the concentrations of K^+, Ca^{2+}, and NO_3^-, and causing ion (Na^+ and/or Cl^-) toxicity; and (c) creating oxidative stress (Zeng et al. 2015). During the salinity stress in plants, a number of biochemical and physiological changes take place within the cells and tissues.

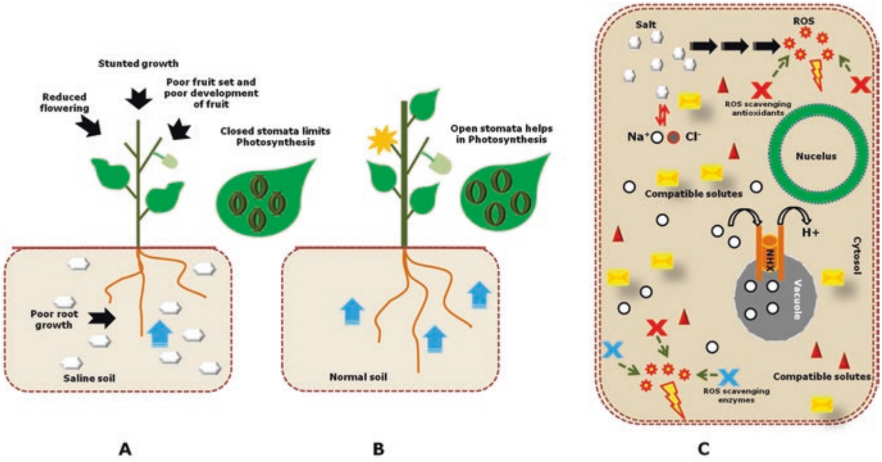

Fig. 1.1 Diagrammatic representation of (**a**) poor stunted growth and development of plant under saline soil, (**b**) healthy plant growth under normal soil, and (**c**) different cellular mechanisms acting to combat salinity stress

1.2.1 Biochemical Changes

1.2.1.1 Osmotic Stress

Higher amount of salts in root zone decreases soil water potential leading to reduced water uptake. Further, plant growth reduces due to toxic accumulation of Na^+ which occurred in leaves (Munns 2005; Munns and Tester 2008; Rahnama et al. 2010) Osmotic shock results in disrupted membrane permeability, reduced relative water content, leaf water potential, transpiration rate, water retention, and water-use efficiency (Munns 2002). Due to reduced soil water potential, the plants are unable to maintain turgor pressure resulting in stomatal closure leading to reduced photosynthetic activity.

1.2.1.2 Specific Ion Toxicity

Plants under salinity stress accumulate higher amounts of sodium, chloride, sulfate, and bicarbonate ion leading to severe ion toxicity. It is known that toxic accumulation of Na^+ takes place more rapidly than Cl^- (Munns and Tester 2008). Toxicity to a specific ion varies with different plant species (Dogan et al. 2010). Ion toxicity may cause changes in the metabolic processes taking place in the cytoplasm and may exert damaging effects on photosynthesis (White and Broadley 2001).

1.2.1.3 Nutritional Imbalance

Salinity can differentially affect the mineral nutrition of salt-stressed plants through nutritional deficiency or imbalance due to the competition of Na^+ and Cl^- with K^+, Ca^{2+}, NO_3^-, and Mg^{2+} (Karimi et al. 2005). Nutrient imbalance under salinity reduces plant growth by affecting the availability, transport, and partitioning of nutrients.

1.2.1.4 Reactive Oxygen Species (ROS)

The primary effects of salinity give rise to a number of secondary effects like oxidative stress due to buildup of reactive oxygen species (ROS) in plant cell (Mittler 2002; Abbasi et al. 2014). High salinity results in increased peroxidation of membrane lipids leading to membrane leakage (Ahmad et al. 2015). Overproduction of ROS enhances protein degradation and DNA mutation along with membrane injury (Pitzschke et al. 2006; Porcel et al. 2012).

1.2.2 Physiological Changes

1.2.2.1 Water Relations

High concentration of salts reduces water and osmotic potential of plants and thereby increases the turgor pressure (Meloni et al. 2001; Romero-Aranda et al. 2001; Gulzar et al. 2003). Under high salt concentration, increase in NaCl within the leaf tissue results in lower osmotic and water potentials. Similarly, transportation of water from roots to the shoots reduces with the reduction in root hydraulic conductance causing water stress in the leaf tissue.

1.2.2.2 Membrane Stability

Due to increased accumulation of Na^+ inside the cell, plants suffer from membrane destabilization and disrupt the membrane of different organelles: vacuole, chloroplast, mitochondria, and endoplasmic reticulum.

1.2.2.3 Leaf Anatomy

Salinity is known to induce anatomical changes in leaves like reduced leaf area and chlorophyll content, faster defoliation, stomatal resistance, photosynthetic activities, etc. (Shannon et al. 2000; Zhang et al. 2016). Thicker epidermis, mesophyll, longer and wider palisade cells, and larger spongy cells under high salinity have been reported (Parida et al. 2004). Under high salinity, reduced intercellular spaces were observed in spinach leaves, while stomatal density reduced in tomato leaves (Delfine et al. 1998; Romero-Aranda et al. 2001).

1.2.2.4 Photosynthesis

Due to osmotic stress and partial closure of stomata, photosynthetic capacity is reduced in many plant species (Ashraf 2001; Kao et al. 2001; Romero-Aranda et al. 2001; Meloni et al. 2003). Salt injury to photosystem II (PSII) is widely reported (Tavakkoli et al. 2011); this in turn contributes to inhibition of photosynthesis. After closure of stomata, activity of a number of enzymes including RuBisCo is reduced due to internal reduction of CO_2 and thus limiting carboxylation and reducing the net photosynthetic rate (Chaves et al. 2009).

1.3 Overview of Mechanism of Salinity Tolerance in Plants

In response to salinity stress, plants exhibit a number of physiological and biochemical strategies to cope with the stress (Fig. 1.1c). The strategies used by plant are briefly described below.

1.3.1 Accumulation of Osmoprotectant/Compatible Solutes

Osmotic adjustment of cell is vital for sustenance under salinity stress. Plants synthesize a number of osmolytes (also known as compatible solutes) to maintain the osmotic balance. Low molecular weight sugars, organic acids, polyols, amino acids, amides, imino acids, ectoine, proteins, and various quaternary ammonium compounds (QACs) are generally known to act as compatible solutes in plants (Suprasanna et al. 2005). Under high salinity, compatible osmolytes accumulate in plant tissues to help plants to survive during hostile condition through osmotic adjustment, stabilization of proteins, and detoxification of reactive oxygen species (Ahanger et al. 2014; Slama et al. 2015). Soluble sugars like trehalose, sucrose, fructose, etc. are well known as osmolyte and osmoprotectant. Under salt stress, amino acids like proline accumulates in higher plants (Mansour 2000). Proline is osmotically very active; it mitigates the effect of NaCl on cell membrane disruption

(Hossain et al. 2011) and maintains membrane stability (Nounjan et al. 2012) by regulating the accumulation of useable N acting as energy and nitrogen reserve during salinity (Tahir et al. 2012). Under salt stress compatible quaternary ammonium compounds (QACs) like glycinebetaine, alaninebetaine, prolinebetaine, choline *O*-sulfate, hydroxyprolinebetaine, and pipecolatebetaine are synthesized and accumulate in plant cells (Mansour 2000). Glycinebetaine (GB) is the most abundant osmoprotective QAC found in chloroplasts and plays an important role in osmotic adjustment and protection of thylakoid membranes, thereby maintaining photosynthetic efficiency, and protection of cellular macromolecules. Polyamines also significantly contribute in salt stress tolerance. Acyclic polyol (mannitol, glycerol, sorbitol) and cyclic polyol (ononitol and pinitol) are known to accumulate in plants during salt stress.

1.3.2 Ion-Selective Absorption and Compartmentalization to Protect from Ion Toxicity

During salinity stress, influx of Na^+ in cells results in ionic imbalance. Plant salinity tolerance is associated with the decreased loading of Na^+ into xylem resulting in exclusion of the Na^+ from shoots (Garthwaite et al. 2005; Munns and Tester 2008). Salt-stressed plants can maintain ionic balance through restricting toxic ion uptake, maintaining the uptake of essential ions, and compartmentalizing toxic ions to vacuoles. Buildup of toxic levels of salts in shoots can be achieved by restricting entry of Na^+ into root cells and then into the transpirational stream. Expulsion of Na^+ from root cells is an important mechanism preventing accumulation of Na^+ within cells and further movement to shoots. Na^+/H^+ antiporters in cell membrane pump out Na^+ from root cells. The cytosolic Na^+ concentration is regulated by compartmentation (Bassil and Blumwald 2014). Vacuolar Na^+/H^+ antiporters (NHXs) compartmentalize Na^+ into the vacuoles, thereby lowering the chances of toxicity and also contributing to osmoregulation.

1.3.3 Scavenging of Reactive Oxygen Species to Protect from Oxidative Stress

ROS produced under stress leads to oxidative damage to constituent lipids of cell membrane, proteins, and nucleic acids. Removal of ROS from cells is a major mechanism used by plants to cope up stress. The antioxidant defense system through scavenging ROS plays an important role in plant adaptation to salinity stress (Ahanger et al. 2014). Plant produces different antioxidants and detoxifying enzymes for efficient removal of ROS. Ascorbate, glutathione reductase (GR), glutathione S-transferase (GST), α-tocopherol, carotenoids, etc. are some of the prominent antioxidants deployed by plants, while superoxide dismutase (SOD), catalase (CAT), peroxidase, and enzymes of the ascorbate-glutathione cycle are the major detoxifying enzymes. SOD converts superoxide to H_2O_2, which is broken down to water and oxygen by the catalase and/or ascorbate-glutathione cycle or by ascorbate peroxidase located in the thylakoid membrane of chloroplast (Wu et al. 2014). Nitric oxide (NO), which is a small volatile gaseous molecule, is also known to be involved in protecting oxidative damage of cellular machineries. NO reacts with lipid radicals, thus preventing lipid oxidation, exerting a protective effect by scavenging superoxide radical (Bajguz 2014).

1.4 Microorganisms in Salt Stress Alleviation in Crop Plants

Due to enormous metabolic diversity and unique ability to sustain under extreme conditions, microorganisms inhabiting the plant rhizosphere play an important role in alleviation of various stress factors including salinity in crop plants. The rhizosphere-inhabiting microorganisms are integral part of biogeochemical cycling of nutrients. They can stimulate plant growth through mobilization of nutrients in soils, production of phytohormones, conferring protection to plants from pathogens through production of antibiotics or other secondary metabolites, improving soil structure, sequestration of toxic chemicals or metals from soil, etc. A number of bacteria from diverse genera such as *Bacillus*, *Paenibacillus*, *Pseudomonas*, *Klebsiella*, *Pantoea*, *Azotobacter*, *Azospirillum*, *Microbacterium*, *Streptomyces*, etc. have been characterized as plant growth-promoting bacteria. Inoculation of such bacteria in plants has been proved to be beneficial for many crop plants. Under saline conditions, the microorganisms can be effectively used for alleviation of salinity stress. Under salinity stress, microorganisms can help plants to maintain the osmotic balance, reduce ROS accumulation, and improve nutrient mobilization which in turn helps to combat the stress. Saline/hypersaline environments or halophytes represent specifically adapted microorganisms and also offer a unique source of salinity stress alleviating microorganisms. Halotolerant bacteria due to their ability to tolerate high concentration of salt have the ability to rapidly proliferate in soils having variable degree of salinity. Such halotolerant bacteria with multiple plant growth-promoting traits have largely been reported to alleviate salinity stress in various plants (Table 1.1). Apart from halotolerant bacteria, arbuscular mycorrhizal (AM) fungi have also been reported to improve the salinity tolerance in some plants. For example, the co-inoculation of *R. intraradices* and *Massilia* sp. RK4 can restore root colonization by AM fungi and nutrient accumulation in maize plants during salinity stress. Such associations of fungi and microbes have significant impact on tolerance of salinity in maize (Krishnamoorthy et al. 2016). Some examples of AM fungi-mediated salinity alleviation are presented in Table 1.1.

1.4.1 Microbial Production and Modulation of Phytohormones for Salinity Tolerance in Plants

Phytohormones of microbial origin can evoke several physiological responses to the plants. Microorganisms are known to produce several phytohormones like indole

Table 1.1 Examples of some halophyte and rhizosphere-associated microorganisms involved in alleviation of salinity stress

Halotolerant bacteria associated with halophytes				
Halotolerant bacteria	**Host halophyte**	**PGP activity**	**Plant response**	**References**
Bacillus, *Pantoea*, *Marinobacterium*, *Acinetobacter*, *Enterobacter*, *Pseudomonas*, *Rhizobium*, and *Sinorhizobium*	*Psoralea corylifolia*	IAA production and siderophore production	Enhanced seed germination and root length of wheat	Sorty et al. (2016)
Bacillus alcalophilus, *B. thuringiensis*, and *Gracilibacillus saliphilus*	*Arthrocnemum macrostachyum*	IAA production, siderophore production, and phosphate solubilization	Mitigated the effects of high salinity on plant growth and physiological performance	Navarro-Torre et al. (2017)
Dietzia natronolimnaea STR1	Not reported	–	Increased wheat tolerance to salt stress by improved wheat growth in terms of plant dry weight and plant height (higher biomass, shoot, and root elongation), increased photosynthetic pigments, enhanced content of enzymes catalase and ascorbate peroxidase, and increased the gene expression of the antioxidants compared to uninoculated plants	Bharti et al. (2016)

(continued)

Table 1.1 (continued)

Halotolerant bacteria associated with halophytes				
Halotolerant bacteria	**Host halophyte**	**PGP activity**	**Plant response**	**References**
Micrococcus yunnanensis, *Planococcus rifietoensis*, and *Variovorax paradoxus*	Seven species of halophytes	N_2 fixation, IAA production, siderophore production, phosphate solubilization, and ACC deaminase activity	An increase in salt stress tolerance, seed germination (%), and plant biomass, and photosynthetic capacity, and a decrease in stress-induced ethylene production at different NaCl concentrations (50–125 mM)	Zhou et al. (2017)
Brachybacterium saurashtrense sp. nov., *Zhihengliuella* sp., *Brevibacterium casei*, *Haererehalobacter* sp., *Halomonas* sp., *Vibrio* sp., *Cronobacter sakazakii*, *Pseudomonas* spp., *Rhizobium radiobacter*, and *Mesorhizobium* sp.	*Salicornia brachiata*	N_2 fixation, IAA production, phosphate solubilization, and ACC deaminase activity	Increase in percent germination at 0–0.5 Mol l^{-1} NaCl concentrations and significant increases in root length, shoot length, vigor index, and the fresh weight of *S. brachiate*	Jha et al. (2012)
Bacillus, *Pseudomonas*, *Klebsiella*, *Serratia*, *Arthrobacter*, *Streptomyces*, *Isoptericola*, and *Microbacterium*	*Limonium sinense*	N_2 fixation, IAA production, phosphate solubilization, and ACC deaminase activity	Significant increase in plant root length, shoot length, leaf number, and leaf area as compared to the non-inoculated control	Qin et al. (2014)
Klebsiella, *Pseudomonas*, *Agrobacterium*, and *Ochrobactrum*	*Arthrocnemum indicum*	N_2 fixation, IAA production, phosphate solubilization, ACC deaminase activity, and HCN production	A significant increase in total N content (up to 76%), maintained ion homeostasis, accumulated less ROS, and enhanced plant growth compared to non-inoculated seedlings	Sharma et al. (2016)

(continued)

Table 1.1 (continued)

Halotolerant bacteria associated with halophytes				
Halotolerant bacteria	**Host halophyte**	**PGP activity**	**Plant response**	**References**
Corynebacterium	*Sesuvium portulacastrum*	–	–	Anburaj et al. (2012)
Serratia marcescens and *B. cereus*	*Aster tripolium*	IAA production, N_2 fixation, siderophore production, and ACC deaminase activity	–	Szymańska et al. (2016)
Chromohalobacter, *Marinococcus*, *Halobacillus*, *Nesterenkonia*, *Halomonas*, *Oceanobacillus*, and *Virgibacillus*	*Salicornia strobilacea*	IAA, N_2 fixation, phosphate solubilization, and ACC deaminase activity	–	Mapelli et al. (2013)
Halotolerant bacteria associated with rhizospheric soil				
Halotolerant bacteria	**Host**	**PGP activity**	**Plant response**	**References**
Bacillus amyloliquefaciens RWL-1	*Oryza sativa*	–	Decrease in abscisic acid (ABA) production with increase in the levels of endogenous salicylic acid was observed	Shahzad et al. (2017)
Streptomyces sp. strain PGPA39	*Solanum lycopersicum*	ACC deaminase activity and IAA production and phosphate solubilization	Significant increase in plant biomass and chlorophyll content and a reduction in leaf proline content	Palaniyandi et al. (2014)
Acinetobacter spp. and *Pseudomonas* sp.	Barley and oats	Production of ACC deaminase and IAA	Increase in plant biomass which led to greater salt uptake and thus decrease in soil salinity	Chang et al. (2014)

(continued)

Table 1.1 (continued)

Halotolerant bacteria associated with halophytes				
Halotolerant bacteria	**Host halophyte**	**PGP activity**	**Plant response**	**References**
Pseudomonas pseudoalcaligenes and *Bacillus pumilus*	Salt-sensitive rice GJ-17	Reduce lipid peroxidation and superoxide dismutase activity	Reduction in toxicity by ROS by reducing plant cell membrane index, cell caspase-like protease activity, and programmed cell death and hence resulted in increased cell viability	Jha and Subramanian (2014)
Pseudomonas putida, *Enterobacter cloacae*, *Serratia ficaria*, and *Pseudomonas fluorescens*	Wheat	–	Enhanced germination percentage, germination rate, and index and improved the nutrient status of the wheat plants	Nadeem et al. (2013)
Rhizobium and *Pseudomonas* sp.	*Vigna radiata*	IAA production and ACC deaminase activity	Dry matter increased up to 1.9-fold and salt tolerance index rose by two-fold	Ahmad et al. (2013)
Brachybacterium saurashtrense (JG-06), *Brevibacterium casei* (JG-08), and *Haererohalobacter* (JG-11)	*Arachis hypogaea*	–	High K^+/Na^+ ratio and higher Ca^{2+}, phosphorus, and nitrogen content	Shukla et al. (2012)
Raoultella planticola Rs-2	Cotton	ACC deaminase activity	Rs-2 reduced the quantities of ethylene and abscisic acid in cotton seedlings and increased indole acetic acid content in cotton seedlings under salinity stress	Wu et al. (2012)
Pseudomonas pseudoalcaligenes, *Bacillus pumilus*	*Oryza sativa*	–	Increased concentration of glycinebetaine (compatible solute)	Jha et al. (2011)

(continued)

Table 1.1 (continued)

Halotolerant bacteria associated with halophytes				
Halotolerant bacteria	**Host halophyte**	**PGP activity**	**Plant response**	**References**
Rhizobium, *Pseudomonas*	Maize	–	Decreased electrolyte leakage and increase in proline production, maintenance of relative water content of leaves, and selective uptake of Kion	Bano and Fatima (2009)
Bacillus subtilis	*Arabidopsis thaliana*	–	Tissue-specific regulation of sodium transporter HKT1	Zhang et al. (2008)

Halotolerant AM fungi			
Halotolerant bacteria	**Host**	**Plant response**	**References**
Funneliformis mosseae, *Diversispora versiformis*	*Chrysanthemum morifolium*	Root length, shoot and root dry weight, total dry weight, and shoot and root N concentration were higher in mycorrhizal treated plant	Wang et al. (2017)
Glomus intraradices	*Phaseolus vulgaris*	Maintains the root hydraulic conductance of the plant	Aroca et al. (2007)
Glomusmosseae	Maize	AM symbiosis raised the concentrations of soluble sugars, reducing sugars, soluble protein, total organic acids, oxalic acid, fumaric acid, acetic acid, malic acid, and citric acid and decreased the concentrations of total free amino acids, proline, formic acid, and succinic acid in maize leaves	Sheng et al. (2011)
Glomus intraradices	Lettuce	Higher relative water content with lower proline content and abscisic acid in the roots	Jahromi et al. (2008)
Glomus mosseae	Maize	Mycorrhizal maize plants had higher dry weight of shoot and root, higher relative chlorophyll content, better water status, higher gas exchange capacity, and higher non-photochemistry efficiency	Sheng et al. (2008)

(continued)

Table 1.1 (continued)

Halotolerant bacteria associated with halophytes				
Halotolerant bacteria	**Host halophyte**	**PGP activity**	**Plant response**	**References**
Glomus intraradices	Tomato	Mycorrhization alleviated salt-induced reduction of P, Ca, and K uptake. Ca/Na and K/Na ratios were also better in AM plants. Mycorrhization improved the net assimilation rates through both elevating stomatal conductance and protecting photochemical processes of PSII against salinity		Hajiboland et al. (2010)
Claroideoglomus etunicatum	*Oryza sativa*	AM symbiosis enhanced the actual quantum yield of PSII photochemistry and reduced the quantum yield of non-photochemical quenching in rice plants subjected to salinity		Porcel et al. (2015)
Glomusgeosporum and *Glomus intraradices*	Tomato	Enhancement of transcript levels of both a tonoplast and a plasmalemma aquaporin gene		Ouziad et al. (2006)
Rhizophagus intraradices, *Claroideoglomus etunicatum*, and *Septoglomus constrictum*	Maize	Improved ion homeostasis		Estrada et al. (2013)

acetic acid (IAA), cytokinin, gibberellin, abscisic acid (ABA), etc. which are known to modulate root development and architecture along with signaling in response to stress. Production of IAA or its derivatives is a very common trait in most plant growth-promoting bacteria and is believed to combat salinity stress in plants. *Pseudomonas* strains showing IAA production increased almost 52% of seedling root growth under 100 mM NaCl as compared to control (Egamberdieva et al. 2010, 2011). Egamberdieva et al. (2011) reported that IAA-producing isolates of *Stenotrophomonas* and *Pseudomonas* could alleviate salinity stress in cucumber plants. Zahir et al. (2010) reported IAA production by *Rhizobium phaseoli* improved growth and yield of mung bean under salinity conditions. IAA-producing *Kocuria turfanensis* strain 2 M4 isolated from rhizosphere of a halotolerant plant showed 17% increase in length and 13% increase in fresh biomass of groundnut plants grown under saline soils (Goswami et al. 2014). Kadmiri et al. (2018) reported that *Pseudomonas* and *Azospirillum* isolates could retain IAA production under hypersaline conditions and could activate the defense mechanisms in wheat plants under saline conditions. ABA acts as an important internal signaling molecule in plants under adverse environmental conditions like drought, salinity, etc. Microorganisms with the ability to produce ABA can interfere with the plant stress response system and can confere tolerance to salinity. It was suggested that growth improvement of plants under saline or other stressed conditions upon inoculation with *Azospirillum* sp. might be due to the ability of the bacteria to produce ABA or increase ABA synthesis in plants. Cohen et al. (2008) and Shahzad et al. (2017) reported that inoculation of ABA-producing bacterial endophyte *Bacillus amyloliquefaciens* RWL-1 could enhance the salinity tolerance in rice. Cytokinin is also known to promote plant growth under stress conditions and help plants to survive under saline conditions. Naz et al. (2009), Kang et al. (2012), and Lubovská et al. (2014) reported stimulation of plant growth and proline content under 20 dS/m NaCl condition in soybean inoculated with cytokinin-producing bacteria isolated from weeds growing in highly saline soils. Gibberellins have also been attributed to plant stress response. In comparison to uninoculated plants, gibberellin-producing endophytic *Aspergillus fumigatus*-inoculated plants showed increased growth and photosynthetic rate under salt stress (Khan et al. 2011).

Microorganisms can modulate production of phytohormones in plants which can help in salinity tolerance. *B. subtilis* and *P. fluorescens* were reported to increase IAA and GA_3 while decreasing ABA in raddish. This modulation was partially attributed in activation of processes involved in the alleviation salt stress (Mohamed and Gomaa 2012; Kang et al. 2014). Kang et al. (2014) reported that plant growth-promoting rhizobacterial strains, viz., *Burkholderia cepacia* SE4, *Promicromonospora* sp. SE188, and *Acinetobacter calcoaceticus* SE370, could enhance salicylic acid and GA4 production which might have reduced the adverse effects of salinity stress in cucumber plants. Khan et al. (2017) also reported regulation of endogenous phytohormones in *Solanum pimpinellifolium* inoculated with plant growth-promoting endophytic bacteria *Sphingomonas* sp. under salinity stress.

1.4.2 Microbial Production of ACC Deaminase in Salinity Tolerance

Under stress conditions including salinity, ethylene level increases in the plant. 1-Aminocyclopropane-1-carboxylate (ACC) is enzymatically converted into ethylene. Many plant growth-promoting bacteria possess an enzyme known as ACC deaminase which can break ACC into ammonia and α-ketobutyrate, thus limiting the synthesis of ethylene which can lead to plant growth inhibition or even death at high concentrations. *Pseudomonas fluorescens* strain TDK1 with ACC deaminase activity could enhance the salinity tolerance in groundnut resulting in higher yield (Saravanakumar and Samiyappan 2007). Jalili et al. (2009) reported that ACC deaminase-producing fluorescent pseudomonads could alleviate the stressful effects on canola. Wu et al. (2012) isolated ACC deaminase-producing *Raoultella planticola* from salinized soil which could increase growth of cotton seedlings by 15.0, 33.7, and 33.3%, respectively. ACC deaminase-producing bacterial isolates belonging to *Bacillus*, *Arthrobacter*, *Isoptericola*, and *Streptomyces* were shown to stimulate the growth of plant under salt stress (Qin et al. 2014). Co-inoculation of ACC deaminase-producing rhizobacterial isolate RHD18 and *Mesorhizobium* resulted in

31.2% increase in dry weight of chickpea under salinity stress (Chaudhary and Sindhu 2017). Win et al. (2018) showed that ACC deaminase-positive endophytic *Pseudomonas* sp. can reduce stress-induced ethylene production in tomato plants under NaCl stress and improve the plant growth. Sarkar et al. (2018) reported that *Burkholderia* sp. MTCC 12259 isolated from coastal rice field can improve the growth of rice through reduction in stress-induced ethylene production.

1.4.3 Microbe-Mediated Improved Osmolyte Accumulation for Salinity Tolerance

Maintenance of internal osmotic balance is key to metabolic activities of plant cells. Production and accumulation of osmolytes in response to salinity is one important strategy employed by plants. Plant-associated microorganisms can also enhance stress tolerance by improving osmolyte accumulation in plants. Zarea et al. (2012) reported higher proline accumulation in wheat upon root colonization with *Azospirillum*. Upadhyay et al. (2012) showed increased proline and total soluble sugar in wheat inoculated with *Arthrobacter* and *Bacillus* significantly contributed to osmotolerance. Kumari et al. (2015) reported that inoculation of *Bacillus* sp. SJ-5 increased proline accumulation in soybean roots which contributed to increased plant tolerance to salinity. Application of *Serratia marcescens* CDP-13 significantly modulated the levels of different osmoprotectants like proline, malondialdehyde, etc. in wheat suggesting their role in adaptation to tolerate salt stressors (Singh and Jha 2016). Yasin et al. (2018) reported that increased accumulation of proline along with upregulation of a number of stress-related genes resulted in improved growth of *Bacillus fortis* strain SSB21-inoculated chili plants under salinity.

1.4.4 Microbe-Mediated Ion Homeostasis for Tolerance to Salinity Stress

Microbes can alter uptake of ions and nutrients through roots mostly via unknown mechanisms. Regulation of Na^+-K^+ influx-efflux through microbial inoculation can control the internal ionic balance required for a number of plant metabolic activities. Reduced efflux of potassium ions from roots and higher concentration in shoots and leaves can be helpful in salt stress adaptations in plants. *Azospirillum* inoculation in wheat could restrict root influx of Na^+ (Ashraf et al. 2004). Inoculating *Arabidopsis* with *B. subtilis* GB03 improved salt tolerance through regulation of the potassium transporter HKT1 (Zhang et al. 2008). Kasotia et al. (2015) also reported reduced Na^+ levels and increased K^+ levels in leaves and roots of *Pseudomonas koreensis*-inoculated soybean plants under salt stress. Yun et al. (2018) reported that inoculation of *Piriformospora indica* improved salinity tolerance in maize through improved stomata operation associated with higher rate of K delivery into the shoots by restricting K^+ efflux from roots and higher K^+ loading on shoots. Inoculation of AMF in cucumber showed increased tolerance against salinity due to a restriction in

Na uptake by roots and to the homeostasis of nutrient uptake (Hashem et al. 2018). Microorganisms can regulate the expression and activity of plant vacuolar transporters involved in Na^+ detoxification. Chen et al. (2017) showed that enhanced salt tolerance in maize and arabidopsis was due to spermidine production by *Bacillus amyloliquefaciens* which in turn upregulated vacuolar transporters responsible for sequestration of Na^+ into vacuoles and expelling Na^+ from the cell to reduce ion toxicity. Chatterjee et al. (2018) showed that *Brevibacterium linens* RS16 regulated the salt accumulation by modulating vacuolar H^+ ATPase activity which provided salt tolerance in rice.

1.4.5 Modulation of Antioxidant Production Through Microbial Inoculation

Production of ROS in cells during the salinity stress can damage the membrane lipids and nucleic acids. Plants deploy scavenging enzymes to eliminate the ROS formed in the cells. Microbial inoculation to plants is known to activate these scavenging enzymes so that the ROS can be efficiently removed. Kohler et al. (2009) showed higher induction in antioxidant enzyme activities in *Pseudomonas mendocina*-inoculated lettuce. Inoculation of *Bacillus* sp. on potato showed improved tolerance to salt through induction of ascorbate peroxidase, catalase, and peroxidase enzymes under 200 mM NaCl (Gururani et al. 2013). Islam et al. (2016) reported increased peroxidase, superoxide dismutase, and catalase activity while improving the growth of *Bacillus cereus*-inoculated mung bean under salt stress. Singh and Jha (2016) reported increase in antioxidant enzyme activities in *Stenotrophomonas*-inoculated sorghum under salinity stress. Vimal et al. (2018) showed that rice inoculated with *Curtobacterium albidum* had enhanced antioxidant enzyme activities which was implicated in reduction of salinity stress.

1.4.6 Microorganism-Induced Root Proliferation and Nutritional Enhancement

As roots are the first to sense the salinity in the soil, they are highly affected by salt concentration in the soil solution. Roots selectively absorb ions, and the amount of ions transported to the shoot is significantly different from those available in the soil. Hence, the characteristics of roots in ion and water absorption are critical in salinity tolerance. In general, higher root growth under salinity stress improves all the functions in the roots that response to salinity (An et al. 2003). Plant growth-promoting bacteria are known to produce a number of growth regulators which can influence the root architecture and growth which may in turn be beneficial for salinity tolerance in plants. Nadeem et al. (2013) showed that inoculation of *Enterobacter*, *Pseudomonas*, and *Serratia* to wheat resulted in higher root growth and mitigated the negative impact of salinity. Goswami et al. (2014) also reported increase in root length of *B. licheniformis*-inoculated groundnut plants growing at 50 mM NaCl.

Effect of inoculation of *Bacillus* sp. Inoculation with *Bradyrhizobium japonicum* and salt-tolerant *P. putida* synergistically enhanced salt tolerance in soybean through alteration of root architecture which in turn facilitated N and P acquisition and nodule formation (Egamberdieva et al. 2017). Chili growing under salinity stress was studied by Wang et al. (2018), and it was reported that the bacterial inoculation could alleviate the salinity stress and increase root length by ~146%.

Nutritional status of plants significantly influences their ability to adapt under a range of abiotic stress conditions including salinity. Nutritional imbalance can restrict development and reduce plant growth and yield. Salinity may affect nutrient availability, competitive uptake, transport, and distribution within the plant (Grattan and Grieve 1999). Salinity is known to reduce N and P uptake/accumulation (Feigin 1985; Sharpley et al. 1992). High sodium concentration during salinity can also reduce the availability of potassium. Hence, plant growth-promoting bacteria which can increase the nutrient uptake in plants can be very useful for alleviation of salinity stress. Particularly, the microorganisms which can fix nitrogen, solubilize phosphates, and mobilize potassium have immense potential in this regard. Yadav et al. (2015) implicated the role of P-solubilizing halophilic archaea in P nutrition of vegetations growing on hypersaline soils. Shahid et al. (2018) reported that inoculation of *Planomicrobium* sp. strain MSSA10 enhanced the K, N, and P uptake in pea plants in order to keep optimum chlorophyll and protein levels, which in turn conferred protection to plants from salt-induced lipid peroxidation of membranes.

1.5 Molecular Mechanisms of Microbial Amelioration of Salinity Stress

Microorganisms can produce a number of phytohormone and their derivatives which can elicit several physiological responses to the plants. Microorganisms are known to produce indole acetic acid (IAA), cytokinin, gibberellin, abscisic acid (ABA), etc. which are known to modulate root development and architecture along with signaling in response to salinity. Higher root proliferation due to microbially derived plant hormones also helps in acquisition of nutrients in a better way. Microbes which can mineralize or solubilize essential nutrients in the rhizosphere can also help to reduce the salinity stress through better acquisition of nutrients. Besides microorganisms can also modulate the phytohormone production in plants which in turn contributes to salinity tolerance. ABA acts as an important internal signaling molecule in plants under adverse environmental conditions like drought, salinity, etc. It has been speculated that aquaporins and hydraulic conductance are affected by ABA and microorganisms can influence the levels of ABA (Aroca et al. 2007). Under salinity stress, *Bacillus megaterium*-inoculated maize plants showed increased hydraulic conductance which was correlated with upregulation of two plasma membrane aquaporin protein (ZmPIP) isoforms (Marulanda et al. 2010). Salinity stress tolerance may be also mediated through ABA-independent pathways. A detailed study on salinity tolerance in *Arabidopsis* through inoculation with *Enterobacter* sp. EJ01 revealed less induction of *RD29B* (a gene known to be regulated only by ABA-dependent pathway) and no induction in *AAO3* (an ABA biosynthetic gene) indicating independence from ABA (Kim et al. 2014). *AUX/IAA* gene family presents genes coding repressors of transcription of auxin-responsive genes (Li et al. 2015). Degradation of AUX/IAA proteins helps in activation of *ARF* transcription factors and subsequently the expression of auxin-responsive genes (Hagen and Guilfoyle 2002). Microbial inoculation can modulate the expression of *AUX/IAA* genes which in turn influence the auxin signaling pathway to protect plant from salinity and other stress conditions (Barnawal et al. 2017).

Salt Overly Sensitive (SOS) pathways participate in mitigation of salinity stress in plants. WRKY transcription factors (WRKY TFs) significantly contribute in regulation of water/drought stress through modulation of cellular osmotic balance, ROS scavenging, and expression of different stress-responsive genes (Agarwal et al. 2011). Microorganisms are known to modulate the expression of *SOS* genes and WRKY TFs which can contribute to plant salinity stress response. Bharti et al. (2016) while studying on salinity stress alleviation in wheat using *Dietzia natronolimnaea* hypothesized that *D. natronolimnaea* STR1 induced salinity tolerance through ABA-mediated and SOS-mediated pathways by upregulating the expression of ABA-signaling cascade genes (*TaABARE* and *TaOPR1*) which in turn induced *TaMYB* and *TaWRKY* expression ultimately resulting in expression of a number of stress-related genes. Modulation of *SOS1* and *SOS4* genes involved in SOS pathway and ion transporters TaNHX1, TaHAK, and TaHKT1 was also observed in *D. natronolimnaea* STR1-inoculated plants. Upregulation of various antioxidant enzyme-encoding genes and elevated proline content in inoculated wheat plants imparted salinity tolerance. Rice inoculated with *B. amyloliquefaciens* SN13 showed increased salt tolerance and upregulation of *SOS1*, *EREBP*, *SERK1*, and *NADP-Me2* genes (Nautiyal et al. 2013).

Microbes can alter root uptake of ions and nutrients mostly via unknown mechanisms. Regulation of Na^+-K^+ influx-efflux through microbial inoculation can control the internal ionic balance required for a number of plant metabolic activities. Reduced efflux of potassium ions from roots and higher concentration in shoots and leaves can be helpful in salt stress adaptations in plants. It is demonstrated that mycorrhiza, *Glomus intraradices*, can selectively take up elements such as K^+, Mg^+, and Ca^{2+} but exclude Na^+ uptake to keep the internal ratios of both Na^+/K^+ and Na^+/Ca^{2+} within the limit despite magnitude of ion concentrations in the surrounding environment. This selective mechanism for ion uptake may partially alleviate the salinity stress in plants (Hammer et al. 2011). The volatile compounds released by PGPB downregulate the gene *hkt1* (high-affinity K^+ transporter 1 protein) expression in root while upregulate it in shoots, orchestrating lower Na^+ levels and recirculation of Na^+ in the plant under salinity stress (Zhang et al. 2008). Chen et al. (2017) showed that salinity tolerance in maize seedlings was associated with upregulation of HKT1, NHX1, NHX2, and NHX3. Yun et al. (2018) reported that inoculation of *Piriformospora indica* improved salinity tolerance in maize through improved stomata operation associated with higher rate of K delivery into the shoots by restricting K^+ efflux from roots and higher K^+ loading on shoots.

1.6 Conclusions and Future Prospects

United Nations has projected that the world population will be growing with addition of 83 million annually to reach almost 10 billion in 2050. Although the population is increasing at very faster rate, globally the total arable land did not change much. In 1995, the total arable land was 10.801% which increased to 10.991% in 2015. Such a disproportionate growth is posing as one of the most serious threats to agricultural sustainability. Moreover, due to various natural and anthropogenic factors, the soil fertility is gradually diminishing and total arable land is shrinking at many regions throughout the world. Soil salinity is one such factor which has affected a huge amount of land over the globe and is considered as one of the major constraints for sustainable agricultural productivity. Despite a considerable effort throughout the world, no effective, sustainable, low-cost strategy for amelioration of soil salinity is available. Management of soil salinity can improve the crop productivity significantly which in turn will definitely contribute to feed the growing population.

Microorganisms due to their enormous metabolic diversity are endowed with the ingenious abilities to survive under harsh environmental conditions including salinity. Microorganisms can survive at hypersaline environments which may have more than ten times salts than seawater. Mining of hypersaline environments can help us to obtain such halotolerant/halophilic microorganisms which can be the new hope for management of soil salinity. Crop plants make an indispensable relation with soil-inhabiting microbes and also contain trillions of microbes inside the plant tissue. Such microbial diversity also contains beneficial halophilic/halotolerant microbes which can help to alleviate the salinity stress. Halophytic plants represent a unique and novel reservoir of such microorganisms which can be effectively utilized for salinity management. With the deeper understanding of plant and soil microbiome, it is now possible to look after possible strategies to transplant and replicate salinity tolerating or alleviating microbiome to salinity-affected plants and soils. Such a strategy can be highly effective but comes with obvious challenges. However, any such microbe-based salinity management strategy necessitates long-term trials under salinity-affected soils to find out their real potential.

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2 Adaptive Mechanisms of Plants Against Salt Stress and Salt Shock

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Abstract

Salinization process occurs when soil is contaminated with salt, which consequently influences plant growth and development leading to reduction in yield of many food crops. Responding to a higher salt concentration than the normal range can result in plant developing complex physiological traits and activation of stress-related genes and metabolic pathways. Many studies have been carried out by different research groups to understand adaptive mechanism in many plant species towards salinity stress. However, different methods of sodium chloride (NaCl) applications definitely give different responses and adaptive mechanisms towards the increase in salinity. Gradual increase in NaCl application causes the plant to have salt stress or osmotic stress, while single step and high concentration of NaCl may result in salt shock or osmotic shock. Osmotic shock can cause cell plasmolysis and leakage of osmolytes in plant. Also, the gene expression pattern is influenced by the type of methods used in increasing the salinity. Therefore, this chapter discusses the adaptive mechanism in plant responding to both types of salinity increment, which include the morphological changes of plant roots and aerial parts, involvement of signalling molecules in stress perception and regulatory networks and production of osmolyte and osmoprotective proteins.

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2.1 Introduction

Salinity is a condition where soluble salts like sodium, calcium, potassium, magnesium, chlorides, nitrates, sulphates, bicarbonates and carbonates are higher than the normal range in affected soil. Like other abiotic stresses, high salinity can decrease and limit the productivity of food crops due to plant sensitivity towards saline soil (Hanin et al. 2016). Several factors can increase soluble salt content in the soil such as irrigation with water containing high levels of salt, irrigation with water from salt-impacted wells or saline industries and intrusion of seawater into low-lying coastal areas and freshwater (Alam et al. 2017). In 2017, many irrigated lands (approximately 45 million ha) were severely affected by the salinity problem. This situation definitely gives negative impact to the world's food production (Machado and Serralheiro 2017). Hence, many researches have been conducted to reveal the complex salt adaptive mechanisms of many plant species, especially the food crops like rice, wheat and maize. The information gained can be further used by breeders to breed new salt-tolerant varieties using conventional or molecular breeding (Shrivastava and Kumar 2015).

Arabidopsis thaliana (Zhang et al. 2018a), *Glycine max* (Wang et al. 2018a), *Oryza sativa* (Hussain et al. 2017), *Zea mays*, *Elaeis guineensis* (Henry and Hak Wan 2012), *Phoenix dactylifera* (Yaish and Kumar 2015) and *Hordeum vulgare* (Ma et al. 2019) are among the plants found to have the ability to develop adaptive responses towards salinity. Generally, the changes in biomass and chlorophyll content as well as development of new roots are among physiological responses observed in salinity-treated plants (Cheng et al. 2018a, b). Further, the activation and inhibition of salinity-responsive genes [e.g. *late embryogenesis abundant* (*LEA*), *metallothioneins* (*MTs*)], accumulation of salinity-responsive proteins (e.g. enzymes and transcription factors) and production of salinity-responsive metabolites (e.g. trehalose, sucrose, proline, glycine betaine and myo-inositol) are common responses when the plants are responding to abnormal salinity (Kumari and Parida 2018; Sun et al. 2018). However, the physiological and biochemical responses eternally rely on NaCl application methods. Gradual plant exposure to low and high NaCl can cause the plants experiencing salt stress, while sudden exposure to high concentration of NaCl can trigger plants to experience salt shock (Shavrukov 2013). In this chapter, we therefore describe the adaptive mechanisms of plants grown in salt stress and salt shock conditions.

2.2 Definitions of Salt Stress and Salt Shock

Different approaches have been used by scientists to mimic salinity condition in soil, which usually depend on NaCl concentrations and application methods. Both factors can cause plants experiencing either salt stress or salt shock. Salt stress is defined as a continuous exposure of plants to low NaCl concentrations, usually 25 mM or maximum 50 mM increments to high NaCl concentrations. The exposure of plants only to low concentrations of NaCl may also lead to salt stress condition. This method is also known as progressive imposition or salt acclimation or gradual step acclimation or salt adaptation, which usually can mimic the real salt stress in nature (Park et al. 2016). The extreme exposure of plants to high concentration of NaCl from normal growth solution (without NaCl) may lead to salt shock. Losing water or plasmolysis can be observed once the plants are experiencing salt shock usually after direct application of concentrated NaCl (>150 mM). Intermediate response between osmotic stress and osmotic shock is recorded after the plants are treated with 100–150 mM of NaCl. At low concentrations of NaCl (≤50 mM), only osmotic stress is recorded in treated plants (Shavrukov 2013).

Osmotic shock and plasmolysis are among early responses that occur in root. This situation can result in protoplast shrinkage and detachment from the cell wall. Rapid changes in the osmotic potential in plant and external environment can trigger water deficits and wilting. Afterwards, the ionic stress in salt shock treated plants when accumulation of Na^+ reaches toxic level (Shavrukov 2013; Park et al. 2016). Gradual increment of NaCl concentration induces salt stress response in plants. Salt stress stimulates small changes in osmotic pressure or osmotic stress inside and out of the plant cell several times along the salt stress treatment. The plant cell undergoes an osmotic adjustment by alteration of the salt concentrations and reducing changes in pressure potential (Negrao et al. 2017). After sometimes which usually takes 1–3 days, the plant experiences ionic stress caused by the quick influx of Na^+ into the plant cell and transportation of Na^+ from root to the leaf (Assaha et al. 2017). Nevertheless, plant species and plant age are also important parameters that need to be considered when determining the severity of salt stress and salt shock. Overall mechanism involved in salt stress and salt shock response in plants is illustrated in Fig. 2.1.

2.3 Morphological Changes of Roots and Aerial Parts as an Adaptation to Salt Stress and Salt Shock

Plant roots regulate water and nutrient absorption from soil, while the aerial parts are involved in carbohydrate synthesis (Sathiyavani et al. 2017). These significant functions of roots and leaves can efficiently occur in plants grown under normal condition. When the plants are exposed to abiotic stresses like salinity, both vegetative organs can undergo morphological changes, which further clarify their pivotal involvement in maintaining water and food contents in plants. These morphological changes are usually depending on the NaCl application methods and plant species

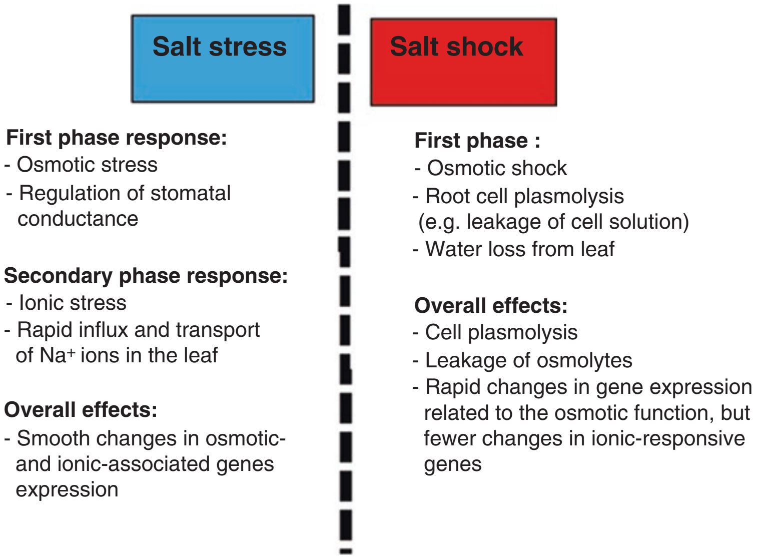

Fig. 2.1 Overall responses of plant exposed to salt stress and salt shock treatments

(Shavrukov 2013). The single application of 50 mM and 100 mM NaCl in wheat plant reduced the length of the root hair by approximately 25% and the density of the root hair by 40% compared to the untreated plant (Robin et al. 2016). The root alteration suggests the effects and involvement of this organ in detecting changes of environmental signals, therefore urging plants to undergo biochemical adjustment to ensure they are still alive under stress condition (Wang et al. 2007). The decrement of root cross-sectional area was detected in salt shock (200 mM NaCl)-treated maize, suggesting the reduction of cortical parenchyma and vascular cylinder (Farhana et al. 2014). Increase of root/leaf ratio of *Citrus* plants grown in 90 mM NaCl for 6 months indicated an improvement of the source/sink ratio of water and nutrients. This scenario is suggesting the plants are counteracting with osmotic stress by maintaining homeostasis of sugar content (Rewald et al. 2012). Exposure of barley plant to salt shock at 500 mM NaCl caused its roots to experience apoptosis-like cell death (Shavrukov 2013).

For aerial part of plants, the young leaves are the most susceptible organ to be affected by the salinity. This can be observed by the inhibition of the leaf cell expansion that definitely reduces the leaf-specific area (Negrao et al. 2017). Apart from that, severe injuries and quick senescence have also been documented in plants exposed to high NaCl concentrations. The exposure may lead to a rise of Na^+ and Cl^- concentrations in the transpiring leaves (Hanin et al. 2016). Different amaranth genotypes showed leaf number and leaf area reduction when the plants are responding to salt stress. The reduction of leaf number can minimize salinity toxicity because it can reduce the toxic ion transportation to aerial parts (Omamt et al. 2006). In gooseberry, the reduction of leaf number and leaf blade thickness was observed in 1.0 g/L NaCl-treated plants (Rezende et al. 2018). Also, the leaf thickness phenotype was observed in salt-tolerant species of *Atriplex patula* treated with different concentrations of NaCl solution at 0.05, 0.1, 0.2, 0.3 and 0.4 M, respectively (Acosta-Motos et al. 2017). Based on these observations, aerial parts are sensitive to the salt toxicity. Morphological changes are one of the ways the plants can use to minimize the salt toxicity and maintain photosynthesis, hence supporting plant growth under unfavourable condition.

2.4 Activation of Plant Stress Signalling and Regulatory Networks Under Salt Stress and Salt Shock

2.4.1 Plant Hormones as Mediated Compounds

Plant hormones are centrally mediated compounds that link and reprogramme the plant developmental process and stress adaptive signalling cascades under abiotic stresses. Among all phytohormones, abscisic acid (ABA) is a significant regulator involved in the activation of cellular adaptation to salinity. ABA can mediate stomatal activities and later initiate stress signalling response (Jia et al. 2002; Osakabe et al. 2014). Other phytohormones like ethylene, cytokinins (CKs), auxins, brassinosteroids (BRs), gibberellic acids (GAs), salicylic acid (SA) and jasmonic acids (JAs) have also been reported to be involved in adaptation process and alleviate adverse effects of salinity (Fahad et al. 2015).

Water deficit is a common symptom suffered by plants grown under high salinity condition. ABA is known as a dehydration-responsive hormone, and it has been suggested that ABA production in the salinity-treated plants is owing to dehydration symptom (Javid et al. 2011). The rise of endogenous ABA concentration was observed in many plant species treated with different severities of salinity like in maize (Jia et al. 2002), soybean (Yoon et al. 2009) and *Arabidopsis* (Ruiz-Sola et al. 2014). Meanwhile, the decrement of ABA concentration was observed in *Salicornia bigelovii* shoot. This condition restricted stomatal opening and photosynthesis, therefore stunting the *S. bigelovii* growth (Ohori and Fujiyama 2011). In other experiments, the exogenous application of ABA to salinity-treated plants reduced the ethylene production and leaf abscission probably due to the decreasing of Cl^- ion concentration in the leaves (Javid et al. 2011). These findings suggest the vital role of ABA regulation in the salinity adaptation.

The interaction of ethylene to its receptor can inactivate the interaction of an ethylene with a negative regulator CONSTITUTIVE TRIPLE RESPONSE1 (CTR1) under saline condition. Inactivation of CTR1 triggers dephosphorylation and cleavage of ETHYLENE-INSENSITIVE2 (EIN2). Afterwards, the truncated EIN2 enters the nucleus and stabilizes EIN3/ETHYLENE INSENSITIVE-LIKE (EILs), hence further activating ethylene-responsive factors such as plant APETALA 2/ethylene-responsive element-binding factor (AP2/ERF) family (Tao et al. 2015; Abiri et al. 2017). Upregulation of *ERF96* was detected in NaCl-treated *A. thaliana*, and overexpression of this gene enhanced plant tolerance to salt stress (Wang et al.

2017). In other findings, tomato overexpressing *SI-ERF.B.3* showed decreased stem expansion and enhanced tolerance to salt shock treatment at 200 mM NaCl (Klay et al. 2014).

Production of reactive oxygen species (ROS) in stressed cell activates auxin metabolism. *Small auxin-upregulated RNA 75* (*TaSAUR75*) was downregulated in wheat root when the plant was exposed to salt shock treatment (342 mM) (Guo et al. 2018). *Auxin response factor* (*ARF*) was differentially expressed in shock-stressed vegetative tissues of tomato plants treated with 250 mM NaCl (Bouzroud et al. 2018). A single application of NaCl at concentration of 50 mM and 150 mM, respectively, affected expansion of xylem vessels due to modification of total indole-3-acetic acid (IAA), which includes free and conjugated IAA levels in developing xylems of poplar plant (Junghans et al. 2006).

The reduction of CK level in stressed plants has proven the involvement of CKs in regulating plant adaptation process to salinity. Overnight salt shock treatment using 200 mM NaCl on CK-deficient *A. thaliana* demonstrated a strong salt-tolerant phenotype. Thus, further suggesting the significant reduction of CK level in stressed plant is crucial in order to enhance salt tolerance (Nishiyama et al. 2012). A study conducted by Wang et al. (2015) further confirmed that the salt resistance definitely relies on CK content. However, an increment of CK content was observed in transgenic *A. thaliana* harbouring *AtIPT8*. Overaccumulation of CKs was observed in transgenic plants responding to salt stress when they were exposed to 100 mM NaCl for 10 days. The high content of CKs inhibited primary root growth and true leaf emergence as well as reduced chlorophyll content in transgenic *Arabidopsis*. The short-term salinity treatment (6 h) with 150 mM NaCl on tomato seedlings however resulted in 25% increment of total CKs and accumulation of active and inactive forms of CKs (Keshishian et al. 2018). Hence, both salt stress- and salt shock-treated plants have distinctive ways to regulate total CK content.

The involvement of BRs in salt-treated plants was observed in barley when upregulation of BR-related gene (*DWARF4*) was observed in plants treated with NaCl at 150 and 250 mM (Marakli and Gozukirmizi 2017). In other studies, the exogenous application of BR increased the activities of antioxidant enzymes (superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), ascorbate peroxidase (APX) and glutathione peroxidase (GPX)), therefore reducing ROS and lipid peroxidation (Vardhini and Anjum 2015). Apart from that, the exogenous application of BR enhanced the capacity of alternative oxidase (AOX) and the ethylene biosynthesis. The AOX has the capability to scavenge ROS, thus increasing plant stress tolerance (Wei et al. 2015). At cellular levels, BRs regulate the expression of *R2R3-type MYB*, the *MULTIPASS* (*OsMPS*) and *BRASSINOSTEROIDS AT VASCULAR AND ORGANIZING CENTER* (*BRAVO*). OsMPS regulates plant growth by reducing cell size, while BRAVO modulates plant stem cell quiescence in salt-treated plants (Sharma et al. 2017).

Among plant phenolics, salicylic acid derived from phenylpropanoid and isochorismate pathway is the only phenolic compound that plays a crucial role as plant growth regulator. The salicylic acid regulates many physiological processes and provides protection not only for plants responding to biotic stresses but to abiotic stresses including high salinity (Jini and Joseph 2017). Under salt stress, at right SA concentration, this phytohormone can promote seed germination. The use of SA more than 100 μM inhibited seed germination, while application of SA less than 10 μM increased photosynthetic rate, stomatal conductance, carbon fixation, transpiration and antioxidant activity in many plant species (Jayakannan et al. 2015). Also, the exogenous application of SA can recover shoot reduction of salt stress- and salt shock-treated plants (Suhaib et al. 2018).

Under salinity condition, jasmonates (methyl jasmonate (MeJA) and jasmonic acid (JA)) activate regulatory transcriptional programme to control root growth (Yoon et al. 2009; Valenzuela et al. 2016). The root inhibition was observed in soybean seedlings exposed to a gradual increment of NaCl (50–200 mM) (Neves et al. 2010). Many JA-signalling genes were reported to be expressed in many plant species grown under high salinity. Some of the expressed genes are *AOC1*, *AOC2*, *LOX3* and *OPR3* (Valenzuela et al. 2016). In other reports, the pretreatment of soybean seedlings with 20 and 30 μM MeJA countered undesirable effects of salt stress like increment of chlorophyll content, proline content, photosynthetic rate and leaf transpiration rate (Yoon et al. 2009). A single application of NaCl (0, 110, 220, 330 mM) on rapeseed seedlings together with 100 mM MeJA counteracted the toxic effects of NaCl on plant growth by increasing photosynthesis rate, soluble sugar content and relative water content (Ahmadi et al. 2018).

2.4.2 Function of Ca^{2+} and ROS

Calcium ion (Ca^{2+}) and ROS are second messengers that are very important in stress signal transduction. ROS can act as a long-distance signal through a cell-to-cell communication cascade, while Ca^{2+} can activate Ca^{2+} sensors (Ca^{2+}-binding calcineurin B-like proteins (CBLs) and CBL-interacting protein kinases (CIPKs)) and calcium signalling pathway (Nath et al. 2016, 2018). In saline condition, the formation of plant CBL-CIPK complex is crucial because it can regulate ion homeostasis.

ROS generation mediated by NADPH oxidase (Nox) induces oxidative stress and adversely affects the native structure and function of enzymes, membrane and cell wall, hence ultimately causing severe injuries to the root and shoot tissues (Nath et al. 2018). In contradiction to the ROS functions, Ca^{2+} plays a vital part in preserving plant membrane structural and functional integrity, cell wall structure and stabilization, ion transport selectivity and regulation and enzyme activity regulation (Shoresh et al. 2011).

In saline soil, extracellular NaCl induces Ca^{2+} influx in plant roots. This mechanism can elevate cytosolic-free Ca^{2+} and increases Na^+/H^+ antiporter SOS1 activity, hence enabling plants to undergo detoxification process. In *Arabidopsis*, activation of the AtSOS3/calcineurin B-like4 Ca^{2+} sensor increases *SOS1* expression. The production of ROS mediated by Nox further helps plants in stabilizing *AtSOS1* transcript (Laohavisit et al. 2013). In *Arabidopsis* there are ten *Nox* genes (*RbohA-J*) present in its genome. Among them, the expression of *RbohD* and *RbohF* was highly upregulated by salinity stress (Kurusu et al. 2015).

2.4.3 Soluble Sugars as Signalling Molecules

Excess accumulation of Na^+ and Cl^- in the leaves leads to the inhibition of photosynthesis, which may interfere with plant carbohydrate metabolism due to restriction of CO_2 fixation into chloroplast via limitation of stomatal opening and CO_2 transport in the leaf mesophyll cells. This process is mediated by shoot- and root-generated hormones like ABA, CKs and IAA (Chaves et al. 2009; Acosta-Motos et al. 2017; Wungrampha et al. 2018).

Instead of converting CO_2 to sucrose and starch under normal condition, plants use catabolism processes to convert starch and sucrose into soluble sugars like glucose and fructose under unfavourable conditions. Like hormones, these soluble sugars can play a role as primary messengers; hence, they can regulate internal signals related to carbohydrate metabolism by controlling gene expression and enzyme activity associated with carbohydrate metabolism, such as ADP-Glc pyrophosphorylase (AGPase), sucrose phosphate synthase (SPS), sucrose synthase (SuSy) and invertase (INV). For that reason, low sugar status appears to be an important indicator in plant signalling response under unfavourable condition, because it can control photosynthesis activity and modulate reserve sugar mobilization and exportation. The high sugar concentration can promote plant growth and carbohydrate storage, hence enhancing survivability of plants under salinity stress and shock (Rosa et al. 2009).

2.4.4 Involvement of Transcription Factors in Activation of Salinity Transcriptional Networks

Transcription factors are regulatory proteins that are involved in the transcriptional process in the nucleus. These proteins can interact with cis-acting elements located at the promoter region. The interaction can further activate or inhibit gene expression from various pathways under abiotic stress including saline condition. In plants, amino acid sequence WRKYGQK (WRKY), DREB1A (dehydration-responsive element-binding protein 1A), AP2/ERF (APETALA2/ethylene response factor), bHLH (basic helix-loop-helix), AREB (ABA-responsive element-binding proteins), MYB2 (myb proto-oncogene protein) and NAC (NAM/ATAF/CUC) are among TFs that have been recognized to be involved in salt stress and salt shock responses (Guan et al. 2017).

Expression of *MYB-like protein*, *NAC*, *bHLH-30-like protein* and *WRKY* was observed in the leaf of salt-treated chrysanthemum at 120 and 200 mM NaCl. Among them the *bHLH* family was the only transcription factor that showed high expression in the leaf of 200 mM NaCl-treated chrysanthemum (Cheng et al. 2018a). In wheat, the expression of *MYB* in salinity-treated plant suggests the involvement of this transcription factor in the activation of genes involved in salinity responses and flavonoid biosynthesis (Shoeva and Khlestkina 2015; Yu et al. 2017). Also, the MYB was reported to be involved in the cell wall biosynthesis in salinity-treated rice (Gall et al. 2015). The NAC was reported to participate in improvement of root growth and seed germination (Yang et al. 2018), while the bHLH was reported to be involved in proline biosynthesis, reducing ROS accumulation and reducing water loss in plants grown under salt stress (Sun et al. 2018). In addition to this, WRKY family was reported to be involved in detoxification of ROS and accumulation of oxylipins such as JA and MeJA (Lim et al. 2015; Kumar et al. 2017).

Different incubation times of *Populus euphratica* (0, 4, 8, 24, 48 and 96 h) in 200 mM NaCl solution induced expression of different types of *NAC* genes in different tissues. The *PeNAC036* expression was observed in all *P. euphratica* vegetative tissues, while the expression of *PeNAC034* was suppressed in the roots and stems. Further, the inhibition of *PeNAC045* expression was determined only in the roots (Lu et al. 2018). The transcriptomic response of chrysanthemum leaves to gradual increase of NaCl concentrations (100–400 mM) showed the expression of different transcription factor genes like *MADS*, *MYB*, *WRKY*, *AP2* and *TAG* (Wu et al. 2016). Prolonged salinity stress treatment of *Aegilops tauschii* to final concentration of 200 mM NaCl induced expression of 39 families of transcription factors in the leaf. Among them, the *FAR1*, *bHLH*, *C3H*, *NAD* and *MYB*-related were the major TF families found in the treated plants (Mansouri et al. 2018). Salinity shock treatment of wheat at 250 mM NaCl resulted in differential expression of *R2R3-MYB* gene in leaves, stems and roots at different time intervals (Yu et al. 2017).

2.5 Expression of Other Salinity-Associated Genes in Plants Responding to Salt Stress and Salt Shock

Rapid activation of many genes can be observed when plants are exposed to salt shock and salt stress treatments, where some have been discussed in the previous subtopics. Gradual increase of NaCl concentration from 100 to 400 mM to salt-intolerant chrysanthemum (*Chrysanthemum morifolium* cultivar Jinba) for 3 days induced expression of 126,646 differentially expressed transcripts in leaves. Some of the expressed transcripts were reported to be involved in osmotic adjustment, such as proline biosynthesis (*P5CS*, *delta-1-pyrroline-5-carboxylate synthetase*), carotenoid biosynthesis (*phytoene synthase*, *beta-ring hydroxylase*, *beta-carotene 3-hydroxylase*) and flavonoid biosynthesis. Others were involved in ionic signalling pathway such as ion transporters (*Ca^{2+}/H^{+} antiporter*, *Na^{+} symporter*, *V-type H + -APase*) (Wu et al. 2016). In contrast to these findings, a recent study conducted by Cheng et al. (2018a) revealed the upregulation of 4348 and 3473 transcripts in *C. morifolium* Jinba leaves when the plants were exposed to single application of NaCl at 120 mM and 200 mM for 12 h, respectively. Also, these treatments induced expression of 41,687 and 42,702 transcripts in 120 and 200 mM NaCl-treated roots, respectively. Among the expressed genes, the *LEA* was upregulated in both leaves and roots, while *betaine aldehyde dehydrogenase* was only upregulated in the leaves for both treatments. These two genes have been reported to be involved in osmotic adjustment when plants are responding to osmotic stress. The *high-affinity potassium transporter 1* (*HKT1*), *Na^+/H^+ antiporters 1* (*NHX1*), *Salt Overly Sensitive 1* (*SOS1*) and *K^+ transport* genes were only upregulated in the stressed roots when they were responding to both treatments. Hence, these findings have shown the adaptive mechanism of roots at the molecular level in order to maintain osmotic and ionic stresses when plants are responding to different severities of salinity.

Exposure of salinity-tolerant and salinity-intolerant peanut plants to salinity shock at 250 mM NaCl for 6, 12, 24 and 48 h induced expression of 86 salinity-responsive genes in leaves of both varieties. These genes encode stress-responsive proteins like LEAs, MTs, major intrinsic proteins (MIPs) or aquaporins, lipid transfer protein (LTP), 9-cis-epoxycarotenoid dioxygenase (NCED), calcineurin B-like protein-interacting protein kinases (CIPKs) and oleosin (Sui et al. 2018). Exposure of salt stress-sensitive jute plants (*Corchorus* spp.) to salt shock treatment (250 mM NaCl) for 12 h induced expression of 1811 and 8427 transcripts in the leaf and root, respectively. Meanwhile, the expression of 631 and 4612 of leaf and root transcripts, respectively, was detected in salt stress-tolerant jute. Most of the expressed transcripts were revealed to be involved in ABA signalling, amino acid metabolism, carbohydrate metabolism, secondary metabolite metabolism and fatty acid metabolism (Yang et al. 2017). In other studies, the involvement of heat shock proteins (Hsps) was profiled in poplar plants treated with salinity shock treatment using 200 mM NaCl. Among the *Hsps*, the expression of *PtsHsp-11*, *PtsHsp-21*, *PtsHsp-36*, *PtHsp40-113*, *PtHsp40-117*, *PtHsp60-31*, *PtHsp60-33*, *PtHsp60-38*, *PtHsp60-49*, *PtHsp70-09*, *PtHsp70-12*, *33*, *PtHsp90-09*, *PtHsp90-12*, *PtHsp100-21* and *PtHsp100-75* was found to be increased in the leaves of treated plants. Each group of Hsp has been reported to have specific function in plants, but the general functions of Hsps are their involvement in protein disaggregation and protein refolding (Yer et al. 2018). Thus, the expression of Hsps in plants grown under saline condition is to maintain tertiary structure or correct folding of proteins.

Previous researchers also found that plants emit volatile compounds when they are responding to abiotic stresses like high light intensity, high temperature and salinity. Most of the volatile compounds possibly functioned as signalling molecules or specific products from stress response pathways in plants (Holopainen and Gershenzon 2010; Loreto et al. 2014). Recently, a total of 7210 genes was detected in tomato seedlings grown under salinity stress (75 mM). Out of this number, about 6200 and 1208 belong to downregulated and upregulated genes, respectively. Among these genes, 18 differentially expressed genes were found to be associated with the volatile compound biosynthetic genes such as *farnesyl pyrophosphate synthase*, *divinyl chlorophyllide a 8-vinyl-reductase*, *squalene synthase*, *mevalonate kinase*, *geranyl pyrophosphate synthase*, *mevalonate disphosphate decarboxylase*, *geranylgeranyl transferase-2*, *geranylgeranyl transferase type-1*, *geranylgeranyl transferase type-2*, *(−)-germacrene D synthase*, *epidermal germacrene C synthase*, *(−)-camphene/tricyclene synthase*, *vetispiradiene synthase 1*, *beta-phellandrene synthase*, *sesquiterpene synthase*, *cinnamoyl-CoA reductase*, *allene oxide synthase* and *allene oxide cyclase* (Zhang et al. 2018b). Functional studies of salt-responsive genes have revealed their ability to improve salt tolerance in many transgenic plants as mentioned in Table 2.1.

Table 2.1 Enhancing plant salt tolerance using genetic engineering approach

Transgenic plant	Gene engineered	Source	NaCl treatment	Improved tolerance under salinity treatment	Reference
Arabidopsis	*ZmMYB3R*	Maize (*Zea mays*)	Single application of 250 mM NaCl for 2 weeks	Enhanced growth performance and higher survival rates	Wu et al. (2019)
				Elevated catalase (CAT), peroxidase (POD) and superoxide dismutase (SOD) enzyme activities	
				Increased sensitivity to ABA	
				Regulation of the stomatal aperture	
Tobacco	*Glutamyl-tRNA reductase1* (*HEMA1*)	Peanut (*Arachis hypogaea*)	Single application of 200 mM NaCl 24 h	High 5-aminolevulinic acid (ALA) and chlorophyll contents	Yang et al. (2019)
				High superoxide dismutase (SOD) and ascorbate peroxidase (APX) enzyme activities	
				Lower content of reactive oxygen species (ROS)	
				Slighter membrane damage	
Tobacco	*Ferrochelatase1* (*FC1*)	Peanut (*Arachis hypogaea*)	Single application of 200 mM NaCl 24 h	High CAT, POD and APX enzyme activities	Yang et al. (2019)

(continued)

Table 2.1 (continued)

Transgenic plant	Gene engineered	Source	NaCl treatment	Improved tolerance under salinity treatment	Reference
Arabidopsis	*ABRE-binding protein 2* (*ABP2*)	Maize (*Zea mays*)	Gradual increment of NaCl concentrations (50, 100 and 200 mM) at 3-day intervals and maintained at a concentration of 200 mmol L^{-1} for 14 days	Decreased ROS levels	Zong et al. (2018)
				Enhanced expression of stress-responsive and carbon metabolism-related genes	
Tomato	*Choline oxidase gene* (*codA*)	*Arthrobacter globiformis*	Gradual increment of NaCl concentrations (50, 100, 150, 200 mM NaCl) each day and maintained at a 200 mM NaCl for 7 days	Accumulation of glycine betaine (GB)	Wei et al. (2018)
				High photosynthetic rates and antioxidant enzyme activities	
				Low reactive oxygen species (ROS) accumulation in the leaves	
				Low Na^+/K^+ ratios	
Arabidopsis	*MdERF4*	Apple (*Malus hupehensis*)	Single application of 100 mM NaCl	Modulating ethylene response	An et al. (2018)

2.6 Activation of Ion Transporters and Detoxification Mechanism in Response to Salt Stress and Salt Shock

Osmotic stress is an initial stress experienced by plants when they are exposed to saline soil. Subsequently, an ionic imbalance or ionic stress occurs when salt content reaches a maximum level. This condition can disturb essential nutrient balance in plants, due to competition of Na^+ and Cl^- with essential plant nutrients such as Ca^{2+}, K^+ and NO_3^- (Liang et al. 2018). The balance of K^+ is important in plants due to its involvement in plant physiological and biochemical processes. As an example, the K^+ is known to be involved in enzyme activation, charge balance and osmoregulation (Wakeel et al. 2011; Zhang et al. 2018c). Hence, the plant growth continuity under saline condition always depends on the homeostasis of these ions including K^+/Na^+ ratio.

Salt sensitivity and tolerance response not only vary considerably among different plant species but also depend on the salt uptake at the root level, long-distance transport of salt, cellular compartmentation and cellular K^+/Na^+ ratio. Further, the SOS1 (salt overly sensitive 1) Na^+/H^+ antiporters, histidine kinases and AHK1/ATHK1 are among important proteins that function as salt sensors or osmosensors when the plants respond to salinity stress (Wu 2018). The participation of SOS pathway in cellular Na^+ detoxification involves three SOS genes (*SOS1*, *SOS2* and *SOS3*), which signify their importance in maintaining ion homeostasis during salt stress (Nath et al. 2016). Among the *SOS* genes, the *plasma membrane* Na^+/H^+ *antiporter SOS1* is generally found in higher plants. For instance, *SOS1* from *A. thaliana* was reported to concentrate in the root tip epidermis and the root/stem/leaf parenchyma xylem-symplast boundary, which indicated *SOS1* involvement in exporting and controlling the long-distance transport of Na^+ (Cheng et al. 2018b). Wu (2018) further reported that some putative salt stress sensors/proteins (SOS1 Na^+/H^+ antiporters, NCX Na^+/Ca^{2+} exchangers, NSCC/NADPH oxidase tandem, mechanosensory channels and transporters, annexins and H^+-ATPase/GORK tandem) have participated in early signalling events when the plants are responding to salinity stress. The vital role of plant SOS1 is to exclude Na^+ from the intercellular space and minimize the toxic effects to the plant cytoplasm (Mansouri et al. 2018).

Six ion transporter families (ABC (ATP-binding cassettes) transporters, HKTs (high-affinity potassium transporters), NHXs (sodium hydrogen exchangers), Ca^{2+}ATPases (calcium ATPases), CAXs (cation hydrogen exchangers) and sugar transporters) were observed in pearl millet when they are responding to salinity shock treatment (250 mM) for 6 days (Shinde et al. 2018). In response to the salinity stress experienced by *Aegilops tauschii* at 200 mM, several ion transporters and channels like *SOS1*, *NHXs*, *HKTs* and H^+*-ATPase* were expressed in the leaf suggesting their involvement in controlling cellular K^+, Na^+ and Ca^{2+} (Mansouri et al. 2018).

The root of salt-susceptible and salt-tolerant alfalfa showed upregulation of K^+/H^+ *antiporter* and *vacuolar* H^+*-pumping ATPase* genes. The antiporter proteins help in salt tolerance, while H^+-ATPases help in osmotic adjustment under salt stress (Postnikova et al. 2013). A study on a salt-tolerant *Iris halophila* Pall further revealed the expression of K^+ transporters, K^+/Na^+ *ion transporters* (*KUP*, *KAT*, *HKT*, *KCNKF*), *chloride channel* (*CLC*), *cation/proton (H^+) exchanger* (*CHX*) and *cation exchanger* (*CAX*) genes in shoots when the plant was responding to salt shock treatment at 300 mM NaCl. This finding shows significant function of ion transporters and exchangers in regulating Na^+ and Cl^- in *I. halophila*, hence enhancing salt tolerance compared to wild plant (Liu et al. 2018).

2.7 Production of Osmolytes and Osmoprotective Proteins in Response to Salt Stress and Salt Shock

Excess Na^+, Mg^{2+}, Ca^{2+}, K^+, Cl^-, SO_4^{2-} and NO_3^- concentrations in the soil decrease osmotic potential and water uptake by the plant root, hence encouraging plant to develop osmotic stress. In response to this condition, plants accumulate compatible solutes or osmolytes and osmoprotective compounds like proline, glycine, betaine, sorbitol, mannitol, phenolics, amino acids and proteins (Rasool et al. 2013; Gupta and Huang 2014). These compounds can balance the osmotic differences in plant cell by increasing capability of the cell to retain water without disturbing normal cellular functions. Under osmotic stress, glycine betaine has been reported to be involved in the osmotic stress adjustment, protein stabilization (RuBisCo), photosynthetic apparatus protection and ROS reduction (Wani et al. 2013). Accumulation of proline can counteract plant cell from osmotic stress (Huang et al. 2013), while degradation of proline can generate energy and nitrogen pool, which later can be used for plant growth once the stress is relieved instead of participation in enhancing antioxidant enzyme activities (Gupta and Huang 2014; Wang et al. 2018b). Mannitol and sorbitol are involved in increasing chlorophyll content, photosynthesis activity, total carbohydrate and mineral content (Gupta and Huang 2014; Khalid and Cai 2011). LEA, pathogenesis-related and thioredoxins are examples of proteins with osmoprotective functions (Chourey et al. 2003; Kosova et al. 2013; Calderon et al. 2018).

In strawberry, gradual application of NaCl up to 20 mM for 5 weeks induced accumulation of phenolic compounds like ferulic acid, caffeic acid, p-coumaric acid, gentisic acid, gallic acid, ellagic acid and methyl gallate (Jamalian et al. 2013). In response to salinity shock at 200 mM and 500 mM NaCl, *Sesuvium portulacastrum* accumulated different types of phenolics in leaves and roots, respectively (Benjamin et al. 2018). Hence, this observation shows phenolics play vital role in adjusting osmotic stress in stressed plants.

Total proline content was observed in many plant species when they were grown in salinity shock or salinity stress treatments, such as in barley (Arias-Baldrich et al. 2015), sorghum (Nxele et al. 2017), tomato (Alves et al. 2018) and rapeseed (Xiong et al. 2018). Foliar spray of proline has further proven the participation of proline in enhancing stress tolerance to salinity, such as in tomato plant (Kahlaoui et al. 2018). In contrary with proline, the decrement of amino acids like arginine, cysteine and methionine was also observed in plants exposed to salinity stress (Gupta and Huang 2014). Meanwhile, the accumulation of amino acids in stressed plants is reported, probably due to their participation in redox homeostasis, ion transport regulation, stomatal aperture modulation and heavy metal detoxification (Rai 2002).

In other experiments, glycine betaine content was found to increase in sorghum seedlings treated with 100 mM NaCl (Saneoka et al. 2001). The exogenous application of glycine betaine on soybeans improved their growth under 150 mM NaCl treatment due to the reduction of proline, malondialdehyde (MDA) and Na^+ content. The catalase (CAT) and superoxide dismutase (SOD) activities however increased (Malekzadeh 2015). Rady et al. (2018) also reported that foliar application of glycine betaine increased growth indices, bulb yields, water-use efficiency (WUE), leaf chlorophyll content, stomatal conductance, endogenous osmoprotectants, non-enzymatic antioxidants and enzymatic antioxidants in onions grown under salinity (EC = 4.80 dS m^{-1}). The increment of sorbitol concentration was observed in vasculature and mesophyll *Plantago major* plants treated with salt stress (400 mM NaCl) (Pommerrenig et al. 2007). The production of mannitol was observed in roots of lentil when the plants were exposed to salt stress (Skliros et al. 2018).

2.8 Conclusions and Future Perspectives

Plants give different responses when they are exposed to salt stress and salt shock treatments. This can be seen by accumulation of different types of genes, proteins and secondary metabolites when they are responding to different salinity treatments. In real condition, salt stress definitely mimics the real salinity toxicity in plants compared to salt shock. Salinity shock causes osmotic shock which rarely occurs in real situation. Hence, the knowledge on morphological, cellular and molecular responses towards salt stress is more important for breeding programme. Therefore, this chapter would be useful for breeder to breed salt-tolerant plants that can grow well under salinity condition and at the same time produce higher yield.

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Molecular Approaches and Salt Tolerance Mechanisms in Leguminous Plants

3

Sagar S. Datir, Mohit Kochle, and Shruti Jindal

Abstract

Legumes are one of the most important food crops not only serving an excellent source for human nutrition around the world but also improving soil quality through biological nitrogen fixation. Its production and yield is mainly hampered by abiotic stresses. Among them salt stress is threatening legume production worldwide. Hence, considering the importance of legumes, it is essential to understand and develop the strategies to improve their performance under salt stress. To develop the salt stress-tolerant legume varieties requires knowledge of morphological, physiological as well as key biochemical processes along with molecular controls of salt traits at different plant development stages. Molecular approaches such as transgenics, molecular marker-assisted selection, gene expression microarray, genomics, transgenomics, transcriptomics, and proteomics have identified several key factors, proteins, and genes in different cellular pathways underlying salt stress tolerance in leguminous crops. Both genetic engineering and molecular marker-assisted selection are efficient approaches to the development of salt-tolerant legumes. Omics approaches led to the identification of various metabolites as well as functional and regulatory genes in response to salt stress which not only provide new avenues to understand key molecular mechanisms underlying salt stress tolerance but also eventually beneficial to produce stress-tolerant legumes with improved characteristics. The aim of this chapter is to provide an overview on salt tolerance mechanism and also briefly discuss about molecular approaches in improvement of leguminous crops.

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Legume · Molecular mechanisms · Proteomics · Salt · Transcriptomics · Transgenic

Abbreviations

APX	Ascorbate peroxidase
ISSR	Inter simple sequence repeat
NGS	Next-generation sequencing
POX	Peroxidase
QTL	Quantitative trait loci
RAPD	Rapid amplified polymorphic DNA
RILs	Recombinant inbred lines
SNPs	Single nucleotide polymorphisms
SOD	superoxide dismutase
ST	salt stress

3.1 Introduction

Legume, an important member of Fabaceae family, not only provides a key source of food, fodder, oil, and fiber products but also fixes the atmospheric N_2 in root nodules using a specialized symbiotic nitrogen-fixing bacteria (Varshney et al. 2013). The multifaceted use of leguminous species has been summarized by Pandey et al. (2016). For example, the grain legumes are more popular as they provide proteins, various minerals, vitamins, and several important nutrients such as calcium, iron, magnesium, and zinc. They also provide an important source of omega-3 fatty acids, while the oil seed leguminous crops such as groundnut and soybean are mainly used in cooking oil production and manufacturing of several other confectionery products. As legume crops have the ability to fix the atmospheric N_2, their indispensable role in sustaining the farming system makes them very unique member of the ecosystem. Moreover, they also provide an important source of nutritive fodder to livestock which helps in increasing the productivity of animals (Lüscher et al. 2014). Besides, due to their high oil content, crops like soybean and *Pongamia pinnata* have been recognized as potential biofuel crops (Kesari and Rangan 2010). However, salinity stress (ST) resulted due to the excess accumulation of salts in soil as well as groundwater, which badly not only affects the growth but also the production of leguminous crops worldwide. Moreover, at least 50% of the legume production is estimated to be lost because of soil salinity (Farooq et al. 2017). It has been predicted that, due to climate change on a global level, severity of salinity stress problem will probably exaggerate in the near future (Zhang and Shi 2013). As salinity problems are increasing and it limits food production worldwide, improving ST tolerance in legumes is a major objective of the plant scientists. As leguminous crops are sensitive to ST, therefore, it is crucially necessary to understand the impact of increasing soil salinity on legume crop health and productivity (Anjum 2016). To develop and introduce a legume crop in salty agriculture environment necessitates immense efforts to enhance ST tolerance of existing legumes using molecular breeding/genetic engineering approaches or cultivation of naturally occurring legumes tolerant to soil salinity (Bruning et al. 2015).

ST is very complex phenomenon which includes integration of various cellular mechanisms and their genetic controls as well as their relations in response to environmental fluctuations. For this, large-scale omics experiments are required which include complete genetic (genomic), structural and the functional mechanisms (transcriptomics), identification of proteins and various metabolites using proteomics, and metabolomics approaches. Omics methods are regularly used in ST studies of leguminous crops (Deshmukh et al. 2014). A massive data is produced through next-generation sequencing (NGS) technology which not only leads in detection of novel candidate genes and key genomic regions but also generates huge numbers of high-density molecular markers (Sha et al. 2015). These markers can be then utilized in marker-assisted breeding. Moreover, genome-wide expression analysis studies obtained through high-throughput sequencing offer breeders to discover the molecular processes underlying the various complex characters. Additionally, both the genomic approaches like targeting induced local lesions in genomes (TILLING) as well as EcoTILLING proved potentially important in screening of mutants and germplasm collections which detects novel allelic variations of key target genes (Sha et al. 2015).

Two important legumes such as chickpea and pigeon pea are susceptible to ST, and therefore, differences at genetic levels in these crops for salinity stress provide opportunity for selecting ST-tolerant genotypes (Choudhary et al. 2018). However, the development of salt-tolerant legume requires the knowledge of molecular controls contributing to salinity traits. Advances in biotechnological and omics tools have provided substantial insights into identification of various molecular processes of ST in legumes. Fields of genomics and bioinformatics provide opportunities for dissecting and developing ST tolerance traits which can be used to manipulate the key candidate genes. It has been revealed that the tolerance of legume crops to saline conditions is accompanied by several molecular events such as sequestration of sodium ion (Na^+); accumulation of proline and quaternary amino acid, i.e., glycine betaine; induction of antioxidant stress responses; and hormonal biosynthesis (Faghire et al. 2013; Farissi et al. 2013; Salwa et al. 2013; Farissi et al. 2014; Latrach et al. 2014). Therefore, understanding the responses of leguminous crops to salinity requires a thorough knowledge of morphophysiological along with molecular mechanisms. Moreover, it is crucial to understand the association of these events with functional genomics that could further lead in discovering several key candidate regulators (Bargaz et al. 2015). For instance, ST produces several reactive oxygen species (ROS), and in turn, several antioxidant enzymes are produced. It has been shown that, in salt-treated roots, the expression of antioxidant genes was found to be correlated with antioxidant enzymatic activities in *Medicago truncatula* (Mhadhbi et al. 2011). Chickpea is nutritionally important legume and sensitive to salinity. Despite the fact that chickpea cultivars possess broad genetic variations and under saline conditions exhibit good yield and productivity, for sustainable production, further improvement is required. Therefore, it has been suggested that for chickpea improvement under saline conditions, it is crucially important to understand the molecular processes and discover the key candidate genes which can further allow overcoming its phenotypic plasticity (Khan et al. 2015; Kaashyap et al. 2018). ST responses are majorly reflected at both transcriptional and posttranscriptional levels. Therefore, to understand ST responses at these levels, diverse (expressed sequence tag) EST/cDNA collections have been utilized to study the expression pattern of several genes (Liu et al. 2017). In legumes, the role of miRNA and their target genes involved during ST highlighted the importance of miRNA-mediated posttranscriptional gene expression (Alejandra and Reyes 2010). For example, in chickpea, miRNA expression profiling in root apex was conducted to explore the role of miRNA in posttranscriptional control of gene expression under ST (Khandal et al. 2017). Therefore, identification of such genes will help to elucidate the various processes underlying the efficiency of symbiosis between legume and rhizobium (Bargaz et al. 2015). It is also essential to establish the connections between transcriptional and biochemical responses of ST with that of other regulatory processes involved in quantitative trait loci (QTLs) associated with ST-tolerant legumes. Fig. 3.1 explains molecular approaches and mechanisms in developing

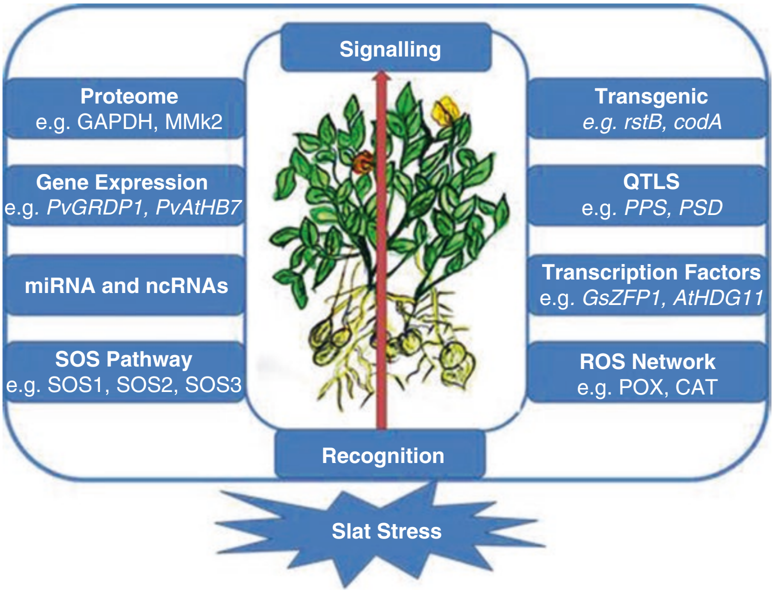

Fig. 3.1 Molecular approaches for developing salt-tolerant legumes. GAPDH-glyceraldehyde-3-phosphate dehydrogenase, MMk2-mitogen-activated protein kinase homolog, *rstB Rhizobium* salt tolerance B, *codA* choline oxidase, *GsZFP1* Cys$_2$/His$_2$-type zinc-finger protein, *AtHDG11 Arabidopsis* homeodomain-leucine zipper transcription factor, *SOS* salt overly sensitive, *ROS* reactive oxygen species, *POX* peroxidase, *CAT* catalase, *PvGRDP1* glycine-rich domain protein 1, *PvAtHB-7* homeodomain-leucine zipper, *miRNA* microRNAs, and *ncRNAs* long ncRNAs

salt-tolerant legumes. The aim of this chapter is to provide an overview on salt tolerance mechanism and also briefly discuss about molecular approaches in improvement of leguminous crops.

3.2 Molecular Approaches in Salt Stress Improvement in Leguminous Plants

3.2.1 Transgenic Approaches for Developing Salt-Tolerant Legumes

Plant's molecular responses to ST have facilitated the development of salt-tolerant legumes with greater yield and productivity using transgenic approaches. Transgenic approaches for the development of ST-tolerant leguminous crops include identification and overexpression of the candidate gene(s) conferring salt tolerance. For this the knowledge of genetic variation underlying salinity traits and translational genomics is essential. These approaches are used to discover genes in model plants which are known to increase the productivity of crop plants under ST and which can be further used as a transgene in the leguminous crops (Banavath et al. 2018). These genes can function as transcription factors that regulate the expression of genes involved in ST, or they might be directly involved in metabolic pathways that are involved in ST tolerance. The following are some examples of transgenic leguminous crops transformed to produce salt-tolerant species. Table 3.1 contains examples of transgenic approaches for developing salt-tolerant legumes.

Table 3.1 Transgenic approaches for developing salt-tolerant legumes

Plant species	Candidate gene	Function	NaCl treatment	Reference
Medicago sativa L.	*GsZFP1*	Transcription factor that regulates the expression of genes involved in abiotic stress response	250 mM	Tang et al. (2013)
	rstB	Salt tolerance gene	120 mM	Zhang and Wang (2015)
Arachis hypogaea L.	*AtHDG11*	Transcription factor that upregulates various stress-responsive genes	250 mM	Banavath et al. (2018)
	AtNHX1	Sodium/proton antiporters in vacuolar membrane to sequester Na^+ in them	150 mM	Banjara et al. 2012
	AVP1	Functions as a proton pump across vacuolar membrane	120 mM	Qin et al. (2013)
Vigna radiata L. Wilczek	*AtNHX1*	Sodium/proton antiporters in vacuolar membrane to sequester Na^+ in them	200 mM	Kumar et al. (2017)
	codA	Responsible for the formation of glycine betaine by the oxidation of choline via COD pathway	50–200 mM	Baloda et al. (2017)

GsZFP1 identified as a candidate gene from wild leguminous plant, *Glycine max* (soybean), encodes for a Cys_2/His_2-type zinc-finger protein, showed increased expression during abiotic stress (Ge et al. 2010). Therefore, to elucidate the function whether *GsZFP1* plays a role in ST, Tang et al. (2013) overexpressed this gene in forage legume alfalfa (*Medicago sativa* L.) using *Agrobacterium*-mediated transformation. The gene was placed under the control of constitutive cauliflower mosaic virus (CaMV) 35S promoter. Transgenic *Medicago* lines transformed with *GsZFP1* gene were irrigated with 250 mM NaCl and under such high ST transgenic lines did not show significant differences in growth characteristics when compared with that of wild type (WT). The osmoprotectant (free proline and soluble sugars) contents were greater in transgenic lines under ST when compared with WT plants. Also both the relative membrane permeability and malondialdehyde (MDA) content were significantly higher in WT plants depicting more cell membrane damage in WT and more production of reactive oxygen species under ST due to lipid peroxidation. These studies clearly demonstrated the role of *GsZFP1* in salt tolerance in alfalfa. Yet in another study, transgenic alfalfa lines were produced by the overexpression of *rstB* (*Rhizobium* salt tolerance B) gene using *Agrobacterium*-mediated transformation (Zhang and Wang 2015). Their results revealed no abnormal phenotypes in transgenic lines when compared with that of WT. Moreover, the transgenic lines showed significant increase in the resistance to salt shock treatment, and their next generations showed enhanced germination as well as better seedling growth when treated with ST. As alfalfa has great agronomic importance worldwide, breeding for ST-tolerant alfalfa is essential for improving its land use efficiency. Therefore, overexpression of salt-tolerant genes such as *GsZFP1* and *rstB* can be further used to produce transgenic alfalfa for increased ST tolerance.

Peanut (*Arachis hypogaea* L.) is an important oil seed crop worldwide and has a high nutritional value, but its yield and production is severely limited because of ST; therefore, it is highly desirable to produce ST-tolerant peanut varieties using genetic engineering approaches. Banavath et al. (2018) developed transgenic peanut plants by transforming them to express *Arabidopsis* homeodomain-leucine zipper transcription factor (*AtHDG11*). This gene was placed under the control of stress-inducible promoter rd29A (responsive to dehydration 29A) from *Arabidopsis*. Increased yield and a three fold enhanced free proline content were noticed in transgenic plants as compared to WT plants under ST in fields (250 mM NaCl). Also the MDA content was more in the case of WT plants due to lipid peroxidation. As compared to WT, transgenic plants exhibited increased SOD activity which protects the plants from oxidative damage, hence making them more stress tolerant. They also identified various ST-responsive genes which were regulated by *HDG11* and highly induced in transgenic plants as a mechanism to fight against the abiotic stress such as ST in this case. The use of constitutive promoter for the expression of such genes may cause undesirable phenotypes in the transgenic crops; therefore, using a stress-induced promoter is a better choice for developing such plants to improve their performance under ST conditions. In another study, transgenic peanut plants were produced when transformed with *Arabidopsis* sodium/proton antiporter gene *AtNHX1* (Banjara et al. 2012) or vacuolar H^+ pyrophosphatase gene *AVP1* (Qin et al. 2013) by *Agrobacterium*-mediated transformation under constitutive promoter. Both of these genes encode for a vacuolar membrane protein. *AVP1* functions as a proton pump, and the overexpression of this gene in transgenics causes a rise in transport of protons inside the vacuole developing an increase in proton chemical gradient across vacuolar membranes which further activates the secondary transporters such as sodium/proton antiporters (*AtNHX1*) to allow Na^+ to flow inside the vacuoles and hence enhanced ST tolerance in transgenic plants. Studies demonstrated that overexpression of such genes results in production of more ST-tolerant plants with improved yield. Therefore, these approaches can prove to be of great importance to increase the yield along with good quality of peanut plants under ST conditions.

Studies on transgenic salt-tolerant mung bean (*Vigna radiata* L. Wilczek) were also performed as it is considered as the most important pulse crop whose productivity has been limited mainly due to ST. Kumar et al. (2017) produced transgenic variety of mung bean by expressing *Arabidopsis* sodium/proton antiporter, *AtNHX1* gene placed under rd29A promoter which is a stress-inducible promoter. This experiment resulted in the more ST-tolerant species when compared with that of WT when exposed to salinity stress (200 mM NaCl). The roots of transgenic plants showed the sequestration of Na^+ due to sodium/proton antiporter in the vacuolar membrane which blocks its influx into shoots. Hence, this gene proved to be an important candidate for developing the transgenic varieties of mung bean. Other methods of developing salt-tolerant transgenic varieties of legumes include the overexpression of the abiotic stress-tolerant genes. For instance, Baloda et al. (2017) introduced *coda* gene (choline oxidase) in mung bean. This gene is responsible for the formation of glycine betaine by the oxidation of choline via COD pathway. This compound is expressed in plants under stress conditions and works as an effective osmoprotectant and accumulates in plants increasing their tolerance to abiotic stress. To check the tolerance of transgenic plants to ST, disc assay was performed in which small leaf discs (1.0 cm in diameter) were cut from transgenic as well as non-transgenic plants. These discs were allowed to float on NaCl solution (50–200 mM) for 4 days, after which it was observed that the leaves from transgenic plants were green as compared to non-transgenic plants which showed complete senescence. Based on these studies, it can be inferred that overexpression of abiotic stress tolerance genes can enhance the ST tolerance in leguminous plants.

3.2.2 Molecular Marker-Assisted Selection and Salt Tolerance

Considering the global food security and malnutrition problems, efforts in developing high-yielding legumes to combat with various abiotic stresses using conventional breeding approaches are underway. However, it is essential to produce varieties using genome-assisted breeding (GAB) as it utilizes the combination of both genomic tools with that of conventional breeding. GAB has immense potential and offers new avenues to produce crop varieties with superior quality (Varshney et al. 2013). For this thorough knowledge and understanding of DNA-based molecular markers, genetic maps, marker-assisted breeding, map-based cloning, genetic diversity and quantitative trait loci (QTL) mapping assessment are prerequisites (Kant et al. 2017). More recent progressions in high-throughput sequencing and next-generation sequencing (NGS) methods could further accelerate the discovery of useful QTLs, and underlying candidate genes which are specifically involved in various abiotic stresses including ST resistance can be introgressed from wild legumes into cultivated legume varieties. Therefore, these approaches can be useful and employed to expand the legume breeding programs (Abdelrahman et al. 2017; Patil et al. 2016). Development of single nucleotide polymorphisms (SNPs) and identification of QTLs associated with ST have been developed in field pea (Leonforte et al. 2013) and soybean (Patil et al. 2016).

Soybean (*Glycine max* [L.] Merr.) is identified as a semi-salt-sensitive glycophyte (Tuyen et al. 2010); hence, it has been suggested that identification of QTLs underlying ST tolerance is very essential for salt-tolerant breeding using marker-assisted selection process (Shi et al. 2018). Complete availability of genomic sequence led to the development of locus-specific markers which made marker-assisted breeding more sophisticated in soybean (Schmutz et al. 2010; Song et al. 2010). Recently, rapid amplified polymorphic DNA (RAPD) and inter simple sequence repeat (ISSR) markers were developed to detect salinity stress tolerance in six different soybean cultivars, namely, Giza 21, Giza 22, Giza 35, Giza 82, Giza 111, and Crawford (Mahgoub et al. 2016). In another study, to expedite the development of ST-tolerant soybean using marker-assisted selection program, Patil et al. (2016) identified SNP markers using high-quality (15x) whole-genome sequencing approach. These findings concluded that studies on salt tolerance mapping using molecular markers are essentially important to soybean breeders not only in developing salt-tolerant soybean varieties but also gaining deeper understanding of salt tolerance genetics.

Like soybean, growth and yield is severely hampered by ST in field pea (*Pisum sativum* L.); hence, advancement of high-throughput systems directed toward detection of SNP markers that further allow construction of genetic linkage maps which will help in the identification of major QTLs associated with ST (Leonforte et al. 2013). A comprehensive linkage map was developed using SNP markers associated with ESTs from 134 recombinant inbred lines (RILs) established from a cross made from ST-sensitive Kaspa and moderately ST-tolerant Parafield cultivars. Linkage map was constructed using a final set of 53 SSRs (EST-derived SSRs) and 414 SNPs (Leonforte et al. 2013). Further studies led to the identification of QTLs for ST tolerance on Ps III and VII linkage groups with flanking SNP markers which were more suitable in the selection of cultivars resistant to ST. Moreover, the candidate genes were identified from field pea associated with ST tolerance by comparing it with the marker-associated sequences from *M. truncatula* genome. This further detected key candidate genes with known functions such as receptor-like protein kinase, histone deacetylase, 14–3-3-like protein, and glutamine synthetase, which were involved in ST resistance in plants (Aydi et al. 2010; Ouyang et al. 2010; Yaish et al. 2011). Such studies concluded that genetic resources such as SNPs and EST markers will be helpful in other studies which will further facilitate the transfer of information from allied legumes for future field pea breeding. Moreover, these studies provide an important information for marker-assisted selection (MAS) and field pea varietal improvement programs.

Response to ST in genus *Medicago* includes reduced seed germination, vegetative growth, as well as yield. *M. truncatula* is a model leguminous crop used to study genetics and genomics aspects (Tang et al. 2014). QTL mapping studies for various physiological traits specifically associated with ST tolerance have been conducted in *M. truncatula* RILs (Arraouadi et al. 2011). In this study, 12 parameters such as stem dry weight, root dry weight, leaf dry weight, etc., related to Na^+ and K^+ concentrations in tissue samples from stems, leaves, and roots, were analyzed from 133 RILs. A total of 13 QTLs were mapped, of which 6 were mapped in ST untreated (control), further 2 in ST, and 5 for salt sensitivity index. However, no major QTLs were detected, and this study did not investigate if any of the co-localizations of these QTLs were due to key genes which were closely linked or because of the pleiotropic effect of same genes on ST tolerance traits. Nevertheless, the information provided in this study may be useful in functional characterization of ST tolerance key genes in this crop and for improved understanding of ST tolerance; QTLs can be further explored using development and near-isogenic line evaluation. This step might set the stage for the positional cloning of ST tolerance candidate genes in *M. truncatula* (Arraouadi et al. 2011).

Several experiments concentrated on the development of molecular markers and identification of major QTLs under salt stress, however, the further validation of these markers in diverse environments is needed. More studies are needed to investigate whether the co-localizations of identified QTLs are due to closely linked genes or because of pleiotropic effects of these genes (Arraouadi et al. 2011). Moreover, there is also a need to explore this information in development of salt stress-tolerant crops in marker-assisted breeding programs.

3.2.3 Gene Expression and Transcriptomics for Salt Tolerance

New discoveries and advancements in technologies such as high-throughput transcript profiling and NGS are widely used to identify specific transcripts expressed during salt stress so that the new ST-tolerant crop varieties can be produced (Wang et al. 2015; Pandey et al. 2016). ST is a complex phenomenon, and hence, it has been revealed that to understand the genetic basis of abiotic stress responses and adaptation in legumes, much more efforts are required especially at molecular level. Therefore, identification and understanding of transcriptional network in important leguminous crops exposed to various environmental stresses is essential and required for clearer perception of various molecular processes related not only with ST adaptation but also in resistance in the legumes (Abdelrahman et al. 2017).

For instance, growth and productivity of common beans is limited by ST (Hiz et al. 2014), and hence, it is essential to identify key genes involved in ST tolerance. Therefore, to better understand the fundamentals behind ST tolerance and to generate the information for functional genomic studies, differentially expressed genes (DEGs) and its related metabolic pathways using comprehensive analysis of transcriptome have been conducted in *Phaseolus vulgaris* (common beans). Comprehensive transcriptome analysis of leaf and root tissue samples from ST-tolerant genotype grown under ST treated and ST untreated, i.e., control conditions, revealed that a total of 6422 unigenes in leaf and 4555 unigenes in root were differentially expressed when subjected to ST treatment. When DEGs subjected to GO and KEGG databases, they observed that leaf as well as root tissues were mainly annotated to energy metabolism, secondary metabolites, and transmembrane transport activity to withstand ST. Moreover, further analyses identified a total of 2678 putative transcription factors (TF) from common bean, and among them 441 were salt responsive (Hiz et al. 2014). In other studies, to unravel various molecular mechanisms involved in ST tolerance, 67 unigenes were identified in leaves when seeds of stress-tolerant bean cv. Pinto Villa were exposed to 200 mM NaCl. Of these 67 unigenes, differential expression levels of 6 stress-related unigenes, namely, *PvGRDP1* (glycine-rich domain protein 1); *PvHSP90*, a heat shock protein 90; *PvLEA-18* known as late embryogenesis abundant-18; *PvERD10*, an early response to dehydration-10; *PvAtHB-7* (homeodomain-leucine zipper); and *PvDnaJ3*, which is a heat shock protein 40, were studied under ST (Hernández-Lucero et al. 2014). It can be concluded that the comprehensive transcriptomics and analysis of DEGs or transcriptional network involved in different common bean cultivars under ST might provide a basis for ST tolerance breeding programs of common beans.

Garg et al. (2016) performed transcriptome analyses to reveal molecular responses of chickpea (*Cicer arietinum* L.) genotypes under ST with contrasting phenotypes differing in their salinity tolerance. Genotype JG 62 is a ST tolerant, while genotype ICCV 2 is identified as ST-sensitive. For this, RNA sequencing was carried out from ST treated and ST untreated, i.e., control roots at vegetative and reproductive stages of these genotypes grown under greenhouse conditions. These studies identified a total of 5545 genes which were especially found regulated in chickpea genotype tolerant to ST. In addition, genes encoding various enzymes involved in carbohydrate metabolism, lipid metabolism, photosynthesis, etc., as well as TF families such as myeloblastosis (MYB), APETALA2/ethylene-responsive element-binding protein (AP2/EREBP) along with both NAC and WRKY, were found to be affected/upregulated by salinity stress. The TF play a role in ST. Overexpression of bHLH-type TF in transgenic *Medicago truncatula* resulted into enhanced root growth when subjected to ST (Zahaf et al. 2012). Transcriptome analysis of roots of *M. truncatula* identified AP2-EREBP-type TF which showed improved ST tolerance in *Arabidopsis* and *M. truncatula* roots (Li et al. 2011). Likewise, in chickpea roots and nodules, the transcriptome in response to salt has been conducted by combining two technologies, i.e., high-throughput transcript profiling and next-generation sequencing technologies (deep Super SAGE) (Molina et al. 2011). They studied the expression pattern of 86,919 transcripts representing 17,918 UniTags and 57,281 transcripts representing 13,115 UniTags (root and nodule tissues, respectively) from INRAT-93 (ST variety). Furthermore, out of these 144,200 analyzed 26 bp tags (roots and nodules), a total of 21,401 distinctive transcripts were discovered. Of these uniquely identified transcripts, 363 and 106 transcripts specifically found up- or downregulated in roots and nodules, respectively, after 2 hours of treatment with 25 mM NaCl in both organs. Therefore, based on these studies it can be concluded that transcriptome data obtained using high-throughput technologies combined with NGS technologies is not only helpful in identification of salt stress-responsive transcripts but such data can also be utilized in molecular marker development for producing ST tolerance in leguminous crops.

Recent discoveries in role of noncoding RNAs may pave new insights in development of salinity-tolerant legumes. Genome-wide high-throughput sequencing led to the identification and furthermore the characterization of noncoding RNAs (ncRNAs) in ST in legumes (Kohli et al. 2014; Wang et al. 2015). ncRNAs such as microRNAs (miRNAs) and long ncRNAs (lncRNAs) are known to be involved in the gene expression at both transcriptional and posttranscriptional levels during plant stress responses (Lv et al. 2016; Xu et al. 2016; Kant et al. 2017; Wang et al. 2017). For example, Kohli et al. (2014) conducted high-throughput sequencing and identified several conserved as well as novel miRNAs associated with gene regulation during salinity stress from chickpea. Similarly, genome-wide high-throughput sequencing of ST-treated leaf and root tissue samples identified a total of putative 7361 and 7874 lncRNAs, respectively, from *Medicago truncatula*, a model legume species. These findings further concluded the possible involvement of lncRNAs in regulation of ST complex controlling networks along with key genes (Wang et al. 2015). To ensure the global food security in the future, the knowledge gained from transcriptome analysis from NGS-based technologies and miRNA regulatory networks associated with legume adaptation to salt stress will enable the development of ST-resistant legumes (Abdelrahman et al. 2017). ST is a complex trait linked not only with physiological and biochemical events but also with several molecular factors. So far very little studies have been conducted to understand these interrelations with respect to noncoding RNAs. Although the knowledge and studies on role of noncoding RNAs during salinity stress are limited, such studies may provide new avenues in understanding the role of noncoding RNAs and elucidating key molecular processes of ST in important leguminous crops.

Overall studies revealed that transcriptomics studies have identified several transcripts in response to ST conditions using high-throughput and NGS technologies. Moreover, the knowledge and comprehensive analyses of such transcripts for the identification of key candidate genes underlying photosynthesis, ion uptake/homeostasis, oxidative stress, etc. are important mainly because these genes could be further useful in developing ST-tolerant legumes using different genetic engineering techniques.

3.2.4 Proteomics for Salt Tolerance

Proteomics envisage quantitative profiling of proteins, profiles of regulatory modification, and protein-protein interaction networks at molecular level which utilizes gel-based 2-DE (two-dimensional electrophoresis) proteomic studies combined with gel-free MS-based (mass spectrometry) quantitative proteomics along with well-established bioinformatics tools. Such approaches led in the discovery of intricate network of various proteins involved in a number of crop plants under abiotic stresses such as ST (Hu et al. 2015). Proteomics approaches are used for increasing the productivity and ST resistance in pea, soybean, *Medicago* species, etc. (Hu et al. 2015; Ji et al. 2016; Long et al. 2016). Abiotic stress conditions cause protein dysfunction, and for cell survival under stress, it is crucially important to maintain the functional conformation and prevention of aggregation of non-native proteins (Joseph and Jini 2010). Therefore, understanding and identification of such intricate network of proteins may enable the development of novel breeding strategies that can further be utilized to produce improved crop productivity and environmental performance (Hu et al. 2015). Table 3.2 summarizes the examples of some important proteomic studies in identified ST-tolerant proteins in leguminous crops.

Ma et al. (2014) conducted comparative studies of ST response of the soybean (*Glycine max* L. Merr.) roots from two genotypes, namely, Lee and Jackson. Their

Table 3.2 Some important biomarkers in identification of salt-tolerant legumes

Plant species	Plant part	NaCl treatment	Potential biomarker	Reference
Glycine max L. Merr.	Leaf, hypocotyl, and root	40 mM	Glyceraldehyde-3-phosphate dehydrogenase (GAPDH), stem 31 kDa glycoprotein precursors, fructokinase 2	Sobhanian et al. (2010)
Glycine max L. Merr.	Root	150 mM	V-H(+)-ATPase subunit A, phosphoglycerate kinase, and cysteine synthase	Ma et al. (2014)
Glycine max L. Merr.	Leaf and root	200 mM	14–3-3 protein, mitogen-activated protein kinase homolog MMK2, protein phosphatase 1, thioredoxin H	Ji et al. (2016)
Medicago sativa L. cv. Zhongmu-1 and *Medicago truncatula* Gaertn. cv. Jemalong A17	Root	300 mM	Ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO), cytosolic malate dehydrogenase (cMDH), glyceraldehyde-3-phosphate dehydrogenase (GAPDH)	Long et al. (2016)
Medicago sativa L. cv. Zhongmu-1	Shoot	100 mM and 200 mM	RuBisCO activase protein, glyceraldehyde-3-phosphate dehydrogenase (GAPDH)	Xiong et al. (2017)
	Root	100 mM and 200 mM	APX, glutathione peroxidase (GPx), and quinone reductase family protein	

results identified 68 differentially expressed proteins (DEPs) from soybean seedlings in root tissue in both Lee and Jackson genotypes under 150 mM NaCl stress exposed for 1, 12, 72, and 144 hours using 2-DE and MALDI-TOF-TOF-MS proteomics techniques. These 68 proteins identified using proteomics were mainly involved in 13 pathways such as signal transduction, redox homeostasis, carbohydrate metabolism, secondary metabolite synthesis, amino acid and nitrogen metabolism, protein synthesis, etc. Out of these 68 DEPs, phosphoglycerate kinase and cysteine synthase were crucially important as indicated by the functional protein network generated from STING 9.0. Furthermore, they revealed that, from 56.5% of differentially expressed proteins, 20.3% were associated with redox homeostasis, 18.8% were associated with carbohydrate metabolism, and 17.4% were found to be associated with the secondary metabolite biosynthesis, suggesting the key importance of these pathways in salt tolerance. Moreover, six of the total ROS-scavenging proteins identified were found to be more abundant in the Lee 68 genotype compared to Jackson and had shown better ROS-scavenging ability in seedling roots as compared to genotype Jackson which was further validated by checking the accumulation of H_2O_2 among them. Under salt stress, the genotype Jackson showed significant downregulation, whereas genotype Lee 68 showed significant upregulation of V-H (+)-ATPase subunit A indicating that Lee 68 genotype has better salt tolerance capacity over Jackson genotype.

Based on salt stress studies performed on roots of Lee 68 and Jackson genotypes, it can be concluded that such studies can be helpful in identifying the significant proteins involved in signaling, cellular, and metabolic pathways that provide ST tolerance in soybean (Ma et al. 2014). Similarly, leaves and roots of soybean were treated with 200 mM of NaCl and proteomic analysis was performed using iTRAQ approach (Ji et al. 2016). A total of 6610 DEPs were detected and out of which 278 and 440 proteins were identified from leaves and roots, respectively. They have also noted that 50 proteins were commonly expressed in leaves and root tissue samples. Identified proteins were then further classified into photosynthesis and carbohydrate metabolism, stress and defense mechanism, membrane and transport processes, etc. Based on protein-protein interaction studies, they concluded that these proteins were mainly engaged in carbohydrate as well as energy metabolism, protein synthesis, and redox homeostasis which can be further attributed to four high ST-responsive networks. Validation of some of the proteins such as 14–3-3, MMk2, PP1, and TRX-h using semiquantitative RT-PCR revealed that these identified key proteins were also regulated at transcription level in response to ST (Ji et al. 2016). Likewise, Ma et al. (2014) performed proteomics at seedling stage of various ST-tolerant soybean genotypes when treated with ST. Their studies identified several potential candidate proteins for enhancing ST tolerance in soybean (Ji et al. 2016). Hossain et al. (2013) revealed that the salinity response of soybean depends on the severity and duration of the stress as well as targeted tissue. They claimed that these factors further affect the proteome and makes the understanding of ST mechanism more challenging. Such studies provide a very comprehensive analysis of proteins expressed especially during ST; hence, results obtained can be further utilized for studying the functions of specific genes expressed during the stressed conditions so as to clarify the molecular mechanism underlying ST in soybean. For example, overproduction of ROS, which further oxidizes macromolecules such as proteins, lipids, carbohydrates, and nucleic acids (DNA), is produced in response to ST which permanently damages plant cells (Gill and Tuteja 2010). To scavenge these ROS, plant cells produce several antioxidant enzymes such as peroxidase (POD), glutathione S-transferase (GST), ascorbate peroxidase (APX), and SOD. Therefore, it can be concluded that the alterations in protein expression levels indicate that the antioxidative defense mechanism is activated in soybean seedling under salt stress (Ji et al. 2016).

In another organ-specific proteomic profiling, Sobhanian et al. (2010) observed the plant response under 40 mM NaCl ST using 2-DE and MALDI-TOF and identified 19, 22, and 24 DEPs in soybean leaves, hypocotyl, and roots, respectively. The proteins were overexpressed or repressed except glyceraldehyde-3-phosphate (G3P) dehydrogenase, fructokinase 2, and stem 31 kDa glycoprotein precursors which were found to be engaged in either leaf, hypocotyl, or roots under ST elucidating their key importance in generating the ST tolerance. The study revealed that there is downregulation of important photosynthetic related proteins in leaves like calreticulin, fructose-bisphosphate aldolase, 50S ribosomal protein, RuBisCO small and large subunits, etc. In addition to this, there is also downregulation of important metabolic-related protein in hypocotyl and roots such as kinesin motor protein, methionine synthase, transketolase, NADPH:isoflavone reductase, etc. This study found the suppression of the glyceraldehyde-3-phosphate dehydrogenase in leaves and hypocotyl which is one of the most important and key enzymes generating NADH by NAD^+ during the glycolysis leading to ATP reduction and further plant growth under ST in soybean. The study suggests that G3P could be the candidate gene to improve ST tolerance of soybean.

Using 2-DE and MALDI-TOF/TOF, Long et al. (2016) studied the proteins affected by 300 mM NaCl ST in *Medicago sativa* (cv. Zhongmu-1) and *Medicago truncatula* (cv. Jemalong A17) and positively identified 60 and 26 deferentially expressed proteins (DEPs), respectively. These proteins were involved in antioxidation, photosynthesis process, signal transduction mechanism, protein synthesis and processing, etc. The functional classification of these DEPs showed molecular binding and catalytic activity were the primary role exhibited by them like transferase activity, hydrolase activity, protein binding, nucleic acid binding, small molecule binding, translation factor activity, transporter activity, hydrolase activity, kinase activity, transferase activity, enzyme regulator activity, etc. Under ST condition the small and large subunits of RuBisCO were upregulated in the Zhongmu-1 indicating the increased photorespiration and better adaptation during moderate ST. Xiong et al. (2017) also observed the effects of ST in *Medicago sativa* cv. Zhongmu-1 by 2-DE and MALDI-TOF-TOF MS and identified 27 and 36 proteins in shoots and roots, respectively, under 100 mM and 200 mM NaCl stress. Furthermore, they discovered some of the novel proteins such as CP12, PR protein 2, harvest-induced protein, isoliquiritigenin 2′-O-methyltransferase which were found related to ST tolerance mechanism. Majority of these DEPs were participated in photosynthesis process, stress and defense mechanism, carbohydrate and energy metabolism, secondary metabolism, protein metabolism, transcriptional regulation, cell wall and cytoskeleton metabolism, ion transport, and signal transduction mechanism. The major ROS-scavenging protein like ascorbate peroxidases, glutathione peroxidase, 1 ferritin protein, and quinone reductase family protein were all upregulated under increased NaCl concentration. However, most of these are found in roots compared to shoots, indicating roots have better and stronger ROS-scavenging ability. Their study concluded that the ST primarily affects photosynthesis, detoxifying and antioxidant, secondary metabolism, and ion transport mechanism and shows different tissue-specific responses. The novel proteins identified can further enhance the understanding of molecular processes underlying ST tolerance responses of legumes.

Overall, very few studies on proteomics and expression of these proteins during ST tolerance in legumes are reported. Therefore, it can be concluded that this knowledge is very limited, and the validation of various expressed proteins which are upregulated or downregulated under ST or which transcripts are expressed individually or in combination with others is needed. Various proteins identified by proteomics can be used as biomarkers that can be helpful in identification of genetic diversity, ST tolerance of cultivars, landraces, and wild relatives of major legume crops which can be further utilized for yield improvement (Barkla 2016). Developing biomarkers for isolation of key candidate genes can be integrated through marker-assisted selection based on proteomics and marker-based gene pyramiding (Hu et al. 2015). Studies on proteomics will also help in understanding various pathways engaged in ST tolerance, and therefore, improving the knowledge of key proteins involved in stress tolerance can be utilized in genetic engineering and transgenic legume development studies (Ahmad et al. 2016).

3.3 Conclusions and Future Perspectives

Increasing salinity problems and limited crop productivity created an alarming situation to develop salt stress-tolerant legumes. Therefore, to uncover the mechanisms underlying salt stress tolerance, knowledge and omics-based technologies are required to accelerate the breeding programs in important leguminous crops. Furthermore, whole-genome sequencing projects if combined with transcriptomics and proteomics will help to discover the key candidates of ST tolerance mechanism. Identified key candidates or genomic regions can either be used to produce transgenic legumes with improved productivity under salt stress conditions or in marker-assisted breeding programs. Although limited studies have been conducted and confirmed various proteins involved in ST tolerance in important legumes, the further network and regulation of these proteins under salt stress needs further investigation. Meager studies identified and demonstrated the salt stress-induced microRNAs, but what type of a particular microRNA is produced and involved under such conditions in important legume crops remains unclear. Considering their importance in human nutrition and environment, it is essential to develop more effective molecular strategies for promoting sustainable legume production under salt stress conditions.

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Crop Improvement Through Microbial Biotechnology: A Cross Talk

4

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Abstract

Salt is a basic necessity of all living beings; however, a paradoxical increase in salt concentration has a detrimental effect on almost all life forms. Increasing cellular salinity induces bimolecular deformations that result in growth inhibition and cell death in both plants and animals. Despite the detrimental effects of salinity, microorganisms evolved mechanisms to successfully survive in extreme salt stress environments like salt brines, saline lakes, sea, effluent treatment plants, saline soils, etc. Microbes were identified to harbor various gene/gene clusters connected to salt stress tolerance, some of which were employed to develop salt stress tolerant/osmotolerant transgenic crops. The goal of this chapter is to summarize the soil salinity, mechanisms of microbial salt stress tolerance, and biotechnological applications of the salt stress tolerance for crop improvement.

Keywords

Genes · Osmotolerance · Salinity · Salt stress tolerance · Transgenic crops

4.1 Introduction

Soil represents one of the complex ecosystems with huge prokaryotic diversity in comparison to any environment (Roesch et al. 2007). Even as small as 1 g of the soil may contain an approximate ten billion microorganisms of thousand diverse species (Knietsch et al. 2003). This diversified microbiota plays various significant roles in soil ecosystems such as in the foundation of soil structure and soil texture

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(Garca-Orenes et al. 2010), breakdown and mineralization of organic matter (OM), energy transformation, and nutrient cycling (Yan et al. 2015a, b). These microbial processes enhance nutrient availability to the plants. Thus, it is important to have high microbial biomass and maintain high microbial activities in various ecosystems (Rontein et al. 2002). However, natural and anthropogenic processes such as improper irrigation, deforestation, chemical contamination, water drainage, etc. increase accumulation of water-soluble salts within soil layers and induce salt stress in soil. Salinity is an environmental factor that became a hazard to plant cultivation worldwide (Gill et al. 2009). Salinity not only decreases growth of plant and crop production but also disturbs environmental health, economic welfare, and agricultural production (Rengasamy 2006). Salinity has a contrary effect on biochemical and physicochemical properties of soil (Singh et al. 2013). It retards plant growth because plants focus their energy to conserve water and to maintain ionic balance and soil microbial activity in osmotic stress (Allakhverdiev et al. 2000). Increased soil salinity also has harmful effect on plants' physiological processes like seed germination, flowering, photosynthesis, etc. (Parida and Das 2005). Salinity has also a profound effect on microorganisms residing in soil ecosystems. Salinity has direct lethal effects either on bacterial biomass (Rietz and Haynes 2003) or on various bacterial physiological activities such as nitrification, ammonification, denitrification, nitrogen fixation, etc., cumulatively resulting in the reduction of OM inputs in the soil (Mavi et al. 2012). Thus, salinity has detrimental effects on microbes and affects soil natural organic materials and its stability (Singh et al. 2016). Salinity affects microbial growth by dehydrating the microbial cell by exosmosis phenomenon (Rengasamy 2008; Guenon et al. 2013). Looking at its detrimental impact on an ecosystem, salinity has become a serious issue; however, our understanding about the impact of salinity on the soil microbiome structure and its functioning is still fragmented. Further microorganisms present in soil having high salt concentration have evolved with different adaptations that make them able to live in saline conditions (Empadinhas and da Costa 2008) by upregulation of some specific genes that impart them halotolerance. These genes primarily can enhance salinity stress tolerance in plants. The goal of this chapter is to summarize the soil salinity, mechanisms of microbial salt stress tolerance, and biotechnological applications of the salt stress tolerance for crop improvement.

4.2 Soil Salinity

Soil salinity is not easy to define, as it depends on the salt species, a variety of environmental factors, and nature of the salts. Saline soils are those which contain an ample amount of salt concentration in root zones, which restricts the crop plant's growth (Ponnamperuma 1984). On the basis of electrical conductivity, soils which have an electrical conductivity (EC) rate of more than 4 deciSiemens/square meter (dS/m^2) in saturated phase generally behave as saline soils (Richards 1954). In 2008, FAO has classified soil salinity on the basis of irrigation water in four levels (Table 4.1).

Table 4.1 Classification of saline soils

Classification of saline soil	EC value (dS/m^2)	Level of effect
Low saline	1.5–2.0	Minimal effect of salinity
Intermediate saline	2.0–6.0	Restriction of the yield of salt-sensitive plants
Hypersaline	6.0–15.0	Salt-tolerant plants can yield satisfactory effect
Very highly saline	>15.0	Few salt-tolerant plants yield satisfactory effect

Note source: FAO Land and Plant Nutrition Management Service (2008)

4.2.1 Major Salts Involved in Soil Salinization

Soil salinization is mainly caused by the accretion of water-soluble salts within soil layers above a certain level. Both cationic and anionic soluble salts deposit in the soil and induce the soil salinity. Out of these salts, common salt ions are chloride (Cl^-), sulfate (SO_4^{2-}) and carbonate (HCO_3^-), potassium (K^+), sodium (Na^+), magnesium (Mg^{2+}), and calcium (Ca^{2+}) (Shi and Wang 2005; Yadav et al. 2011). However, sodium is the major culprit, as its accumulation in higher concentration leads to soil modification and also affects the physio-chemical structure of the soil (Yadav et al. 2011).

4.2.2 Causes of Soil Salinity

Soil salinization is described and characterized in terms of the composition of the water-soluble salts in the soil above a certain level that adversely affects interaction of natural environment health, crop production, microbial biomass, and its activities. Salinization occurs in the environment through various natural and anthropogenic processes.

4.2.2.1 Natural Processes

Soil salinity is induced by various geological, hydrological, and pedological processes (Chaitanya et al. 2014). Geological processes such as weathering of different types of rocks (igneous rocks, sandstones, and volcanic rocks), water drainage from zones, and evapotranspiration increase salt concentration in soil surface as well in groundwater (Wanjogu et al. 2001). Hydrological and pedological processes such as the intrusion of saline seawater into rivers are the main cause of salinity in coastal areas (Cyrus et al. 1997). Some salts like sodium chloride (NaCl) will be carried by the wind during cyclones and deposited by rainfall which also increases salinity (Chaitanya et al. 2014).

4.2.2.2 Anthropogenic Factors

Secondary salinization occurs due to human-made activities such as improper irrigation, chemical contamination, deforestation, etc. (Oldeman et al. 2017).

1. Improper Irrigation

Irrigation is a widely used agricultural practice throughout the world, mainly in hot, dry climate areas (tropical regions) (Lioubimtseva et al. 2005; Boutraa 2010). In such areas, water requirement of the crops during the complete vegetative cycle cannot be met through rainwater. Thus, irrigation is necessary to fulfill the hydric needs of the crop in such areas for better agricultural harvest. All types of natural waters like surface water, meteoric (rain), or subterranean origin, contain variable amounts of soluble salts. Thus, irrigation water increases soil salt concentration (Szaboles 1992). Inadequate management of the irrigation is also responsible for increasing soil salinity in irrigated areas. Improper timing of irrigation, use of inadequate amount and quality of irrigation water, and incorrect and excessive use of chemical fertilizers are the major factors for increased soil salt concentration (Homaee et al. 2002).

2. Deforestation

It is one of the major causes of soil salinization. Deforestation allows migration of salt ions in both the topmost and lower horizons of soil (Said-Al and Omer 2011). Due to soil erosion, sedimentation and a greater amount of salt runoff increased in the streams and rivers (Pitman and Lauchli 2002).

3. Salt Accumulation in Air or Water in Soil

Chemicals emitted from industries having various forms of salts can accumulate on the upper surface of the soil (Szaboles 1994). Wastewater with considerable salt concentration from municipalities and upper soil may be contaminated by sludge which causes salinization of soil (Bond 1998).

4. Chemical Contamination

Modernization in agriculture leads to salinization of the soil due to chemical contaminants observed in the case of greenhouses and advanced farming approaches (Omamt et al. 2006).

5. Overgrazing

Soil salinization may increase in arid and semiarid areas where animals hardly fulfill their food requirements. Thus, due to overgrazing, natural vegetation will be reduced, and natural soil cover will be poor and salinization will develop continuously (Yadav et al. 2011).

4.3 Impacts of Soil Salinization

4.3.1 Impact of Salinity on Soil Microorganisms

In soil, activities of complex microbial communities play a major role in soil organic matter (SOM) and in nutrient cycling and various processes such as nitrogen fixation, ammonification, nitrification, oxidation, etc. (Yan et al. 2015a, b). Microbial bioprocess allows decomposition of organic matter that releases essential inorganic plant nutrients like nitrogen, sulfur, phosphorus, etc. in the soil (Mavi et al. 2012). Thus, microbes play a significant role in maintaining healthy soil ecology and became essential in understanding how the microbial flora respond to various environmental stresses. Amidst various environmental stresses, soil salinity induces stressful conditions for the soil microbiome and has detrimental effects on soil microbiome functions (Sardinha et al. 2003; Morrissey et al. 2014). Deposition of higher amounts of water-soluble salts in soil affects sensitive microbes mainly by two principal mechanisms: specific ion effects and osmotic effects. As salt concentration increases in the environment, osmolarity outside the microbial cell increases. This results in drawing water out from soil microbial and root cells through plasmolysis leading toward dehydration and cell death (Oren 1999). Thus, plants and microbes need to maintain their cytoplasmic osmotic potential (OP) with respect to the external environment. Accordingly, halophilic microorganisms have evolved two protective strategies: exchange of ions across membrane and synthesis and bioaccumulation of osmolytes inside the cells (Empadinhas and da Costa 2008).

As part of a survival strategy, prokaryotic halophiles take up less toxic ions (mainly potassium ion) over sodium ions by selective ion pumps (Ventosa et al. 1998). In halophilic bacteria, Na^+ ion concentration is lower in the cytoplasm in comparison to its surrounding medium, while K^+ concentration in the cytoplasm is frequently higher than in the surrounding medium. Thus, import of K^+ from the outside to the cytoplasm and export of Na^+ from the cytosol to the outside must be against its concentration gradient, thus requiring energy (Oren 2008). The second, most common strategy in halophilic organisms is the synthesis and gathering of naturally occurring organic osmolytes or osmoprotective compounds like ectoine, glycine betaine, amino acids, etc. inside the cell that enables water retention within the cell (Oren 2008). Both strategies of osmoregulation require a large amount of energy (Oren 2001). In extrusion and uptake of ions and synthesis of an osmotic solute, microorganisms use more energy (between 23 and 79 ATP molecules), while these microorganisms use approximately 30 ATP molecules to synthesize cell walls (Oren 2002; Jiang et al. 2007). This expansion of large energy in osmoregulation impacts microorganisms' growth and activity (Wichern et al. 2006). In this way, activity and growth of complex microbial communities retarded during salt stress conditions.

Soil organic matter (SOM) possesses different elements out of which the microbial biomass plays a major role in the formation of the SOM. Microbial biomass helps in recycling and transformation of the soil nutrients and organic matter. Microbial biomass also acts as a basis of various minerals and enzymes in the soil for the plants. Soil salinization near the soil surface disturbs successions of microbial processes of soil surface microbial biomass. Increasing the amount of soluble salt in soil diminishes microbial biomass and its activity and growth and changes microbial community structure (Andronov et al. 2012; Rousk et al. 2011; Setia et al. 2011). Soil water potential also depresses the growth and activity of complex microbial biomass in soil. Total soil water potential depends on both osmotic potential and matric potential (Liu et al. 2009). During soil dryness, water is held more tightly with soil aggregates, resulting in decreased water availability for soil microbes followed by microbial dehydration and cell death. So, there is a huge reduction of microbial biomass during salinity (Rietz and Haynes 2003; Yuan et al. 2007).

Since each microbe has its own different tolerance power to overcome small osmotic potential (OP) (Llamas et al. 2008), hereby an increased soil salinity modulates soil microbial community structure (Llamas et al. 2008; Chowdhury et al. 2011). It has been reported that bacteria are less susceptible to osmotic stress than fungi (Wichern et al. 2006). Increasing soil salinity due to various salts (NaCl, KCl, etc.) in different concentrations results in reduction of total fungal counts and bacterial and actinobacterial counts (Ibekwe et al. 2010). Osmotic stress results in the reduction of fungal genetic diversity on a long-term basis (Hohmann 2002). Within fungal species, low osmotic potential has various adverse effects, such as a decrease in hyphal growth, spore germination, varied texture, mode of transcription, and thick-walled spore formation (Mandeel 2006).

Rhizospheric microorganisms play a critical role in plant growth by regulating the availability of nutrients for plants. Thus, some agents disturbing rhizospheric association structure and function will also influence the nutrients available for plant growth. Microbes regulate the nutrient availability for plants in many ways, such as nitrogen recycling through re-mineralization and immobilization and nitrification. Salinity has adverse effects on re-mineralization/immobilization in both forms of nitrogen. In the presence of salts, nitrification is more or less inhibited (Omar and Ismail 1999) that results in ammonia accumulation and affects nitrogen cycling. The presence of salt in the soil also retards the nitrogen immobilization process (Azam and Ifzal 2006). Thus, salinity retards both re-mineralization and nitrification, a major component of nitrogen biogeochemical cycling. It reduces nutrient bioavailability to plants followed by retarded plant growth.

4.3.2 Impact on Enzymatic Activities

Biochemical transformations in the soil mostly depend on or are related to microbial enzymes (soil enzymes) and their activities. Soil enzymes act as a catalyst in various reactions involved in nutrient cycling, decomposition of organic residues, and formation of organic matters (Singh 2016). Moreover, soil enzymes play an important role in maintenance and functioning of living beings by catalyzing intercellular metabolic reactions. Enzymes have their biotechnological potential with various industrial applications. The origin of enzymes is generally from microorganisms but can originate also from animals and vegetables. Various soil enzymes such as β-glucosidase, dehydrogenase, urease, and phosphatases have a fundamental role in various aspects of ecosystems mainly in the transformation of different nutrients for plants via biogeochemical cycles (carbon cycle, nitrogen cycle, phosphorus cycle). Dehydrogenase activity among soil microbes could be used for the calculation of the overall oxidative capacity of the microbial community (Chu et al. 2007). β-Glucosidase activity is a prime signal of soil quality as it breaks down the glycosidic bond and produces the glucose that will be used as an energy source in the microbial composition (Makoi and Ndakidemi 2008). Phosphatase enzymes transform organic phosphorus into an inorganic form that acts as a macronutrient for a plant. Urease is the key enzyme of the nitrogen cycle, catalyzing the breakdown of urea (carbamide) into carbamate and ammonia (NH_3), which depends on the carbon dioxide concentration and pH of the soil (Bittsanszky et al. 2015). Catalase and urease enzymes were also involved in the decomposition process that transforms vegetable residues into humus and nutrients. Amylase activity was found to be correlated with the productivity of crop yield by hydrolyzing the complex sugars such as starch into simpler sugar elements (Kuhad et al. 2011). The effect of salinity varies from enzyme to enzyme, as salinity strongly inhibits the activities of urease, alkaline phosphatase, and β-glucosidase (Pan et al. 2013) while having fewer effects on dehydrogenase and catalase (Garcia and Hernandez 1996).

4.4 Halotolerance Mechanisms

The basic feature of all halophiles is that they need to maintain cytosolic osmotic balance with the environment. The plasma membrane is permeable to water, and inward active transport of water, which is lost by exosmosis, is energetically not favorable (Oren 2008). Increase in salt concentration in the surrounding environment generated a challenge for the microorganism by lowering the water potential of the surrounding environment and producing toxic effects on cellular metabolism. Living cells uptake water by osmosis, determined by water potential. A cell with low water potential uptakes water from its surroundings; however, abundance of solutes such as NaCl (<100 mM) lowers the water potential of the solution. Decreasing water potential of the solution to a degree makes it difficult for a cell to maintain its cytoplasmic volume or retain water from its surroundings without undergoing certain adaptations. Hence, to circumvent the toxic effect of ions and to balance/restore osmotic balance, cells are evolved with different adaptations that allow organisms to exist in harsh saline environments. The adaptation strategies include (1) ion homeostasis; (2) synthesis, uptake, and accumulation of compatible solutes; and (3) general salt stress tolerance proteins.

4.4.1 Ion Homeostasis

4.4.1.1 Na^+/H^+ Antiporters

It is necessary to balance ionic concentrations inside the cytosol of a living cell. Cytosolic ionic concentrations are regulated by the balanced functioning of various pumps and membrane-bound protein transporters. Na^+/H^+ antiporters are transmembrane proteins existing in the plasma membrane of almost every cell through the living world, from unicellular bacteria to complex multicellular organisms such as in plants and humans (Brett et al. 2005; Lentes et al. 2014). Na^+/H^+ antiporters play a key part in the maintenance of intracellular pH, volume of cell and cellular sodium (Na^+) concentration, and homeostasis of the cell (Alkoby et al. 2014). These are the type of secondary active transporters which exchanges Na^+ ions with H^+ ions across the membrane. In *E. coli*, two genes code for two different Na^+/H^+ antiporters NhaA and NhaB. These two types of Na^+/H^+ antiporters are electrogenic transporters that intake H^+ ions and extrude Na^+ ions. NhaA transports two H^+ ions in exchange for one Na^+ ion, while NhaB transports three H^+ ions in exchange for two Na^+ ions (Padan 2014). H^+ translocating ATPase or respiratory chain generates proton electrochemical gradient for the functioning of the Na^+/H^+ antiporters. The homeostatic activity of each type of Na^+/H^+ antiporters is pH dependent and varies from species to species. In *E. coli*, NhaA transporter (EcNhaA) activity increases within pH range of 7.0–8.5 (Taglicht et al. 1991; Tzubery et al. 2004), while in *Helicobacter pylori*, NhaA transporter (HpNhaA) activity is active at pH range from 6.0 to 8.5 (Inoue et al. 1999). At pH above 8.5, transport activity of both EcNhaA and HpNhaA declines due to exhaustion of H^+ substrate from the cell (Mager et al. 2011). NhaA transporter is highly selective for extrusion of Na^+ (Taglicht et al. 1993) and responsible for salt tolerance in bacteria at basic pH range of 7–8 in the presence of sodium (Na^+) or lithium (Li^+) ions (Padan et al. 2004). In alkaline conditions, Na^+/H^+ antiporters are also responsible for enhancing the growth of bacteria due to acidic cytoplasm in relation to the external environment (Padan et al. 2005). It has been characterized that Na^+/H^+ antiporters are responsible for maintaining pH homeostasis possibly due to extrusion of Na^+ ions from the cytoplasm in some of the extreme aerobic alkaliphiles gram-positive bacteria such as *Bacillus pseudorfirmus* OF-4 and *Bacillus halodurans* C-125 (Swartz et al. 2005). It has been shown that some cyanobacterial species like *Synechocystis* sp. express their optimal growth at high alkaline pH range from 7.5 to 11 due to Na^+/H^+ antiporters (e.g., NhaS2 and NhaS4). Inactivation of these Na^+/H^+ antiporters (NhaS2 and NhaS4) in *Synechocystis* sp. strain PCC 6803 makes it sensitive to alkaline conditions. All these prove that Na^+/H^+ antiporters are responsible for maintaining pH and ion homeostasis inside the cells.

4.4.1.2 Potassium Ion (K^+) Transporters

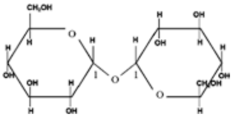
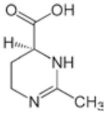
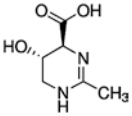
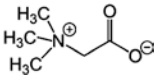
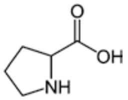
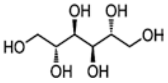
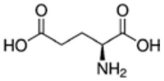
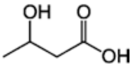
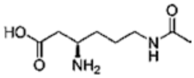
Potassium ion plays a key role in maintaining turgor pressure in the bacterial cell under stress conditions. Stimulation of K^+ intake is the first rapid response to an osmotic shift by a bacterial cell to balance water loss and intake of noxious Na^+ ions during osmotic stress (Sleator and Hill 2002; Epstein 2003). In response to salt stress, K^+ accumulates in large amount through turgor responsive transport system. Many K^+ transport systems have been characterized in bacteria which have different K^+ transport affinities. There are three uptake systems known for K^+ uptake: Kup system, which is constitutively active and maintains low status of K^+ intake and not disposed by osmolality, and Trk and Kdp systems, which are multi-component systems activated after osmotic upshock (Sleator and Hill 2002). Kdp has higher affinity with Km (2 μM), and Trk has a relatively low affinity with Km (1.5 mM) (Epstein 1986; Serrano 1996). Recently, potassium transporter OsHAK21 has been reported in rice plant that functions to maintain ion homeostasis in rice plants in salt stress conditions (Shen et al. 2015).

4.4.2 Synthesis, Uptake, and Accumulation of Compatible Solutes

Compatible solutes are low-molecular-weight, small soluble organic molecules that accumulate in the cytosol of bacterial cells and act as osmoprotectant (Rhodes and Hanson 1993). Osmolytes have no influence on the cellular metabolism of organisms even if they accumulate in high concentration. Osmolyte accumulation makes bacteria versatile in their adaptation to salinity (Ventosa et al. 1998). Some osmolytes help to increase the protein stability by acting as molecular chaperones by folding and unfolding of protein in cells (Chattopadhyay et al. 2004; Yancey 2005). Compatible solutes don't change the structure of protein itself but exert their effects by changing the solvent structure or change in dynamic properties of proteins. Osmolytes also influence protein–DNA interaction by linking with nucleic acid (Paul et al. 2007; Kurz 2008). Compatible solutes, when accumulated in high concentration, provide the highest level of halotolerance and prevent the bacterial cells from plasmolysis by lowering the osmotic potential of the cell during adverse salt stress environmental conditions (Kempf and Bremer 1998). Due to the property of stabilizing biomolecules such as enzymes, DNA, proteins, stress protective agents, etc., compatible solutes have been used in various biotechnological applications. As per compatibility hypothesis, mostly osmolytes are neutral at physiological pH, while some archaeal and bacterial osmolytes such as diglycerol phosphate are anionic and neutralized by pairing with K^+ ions. Due to a variety of compatible osmolytes, a cell has multiple options for its osmotic protection (Yancey 2005). Compatible solutes are accumulated in two ways: uptake from environment/culture medium and de novo synthesis. De novo synthesis of solutes is less preferable over uptake of solutes from an environment. Major compatible solutes are categorized into two groups: (1) sugars and polyhydric alcohols and (2) α-amino acids, β-amino acids, and their by-products (e.g., glycine betaine, Nε-acetyl-β-lysine). Some osmolytes are listed in Table 4.2.

ProP and ProU are the transporters that enable the uptake of osmoprotectants across the cytoplasmic membrane in *Escherichia coli* (*E. coli*) and *Salmonella typhimurium* (*S. typhimurium*). Originally these membrane transporters were recognized as osmotically induced uptake systems for amino-acid derivatives such as

Table 4.2 Description of various osmolytes and osmoprotective compounds involved in osmotolerance

Solute	Chemical structure	Occurrence	References
Trehalose ($C_{12}H_{22}O_{11}$)		*Pyrobaculum aerophilum*, *Stenotrophomonas* sp., *Chromohalobacter israelensis*	Kempf and Bremer (1998)
Ectoine ($C_6H_{10}N_2O_2$)		*Halomonas elongata*, methanotrophs, *Vibrio cholerae*, *V. costociola*	Reshetnikov et al. (2011)
Hydroxyectoine ($C_6H_{10}N_2O_3$)		*Halomonas elongata*, *Nocardiopsis halophila*, *Pseudomonas stutzeri*	Yancey (2005)
Betaine $C_5H_{11}NO_2$		*Methanosarcina thermophila*, *Actinopolyspora* sp.	Rhodes and Hanson (1993)
Proline ($C_5H_9NO_2$)		*S. typhimurium*, *Escherichia coli*	Rhodes and Hanson (1993)
Mannitol ($C_6H_{14}O_6$)		*Pseudomonas putida*	Kempf and Bremer (1998)
β-Glutamate ($C_5H_9NO_4$)		*Nocardiopsis halophila*, *Methanotorris igneus*	Kempf and Bremer (1998)
Hydroxybutyrate ($C_4H_8O_3$)		*Photobacterium profundum*, *Methylarcula marina*	Roebler and Muller (2001)
N-acetyl-β-lysine		*Methanosarcina thermophila*, *Methanothermococcus*, *Thermolithotrophicus*, *Methanohalophilus*	Ventosa et al. (1998)

proline; however, successive studies had approved them responsible for the transport of a wide variety of organic solutes such as proline, glycine betaine, and ectoine (Kempf and Bremer 1998). ProP is a single component system and a secondary H^+/proline symporter. Although it was identified as a proline transporter, it transports a wide variety of organic solutes having structural similarity to proline and glycine betaine, e.g., L-pipecolate, dimethylglycine, carnitine, and ectoine. ProP activity is enhanced by high osmolality, suggesting it as a sensor of high osmolarity. The second osmoprotectant transporter, ProU, is a member of ATP-binding cassette (ABC) superfamily of transporters, having three subunits: ProV, ProW, and ProX. ProV is a membrane-bound ATPase, ProW is the integrated membranous protein, and ProX is the periplasmic substrate-binding protein. Like ProP, ProU transports a variety of the substrates like proline, proline betaine, glycine betaine, choline, etc. with a preference for glycine betaine and proline betaine. A sudden increase in the osmolality of the medium causes an increase in the ProU transcription proportionally to the medium osmotic concentration, and ProU expression remains enhanced till osmotic stimulus persists (Kempf and Bremer 1998). OmpF and *O*mpC porins are present in *E. coli* and *S. typhimurium's* outer membrane and are responsible for permeation of osmoprotectants across the outer membrane. Expression of both *OmpC* and *OmpF* is regulated by medium osmolarity in reciprocal fashion as synthesis of *OmpC* is found predominant in hypertonic environment, while *OmpF* synthesis is found predominant in hypotonic environment. *Envz* (a membrane-embedded sensory kinase) and *OmpR* mediate the osmoregulation of *OmpC* and *OmpF*.

B. subtilis have several compatible solute import systems like *OpuA-E*, *OpuA*, *OpuB*, and *OpuC*. These help in the transport of a wide variety of organic solutes such as glycine betaine, proline, proline betaine, ectoine, choline, etc. *OpuA* is restricted to the uptake of glycine betaine and proline betaine only, while *OpuC* has a wide range of compatible solutes to be imported from the environment and transports choline with low affinity (Roebler and Muller 2001). Choline is transported with high affinity by OpuB which will further be converted into glycine betaine. *OpuE* is dedicated to proline uptake, while *OpuD* transports only glycine betaine (Kempf and Bremer 1998).

4.4.3 General Salt Stress Tolerance Proteins

Any shift in environmental parameter affecting cell growth would result in induction or downregulation of genes associated with that parameter. Change in media salt concentration also leads to change in the expression profile of certain genes which are expected to be involved either directly or indirectly in salt stress tolerance mechanisms. Many of the genes involved in salt tolerance were identified by their salt-linked expression, and many of such proteins were found to be involved in general stress tolerance mechanisms such as refolding of denatured proteins. Genes *brp*A, *gal*E, *mur*B, and *maz*G have been identified from human gut microbiome (Culligan et al. 2012a, b), and genes *gsp*M and *ech*M have been identified from the freshwater pond water by functional metagenomics approach (Kapardar et al. 2010a, b). Additionally a novel osmotolerance gene *brp*A sharing homology with a brp/blh-family beta-carotene 15,15′-monooxygenase protein encodes a carotenoid-modifying enzyme (Culligan et al. 2012a). Gene *gal*E encodes for UDP-glucose-4-epimerase or GALE that catalyzes the reversible conversion of UDP galactose to UDP glucose, and UDP glucose plays part in the osmoprotectant trehalose biosynthesis. Gene *mur*B encodes for UDP-N-acetylenol pyruvoyl glucosamine reductase and forms UDP-N-acetylmuramic for peptidoglycan synthesis (Griffiths and Gupta 2006), essential for growth of *B. subtilis*, *E. coli*, and *S. aureus*. Another osmotolerance gene *maz*G encodes for nucleoside triphosphate pyrophosphohydrolase that hydrolyzes dNTPs to pyrophosphate (PPi) and their corresponding dNMPs and also removes abnormal dNTPs from nascent DNA strands. Osmotolerance gene *gsp*M is quite similar to the general stress proteins, while *ech*M gene encodes enoyl-CoA hydratases (Kapardar et al. 2010a, b).

Similarly, Clp protein constitutes a universally conserved family of the proteins having ATPase activity that acts as a molecular chaperone and is involved in regulating ATP-dependent proteolysis. In *Bacillus subtilis*, various stresses like heat shock induce expression of the *clpC* gene. Its product was identified as a general stress protein. BBC1 protein of alga *Chlamydomonas* sp. W80 showed the tolerance for freezing and salt stress with expression in *E.coli* (Tanaka et al. 2001). In *Listeria monocytogenes*, it was observed that genes regulating general stress protein and metabolic protein were induced after salt stress (Tremoulet et al. 2002).

4.5 Biotechnological Applications of Salt Tolerance Genes for Crop Improvement

Tailor-made microorganisms with improved capabilities for inactivating toxic substances were constructed by genetic engineering. This assures salt tolerance genes for wide applications in the field of biotechnology, such as bioremediation, wastewater treatment, and plant biotechnology. Water and soil are being contaminated by anthropogenic sources such as pesticides and discharge of hypersaline effluents produced by various industries like leather, pharmaceuticals, tanneries, oil refineries, pesticides, etc. Waste containing organic pollutants with a high percentage of salt is difficult to treat by the conventional biological methods, as the high salt concentration will plasmolyze bacterial cells. To improve treatment strategies, salt stress tolerance genes could be engineered into the organic pollutant-degrading bacteria. Till now, a very few bacterial strains are known for degrading organic pollutants in the saline environment (Kim et al. 2003). It is possible to transfer the salt tolerance genes into the desired host by genetic engineering methods for improving the growth of plants in saline conditions (Table 4.3). Both plant- and microbe-originated salt stress genes have been used to provide salt tolerance in plants. Gene *TmHKT1;5-A* isolated from *Triticum monococcum* when introduced in the *T. turgidum* ssp. *durum* results in increased yield even in high salinity conditions (James et al. 2012; Munns et al. 2012). Genes *otsB* and *otsA* isolated from *Escherichia coli* are responsible for trehalose synthesis and transformed into *E.coli* to make recombinant *E. coli M15* (Joseph et al. 2010). Bacterial *mtlD* gene involved in mannitol production was transformed into *Solanum tuberosum* (Rahnama et al. 2011) and into *Arabidopsis thaliana* (Thomas et al. 1995) to protect them from salt stress. It has been also reported that *mtlD* gene from *Escherichia coli* shows overproduction of mannitol in tobacco plant (Tarczynski et al. 1992). The *codA* gene of *Arthrobacter globiformis* was transformed into *Arabidopsis thaliana*, resulting in glycine betaine accumulation

Table 4.3 Salt stress tolerance genes used to develop transgenic plants/bacteria

Gene isolated from	Transgene	Transgenic organisms	References
Triticum monococcum	TmHKT1;5-A	Wheat	Munns et al. (2012) and James et al. (2012)
Porteresia coarctata	*MIP*	Tobacco	Roy et al. (2014)
Hallobacillus sp. and *Bacillus halodenitrificans*	*acd*S	Wheat	Ramadoss et al. (2013)
Methanohalophilus portucalensis	*Mpgsmt-sdmt*	Recombinant *E. coli*	Lai and Lai (2011)
Arthrobacter globiformis	*codA*	*Arabidopsis*	Hayashi et al. (1997)
		Rice	Mohanty et al. (2002)
		Tomato	Goel et al. (2011)
Escherichia coli	*mltD*	Tobacco	Tarczynski et al. (1992)
	mltD	Potato	Rahnama et al. (2011)
	betB	Tobacco	Pilon-Smits et al. (1998)
	betB	*Arabidopsis*	Thomas et al. (1995)
	otsA and otsB	Recombinant *E. coli*	Joseph et al. (2010)
	CodA	Rice	Sakamoto and Murata (1998)
Pseudomonas syringae	*betT*	Tomato	Chen and Beattie (2008)
Glycine max	*Ncl*	Soybean	Do et al. (2016)
Arabidopsis	(SOS1)	Tobacco	Shi et al. (2000)
	ATNHX+ (AVP1)	Cotton	Pasapula et al. (2011)
		Cotton	Shen et al. (2015)
		Tomato	Gouiaa and Khoudi (2015)
	Na^+/H^+ *antiporter (AtNHX1)*	Tomato	Zhang and Blumwald (2001)
	AVP1	Barley	Schilling et al. (2014)
	YHem1	*B. napus*	Sun et al. (2015)
	AtLEA4-1	*Brassica juncea*	Saha et al. (2018)
	APX, SOD	*B. oleracea*	Metwali et al. (2012)
	BrGI	*B. rapa*	Kim et al. (2016)
	DREB	*B. napus*	Qamarunnisa et al. (2015)
	P5CS	Tobacco	Kishore et al. (1995)
Oryza sativa	*SNAC1*	Rice	Hu et al. (2008)
Oryza sativa	*OsCDPK21*	Rice	Asano et al. (2012)
Gossypium sp.	*GhMpK2*	Tobacco	Zhang et al. (2011)
B. juncea	*ECS*	Rice	Bae et al. (2013)
Aeluropus littoralis	Na^+/H^+ *antiporter gene*	Soybean	Liu et al. (2014)
Cicer arietinum	*Lectin*	*B. juncea*	Kumar et al. (2015)

(continued)

Table 4.3 (continued)

Gene isolated from	Transgene	Transgenic organisms	References
Gossypium hirsutum	*GhSOS1*	*Arabidopsis*	Chen et al. (2017)
Chrysanthemum crassum	*SOS1*	*Arabidopsis*	An et al. (2014)
Helianthus tuberosus	*SOS1*	*Arabidopsis*	Chen et al. (2017)
Salicornia brachiata	*SOS1*	Tobacco	Yadav et al. (2012)
Saccharomyces cerevisiae	*HAL2*	Citrus	Cervera et al. (2000)
Saccharomyces cerevisiae	*HAL2*	Tomato	Arrillaga et al. (1998)

(Hayashi et al. 1997). Transgenic salt-tolerant plants of indica variety Basmati and tobacco *Lycopersicon esculentum* were generated by the transformation of a *Arthrobacter globiformis* gene coding for choline oxidase (Mohanty et al. 2002; Goel et al. 2011). The *beta* gene isolated from *E.coli* transformed into tobacco plants made them tolerant to high salt concentration. The transformation of *bet*B gene from *Escherichia coli* to tobacco plant showed that increased betaine aldehyde dehydrogenase (BADH) has increased its osmotolerance (Holmstrom et al. 2000).

Vacuolar H^+ pyrophosphatase gene (*AVP1*) when overexpressed in *Arabidopsis* increased drought resistance and salt tolerance in the cotton plant (Pasapula et al. 2011). The seedlings of the transgenic cotton plants with *AVP1* gene expression possessed more dry weight than the wild-type plants. *AVP1* when overexpressed shows an increase in proton electrochemical gradient that enhances sugar and vacuolar sequestration of ions and hence decreased water potential, which results in increased salt and drought resistance as compared to wild types in hydroponic growth conditions (200 mM NaCl) for a week (Pasapula et al. 2011). Tomato being moderately tolerant to salinity stress could cause severe crop loss. In a study made by Gouiaa and Khoudi (2015), transgenic tomato plants generated by overexpression of Na^+–K^+/H^+ exchanger gene (*TNHXS1*) along with H^+-pyrophosphatase (H^+-PPiase) gene showed more tolerance to salt than the wild-type tomato plants. Do et al. (2016) identified the *Ncl* gene that was responsible for providing tolerance in case of salinity stress in soybean. The transformation of the *Ncl* gene into a soya bean cultivar Kariyutaka has enhanced its osmotolerance, shoot dry weight, and yield. Asano et al. have determined the role of calcium-dependent protein kinases (CDPKs) during both biotic and abiotic stress responses. Plants with overexpression of *Oryza sativa CDPK (OsCPK12)* showed increased salt tolerance through the reduction of the level of ROS generation. OsAPx2 and OsAPx8 genes encoding ROS-scavenging enzymes were expressed more in plants with increased expression of *OsCPK12* (Asano et al. 2012). Tomato plants with increased expression of vacuolar Na^+/H^+ antiport were able to grow and survive in 200 mM NaCl concentration. It has been possible to generate crops with salt tolerance by the combined application of transgenesis and breeding (Zhang and Blumwald 2001).

Due to the recent developments in biotechnology methods, it is now possible to design a salt-tolerant *Brassica* species (Kumar and Srivastava 2016). A new strategy was developed to develop transgenic species. Till now, many high-salinity surviving transgenic *Brassica* species have been designed. The concept of transgenesis in creating salt-tolerant species became stronger by the administration of *YHem1* gene into *Brassica napus* genome. One example of this is the gene *YHem1*, i.e., yeast Hem1 gene, working under the influence of a gene promoter *Arabidopsis HemA1*. The stress tolerance gene has also been transformed into *Brassica oleracea* which ultimately confirmed the stress tolerance (Metwali et al. 2012). A crop *Brassica juncea* conferred tolerance against salinity by transgenic expression of chickpea lectin (Kumar et al. 2015). It shows that the lectin gene involved intolerance against salinity. The salt tolerance in *Brassica rapa* has been increased through the reduction in expression of *GIGANTEA (GI)* using RNAi-mediated method. In a study, expression of *GI* has actually increased tolerance to salt (Kim et al. 2016). The role of transcription factors in expressing salt-tolerant genes is significant. For example, DREB proteins bind to cis-acting dehydration response element (DRE) which is a promoter element present in various stress response genes and turn on their expression (Shinozaki et al. 2003). Qamarunnisa et al. successfully introduced *DREB* gene in *Brassica napus* var. Westar that increased the plant proline content under high salt conditions (Qamarunnisa et al. 2015).

GhSOS1 salt tolerance gene was isolated from *Gossypium hirsutum* L. *GhSOS1* is a Na^+/H^+ antiporter gene present in the plasma membrane that provides a control in increased NaCl concentration in the cotton plant. The clone of cotton salt-tolerant genotype is *GhSOS*1. So properties of *GhSOS1* gene expression level increase under salinity conditions and enhance tolerance to salinity. It is isolated and transformed into *Arabidopsis* where its overexpression increased salt stress tolerance (Chen et al. 2017). *Chrysanthemum crissum* and *Helianthus tuberosus* are some of the plant species from which the *SOS1* gene was extracted and cloned. *SOS1* may be a target gene for enhancing the salt tolerance of transgenic plants (An et al. 2014).

Salicornia brachiata is a salt-tolerant plant that lives in extreme saline environment. It contains a salt overly sensitive 1 (*SOS1*) gene that has a significant role to adapt halophyte plant in a saline environment. The overexpression of the *SbSOS1* gene isolated from *S. brachiata* provides high salt tolerance in tobacco, maintains the K^+/Na^+ ratio, and increases proline and amino acid than the plants with normal gene expression (Yadav et al. 2012). There is a reduced leakage of electrolytes in tobacco plants with transgene in extreme saline environment. *Carrizo citrange* is very sensitive to salt stress. So, in the salty soil environment, *C. citrange* plant restricts its use and is unable to grow. Using recombinant technology can enhance its ability to grow easily in saline stress condition. Transgenic crops like *C. citrange* (Cervera et al. 2000) and *Lycopersicon esculentum* (Arrillaga et al. 1998) with *HAL2* gene show a significant increase in salt tolerance. *HAL2* gene is a salt tolerance gene isolated from yeast and successfully transformed into *C. citrange* plant. Transgenic *C. citrange* plant has been produced with stable *HEL2* gene integration and expression. A transgenic plant with *Hal2* gene shows improvement in growth under salt stress condition (Cervera et al. 2000).

4.6 Conclusions and Future Prospects

The microbial world is highly diverse and evolved biological machinery to cope up various abiotic stresses like salt-induced stress. Various studies have cataloged a diverse set of genes extending salt stress tolerance to the host. Plant genetic engineers have used some of these genes to develop salt stress tolerance crops and received success up to a limit. Identification of novel salt stress tolerance genes is a never-ending quest, and using culture-independent methods is playing a strong role in achieving this target. Despite ongoing discoveries, the pace of this search is slow and requires extensive efforts to catalog candidate novel genes involved in salt stress tolerance to develop salt-tolerant crops and salt-tolerant industrially important microbes.

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Role of Phytohormones in Recuperating Salt Stress

5

Syed Uzma Jalil and Mohammad Israil Ansari

Abstract

Plant hormones are the chemical compounds that naturally synthesize in plants, regulating growth and different physiological metabolisms at the location away from its site of synthesis and work in low concentration. Plants are frequently affected with several abiotic stresses, among them being salt stress as the prime factor that reduced agricultural production. Salt in the soil water generally hinders the metabolism by decreasing the capability of plant to absorb water. The extreme concentration of salt moves to the plant through transpiration process that results into cell wounding which significantly disturbs the transpiration and leads to osmotic stress. Crop plants are required to adapt to adverse external stress generated by environmental conditions with their native biological mechanisms defeated which their growth, development, and productivity endure. Plant hormones are chemical messengers and act as signal compounds; their intricate hormone signaling systems and capability to crosstalk make them perfect candidates for facilitating defense responses. Plants have developed mechanisms that recognize the stress signal, promote optimum growth response, and adapt to adverse environmental conditions and play pivotal roles in facilitating the plants to acclimatize against salinity stress. This chapter summarizes various roles and mechanisms of phytohormones for salinity stress resistance in various plant systems.

Keywords

Salinity stress · Phytohormones · Agricultural productivity · Defense response

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5.1 Introduction

Abiotic stress conditions adversely affect the plants. Adequate amount of salt are required for various physiological processes of plants, but excessive salt causes salinity stress that negatively affects the crop by limiting the agricultural productivity (Munns 2005). Plants have developed different mechanisms in response to salinity stress. Salinity in the soil usually inhibits the metabolism by reducing the water absorption capability of the plants (Rozema and Flowers 2008). The extreme concentration of salt affects plants, which causes cell injury. High salinity is the key feature that restricts the spread of plant in their natural habitats (Rahnama et al. 2010).

Salinity stress implicates modifications in different metabolic and physiological processes, dependent on the sternness and period of the stress, and finally hinders the production of crops (James et al. 2011; Bai et al. 2018). Salinity stress results to osmotic stress followed by ion leakage that represses plant growth (James et al. 2011; Ansari et al. 2011). Salinity stress is expressed as hyperosmotic stress because during the preliminary levels of salinity stress, roots decrease the water immersion ability and leaves enhance water deficit due to osmotic stress in plants (Munns 2005). Salinity stress leads to different physiological variations in plant cell, involving nutrient imbalance, interruption of membranes, alterations in the antioxidant enzymes, impairment in the capability to detoxify ROS, and reduced photosynthetic events (Munns and Tester 2008; Barnawal et al. 2017).

When crops are grown in soils containing extreme NaCl levels, increases in Na^+ and Cl^- in plants result in excessive ion absorption and disturb ion equilibrium, leading to substantial physiological disorders in plant that is the most negative consequence of salinity stress (Mahajan and Tuteja 2005; Ansari and Lin 2010). An increase in Na^+ level inhibits uptake of K^+ ions that is an important component for physiological processes of plant, which leads to lesser yield and may cause death of plant (Ahmad and Umar 2011). During high salt condition, the generation of ROS is elevated (Apel and Hirt 2004; Ahmad 2010). Salinity stress induces free radicals that lead to oxidative damages, which degrades biomolecules, *viz.*, DNA, proteins, and fatty acids, in various cell compartments that disturb vigorous cellular functions of plants (Ahmad and Prasad 2012) (Fig. 5.1).

Phytohormones have significant role in mediating plant responses against abiotic stresses by establishing several physiological and biochemical mechanisms. Plant hormones are engaged in agriculture to increase production of plants. These phytohormones were used for protecting the plants against various abiotic stress responses. In the present scenario, phytohormones, *viz.*, auxin, ethylene, salicylic acid, abscisic acid, cytokine, brassinosteroid, and methyl jasmonates, have to not only regulate developmental process but also involve in defense mechanism of plants against different abiotic stresses. This chapter summarizes various roles and mechanisms of phytohormones for salinity stress resistance in various plant systems (Table 5.1).

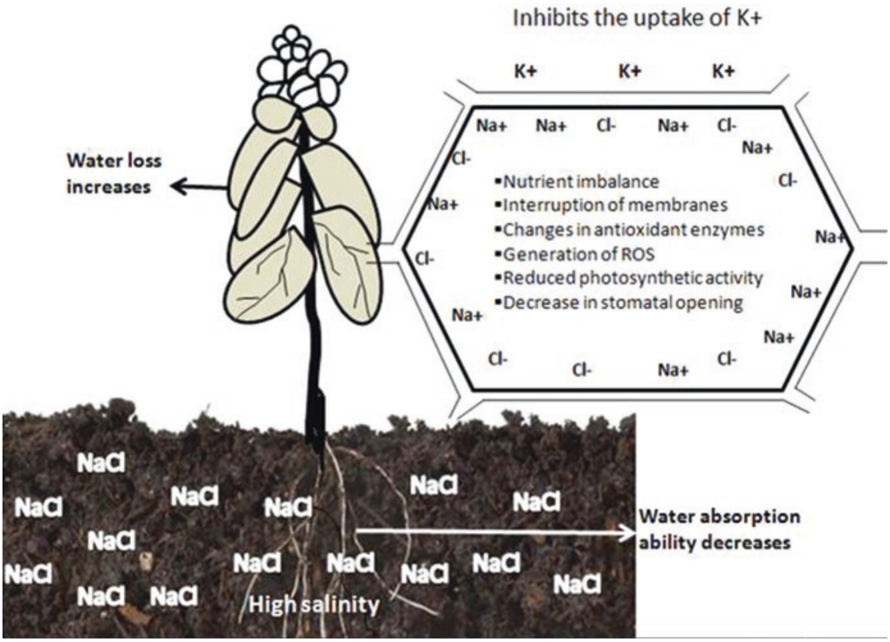

Fig. 5.1 Salinity stress effects on physiological and developmental process of plants

5.2 Role of Abscisic Acid in Salinity Stress Tolerance

Abscisic acid is a sesquiterpenoid phytohormone involved in stimulating seed dormancy and leaf abscission. Abscisic acid is involved in many functions during different stages of the plant life span; it mediated plant responses against abiotic stress conditions and is also involved in endogenous signaling responses in plants against abiotic stresses. Abscisic acid has been proposed to significantly involve in stress responses or adaptation. During high salinity conditions, abscisic acid is substantially synthesized that is essential for defensive mechanisms of plant. The synthesis and remobilization of abscisic acid is the quick action of plants against salinity stress that leads to closure of stomata, thus decreasing water deficit through transpiration and ultimately limiting cell growth. Abscisic acid is an important endogenous envoy of plant responses against salinity stress, and it explained an inimitable hormone perception mechanism, which binds the abscisic acid to abscisic acid receptor that leads to inactivation of phosphatases, and these protein phosphatases target abscisic acid gene expression and ion channels.

Plants exposed to salinity induced surge in abscisic acid level that is in maximum study, proposing that increased level of endogenous abscisic acid under salt condition is because of water deficiency (Zhang et al. 2006). This cannot favor the protracted elevating of abscisic acid concentration that might arise in proportional to gradually enhancing salt content in the environment or condition of field (Etehadnia et al. 2008). Furthermore, the exposure to salinity stress enhances abscisic acid

Table 5.1 Roles and mechanisms of phytohormones against salinity stress in different plants

Plant hormones	Plants	Roles and mechanisms	References
Abscisic acid	Brassica	Endogenous abscisic acid level in leaf	He and Cramer (1996)
	Zea mays	Enhanced Na^+ efflux and H^+ influx	Cramer and Quarrie (2002) and Zhang et al. (2006; 2016)
	Phaseolus vulgaris	Endogenous abscisic acid level increases in leaf	Cabot et al. (2009)
	Rice	Salinity stress controlled to enhance the abscisic acid level	Kang et al. (2005)
	Barley	Abscisic acid present in the roots accumulates ion in root vacuoles required for salinity stress tolerance	Jeschke et al. (1997) and Fukuda and Tanaka (2006)
	Citrus	Stimulates ion accumulation in vacuoles and activates genes for salinity and osmotic stress mitigation	Gomez et al. (2002)
	Sorghum	Reducing the production of toxic Cl^- ions in leaves	Amzallag et al. (1990)
	Wheat	Deferred toxic effect of NaCl and protect from ionic stress	Keskin et al. (2010)
		Stress-responsive genes were highly expressed after the application of abscisic acid	
Auxin	Rice	Decline in IAA level in the leaves as high concentration of IAA causes growth inhibition	Prakash and Prathapasenan (1990) and Nilsen and Orcutt (1996)
	Tomato	Salinity results in 75% reduction of IAA levels.	Dunlap and Binzel (1996)
	Wheat	Improved the fresh and dry weight of seedling, hypocotyl length, and its dry weight	Balki and Padole (1982), Gulnaz et al. (1999), Sastry and Shekhawa (2001), Afzal et al. (2005), and Akbari et al. (2007)
Ethylene	*Thellungiella salsuginea* and *Cakile maritima*	Accumulated more ACC	Ellouzi et al. (2014)
	Tomato	ACC accumulation was increased that decreases the events of leaf senescence	Ghanem et al. (2008)
Jasmonates	Broccoli	Increasing leaf CO_2 assimilation, plant dry weight, and root respiration	del Amor and Cuadra-Crespo (2011)

(continued)

Table 5.1 (continued)

Plant hormones	Plants	Roles and mechanisms	References
	Soybean	Reducing salinity stress symptoms	Yoon et al. (2009)
	Barley	Reducing the effect of salinity stress on plant development and photosynthetic activity	Tsonev et al. (1998)
Cytokinins	Eggplant	Increase proline level, alleviating the salinity stress	Wu et al. (2013)
	Tomato	Leaf xylem CKs regulate photosystem II efficiency and enhanced growth and productivity of plant	Albacete et al. (2010) and Ghanem et al. (2011)
Gibberellic acid	Rice	Regulating some salt-regulated proteins	Wen et al. (2010)
	Wheat	GA application induced inflection of ion and phytohormone homeostasis	Iqbal and Ashraf (2013)
	Linseed	Enhancing the growth, physio-biochemical parameters, amino acid and antioxidant enzyme activity	Khan et al. (2010)
	Soybean	Plant length and plant fresh/dry biomass and development	Hamayun et al. (2010)
Salicylic acid	Rice	Increase in the activity of salicylic acid biosynthetic enzyme	Sawada et al. (2006)
	Arabidopsis	Restoring membrane potential and preventing K+ loss via GORK channel	Jayakannan et al. (2013)
	Barley	Increases the content of leaf pigments and sustains membrane permeability	El-Tayeb (2005)
	Mung bean	Alleviates decreases in photosynthetic activities by increasing N_2 and S assimilation and antioxidant activity	Nazar et al. (2011)

concentration in the leaf of *Brassica* and *Zea mays* (He and Cramer 1996; Cramer and Quarrie 2002) which is intensely related to growth restriction. During the salinity stress, the abscisic acid level of rice enhanced in moderate NaCl concentration than the control plant (Kang et al. 2005). When root growth continues, the abscisic acid concentration increases (Jia et al. 2002); it proposes that growing roots may have diverse responsiveness to the limited level of abscisic acid not only in endogenous form but also in exogenous form (Creelman et al. 1990). The shoot and root synchronized stress responses via enhanced level of phytohormones mobilizing in xylem sap (Davies 2004). However, it is suggested that abscisic acid acts as the root-to-shoot stress signal, which arbitrates the impact of stress (Jia et al. 2002).

A study has revealed that in the salinity condition, abscisic acid supports the plant to increase the water uptake and water potential of xylem tissues and generally inhibition of leaf growth (Jeschke et al. 1997; Fricke et al. 2004). Salinity stress encouraged the synthesis of abscisic acid in roots and transfer to its xylem, which is well associated with the responses of the stomata. This is elucidated when salt is exposed to the barley root; abscisic acid present in the roots accumulates ion in barley root vacuoles that can require for salinity stress tolerance (Jeschke et al. 1997). Abscisic acid plays an important role in signaling for the closure of stomata and prompts rapid depolymerization of actin filaments and reduced development of new filaments, which subjectively is present all over the cell. The modification in actin regulation is required in signaling pathways for stomatal closing; subsequently actin adversely restricts with regular stomatal closing responses to abscisic acid (Jae-Ung and Youngsook 2001). Further, exogenous application of abscisic acid in citrus decreases ethylene synthesis to decrease the cytotoxic Cl^- ion production (Gomez et al. 2002). Furthermore, stress-induced abscisic acid facilitated the leaf development inhibition and restricted Na^+/Cl^- accumulation in foliage (Cabot et al. 2009).

Abscisic acid deferred the toxic impact of NaCl stress in sorghum and developed tolerance against salt (Amzallag et al. 1990). Generally, the upregulation of abscisic acid occurs under salinity stress, which activates genes that are involved in osmotic stress and salt mitigation (Wang et al. 2001; Ansari and Silva 2012). Furthermore, quantification of transcript of the vacuolar H^+ inorganic pyrophosphatase (HVP1 and HVP10) genes and vacuolar H^+ ATPase (HvVHA-A) revealed that abscisic acid regulated these gene expressions in barley (*Hordeum vulgare* L.) under extreme salinity condition (Fukuda and Tanaka 2006). In addition, stress-responsive genes were highly expressed in wheat after the exogenous application of abscisic acid (Keskin et al. 2010). Therefore, abscisic acid is significantly involved in signal transduction and triggering of downstream responses.

5.3 Role of Auxin in Salinity Stress Tolerance

Auxins are influential phytohormones synthesized by plants and promote various growth and developmental processes in plants. However, limited studies reported on the association of auxin content with salinity stress in plant and involvement of auxin in mitigating salinity stress. Indole acetic acid (IAA) is a highly copious and an indigenous auxin, essential for plant growth regulations (Wang et al. 2001). It also is involved in the defense mechanism of plants against high salinity conditions.

The deviations in IAA level during salinity stress seemed similar as abscisic acid (Ribaut and Pilet 1991). The enhanced concentration of IAA has allegedly been associated with growth inhibition (Ribaut and Pilet 1991). Thus, decrease in the growth of plant during salinity stress may be due to the alteration in hormonal balance. Therefore, exogenous exposure of IAA to plants is a good strategy to offset the effect of stress conditions. It has been studied that NaCl affected a substantial decline in IAA level in rice seedlings (Prakash and Prathapasenan 1990). In this study, the exposure of gibberellic acid (GA_3) during the saline condition relatively disables the influence of salinity stress on decreasing IAA concentration and indicates that salinity stress may impact phytohormone balances by hindering the developmental process of plant. Progressive decline of auxin levels in *Oryza sativa* was also observed after NaCl treatment (Nilsen and Orcutt 1996). High salt condition results in the decline of auxin level in tomato (Dunlap and Binzel 1996). Further, salinity stress causes a substantial reduction in the IAA concentration in the plant roots (Sakhabutdinova et al. 2003). Other studies also show that pre-sowing wheat with IAA lessened the effect of salinity stress on inhibition of growth (Sastry and Shekhawa 2001; Afzal et al. 2005). Moreover, seed germination of wheat reduced with enhanced salt concentration, whereas the antagonistic consequence of salinity stress was mitigated by the treatment of auxin such as IAA or NAA on seeds (Balki and Padole 1982; Gulnaz et al. 1999). In other experiments, it shows that treatment with auxin on different cultivars of wheat plants enhanced the fresh/dry weight of seedling and hypocotyl length under high salinity condition (Akbari et al. 2007).

Auxin activates the transcription of various genes known as primary auxin response genes in Arabidopsis, rice, and soybean (Hagen and Guilfoyle 2002). It has been studied that auxin hinders the growth of tiller in *Oryza sativa* that is mediated via downregulation of OsIPT gene and synthesis of cytokinin (Liu et al. 2011). Thus, the identification of new gene that responds in high salt conditions offers the source for investigators to establish gene editing approaches to develop salinity stress tolerance varieties (Zhu 2002).

5.4 Role of Ethylene in Salinity Stress Tolerance

Ethylene, a gaseous phytohormone, is regarded as a stress hormone and involved in plant developmental process, particularly fruit ripening, senescence, and leaf abscission (Gamalero and Glick 2012; Groen and Whiteman 2014). Many studies reported the function of ethylene in plant adaptation against salinity stress condition (Cao et al. 2007), mainly by increasing the ROS scavenger expression (Peng et al. 2014). Furthermore, during salinity stress and other abiotic stresses, the level of ethylene and 1-aminocyclopropane-1-carboxylic acid (ACC) could be induced in various plants (Morgan and Drew 1997). Furthermore, in high salinity condition, leaves and roots of halophyte plants *Thellungiella salsuginea* and *Cakile maritima* accumulated more ACC in comparison to *Arabidopsis* (Ellouzi et al. 2014). Moreover, proteomic analysis reported that various constituents of ethylene biosynthesis were highly accumulated in the soybean salt-tolerant genotype Lee 68 rather than salt-sensitive genotype Jackson in salinity stress condition (Ma et al. 2012).

The studies focused on functional and molecular characterization of ethylene synthesis and its signal transduction pathway that are responsible for salinity stress tolerance in plants. Usually, elevation of these pathways could improve plant resistance to salinity stress, whereas hindrance of it prompts expanded plant sensitivity against salinity stress condition. However, other studies revealed that ACC might assume a negative part in the development of tomato seedlings in salinity stress (Albacete et al. 2009). Furthermore, ACC accumulation was increased in the leaves of tomato preceding Na^+ accumulation and was related to the events of leaf senescence in salinity stress condition (Ghanem et al. 2008), although these outcomes did not demonstrate the immediate impacts of ACC on plant against salinity stress. It was reported that ethylene is not the essential component in salinity stress-mediated growth restriction of plant (Shibli et al. 2007); otherwise, it adversely affects the plant against salinity stress condition at specific developmental stage.

5.5 Role of Jasmonates (JAs) on Salinity Stress Tolerance

Jasmonates (JAs) are cyclopentanone hormones synthesized by fatty acid metabolism that is involved in many diverse developmental pathways and regulates various mechanisms of plants such as fruit ripening, germination, flowering, senescence, and photosynthesis (Cheong and Choi 2003). However, JAs are involved in plant defense against herbivory and abiotic/biotic stress conditions (Dar et al. 2015). Exogenous exposure of methyl jasmonate (MeJA) alleviated the salinity stress of broccoli plants by increasing leaf CO_2 assimilation, plant dry weight, and root respiration (del Amor and Cuadra-Crespo 2011). Further, the exogenous exposure of MeJA effectually decreases the symptoms by salinity stress in seedlings of *Glycine max* (Yoon et al. 2009). Moreover, endogenous levels of JA are enhanced in the roots of *Oryza sativa* during high salt conditions and are observed to respond the harmful impact of salinity stress (Moons et al. 1997; Wang et al. 2001; Wasternack and Hause 2002). Furthermore, JA treatment on barley alleviates the influence of salinity stress on photosynthetic rate and growth inhibition (Tsonev et al. 1998).

5.6 Role of Cytokinins (CKs) on Salinity Stress Tolerance

CKs are involved in various physiological and developmental mechanisms of plant system, e.g., nutrient mobilization, shoot differentiation, apical dominance, cell division, anthocyanin production, chloroplast development, and photomorphogenesis (Nishiyama et al. 2011; Fahad et al. 2015). It was described that exogenous exposure of CKs increases proline level in eggplant, alleviating the effect of salinity stress (Wu et al. 2013; Per et al. 2017). In a report, primary component analysis of tomato plants (cv. Boludo F1) grown under moderate salinity showed that leaf CKs regulate growth and photosystem II activity of leaf that improved crop productivity (Albacete et al. 2010). Furthermore, increasing the root CK synthesis in tomato plants altered both shoot hormonal and ionic status, thus improving growth of plant in response to salinity stress (Ghanem et al. 2011). CK signaling pathway triggers CK-responsive genes in high salinity condition (Ha et al. 2012).

5.7 Role of Gibberellic Acid (GA) on Salinity Stress Tolerance

GA is involved in plant metabolism especially cell elongation. A vital role of GA against abiotic stresses is regularly evident, and its reduction has been shown to contribute to plant growth in abiotic stress conditions (Colebrook et al. 2014). Furthermore, GA alleviates NaCl-induced growth inhibition in rice, by regulating some salt-regulated proteins, which reveals the moderating influence of GA on salinity stress in rice (Wen et al. 2010). GA-priming-induced surge in grain yields two wheat cultivars. This increase in productivity is accredited to the inflection of ion and hormone homeostasis induced by GA priming in salinity stress (Iqbal and Ashraf, 2013). The treatment of GA in combination with $CaCl_2$ alleviated the antagonistic effect of salinity stress by enhancing the growth, biochemical parameters, amino acids, and antioxidant enzyme activity in linseed (Khan et al. 2010). GA reduced the negative effects of salinity stress in other crop plants (Hamayun et al. 2010).

5.8 Role of Salicylic Acid (SA) and Brassinosteroids (BRs) in Salinity Stress Tolerance

SA is a naturally occurring phenolic compound having hormonal properties, and brassinosteroids (BRs) are polyhydroxy steroidal phytohormones, both being involved in the regulation of plant development and for mitigating abiotic stress responses (Clause and Sasse 1998; Fragnire et al. 2011). Endogenous SA increased followed by increasing the SA biosynthetic enzyme activities in seedling of rice during salinity stress condition (Sawada et al. 2006). In addition, SA increases salinity stress adaptation in *Arabidopsis* via reinstating membrane potential and inhibiting K^+ ion loss (Jayakannan et al. 2013). Pretreatment of SA on seedlings of *Arabidopsis* upregulated the H^+-ATPase activity by enhancing K^+ ion accumulation under high salinity condition and by reducing the accumulation of Na^+ (El-Tayeb 2005; Jayakannan et al. 2013). Furthermore, SA improves photosynthesis in salinity condition via increasing antioxidant metabolism and N_2 and sulfur assimilation in different cultivars of mung bean (Nazar et al. 2011). BR may also alleviate the negative results of salinity stress (Anuradha and Seeta Ram Rao 2001; Krishna 2003; Ashraf et al. 2010; El-Mashadand and Mohamed 2012). Exposure of BR increased the antioxidant enzymes activity and non-enzymatic antioxidant compounds (El-Mashadand and Mohamed 2012). Both SA and BRs are abundant in the plant and regulating various biochemical and physiological processes that improve stress tolerance (Ashraf et al. 2010).

5.9 Conclusions and Future Prospects

Plants involve intricate responses at cellular, molecular, physiological, and metabolic levels for tolerance against salinity stress by mediating osmotic regulation, antioxidant metabolism, stress signaling, and hormone metabolism. Researchers are interested to work on the complete profiling of metabolites, proteins, and genes by using proteomic, transcriptomic, metabolomics, and genomic approaches of different plant species involved in salinity stress tolerance. However, due to the lack of information on these approaches, it is important to work on the combined approach, which is essential for the determination of the key pathways and mechanisms involved in controlling salinity stress tolerance. In addition, in spite of the substantial advancement in understanding the stress responses of plants, there is still unclear information about transmembrane ion transport, sensor, and receptor in the signaling transduction. The future focus ought to be on the investigation of intercellular and intracellular molecular interaction for salinity stress responses. Genetic engineering has been shown to be an efficient method for developing salinity stress-tolerant plants, and this approach will become more influential as more applicant genes related to salinity stress tolerance are identified and extensively utilized.

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An Enigma in the Genetic Responses of Plants to Salt Stresses

6

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Abstract

Soil salinity is one of the main factors restricting crop production throughout the world. Various salt tolerance traits and the genes controlling these traits are responsible for coping with salinity stress in plants. These coping mechanisms include osmotic tolerance, ion exclusion, and tissue tolerance. Plants exposed to salinity stress sense the stress conditions, convey specific stimuli signals, and initiate responses against stress through the activation of tolerance mechanisms that include multiple genes and pathways. Advances in our understanding of the genetic responses of plants to salinity and their connections with yield improve-

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ment are essential for attaining sustainable agriculture. Although a wide range of studies have been conducted that demonstrate genetic variations in response to salinity stress, numerous questions need to be answered to fully understand plant tolerance to salt stress. This chapter provides an overview of previous studies on the genetic control of salinity stress in plants, including signaling, tolerance mechanisms, and the genes, pathways, and epigenetic regulators necessary for plant salinity tolerance.

Keywords

Salinity stress · Osmotic tolerance · Tolerance mechanism and signaling pathways

6.1 Introduction

Saline soil conditions are a developing problem for agriculture throughout the world. Accumulation of salt in cultivable soils mainly results from seawater and irrigation water with traces of sodium chloride (NaCl) (Tester and Davenport 2003). Increased soil salt content causes a reduction in water absorption by plants. Plant growth is affected by the uptake of large amounts of both Na^+ and Cl^-, which diminishes photosynthetic productivity and interrupts metabolic processes (Mäser et al. 2002; Deinlein et al. 2014). These responses of plants to salinity depend on acclimation, phenotypic plasticity, and environmental variation (Hasegawa et al. 2000; Debat and David 2001; Zhu 2002; Munns and Tester 2008; Taiz and Zeiger 2010). Acclimation is more likely to be an inherent genetic trait involved in epigenetic responses (Boyko and Kovalchuk 2011; Mirouze and Paszkowski 2011; Sunkar et al. 2012; Oh et al. 2013). Important information on the physiological mechanisms in plant responses to salinity stress has been discovered by studies of plant species native to saline conditions (halophytes) (Flowers et al. 2010) and genetically modified model plants sensitive to Na^+ and Cl^- (glycophyte), especially *Arabidopsis thaliana* (Zhu 2002, 2003; Flowers and Colmer 2008; Munns and Tester 2008). Many identified genes responsible for salt tolerance in halophytes, glycophytes, and crops encode transport determinants that mediate Na^+ homeostasis (Apse and Blumwald 2007; Horie et al. 2009; Craig Plett and Møller 2010; Dassanayake et al. 2011; Pardo and Rubio 2011; Peleg et al. 2011; Munns et al. 2012; Oh et al. 2013).

Salinity stress is divided into an initial osmotic stress phase in plants and an Na^+ accumulation stress phase (Sahi et al. 2006; Munns and Tester 2008), followed by a Cl^- stress phase (Teakle and Tyerman 2010). Plants first alleviate osmotic stress by improving the water retention system, while increasing the amount of water uptake. In the next step, plants minimize the destructive consequences of ionic Na^+ stress by removing Na^+ from the leaves, sequestering Na^+, and transporting Na^+ mostly into the vacuole (Blumwald 2000; Munns and Tester 2008). Despite these tolerance response mechanisms, salinity stress continues to reduce crop yields and the nutritional resources of humans around the world (Nations 2013; Schroeder et al. 2013). The use of genetically modified crops is a promising approach to improve these reductions in yield. This chapter provides an overview of previous studies on the genetic control of salinity stress in plants, including signaling, tolerance mechanisms, and the genes, pathways, and epigenetic regulators necessary for plant salinity tolerance.

6.2 A General View of Salinity Tolerance Mechanisms in Plants

Plants use multiple mechanisms to cope with salinity stress because of the different effects caused by this abiotic stress (Tuteja 2007). Plants commonly use three main mechanisms for salt tolerance: (i) osmotic tolerance, (ii) ion exclusion, and (iii) tissue tolerance (Munns 2005; Munns and Tester 2008). Long-distance signals are responsible for regulating osmotic tolerance, leading to a decrease in shoot growth just before Na^+ accumulation in the shoots of plants (Munns and Tester 2008). Then, the Na^+ and Cl^- transportation process as part of ion exclusion in plant roots results in a decrease in the accumulation of Na^+ and Cl^- (toxic concentrations) in plant leaves. In the final step, the high concentration of salt found in the leaves is transported at both the cellular level, specifically into the vacuole, and the intracellular level, leading to plant tissue tolerance (Munns and Tester 2008; Fig. 6.1).

Current knowledge about the osmotic phase is lacking. It has been noted that the osmotic phase contains fast and long-distance signaling, probably through a

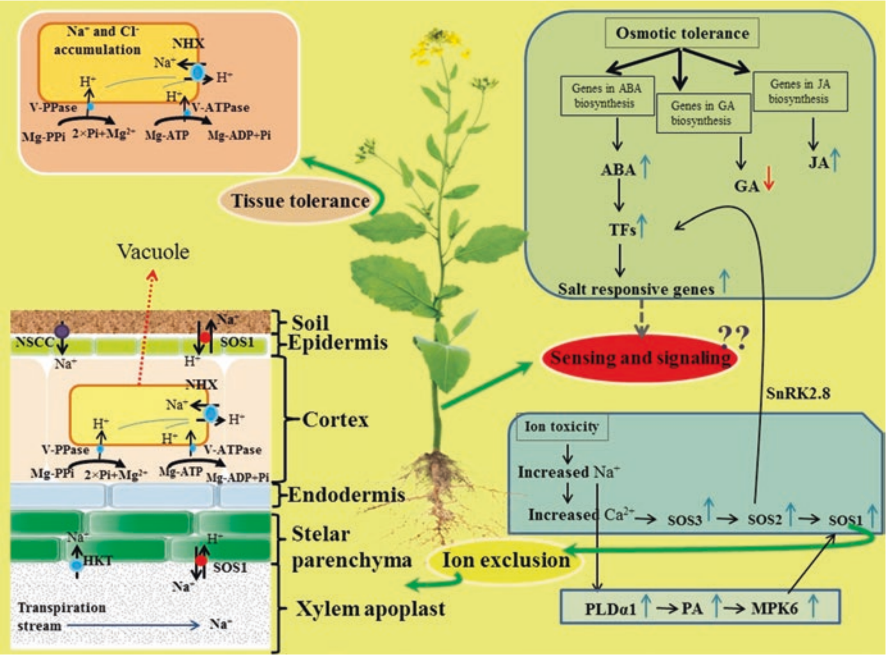

Fig. 6.1 Mechanisms involved in plant salinity tolerance

long-distance electrical signaling process (Maischak et al. 2010) or through reactive oxygen species (ROS) waves (Mittler et al. 2011). There may be different reasons for variation in osmotic tolerance, such as differences in the initial salt perception, differences in long-distance signaling, or differences in the reaction to signals. Due to many unknowns in the field of salinity stress research, more study is needed to understand osmotic tolerance in detail (Fig. 6.1), such as our current understanding of the ionic phase, which is better understood because Na^+ and Cl^- accumulation in leaves can be phenotyped quite easily. The ionic phase helps plants to lessen toxicity by decreasing toxic ion (Na^+ and Cl^-) accumulation in leaf blades and enhancing the plant's ability to bear salts that it could not transport from the shoots by transporting the ions into vacuoles, which is called "tissue tolerance" (Fig. 6.1). It has been reported that a wide range of transporters and proteins that control transport activity are involved in these two processes, acting at both the tonoplast and plasma membrane (Tester and Davenport 2003; Craig Plett and Møller 2010). Tissue tolerance might play a vital role in synthesizing compatible solutes of Na^+ removed from the cytosol and sequestering them in the vacuole to avoid the detrimental effects of these ions on cellular processes. Thus, tissue tolerance involves the control of ion transport as well as related biochemical processes, carrying an important role in osmotic protection and adjustment (Flowers and Colmer 2008; Munns and Tester 2008) (Fig. 6.1). Different plants may utilize either several or few mechanisms for salinity tolerance. There is no evidence so far to indicate that these salinity tolerance mechanisms are mutually exclusive. For example, ion exclusion does not necessarily stop tolerance based on the osmotic phase during salt toxicity. Additionally, any particular plant employs multiple approaches to cope with salinity stress. For instance, at moderate salinity, a plant with ion exclusion as its main tolerance mechanism may demonstrate tissue tolerance as its key tolerance mechanism once the exclusion processes are saturated at higher levels of salinity stress. It seems that some salinity tolerance mechanisms may be more efficient under certain conditions. For example, it has been reported that Na^+ exclusion at a higher salinity level is probably more efficient for plants (Munns et al. 2012), whereas osmotic tolerance may be more effective at a moderate salinity stress level. Other abiotic stresses, such as water deficit, may have consequences in interaction with saline stress. It has been reported that salinity tolerance is complex and involves a multigenic system (Dewey 1962; Flowers and Yeo 1995), leading to frequent failures in traditional breeding programs designed to precisely introgress salinity tolerance. Therefore, it seems that carrying out more research on the mechanisms that are theorized to contribute to salinity tolerance is more important than studying the molecular genetic basis contributing to salinity tolerance as a specific trait in itself. Among these traits, Na^+ exclusion from plant leaf blades has been widely studied due to its direct impact on plant phenotypes. Hence, significant improvements in salinity tolerance of durum wheat (with poor Na^+ exclusion) have been obtained as measured by yield while growing under high salinity via focusing on Na^+ exclusion (Dewey 1962; Flowers and Yeo 1995; James et al. 2012; Munns et al. 2012). Therefore, trait modification should be associated with variances in tolerance, comparing yields (performance in the field) under high and low saline conditions. Allowing different traits to be quantified and measured by manipulating experimental conditions, as described above for achieving osmotic tolerance, and examining the effects of the trait being screened on salinity tolerance in field conditions are key.

6.3 How Plants Sense Salt Stress

Abiotic and biotic stresses are sensed by different plant sensors and stimulate complex signaling pathways including plant hormones, signal transducers, and transcriptional regulatory factors (Gilroy et al. 2014; Zandalinas et al. 2018). These signaling pathways activate stress-responsive genes that have arisen from the adaptation of plant tolerance against specific stresses (Casaretto et al. 2016). The complicated signaling pathways that control physiological, developmental, and biochemical variations during salt stress, as well as the expression of salt-responsive genes that help plants survive, are not fully understood. Nonetheless, recent studies have highlighted the involvement of salt networks, processing, and transferring mechanisms in the transduction pathway (Deinlein et al. 2014). The three main signaling pathways that make up the main transduction pathway throughout salinity stress include a calcium-dependent signaling pathway, an oxidative/osmotic stress signaling pathway, and the calcium-dependent signaling of the salt overly sensitive (SOS) pathway. The calcium-dependent signaling pathway activates the dehydration responsive element/C-repeat (DRE/CRT) (stress-responsive genes) or late embryogenesis abundant (LEA)-like proteins. In addition, the oxidative/osmotic stress signaling pathway plays a role in mitogen-activated protein kinase (MAPK) cascades that are involved in the production of antioxidants and compatible osmolytes and regulation of the cell cycle. Furthermore, the SOS-calcium-dependent signaling pathway regulates ion homeostasis (Zhu 2001). Root cells have an early reaction to changes in osmotic and ionic balances caused by increased internal and/or external concentrations of salt. Only seconds are needed for plants to respond to salt stress, as plasma membrane-localized proteins accompanied by other receptors function as sensors that recognize salinity stress signals (Tracy et al. 2008). Plasma membrane-resident proteins activate the defense responses of plants against salinity stress by detecting and conveying stress signals and consequently triggering a signal transduction pathway (Scandalios 2005; López-Pérez et al. 2009). Subsequently, secondary messengers, such as calcium, reactive oxygen species (ROS), and inositol phosphate, are activated as results of stress signal detection and transduction. Therefore, the initial response of plants is an increase in the extracellular (tonoplast) and intracellular (cytosol) concentrations of calcium (Ca^{++}) once signals are perceived at the plasma membrane (Moore et al. 2002; Tracy et al. 2008). The extracellular level of Na^+ alters the flux of Ca^{++} into the cytosol, which is additionally modified by secondary messengers (Tracy et al. 2008). These disturbances in Ca^{++} flux in the cytosol are detected by Ca^{++}sensors (specific Ca^{++}-binding proteins). These Ca^{++}-binding proteins undergo conformational changes in Ca^{++}-dependent pathways, as demonstrated by deficiencies in some enzymatic activities (Luan et al. 2002). In *Arabidopsis*, expression of aequorin, a Ca^{++}-binding protein, has been observed to increase Ca^{++} flux in root cells during salt stress (Kiegle et al. 2000). Ca^{++}-binding sensory proteins in association with their allied components trigger phosphorylation cascades. Phosphorylation cascades regulate the expression of major stress-responsive genes through the activation of transcription factors. The expression of stress-responsive genes helps plant cells survive the stress (Cheong et al. 2003). Therefore, plants respond to stresses in two ways: as single cells and synergistically as intact organisms. Stress-responsive genes can be categorized as early stress-induced genes, which are activated promptly after sensing stress signals (for a short time), and late stress-induced genes, which are expressed several hours after receiving stress signals (with constant expression) (Gilroy and Trewavas 2001; Chaves et al. 2009). Activation of early-stress induced genes, which include numerous transcription factors, does not depend on new protein synthesis as a prerequisite (Gilroy and Trewavas 2001). However, antioxidants, late LEA-type proteins, osmolytes, and proteins that are related to membrane stabilization are classified as late stress-induced genes (Chaves et al. 2009). Recent advances in understanding plant-stress signaling and compatible responses have verified that multiple factors are involved in the initiation of plant responses to salt stress. Signaling responses of plant to stresses act as an individual signaling factor that either activates or deactivates the next signaling factor. Signal transduction of salinity stress contains osmotic and ionic homeostasis signaling pathways together with growth-regulation pathways. The signals of the adaptation cascade principally contain hormonal regulatory pathways (ABA and GA signaling pathways) and toxicity signaling pathways (SOS and calcium signaling pathways) (Fig. 6.1). These hormones work as signal transducers, intensifying earlier signals and triggering a new phase of signaling that may involve the same signaling pathway or even involve other of transduction pathways (Xiong and Zhu 2002; Mahajan and Tuteja 2005). Molecules that are not directly involved in signal transduction pathways but play important roles in changing and controlling signaling components are known as accessory molecules (Zhu 2002).

6.3.1 Transcriptional Regulatory Factors Contributing to Plant Salinity Tolerance

Gene expression regulation in response to salinity stress consists of a wide range of mechanisms and pathways that regulate the production of specific gene products, either proteins or RNA (Cheong et al. 2003; Mazzucotelli et al. 2008; Gill and Tuteja 2010). Gene expression regulation has been observed in studies of the initial transcriptional response, RNA processing, and post-translational changes to proteins (Mazzucotelli et al. 2008). Many transcription factors (key regulators of gene expression) and tolerance genes that are up-/downregulated in plants in response to salinity stress have been identified and characterized via transcriptomics and genomics approaches (Ma et al. 2006; Mantri et al. 2007; Fernandez et al. 2008). Many stress-related genes can be found among transcription factor family genes, such as *bZIP*, *WRKY*s, *AP2*, *C2H2 zinc finger (C2H2ZF)*, *NAC*, and dehydration-related transcription factors (*DREB*) (Lata et al. 2011) (Table 6.1). Transcription factors control the

Table 6.1 Genes and transcription factors differentially expressed under salt stress in plants

Genes	Function(s)	Species	References
AtSKIP	Transcription factor, transcriptional pre-initiation, splicing, and polyadenylation	*Arabidopsis thaliana*	Lim et al. (2010)
JcDREB	Transcription factor	*A. thaliana*	Tang et al. (2011)
Annexin 1 (AnnAt1)	A member of a multigene family of Ca^{2+-} dependent membrane-binding osmotic stress-responsive proteins	*A. thaliana*	Lee et al. (2004)
AtALDH3 (aldehyde dehydrogenase)	Maintain membrane integrity under salinity stress	*A. thaliana*	Sunkar et al. (2003)
PRP	Proline-rich proteins and cell-wall protection; senescence-associated genes, regulatory processes, and cellular signal transduction; heat-shock proteins, protein stabilizing	*Oryza sativa*	Roshandel and Flowers (2009)
SAG			
HSPC025			
OsHSP23.7	Heat-shock proteins, molecular chaperones, folding, assembling, and transporting proteins	*O. sativa*	Zou et al. (2009)
OsHSP71.1,			
OsHSP80.2			
OsHsp17.0,	Heat-shock proteins, molecular chaperones, and folding, assembling, and transporting proteins	*O. sativa*	Zou et al. (2012)
OsHsp23.7			
SOS1	Plasma membrane Na+/K+ antiporter; protein kinase; calcium-binding protein; vacuolar Na+/K+ antiporter	*Brassica juncea* and *B. campestris*	Chakraborty et al. (2012)
SOS2			
SOS3			
AtNHX1			
DcHsp17.7	Cell viability and membrane stability under heat stress	Carrot	Song and Ahn (2011)
TaODORANT1 (R2R3-MYB)	Keep lower Na^+ accumulation in leaves	Wheat	Wei et al. (2017)

expression of stress-related genes by binding to the *cis*-acting components in their promoters (Azizi et al. 2016). Overexpression of *bZIP* genes has been reported in salt-treated wheat plants susceptible to salinity stress, whereas they are downregulated in wheat salt-tolerant varieties (Johnson et al. 2002). Moreover, upregulation of *NAC* genes in both salt-treated rice and wheat plants confirms the importance of transcription factors in plant salinity tolerance (Nakashima et al. 2007).

Several transcription factors have been reported to be involved in tolerance to abiotic stresses in rice, including *DREB1/CBF*, *DREB2*, and *AREB/ABF* (Ito et al. 2006; RoyChoudhury et al. 2008; Fujita et al. 2011; Mizoi et al. 2012; Fujita et al. 2013), and many more have been shown to play roles in salinity stresses, such as *OsNAC5* and *ZFP179* (Song et al. 2011). It is assumed that the transcription factors *OsNAC5* and *ZFP179* are involved in salt-tolerance responses of plants through the regulation of proline, sugar, and LEA protein synthesis and accumulation (Song et al. 2011). In *Arabidopsis*, *AtWRKY8* is a transcriptional regulatory element that directly binds to the *RD29A* gene and modulates its expression. High expression of *AtWRKY8* under salinity stress has been previously reported (Hu et al. 2013). Another transcription factor, *SALT-RESPONSIVE ERF1 (SERF1)*, has been shown to be involved in salinity tolerance in rice. *SERF1* expression results in the specific induction of genes encoding members of a MAPK cascade in the roots of rice in response to salinity and H_2O_2 stresses (Schmidt et al. 2013). The *SERF1* controls the expression of itself, as well as *MAP3K6*, *MAPK5*, *DREB2A*, and zinc finger protein 179 (ZFP179) by binding to their promoters (Schmidt et al. 2013). These researches also showed that plants with *SERF1* suppression are more susceptible to salinity stress compared with control plants.

Several transcription factors regulated by kinases have been found to be major elements involved in plant resistance against salt stress. For instance, a receptor-like kinase (RLK) encoded by *OsRMC* in rice is reported to be a negative controller of salinity stress responses (Serra et al. 2013). Two AP2/ERF family members, *OsEREBP1* and *OsEREBP2*, negatively regulate the expression of *OsRMC* through a GCC-like DNA motif in its promoter. It is assumed that *OsEREBP2* plays a central role in regulation of plant responses to abiotic stresses because it shows higher induction of expression compared with *OsEREBP1* after various treatments (Serra et al. 2013). Similarly, *OSBZ8*, a bZIP ABRE-binding transcription factor, has been shown to be expressed more highly in salt-resistant rice cultivars compared with susceptible cultivars (Mukherjee et al. 2006). Further study of *OSBZ8* has revealed that this transcription factor is activated and phosphorylated using an SNF-1 class of serine/threonine kinase accompanied with Spd under salt stress (Mukherjee et al. 2006).

6.3.2 Plant Salt Tolerance Genes

Numerous genes and transcription factors with different functions are upregulated under salinity stress in various plant species (Table 6.1). Salt-responsive genes are categorized into four main groups: (i) heat-shock proteins (HSPs)/molecular chaperones (such as *HSP* genes), (ii) ion transport or homeostasis genes (such as *SOS* genes, *AtNHX1* and H^+*-ATPase*), (iii) senescence-associated genes (such as *SAG*), and (iv) dehydration-related transcription factors (such as DREB) (Gupta and Huang 2014).

6.3.2.1 Heat-Shock Proteins (Hsps)/Molecular Chaperones

Molecular chaperones are key elements that contribute to cellular homeostasis in both ideal and divergent growth conditions by assisting in protein folding, assembly, transport, and degradation and are involved in a wide range of cellular processes. They are also responsible for stabilizing proteins and membranes as well as assisting in protein refolding under stress conditions. A wide range of molecular chaperones are stress proteins, and many are heat-shock proteins (Hsps) (Morimoto et al. 1997; Kim and Yenari 2017). There are five main categories of Hsps/chaperones: the chaperonins (GroEL and Hsp60), the Hsp70 (DnaK) family, the Hsp90 family, the Hsp100 (Clp) family, and the small Hsp (sHsp) family (Huang and Xu 2008). Along with these main families, there are several proteins (e.g., disulfide isomerase and calnexin/calreticulin) with chaperone functions that contribute to protein folding in the endoplasmic reticulum (ER) (Kleizen and Braakman 2004). The Hsps/chaperones are localized to the cytoplasm and organelles (the nucleus, chloroplast, mitochondria, and ER) (Trivedi et al. 2016), where they bind to specific non-native substrates and sites. These molecular chaperones are not covalently bonded to their targets and are not included in the final product. It has been confirmed that Hsps/chaperones play critical roles in protecting plants against stresses and reestablishing cellular homeostasis (Bukau and Horwich 1998). Many Hsp 70 proteins are well-known to contribute to salt tolerance in plants (Wahid et al. 2007; Tang et al. 2016). At least 18 genes encoding Hsp70 family members are present in the genome of *Arabidopsis*, of which 14 belong to the DnaK and four to the Hsp110/SSE subfamilies (Wahid et al. 2007). Hsp70 genes positively impact salinity, drought, and heat tolerance of plants (Wang et al. 2004; Leng et al. 2017), but the cellular mechanisms of their function in stress response are not clearly defined. Transfer of a Group II chaperonin, CCTα, from mangrove plants to *E. coli* enhanced salt and osmotic-stress tolerance (Yamada et al. 2002). Hsp90 (and its signal transduction protein substrates that include steroid hormone receptors and signaling kinases), Hsp100/Clp genes (members of the large AAA ATPase superfamily), and sHsps are constitutively expressed in almost all organisms, and the expression of these chaperones is increased in response to wide ranges of stresses in both eukaryotes and prokaryotes (Agarwal et al. 2001; Krishna and Gloor 2001; Guo et al. 2016). For instance, the expression of Hsp90 and Hsp100/Clp genes is increased in response to salt, heat, cold stress, and light and dark alteration in *Arabidopsis* (Milioni and Hatzopoulos 1997; Queitsch et al. 2000; Agarwal et al. 2001; Krishna and Gloor 2001). In addition, mitochondrial electron transport is increased in maize cells expressing mitochondrial sHsps, which mainly occurs through activation of NADH:ubiquinone oxidoreductases (Complex I), where the association of these enzymes with Complex II is disrupted (Hamilton and Heckathorn 2001). Hsp90 performs its roles in a multi-chaperone machinery accompanied by Hsp70 and co-chaperones. This complex contains Hip as the Hsp70-interacting protein, Hop as the Hsp70/Hsp90 forming protein, p23 and Hsp40 as DnaJ homologs, two immunophilins FKBP51/54 and FKBP52, and Cdc37/p50 (Breiman 2014). *GmHop-1* was isolated from soybean under normal (with constitutive expression) and stress conditions (with an increased level of expression) and found to be a co-chaperone homolog of the Hop protein in mammalian systems (Zhang et al. 2003). In rice, overexpression of *OsHSP90-2* and *OsHSP90-4* is observed under cold, heat, drought, and salt stresses (Zhang et al. 2016). Moreover, an *E. coli* line expressing *OsHSP90-2* was established and found to be resistant to drought, high salinity, and heat stresses (Zhang et al. 2016). Similar variations have been observed in the expression levels of HSPs and HSFs in response to drought, salinity, and heat stress in tomato (Fragkostefanakis et al. 2015).

(a) Transcriptional Regulators of Plant Hsps/Chaperones Under Salinity Stress

Heat-shock factors (HSFs) are the main inducers of Hsps/chaperones and are classified into three groups: A, B, and C (Nover et al. 2001; Scharf et al. 2012; Guo et al. 2016). They are present in eukaryotes and in plants in high numbers (38 in soya bean (*Glycine max*), 25 in rice (*Oryza sativa*), and 21 in *Arabidopsis* (*Arabidopsis thaliana*)) (Fujimoto and Nakai 2010). Due to this diversity, studying the HSF family in plants is difficult, but the analysis of their sequences and expression patterns reveals both diverse and overlapping purposes in stress resistance (von Koskull-Döring et al. 2007). All HSFA1 genes are involved in osmotic stress tolerance, including HSFA1d and HSFA1e (Nishizawa-Yokoi et al. 2011; Yoshida et al. 2011). An *Arabidopsis* quadruple mutant, *Hsfa1a*, *b*, *d*, and *e*, is sensitive to salinity stress and has a deficiency in intact seed production (Bechtold et al. 2013; Liu and Charng 2013). However, this deficiency is moderately and totally complemented by upregulation of *HSFA2*, a target of HSFA1d and e (Nishizawa et al. 2006; Ogawa et al. 2007). In addition to HSFA1, many other HSFs are involved in salinity stress signaling, and their expression can be induced by ABA-dependent and ABA-independent signaling pathways (Huang et al. 2012). In addition, overexpression of *HSFA2* using the E12 promoter improves the resistance of plants to osmotic, heat, and salt stress (Ogawa et al. 2007). Furthermore, the expression of *HSFA3*, a target of DREB2A, and HSFA4a is triggered by several stresses including cold, heat, salt, and drought and even by oxidative stresses, wounding and UV-B light (Winter et al. 2007; Yoshida et al. 2008; Pérez-Salamó et al. 2014). However, overexpression of tomato *SIHSFA3* in *Arabidopsis thaliana* resulted in plants with heat tolerance and salt sensitivity (Mishra et al. 2002). The specific induction of *AtHSFA6a* and *AtHSFA6b* under salt, ABA, osmotic, and drought stress has been confirmed (Hwang et al. 2014; Huang et al. 2016). The presence of two ABA-responsive elements (AREs) and binding of three ABA-responsive transcription factors (AREB1, ABF3, and AREB3) have been observed in the promoter of *AtHSFA6a* (Hwang et al. 2014). Additionally, there are several ABREs in the promoter of *AtHSFA6b* that may be bound by *AREB1*. The specific roles of these genes as response amplifiers and in stress adaptations make them potential candidates for genetic engineering of plants to improve resistance.

6.3.2.2 Ion Transport and Homeostasis Genes

The regulation of Na^+ transport within plants is markedly influenced by the *high affinity potassium transporter* (*HKT*) gene family (Munns and Tester 2008; Horie et al. 2009; Hauser and Horie 2010; Ali et al. 2012) and the salt overly sensitive (SOS) pathway (Mahajan et al. 2008; Weinl and Kudla 2009; Kudla et al. 2010; Ji et al. 2013). To date, many studies have shown that Na^+ accumulation in plant shoots can be altered by genetic manipulation of these genes that leads to changes in their expression (Feki et al. 2014; Suzuki et al. 2016; Li et al. 2017; Han et al. 2018). Nevertheless, practical use of this information for generating improved crops in the field has some limitations. Improvement of salinity tolerance in crops monitored by phenotyping for Na^+ exclusion or salt tolerance in mutants and mapping populations is highly linked to the *HKT1* group of *HKT*s, which appear to be the most likely candidate gene family in related quantitative trait loci (QTL) with a strong potential to improve salinity tolerance in crops (Ren et al. 2005; James et al. 2006; Rus et al. 2006).

Marker-assisted selection (MAS) has been reported to be useful for improving saline-stress tolerance of durum wheat through incorporating of HKT alleles across *Triticum monococcum* (James et al. 2012; Munns et al. 2012). In addition, transforming *HKT1s* through transgenic methods to improve salinity tolerance has been reported to be relatively successful (Suzuki et al. 2016; Han et al. 2018). Another study, focusing on *HKT2*, showed that this gene plays an important role in salinity tolerance through a mechanism other than Na^+ exclusion (Mian et al. 2011). The activity of this gene depends on cell-type-specific expression (Møller et al. 2009). Thus, improvement of salinity tolerance in crops may be achievable if cell-type-specific expression and stress inducibility of these genes can be effectively achieved in crops. The SOS gene family plays a fascinating role in ion homeostasis, which is related to salt tolerance (Liu et al. 2000; Shi et al. 2000, 2002); Table 6.1). In numerous plant species, genes involved in ROS scavenging and osmotic homeostasis are upregulated in response to salinity stress. For instance, *glutathione S-transferase* and *ascorbate peroxidase* are two active components of ROS scavenging, and high expression of these genes has been observed in rice plants treated with salt. Similarly, upregulation of metallothionein and water channel proteins has also been detected (Kawasaki et al. 2001).

6.3.2.3 Senescence-Associated Genes

Senescence is a biological aging process that finally leads to the death of the plant. In annual crops, this aging process overlaps with the reproductive phase of plant development. Quick senescence and leaf abscission are two escape strategies plants use to minimize plant canopy size in reaction to various stresses (Kooyers 2015). During the aging process, nutrients are transported from source to reproductive organs, a process that is regulated by complex systems controlling biological and molecular processes in plants (Munné-Bosch and Alegre 2004). Although the processes related to plant senescence are well-characterized, the molecular mechanisms regulating stress-induced aging are not well-understood. Many transcription factors, including WRKY, NAC, MYB, bZIP, and AP2/EREBP families, have been reported to be involved in the regulation of aging (senescence)-related genes in various plants, including rice (Liu et al. 2008), *Arabidopsis* (Breeze et al. 2011; Woo et al. 2013), wheat (Gregersen and Holm 2007), barley (Parrott et al. 2007), *Medicago truncatula* (De Michele et al. 2009), and aspen (Anderson et al. 2004). As described previously, these transcription factors are involved in regulating stress-responsive genes. For instance, delayed senescence has been observed in *anac092* (a member of *NAC* transcription factor family) knock-down plants. Additionally, Balazadeh et al. (2008) observed that in comparison with two other stresses, drought and wounding, salinity stress affected the expression levels of transcription factor genes and senescence-associated genes (*SAGs*). It can be concluded that transcription factors are elements in the regulation of salinity stress-induced plant senescence. Furthermore, modifications of the plant hormonal balance and hormonal crosstalk play roles in stress-induced senescence. Two plant hormones, cytokinin (CK) and ABA, show antagonistic effects on leaf senescence (Yang et al. 2003; He et al. 2005; Verslues 2016). Decreased concentrations of bioactive CKs have been observed in the plant leaves under water shortage and salt stress (Buchanan-Wollaston 1997; Havlov et al. 2008). Additionally, degradation of chlorophyll and disassembly of the photosynthetic machinery can be postponed by exogenous application of CK (BADENOCH-JONES et al. 1996). In contrast, ABA promotes leaf senescence in plants such as rice (Ray et al. 1983; Aneja et al. 1999), reduces chlorophyll content, and affects the expression of nuclear and chloroplast photosynthesis-related genes in barley (Yamburenko et al. 2013). The *OsNAC2* transcription factor regulates the expression of ABA-dependent genes and ABA biosynthesis, stimulating leaf senescence through the activation of chlorophyll degradation-related genes, which leads to increased plant sensitivity to stress conditions (Mao et al. 2017; Shen et al. 2017). Ethylene, having antagonistic effects on ABA action, is also synthesized under biotic and abiotic stresses and is associated with stress-induced senescence (Anderson et al. 2004; Kazan 2015). Therefore, any interruption in ethylene biosynthesis and/or signaling impacts plant stress tolerance.

The association of stress-induced senescence, chlorophyll metabolism, and chloroplast stability has been confirmed by the discovery of the *STAYGREEN* (*SGR*) genes (Sakuraba et al. 2014). Stay-green refers to the postponed foliar senescence and persistent leaf greening observed in mutant plants (Thomas and Ougham 2014). In plant stress responses, SGR morphologies mostly arise from changes in chlorophyll metabolism, including late chlorophyll breakdown or over-production of chlorophyll (Hörtensteiner and Kräutler 2011; Jagadish et al. 2015). In addition, leaf senescence and chlorophyll degradation are significantly regulated by SGR-related genes (such as *SGR2* in *Arabidopsis*) under biotic and abiotic stresses (Sakuraba et al. 2014). Knocking down *NONYELLOWING1* (*NYE1*) and *STAYGREEN-LIKE* (*SGRL*) gene expression in *Arabidopsis* leads to persistence of green color in plant leaves under drought, high salinity, heat, and light stresses. Therefore, these genes are certainly involved in the mechanism of stress-induced senescence (Ren et al. 2007; Sakuraba et al. 2014). Chloroplasts are the dominant sources of nutrient remobilization throughout senescence (Diaz-Mendoza et al. 2016), and the main sink of chloroplast products is proteolysis in the plant central vacuole, which is accomplished through several vesicular trafficking pathways (Zhai et al. 2016). Autophagy, as with vesicular trafficking pathways, has been assumed to specifically transfer stromal proteins to the vacuole during senescence (Xie et al. 2015). Autophagy genes have been verified to be upregulated in plants in response to abiotic stresses (Li et al. 2015; Wang et al. 2017), which means downregulation of autophagy genes negatively affects plant abiotic stress response and resistance. In addition, all autophagy mutant plants undergo leaf senescence much sooner than control plants in response to stress conditions, ultimately ending with their death (Xiong et al. 2005; Avila-Ospina et al. 2014; Zhai et al. 2016). After autophagy, the second pathway for chloroplast degradation is controlled by proteolytic senescence-associated vacuoles (SAVs), which play a role in the trafficking of stromal proteins and age-induced chloroplast degradation (Otegui et al. 2005; Matsui et al. 2008). Chloroplast vesiculation (CV), the third pathway for chloroplast degradation, was identified in recent years and is induced under various abiotic stresses, including salt, drought, oxidative, and light stresses (Wang and Blumwald 2014). Chloroplast degradation products obtained from stromal and thylakoid proteins are present in CV-containing vesicles (CCVs), which traffic proteins from the chloroplast to the vacuole. Plants with higher *CV* expression show earlier senescence and chloroplast degradation, whereas knocking down *CV* results in plants with enhanced tolerance to abiotic stress (Wang and Blumwald 2014).

6.3.2.4 Dehydration-Related Transcription Factors

*Dehydration-related transcription factors (DREB*s*)* are members of the ethylene response factor (ERF) family (transcription factors). They bind to the dehydration responsive element DRE/CRT in the promoter region of many abiotic and biotic signaling genes in plants. In the last decade, comprehensive knowledge on the regulatory roles of DREBs in plant responses to the abiotic stresses has been attained. Specifically, the ERF proteins are members of the APETLA2 (AP2)/ethylene-responsive element binding proteins (EREBP). This ERF family contains two subfamilies, including EREBP with single AP2 domain and AP2/family with double AP2 domains. The EREBP protein subfamily is divided into two clusters, ERFs and DREBs/CBFs. The ERFs bind to the GCC box of pathogenesis-related (PR) gene promoters and confers ethylene sensitivity (Gu et al. 2000). In contrast, DREBs/CBFs bind to the DRE/CRT promoter of cold and *dehydration-responsive LEA* genes (Yamaguchi-Shinozaki and Shinozaki 1994; Kasuga et al. 1999). DREBs/CBFs are classified into two subgroups, including DREB1/CBF (induced by cold) and DREB2 (induced by dehydration stresses) (Agarwal et al. 2017).

Although these genes are induced by cold and dehydration stresses separately, their expression has been observed in the complex response of plants to abiotic stresses. The expression of *DREB1A* and *DREB2A* is induced by cold stress and by dehydration and salt stresses, respectively, in *Arabidopsis* (Liu et al. 1998). In addition, *BnCBFs 5*, *7*, and *16* were found to be induced under cold stress (Gao et al. 2002), whereas *BjDREB1B* was induced by salt, drought, and low temperature in *Brassica napus* (Cong et al. 2008). Furthermore, cold stress stimulates the expression of *OsDREB1B*, *OsDREB1A*, and *OsDREB1F* in rice, whereas the expression of *OsDREB2A* and*OsDREB1F* is induced in response to salt stress (Dubouzet et al. 2003; Wang et al. 2008). Various DREB homologs from different plants have been shown to be involved in response to abiotic stresses, including salinity (Table 6.2).

6.4 Salinity Responses of Plants via Shoot Tissue Tolerance

Three mechanisms have been shown to be involved in shoot tissue tolerance, including (i) Na^+ accumulation in the vacuole, (ii) compatible solute synthesis, and (iii) reactive oxygen detoxification by catabolic enzymes (Bhowmick 2012). Different success rates for improving salinity tolerance of crops have been observed depending on the abundance of AVP1, one of vacuolar H^+ pyrophosphatases; NHX, a vacuolar Na^+/H^+ antiporter (Ashraf and Akram 2009); enzymes responsible for detoxifying ROS (Das and Roychoudhury 2014); and some amino acids such as

Table 6.2 DREB/DREB homologs identified in various plant species

Plant species	Gene	Stress stimulation	References
Atriplex hortensis	*AhDREB1*	Salt	Shen et al. (2003)
Glycine max	*GmDREBa, GmDREBb, GmDREBc*	Salt, drought, cold	Li et al. (2005)
Brassica juncea	*BjDREB1B*	Drought, salt, low temperature	Cong et al. (2008)
Gossypium hirsutum	*GhDBP2*	Drought, salt, low temperature	Huang and Xu (2008)
Oryza sativa	*OsDREB2A*	Salt, dehydration	Dubouzet et al. (2003)
Oryza sativa	*OsBZ8*	Salt	Mukherjee et al. (2006)
Oryza sativa	*OsDREB1F*	Salt, drought, cold	Wang et al. (2008)
Hordeum vulgare	*DREB2*-type *HvDRF1*	Drought, salt	Xue and Loveridge (2004)
Triticum aestivum	*TaDREB1*	Low temperature, salinity, drought	Shen et al. (2003)
Zea Mays	*ZmDREB2A*	Cold, dehydration, salt, heat	Qin et al. (2007)
Physcomitrella patens	*PpDBF1*	Salt, drought, cold	Liu et al. (2007)
Pennisetum glaucum	*PgDREB2A*	Salt, dehydration	Agarwal et al. (2007)

proline and glycine betaine that are responsible for the synthesis of compatible solutes (Roy et al. 2014). Expression of *NHX*, as well as vacuolar pyrophosphatase genes, appears to play important roles in salinity tolerance in plants; however, it is not clear which ions may be transported by NHX proteins (Bassil et al. 2011a, 2011b; Barragán et al. 2012) (Fig. 6.1). Nevertheless, it has been shown that these proteins are potentially important for the transport of Na^+, H^+, and K^+ (Barragán et al. 2012). Despite many successful attempts to improve crop tissue tolerance via enzymes active in the metabolism of reactive oxygen and the synthesis of compatible solutes, transgenic lines are often reported to have low performance in low stress conditions (Cortina and Culiáñez-Macià 2005; Hmida-Sayari et al. 2005; Suárez et al. 2009). It has been reported that these negative impacts might be avoided by using tightly regulated “stress inducible promoters” (Sheveleva et al. 1997; Su and Wu 2004; Vendruscolo et al. 2007). There have been many successful reports of enhancing salinity tolerance of plants via improving tolerance of shoot tissues, mainly through improving Na^+ accumulation in vacuoles (Shi et al. 2003; Xue et al. 2004), so this method might be considered an ideal mechanism to improve crop performance. However, because of inadequate field trial and quantitative data, we are still a long way from achieving this goal.

Salinity tolerance is affected by HKT1 proteins because they recover Na^+ from the xylem in plant roots (Byrt et al. 2007; Horie et al. 2009; Hauser and Horie 2010; Xue et al. 2011). However, this process only occurs with genes encoding transporters that are involved in Na^+ transport through the plasma membrane, and these genes cannot be constitutively overexpressed, unlike transporter genes involved in ion-tissue tolerance that are responsible for Na^+ transportation through the vacuole. The fundamental effect of Na^+ transport through the plasma membrane on the total ion accumulation in plants depends on the cell type where transportation occurs (Tester and Davenport 2003). Enhanced Na^+ influx within stellar cells and recovery of Na^+ from root xylem are an undesired phenotype in root epidermal cells, where Na^+ influx should essentially be reduced. Currently, the cell-type-specific expression that would be essential for effective manipulation of transporter gene(s) that control the processes of ion exclusion has not been achieved in most crops.

6.5 Plant Responses to Salinity Stress Through Osmotic Tolerance Mechanism

Our knowledge about candidate genes involved in osmotic tolerance is limited. Controlling cell cycles and processes relating to perception of signals coming into shoots from the roots and long-distance signaling are more likely to be the main drivers of alterations in osmotic tolerance. For several reasons, including that crop plants are often exposed to low salinity levels during the growing season and that salinity levels start low at the beginning of growing season and build up at the end of season, genes involved in osmotic tolerance traits are significantly more effective for crop salinity tolerance than genes that control ion exclusion. It has been reported that transferring the *TmHKT1;5-A* gene into the Tamaroi (durum wheat) from *T. monococcum* leads to considerable improvement of grain yield in both field grown durum under salinity stress via enhancing ion exclusion and pots with extremely saline soils (James et al. 2012; Munns et al. 2012). However, the yield obtained from Tamaroi without TmHKT1;5-A was similar to the yield of the Tamaroi cultivar with the introgressed gene under moderate as well as low salinity conditions (James et al. 2012; Munns et al. 2012), suggesting that ionic stress has a lesser impact on the yield of plants grown under low to moderate salinity conditions, compared to osmotic stress. Therefore, we should prioritize the identification of genes involved in osmotic tolerance for the improvement of crop salinity tolerance in soils with low to moderate saline levels.

6.6 Epigenetic Regulations of Plant Responses to Salt Stress

Gene expression induced by a range of stress signals in plants largely depends on nucleosome histone post-translational modifications and even on DNA methylation. Most of these stimulus-induced modifications are found at the lowest level when the stress is applied. Some of the modifications are persistent, transmitted as “stress memory” and even inherited through mitotic or meiotic cell divisions. Subsequent plant stresses can be avoided through epigenetic stress memory. A comprehensive study of stress responsive epigenomes and transcriptomes will improve our knowledge about stress adaptation in plants.

6.6.1 Epigenetic Changes and Chromatin Remodeling in Plants in Response to Salinity Stress

Regulation of chromatin is an indispensable part of gene modification and genome activity (Kurdistani et al. 2004; Pokholok et al. 2005). The expression of stress-responsive genes is coordinated with changes in histone modifications and DNA methylation to acclimate plants to environmental changes. Several chromatin regulators, specific histone modification sites, and histone modifiers have been identified through genetic and biochemical approaches to regulate major stress-responsive genes. These findings confirm the importance of chromatin regulation in plant responses to various stresses. In fact, epigenetic regulation is a key mechanism involved in many biological processes, such as genome stability, development, chromatin regulation, gene expression, small RNA (miRNA)-mediated regulation, etc. (Grewal and Jia 2007; Feng et al. 2010). In the past decade, several histone modifications, including H3K4me3, H3K9me2, H3K9ac, H3K27ac, H3K23ac, H3K27me3, and H4ac, together with DNA methylation have been shown to be involved in the regulation of gene expression in response to abiotic stresses, such as high salinity, water deficit, and temperature alterations (Kim et al. 2008; Luo et al. 2012). Many histone modifications change quickly to react to environmental stresses, whereas others change slowly, accompanied by modifications in gene expression, to regulate physiological homeostasis and development under abiotic and biotic stresses (Kim et al. 2008, 2012). It is unclear which occurs first, transcriptional or chromatin modifications, and how they are connected.

6.6.2 Epigenetic Histone Modifications of Plants in Salt Stress Reactions

The importance of histone modifications in the salinity responses of plant has been increasingly seen, and three different histone modifications, methylation, acetylation, and phosphorylation, have been identified as having effects on the salinity responses of plants. Histone acetylation is regulated by the antagonistic functions of HAT and HDAC proteins and is usually associated with gene expression. Overexpression of *ZmEXPB2* and *ZmXET1*, two cell wall-related genes, is correlated with greater amounts of H3K9 acetylation in the promoter and coding regions, which are supposed to be essential for high salinity response in maize plants. Because of the high transcription levels of two *HAT* genes, *ZmHATB* and *ZmGCN5,* under salinity stress simultaneously with *ZmEXPB2* and *ZmXET1*, it is assumed that their upregulation is correlated (Li et al. 2014). HAT activity is regulated by a transcriptional adaptor, ADA2b, which is hypersensitive to salt and involved in salinity tolerance in *Arabidopsis* (Kaldis et al. 2011). Nevertheless, mutants in HDAC proteins (such as HD2C), histone deacetylase 19 (HDA19) and histone deacetylase 6 (HDA6), which normally increase histone acetylation, result in hypersensitivity to salt in *Arabidopsis* (Chen et al. 2010; Luo et al. 2012). In *Arabidopsis*, histone deacetylase complex 1 (HDC1), *Arabidopsis* Swi-Independent 3 (AtSin3), HDA6, and HDA19 are activated as an HDAC complex (a functional complex of multiple proteins) that regulates the expression of abiotic stress-responsive genes (Perrella et al. 2013). In addition, upregulation of *RAB18*, *ABA1*, and *ABA3* has been observed in *Arabidopsis hdc1* mutants, but the expression of *RAB18*, *ABA1*, and *ABA3* is suppressed by overexpression of *HDC1* in response to salinity stress (Perrella et al. 2013). Therefore, these findings show that histone modifier complexes, including HATs and HDACs, might be essential for fine adjustment of histone acetylation for plant adaptation and response to high salinity stress. Although various studies on the salt stress responses of plants have revealed connections between histone acetylation modifications and regulation of gene expression, it has generally been an accepted fact that histone methylation interacts with acetylation in plant responses to environmental stress (Asensi-Fabado et al. 2017). Various studies have been carried out to understand the central salt-tolerance mechanisms in plants (Deinlein et al. 2014). A ChIP-seq study of four histone modifications showed that an Na^+ priming treatment alters the epigenomic state of plants, including shortening and fractionation of H3K27me3 islands, whereas other islands (H3K4me2, H3K4me3, and H3K9me2) were only slightly altered (Sani et al. 2013). Continual loss of H3K27me3 results in the suppression of gene expression in these islands and consequently increases the level of HKT1 mRNA and chromatin transition (Sani et al. 2013). In addition, it has been confirmed that shortening and fractionation of H3K27me3 in many genes, including *HKT1*, play a major role in somatic memory triggered by salinity stress (Sani et al. 2013). Fast, transient upregulation of histone H3 Ser-10 phosphorylation, H3 phosphoacetylation, and H4 acetylation followed by stress-related-specific gene expression has been reported in *Arabidopsis* and tobacco (Sokol et al. 2007).

The contribution of DNA methylation to plant responses to stresses is still poorly understood, even though global changes in DNA methylation, hyper- and hypomethylation, in the responses of several plant species to abiotic stresses have been observed (Bilichak et al. 2012; Wang et al. 2014). Nonetheless, demethylation and transcriptional activation of *NtGPDL* (glycerophosphodiesterase-like protein) have been observed in tobacco in response to oxidative stresses (Choi and Sano 2007). Furthermore, decreased DNA methylation and inhibited transcriptional activation of specific salt stress-related genes encoding transcription factors have been observed in soybeans under salinity stress treatment (Song et al. 2012). Thus, DNA methylation and modification may play critical roles in the transcriptional regulation of stress-responsive genes in plants.

6.7 Conclusions and Future Prospects

The challenges of salinity stresses for plant development remain and should be alleviated. Salt stress has detrimental impacts on plants in different ways as described above, but a comprehensive understanding of tolerance mechanisms is still lacking. For plants to respond to salt stress, they activate diverse signaling pathways that give rise to salt tolerance traits (osmotic tolerance, ion exclusion, and tissue tolerance) through the genes controlling these traits. Many studies have reported either upregulation or downregulation of genes involved in various tolerance pathways that help plants survive high-salinity stress. In the last decade, many studies have focused on the involvement of epigenetic mechanisms in plant responses to salt stress. However, information is still incomplete, and promising results should be considered by plant breeders and biotechnologists for plant improvements.

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Application of Microbial Biotechnology in Improving Salt Stress and Crop Productivity

7

Maneesh Kumar and Mohd Sayeed Akhtar

Abstract

Soil salinity is the principal detrimental abiotic stress that globally impedes crop yield. It affects a wide range of biochemical, morphological, physiological, and molecular changes and is responsible for inducing ion toxicity, hormonal disturbance, water uptake, homeostasis disturbance, and oxidative stress. To evade this abiotic stress, many genes are identified, and their mechanisms have been elucidated in *Arabidopsis thaliana* through the transgenic approaches and also in other plants like *Prunus cerasifera*, *Brassica juncea*, *Ipomoea batatas*, tobacco, etc. Modern tools revolutionized microbial biotechnology by providing a better choice for plant scientists to select or incorporate genes of interest into preferred species or cultivars. Transgenics may regulate the various metabolic pathways including biosynthesis of chlorophyll and osmolyte, ion exchange homeostasis, antioxidant defense mechanism, and additional frontier defense corridors against salinity stress. Exclusively using such gene manipulations, many genetically modified crop varieties like canola, cotton, maize, rice, and soybean are being developed. Many techniques have been introduced for establishing possible sustainability against soil salinity. Apart from this, it also incorporates some receptor genes in crop plants that may sense or escape any changes in soil salinity under environmental condition. Thus, the aim of this chapter is to enlighten the basic importance and modern application of microbial biotechnology to understand the behavior of transgenic crop plants in saline soil. The study also elaborates understanding of molecular machinery for healthy crop production.

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7.1 Introduction

Diversity in an agricultural environment plays a sustainable approach to improve the livelihood of farmers in their communities. Any loss in diversity reduces farmers' capacities to deal with severe abiotic factors including temperature, drought, and salinity. Among these stresses, salinity causes a major problem in growth and productivity of crop plants. It is a crucial limiting factor in plant development as many of them are highly prone to salinity (Hasanuzzaman et al. 2013; Shukla et al. 2017). Terribly, the stress could also affect the qualitative features of agricultural crops (Waśkiewicz et al. 2016). It occurs mainly due to a high concentration of suspended salt ions in agricultural soils (Shrivastava and Kumar 2015). The high concentration of salt ions eventually leads to unbalance of various physiological and molecular mechanisms of many essential food crops (Wang et al. 2013b). It also measures the presence of water extract in soils (Rengasamy 2010; Tavakkoli et al. 2010).

Dryland salinity is another major issue that causes land degradation and low crop productivity in some parts of the world, especially in southern Australia (Tester and Davenport 2003; Munns et al. 2006). Under such situation, high plant yield is always possible through multifarious sustainable approaches. There will be sufficient increase in food demand for an increasing world population at the end of 2050, which is one of the major challenging issues (Gilbert et al. 2010). Many modern and advanced agricultural practices need influential focus on the use of arable farmland (Coleman-Derr and Tringe 2014; Alptekin et al. 2017). An approach is being developed to utilize unused land in arid regions where water availability is limited (Coleman-Derr and Tringe, 2014).

Frequent watering with high-salt water obstructs efficiency of plants to grow at quantitative and qualitative levels. Also, water scarcity and other unpredictable environmental stresses are compounded with salinity and drought is predominantly reducing the soil fertility and crop productivity (Coleman-Derr and Tringe 2014; Thao and Tran 2016). Irrigated and fertilized lands are substantially influenced by salinity (Munns 2002; Flowers 2004). However, at global priority, vegetable crops including edible herbaceous species (flowers, fruits, leaves, shoots, stems, tubers, and roots) are extensively being produced and consumed. Vegetables and other plant products, even though they are at great risk of salinity, have a significant role in the nutrition and health of humans (Tuna et al. 2007). The high productivity of vegetable crops basically relies on per unit water applied in comparison to the economic value of field crops. It is important for small farmers as they produce vegetable crops in small areas, under intensive procedures. Vegetable crops, which are grown in dry and hot regions with little rainfall, need more attention for they need a larger amount of fertilizers and irrigation facility (Ahmad et al. 2012).

However, a comparatively higher chance of soil and water salinity is closely related to irrigation and fertilization practices (Munns 2002).

Although a significant fertility of agricultural land areas eventually deteriorates with time due to salinity, there has been increased in the need for salt-tolerant crops to sustain food production for the growing population (Pitman and Läuchli 2002). Salt tolerance might hold its greatest impact on agricultural crops which are grown naturally in soil or subsoil salinity in the arid and semiarid regions (Abrol et al. 1988). Salinity tolerance comes from the molecular behavior of genes that limit or regulate salt uptake and its transport, adjustment of osmotic equilibrium of cells which are present in roots and shoots, leaf growth, and the onset of senescence (Munns 2005; Shrivastava and Kumar 2015). The importance of stress-tolerating plants extensively increases in salt-affected areas. However, any loss of salt tolerance should be taken into account to mitigate the detrimental effects in plant growth and improve the global food security by using microbial biotechnology (Dikilitas and Karakas 2014; Lamaoui et al. 2018). Stress could lead to alteration in gene expression in plants on a large scale (Munns 2005; Xu et al. 2015). Significantly, many active genes have been reported in different plant cells and tissues that are highly adapted for specific functions. These genes are importantly expressed in specific cell types. Recently with the use of modern biotechnology (marker-assisted selection or genetic engineering), many salt-tolerant genes have been introduced into elite crop cultivars. Especially, the consequences of incorporation of the exact combination of genes including various traits enable to establish tolerance capacity and crop production against the deleterious effects of soil salinity. Many salt-tolerant known proteins have actively behaved under distinct physiological responses (Roy et al. 2014). Little information about biological mechanisms has also been evaluated by molecular studies of gene expression under salinity tolerance. So, extensive molecular approaches have been developed to integrate foreign gene(s) with desirable behaviors that potentially sustain stress conditions. Experimental approaches have been adopted to develop stress signaling pathways in transgenic plants. New tools and techniques have now been available to understand the genetic performance and the complexity of stress tolerance or responses on large-scale farming through genome-wide expression profiling (Munns 2005; Shahbaz et al. 2012; Shrivastava and Kumar 2015).

Initially, the symptomatic impacts of abiotic stress haven’t been taken seriously, but later these significant physiological changes have been experimentally considered (Sevillano et al. 2009). These stresses pleiotropically limit the crop growth and productivity at physiological and biochemical stages (Ciobanu and Sumalan 2009). Under different physiological stages, including seed germination, leaf area and size, shoot and root length and weight, stem thickness, plant height, fruit initiation, and maturity of important agricultural crops are being dramatically affected by abiotic stresses (Zhu 2001; Rodriguez-Uribe et al. 2011). In order to improve the quality of crops under such conditions, attention has been given to establishing a relationship between plants and abiotic stresses in minimizing any negative influence during crop production (Munns 2002; Ashraf 2002; Dikilitas and Karakas 2014).

An understanding of the impact, significant adaptations, and mitigation strategies leads to approaches that sustain crop productivity and profitability in order to cope with such abiotic impact. These strategies address the changing effects due to abiotic stresses on different crops. Focus has to be given on the adaption strategies based on scientific knowledge which could be generated by agricultural experts in different regions in India (Shrivastava and Kumar 2015). Multiple adaptations to soil with high salt ion content are manifested with a wide range of cellular specializations, i.e., membrane permeability and transport, biochemistry, and gene transcription. Biological mechanisms develop within a particular plant to manage salt ions present in soils and also accompany a large number of taxonomic variations (Munns et al. 2006; Shrivastava and Kumar 2015). The harsh circumstances might be created by humans, due to use of excessive agrochemical fertilizers. These nondegradable synthetic fertilizers along with other unwanted agricultural practices ultimately accelerate the soil and water contaminations on a large scale. Therefore, some superficial approaches have to be taken to fulfill the requisite demand of healthy foods and feed, in spite of the presence of multiple stresses, for human and animal survival (Thao and Tran 2016; Singh et al. 2018). Understanding plant molecular mechanisms with respect to salt stress is a challenging effort for many biologists and plant breeders. Plants itself develop much physiological adaptations against soil salinity through various changes in genes that are mediated under stress response at the molecular and biochemical level (Gupta and Huang 2014).

Under unpredictable climatic changes mainly including altered rainfall pattern, temperature and CO_2 concentration may enhance abiotic stresses and may have a negative impact on crop productivity. Extensive chemical fertilizers have been used for excessive food demand but the approach has created serious environmental and health threats (Brozynska et al. 2014; Abberton et al. 2016; Rasheed et al. 2017). Many established agro-technological management systems effectively face these challenges, but it would still require more efficient biological applications to accomplish the agricultural products for global inhabitants (Thao and Tran 2016). Mostly, plants in its own habitat are well adapted to recognize external stimuli and they also develop their own defense mechanisms under extreme abiotic stresses (Cramer et al. 2011). These external stresses have not only influenced the phenotypic characters of crops but also reconciled its growth, development, and mortality rate (Mariani and Ferrante 2017). Thus, the aim of this chapter is to enlighten the basic importance and modern application of microbial biotechnology to understand the behavior of transgenic crop plants in saline soil. The study also elaborates the understanding of molecular machinery for healthy crop production.

7.2 Current Prospective About Salt Stress

Salt stress is the main restraint to agriculture that lowers the average crop productivity. This is one of the vast problems for agriculturists and plant biologists (Tran et al. 2016). It has shocking effects on soil crop production and yield. It extremely affects mineral nutrient balance; physiological, biochemical, and morphological responses;

and gene expression of plants. Plants responding to saline conditions have usually been determined by measuring biomass, emergence, growth, maturity, phenology, and commodity yields. Around 5.2 billion hectares of agriculture land have lost its fertility due to erosion, salinity, and soil degradation. Economically, salt stress critically affects the agriculture productivity of any country (Numan et al. 2018). Presently, modern breeding techniques have been introduced that include the crops' quality and its yield and resistance to both biotic and abiotic stresses (Varshney et al. 2017). The breeding method would adopt a mode to sustain the productivity of agricultural crops under multiple stresses. In the future, practical breeding platforms would demand automated genotyping technologies that possess quantitative traits under extensive inherited conditions in crops. A potential approach faces severe confrontation at the technological level, i.e., genotype–phenotype gaps in crop plants (Rasheed et al. 2017).

Drought, excessive light, flood, heavy metals, nutrition deficiencies, pollutants, salt, and temperature are the major abiotic stresses (Fig. 7.1) that are highly responsive in limiting agricultural growth (Akpinar et al. 2013; Budak et al. 2013). Along with these abiotic issues, the physical properties of the soil profile are often considered for any salt load. Leaching and inadequate drainage facilities lead to accumulation of salt ions in soils. These soluble salt ions may contribute to the salinity hazard of crops and to the salt load of agricultural drain water. During irrigation, this soluble salt may be deposited in the soil. Saline soils that extensively occur in the dry and partially dry provinces comparatively need more attention to uphold their long-term efficiency. A comparative assessment of saline soils has been made with

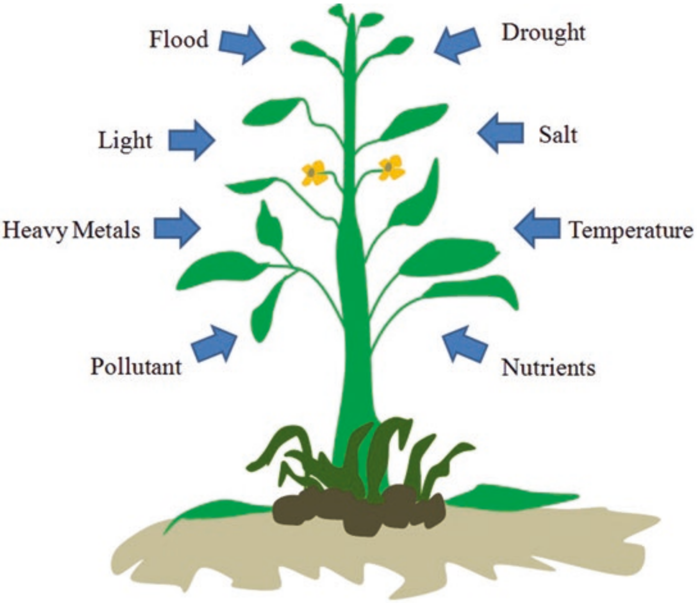

Fig. 7.1 Green plant experiencing abiotic stresses

ordinary healthy soils for any physical changes. In order to achieve this, we have to keep proper knowledge about the physicochemical characteristics of salt-affected soils. This is the foremost issue that needs extra attention to solve the problems regarding soil salinity and crop production (Numan et al. 2018; Rengasamy 2006). Due to such rational concern, many biological techniques are being introduced to uphold the productiveness of agricultural soil. Phytoremediation is one of the healthy biological practices which are being used on a large scale to improve the fertility of soil (Budak et al. 2015; Imadi et al. 2016).

Microorganisms have played a decisive role in crop development (Kumar et al. 2018b). In the future, improvement of crop production would be possible by using certain techniques of microbial biotechnology to manipulate plants to sustain them to grow and develop under any stress condition. For the last two decades, many stress-tolerant genes from microbes have been introduced that potentially improve crop plants (Kumar et al. 2018b). Focus has been given to commence potential transgenic stress-tolerant genes in crop plants through genetic engineering. With this, many active microbes are being utilized in food and therapeutic industries and in genetic transformation studies. These genetic transformation technologies significantly attribute to the development of sustainable agriculture for human society. Traditionally, crop genetics and breeding efforts through conventional methods had largely been used to control the traits of essential crops (Kawashima and Berger 2014, Baloglu et al. 2018). The modern approach of non-Mendelian genetics like methylation and histone modifications is also being highly used to improve crop productivity against salt stress (Kawashima and Berger 2014).

Among all the mentioned abiotic stresses, salinity causes a major threat by imposing both ionic and osmotic pressure to agricultural crops. These stresses can be differentiated at several tolerance levels. Plants sensitive to salinity permanently cause a great diminution in physiological activities in shoot and root growth within a few hours. The reduction in physiological behavior also depends upon the amount of salt ion (Na^+) concentration in growing plant tissues. High salt ion (Na^+) concentration also results in cellular toxicity, osmotic damage in the leaf apoplast, and nutrient deficiency (Tester and Davenport 2003). Soil under the effect of salinity influences water uptake of plant roots by decreasing the water potential of the soil. Any deficiency in existing water in saline soil would potentially dehydrate root cells, creating osmotic pressure due to salinity. Higher concentrations of Na^+ and Cl^- ions in soils disturb the ionic balance, hydraulic conductivity, etc. and reduce the absorption of other essential ions including K^+, Ca^{2+}, and Mn^{2+} (Hasegawa et al. 2000; Sabir et al. 2009; Elhindi et al. 2017). *Phaseolus vulgaris* (salt sensitive) and *Sesbania aculeata* are the two leguminous species which possess nodules in its roots that play a sustainable role in salt tolerance. These leguminous plants have effectively evolved themselves through many metabolic changes that enable them to resist salt stress. Although the dry weight of root and shoot has been decreased by a certain amount of NaCl, the dry weight of nodules is not much altered. It was observed that under salt stress the amount of nodules present in *P. vulgaris* was low compared with those in *S. aculeata*. Interestingly, it was the opposite in terms of nodular size. Along with such detrimental effects, the amount of proline and glycine betaine was increased, whereas the quantity of soluble proteins diminished in plant parts of both leguminous plants (Ashraf and Bashir 2003; Marks et al. 2018).

Andreu and his colleagues (2011) studied about salt tolerance, showing its variation between species and genotypes. They analyzed different degrees of salt tolerance in roots of *Prunus* species which are highly associated with many genotypic differences. However, such responses in salt tolerance have not been observed in wheat and barley for a short period of time (a few days). The osmotic effects and toxic ionic effects due to salt treatment have been observed only after a period of 3 weeks (Munns and Tester 2008). Apart from root growth, accumulation of starch in the maturation zone of roots between both rootstocks also showed resistance with different degrees of salt tolerance of the same rootstock at different salt concentrations (Andreu et al. 2011). Yin et al. (2009) worked on tomato during its early developmental stages. They observed that accumulation of carbohydrates made the tomato plant more resistant to salinity stress. Later, they detected the ADP-glucose pyrophosphorylase encoded by *AgpL1* and *AgpS1* genes, which were further upregulated under salinity stress.

Globally, about more than 7% of the earth's surface constitute saline soils that steadily increases barren regions (Wang et al. 2003). This stress is one of the limiting abiotic stresses that continuously attracts the attention of researchers in relation to plants. This stress cannot be ignored as high salt concentration significantly reduces the plant biomass growth and crop productivity (Dehghan et al. 2013; Kayikcioglu 2012). With the advancement in the agricultural system, many innovative agronomic tools have been introduced in improving crop productivity (Vinocur and Altman 2005). The environmental burden causes immense morphological, physiological, biochemical, and other changes that lead to programmed cell death in plant tissues (Wu et al. 2016).

The emergence of excessive abiotic stress negatively impacts the hormonal and nutritional balance in crops and hampers its productivity (Nadeem et al. 2014). Homeostasis and compartmentalization play a crucial role in a normal plant to detox the reactive oxygen species (ROS) and sustain and grow through all salt stress (Niu et al. 1995; Hakeem et al. 2016). Evolutionarily adapted plants might classify as either halophytes or glycophytes. Halophytes have the capability to accumulate salts in leaves to sustain its survival in salinity, while glycophytic plants cannot endure salinity and eventually die under such harsh condition. Majority of terrestrial plants are being modified and categorized as glycophytes. They grow naturally and adapted themselves in both biotic and abiotic environments (Munns and Tester 2008; Flowers 2004; Läuchli and Grattan 2007). Irrespectively, to a certain extent, glycophytes and halophytes are unable to uphold high salinity in its cellular cytoplasm. At this insensitive state, these plants develop certain mechanisms to get rid of salt stress. Plants transport excess absorbed salt ions to the large vacuole or sequester it to older plant tissues as sacrifice to protect themselves (Reddy et al. 1992; Chen and Gu 2015). In another case, plants which are grown in marshy places or under extreme saline conditions always experience the stress which might possibly be characterized as high osmotic pressures. Many ionic salts including bicarbonate (HCO_3^-), sulfate (SO_4^{2-}), chloride (Cl^-), and carbonate (CO_3^{2-}) are found in soil along with sodium (Na^+) and potassium (K^+) and are highly water-soluble through salinization. Moreover, sodium chloride constitutes a huge amount and their chloride ions are considerably toxic to plants, which severely impacts plant growth and development (Hasegawa et al. 2000; Zhu et al. 2005; Boyko and Kovalchuk 2008).

Salinity stress critically facilitates plants to survive by releasing diverse hormones, activates antioxidant cellular enzymes, regulates their ion uphold, and regulates many other biochemical pathways at a genetic and molecular level. Plants are essentially prepared through developing stress tolerance capability to grow effectively. Many plant inherent mechanisms from the plant growth-promoting bacteria (PGPB) facilitate them to uphold the growth of crop plants by tolerating salt stress. These cost-effective bacteria can be used as economical tools and are involved in producing some siderophores scavenging nitrogen (N) and many soluble organic and inorganic phosphate (Parnell et al. 2016; Van Der Heijden et al. 2016; Marks et al. 2018). Hence, fulfilling an excessive demand for crop products becomes one of the most important confronts for many agriculturalists and other biologists. They are continuously generous about healthy crop yield (Kolodyazhnaya et al. 2009; Miransari 2014; Ahanger et al. 2017). Despite having several tolerance mechanisms, many crops couldn't sustain and generally fail to survive under different environmental stresses (Roy et al. 2011). The perceptive progress in knowledge of molecular interactions between plant and salt and developing salt-tolerant crop varieties are the major challenges. Many reports have been reviewed; the molecular components that are participating in response to salt varied widely among plant species (Shokri-Gharelo and Noparvar 2018).

Currently, in improving crop performance, genetic engineering plays a crucial part in making transgenic plants sustain salt tolerance. In order to develop such transgenic plants, proper knowledge of the mechanisms for salt tolerance is essentially required (Hasanuzzaman et al. 2013; Deinlein et al. 2014; Gupta and Huang 2014). Many microorganisms could play a distinctive role in exploiting their properties in producing plant growth hormones, genetic diversity, and synthesis of compatible solutes in order to tolerate salinity (Shrivastava and Kumar 2015). Many functional genes which participate in molecular networks for stress awareness, internal signal transduction, and other metabolic pathways demand upregulation of genes that protect the plant's cellular structure under abiotic stresses (Vinocur and Altman 2005; Bhatnagar-Mathur et al. 2008; Bartels and Sunkar 2005). Complementary studies through integrated approaches are essentially used as key factors in plant genetic engineering. Proteomic, transcriptomic, genetic engineering, and genetic studies and others are the recent approaches to understand the salt response of various plants. However, many aspects remain unclear about the cellular and molecular responses of plants to salinity. Especially, the chromatin structure has highly influenced genome expression. The genetic expression is often associated with epigenetic regulation. Alternative splicing, DNA methylation, histone modification, and posttranslational modifications are essential molecular mechanisms used in epigenetic regulation (Bender 2004; Zhang 2008; Liang et al. 2018; Shokri-Gharelo and Noparvar 2018). Arginine and lysine methylation is being widely utilized in regulating transcription (Liu et al. 2010). Many facts have been experimentally reported indicating that DNA methylation and siRNA dynamically contribute in the gene regulation of plants under many abiotic stresses (Chinnusamy and Zhu 2009a; Zhang et al. 2013). The genotypic differences would help to develop tolerance against high levels of salt in main crop plants that could prospectively improve crop yield and technical support to the farmer.

7.3 Role of Genetic Engineering in Tolerating Salt Stress

Plant breeding is being known for the past 10,000 years but, in the last 35 years, many decisive and significant changes in agriculture have observed. Many genetically modified plants are being used as a transgenic organism. They are generated by an alteration in genetic structure (i.e. introducing, deleting, or silencing a gene or group of genes of interest) of crops utilizing the recombinant DNA technology (RDT). These engineered crops possess enhanced agronomic traits that have made through exclusive changeover from laboratories and greenhouses to fields all around the globe. Commercially, the modern technologies have been evolved as science in making healthy and stress tolerance agricultural crops of tomorrow. The technology generally equipped with genomic information and nanotechnology which are being exploited by plant molecular biologists to redesign the various molecular tools to engineer agricultural crops in a précised way. The alteration in the genetic makeup of crops with selected desirable features significantly contributes to the modern plant breeding (Moeller and Wang 2008).

Genetic engineering is a potential molecular approach to improve the tolerance capacity of plants against abiotic stress more quickly through various genetic modifications. The beneficial outcomes of these genetic modifications contain increased food production, consistency, and yields. The plants which are generated through genetic engineering are considered to be genetically modified (GM). These modified plants acquired the improved traits which enable them to sustain in the stress conditions or any other external stimuli (Datta 2013). During the period of green revolution in India, proficient use of many agrochemicals and high-yielding agricultural crops that have improved throughout conventional plant breeding practices. This approach significantly improved crop productivity in India. Later, agricultural biotechnology comes with a modern technique which behaves like a potential tool and effectively complements the conventional methods to fulfill the shortage of food. Although there is a massive improvement in the use of biotechnological tools, economically gene pools are being utilized countless beneficial genetic traits in chief food crops. These crops can help to meet the demand of biotic and abiotic stress tolerant crops along with nutritionally-balanced and high-yielding crop varieties (Datta 2013; Cabello et al. 2014). The comparative functional studies of healthy and transgenic crop production in abiotic stress relied on known physiological and phenotypic parameters. Such concepts and strategies of parameters of engineered stress tolerance mainly depend upon the genes carrying traits that control many physiological roles including ion omission, osmotic pressure and tissue tolerance against salinity (Roy, et al. 2014; Volkov 2015).

As the relevant challenges of plants are to grow in natural environments in combination with abiotic stresses, drought, salinity, and heat which direct to synergistic, unbalanced or even hostile effects. The abiotic stress conditions i.e. drought, salinity, and extreme temperatures actively induce complex regulatory networks within crops and pose severe constraints in its productivity. These stresses lead to establishing a self-protection mechanism within the crops. Many sensors inside the cells activate the downstream signaling and also control many transcriptional cascades, among several groups of transcription factors (TFs), under the program through gene-expression at cellular. The cellular program is used many hormones which are integrated into many biological mechanisms for plant physiological growth and development within the environmental cues (Mittler and Blumwald 2010). An improved and exclusive knowledge of the multifarious mechanisms involved in the stress responses are provided diverse ‘omics’ platform to recognize novel points to develop as the main effective scheme for crop production.

Several plants are being evolved into various molecular mechanisms within themselves to tolerate salt. The gene manipulation occurs during molecular activities involve some signaling components for post-translational modification. This epigenetic control mechanism is highly influenced by small RNAs during gene expression and the entangled effects of various plant hormones. The complex molecular networks enable the researchers to ensure genetic control within plants to tolerate environmental salt stress. The possible incorporation of synthetic natural science approaches into existing genetic engineering methods, lead to innovative outlooks. This meant for the lucid intend of molecular tools and plans to advance stress tolerance potential (Cabello et al. 2014). Several environmental stresses are responsible for producing many reactive oxygen species (ROS), which lead to oxidative harm to biomolecules at the cellular level. The effectors’ proteins are present in stress tolerance cells that activate many stress-responsive mechanisms for the concern of cellular homeostasis. These proteins are also participated in removing other toxic compounds, and defend and mend any damage occurred in cellular membranes. Molecular modifications under the stress response are maintained by post-translational modifications of proteins through, phosphorylation sumoylation, and ubiquitination. Later also some stress-inducing small RNAs (miRNAs and siRNAs) are engaged for the post-transcriptional regulation in abiotic stress (Cabello et al. 2014). Therefore, a traditional move towards acquiring any stress tolerance basically relies on endogenous enzymatic and non- enzymatic behavior of ROS scavenging systems within the cell (Gill and Tuteja 2010; Miller et al. 2010; Cabello et al. 2014). Current molecular progress simultaneously facilitates the overexpression of some genes that are transcribing the enzymes having antioxidant properties including dehydroascorbate reductase, glutathione-S-transferase, and glutathione reductase (Le Martret et al. 2011; Luo et al. 2013). For such features, *Escherichia coli* is being used through in tobacco and *Arabidopsis* together to synthesize these antioxidant enzymes, proline as a metabolite and some osmoprotectant through antisense technology (Stein et al. 2011; Cabello et al. 2014).

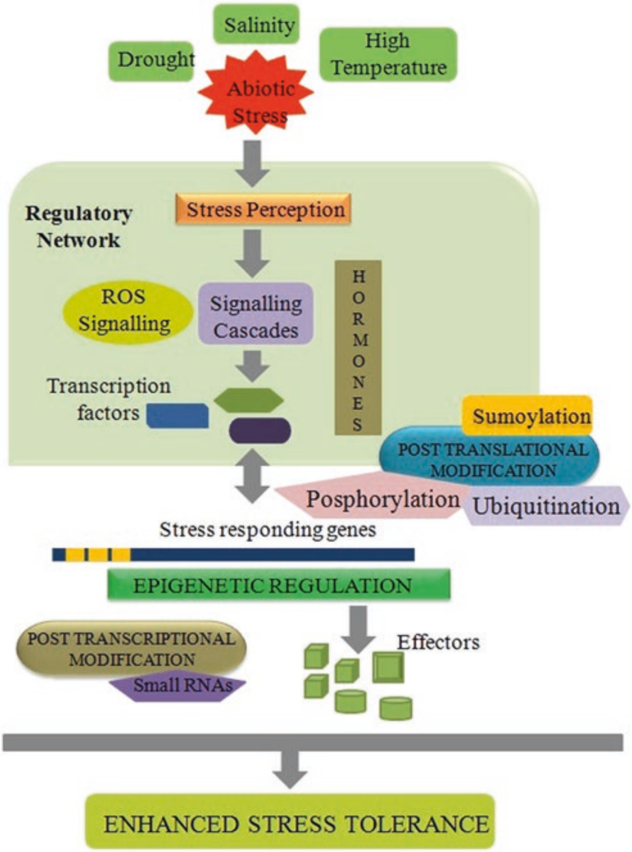

Fig. 7.2 The biological mechanism of plants to resist abiotic stress using genetic engineering strategies

High regulatory networks in plants in response to environmental stress comprise a capable tactic on the way to accomplishing broad-range greater stress tolerance (Fig. 7.2). Therefore, overexpression of any stress sensor proteins like ion channels, Ca^{2+} binding proteins, and parts of kinase families and signal transducers are widely investigated (Agarwal et al. 2013). There are more than 50 families of TFs have been reported including AREB, bZIP, DREB, MYC/MYB, NAC and WRKY, which regulate the complex cellular networks as downstream integrators. They all effectively express the stress-responsive genes in a combinatorial and amplificatory manner (Hirayama and Shinozaki 2010; Cramer et al. 2011). The ectopic constitutive gene expression for TFs proficiently improves the tolerance capacity of abiotic stresses in the plants but frequently results with some dwarf phenotypes and low crop yield (Hussain et al. 2011; Cabello and Chan 2012; Agarwal et al. 2013). The constitutive gene expression of HaHB1 and WRKY30 in *A. thaliana* have been reported when pleiotropic effects are absent and enable the abiotic stress tolerance (Cabello et al. 2012; Scarpeci et al. 2013).

The advantage of tolerant genotype and the comparative study of the, biochemical/physiological and/or molecular/cellular level of any crop plants would pave the perceptive knowledge along with sorting out the novel existence mechanism under any abiotic stress condition. The genus *Brassica*, is very close to *Arabidopsis thaliana*, is an efficient model plant. This includes numerous crops which are widely adapted to survive in varied agro-climatic conditions (Morinaga 1934; Bohnert et al. 2006). There are lots of important inter- and intra-specific distinction have been reported in many species of *Brassica* genera for salt tolerance. Researchers have extensively investigated the relationship between the genetic system and salinity tolerance. They found many tissue-specific SOS genes that show key involvement in Na^+ regulation (Bohnert et al. 2006). The ruling of the SOS path considerably controls both the ion exchange homeostasis in *A. thaliana* and any alteration in the respective genes of this pathway make the plant sensitive towards soil salinity (Zhu 2003). The SOS pathway manages the growth of the plant root in defined media. The experimental observation had shown a well-built association among SOS transcript accretion and other physiological parameters of diverse cultivars of *Brassica* species in influence to salt stress. Two reported cultivars of *B. juncea* (var. RH8813 and CS52) responded differently under the salt stress. During salt stress condition *B. juncea* (var RH8813) comparatively showed about 30% reduction in root length, with *B. juncea* (var CS52) which showed minimal effect, over a stress period of 24–72 hrs. The researchers found that *B. juncea* var CS52 possesses salinity tolerance genes which constitutively the transcription level of SOS genes. Also, it was also observed that a large amount of proline accumulation in the genotype of *B. juncea* var. CS52 was found in under salinity stress (Hasanuzzaman et al. 2017). It was also stated about these regulatory genes which are highly conserved in all **Brassica** but work different concentration of salt in the soil (Kumar et al. 2009) (Fig. 7.3).

Ipomoea batatas (L.) Lam, (sweet potato) is the main food item, and also an alternative source of bioenergy. The plant bears Iron-sulfur cluster which responsible for biosynthesis of the nitrogen fixating (Nif) proteins. The gene cluster from a salt-tolerant sweet potato line LM79 possesses a scaffold protein gene, *IbNFU1*. Although there was no any crucial role of *IbNFU1* gene has been reported in sweet potato but when allowing the overexpression of *IbNFU1* gene in the tobacco plants improved salt tolerance level (Wang et al. 2013a; Liu et al. 2014). The *IbNFU1*-overexpressing sweet potato plant significantly shows accumulation of proline residues and allows ROS scavenging. The overexpression of the gene also enables the proper photosynthetic rate, promote stomatal conductance, transpiration rate and chlorophyll related content in comparison to wild-type crop plants in salinity condition.

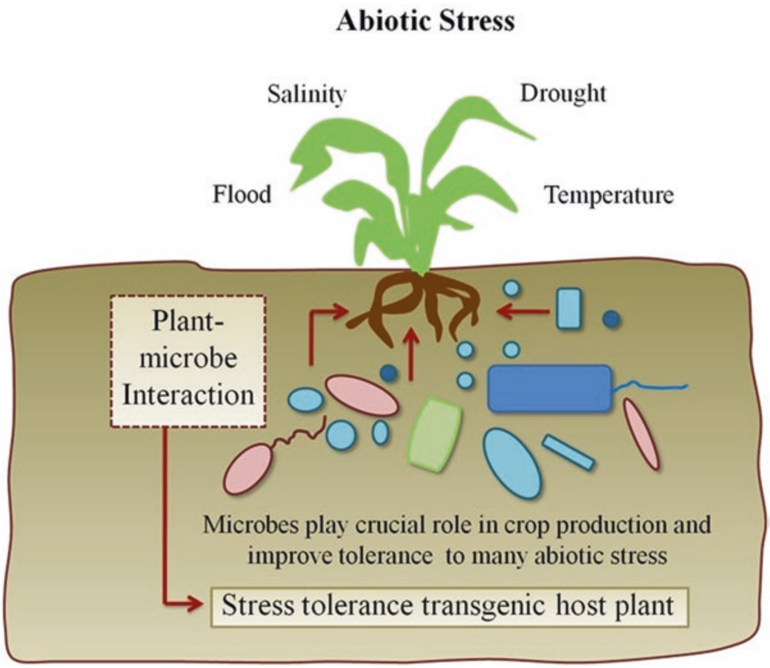

Fig. 7.3 Association between plant and microbes under salt stress

7.4 Plant–Microbe Interactions

Microbes are cost-effective ecological engineers that are probably concerned about environmental stresses and are responsible for sustainable agricultural production. They are the backbone of almost all ecosystems that revolutionized the perspective approach to analyze their principal role in the biosphere (Parnell et al. 2016). They are potentially engaged in regulating physical and biochemical mechanisms including plant growth, biosynthesis of hormones, improved nutrition, siderophore production, and improved antioxidant system. In nature, plants live together with many complex and valuable microbial consortia and effectively impact the soil fertility for plant productivity. Many efforts have been made to explore the benefits of multi-stress-tolerant microorganisms (Kumar and Verma 2017; Kumar et al. 2018a). To sustain abiotic tolerance, plant–microbe interactions are essentially needed for qualitative crop production. This new move toward crop production has been widely used under plant microbiome research. Interestingly, this would establish the foundation stone for the next green revolution (Vessey 2003; Finkel et al. 2017). Several studies are being reported about the biological effectiveness of microbiota members for healthy plant growth including nutrient acquisition (Van Der Heijden et al. 2016), enhanced tolerance capacity for abiotic stresses (Rolli et al. 2015), adaptation to environmental variations (Haney et al. 2015), and establishment of mycorrhizal associations. Plants involved in microbial colonization are highly selective in the microbial niche. This microbial colonization also properly elaborates the taxonomic knowledge of a variety of soil microorganisms (Lundberg et al. 2012; Bulgarelli et al. 2013; Ofek-Lalzar et al. 2014; Cao et al. 2015).

Microbial diversity in soil highly influences plant physiology and the general crop management practice in arid regions. Many significant microbes endorse the development of crop plants within the rhizosphere in arid soils which exist under water stress. Water scarcity is a serious problem around the world. Although drought and salinity are some of the major environmental factors known for severe desertification of agricultural land, irrigation is another basic reason in soil salinization. An association between soil and the rhizosphere microbes from these regions is probably the main agent for neutralizing such abiotic stresses in plants (Bartels and Sunkar 2005; Daffonchio et al. 2015). Gonzalez along with his colleagues introduced the importance of *Azospirillum brasilense*. *A. brasilense* effectively attenuated the effect of salinity in jojoba plant. They observed an undesirable reduction of a saline condition by *A. brasilense* on the jojoba rooting (Gonzalez et al. 2015). In the same year, Fasciglione et al. (2015) used *Azospirillum* to report lettuce growth in saline condition. Interestingly, this quality of lettuce and life of lettuce are highly improved (Kumar et al. 2017).

Arbuscular mycorrhizal fungi (AMF) are ubiquitous in nature that generally form a mutual relationship with roots of various plants. They are well governed in rhizosphere soil by certain climatic features (Wang et al. 2018). They are the bio-ameliorators of selected strains of fungal microbes which are potentially enabled plants that sustain under soil salinity. Wang et al. (2018) also examined a colonial effect of two different AMF, *Funneliformis mosseae* and *Diversispora versiformis*. Both enhance the growth ability and nutrient absorption of many perennial herbaceous medicinal plants including *Chrysanthemum morifolium* (Hangbaiju) under the salt stress condition. *C. morifolium* has a moderate salt tolerance capacity. The symbiotic association of *D. versiformis* fungi with the Hangbaiju plant enhances the uptake of nitrogen through the root. They are environmental-friendly symbiotic organisms that also lower the pollution at some level. This would be useful for many biotechnological practices in the future to enable essential crops to tolerate soil salinity under such symbiotic association (Sadhana 2014; Wang et al. 2018). Mycorrhizal inoculation drastically improved the amount of chlorophyll and water-holding efficiency in saline soil. It was found that sweet basil plants with AMF, *Glomus deserticola*, showed enhanced growth of the plant, efficiency of photosynthesis, gas exchange, and efficient utilization of water under salt stress (Saeed Akram et al. 2009). The study was performed to elucidate the AMF which can ease salinity. This has effectively influenced the growth and biomass productivity of sweet basil plants (Elhindi et al. 2017). So, the potential use of AMF in maintaining soil salt tolerance would be utilized for the production of other essential agricultural crops.

7.5 RNA Interference and Salinity

As plants face various forms of stress conditions during their life cycle, a relevant molecular mechanism of RNA interferences was smartly developed to sustain plants in such stressful environment. Presently, it has extensively been used in all parts of the molecular laboratory. This highly complex molecular mechanism is well conserved across many crop plants and has also been commonly documented in almost all living organisms (Allen et al. 2004; Eamens et al. 2010; Rajwanshi et al. 2014). The new technology is highlighted and well widely diversified to manage different functional needs to regulate themselves in a high degree of salt stress. RNA interferences are broadly evolved at an extraordinary level of complexity. They are predominantly retrieved from small RNAs that are tempted from prepared double-stranded RNA (dsRNA) or hairpin-structured RNA (hpRNA) (Hannon 2002; Baulcombe 2004; Meister and Tuschl 2004; Chapman and Carrington 2007). They are present in almost all eukaryotes and are exclusively involved in chromatin modification in crop plants. In such agricultural crop plants, these small RNAs also take part in genetic improvement and ensure regular growth and progress under stress conditions (Eamens et al. 2008; Eamens et al. 2010; Rajwanshi et al. 2014). Over the last decade, small RNAs establish its importance in controlling gene expression, epigenetic alteration in genomes, and defense against viruses. There are 21–23 nucleotide (nt) RNAs that significantly emerged as a posttranscriptional regulatory constituent (Chapman and Carrington 2007; Ghildiyal and Zamore 2009). Based on diverse biogenesis machinery, these small RNAs have been recognized and classified into four broad classes. MicroRNAs (miRNAs), small interfering RNAs (siRNAs), long noncoding RNAs (lnc RNAs), and Piwi-interacting RNAs (piRNAs) emerged as key gene regulators that work together with several accomplice proteins at the cellular level (Ghildiyal and Zamore 2009; Malone and Hannon 2009; Xie et al. 2010; Mateos et al. 2011; Rajewsky et al. 2017). The mode of gene expression, preservation, and deviation of miRNAs are well explained by next-generation sequencing. Moreover, the functional activities of these well-known miRNAs elucidate its role in many physiological growth including flowering and food development (De Fátima and de Folter 2017).

MicroRNAs (miRNAs) comprise a group of small RNAs that are exclusively known for controlling various gene expressions. These endogenous noncoding sequences constitute 20–24 nucleotides that are involved in negative regulation during protein-coding nucleotide sequences through specific interactions (Wang et al. 2014). They potentially cleave mRNA or repress the translation process by using many regulatory components developed in plants to respond to various biotic and abiotic changes. They are evolutionarily conserved and are considered a dynamic area of modern research (Jones-Rhoades and Bartel 2004). They are generally involved in repressing gene expression. They actively encode transcription factors or proteins that are involved in RNA metabolism and lead to the growth of plants at a physiological level. In the past two decades, knowledge is being well explored regarding the molecular behaviors of miRNAs in different biological procedures in plants (Jha and Shankar 2011). They participate in regulating various gene expressions at the posttranscriptional stages by targeting mRNA transcripts that further represses mRNA translation (Bartel 2004; Jones-Rhoades et al. 2006; Xie et al. 2010). Recently, many stress response microRNAs (miRNAs) have been reported from many plants. They regulate several biological roles in plant development against different stress-related environmental factors by targeting mRNAs that delay or restrict the gene expression by doing a translational modification (Zhang et al. 2007; Liu et al. 2008). They also carry out negative posttranscriptional processes that affect hormone (auxin) signaling and development of plant organs (Pasquinelli and Ruvkun 2002; Chen 2005; Mallory and Vaucheret 2006). Transgenic plants are being extensively used for the study to reduce the salt and drought stress or to develop the tolerance capability (Bian et al. 2012; Xia et al. 2012).

7.6 Role of DNA Methylations Against Salinity

Many plants would adapt themselves to salt stress due to many genetic alterations. These are some of the unique biological features that enable plants to sustain under environmental stresses. Numerous physiological changes occur in plants (Yang et al. 2015). Photosynthesis, cell structure modification, toxic ion compartmentalization, and hormonal regulation are some of the important biological mechanisms that extensively evolved (Munns and Tester 2008; Zhang et al. 2011; Ditta 2013). Efficacy of physiological changes is mainly based on the pattern of gene expressions under molecular procedure within the cells.

Mammalian and plant genomes possess a particular type of DNA sequences which are involved in transcribing noncoding RNAs (ncRNAs). These transcribed functional RNA molecules couldn’t translate into protein but highly regulate gene expression at both transcriptional and posttranscriptional levels. Such breakthrough principal roles of these ncRNAs have revolutionized perceptive view of molecular, cellular, developmental, and evolutionary biology. These ncRNAs are of diverse sequences, sizes, genomic loci, and biogenesis. They are also known for their specific gene silencing (Chinnusamy and Zhu 2009a; Mahfouz 2010). The ncRNAs are directly responsible for a wide range of epigenetic modifications including methylation and possess some histone-modifying complexes (Yan et al., 2010; Gursanscky and Carroll 2012; Peschansky and Wahlestedt, 2014; Tao et al. 2015; Al-Harrasi et al. 2018). A close correlation has been found between DNA methylation and abiotic stresses (Zhao et al. 2010; Xu et al. 2015).

Within all multicellular organisms, DNA methylation participates as a main part in gene expression. It is a conserved epigenetic modification that contributes to genome stability, adaptation, and evolution. It generally occurs or is changed in plants by diet or stressful external conditions (Lim and Song 2012; Solis et al. 2012). The methylation of cytosine residues sustains usual growth and development and proper responses to any disease states and other environmental cues (Mahfouz 2010). The interruption in different DNA methyltransferases results in developmental abnormalities (Li et al. 1992; Finnegan et al. 1996). It also significantly reduces the biological mechanisms within wild-type plants that grow in soil salinity (Yan et al., 2010). It takes place in the perspective of CG, CXG, and CXX (where X is adenine, cytosine, or thymine) in crop plants (Lindroth et al. 2001; Cao and Jacobsen 2002). Some enzymes like methyltransferase-1 (MET1), chromomethylase-3 (CMT3), and domains rearranged methyltransferase (DRM)-2 are very crucial and are exclusively being expressed as DNA methyltransferases. They effectively transmit a methyl group to the cytosine bases of DNA to give 5-methylcytosine (Ronemus et al. 1996; Lindroth et al. 2001; Cao and Jacobsen 2002; Popova et al. 2013). MET1 and CMT3 are, respectively, responsible for the repair of CG and CHG methylation, whereas DRM2 is important for de novo DNA methylation and prominently shows in CHH methylation (Cao et al. 2003; Miura et al. 2009).

The molecular procedure of DNA methylation is effectively based on two different mechanisms, i.e., one is RNA-directed DNA methylation (RdDM) and the other is histone posttranslational modification (Yan et al. 2010; He et al. 2011; Fulneček and Kovařík 2014). RdDM is a popularly known epigenetic mechanism that is frequently responsible for endogenous CG methylation of genes with influence toward either intracellular or extracellular stimuli (Mahfouz 2010; Matzke and Mosher 2014). It is one of the fundamental marks first revealed in viroid-infected tobacco plants; however, information on methylation marks are intended for certain parts of the vague genome (Bird 2002; Bender 2004). It might be a part of the regulatory event that eventually represses epigenetic modifications. The modifications trigger the formation of transcriptionally silent heterochromatin. The mechanism effectively involves many transcription factors that are specifically targeted through a sequence-specific approach on their own (Mathieu and Bender 2004; Matzke and Mosher 2014).

The siRNAs usually mediate gene silencing in plants at the transcriptional level (Law and Jacobsen 2010; Gursanscky and Carroll 2012). RdDM starts with biosynthesis of small single-strand 24-nt siRNA transcripts which are generally transcribed by the specific RNA polymerase IV (Pol IV) present in plants (Huang and Ecker 2018). This single-strand 24-nt small siRNAs are transformed into double-stranded RNAs under the effect of another polymerase enzyme, RNA-dependent RNA polymerase 2 (RDR2) transcription factor. Afterward, this double-stranded RNA cleaved to 24-nt siRNAs by using Dicer-like 3 (DCL3). Another protein, AGO4, is attached to one strand of siRNA to create an AGO4-guide RNA complex. The AGO4-guide RNA complex somehow interacts with the scaffold ncRNAs of various target loci, which are transcribed through RNA polymerase V (PolV) present within a plant (Chinnusamy and Zhu 2009b). This effect recruits domains rearranged methyltransferase 2 (DRM2) that performs the methylation of target loci (RDR2-RdDM, Fig. 7.4). Transcriptionally, RdDM suppresses a subset of transposons and genes which are concerned with pathogen defense, stress responses, and reproduction (Matzke and Mosher 2014).

Currently, numerous sensitive genes and proteins are being introduced through both forward and reverse genetics approaches which are highly sensitive to salt stress. Some transcriptional factors like CCAAT-binding transcription factors (CBFs); NAM, ATAF1/2, CUC2 transcription factors (NACs); MYB; and WRKYs are some of the functional transcriptional regulatory proteins that play defensive

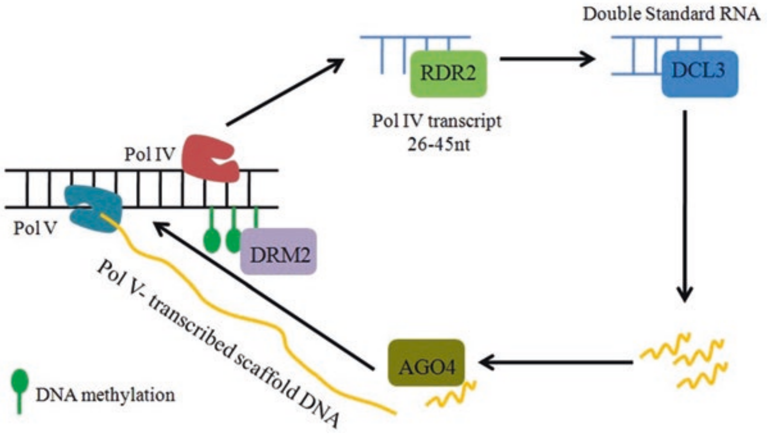

Fig. 7.4 RNA-directed DNA methylation (RDR2-RdDM)

roles during salt stress in plants (Jin and Martin 1999; Singh et al. 2002; Yan et al. 2012; Xu et al. 2015). The plants which experienced stresses show fewer methylation profiles irrespective of DNA replication. With regard to tobacco under salt stress, it has been reported that methylation in a coding region of glycerophosphodiesterase gene would reduce the plant size (Choi and Sano 2007). Differential expression levels in the genome and de novo DNA methyltransferases were also observed in rice and other plants subjected to salt stress (Sharma et al. 2009). Posttranslational histone tail modifications are other conserved epigenetic regulatory modifications, known in the plant kingdom to also interact with DNA methylation and regulate chromatin structure. Both epigenetic events, i.e., DNA methylation and histone modifications, are reversible and together participate in the execution of dynamic transcriptional profiles throughout the whole plant life cycle (Hsieh 2016).

Apart from functional transcriptional regulatory proteins, an abundant class of siRNAs includes heterochromatic siRNAs found in plants that are also activated by various environmental stimuli. Various evidential reports about the biological activity of siRNAs have been studied that would affect the chromatin structure (Furini et al. 1997). The gathering of three main siRNAs within wheat seedlings has been found during saline condition (Boyko et al. 2010). Interestingly, proline accumulation in *Arabidopsis* was observed due to the involvement of 24-nt SRO5-P5CDH just like naturally occurring antisense transcript siRNA. This siRNA is actively involved in the cleaving of P5CDH mRNA under salt stress, which is a good example for developing many salt-tolerant plants (Borsani et al. 2005). Proline accumulation in a plant under salt stress is a significant method for osmotic regulation. Other stress-regulated siRNAs possibly show the way to genetic transformation due to DNA methylation and histone modifications. Microarray analysis establishes that abiotic stresses dramatically influence the genes concerned with RdDM pathways in *Arabidopsis* (Chinnusamy and Zhu 2009a; Huang et al. 2013).

The methylation-sensitive amplified polymorphism (MSAP) method is a new conceptual model to control and to establish the significance of methylation in salinity. It has some quantitative features which enable maintenance and conservation of methylation condition of CCGG sequences along with another process including demethylation and de novo methylation (Bednarek et al. 2017). Under a high salt-tolerant capacity, cytosine in CCGG sites gets methylated in cotton line. This property has been utilized at different methylation levels in another plant (Zhao et al. 2010; Cao et al. 2011). Moreover, this can enumerate actions which are mainly involved at CG and CHG sequences sites, providing a crucial approach for the purpose of epigenetic changes in respect to abiotic stresses in plants (Bednarek et al. 2017).

7.7 Conclusions and Future Prospects

Abiotic stress factors belong to a very diverse environmental condition that drastically affects the growth and production of crop plants. Salinity behaves as natural attributes of ecosystems and is usually induced by illegal anthropogenic activities. Globally, around 20% of the cultivating lands are being influenced by soil salinity. Such a hypersaline situation is a significant limiting factor to essential crop production. Research on salt tolerance mechanisms or techniques has been developed for sustainable agriculture. Microbiomes have been introduced which possess many salt-loving microbes. Any reduction in salt stress results in accumulation of organic carbon and microbial biomass and improves the fertility of the soil.

Organic manures efficiently incorporate with hypersaline soil to improve its microbial and enzyme activities over the years and contribute to the function of nutrient cycling. The use of promising low-cost eco-friendly bacteria for plant growth under salinity stress led to sustainable agriculture. Bacterial performance against stress would facilitate scientists to understand their behavior for large-range applications for the growth of agriculture. RNA interference is another high-throughput technique, including miRNA and siRNA, which involved regulation of many molecular mechanisms at the genetic level for proper plant development and propagation during abiotic stress conditions. In the coming future, miRNA- or siRNA-mediated RNA interference will enable the transgenic crop plant to nourish under stress conditions. With the aim to improve crop production under salinity stress, certain contemporary techniques can be used to influence plant productivity. Focus has been given to incorporating genes with novel phenotypic properties into crop plants including the use of microbes at each step through genetic engineering. Many potential salt-tolerant genes are available in nonessential crops which need to be isolated and incorporated in essential crops to meet the requirement for food and feed items. Microbial biotechnology in sustainable agriculture not only improves crop productivity under stress conditions but also simultaneously develops tolerance capacity.

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Consequences of Bioinoculants and Intercropping Approach to Alleviate Plant Drought and Salinity Stress for Sustainable Agriculture

8

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Abstract

Saline conditions have created severe negative influence on agricultural productivity and salt accumulation in soil leading to significant yield losses. According to an estimate, approximately 5.2 billion hectares (ha) of agricultural land is subject to soil degradation, erosion, and salinity. The salt-affected soil in India is about 8.1 million ha, with 3.1 million ha coastal saline soil, 2.8 million ha sodic soil, and the remaining 2.2 million ha saline soil located inland. Even though salinity has already significantly affected the fertile lands, the land area under salinity is still increasing due to various anthropogenic activities as artificial irrigation, improper water management, blocking of natural drainage system, and similar human interferences with the environment. In saline-affected soils, the rhizosphere environment becomes unfavorable and inhospitable for growth of plants and microbes, although there may be sufficient amount of water and nutrients in soils. Overuse of artificial fertilizers and chemical pesticides causes long-term degradation of natural soil fertility and creates environmental pollutions. The degradation of soil fertility, in combination with an estimated rise of the world population to 8.5 billion over the next 25 years, calls for additional strategies to ensure the worldwide requirement of food supply. The development of sustainable and safe means for agriculture production will be necessary, which includes enhancing the output on arid and saline areas to avoid further loss of cultivable land. The application of bioinoculants like plant growth-promoting rhizobacteria (PGPRs) and arbuscular mycorrhizal fungi (AMF) has the potential

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to enhance plant growth under abiotic stress conditions and to avoid soil degradation at the same time. This kind of plant-microbe interaction is based on biological processes and has the potential to change conventional agricultural practices to a vital and sustainable agriculture. In such a world, microbes take over an important role as an ecological actor to resolve environmental stress problems. Another natural way to enhance plant growth and marginal land use is in planting different species simultaneously at the same space (intercropping), as, e.g., intercropping of legumes and cereals. Highly promising are techniques where plants with different root systems are grown together, which do not compete for the same space in the soil. The different abilities of intercropping plant species to use ephemeral or permanent water sources strongly affect physiological performance and species coexistence in water-limited ecosystems. Therefore, the present chapter highlights the various techniques to counteract crop loss in marginal soil and explore various beneficial biofertilizers and their modes of action in terms of abiotic stress tolerance and reduction to enhance agricultural production in a sustainable way.

Keywords

ACC deaminase · Biofertilizer · Hydraulic lift · Intercropping · Salt stress · AMF · PGPR

8.1 Introduction

The world population is expected to reach 9 billion by year 2050, and the global food supply needs to be increased by 70% to meet the rising demand (Smol 2012). Changes in the global climate scenario create abiotic stresses which is a pivotal factor for suppressing agricultural productivity (Grover et al. 2010; Larson 2013). Global arid and semiarid agricultural lands are decreasing 1–2% every year due to abiotic stresses (Kafi and Khan 2008) with estimated associated economic losses in the value range of US$ 12 billion per annum globally and on a rising scale (Qadir et al. 2008; Dodd and Perez-Alfocea 2012).

Diminishing soil fertility and abiotic stress conditions like temperature extremes, drought, salinity, and acidic conditions affect yield of several crops cultivated in different agroclimatic conditions in a significant amount (Saravanakumar and Samiyappan 2007; Yaish and Kumar 2015; Ahmad et al. 2016; Barnawal et al. 2017; Ilangumaran and Smith 2017; Mavrodi et al. 2018). The most dominant abiotic stresses are drought and salinity, which severely affect plant growth and biomass production with associated yield loss of 50–82% (Christensen et al. 2007; Sattar et al. 2010). Drought stress already affects 64% of the global land area and salinity affects already 6%, which accounts for almost 1 billion ha worldwide (Metternicht and Zinck 2003; Cramer et al. 2011). Currently, soil salinity is a major problem which degrades agricultural land, and globally the rate of soil degradation by salinity is steadily increasing, particularly in the arid and semiarid regions. Drought is another potential stress factor which affects germination, plant growth, and yield and is also associated with reeducation of cultivation land. Climate models predicted changes in weather patterns, leading to increase in the severity and frequency of drought further reducing the area of agricultural land (Lesk et al. 2016; Walter et al. 2011). With changing environmental condition, plants undergo stress conditions which put the plant metabolism out of homeostasis and unsuccessful for protection (Foyer and Noctor 2005; Simontacchi et al. 2015).

Recent methods in the development of abiotic stress-tolerant plants are challenging. Classical breeding and modification of the host genome have a lot of restrictions and limitations for cultivation in the field, despite significant efforts, and abiotic stresses affect several aspects of plant gene regulation and physiology (Dodd and Perez-Alfocea 2012; Krishna et al. 2015; Kumari et al. 2015). Bioinoculants like plant growth-promoting rhizobacteria (PGPRs) and arbuscular mycorrhizal fungi (AMF) help to alleviate plant abiotic stresses through inducing systemic tolerance (Wang et al. 2012; Jegan et al. 2016; Singh and Jha 2017). Intercropping is a traditional agronomy approach of cultivating two or more plant species simultaneously on the same field and establishing a mutual relationship between two plants which enhances the yield and soil fertility and alleviates drought stress (Brooker et al. 2015; Xue et al. 2016). The combination of bioinoculants and the intercropping system will be a holistic approach to alleviate abiotic stresses and crop productivity. Therefore, the present chapter highlights the various techniques to counteract crop loss in marginal soil and explores various beneficial biofertilizers and their modes of action in terms of abiotic stress tolerance reduction to enhance agricultural production in a sustainable way.

8.2 Impact of Abiotic Stress on Crop Growth and Yield

Abiotic stress like drought and salinity affects plant growth and productivity of agricultural crops and causes huge economic losses. Globally more than 800 million hectares of agricultural land, which is more than 6% of the world's total land area, is affected by salinity (Shrivastava and Kumar 2015). Soil salinity increases cellular Na^+ and Cl^- concentrations in plants that suppress the growth and yield by blocking cellular and metabolic activities like cell expansion, photosynthetic activity, and cytotoxic effects and decreasing carbon assimilation level (Hasegawa 2013; Roy et al. 2014). High salt concentration causes accumulation of salt ions in cells that is toxic and is manifested by chlorosis and necrosis of the leaf tissues (Hasegawa et al. 2000; Wahome et al. 2001). Considerable attention has been directed towards genetically engineered plants for saline tolerance but with moderate success (Apse et al. 1999). The variations in growth response to saline exposure reflect in the plant's ability to tolerate salinity. The most sensitive among the cereals is rice (*Oryza sativa*), with barley (*Hordeum vulgare*) being the cereal with highest salt tolerance. Salinity exposure reduces the nitrogen uptake as well as the translocation of nitrogen from the roots to shoots and also the nitrate assimilation in leaves (Peuke et al. 1994). Accumulation of low molecular weight osmolytes like sugar, alcohols (e.g., glycerol, sorbitol, and mannitol), and specific amino acids (proline and glycine betaine, a quaternary ammonium compound) is a plant response to salinity stress. This osmolyte accumulation is the major underlying mechanism of plants to osmotic stress tolerance adaptation (Rhodes and Hanson 1993; Hayashi et al. 1997).

Drought is recognized as one of the most serious environmental stresses worldwide, limiting plant growth and hence agricultural productivity (Oyewole et al. 2017; Farooq et al. 2009). It affects many biological processes in plants as water and nutrient relations, photosynthesis, phenology, assimilate partitioning, and respiration, all influencing plant growth and leading ultimately to economic and other numerous implications in human society. Drought stress reduces the rate of cell expansion and division, causes smaller leaf size and shorter stem elongation and root proliferation, and lowers crop productivity by disturbing stomatal oscillations (Farooq et al. 2009; Li 2009). Several environmental models predict an increase of drought frequency and intensity as a consequence of global climate change. Additionally, plant genetic mechanisms involved in environmental stress tolerance are often lacking a significant portion of variance, which is again attributable to the environment, in “genotype-environment” interactions (Lesk et al. 2016; Xu 2016).

8.3 Bioinoculants as a Sustainable Tool

Bioinoculants refer to plant beneficial microbes which are introduced or resident living organisms in the rhizosphere (or phyllosphere) to control phytopathogens and enhance plant growth (Ilangumaran and Smith 2017; Singh and Jha 2017). Rhizosphere microorganisms are potential bioinoculants, a sustainable resource for enhancing plant productivity by alleviating plant biotic and abiotic stress through induced systemic tolerance (IST) in plants by altering physical and chemical processes without affecting plant metabolism (Yang et al. 2009; Wang et al. 2012; Singh et al. 2013; Jegan et al. 2016). Potential plant growth-promoting rhizobacteria (PGPR), belonging to genera *Rhizobium* (Peláez-Vico et al. 2016; Kamran et al. 2017), *Pseudomonas* (Saravanakumar and Samiyappan 2007; Mavrodi et al. 2018; Sekar et al. 2018), *Azospirillum* (Dardanelli et al. 2008; Naqqash et al. 2016), *Pantoea* (Kamran et al. 2017), *Bacillus* (Wang et al. 2012; Thijs et al. 2014; Giri et al. 2017), *Enterobacter* (Nadeem et al. 2007; Naqqash et al. 2016), *Azotobacter* (Mishra et al. 2016), *Bradyrhizobium* (Aliasgharzad et al. 2006; Afzal et al. 2010), *Methylobacterium* (Dourado et al. 2013), and *Burkholderia* (Ait Barka et al. 2006; Thijs et al. 2014), are reported to be involved in the alleviation of plant biotic and abiotic stresses.

Arbuscular mycorrhizal fungi (AMF) is a potential beneficial soil fungi which forms a symbiotic relationship with plant by colonizing in roots and plays a crucial role in the promotion of plant growth (Igiehon and Babalola 2017; Saxena et al. 2017). Particularly, AM structures (e.g., arbuscules and hyphae) are involved in H_2O_2 accumulation within the roots and transfer of phosphate to the host plant (Huang et al. 2017; Mishra et al. 2016; Oyewole et al. 2017). For the past few decades, plant-microbe interaction which revealed application of bioinoculants increased crop biomass, disease suppression, and alleviation of abiotic stress (Singh et al. 2013; Grover et al. 2010; Yang et al. 2009). Particularly combined application of potential bioinoculants in a "microbial consortium," a synergistic mixture of potential microbes, holds higher efficacy and a potential solution for sustainable eco-friendly agriculture (Jain et al. 2013; Thijs et al. 2014; Wang et al. 2012; Sekar et al. 2016).

8.4 Bioinoculant-Mediated Drought and Salinity Stress Protecting Strategies

Bioinoculants are reported to potentially alleviate plant abiotic stresses by inducing IST through mechanisms like ACC deaminase production (Glick 2005; Barnawal et al. 2017), accumulation of carbohydrates (Al-Garni 2006), high stomatal conductance (del Amor and Cuadra-Crespo 2012), osmolyte accumulation (Singh and Jha 2017), exopolysaccharide (EPS) production (Sandhya et al. 2009; Kamran et al. 2017), increased water uptake and aquaporin-mediated distribution to plant tissues (Saxena et al. 2017), and production of ectoines, compatible solutes, trehalose, and glycine betaine by several PGPR and AMF, which aid in protecting plants and help plants in recovering from stress conditions (Mayak et al. 2004; Park et al. 2007; Wood 2011). AMF potentially limits the overproduction of reactive oxygen species (ROS) in plants under drought conditions and enhances antioxidant enzymes like superoxide dismutase and catalase activities and also nonenzymatic antioxidants like ascorbate and glutathione concentrations (Huang et al. 2017).

8.4.1 Hydraulic Lift

Deep-rooting plants under drought conditions may transport water upward, from the more moist subsurface soil layers through the root axis to the dry topsoil along the water potential gradient, a process known as "hydraulic lift" (HL). Hydraulic lift potentially works in intercropping systems, particularly in combination of deep- and shallow-rooting planting systems. In the top soil, intercropped shallow-rooting plants may profit from the lifted water (Prieto et al. 2012; Liste and White 2008), a process that has also been called, quite fittingly, "bio-irrigation" (Liste and White 2008; Saharan et al. 2018). This term is frequently used by marine biologists for a process more adequately called "bio-turbation," i.e., the supply of water to deep sediment layers in water bodies through the activity of marine animals (Kristensen et al. 2012).

The potential use of the so-called hydraulic lift, also called more precisely hydraulic redistribution (Burgess and Bleby 2006), is one interesting aspect of intercropping deep-rooting and shallow-rooting plants. In this context, AMF used as "biofertilizers" may play a fundamental role in the redistribution and use of available water. On the one hand, AMF may form a common mycorrhizal network (CMN) and a bridge between the deep-rooted and the shallow-rooted plants and thereby provide water to the latter. The potential of such a CMN for water delivery was shown in several studies (Egerton-Warburton et al. 2007; Querejeta et al. 2011), and theoretical considerations indicated that the hyphae of AMF may redistribute water at a much higher rate than commonly thought (Allen 2007; Akhtar and Siddiqui 2008). On the other hand, the water redistributed by AMF may also be delivered to the dry soil to promote the activities of PGPRs and help them to mobilize nutrients, a possibility that has not received much attention yet. Two recent model studies with mycelia of *Agaricus bisporus* and *Pythium ultimum* have experimentally verified the potential of fungal hyphae to move water along a water potential gradient from moist soil to dry soil and to promote water uptake and metabolism of bacteria in the latter (Guhr et al. 2015; Worrich et al. 2017).

8.4.2 Molecular Mechanisms Involved in Plant Stress Tolerance Induced by Bioinoculants

Despite adequate knowledge about salinity and drought-tolerant genes involved in plant system and overexpression in other plants, knowledge about these genes in the bacterial system that influence plant growth under abiotic stress is little. PGPRs are reported to stimulate tolerance level of plant to abiotic stresses, like drought, salinity, and nutrient deficiency, through several potential mechanisms by stimulating host systematic pathway and exoproducts (Yang et al. 2009). Tolerance to abiotic stress, especially for salinity, is a complex trait which is controlled by multiple genes (Navin et al. 2014), and proteins encoded by these genes protect the bacterial cells from these stresses (Qiu et al. 2011). In transgenic tobacco plants, *E. coli betA* gene was expressed encoding choline dehydrogenase alone or in combination with the *E. coli bet B* gene encoding betaine aldehyde dehydrogenase. The expression of these genes was shown to accumulate glycine betaine and exhibit increased tolerance to salt stress (Holmstrom et al. 2000). In *Arabidopsis* transformation of *codA* gene modestly increased the levels of ascorbate peroxidase, H_2O_2, and catalase in the genetically edited plants, by stimulated expression of H_2O_2 scavenging enzymes (Alia et al. 1998).

Relatively few mechanisms have been unequivocally demonstrated in explaining the increased resistance to environmental stress of plants treated with PGPB. *Paenibacillus polymyxa* B2-treated *A. thaliana* showed increased expression of a drought-response gene, *Early Response to Dehydration 15* (*ERD15*), than control plants (Timmusk and Wagner 1999). Co-inoculation of *Rhizobium tropici* and *P. polymyxa* strains in *Phaseolus vulgaris* stimulates overexpression of trehalose 6-phosphate gene which increased nodulation, N content, and plant growth (Figueiredo et al. 2008). The members of the high-affinity K^+ transporter (HKT)/channel family mediate important stress tolerance mechanisms in plants. Bioinoculants are known to stimulate the nutrient exchange of both macro- and micronutrients and alleviate nutrient imbalance triggered by the high influx of Na^+ and Cl^- ions, particularly in tissue-specific regulations of stressed plants (Zhang et al. 2008). Bioinoculants play a vital role in balancing ion homeostasis and high

K^+/Na^+ ratios in shoots by altering Na^+ and Cl^- accumulation in leaves, enhancing Na^+ exclusion via roots and stimulating the activity of high-affinity K^+ transporters (Ilangumaran and Smith 2017). *B. subtilis* GB03 confers salinity tolerance level in *A. thaliana* by concurrently upregulating and downregulating *HKT1* gene expression in shoots and roots, resulting in the reduction of Na^+ accumulation in plants compared to controls (Zhang et al. 2008). However, Niu et al. (2016) reported that application of PGR to *Puccinellia tenuiflora* showed less Na^+ accumulation validating the upregulation of PtHKT1 and PtSOS1 genes under high salt concentrations (200 mM NaCl).

Estrada et al. (2013) first reported that AMF colonization regulates host maize transporter genes under saline conditions by regulation of genes ZmAKT2, ZmSOS1, and ZmSKOR significantly increasing K^+ and decreasing Na^+ accumulation as compared to non-AMF plants. Colonization of *C. etunicatum* in rice plants enhances salinity by upregulation of OsNHX3, OsSOS1, OsHKT2;1, and OsHKT1;5 genes (Porcel et al. 2016). *Rhizophagus irregularis* (AMF)-colonized black locust plants have been reported to have significantly increased relative water content, decreased shoot/root ratio of Na^+, and upregulated the expression of chloroplast genes (*RppsbA, RppsbD*, and *RprbcL*), aquaporin genes (*RpPIP1;1, RpPIP1;3, RpPIP2;1*, and *RpTIP1;1*), and membrane encoding transport genes (*RpSOS1, RpHKT1*, and *RpSKOR*) involved in K^+/Na^+ homeostasis in roots under salinity conditions (Chen et al. 2017).

8.4.3 Amelioration of Plant Stress by Extracellular Metabolites

8.4.3.1 Extracellular Enzymes

PGPRs produce 1-aminocyclopropane-1-carboxylate (ACC) deaminase (EC 4.1.99.4), which catalyzes the deamination of ACC, an immediate precursor of ethylene synthesis in all higher plants, to produce α-ketobutyrate and ammonia (Glick 1995). ACC deaminase production was not observed in plants, and hence, the presence of ACC deaminase-producing bacteria regulates the stress ethylene level, and most of the plants harbor the ACC deaminase-containing bacteria in seeds, roots, and leaves (Penrose and Glick 2003). Plants grown under stress treated with PGPR showed enhanced ability to overcome stress by lowering stress ethylene levels and preventing inhibition of plant growth in pepper and tomato plants under high concentrations of salt (Mayak et al. 2004). Cheng et al. (2007) have shown the efficiency of ACC deaminase-producing *P. putida* UW4 in facilitating plant growth under salt stress. *P. fluorescens* possessing ACC deaminase activity enhanced the saline tolerance in *Arachis hypogea* plant, which resulted in increased yield compared to the plant treated with *Pseudomonas* strains not having ACC deaminase activity (Saravanakumar and Samiyappan 2007). ACC deaminase-producing *Arthrobacter protophormiae* decreased salinity stress in *Pisum sativum* plants and enhanced nodulation and AMF colonization (Barnawal et al. 2014). Similarly, Gamalero et al. (2008) reported *P. putida* UW4 AcdS$^+$ increased AMF colonization of *G. rosea* in cucumber plant growth than *AcdS* mutant. AMF-colonized plants have potential to increase the activities of superoxide dismutase, glutathione reductase, ascorbate peroxidase, ascorbic acid, total carotenoids, and glutathione which help plants to withstand drought-induced oxidative stress (Pedranzani et al. 2016). Zhu et al. (2012) reported that the AMF *Glomus etunicatum* is capable of alleviating the damage caused by stress by reducing membrane lipid peroxidation and membrane permeability and increasing the accumulation of osmotic adjustment compounds and antioxidant enzyme activity.

8.4.3.2 Exopolysaccharides (EPS)

PGPRs secrete an exopolysaccharide (EPS) substance which is an important material in biofilm formation. The EPS are mainly composed of a heterogeneous mixture of proteins, lipids, polysaccharides, and nucleic acids and denote a highly significant part of the extracellular matrix with a share between 40 and 95% total organic matter in the biofilm (Wingender et al. 1999). They form an enclosed matrix of microcolonies, which confer protection against environmental fluctuations, water and nutrient retention, and epiphytic colonization and make cations, including Na^+, unavailable to plants in binding to them under saline conditions (Alami et al. 2000; Grover et al. 2010). EPS-producing bacteria binds with soil and forms soil particles building microaggregates and macroaggregates which aid in maintaining elevated soil moisture content, stabilizing soil structures, and increasing the water-holding capacity and cation exchange capacity (Naseem et al. 2018). The EPA *P. putida* strain GAP-P45 producing exopolysaccharides (EPS) showed, on sunflower seedlings, to alleviate drought stress by increasing the plant survival rate, biomass, and root-adhering soil/root tissue ratio of sunflower seedlings, subjected to drought stress (Sandhya et al. 2009). Inoculating wheat seedlings with EPS-producing *B. amyloliquefaciens* influences sodium uptake restriction and stimulates plant growth under high salinity stress conditions (Ashraf et al. 2004). Alami et al. (2000) reported an increase in the *root-adhering soil* and *root tissue* ratio (RAS/RT ratio) on EPS-producing *Rhizobium* sp. YAS34-treated sunflower plants under drought conditions. Similarly, wheat plants treated with *Pantoea agglomerans* showed enhanced growth and yield under salt conditions (Amellal et al. 1998). Co-inoculation of EPS-producing *P. mendocina* and *G. intraradices* to *Lactuca sativa* enhanced the aggregation of the soil matrix (Kohler et al. 2006).

1-Aminocyclopropane-1-Carboxylate (ACC) Deaminase

PGPRs produce 1-aminocyclopropane-1-carboxylate (ACC) deaminase (EC 4.1.99.4), which catalyzes the deamination of ACC, in all higher plants the immediate precursor of ethylene, to produce α-ketobutyrate and ammonia (Glick 1995). ACC deaminase production was not observed in plants, but many plants harbor ACC deaminase-containing bacteria in seeds, roots, and leaves as their presence regulates the stress ethylene level (Penrose and Glick 2003). Plants grown under stress treated with PGPR showed enhanced ability to overcome stress by lowered stress ethylene levels and prevented inhibition of plant growth in pepper and tomato plants under high salt concentrations (Mayak et al. 2004). The efficacy of ACC deaminase-producing *P. putida* UW4 to facilitate plant growth under salt stress conditions was shown by Cheng et al. (2007). The saline resistance in groundnut plants (*Arachis hypogea*) was enhanced by *P. fluorescens* possessing ACC deaminase activity, resulting in yield increase compared to groundnuts treated with *Pseudomonas* strains lacking the ACC deaminase activity (Saravanakumar and Samiyappan 2007). ACC deaminase-producing *Arthrobacter protophormiae* decreases salinity stress in *Pisum sativum* plants, enhancing nodulation and AMF colonization (Barnawal et al. 2014). Similarly, Gamalero et al. (2008) reported *P. putida* UW4 AcdS$^+$ increased AMF colonization of *G. rosea* in cucumber plant growth than *AcdS* mutant.

8.4.3.3 Osmoprotectants

Plants maintain the osmotic stress through altering biological and physical systems like enhancing the antioxidant systems and osmolytes. Production of antioxidant and accumulation of osmolytes are indicators of salinity, and other abiotic stress leads to production of polyols (mannitol, inositol, glycerol), amino acids (proline), and betaines (glycine betaine) that function as osmolytes, which accumulate in the cytosol to maintain osmotic balance in the cell and function as osmoprotectants by inhibiting dehydration of membranes and stabilizing dehydrated enzymes rather than playing a role in osmoregulation (Kempf and Bremer 1998). PGPR produces and accumulates large quantities of osmoprotectants like proline, glycine betaine, and trehalose in their cytosol under osmotic stress which is faster than associated host plant (Ilangumaran and Smith 2017).

A fundamental phenomenon of osmoregulation is exhibited by bacteria to uphold the osmotic balance between cellular fluids and the external environment (Wood 2011). Secretion of osmolytes by plant-associated PGPR aids in maintaining osmotic balance in plants and protecting plants from cellular oxidative damage under saline conditions. Under stress conditions, plants are reported to accumulate glycine betaine (GB) and proline that are often synthesized by plants in response to various abiotic stresses, mediating osmotic adjustment, free radical scavenging, and subcellular structure stabilization (Ashraf and Foolad 2007; Kumari et al. 2015). PGPR-inoculated plants showed a significant concentration of GB and proline with higher biomass than control plants and the osmoprotectants are not group specific (Farwick et al. 1995; Ait Barka et al. 2006; Ashraf and Foolad 2007; Kumari et al. 2015).

Ectoine is a common compatible solute in halophilic bacteria (Csonka and Epstein 1996) and is reported to be involved in the maintenance of root function for the uptake of water and distribution to shoot, enhance transpiration rate through nitrogen supply to leaves, and protect Rubisco proteins which improve the photosynthesis process particularly under saline conditions (Moghaieb et al. 2006). Several studies showed exogenously provided ectoine alleviates osmotic stress in several microbial and plant systems, and also ectoine biosynthesis in tobacco plant showed tolerance to hyperosmotic shock (Nakayama et al. 2000; Moghaieb et al. 2006; Widderich et al. 2014).

Mycorrhizal colonized plants often have greater tolerance to drought than nonmycorrhizal plants, and several nonnutritional mechanisms have been proposed for explaining host plant protection by AMF symbiosis against drought-induced detrimental effects (Al-Karaki et al. 2004). Many reports showed AMF stimulates plant growth under salt and drought stress conditions by enhancing host plant nutrition uptake, higher K^+/Na^+ ratios in plant tissues, and a better osmotic balance by accumulation of compatible solutes such as proline, glycine betaine, or soluble sugars (Porcel et al. 2012; Igiehon and Babalola 2017; Saxena et al. 2017; Saharan et al. 2018). Dual inoculation of *Rhizobium* and AMF enhanced plant nutrition, growth parameters, and proline concentration in *Acacia saligna* under salt stress condition (Soliman et al. 2012). Co-inoculation of *Glomus clarum* and *A. brasilense* increased plant nitrogen content in cowpea by 230% against 151 and 94% in plants inoculated separately with nitrogen-fixing bacteria and AMF, respectively, at 7.2 dS/m salinity (Rabie et al. 2005). Porcel et al. (2016) reported inoculation of *C. etunicatum* in rice plants enhanced salinity stress by decreasing the Na^+ concentration from root to shoot and increasing accumulation of Na^+ in the roots.

8.5 Intercropping System

Intercropping or mixed cropping describes a farming system where two or more crop species or genotypes are grown together and coexist on the growing area for a certain time. It is an ancient farming practice and is still practiced by many subsistence farmers. Such practices have not survived the introduction of modern highly mechanized intensive agriculture. However, now mixed cropping or intercropping is discussed again as an option for a sustainable intensification of agriculture (Brooker et al. 2015). It increases the diversity in agriculture by combining two or more crop species or genotypes. They are planted at the same time or they overlap for a certain time, also called relay cropping. Plants can be sown by hand or in regular rows. This practice can increase yields on a given piece of land compared to monocropping of one of the crops, and this is called overyielding. Interspecific plant interactions change the use of resources by making complementary use of them or by facilitating the uptake for the other crop (Vandermeer 1989; Zhang and Li 2003). A complementary use of resources can happen through various mechanisms like complementary root architecture which enables the plants to make the best use of space for taking up nutrients and water. Facilitation can be achieved by combining cereals with legumes which can fix nitrogen and may make a part of this nitrogen available to cereals (Hauggaard-Nielsen and Jensen 2005; Li et al. 2007). But also pest and pathogen pressure is reduced in intercropping systems: Mixing genotypes of a given crop may combine different modes of resistance to pathogens and reduces yield losses (Mundt 2002). But the coexistence of two crops also depends on abiotic factors like nutrient availability, soil type, or climate (Wang et al. 2007). Mixed cropping can act as a buffer against extreme events when one crop is more resilient than the other which stabilizes yields over time. Because of the increased resilience it is often discussed to be especially suited for marginal lands (Qiao et al. 2015).

8.6 Bioinoculants in Alleviating Biotic and Abiotic Stress

A number of species of PGPR are reported to mediate abiotic stress tolerance in plants, e.g., *Azospirillum brasilense* mediated salt tolerance in pea (Dardanelli et al. 2008), lettuce (*Lactuca sativa*) (Barassi et al. 2006), maize (*Zea mays*) (Hamdia et al. 2004), chickpea (*Cicer arietinum*), and faba beans (*Vicia faba*) (Hamaoui et al. 2001); *Pseudomonas* spp. conferred salt tolerance in maize (Nadeem et al. 2007); and *P. fluorescens* in groundnut (*Arachis hypogea*) (Saravanakumar and Samiyappan 2007). Saline-tolerant *Halobacillus* sp. SL3 and *Bacillus halodenitrificans* PU62 increased dry weight (17.4%) and root elongation (90%) on wheat seedlings grown at 320 mM NaCl stress, in comparison to uninoculated seedlings, indicating a significant reduction of the deleterious effects of NaCl (Ramadoss et al. 2013). Salt-tolerant isolates *Bacillus pumilus* and *Arthrobacter* sp. enhanced the growth of wheat under salinity stress (Tiwari et al. 2011). Jha and Subramanian (2013) reported treatment of *P. pseudoalcaligenes* and *B. pumilus* in the salt-sensitive GJ17 rice cultivar showed potential growth promotion than control under salinity stress by reducing lipid peroxidation and superoxide dismutase activity, ROS by plant cell membrane index, cell caspase-like protease activity, and programmed cell death.

8.7 Cross-Kingdom Communication

The communication between trans-kingdom is an important instrument in maintaining the balance and relationship among a diverse group of macro- and microorganisms. Particularly, bacteria secrete a variety of signal molecules for communication across interspecies, intraspecies, or intra-kingdom (Atkinson and Williams 2009). Gram-negative PGPRs synthesize small signal molecule *N*-acyl-l-homoserine lactone (AHL), while gram-positive PGPRs synthesize short peptides, and this process of signals is referred to as quorum sensing (QS) (Boyer and Wisniewski-Dye 2009; Hartmann et al. 2014). QS enables bacteria to intercommunicate and to act as a multicellular organism, attaining benefits that could not be obtained when bacterial cells function alone (Bassler and Losick 2006). AHL-producing bacteria obtain an important role in growth and health of plants by regulating phenotypic traits such as root colonization or inducing systemic resistance and in the production of antibiotic and antifungal compounds regulated by AHL molecules (Schuhegger et al. 2006; Schenk and Schikora 2014). QS signals play a vital role in bacterial gene expression for the survival, establishment, and interaction with other communities (Boyer and Wisniewski-Dye 2009).

The AHL signals modulate the signal transduction process in mammalian and plant cell (Telford et al. 1998; Mathesius et al. 2003). Signals from host regulate the expression of bacterial genes (Gao et al. 2003; Sperandio et al. 2003). The rhizosphere habitat turns out to be a privileged environment for QS signaling since it harbors a significant density of microorganisms. QS molecules significantly influence plant to respond more robustly and rapidly to protect from stress conditions (Schenk and Schikora 2014). AHL signals from *Serratia liquefaciens* MG1 and *P.*

putida IsoF highly induced systemic resistance in tomato plants against *Alternaria alternata* by activating the defense genes involved in the ethylene production of salicylic acid (Schuhegger et al. 2006). QS molecule oxo-C14-HSL-triggered oxylipin/salicylic acid signaling pathway induces stomata defense responses to protect the plant from drought stress and acts as a physiological barrier, preventing the entry of pathogens by strengthening the cell wall (Melotto et al. 2008; Schenk and Schikora 2014). Consortia of PGPR and AMF increase the colonization efficiency of AMF compared to AMF alone (Vosátka and Gryndler 1999; Hernández and Chailloux 2004), and the nodulation efficiency of rhizobia is enhanced by the interactions between PGPR and rhizobia (Guiñazú et al. 2009). Roots from host plants secrete compounds, mimicking quorum sensing (QS) signals of bacteria for stimulation or repression of QS-regulated responses of associated bacteria. Gao et al. (2003) identified 15 AHL mimicking compounds from the exudates of *M. truncatula* seedlings and in several other plants, having specific effects on QS-regulated behavior in bacteria (Teplitski et al. 2000; Daniels et al. 2002; Sanchez-Contreras et al. 2007).

Strigolactones (SLs) are multifunctional molecules which are important signaling factors in controlling the developmental and functional regulation processes in plant. They are crucial in the cross-kingdom communication by triggering microbial gene regulation, especially in the regulation of hyphal branching in arbuscular mycorrhizal fungus (AMF) symbiosis (Proust et al. 2011). SLs are carotenoid-derived compounds that are considered plant hormones and are generally involved in the morphological changes in plant, such as regulation of stem branching, root elongation, root hair formation, increase of stem thickness, and boost of leaf senescence (Rameau 2010; Delaux et al. 2012; Al-Babili and Bouwmeester 2015). These molecules are exuded by plant roots when plants face nutrient-deficient conditions, especially phosphate limitation. Many studies showed that SLs enhance the AMF colonization under phosphate-deficient conditions and also enhanced nodule formation (Rameau 2010; Proust et al. 2011; Peláez-Vico et al. 2016; Dawwam et al. 2013). Subramoni et al. (2011) reported AHL mimicking compound from plant regulates the expression of genes that are engaged in the production of antibiotic AHL-negative *P. fluorescens.* The bacteria *P. fluorescens* 2–79 shows an antagonistic activity against several phytopathogens and was isolated from the rhizosphere of wheat.

8.8 Consortial Products in the Alleviation of Plant Abiotic Stress

Sustainable systems require a deep understanding of plant-microbe interactions with different combinations to alleviate plant stress and enhance growth promotion. Several studies reported combination of bioinoculants enhanced the plant abiotic tolerance level than the single and non-inoculated without affecting growth and yield of the plant (Ruiz-Sanchez et al. 2011; Thijs et al. 2014; Singh et al. 2013; Sekar et al. 2016). Potentially, AMF-colonized plants significantly interact with multiple PGPRs and showed a promising approach to enhance plant tolerance level to drought and salinity stress and productivity (Singh et al. 2013; Wang et al. 2012; Barnawal et al. 2014; Mishra et al. 2016).

Co-inoculation of AMF with PSB significantly enhances yield and growth of tomato than control treated plants (Bona et al. 2017). Consortia of PGPR and AMF increase the colonization efficiency of AMF than AMF alone (Vosátka and Gryndler 1999), and the interactions between PGPR and rhizobia significantly increase the nodulation efficiency (Guiñazú et al. 2009). Consortium of ACC deaminase-producing PGPR *P. putida* UW4 and *Gigaspora rosea* stimulates the colonization of AMF and abundance of arbuscule, enhanced plant growth and leaf area, and increased the photosynthetic performance index of cucumber (Gamalero et al. 2008). Dual inoculation of *Glomus intraradices* and *A. brasilense* protects rice plant from drought stress by enhanced ascorbate accumulation (Ruiz-Sanchez et al. 2011).

8.9 Development of Potential Products and an Alternative Approach

The application of biofertilizers is an alternative and environmentally friendly path to a sustainable agriculture, with plant growth-promoting rhizobacteria as members of such a potent group of microorganisms. Numerous fungi and bacteria are able to establish symbiotic or close associations with plants, improving therewith nutrient uptake, pathogen immunity, or stress tolerance and by this the general development and growth of colonized plants. Therefore, a clear understanding of the various interactions and mechanisms that govern the mutual benefits between these microorganisms and the host plants is required to make use of these microbes as beneficial biofertilizers. Efforts to develop and optimize biofertilizers are ongoing, and various properties of plant growth-promoting microbes to improve the bioformulations for application in integrated soil management are discussed widely.

The actually emerging field of biofertilizer application as an integral component of agricultural practice has started with the successful use of microorganisms in few countries of the developing world and is expected to gain wider attention and acceptance with the course of time (Weekley et al. 2012). A reasonable expectation for future development is that intense use of microorganisms will offer several strategies for an overall agricultural crop management based on biofertilizers. Commercial biofertilizer products are already available on the market and in use to improve crop production efficiency. A further, more widespread application of microbe-based biofertilizers requires to address open issues with more attention and a clear focus to resolve them (Gamalero et al. 2008):

(i) Effective and commercial competitive multifunctional biofertilizers are to be developed and selected for a broad variety of important crops.
(ii) The upscaling of biofertilizer application from scientifically guided laboratory and greenhouse experiments for use in industrial or privately owned farming requires several new approaches for production, distribution, warehousing, and training/marketing of these microorganism-based formulations.

(iii) Education of farmers and potential distributors is required to inform and convince them about the long-term benefits of using biofertilizers instead of using chemical fertilizers. The adverse and life-threatening effects of prolonged use of chemical fertilizers should also be highlighted. The misconception about bacteria – that they only act as agents of diseases – needs to be corrected before the public starts accepting the deliberate release of biofertilizer into the environment on a large scale.

(iv) While the first generation of biofertilizers will be out of non-transformed bacterial strains, selected for certain positive traits, invention of genetically engineered strains which are more efficacious in stimulating plant growth is required. However, scientists will need to prove, to both the public and regulatory agencies worldwide, that genetically engineered strains do not present any new hazards or risk.

(v) A stringent quality control system must be put in place for the production of inoculants and their application in the field, ensuring safe products and exploring the benefits of plant-microorganism symbiosis. A “Biofertilizer Act” and strict regulation for quality control in markets and application have to be established. The microbial persistence of biofertilizers in soil environment under stressful conditions should be studied. Agronomic, soil, and economic evaluation of biofertilizers for diverse agricultural productions has to be done.

8.10 Conclusions and Future Prospects

Till date, several high-quality researches indicate significant osmoprotectant functions in plants, grown under abiotic stress of drought and saline conditions. For example, levels of endogenous osmoprotectants or establishing gene(s) of their biosynthetic pathways appear to initiate a broad band of biochemical pathways, which may increase the drought and salt tolerance of plants. The evolution of adaptive mechanisms, evolutionarily developed by plants as defense mechanisms against extreme environment conditions, may be utilized as a valuable resource for the development of crops safeguarded from extremities. The use of technologies like genomics, metabolomics, or proteomics has proven highly valuable in the research and identification of the underlying genetic structures governing the drought and saline tolerance mechanisms of plants. Genetic research on saline-tolerant species and their individual regulation of the stress tolerance mechanisms will be obligatory and will enable to translocate these protective mechanisms onto other drought- or saline-sensitive crops and to augment their abiotic tolerance level for higher yield on irrigated and marginal soil. Additional research in the regulation of endogenous osmoprotectant metabolism, at physiological and biochemical level, will honor the role of osmoprotectants in many plant functions and will contribute to dealing with adverse drought and saline stress conditions.

The declining crop productivity is a detrimental consequence of the rising environmental stress exerted onto the agricultural production area, which is becoming a serious problem. The overdependency on pesticides and chemical fertilizers is a result of the need to comply with the nutritional demand of the rising population and the short-term solution from the industry in the form of artificial pesticides and fertilizers, not considering the holistic approach of sustainability. These chemicals not only develop negative side effects in the midterm and influence the ecological balance in a unfavorable way but may become also hazardous for humans as they partly accumulate in nature and find their way to the consumers of agricultural products. In this derogatory situation, the use of biofertilizer and biologically based pesticides (biocontrol agents) may become a viable alternative to produce the needed nutrition for the expanding population but also has the potential to turn to a sustainable food production where mankind do not constantly need to solve the problems created one generation before. For this it is mandatory to realize the beneficial aspects and emphasize the importance of widespread biofertilizer application nowadays in agriculture. Even though biofertilizers show a huge potential for boosting agricultural productivity, the most critical factor for a sustainable augmentation of farming yields is the holistic approach to determine the most suitable microorganism for a fruitful interaction with plants in a particular environmental situation.

Even though biofertilizer utilization is accelerating and prospering, the technology is still in the evolutionary stage. The new possibilities resulting from the valuable findings of molecular biotechnology research will elucidate the interactions between plants and related microorganism. The combination with the current progress in microbial science technology, plant-pathogen relationships, genetic engineering, and genomics will additionally accelerate the development and optimization of the needed procedures to broaden biofertilizer usage.

The broader acceptance of biofertilizer usage will rely on innovations related to beneficial microorganisms and the invention of strategies for their simple and efficient application in the daily farming business. This includes the need for additional research and extensive development of temperature-resistant strains and bioformulations allowing a successful marketing and application of end products. The requirement to address the efficacy of the current biofertilizer mechanisms towards exploitation in the actual agricultural environment and the need to identify the properties and function of various biofertilizer strains will create the most important and challenging part of future research.

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9

Role of Signaling Pathways in Improving Salt Stress in Plants

Rabia Amir, Faiza Munir, Ghulam Kubra, Irum Nauman, and Norina Noor

Abstract

Salinity is one of the major agricultural constraints exhibiting severe damage to crop productivity. Multivariate signaling pathways trigger plant's tolerance against salt stress and promote survival via restoring cellular homeostasis. These signal transduction pathways comprise of osmotic and ionic homeostasis pathways, detoxification response pathways for repair and injury control and the growth regulation signaling pathways. The salt overly sensitive (SOS) pathway represents another prominent signaling pathway, arising as a major defence strategy in controlling ion homeostasis. Additionally, the downstream adaptive responses against salinity stress are prompted by mitogen-activated protein kinase (MAPK) and sucrose non-fermenting 1-related protein kinase 2 pathways. Recent investigations suggest that reactive oxygen species (ROS) have a major role in regulating the crosstalks of stress-induced hormonal signaling and endogenously elicited redox and metabolite signals. Thus, the aim of this chapter is to focus on the salt stress-mediated signal transduction pathways including major plant transcription factor (TF) families coordinating regulatory networks underlying salt stress tolerance.

Keywords

Salt stress · Signal transduction pathways · SOS · ROS · Transcription factor

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9.1 Introduction

Plants have adopted complex biochemical, molecular and physiological mechanisms in order to perceive and respond to multiple abiotic stresses (Zhu 2016). Plants are exposed to a variety of abiotic stress factors such as drought, cold and salinity which are the main reasons in reduction of crop yield and limiting factors for their growth and production (Savvides et al. 2016). Plants may share common molecular and biochemical signaling pathways to cope with multiple stress factors. Due to extensive crosstalk between the regulatory pathways against abiotic stresses, it is difficult to understand and comprehend the underlying mechanisms of stress acclimation in plants. However, the perception of stress factor is accomplished by more than one sensor (Foyer et al. 2016).

Once the signal reaches multiple primary sensors, secondary signals then initiate the cascade of signaling events. The early signaling events involve cytoplasmic free Ca^{2+} concentration and production of secondary signaling molecules as reactive nitrogen forms (RNF), abscisic acid (ABA) and reactive oxygen species (ROS) (Nguyen et al. 2016). They activate the MAPK cascade, phosphatases and calcium-dependent protein kinases (CDKPs) that in turn can dephosphorylate or phosphorylate specific proteins and finally initiate the activation of different TFs (Ranty et al. 2016). Plant responses in later stages of stress comprise activation of stress-responsive genes or the regulation of signaling proteins by the initiation of their synthesis or deprivation according to the stress stage, further activating other advanced defence mechanisms (Zhu 2016). Among other abiotic stresses, salinity is one of the main limiting factors of plant growth and crop productivity. Salts occur naturally in the soils and salinization may be produced by natural methods such as steady withdrawal of an ocean or by mineral weathering or by the use of irrigation water (Negrão et al. 2017). Around the world soils affected by salinity are estimated to be approximately 830 million hectares and about 20% of all irrigated lands are salt affected. Globally, more than 30% of agricultural products come from irrigated cultivated land which contribute to about 17% of the total land.

Soil salinity affects plant growth and yield distressing agriculture globally. Salt build-up in agricultural lands is mostly resulting from irrigation water that contains trace amount of salt (El Naim et al. 2012). This salt accumulation results in decreased capability of plants to absorb water resulting in dehydration. Similarly elevated salinity levels lead to ion (Na^+) toxicity, hyperosmotic stress and secondary stresses, for instance, oxidative damage (Zhang et al. 2016). In plants, roots are the primary organs which sense the increase in Na^+ ions and the hyperosmotic component. The increase of Na^+ and Cl^- in root tissues negatively affects growth by impairing photosynthesis and other metabolic processes (Chhabra 2017). Hence, salt stress has both ionic and osmotic stress components. Ionic stress occurs when salts enter into plants and alter the K^+ to Na^+ ratios and increase Na^+/Cl^- ion concentration which are detrimental because of their negative effects on important processes, including enzymatic activity, protein metabolism and balance of plant growth regulators (Gupta and Huang 2014). When salt concentration increases inside the plant, the salt starts to accumulate inside the older leaves and eventually they die. If these older leaves die at a rate more than that at which new leaves generate, it reduces the capacity of plants to provide the carbohydrate necessity for younger leaves, ultimately reducing the rate of growth (Negrão et al. 2017). This phase may be recognized by the appearance of some specific symptoms of plant damage in leaves such as colour change, tip burn, marginal necrosis and succulence. The shortening of lifetime of individual leaves results in growth and yield reductions in plants which ultimately lead to reduction in overall crop productivity (Parihar et al. 2015). Similarly, osmotic stress is severe than ionic stress, except in resistant plant species which lack the ability to store Na^+ in the cell vacuole or in cases where salt concentrations are very high in the growth medium (Sathee et al. 2015). In addition, salinity brings about several nutritional disorders in plants by affecting nutrient availability, their transport and portioning within plants. Plant osmotic sensors possibly localize adjacent to Ca^{2+} channels because Ca^{2+} increases in response to NaCl are very rapid within seconds. Therefore, it is reported that osmosensor may be closely coupled to Ca^{2+} channels (Kurusu et al. 2015). In recent studies, one plasma membrane protein OSCA1 was proposed to be an osmosensor. OSCA1 is capable of forming hyperosmolality-gated calcium-permeable channels responsible for Ca^{2+} increase, induced by osmotic stress (Demidchik and Shabala 2018). When osmotic stress stage is reached, it lowers the rate of generating new leaves, leaf expansion reduces and the lateral buds develop resulting in lesser branching or generation of lateral shoots leading to fewer branches or lateral shoot formation in plants (Hazman et al. 2016). When salts accumulate in the soil medium, its osmotic potential is reduced, preventing the flow of water and nutrients over the root membrane, thus reducing the rate of plant growth and development. Moreover, osmotic stress includes lowering of stomatal conductance and reduced rate of photosynthesis (Parihar et al. 2015). Thus, the aim of this chapter is to focus on the salt stress-mediated signal transduction pathways including major plant transcription factor (TF) families coordinating regulatory networks underlying salt stress tolerance.

9.2 Salinity Stress in Crops

One of the main abiotic stresses is salinity which negatively impacts crop production throughout the world (Qados 2011). Salt is damaging the cultivable land gradually and inhibits the nutritional potential of agricultural yield; thus recovery of such affected soils is necessary (Läuchi and Epstein 1984). Salt affected soil and irrigation water are harmful for crops as it creates stressed condition in them. For example, wheat, rice and maize, the world-renowned staple crops, are severely affected by high salinity. Out of these major crops, rice is inherently highly sensitive to salt stress; advanced salinization is becoming an enormous constraint in rice production. Specifically at early stages of vegetative production and late stages of reproduction, rice crop shows more vulnerability to such stress. It has been reported that rice shows resistance to stresses at the reproductive and grain filling stage as compared to vegetative and germination stages (Espe et al. 2017).

Studies suggest that in rice, salinity affects seedling development, its growth and grain yield components negatively. Moreover, intense salt stress in rice significantly changes the leaf anatomy, thus reducing chlorophyll content and permeability of the membrane. In the case of wheat which is the most important food grain all over the world, salinity affects its growth phase by compelling wheat to mature early. Root is the potential fundamental plant organ which can shift in order to avoid salt stress (Singh et al. 2017b). A study conducted on root development in wheat indicated that the root is more responsive to salinity. Furthermore, it is reported that under salt stress the rate of leaf expansion and area of flag leaf shrink (Shaar-Moshe et al. 2017). In maize crop under salinity, there exists excessive accretion of Na^+/Cl^- ions in the rhizosphere thus resulting in the disruption of nutritional balance because of the interaction of these ions with other minerals such as zinc, manganese, phosphorus, magnesium, iron, nitrogen, calcium, copper and potassium (Celik et al. 2010). Uptake of these nutrients is low during salt stress (Hadi and Karimi 2012). Likewise in maize, stomatal movements are disturbed by Na^+/K^+ transport causing severe water loss and leaf necrosis (Ferreira et al. 2017).

Reactive oxygen species (ROS) are produced in excess amount in maize against salt stress to lower the damage triggered by oxidation (Miller et al. 2010). Salinity affects the plant in numerous ways including suppression in the amount of nutrients absorbed and osmotic stress. Additionally, it influences biomass allocation patterns, biochemical reactions and physiological processes (Shao et al. 2008), thus disturbing the growth and developmental patterns in non-halophytic plants. Halophytic and non-halophytic plants shows diverse responses to salt stress (Vicente et al. 2004). Predominantly, plants deal with salt stress either by avoiding or tolerating. To cope with osmotic stress and ion imbalance, many plants trigger their defence mechanisms which exclude excessive salts from cells, and some plants tolerate their presence in the cells. Additionally, molecular and biochemical reactions are activated to display their part in improving salt tolerance mechanisms. Secondary effects are detected in plants against hyperosmotic stress and ion imbalance (Chaves et al. 2009; Fig. 9.1).

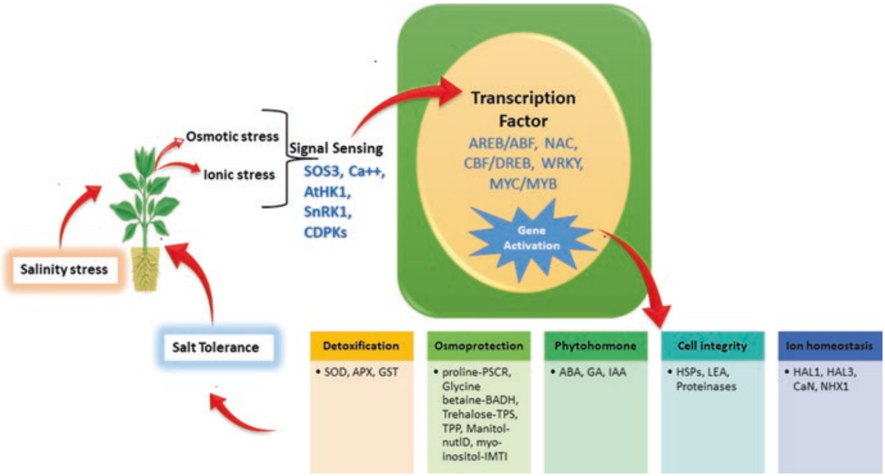

Fig. 9.1 A generalized schematic representation of salinity stress tolerance mechanism in plants

The SOS signaling pathway is recognized as a crucial regulator of plant ion homeostasis and salinity (Mahajan et al. 2008). In the cytosol, plants maintain higher K^+ concentrations and lower Na^+ concentrations under salt stress, and this is done by activating and modifying K^+/Na^+ transporters and H^+ pumps which are main regulators for transportation processes (Parida and Das 2005). Studies showed that protein kinase complex comprised of a calcium-binding protein SOS3 and salt stress-elicited Ca^{2+} signal initiates the SOS2 serine/threonine protein kinase. After that the compound phosphorylates and helps in activating several ion transporters, for example, Na^+ and H^+ antiporter SOS1 of plasma membrane (Sun et al. 2018).

9.3 Salt Uptake, Partitioning and Remobilization

Highly saline soil is one of the key environmental constraints to crop yield. Highly saline soils hold a variety of salts, for example, $MgSO_4$, KCl, Na_2SO_4, Na_2CO_3, $MgCl_2$ and $CaSO_4$, but normally dominated by NaCl (Khot and Joshi 2016). Growth inhibition is among the most popular effects of soil salinity for numerous reasons; however, it is frequently linked with K^+ deficiency as well as high internal Na^+ concentration. Thus plants have established a specific mechanism for transporting sense and react against multiple Na^+ circumstances. Thus, in the presence of excess Na^+, plants will attract Na^+ from soil and spread it throughout the plant body (Deinlein et al. 2014).

9.3.1 Sodium Ion (Na^+) Influx at the Plant-Soil Interface

Under a normal physiological state, plants have a mechanism of maintaining high cytoplasmic K^+/Na^+ ratio and negative membrane potential (approximately -140 mV). However, under salinity stress an electrochemical gradient is established which facilitates passive transport of Na + ions from soil to the cytoplasm. Influx of Na^+ ions from soil to root occurs through different transporter and channels (Freedman et al. 2014). The Na^+ influx occurs through specific channels which are non-selective cation channels (NSCCs), including the glutamate receptors (GLRs) and cytosolic nucleotide-gated channels (CNGCs). In plants, it is reported that aquaporins are involved in Na^+ absorption. Roots absorb Na^+ which is then delivered to the xylem with the help of other channels and transporters and ultimately transported to leaves. It is reported that there are about 20 GLRs and CNGC3 in glycophytic *Arabidopsis* plant genome (Shabala et al. 2015). Among these CNGC1, CNGC13 and CNGC4 have been reported to influx Na^+ and traces of K^+, while CNGC2 is involved in K^+ intake over Na^+. However, it is also suggested that CNGC10 is responsible for the conduction of K^+ and Na^+ ions (Julkowska and Testerink 2015). In one study it is reported that CNGC19 and CNGC20 are involved in ionic salt stress in some plants, while in other studies it is reported that these two are involved in reordering of Na^+ ions in plants against salinity rather than uptaking it from soil (Jha et al. 2016).

GLRs are ligand-sensitive NSCCs and are gated by glutamate which may play an important role in Na^+ ion flux in crop plants. It is evident from the experiments using *Xenopus laevis* oocytes that GLRs are involved in uptake of Na^+ ions. Evidence suggested that specific ion pore domains of AtGLR1.4 and AtGLR1.1 possibly play roles in Na^+ ion absorption (Shabala et al. 2015). Similarly, in oocytes the AtGLR3.7 expression endorsed Na^+ permeable influx. Similarly, high-affinity K^+ transporters (HKTs) associate with a transporter family present in plants, fungi and bacteria (Deng et al. 2016).

9.3.2 Long-Distance Transport of Na^+

Xylem is a long-distance transporter of Na^+ to the shoot depending upon Na^+ uptake into the xylem. The overall transport mechanism for xylem stocking of Na^+ is not fully studied but is thought to comprise passive loading (Kumar and Mosa 2015). Another plant system, cation antiporter CHX (cation/H^+ exchanger), is also associated with influx of Na^+ ions in the xylem. Previously, AtCHX expression was found in the endodermal root cells and its has the ability to lose its functioning by decreasing the level of Na^+ in the xylem sap without disturbing Na^+ concentrations in the phloem. However, it is reported that K^+ homeostasis is attained by CHX21 and its homologue CHX23 (Adams and Shin 2014).

When Na^+ reaches the stele in roots, it is then loaded into the xylem, and hence this lengthy delivery of Na^+ occurs via transpiration stream from the root to the shoot. It was proposed that Na^+ loading in the xylem is carried out by transporters which prevail in the plasma membrane. After the apoplastic barrier, there is a supplementary central point where Na^+ ion transport to shoots from the roots can be controlled in plants (Kumar and Mosa 2015). At average salt stress, SOS1 facilitates xylem piling of Na^+ ions, and this loading in xylem is evident by experiments in *Hordeum vulgare* concluding that the acidification in the xylem results in increasing Na^+ accumulation in the xylem sap (Zhu et al. 2015). At high salinity, Na^+ loading in the xylem is carried out by passive transport. The passive loading occurs through the ion channels which are permeable to the Na^+ located at the xylem parenchyma surface, while active loading is carried out by Na^+/H^+ exchange channels (Bojórquez-Quintal et al. 2014). During passive transport depolarized plasma membrane and high cytoplasmic Na^+ accumulation in xylem parenchyma cells would support the piling of Na^+ into the xylem. The crop plants can recover Na^+ ions from the xylem to cells in roots so they prevent higher Na + concentration in plant leaves (Pandolfi et al. 2016). This retrieval of Na^+ is reported at the base of the roots and shoots in crops such as *Glycine max* (Vaishnav et al. 2015), *Phaseolus vulgaris* (Salama et al. 2015) and *Zea mays* (Chen et al. 2017; Salama et al. 2015).

9.3.3 Compartmentation of Sodium Ion (Na^+)

The compartmentation of Na^+ into the vacuoles provides an effective method of overcoming the injurious effects of Na^+ in the cytoplasm. Compartmentation of sodium ion in the vacuoles of plant cells possibly happens in all tissues, and it is the key mechanism to purify Na^+ ion effects on plant. But in normal conditions Na^+ has its own importance as an inexpensive osmolyte in lowering the H_2O potential (Bojórquez-Quintal et al. 2014). However, Na^+/H^+ antiporter mediates the influx of Na^+ into the vacuole by proton-generated electrochemical gradient through H^+-PPiase and H^+-ATPase. Similarly, AtNHX1 overexpression considerably improved the salt tolerance in *Arabidopsis.* However, the role of NAX1 in salt tolerance is observed in other crops like *Solanum lycopersicum*, *Triticum aestivum* and *Oryza sativa* by little manipulation of the expression of NAX1 orthologues through genetic engineering (Suzuki et al. 2016a). Under normal conditions with low Na^+, NHX transporter mediates H^+:K^+ exchange rather than H^+:Na^+ exchange. This is a distinguishing feature of NHX to function in a dual manner and play an important role during salinity stress as it directly impacts cytoplasmic K^+ and Na^+ and stomata and indirectly amends K^+ and Na^+ translocation (Singh et al. 2017a). Cytoplasmic Na^+ ions are additionally regulated through the H^+-driven antiporters like SOS1 that are highly expressed in tip cells of roots. As such cells lack vacuole so vacuolar compartmentation of Na^+ is not possible. So for extrusion of cytosolic Na + ions in apoplast, SOS1 plays a key role in the tissues (Zhu et al. 2015).

9.4 Response of Plants to Salt Stress

The effect of salt stress on plant growth is a two-stage mechanism: the growth of plant is first reduced by a reduction in the soil water potential that is known as osmotic phase, and after this phase, the leaves die because of a rapid accumulation of salt in the cell walls or cytoplasm when the vacuoles can no longer withstand the incoming salts – this phase is known as ionic phase (Taş et al. 2016). It is reported that this salt amassing in older leaves causes their demise consequently decreasing the amount of growth hormones and carbohydrates to the meristem, thus constraining growth. The growth of plant is limited by a decrease in the rate of photosynthesis, and uptaking excessive salts alters the assembly of particular metabolites, thus inhibiting plant growth directly (Zandalinas et al. 2018). The root anatomy (length and root diameter) describes the performance of root and uptake of nutrients and water, thus increasing the replacement rate of water lost in plant. A good extensive system improves shoot growth and adaptation of plants to soil as well as enhanced plant yields, since roots act as an interface between the soil and the plant. A flourished root system suits plants, as it permits them to infiltrate deep into the soil to obtain nutrients and water (Ferreira et al. 2017; Table 9.1).

Table 9.1 Physiological, molecular and biochemical responses of plants under salinity stress

Physiological response	Loss of turgor	Yousuf et al. (2016)
	Reduced leaf water potential	Jiang et al. (2014)
	Decrease in stomatal conductance	Munns (2002)
	Reduced internal CO2 concentration	Hajiboland et al. (2014)
	Reduced photosynthesis rate	Jiang et al. (2014)
	Reduced growth	Rao et al. (2006)
	Leaf senescence	Negrão et al. (2017)
Molecular response	Stress-responsive gene expression	Rivero et al. (2014)
	Genes that control salt uptake and ion transport	Hasegawa et al. (2000)
	Genes with an osmotic protective function	Tang et al. (2015)
	Genes that control cell and tissue growth rate	Rejeb et al. (2014)
Biochemical response	Osmolyte accumulation such as proline, glycine betaine and TSS	Rivero et al. (2014)
	Increased antioxidant enzyme activity such as SOD, APX, GR, GPX and CAT	AbdElgawad et al. (2016)
	Changes in photosynthesis pathway	Jha and Subramanian (2016)
	Ion homeostasis	Hossain and Dietz (2016)

9.5 RNF and ROS Production

As soon as stress hits plants, they have to cope with the production of intensive reactive oxygen species (ROS) and reactive oxygen forms (RNF production). ROS play a dual role depending upon its concentration: in low concentrations, they play a role as signaling molecules in plant cells; conversely, if their level is quickly increased by salt stress, defined as an “oxidative burst”, they become toxic to plants (del Río 2015). ROS molecules that play a signaling role are superoxide radical (O_2-●), singlet oxygen ($1O_2$), hydroxyl radical (●OH) and hydrogen peroxide (H_2O_2). In order to exploit reactive oxygen species (ROS) as signaling molecules, their level must be sustained by a fine balance between ROS scavenging and ROS production pathways (Huang et al. 2012). H2O2 is comparatively stable as compared to other ROS; this favours its transport by diffusion to other cell compartments due to its neutral characteristic or through the membranes via aquaporins, which support its signaling function (Gill and Tuteja 2010). Elevated level of H_2O_2 can be perceived either by the oxidation of certain amino acid residues that may affect phosphorylation of the protein depending on their localization or by different TFs like heat shock factors. Similarly, methionine oxidized by H_2O_2 to methionine sulfoxide can be combined with oxidative signals to transformed protein phosphorylation (Golldack et al. 2011). It was reported that oxidation of Met residues in nitrate reductase (acts as kinase substrate) can prevent the phosphorylation of adjacent Ser residues. Once the signal is perceived by the cells, H_2O_2 influences downstream signaling components by the activation of certain TFs (WRKY, NACTFs), MAP kinases (MEK2 pathway) or miRNAs. Major plant TFs are key players against salt stress (Lee et al. 2012). Many members of the AREB (ABA-responsive element-binding protein)/ABF/ABRE (ABA-responsive cis element-binding factor) family are known to be involved in stress signaling. The role of DREB/CBF (dehydration-responsive element-binding/C-repeat binding factors) was previously reported to be involved in salt stress tolerance and drought stress in *Arabidopsis*. Moreover, the *Arabidopsis* bZIP24, which belongs to bZIP-type AREB/ABF, is involved in reprogramming a wide range of salinity gene expression (Yoshida et al. 2015, Kidokoro et al. 2015). Increased ROS level is toxic for plants as it can cause severe cellular damage by peroxidation and membrane lipid deprivation which lead to protein degradation resulting in cell death. However, a lower level of ROS is involved in plant stress responses. It was reported in the study that nitric oxide (NO) and ROS are produced constantly under many abiotic stresses (Corpas et al. 2011). Nitric oxide performs a dual role as it can damage plant cells or functions as a protective molecule depending on the ROS compounds, localization and concentration (Zorov et al. 2014). It also plays a role as an antioxidant and protects cells from oxidative damages, e.g. by activating antioxidant enzymes through NADPH regulation in *Phaseolus vulgaris* roots under salt stress (Schieber and Chandel 2014). In addition, NO also alleviates osmotic and ionic stress because it acts as a signal for high Na^+/K^+ ratio in the activation of plasma membrane Na^+/H^+ antiporter and vacuolar H^+-ATPase and H^+ pyrophosphatase (Gill and Tuteja 2010).

9.6 Osmotic Adjustment and Osmoprotection

Plants conduct numerous changes in various biochemical and physiological processes in response to salinity (Gupta and Huang 2014). A saline environment causes ion disequilibrium and hyperosmotic stress leading to secondary effects (Hasegawa et al. 2000). Primarily, plants either avoid or tolerate salinity stress. In such cases, either crop plants are inactive throughout the salinity stress or they develop advanced cellular adjustment to tolerate salinity stress. The basic strategy of salt tolerance can be characterized as those that play a role in diminishing ion disequilibrium or osmotic stress or boost the subsequent secondary effects triggered by these stresses.

Plants modify different physiological and biochemical processes by signal perceptions and transduction cascades for survival against drought and salt stresses (Ranganayakulu et al. 2013). To combat these stress factors, crop plants adapt osmotic regulation mechanisms by increasing the concentration of osmotically active compounds or osmoprotectants (Shahbaz and Ashraf 2013; Talaat and Shawky 2013). In order to alleviate osmotic stress brought about by high salinity, plants usually synthesize necessary organic solutes that accumulate in the cytoplasm and organelles. These solutes have an important part in osmotic adjustment and osmoprotection (Kader and Lindberg 2010). Nevertheless, solutes that are stored in cells may fluctuate among plant species as well as within other organisms. The accumulation of low-molecular-weight organic compounds called osmolytes, also recognized as compatible solutes, which are highly soluble and are not affected by normal metabolic reactions as they are harmless even at elevated cellular concentrations, is a metabolic consequence of osmotic stress

(Yancey 2005). Osmotic adjustment is a cellular response to turgor reduction in plants. Initially imbalance in water potential is created by the chemical potential of the saline solution between the symplast and apoplast, which results in decreasing turgor and subsequently causes growth retardation (Bohnert and Jensen 1996). In plants the main function of osmolytes is osmotic adjustment (Hasegawa et al. 2000). Commonly organic osmotic solutes comprise of basic sugars such as fructose and glucose, sugar alcohols as glycerol and methylated inositols and complex sugars like trehalose, raffinose and fructans (Esan and Olaiya 2016). Others consist of quaternary amino acid derivatives as glycine betaine, proline, β-alanine betaine and proline betaine, tertiary amines as 1,4,5,6-tetrahydro-2-mehyl-4-carboxyl pyrimidine and sulfonium compounds as choline-o-sulfate and dimethylsulfonium propionate (Nuccio et al. 1999).

Glycine betaine avoids the stability of the plasma membrane as well as thylakoid after being exposed to saline solutions (Rhodes and Hanson 1993). On the other hand, K^+ and Na^+, if compartmentalized in the vacuole, are chiefly compatible inorganic solutes utilized by the plants exposed to salt stress. To activate the defence strategies of plants under osmotic stress and ionic toxicity, plants at first need to detect the stress and then activate the whole signaling pathway, which is initiated by an alleviated level of cytoplasmic Ca^{2+}, either in synchronization with the modifications in cytosolic pH or independently. Furthermore, several osmoprotectants enhance stress tolerance level when expressed in plants as transgene products (Bohnert and Jensen 1996; Zhu 2001). A modified biochemical role of osmoprotectants is considered as ROS scavenging that is produced under ionic and hyperosmotic stresses and results in membrane degradation and finally cell death (Bohnert and Jensen 1996). A mutual characteristic of these solutes is that they can build up in higher concentrations deprived of interrupting with intracellular biochemistry (Bohnert and Jensen 1996). Hence, these compatible solutes have the ability to uphold enzymatic activity that is present in saline solutions. These solutes have a negligible consequence on the pH balance of the cytoplasm and lumen of different organelles in the cell.

9.7 Signaling Pathways for Ion Homeostasis

Plants are somehow capable to adapt in a saline environment by natural behaviour. Studies showed that Na + toxicity of many crops is frequently conversely identified with the degree of Na^+ accumulation in the shoots (Cotsaftis et al. 2011). A unique system that a plant may utilize in order to reduce accumulation of Na^+ in shoots is to bind the quantity of Na^+ that is transferred from the roots towards the shoots. General plant mechanisms to retain ion homeostasis inside the cell under salinity stress comprise of subcellular Na^+ tissue tolerance, Na^+ compartmentalization and intervention of ion fluxes by transmembrane transporter proteins (Munns and Tester 2008). During salinity stress, in the cytosol plants uphold a lower concentration of Na^+ and very high level of K^+. The major responses towards salinity stress include keeping the cellular ion homeostasis by preventing the build-up of toxic sodium (Na^+) (Tester and Davenport 2003).

SOS signaling is a well-studied signaling pathway essential for regulating ion homeostasis. Stimulation of the SOS signaling pathway has been perceived as a crucial component for exclusion of Na^+ and also acts as a regulator for ion homeostasis at cellular level in plants (Zhu 2000). A few overexpressed genes regulated by this pathway are AtNHX1, SOS1 and SOS3; they evidenced to be a main approach to improve salinity stress tolerance in transgenic plants (Yang et al. 2009). Recently, combinatorial approaches have been utilized to disentangle the novel functions as well as components of the SOS pathway and the roles of other complicated regulatory networks which control ion homeostasis in crop plants.

9.7.1 SOS Pathway of Na^+ Elimination and Partitioning

Plants fight against salinity stress through different mechanisms, including Na^+ elimination from tissues and the responses which counteract osmotic factors of stress. Plants' ability to eliminate Na^+ from shoot is a critical issue of salinity tolerance. Moreover, salinity tolerance can be boosted by modifying Na^+-specific transport processes. Different problems related to plant metabolism and osmosis can be caused by high concentrations of Na^+ in shoots. One of the major mechanisms in cereal crops which confer against tolerance to salt is elimination of sodium. These cereal crops include *Hordeum vulgare*, *Oryza sativa* and *Triticum aestivum* (Gorham et al. 1990; Munns et al. 2006). Another primary phenomenon which induces tissue tolerance in plants involves cellular alterations that confiscate Na^+ into the vacuoles (Munns and Tester 2008). When plants are developed at elevated salt concentrations, the SOS pathway controls K^+/Na^+ ion homeostasis and functions to preserve lower levels of cytoplasmic Na^+ ions by confiscating Na^+ into the vacuoles (Ahuja et al. 2010). In the cytoplasm of root cells, an increase in Ca^{2+} levels was noted after salt stress tolerance which stimulated the SOS signal transduction cascades in order to defend the cell from the damage conferred by extreme ionic accumulation (Chinnusamy et al. 2005). Numerous loci which were hypersensitive to salt were recognized by adopting forward genetics (an approach used to identify a gene or a set of genes for a particular phenotype of a plant) criteria (Shi et al. 2000). Among these loci, SOS1 is important as it encodes some antiporters located in the plasma membrane such as Na^+/H^+ antiporters, which play an imperative role in the expulsion of Na^+ from the cytoplasm. This is considered as an important mechanism involved in plant salinity stress tolerance (Shi et al. 2000).

SOS1 protein is formed in a variety of tissues, but studies have reported a greater accumulation of these proteins in the regions of vascular tissues and root epidermis after a few hours or days of salt stress induction. The activity of SOS1 shows a direct response to phosphorylation through SOS2 (a serine/threonine protein kinase) that is associated with the CIPK24 (a calcium-induced protein kinase) (Chinnusamy et al. 2004) and SnRK3 (a sucrose non-fermenting 1-related protein kinase-3) families (Hrabak et al. 2003; Ji et al. 2013). A OS3-like SCaBP8 protein, called as calcineurin B-like (CBL10), which has been revealed as a substitute regulator of SOS2 movement, plays a role mainly in the shoot region of plants (Quan et al. 2007).

CIPK24 is triggered when it interacts with SOS3 (calcineurin-B-like [CBL] calcium sensor CBL4) that is mainly found in the root region (Du et al. 2011). SOS3 converts a myristoylated calcium-binding protein which acts as a primary calcium sensor to detect rise in cytosolic Ca^{2+} activated by increase in Na^+ levels in the cytoplasm. When Ca^{2+} binds, CBL4 forms dimers, permitting it to interact with an NAF (asparagine, alanine, phenylalanine) domain on CIPK24. This binding of CBL discharges the C-terminal autoinhibition domain of CIPK24, which further activates the kinase protein. SCaBP8-SOS2- and CIPK-CBL-mediated phosphorylation at the C-terminus of the SOS1 protein further eliminates the autoinhibitory domain of SOS1, which ultimately activates the antiporter protein (Quintero et al. 2011). Thus it can be deduced that salt-induced Ca^{2+} efflux affects and lapses with the alleviated activity of an antiporter protein which ultimately limits or lessens Na^+ accumulation in the cytoplasm (Quintero et al. 2011). The categorization of Na^+ in vacuoles via SOS1 activity is triggered by NHX1 protein (a vacuolar membrane Na^+/H^+ antiporter) (Apse et al. 1999). In another pH- and Ca^{2+}-dependent pathway, NHX1 selectivity and vacuolar Na^+/H^+ transport activity are controlled by calmodulin-like CaM15 proteins (Yamaguchi et al. 2005). Moreover, the activated complex of SOS2/3 ultimately activates CAX1 (Cheng et al. 2004) that also takes part in vacuolar compartmentalization of Na^+ (Qiu et al. 2004).

9.8 Signaling Pathways for Osmotic Adjustment and Osmoprotection

9.8.1 ABA/JA Signaling

In plants ABA levels affects abiotic stress tolerance and responses like metabolic changes, seed dormancy, germination, senescence, closing of stomata and regulation of stress-responsive genes (Miransari and Smith 2014). During excessive saline conditions, the endogenous ABA performs an important part against stress induced by ABA-dependent processes. The ABA level fluctuates extensively in abiotic stresses specifically salinity and drought (Suzuki et al. 2016b). These stresses induce the activity of numerous ABA biosynthetic genes which include zeaxanthin oxidase (ZEP), ABA aldehyde oxidase (AAO), molybdenum cofactor sulphurase (MCSU) and 9-cis-epoxycarotenoid dioxygenase (NCED) reported to be controlled by calcium-dependent phosphorylation pathway (Zhu 2002). Endogenous ABA accumulation is a feedback mechanism as it stimulates ABA biosynthetic gene expression under stress conditions by the calcium-dependent signaling pathway; also once the plant is able to combat stress, it also triggers ABA catabolic enzymes in order to damage the excessive ABA (Suzuki et al. 2016b). ABA has significant roles, and one of its potential roles is to regulate salt stress to maintain ion homeostasis. Under saline conditions, ABA is usually synthesized in the roots which is then transferred to the leaves of the plants restricting cellular growth and inducing stomatal closure. High salinity creates greater pH in the xylem sap, resulting in changes in stomatal movement and function against salt stress (Amjad et al. 2014). Environmental stresses like salinity stimulate activation of genes like SDR1, NCED, ZEP and AAO which encode vital enzymes on behalf of ABA biosynthesis. ABA is produced from a precursor β-carotene by oxidative cleavage of zeaxanthin to violaxanthin through ABA aldehyde and ultimately biosynthesis of ABA leading to stomatal closure dependent on NADPH oxidase activity (Yoshida et al. 2014). Thus the mechanism is controlled by the Ca^{2+}-dependent phosphorylation cascade. It is also reported that high levels of salt and ABA enhance the intracellular Ca^{2+} level in plants. ABA also acts as plants' endogenous messenger, and ABA signaling mechanism is essential to identify the salinity stress tolerance in plants (Kong et al. 2015). There are three main components in ABA signaling: (1) pyrabactin resistance (PYR)/pyrabactin resistance-like (PYL)/regulatory component of ABA receptors (RCAR); (2) protein phosphatase 2C (PP2C), which acts as a negative regulator; and (3) SNF1-related protein kinase 2 (SNF; sucrose non-fermenting), where SnRK2 acts as a positive regulator which in turn phosphorylates the downstream substrate proteins and prompts the transcription of ABA-responsive genes under salinity and other abiotic stress factors (Chen et al. 2015).

Jasmonic acid (JA) is an important phytohormone with many derivatives collectively known as jasmonates which perform their function in plant physiology, regulating plant responses against any stress including salinity (Qiu et al. 2014). Jasmonates play an important part in induction of tolerance response against abiotic stresses including salt stress in plants. JA are integrated into several signaling pathways produced by ABA, ethylene, salicylic acid and other hormones and molecules (Verma et al. 2016). A component of jasmonic acid signaling pathway is Ca^{2+} as a secondary messenger, JAZ proteins, mitogen-activated protein kinases (MAPKs) and JA-linked TFs. MAPK and Ca^{2+} cascades initiate the regulation of JA biosynthesis (Li et al. 2016a). JA activates MAPKs which regulate the MYC2 expression and repression of JA biosynthesis genes negatively (Javid et al. 2011). JA pathway is associated with numerous salinity stress response for example in *Oryza sativa*, a nucleus-limited JAZ interrelating protein lacks a binding domain, and rice salt sensitive 3 (RSS3) interacts with OsbHLH094 and OsbHLH089 TFs creating a tertiary complex which regulates salinity triggering root cell elongation (Toda et al. 2013). In another study conducted on wheat, overexpression of JA biosynthesis gene OPR1 in *Arabidopsis* reduces salt-mediated root growth prevention (Dong et al. 2013). Many studies report that JA is associated with abiotic stress tolerance. In *Oryza sativa* salt tolerance is enhanced when there is overexpression of the OsCYP94C2b gene encoding an OsCYP94C1-associated enzyme which also catalyzes the conversion of JA-IIe to an inactive form (Kurotani et al. 2015). Moreover, large-scale transcriptomic research revealed that few JA biosynthetic genes (AOS, OPR3, LOX3, AOC1 and AOC2) are upregulated in the roots against salinity stress (Geng et al. 2013). Similarly, pretreatment with methyl jasmonate in *Pisum sativum* attenuates salt stress (Tsonev et al. 1998), and treatment of jasmonate to plants induces the production of proteins called jasmonate-induced proteins (JIPs) in response to abiotic stress including salinity (Sembdner and Parthier 1993).

9.8.2 ROS Signaling

Reactive oxygen species can be interpreted in plant biology from two perspectives: first as lethal by-products of anaerobic metabolism where higher levels of increase under different stress conditions can result in oxidative damage to plants and, second, as important signal transduction molecules controlling growth rate and development and more importantly responding against abiotic and biotic stresses (Choudhury et al. 2017). The site of ROS production is mainly in different organelles including chloroplast, mitochondria, peroxisomes or apoplast (Gilroy et al. 2016). Under normal circumstances, ROS production is minimal, but under abiotic stresses like salinity and drought stress, ROS production increases to drastic levels. Accumulation in organelles depends upon the scavenging and the production of reactive oxygen species (ROS) (Miller et al. 2010). As concerns plants, ROS production against salinity stress causes injury to proteins, membrane lipids and nucleic acids (Gill and Tuteja 2010). In the recent years many reports are generated related to ROS sensing where different MAP kinases, protein phosphatase inhibitors, histidine kinases and different TFs play a very important role (Apel and Hirt 2004). Apart from these, different redox molecules like NADPH oxidases, peroxiredoxins, glutaredoxins and thioredoxins that are involved in cellular redox reactions are also considered as signaling sensors in ROS signaling (Foyer and Noctor 2005).

In plants, in response to salt stress, ROS also work along with secondary messengers like Ca^{2+}. And the reason of this correlation is that in the signaling cascades, ROS (O_2, OH and H_2O_2) have to work along with other secondary messengers to transmit signal to the downstream TFs to initiate the required response. A positive feedback mechanism is constituted by respiratory burst oxidase homologues (Rbohs) and ROS-activated Ca^{2+}-permeable channels that improve ROS and Ca^{2+} signals in the root cells (Takeda et al., 2008). Salinity stress causes upregulation of cytoplasmic calcium by plasma membrane Ca^{2+} influx and also calcium ion discharge from the intracellular Ca^{2+} stocks, and these perform significant roles in salt tolerance and the ROS signaling cascade (Kader and Lindberg 2010). In *Arabidopsis*, Ca^{2+} influx induced by salt stress was noticeably repressed in the double mutant RBOHD/F that implicates the roles of RbohF and RBOHD in salt-activated cytoplasmic calcium rise. The elevation responsible for the activation of Rboh/Nox and apoplastic H_2O_2 accumulation is triggered by salinity-induced Na^+ ion build-up in the cytosol. H_2O_2 forms OH by interrelating with transition metals like Fe in the cell wall (Kurusu et al. 2015). OH is responsible directly for activating both outward-rectifying depolarization channels (GORKs) and K^+-permeable NSCCs, which results in K^+ ion escape from the roots. In order to deliberate salt stress tolerance, the absolute concentration of K^+ ions is an important factor (Shabala and Pottosin 2014).

Moreover, ROS homeostasis and antioxidant defence are regulated in reaction to salt or oxidative stress by the MAPK cascade. In *Arabidopsis*, the MEKK1-MKK1/MKK2-MPK4 cascade performs a crucial role in SA and ROS-induced stress signaling. Mkk1/2 and mpk4 mutant transcriptome analysis showed that the MEKK1-MKK1/MKK2-MPK4 cascades are responsible to control the working of the ROS removal enzyme to maintain ROS balance in plants (Desikan et al. 2001). Against salt stress, different TFs are activated by MAPKs which include WRKY, ZAT and MYB. To maintain the required balance, these TFs (WRKY, ZAT, MYB, etc.) regulate the scavenging and production pathways of ROS (Mittler et al. 2004). According to a study conducted in *Arabidopsis*, under salt stress plants experience oxidative stress and the level of H_2O_2 also starts elevating, which inactivates the *Arabidopsis* protein tyrosine phosphatases (AtPTP1). AtPTP1 can potentially deactivate MPK6 which is an inhibitor of MAPK cascade. So under salt stress, inactivation of AtPTP1 by H_2O_2 will help activate the MAPK cascade and regulate other TFs (Gupta and Luan 2003). From this it can be inferred that MAPKs also play a vital role in ROS signal transduction.

9.8.3 Calcium Signaling

Plant tissue resistance is increased by calcium against several stresses such as biotic and abiotic stresses. Calcium has been known as a universal secondary messenger in plants, for enviroment as well as evolving stimuli, because of its capability to exhibit composite geometries and varying coordination numbers (Gilroy and Trewavas 2001). In plants, salinity stress is sensed by both ionic (Na^+ and/or Cl^-) toxicity and osmotic stress, and these stress factors can also be detected either at the inner or outer surface of plasma membrane by a transmembrane protein, or within the cytosol by enzymes (Zhu 2003). Changes in cytosolic Ca^{2+} are seen by many proteins known as Ca^{2+} sensors. Most of these sensors have classical helix-loop-helix EF-hand motif that is responsible for Ca^{2+}-dependent conformational change. These sensor proteins when bound to Ca^{2+} can either serve as an activator or else attach with another protein group to trigger downstream signaling (sensor transducer) (Qiu et al. 2003). A complete set of these calcium sensor proteins contains Ca^{2+}-dependent protein kinase (CDPKs), calmodulin-like proteins (CMLs), calmodulin (CaM s) and calcineurin B-like proteins (CBLs) (Srivastava et al. 2013).

After the characterization of salinity, a transient, oscillating or stable modification in Ca^{2+} concentration is prompted within seconds. Downstream responses, are activated either via initiation or downregulation of responsive genes (Knight 1999). The cytoplasmic Ca^{2+} probably has a role in encoding specific information of crop plants against salt stress in terms of frequency, amplitude and period of the peak or signal. Particular Ca^{2+} signatures are important in sensing subsequent actions in the process of signaling for plant cells. Calcium sensors manage to identify and interpret the information carried on the calcium signatures and spread it downstream for initiation of a phosphorylation/dephosphorization cascade that leads to adjustment of gene expression (Hadi and Karimi 2012). The signal cascade provides a phenotypic response against stress tolerance via expression of several stress-responsive genes through direct or indirect means. These stress responses are determined by the number and type of genes up- or downregulated, and this response can be either a growth inhibition or apoptosis (Tuteja and Sopory 2008).

In plants, the SOS pathway (salt overly sensitive pathway) emphasizes the importance of Ca^{2+} signal in restoration of ion homeostasis at the cellular level during the course of salt stress (Ji et al. 2013). A total of 10 calcineurin B-like (CBLs) proteins and 26 CBL-interacting protein kinases (CIPKs) have been reported in *Arabidopsis*, and significant advancement is being made to recognize the signaling in SOS3 (CBL4) and SOS2 (CIPK24) that is responsible for assisting salt stress response (Qiu et al. 2003). The cytosolic calcium levels increase which are perceived by SOS3 against the salt stress. The SOS3 protein (sensor) cooperates with SOS2 protein kinase (decoder) to create a complex, and this SOS2-SOS3 complex is then responsible for stimulating the SOS1 protein (a plasma membrane Na^+/H^+ antiporter), thus establishing Na^+ ion homeostasis in cells. The activated calcium sensor/decoder elements, through a cascade of transcription factors like CaM-binding transcription factors (CaMTAs), GT-element-binding proteins (GTLs), NACs, MYBs and WRKYs, finally make their outcome evident (Mahajan et al. 2008). During the course of salinity stress-triggered systemic signaling, Ca^{2+} signaling has a vital role. Ca^{2+} signals accomplish a dominant role in composing the entire plant reaction towards soil-borne signals which are produced due to salinity stress. Subsequently, for the suitable formation of salt-prompted extended-distance signals, the tonoplast localized cation channel TPC1 and the plasma membrane localized NADPH oxidase RBOHD contribute critically. In roots of *Arabidopsis*, during the proliferation of salt stress-initiated systematic Ca^{2+} wave, a cation channel TPC1 is stated to be involved, and it may also contribute in maintaining whole-plant resistance against salt stress (Choi et al. 2014).

9.8.4 Phosphatidic Acid Signaling

Phospholipid acid (PA) is one of the signaling lipids that rapidly accumulates against abiotic stresses together with salinity stress (Zhu 2016). Salt stress generates a vibrant system which yields numerous signal molecules like DAG, IP3 and PA. The phospholipid signaling is assembled on the foundation of phospholipases that will catalyze the production of lipids (Golldack et al. 2014). There are three classes of phospholipases, i.e. PLA2, PLC and PLD, that are believed to have roles in generating lipid and lipid-derived messengers. Lipid messengers like diacylglycerol pyrophosphate (DGPP) and phospholipases 3,5-bisphosphate [PI(3,5)P2] are produced in response to signaling pathways which yield precursors of phospholipases (Julkowska and Testerink 2015). Phospholipase C (PLC) acts as a secondary messenger which catalyzes phosphatidylinositol 4, 5-bisphosphate (PIP2) hydrolysis into DAG and IP3. Phospholipase D (PLD) is known to hydrolyze the phospholipids in order to yield phosphatidic acid (PA) and major groups and is observed in being involved in the responses to several stresses (Yu et al. 2010). Recently conducted studies show that PLD and PA perform significant and complicated functions in resistance against salinity or drought (Bargmann et al. 2008).

Many studies report that in plants PA is a vital signaling molecule under salt stress. According to a study, the key role of salt-induced PA in *Arabidopsis* is attributed to PLD (phospholipase D). PA content in wild plants is considerably decreased by salt treatments, while in plants with mutant PLD1, this effect was highly negligible (Ruelland et al. 2015). The significance of PA in response to salt stress yields additional support from details that PLD1 deficiency and PLD3-deficient *Arabidopsis* mutants have reduced resistance to salinity stress (Hong et al. 2008). In addition, mitogen-activated protein kinase 6 (MPK6) is also recognized as an exact target for PA signaling towards salinity. PA then binds and stimulates MKK6, which phosphorylates SOS1 transporters in vitro. These results have established an association among MAPK cascades, lipid signaling pathways and salinity (Morris 2010).

Salinity may negatively impact the arrangement of plant microtubules. Microtubule tissue is regulated by microtubule-related proteins (MAPs) (Wang et al. 2011). In response to salt tolerance in plant cells, depolymerization and recombination of the microtubule occur, and they are considered necessary to withhold the pressure caused by salt stress and later on survival of the plants. Phosphatidic acid (PA), which is a by-product of phospholipase D (PLD), controls MAP 65–1, which is a microtubule-related protein in reaction to salt strain. PA binds with MAP 65–1, which results in its increased activity by improving microtubule polymerization and binding. In model plant *Arabidopsis thaliana*, excessive expression of MAP 65–1 increases the salinity stress resistance (Zhang et al. 2012).

9.8.5 MAPK Signaling

The adaptation of plant behaviour for salt stress, especially for the concentration of Na^+ in cells, is important for managing plants under stress. MAPK signaling can have a significant effect on this phenomenon by influencing the activity of the proton pump, localizing Na^+ in the vacuole and controlling microtubule alignment (cell cycle). Under salt stress, calcineurin B-like protein SOS3 can detect an increased concentration of Ca^{2+}, which activates the MAPK molecule to regulate Na^+/H^+ SOS1, which is sited in the plasma membrane of SOS2 (Zhu 2003). The task of explaining the MAPK signaling pathway for a specific abiotic stress persists in recognition of initial protein sensors that will identify specific MAP 3 K and MAK2K, which are responsible for activation of MAPK and connection of downstream effector proteins with kinases and physiological outcomes. Possibly, instead of primary osmotic signals, saline- and drought-activated MAPKs are mainly activated by secondary signals (de Zelicourt et al. 2016).

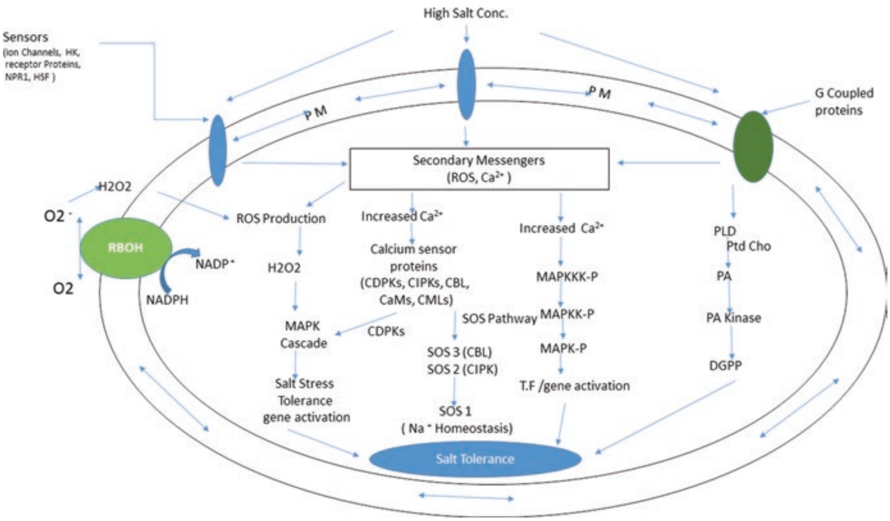

Fig. 9.2 Graphical depiction of signaling pathways and their crosstalk in plants showing cellular responses under salinity stress

9.8.5.1 MAPK Signaling Cascades

Many MAPKs are triggered through salt, osmotic stress, drought and cold. Such circumstances distract the redox balance of plants. The MAPK signaling cascade consists of the MAP kinase kinase kinases (MAPKKKs/MAP 3Ks/MEKKs), MAP kinase kinases (MAPKKs/MAP 2Ks/MKKs/MEKs) and MAP kinases (MPKs/MAPKs). During salinity stress, stimulated plasma membrane activates MAP kinase kinase kinase kinases (MAP 4Ks). MAP 4Ks associate the upstream signaling steps to the core MAPK cascades. MAP 3Ks are serine/threonine kinases phosphorylating two amino acids, S/T-X and S/T motif, of the MAP 2 K activation loop. MAPKS are serine/threonine kinases that aid in the phosphorylation of a broad range of substrates, involving other kinases or other regulatory elements. Different combinations of the MPK cascade provide plant resistance to NaCl and perform their part in cell differentiation, cell biosynthesis and growth (Colcombet and Hirt 2008). Regardless of plant species, signaling includes a phosphorus regeneration mechanism: MAPKKK activates the MAPKK downward, which then triggers the target MAPK. Lastly, MAPK controls the characteristics of the substrate protein by phosphorylating the serine or threonine deposits next to the proline (Turjanski et al. 2009; Fig. 9.2).

MAPKs are categorized as basic serine/threonine protein kinases, on the basis of the amino acid which is phosphorylated in the activation process (Taj et al. 2010). A major step in the start of MAPK is the phosphorylation of the tyrosine and threonine remains. Protein phosphorylation is one method of regulating the activity of enzymes. For the initiation of downstream MAPK, phosphorylation of upstream MAPKK and MAPKKK is necessary and very specific (Pearson et al. 2001). Initially the signal is received at plasma membrane; thus the steps involved in the signaling pathway in the cytoplasmic phase are (i) signal permeability, (ii) signal amplification and (iii) targeting. MAPK signal transduction can activate multiple proteins performing regulatory functions (Imajo et al. 2006). Each substrate signal activates a different MAPK pathway, so this substrate specificity along with other antagonistic and synergistic pathways related to many other external stimuli of MAPK signaling plays an effective role in cellular response. MAPK-specific signals lead to the activation of different cytoplasmic enzymes or proteins and the activation of downstream TFs.

The most important MAPK molecules that are triggered in abiotic stresses are MPK6m, MPK3 and MPK4 (Teige et al. 2004). According to a study, in cold and salt stresses, MPK4 is activated by M2K2. In different strains like salt, heat and cold, activation of MAPK molecules leads towards microtubule recovery. In alfalfa, MAPK of 46 kDa was expressed by saline stress. In *Arabidopsis*, saline pressure causes the expression of AtMPK4, AtMPK3 and AtMPK6 (Miransari et al. 2013). Similarly in maize (*Zea mays*), MAPK, ZmMPK3s, ZmMAPK5 and ZmSIMK1 show increased expression during the course of salt stress (Wang et al. 2010). During stress conditions, like salt or cold stress, AtMPK3 promoter DNA sequence has recently been studied, which helps understand the upregulation of AtMPK3 involved in the molecular pathway (Sinha et al. 2011). OsMAPK5 overexpression of transgenic rice upregulates tolerance and suppresses allergy to various strains including salt stress (Xiong and Yang 2003). Superoxide dismutases (SOD) are equally performing their function in the adaptive reaction of plants towards abiotic and biotic stressors. Reported data suggest that expressions of FSD2 and FSD3 induced under salt stress are influenced by MEKK1 through MKK5–MPK6-coupled signaling.

9.9 Role of Transcription Factors in Salinity Stress

Biotic and abiotic stresses cause various plant responses via genetic modification and cellular metabolism in order to bring modifications in crop yield and the plant's growth and development. Transcription factor (TF) and cis-acting factors, which exist inside the promoter region of various stress-related genes, function accordingly and perceive stress signals, and suppression or overexpression of such genes can improve plant resistance to different types of stress factors (Nuruzzaman et al. 2013). As TFs are important regulators of stress-responsive genes, they may be opted for the procedure of genetic engineering to raise salinity-tolerant crops. Many TFs of the WRKY, AP2/EREBP, NAC, bZIP and MYB families are involved in several abiotic stress regulation, and several transcription factor genes have also been identified that enhance stress tolerance in crops (Wang et al. 2016).

9.9.1 Basic Leucine Zipper (bZIP)

The fundamental leucine zipper (bZIP) family is comprised of conserved bZIP regions which consist of a primary area for nuclear localization and a leucine-rich motif for the dimerization on C-terminus and DNA binding on the N-terminus

(Hurst 1995). Against several abiotic stress circumstances especially in salt stress, bZIP transcription factors play a significant part. To extend the knowledge about bZIP TF under salinity stress, in *Arabidopsis* and in the halophytic *Arabidopsis*-relative species, selective screening of salt-inducible transcripts was done and bZIP24 was identified. bZIP24 acts as a central functioning unit in salt tolerance in plants by activating different stress-adaptive mechanisms in plants (Yang et al. 2009). Additionally, genetic engineering of TFs by introducing some modification can also make TFs more active towards salt stress condition. Like some crop plants including tomatoes and rice, some transgenic changes were done in the group AbZIP TFs which modified and enhanced the tolerance of these crop plants against salt stress (Hossain et al. 2010). Thus the enhanced tolerance towards salinity stress is intervened by the activation of stress-inducible genes which are mainly involved in ion homeostasis in the cytoplasm, growth and development of plants and osmotic adjustments.

9.9.2 WRKY Transcription Factors

WRKY TF has been implicated in numerous processes in plants which include growth, leaf senescence, seed development, biotic responses and abiotic stressors (Rushton et al. 2010). Accumulated evidence has exposed that the WRKY TF plays an evident part in defence against various abiotic stresses such as cold, heat, salt and drought as well as the osmotic pressure (Banerjee and Roychoudhury 2015). The inclusion of WRKY carriers in plant adaptation towards salt stress is demonstrated for WRKY25 and WRKY33, which increases the salinity tolerance and the ABA sensitivity, when overexpressed regardless of the SOS pathway in *A. thaliana* (Jiang and Deyholos 2009). The ability of WRKY-like TFs towards deliberate improved salinity tolerance via expression of transgene is additionally maintained by the diverse salt-prompted activation of WRKY proteins in salt-sensitive rice plants and the halophytic rice-relative model plant species (Diédhiou et al. 2009). Better tolerance to drought and salinity was improved by soybean GmWRKY54 gene of transgenic *Arabidopsis* plants (Zhou et al. 2008).

9.9.3 NAC Transcription Factors

NAC TF also has a large plant-specific family that exists in many plant species. Not only are NAC proteins associated with various methods such as defence, biotic stress adaptation and developmental responses; but they correspondingly play an important part in stress tolerance which includes salt and drought stress tolerance (Puranik et al. 2012). It has been shown that several NAC TFs are linked with abiotic stress reactions. For example, the 33 NAC gene in *Arabidopsis* was significantly altered during salt stress, and 40 NAC genes are altered in rice during drought or salinity stress (Golldack et al. 2011). Better salt and drought tolerance can similarly be attained by transgenic overexpression of various NAC factors stretching from rice and *A. thaliana* to wheat, tomato and chickpea.

9.9.4 MYB TFs

The MYB transcription factors are extensively present in plants and comprise a large family characterized by an extremely conserved MYB domain for DNA binding. Many MYB TFs are identified to function in various important biochemical and physiological processes such as cell cycle, cellular development, hormone synthesis, growth and development and against various biotic and abiotic stresses (Ambawat et al. 2013). MYB TFs are highly involved in reacting against salt stress. In rice, OsMYB2 was induced by salinity stress. In *Arabidopsis*, AtMYB41 of the R2R3-MYB transcription factor is regulated transcriptionally against salinity stress (Lippold et al. 2009).

The AP2/ERFBP family contains an immense group of plant-specific TF and is categorized by the occurrence of highly conserved DNA-binding domain of AP2/ethylene-responsive element binding (ERF). On the basis of similarity and amount of AP2/ERF domains, the AP2/EREBP TFs are assembled into four major families: AP2 (apetala 2), DREB (dehydration-sensitive element-binding protein), ERF and RAV (ABI3/VP1-related). AP2/ERFBP TF has several roles in plant stress responses and development processes which include growth and reproductive ability, cell proliferation, biotic and abiotic stress responses and growth hormone responses (Kizis et al. 2001). One such study suggested that six genes from the DREB family, ScDREB1, ScDREB2, ScDREB4, ScDREB6, ScDREB7 and ScDREB8, were responsive towards drought and salt stress (Li et al. 2016b). The expression of DREB2A and its homologue DREB2B is also reported to be especially induced under high salinity (Agarwal et al. 2006).

Plants' reaction to abiotic stress is a tremendously complex process because of large gene families and the multifaceted communications between TFs and cis elements on the promoter regions of target genes (Tran and Mochida 2010). In addition, a transcription factor can tailor numerous target genes to the corresponding cis elements in promoters, while a multi-cis gene can be adapted to different TF families. Thus, the TF not only functions independently but also intersects against several abiotic stress responses, indicating the complication of plant networks associated with signal stressors (Wang et al. 2016).

9.10 Conclusion and Future Prospects

The damaging consequences of salinity stress on plant's growth and production have intensified the food and environmental issue. The most effective measure to solve this global problem is to improve the ability of plants to tolerate salt stress. The mechanisms of plants towards the adverse effect of salinity stress include antioxidant enzyme formation, ion regulation, biosynthesis of compatible solutes and compartmentalization. Many useful techniques in molecular biology are presented, which make us understand the complex signaling mechanism in plants. Recent studies have shown that the response of plants towards a combination of stress results in a greater degree of complexity in plant response, which are controlled by the cross-talk between their specific signaling pathways. Advanced molecular techniques such as transcriptomics, genomics and proteomics are useful tools for understanding plant stress signaling and provide enhanced perception of tolerance mechanism in crops. Though many TFs are involved in stress tolerance, the role of TFs can be utilized to minimize the gaps of extensive application of genetic engineering in model plants. Thus, the future challenge should be directed towards genetic engineering approaches to utilize it for the improvement of crop production.

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Mechanisms and Molecular Approaches for Salt Tolerance Enhancement

10

Anwar Shahzad and Irfan Bashir Ganie

Abstract

With increasing profound knowledge of biochemical, physiological and molecular responses that are activated by salt stresses, it has now become clear that many of these responses follow the same route of protective mechanism. The mechanism of protection that includes a wide range of responses against salt stress could be achieved by biochemical pathways and mechanisms. Nowadays, soil salinity has become a major tool of curtailment in the production of good yield and growth of various species of crop plants that can feed millions. In this regard, transgenic salt-tolerant plants have been successfully generated with the help of bacterial or plant background salt-tolerant genes; nevertheless, salt tolerance has also been significantly regulated at transcriptional level by means of 'gene overexpression' phenomenon and introduction of transcription factors from salt-tolerant background. Currently, genes from salt-tolerant plants or specific prokaryotes have been transferred to salt-sensitive plants to test their effect or expression. These genes are involved in stress (especially salt stress) responses like signalling, transcriptional control, scavenging of ROS and protein protection. Many halophytic plant species have been discovered that help in probing out the determinants of salt tolerance. Thus, the aim of this chapter is to provide an overview on the various biochemical and molecular mechanisms involved against salt stress.

Keywords

Antioxidative enzymes · Hormone regulation · Ion homeostasis · SOS signalling pathway · Transcription factors

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10.1 Introduction

From the very outset agriculture has been under salinity stress, but it is expediting now with the passage of time, due to human intervention and availability of saline water in the fields which come under the use for crop production. Agricultural production is facing serious repercussions due to uncontrollable increase of salinity stress. Thus, it can be said that salinity has a negative impact on agriculture. Therefore, it is a big challenge to human capacity to overcome such a salient killer and to successfully improve crop production across the globe. Researchers are now putting their efforts, energy and sweat in the field of plant science to unravel the magical importance of salt-tolerant plants, so that new ways might be created for the production of high yield and highly stable crop plants in saline environments. Traits like abiotic tolerance and high yield are difficult to establish in crop plants under saline conditions. However, research fields like cell biology and molecular genetics are providing an inner view of plant response to salinity stress and assist in identification of genetic determinants and other actors that enhance salt tolerance. Recently it has been confirmed that most of the salt-tolerant determinants are omnipresent among plants; this concept led to the use of some species such as *Arabidopsis thaliana* as genetic models to reveal more about salt stress.

Salinity stress has become a global curtailment in food production and has limited the yield of crops. The United Nations Environment Programme has reported that 20% of agricultural land and 50% of cropland are under the conditions of salinity stress. To achieve the goal of salt tolerance enhancement and abundant yield of crop plants, scholars and scientists of different fields need to work coordinately with an aim to develop improved strategies for salt tolerance. Biotechnology, especially its molecular branch, has progressed and fledged well over the past decade and has played a crucial role towards providing necessary knowledge for crop improvement under different stress conditions. Through the involvement of molecular biology and genomics, scientists have successfully identified and characterized the genes involved in salinity stress response, mapped out signalling pathways and ultimately utilized this information for improving the salinity tolerance of existing crops. The availability of new tools and techniques such as gene pyramiding, marker-assisted breeding and genetic engineering has tremendously enhanced our ability to generate stress-tolerant crops. The sophisticated techniques of genome editing such as zinc finger nucleases, TALENs and CRISPR/Cas9 have provided newer and faster avenues for plant biologists to generate precisely engineered crops which display better results.

In the field of agriculture salt stress has become a huge challenge for scientists and scholars as it has greatly reduced global plant productivity. Classical breeding approaches have restricted the wide range of improvisation in salt tolerance; therefore plant biologists are trying hard to find out other ways that can help enhance salt tolerance among various crop plants. Several species of plants and different experimental strategies are underway for the identification of genes, which are contributing to salt tolerance. Besides the utilization of halophytic genes for salt tolerance, genes of other species like yeast, Arabidopsis and glycophytes have also been employed. Though there are various approaches for gene identification, three approaches are very common for the identification of salt-tolerant genes: (i) identification of genes that are involved in a wide range of processes like osmolyte synthesis and ion homeostasis; (ii) identification of genes that are regulated by salinity stress, which can be applicable to many plant species; and (iii) identification of salt-tolerant determinants based on functionality and genetic amenability. Thus, the aim of this chapter is to provide an overview of the various biochemical and molecular mechanisms involved against the salt stress.

10.2 Biochemical and Molecular Mechanisms of Salt Tolerance

10.2.1 Antioxidative Defence System

Reactive oxygen species (ROS) such as hydroxyl radical (•OH), hydrogen peroxide (H_2O_2), superoxide (O_2^-) and singlet oxygen remained a greater threat to the normal physiological and metabolic processes of plants because of its reactiveness with biological molecules. ROS are produced when electrons from ETC in the chloroplast and mitochondria react with O_2 without other acceptors. These reactive oxygen species have a tendency to damage the structural integrity of the cell. However, it has been reported that plants usually have the ability to neutralize such species by activating the antioxidative enzyme system that includes SOD, which have the ability to catalyse the reaction of superoxide dismutation and also prevent metal ion reduction, hence the synthesis of hydroxyl radicals. Plants exposed to salinity stress have increased production of activated species (Mittova et al. 2002). The elevated level of antioxidants among various plant species greatly contributes in tolerance to activated oxygen species (Wise and Naylor 1987). Dionisioses and Tobita (1998) reported that the activity of SOD decreased, while the activity of peroxidase increased in salt-sensitive plants when the antioxidant production was compared between plants which are salt tolerant and salt sensitive in response to salt stress. There was enhancement of Na^+ accumulation, lipid peroxidation and electrolyte leakage in the leaves of salt-sensitive varieties under salt stress conditions. Stevens et al. (1978) and Siegal et al. (1982) claimed that high concentration of peroxidase or increase in the activity of peroxidase may not be the only criterion for the screening of salt tolerance in Brassica species. When cotton was exposed to salt stress, there was significant increase in the concentrations of two antioxidative enzymes, i.e. catalase and tocopherol, thus showing a response of salt tolerance (Gossett et al. 1996). The activities of antioxidative enzymes, particularly of peroxidase and glutathione reductase, increase during salinity stress in salt-tolerant cultivars, while the activity of these enzymes may decrease or remain unchanged in salt-sensitive cultivars. It has been observed that salt-tolerant plants when compared with salt-sensitive plants had a lesser oxidized/reduced ratio of ascorbate and an enhanced reduced/oxidized ratio of glutathione under conditions of salt stress. It has been observed by the researchers that the process of lipid peroxidation increases and decreases in the salt-tolerant lines under salt stress. Garratt et al. (2002) found that glutathione reductase and SOD activities were enhanced in the cultured cells of salt-tolerant cv. Dhumad as compared in those of moderately tolerant (H-14) or salt-sensitive (RAhs-2) cultivars in saline medium. Shalata and Tal (1998) concluded that the activity of antioxidative enzymes might have been increased in the cultivated salt-tolerant tomato varieties than its wild-type *Lycopersicon pennellii*. They reported that activity of antioxidative enzymes like catalase and glutathione in the latter species was low and the level of lipid peroxidation was high, whereas the activities of SOD and ascorbate peroxidase were inherently higher than those in the cultivated tomato species. Working with the same two species of tomato, Mittova et al. (2000) concluded that the activities of SOD and ascorbate peroxidase increased in the salt-tolerant lines under salinity stress. In the contemporary world, this field of plant science is emerging as an interesting subject and would definitely contribute to crop improvement. However, more work needs to be done to decipher more knowledge about the different protective mechanisms of a plant against various activated species of oxygen.

10.2.2 Maintenance of Ion Homeostasis

Accumulation of salt ions (Na^+ and Cl^- ions) is the alarming factor of salinity stress especially when the plant gets exposed to high saline conditions. The ion requirement for plant growth has a limit, and each ion works best at its optimum, but when an excess amount of salt, beyond the limit, makes its way into the cell, severe chances of ionic imbalance may occur which ultimately leads to activation of ROS such as hydroxyl radical, singlet oxygen, superoxide and hydrogen peroxide (Apel and Hirt 2004). Na^+ ion concentration inhibits K^+ ion uptake when its concentration goes beyond the optimum; therefore, the production of crop yield remains low and may even lead to the death of the cell (James et al. 2011). The production of detrimental reactive oxygen species can lead to disruption of various metabolic processes by distorting the chemical structure of various proteins and enzymes; besides this ROS can also damage lipids and the DNA of a plant cell. Like any other species, a plant is supposed to maintain ion homeostasis in order to provide proper support for normal development and growth of the plant during the period of salinity stress. Despite thriving in salty habitats, both halophytes and glycophytes fail to hold salt concentration beyond the optimum; both halophytes (salt-loving plants) and glycophytes (salt-sensitive plants) fail to tolerate beyond optimum level of salt concentration in their cytoplasm. Hence, the excess amount of salt is either thrown out of the cell or compartmentalized within the vacuole, which in turn protects the plant from salinity stress.

The vacuolar membrane or the tonoplast possesses two types of H^+ pumps: V-ATPase (vacuolar-type H^+ -ATPase) and V-PPase (vacuolar pyrophosphatase). Both these pumps have a big role in salt stress responses, but V-ATPase performs better than V-PPase because it is the dominant form of H^+ pump present inside the plant cell. It was noticed that the activity of V-ATPase pump increased when exposed to saline condition, but under the same conditions, activity of V-PPase was almost inhibited; however, in the case of halophytic plant (*Suaeda salsa*), V-ATPase activity was upregulated and V-PPase played a mega role. The solute homeostasis is maintained by these pumps during non-stress conditions; besides this the energization of secondary transport and vesicle fusion is facilitated by these pumps. Under excess saline conditions the tolerating ability of the plant depends upon the V-ATPase pump activity.

Many plants have developed efficient ways to keep their ion concentration low in the cytoplasm. Plasma membrane, vacuolar membrane and other structural elements play an important active role in maintaining ion homeostasis of the cell under salinity stress conditions by regulating the process of ion uptake and transport. Transportation of ions is done by a wide range of proteins such as channel proteins, antiporters and symporters. Maintenance of cellular Na^+/K^+ homeostasis is important for plant survival under salinity stress. It has been reported that *Arabidopsis* NADPH oxidases AtrbohD and AtrbohF function in ROS-dependent regulation of Na^+/K^+ homeostasis in *Arabidopsis* under salt stress. Plants maintain a high level of K^+ within the cytosol of about 100 mM ideal for cytoplasmic enzyme activities. Plant vacuole represents the pool of K^+ ions that play a vital role in maintaining the turgor pressure of the cell. K^+ ions are transported into the plant cell against the concentration gradient via channels and transporters. When the extracellular concentration of K^+ is low, the affinity or the activity of K^+ transporters increases and facilitates its uptake transport; on the other hand a low concentration of Na^+ (about 1 mM or less) needs to be maintained in the cytoplasm. Both ions (Na^+, K^+) share the same route and mechanism of transport but have many responses under salt stress.

10.2.3 Entry of Na^+ Ions and Their Compartmentation

The plasma membrane facilitates the transport of Na^+ ions through high-affinity K^+ transporter HKT1 because of the presence of a negative potential on the plasma membrane. The cation channels which are nonselective in nature mediate Na^+ ion currents across the membrane which are partially sensitive to calcium and may restrict the entry of Na^+ in roots (Tester and Davenport 2003). AtHKT1 and other types of transporter proteins of *Arabidopsis* mediate the entry of Na^+ into the vacuole when allowed to express in heterologous systems like yeast cells and *Xenopus oocytes* (Uozumi et al. 2000; Fig. 10.1).

The transcript levels of vacuolar H^+-ATPase increase among various plant species under salt stress conditions (Dietz et al. 2001). The overexpression of AtNHX1 in rice and *Arabidopsis* (Gaxiola et al. 2001; Apse and Blumwald 2002; Ohta et al. 2002; Yokoi et al. 2002) was reported to increase salt tolerance. In *Arabidopsis* sos1, sos2 or sos3 mutants, the activity of AtNHX1 is not impaired by salt stress. However, ABA deficiency or mutation at ABA gene would lead to synthesis of dysfunctional AtNHX1 protein which certainly has a great role in salt tolerance mechanisms (Shi et al. 2002). That means the aforementioned suggests that

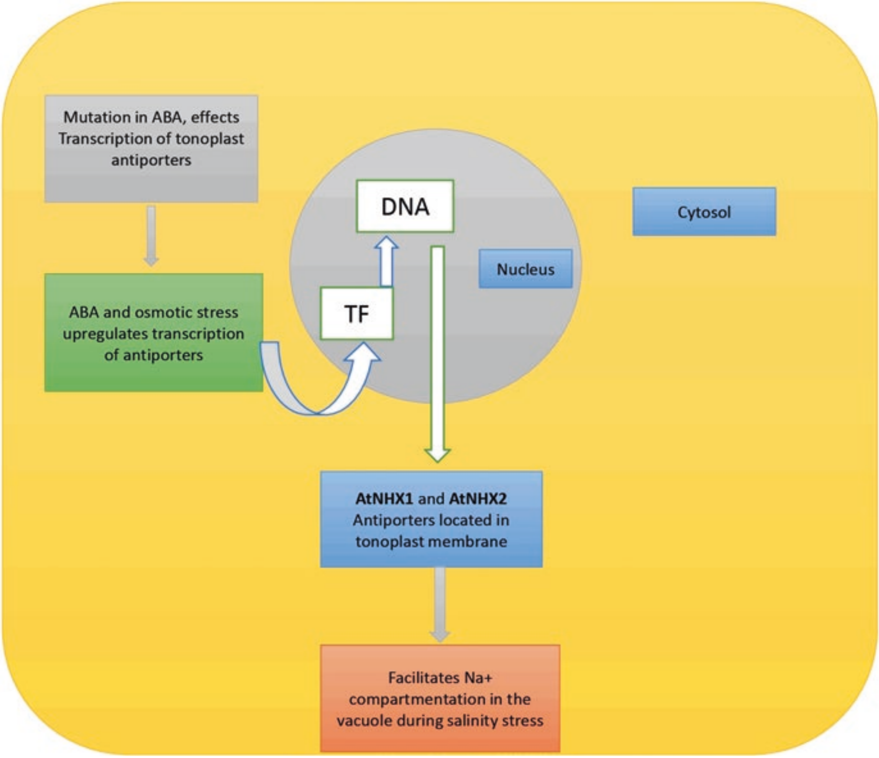


Fig. 10.1 ABA and osmotic stress upregulates transcription of genes that code for tonoplast embedded antiporters

the interdependency between the SOS and ABA pathways has a vital role in regulating the expression of vacuolar antiporters under salinity stress. In addition, many plants have developed different methods to maintain their ion balance. Membrane proteins like antiporters, symporters, carriers and channels facilitate ion homeostasis. Maintenance of cellular Na^+/K^+ homeostasis is important for plants to survive under saline environments. Within the vacuole K^+ concentration ranges between 10 mM and 200 mM. The vacuole serves as the largest pool of K^+ (10 mM and 200 mM) within the plant cell; therefore, the turgor within the cell is maintained by K^+. Class 1 HKT of *Arabidopsis* is a type of Na^+/K^+ transporter which facilitates the transport of K^+ and prevents the accumulation of Na^+ when the concentration of K^+ is low in the environment as compared to Na^+ concentration; thus it can be said that the class 1 HKT transporter works against concentration gradient when the K^+ concentration is lower than the Na^+ in the environment (Fig. 10.2). NHX proteins located in the membrane of tonoplasts are also essential for the active uptake of K^+, turgor regulation and stomatal function.

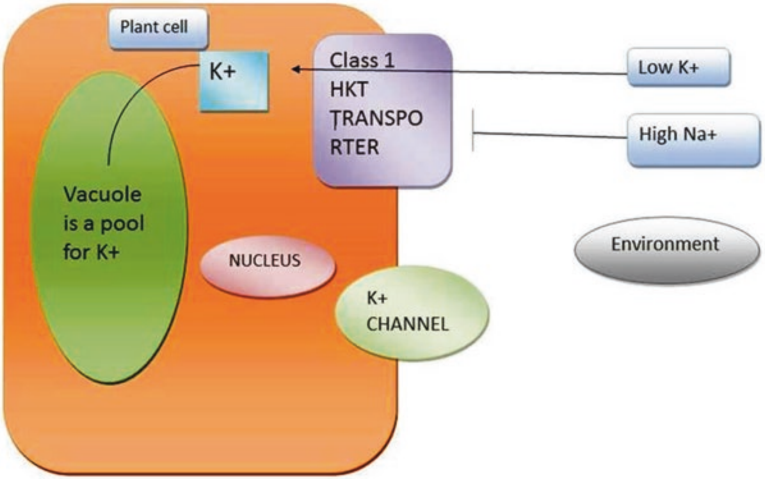


Fig. 10.2 Depicts the role of HKT 1 in transport of K^+ against the concentration gradient

10.2.4 Role of Carbohydrates in Salt Stress Response

Another compound known as pinitol gets accumulated during salinity stress. Two steps are involved in pinitol synthesis. Myo-inositol methylation forms an intermediate compound known as ononitol, which upon the phenomenon of epimerization led to the formation of pinitol. The enzyme, which plays an important role in pinitol formation, is known as inositol methyl transferase, generally encoded by imt gene. Introduction of imt gene into the genome of salt-sensitive plants has successfully enhanced salt tolerance, and similar results were obtained when mltd gene was introduced into the salt-sensitive plant; therefore, from the above observations we can come to the conclusion that the pinitol compound has a major part in salt stress alleviation (Ford 1984). It has been described that the more is the accumulation of polyols, the more is the tolerance to salt stress. Mannitol, sorbitol and myo-inositol have provided salt resistance in a wide range of plants, including microbes, plants and animals (Gorham et al. 1981; Sun et al. 1999). It has been observed that carbohydrates like starch, fructose, glucose and fructans resist salinity stress when subjected to it (Parida et al. 2004). Carbohydrates mitigate salinity stress by activating mechanisms like osmoprotection, carbon accumulation and ROS scavenging. Reducing sugars like sucrose and fructose gets increased within the cell belonging to different species when subjected to salt stress. Besides protecting the plant from salt stress injury, carbohydrates also alleviate the plant from various physical and chemical damages. Due to the higher activity of sucrose phosphate synthase, salt stress responses increase in tomato (*Solanum lycopersicum*) upon exposure to saline conditions (Gao et al. 1998). It has been reported from various rice genotypes that the sugar content both increases and decreases during salt stress (Alamgir and Yousuf Ali 1999). It was reported that the starch content decreases in the root system of rice, while in the shoot system it does not have significant implications under salt stress. Parida et al. (2004) also noted that the starch content decreases, while the content of reducing and non-reducing sugars increases in *Bruguiera parviflora* leaves.

10.2.5 Enhancement of Salt Tolerance Through Compatible Solutes

Compatible solutes, especially those with a low molecular weight, play a vital role in the maintenance of ionic balance and osmotic adjustment under conditions of salinity stress, thereby protecting the plant from salt stress. Compatible solutes include sucrose, trehalose, proline, polyols, prolinebetaine, alaninebetaine, betaine, choline O-sulphate and pipecolatebetaine (Ashraf and Foolad 2004). So far, the most studied compatible solute that is proline plays a defensive role against osmotic stress (Ashraf and Foolad 2004). The intracellular proline which gets accumulated provides tolerance towards salt stress and also contributes in stress recovery by providing organic nitrogen. The amino acid glutamate or ornithine acts as a precursor for proline during osmotic stress (Fig. 10.3). Similarly, glycine betaine is an

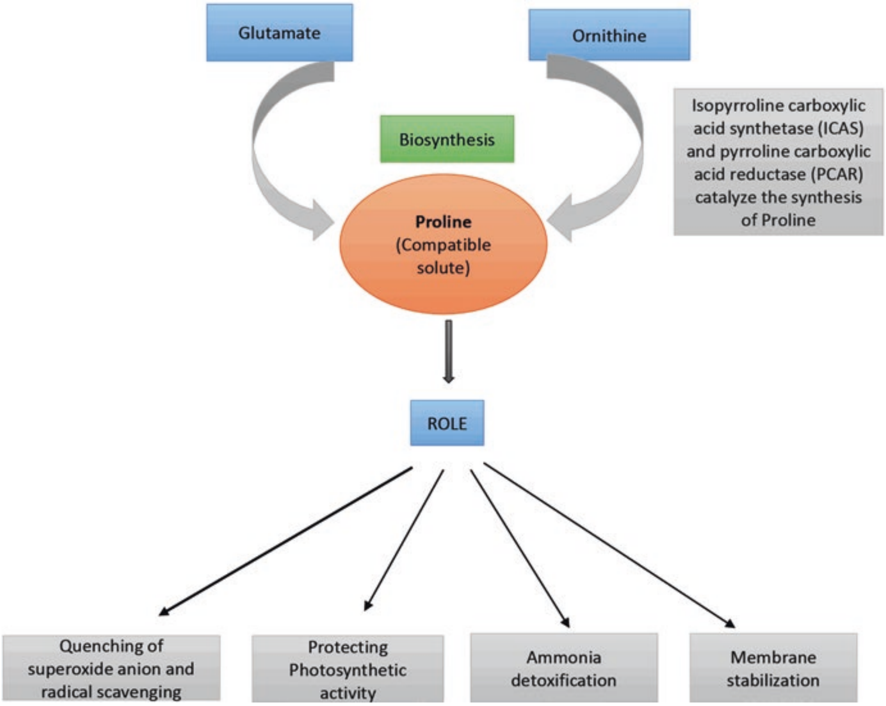
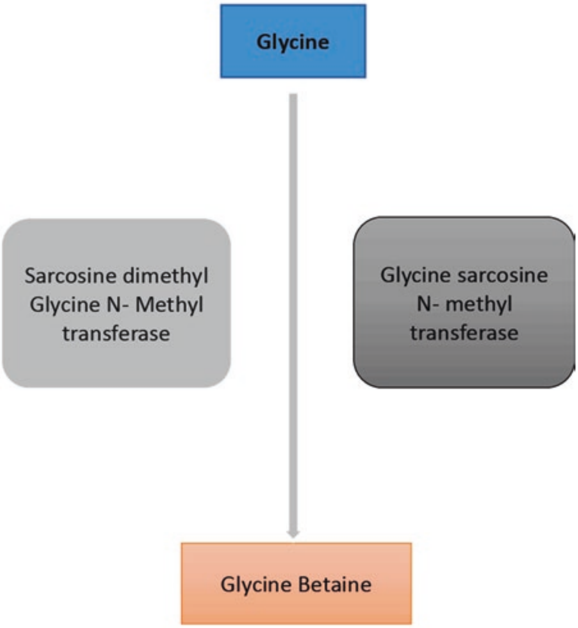


Fig. 10.3 Biosynthetic pathway of proline and its defensive role under the conditions of salt and osmotic stress

Fig. 10.4 Biosynthetic pathway of glycine and its role in halophytic plants



amphoteric molecule present in plants, animals and microorganisms. It shows interactions with compounds containing both hydrophobic and hydrophilic domains, such as enzymes and protein complexes, because of its unique structure. Glycine betaine is believed to act as an osmoregulator, maintaining the structure and activities of key enzymes and proteins that kept membranes at defence position upon the risk of salt stress (Robinson and Jhones 1986). The various pathways of glycine betaine synthesis are illustrated in Fig. 10.4 (Weretilnyk et al. 1989).

10.2.6 Salinity Tolerance by Nitric Oxide

Due to diverse functionality such as short life span, uncharged character and highly permeable factor, nitrogen has successfully made its place in bioscience. It has caught the scientific eye in biological research because of its role in salt tolerance enhancement and mechanism of defence against various types of abiotic stresses. Increasing concentration of salt may cause ionic and osmotic stresses or imbalance, thus debilitating many key metabolic processes that promote development of the plant (Hasegawa et al. 2000; Khan et al. 2010). Salinity over the optimum can impede the activity of many enzymes involved in the assimilation of nitrate (Siddiqui et al. 2009). Salinity stress causes damage to the antioxidative enzyme system at the cellular level. Plant triggers an eclectic range of responses so as to defend oxidative stress, thereby protecting the plant. However, NO involvements in salt tolerance have not yet been unravelled. Sodium nitroprusside (SNP), which is a NO donor, has successfully alleviated the risk of oxidative damage when applied to rice plants exogenously lupin (Kopyra and Gwóźdź 2003) and cucumber (Fan et al. 2007), enhanced seedling growth (Song et al. 2009) and increased the dry weight of maize and *Kosteletzkya virginica* seedlings (Zhang et al. 2006; Guo et al. 2009) under salt stress. During salinity stress many enzymes like endopeptidase and carboxypeptidase get enhanced upon the pretreatment of NO, and this also leads to better carbon and nitrogen metabolism by increasing solubilization of protein (Zheng et al. 2010). It was observed that the plasma membrane protein H^+ -ATPase in plants gets expressed when NO is applied to plants during salinity stress. Zhao et al. (2004) in *Phragmites communis* and Zhang et al. (2007a) in *Populus euphratica* reported that NO enhanced salt tolerance of calluses under salinity by increasing K^+/Na^+ ratio, and this process was H_2O_2 dependent in the increased (PM)H + -ATPase activity. For the proper adaptation of plants to saline conditions the key factor is the balanced transport and regulation of Na^+ import and export across the PM and vacuolar membrane (Rausch et al. 1996). It has been observed that glucose-6-phosphate dehydrogenase has a significant role in NR-dependent NO production and establishment of salt tolerance in red kidney bean roots (Liu et al. 2007). Besides having role in activation of ROS scavenging enzyme system such as peroxidase, superoxide dismutase, ascorbate peroxidase, nitric oxide also promotes accumulation of proline (Pro) (Shi et al. 2007; López-Carrión et al. 2008; Sheokand et al. 2008). It does not only take part in the activation of an antioxidative scavenging system, but it has also participated in the activation of photosynthetic pigments so that photosynthesis would get enhanced during salt stress (Ruan et al. 2002). NO also triggers expression of transcripts or genes (code for sucrose-phosphate synthase and pyrroline carboxylate synthase) that are involved in the establishment of tolerance to various abiotic stresses including salt stress.

NO induces expression of many redox-regulated genes. Superoxide radicals are the biggest threat to cell integrity, and NO has a tendency to prevent their harmful reactions like lipid oxidation by neutralizing lipid radicals; it also exerts a protective effect by scavenging superoxide radicals and helps in the activation of antioxidative enzyme system. NO has both positive and negative effects on plant growth when applied exogenously depending on the concentration of NO. Below the optimum or at optimum, NO promotes salt tolerance by activating antioxidant scavenging system and ROS detoxification system (Nalous et al. 2012). It has been observed that the salt tolerance of NO-treated plants was due to the regulation of membrane proteins such as H^+-ATPase and Na^+/K^+ ratio (Crawford 2006). Increase of Na^+/H^+ exchange may contribute to better balance between K^+ and Na^+ which could lead to ionic homeostasis. Zhang et al. (2007b) tested the activity of H^+-ATPase and the ratio between K and Na in calluses by adding H_2O_2. The results suggested that the impact of H_2O_2 was such that it led to an increase in H^+-ATPase activity which could adjust the plant to thrive well under saline conditions.

10.2.7 Salt Tolerance Through Hormone Regulation

The primary response of plants against salinity stress is the release of the ABA hormone that allows the plant to withstand prevailing abiotic stresses. The release of ABA has been co-related with the deficiency or unavailability of water around the root area (Popova et al. 1995; He and Cramer 1996). It has been described that the inhibitory effect of salinity stress on different metabolic activities can be mitigated by using ABA, because it has been stated on experimental basis that ABA acts as signal modulator of gene expression in case of salt- responsive and water-deficit-responsive genes (Chen et al. 2001; Gurmani et al. 2011). The effects of ABA phytohormone on the expression of two important genes HVP1 and HVP10 which encode for H^+ vacuolar pyrophosphatase, as well as HvVHA-A gene, which codes for subunit A (regarded as catalytic subunit) of H^+-vacuolar-ATPase in *Hordeum vulgare*, have also been studied under salt stress conditions (Fukuda and Tanaka 2006). During salt stress conditions, the treatment of ABA to wheat plants has significantly induced expression of various important genes such as MAPK4-like, GLP 1 and TIP 1 genes which indirectly regulate many key developmental phases (Keskin et al. 2010). In addition to ABA, hormones like salicylic acid (SA) and brassinosteroids (BR) have also been able to generate biochemical and molecular responses (Clause and Sasse 1998; Fragnire et al. 2011). The endogenous level and the activity of salicylic acid and biosynthetic enzymes increase in rice seedlings under salt stress (Sawada et al. 2006). It has been recently reported that SA improves salt tolerance in *Arabidopsis* by means of restoration of the membrane potential and prevention of K^+ leakage (Jayakannan et al. 2013). Although the activity of H^+-ATPase protein successfully increases K+ retention when the seedlings of *Arabidopsis* were pretreated with SA hormone, the accumulation of Na^+ in roots was not prevented as much by SA pretreatment; however, the concentration of Na^+ was significantly reduced in shoots (Jayakannan et al. 2013). Besides maintaining membrane integrity, SA hormone also increases content of chloroplast pigments such as chlorophyll and carotenoid. This phenomenon is associated with upward movement of K^+ and accumulation of soluble sugar, thereby assisting the plant to establish salt tolerance (El-Tayeb 2005). It has been reported that the accumulation of non-enzymatic antioxidant compounds such as tocopherol, ascorbate and glutathione has significantly improved upon the treatment of BR. In spite of that, it has also been observed that the activity of these enzymes has also been increased (El-Mashad and Mohamed 2012). Hormones like BR and SA are thought to be omnipresent among different plant species and have a vital role in salt tolerance enhancement. Ashraf et al. (2010) have demonstrated the role of BRs and SA in various physiological and biochemical processes that possibly led to salt stress impediment provided that these hormones are applied exogenously to the plant.

10.2.8 Signalling/Regulatory Mechanism of Salt Tolerance

The attractive and alternative mechanism for salt tolerance is to manipulate the specific salinity-tolerant pathway or to adjust the global signalling and regulatory salt-tolerant pathways. Alteration or tempering with any component of signalling and regulatory system may have serious repercussions on secondary regulators which contribute largely to the salt tolerance particularly in the process of ion exclusion, osmotic adjustment (Suzuki et al. 2012) and strongly controlling the regulation of Na^+ accumulation in shoot system of the plant (Jiang et al. 2012). Many pathways are initiated or influenced by Ca^{2+} binding to proteins that help in the activation of transcription of genes that respond to salt stress. The signals of saline conditions are received by receptors located on or within the plasma membrane, which further activate the Ca^{2+} signalling cascade, ultimately effecting the regulation of gene expression and protein activities (Kudla et al. 2010).

Due to salt stress intervention, the process of ion homeostasis gets deeply disturbed and disrupted, thereby leading to an accumulation of Na^+ ions in the cytosol. Different ion transporters embedded in the membranes of tonoplast and plasma membrane selectively and/or under a certain limit regulate the Na^+ compartmentation within the vacuole and selectively import K^+ over Na^+ into the plant cell. It has been discussed that plants withstand salinity stress by both the phenomenon of suppression and activation of some ion transporters. Besides, the transcript level of many important genes that may have a significant impact on growth of the plant gets unregulated or downregulated in response to salt stress. Different pathways of salt tolerance and regulation of ion homeostasis have been unravelled from scientific work, especially the molecular and biochemical work that has been carried out from SOS genes of *Arabidopsis*. The gene SOS1 encodes for Na^+/H^+ antiporter (located in the plasma membrane) that plays a vital role in the maintenance of ion homeostasis. SOS3 senses the calcium level of the cytosol especially the level induced by salt stress, and this phenomenon led to the formation of the SOS3-calcium protein complex. For the activation of SOS2, SOS signalling requires both calcium and SOS3 protein provided that the release of calcium should remain consistent under salt stress as it is a secondary messenger in salt stress responses (Wu et al. 1996; Zhu et al. 1998; Halfter et al. 2000; Ishitani et al. 2000; Liu et al. 2000; Shi et al. 2000; Fig. 10.5).

It was proposed that the sequence of amino acids of SOS2 protein is similar to the amino acid sequence of SOS2 protein of *Arabidopsis* putative protein kinase (Guo et al. 2001). It has also been observed that SOS2-like protein kinases (PKSs) interact with certain SOS3-like calcium-binding proteins (SCaBPs) (Guo et al. 2001). Therefore, many PKS-SCaBPs complexes are formed that regulate calcium-induced routes or pathways. These kinase proteins, PKs and SCaBPs, add intricacy to existing calcium regulation through other types of protein kinases. In addition to SOS1 regulation, particularly at transcription level, SOS3-SOS2 protein kinase complex activates plasma membrane-embedded Na^+/H^+ antiporter. Gong et al. (2001) found a number of genes that got expressed in sos3 mutants at higher or lower levels than the wild-type plants through RNA blot analysis and differential

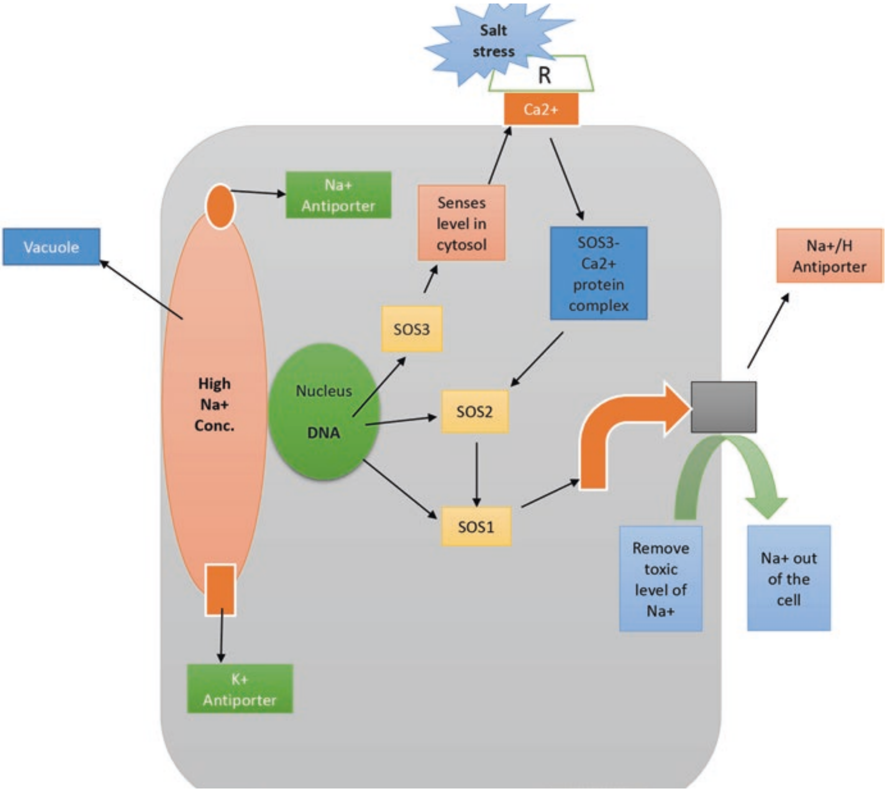


Fig. 10.5 SOS signalling response to salt stress

subtraction screening. In yeast, salt stress along with the phenomenon of calcium signalling affects the downstream protein called calcineurin. Calcineurin is a phosphatase type of enzyme that has a regulatory subunit having similarity in amino acid sequence to that of SOS3 protein. It has also been presumed that other types of proteins could also be involved in SOS pathway (Hasegawa et al. 2000).

10.2.9 Improvement of Salinity Tolerance Through Molecular and Genetic Engineering Approaches

Plant breeding approaches have not been much successful yet, because of the presence of reproductive barriers, and if the breeding would occur, there may have the chances of exchange or transfer of undesirable trait; therefore, so as to elude such hindrance to occur, gene manipulation technique has proved to be a potent tool in establishing salt tolerance in various crop plants, as it deals more specifically with the genes which contribute in salt tolerance. A large number of metabolic activities like osmolyte production, antioxidative enzyme activities and upregulation of genes are involved in stress responses. The responses of salt-tolerant plants to salt stress through a number of mechanisms have been comprehended so as to utilize it for the production of transgenic plants, either by manipulation of stress-tolerant gene(s) to the genome of salt-sensitive crop plant or overexpression of the existing genes by adding a strong promoter. Salt-tolerant genes have been consistently employed in genetic engineering to bring salt tolerance among various salt-sensitive crop species.

It has been studied that the genetic approach towards salinity stress is much diverse and has been classified as halophytes (show tolerance to higher salt concentrations) and glycophytes (are very sensitive to salt concentrations above optimum). Halophytes survive higher salt concentrations by either salt exclusion mechanism or compartmentation of salt ions in plant organelles like vacuoles. It has been reported that overexpression of AtSOS1 gene encoding for Na^+/H^+ plasma membrane antiporter shares sequence similarity with bacterial and fungal Na^+/H^+ antiporters (Shi et al. 2000, 2003). Similarly, genes like HbNHX1, GhNHX1 and BnNHX1 have brought salt tolerance to a number of plant species (Table 10.1). Recently, gene AtNHX5 has successfully delivered a high level of salt and drought tolerance in paper mulberry (Li et al. 2011). The gene AtHKT1 of *Arabidopsis* carry out Na^+ circulation in plants by facilitating Na^+ loading in the phloem of leaf and Na^+ unloading from the phloem of root. It has to be taken into account that a slight mutation or alteration in AtHKT gene may lead to an accumulation of salt ions in the shoot system and salt sensitivity among various plant species. It has also been reported that when HvHKT gene of barley was allowed to overexpress, transgenic plants acquired salt tolerance by increasing the loading of sodium ions into the xylem and were more tolerant to salt due to increased Na^+ loading into the xylem. Therefore, it can be stated that the process of ion translocation can be a great factor for salt tolerance enhancement.

Table 10.1 Salt-tolerant genes for different antiporter membrane proteins in salt tolerant and salt-sensitive plant species

Salt-tolerant genes	Encoding for the protein	Transgenic salt-tolerant species	References
SOD2	Plasma membrane Na^+/H^+ antiporter protein	Arabidopsis	Gao et al. (2003)
nhaA	Na^+/H^+ antiporter	Rice	Wu et al. (2005)
AVP1	Vacuolar H + -pyrophosphatase	Arabidopsis	Gaxiola et al. (2001)
AVP1	Vacuolar H + -pyrophosphatase	Cotton, maize and wheat	Pasapula et al. (2011)
AtNHX1	Vacuolar Na^+/H^+ antiporter	*Brassica* and tomato	Apse et al. (1999), Zhang et al. (2001) and Zhang and Blumwald (2001)
AgNHX1	Vacuolar Na^+/H^+ antiporter	Rice	Ohta et al. (2002)

10.2.10 Improvement of Salt Tolerance Through the Gene Transfer Method

The incremental enhancement in salt response to salt stress due to the accumulation of different osmoprotectants was measured under laboratory conditions and was correlated with manipulated solutes. Flowers et al. (1997) have questioned the applicability of this approach to a wide range of plant species. It has been reported that the protection of various metabolic activities in chloroplast against oxidative damages is provided by an osmoprotectant called mannitol (Shen et al. 1997); since its concentration is too low in the cytoplasm, therefore it could not provide sufficient and significant osmotic adjustment. In addition to desiccation tolerance and tolerance against cold stress, proteins known as late embryogenesis abundant or LEA proteins play a vital role in seed development under salt stress conditions (Close 1997). Rice plants have shown tolerance to salinity stress when the LEA gene HVA1 of barley was transformed in its genome (Xu et al. 1996). Similarly, LEA class genes have significantly improved freezing and salt tolerance in transgenic yeast (Imai et al. 1996). LEA proteins are thought to play an important role in preserving the structural integrity of the cell, but their extent of utility for salt tolerance enhancement needs to be explored. It has been proposed that biotic and abiotic stresses have made oxidative damage a common route of cell destruction. A large number of salt-responsive genes have been cloned, manipulated and overexpressed in various plant species that code for different antioxidative enzymes to test their role in plant defence against various oxidative stresses activated by salinity stress (Foyer et al. 1994). Allen (1995) reported that the overexpression of antioxidative enzymes like glutathione S-transferase and glutathione peroxidase also contributes in plant defence against salt stress in tobacco species (Roxas et al. 1997). Many genes have been successfully transferred to salt-sensitive crop plants that code for enzymes which are strongly involved in the synthesis of osmoprotectants, molecular chaperons and detoxifying enzymes which in turn are involved in debilitating the risk of oxidative damage. In tobacco, mannitol, an osmoprotectant, increased in concentration when its gene was manipulated; similarly *Arabidopsis* have shown the same result (Tarczynski et al. 1993). The salt stress has also been defended by other types of osmoprotectants such as ononitol, fructans, trehalose and proline.

10.2.10.1 Functional Screening of Mannose-1-Phosphate Guanylyltransferase (OsMPG1) Gene

Screening of cDNA clones has been considered as a great molecular technique that can assist in the identification of a particular gene with a specific function. This screening experiment has been successfully carried out on organisms like *E. coli* and yeast cells. The *E. coli* or yeast cells have significantly expressed cDNA clones (involved in alleviation of salinity stress) of plants that were previously identified by screening experiment. Since a great portion of plant genome is left as such which is expected to have role in salt tolerance enhancement. Therefore, research work needs to be done for the identification and characterization of genes that can possibly establish salt tolerance among different crop plants.

A cDNA clone has been identified through a functional screening test from a rice plant variety that allows yeast cells to grow in the presence of 1.2 M NaCl. Further techniques as BLAST and sequencing identified the cDNA clone as mannose-1-phosphate guanylyltransferase (OsMPG1) gene from this rice variety. Three more genes of mannose-1-phosphate guanylyltransferase (OsMPG1) were identified when the rice genome sequence database was analysed. Excluding one MPG gene, all three MPG genes, viz. MPG1, 3 and 4, complement to yeast MPG mutant – YDL055C. With the help of qRT-PCR, transcript profiling was carried out on all members of the MPG family by using two contrasting rice genotypes (IR64 and Pokkali) which had been exposed to different abiotic stresses (salinity, drought, oxidative stress, cold). These MPG genes showed differential expression under various abiotic stresses. The analysis of rice microarray data has shown that the OsMPG1 gene was getting increasingly expressed in tissues like roots, leaves and shoot apical meristem and different stages of seed development, thereby indicating its developmental regulation. When OsMPG1 gene was overexpressed in transgenic, the salinity stress tolerance was enhanced (Kumar et al. 2012).

10.2.10.2 Engineering of Molecular Chaperones

It has been studied that excess salt concentrations led to the formation of different reactive oxygen species which distort chemical structures of different biomolecules because of their tendency to react highly with the substances. Due to the presence of diverse functionalities of molecular chaperons, the ROS somehow fail to distort protein structures (Fig. 10.6). Because of their ability to defend abiotic stresses including salt stress by keeping proteins in their native form and prevent their structural aberration to occur, a number of genes that encode for molecular chaperons

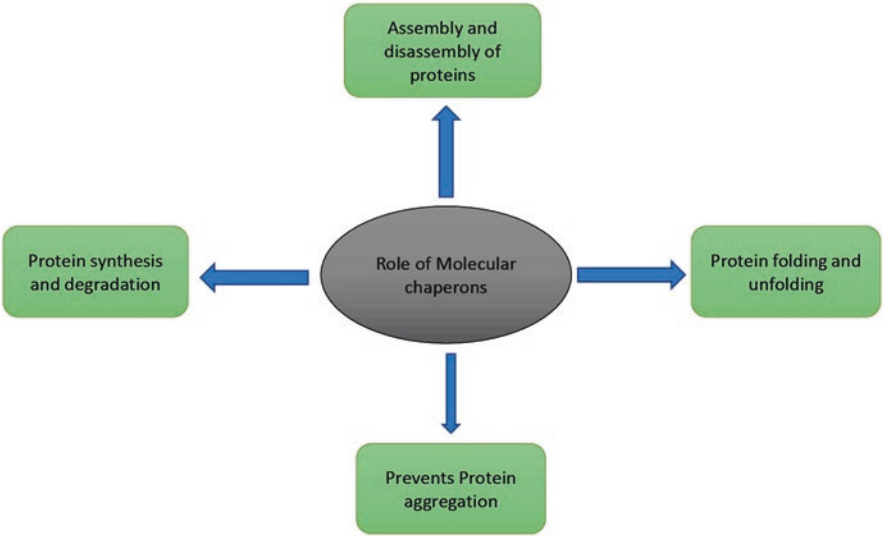


Fig. 10.6 Role of molecular chaperons under salt stress conditions

Table 10.2 Various molecular chaperon genes showing salt stress tolerance in a number of salt-sensitive plant species

Genes and their plant source	Molecular chaperons	Salt-tolerant transgenic plants
pgHsc70 from *Pennisetum glaucum*	HSP70 cytosolic chaperon	Transgenic plants
DcHsp17.7 from *Daucus carota*	Heat shock protein	*E. coli*
RcHsp17.8 from *Rosa chinensis*	Small heat shock proteins	*E.coli*, yeast and Arabidopsis
T30hsp70 from gene *Trichoderma harzianum*	Heat shock protein	Arabidopsis

have been transferred to the genome of salt- and oxidative-sensitive plants which have successfully shown positive results in transgenic plants (Boston et al. 1996; Table 10.2).

10.2.10.3 Salinity Tolerance by Transcriptional Regulation

There is a wide array of mechanisms that assist in the regulation (upregulation or downregulation) and expression of a particular gene or transcript that provides salinity tolerance to the plant when exposed to salt stress. Those genes which contribute to salt tolerance have been regulated at various levels of central dogma, from initiation of transcription to the posttranslational modification of a protein. Transcriptomics is the only field which provides detailed and precise information of gene expression at the mRNA level of a particular gene or transcript involved in salinity stress, and this technique has been widely used to screen out the genes involved in a wide range of abiotic stress responses. A large number of genes and transcription factors which may either get upregulated or downregulated in response to salt stress have been identified with the utilization of modern techniques like genomics and transcriptomics. Transcription factors are the most important regulators that control expressions of a particular gene; by having precise and profound knowledge of a transcription factor, scientists can integrate it in the genome of a sensitive crop plant. There are a huge number of genes that encode for a particular protein which help in the upregulation or downregulation of salt stress response; some of the genes which show salt and other abiotic stresses are given in Table 10.3. The transgenic plant *Trifolium alexandrinum* contains the HARDY gene of *Arabidopsis* and shows good strength of tolerance to drought and salt stress (Abogadallah et al. 2011). Gene MtCBF4 which has been isolated from *M. truncatula* codes for transcription factor that enhances salt tolerance in *Arabidopsis* when allowed to overexpress. Similarly, BrERF4 gene of *Brassica* when allowed to overexpress in *Arabidopsis* shows tolerance to both salt and drought stress (Seo et al. 2010).

Table 10.3 Genes that respond to abiotic stresses in transgenic species

Mechanism	Genes	Species	References
Transcription control	CBF1	*A. thaliana*	Anonymous
	DREB1A	*A. thaliana*	Kasuga et al. (1999)
	CBF3	*A. thaliana*	Gilmour et al. (2000)
	SOS1	*A. thaliana*	Shi et al. (2003)
	BrERF4	*A. thaliana*	Seo et al. (2010)
	MtCBF4	*M. truncatula*	Anonymous
	HARDY	*A. thaliana*	Abogadallah et al. (2011)
	HAL1	*Cucurbita melo*	Bordas et al. (1997)
	CBFs	*Brassica napus*	Jaglo et al. (2001)
	CBF1	*L. esculentum*	Hsieh et al. (2002)
	CBF4	*A. thaliana*	Haake et al. (2002)
	AtMYC2 and AtMYB2	*A. thaliana*	Abe et al. (2003)
	ABF3 or ABF4	*A. thaliana*	Kang et al. (2002)
	HsfA1	*L. esculentum*	Mishra et al. (2002)
	spl7	*Oryza sativa*	Yamanouchi et al. (2002)

10.2.10.4 Salt Tolerance Through Transgenes

This is an important and much needed approach in improving salt tolerance and to establish homeostasis by the process of Na^+ excretion under saline conditions restoring both ionic and osmotic homeostasis. Because of the availability of molecular tools, it has become easy now to introduce gene of desired level of tolerance into the plant tissue. To improve salt tolerance, recently Na^+/H^+ antiporter genes have been introduced in the subcellular sites of plant cells, and this manipulation came out as a major factor of Na + reduction. Vacuolar Na^+/H^+ antiporter gene from Arabidopsis thaliana, AtNHX1, overexpressed in transgenic Arabidopsis has shown significant improvement in salt tolerance (Apse et al. 1999). AtNHX1 has been demonstrated to be a dominant vacuolar cation/H^+ antiporter in the leaves from a study of an *Arabidopsis* T-DNA insertional mutant of *AtNHX1* and also possesses K^+/H^+ antiporter activity (Apse et al. 2003). It has been observed that the vacuolar Na^+/H^+ antiporter gene improved salt tolerance in species like tomato (Zhang et al. 2001), *Brassica napus* (Zhang et al. 2001) and rice (Ohta et al. 2002) when overexpressed under laboratory experimental conditions. It has been observed that the expression of plasma membrane Na^+/H^+ antiporter genes helps in the reduction of cellular level of Na^+ (Shi et al. 2003). These authors have shown that the genes SOS1 and SOD2 which encode for plasma membrane Na^+/H^+ antiporter enhance salt tolerance in transgenic Arabidopsis.

The primary control line or regulators of ionic balance are the membrane proteins that allow or block the passage of different ions. Researchers are producing abiotic stress-tolerant transgenic plants by increasing plasma membrane and vacuolar membrane transport proteins which possess a vital role in maintaining ionic balance during salinity stress. Some of the genes which encode for different transport proteins and help in maintaining ionic balance are gene *AtCLCd* that encodes for tonoplast chloride channel protein involved in cation detoxification and the gene

AtNHXI which confers salt tolerance in plants by compartmentalizing Na^+ ions in the vacuoles that has been cloned and overexpressed in Arabidopsis (Hechenberger et al. 1996). Similarly, overly sensitive I (SOSI) locus in *A. thaliana* was cloned and overexpressed using CaMV 35S promoter (Shi and Zhu 2002). The upregulation and Na^+ tolerance of SOSI gene were found to be consistent in function, which enhances or increases the activity of proton motive force necessary for Na^+/H^+ antiporter activities (Shi and Zhu 2002). There are a large number of genes that encode for proteins like proton pumps, antiporters and ion transporters. Some of the genes are AtMRP4 (ABC transporter protein of guard cell plasma membrane), *AtNHX1* (vacuolar $Na^{+/}H^+$ antiporter), *AtNHX2*, *AtNHX5* (vacuolar Na^+/H^+ antiporter), *AVP1* (AVP1 proton pump overexpression), *GmCAX1* (cation/proton antiporter) and HKT1 (potassium transporter), which could be utilized for the production of transgenic salt-tolerant plants.

10.3 Conclusions and Future Prospects

With untiring efforts plant biologists have unravelled or identified many transporters for elements like H^+, K^+ and Na^+ that play a crucial role in ionic balance and plant metabolism. It is now evident from various research efforts that SOS signalling regulates the expression and activities of various important membrane transporters. Future efforts need to be invested to discover the elusive salt stress sensors and identify additional vacuolar membrane transporters that may assist in Na^+ compartmentation. Research should be carried out on the membrane transporters and ion channels that assist in excreting Na^+ ions from the cell but at the same time must be helpful in K^+ uptake to maintain balance during salinity stress. As time advances, new signalling components take part in the salinity stress, level of expression and the activity of salt-tolerant genes varies in accordance with the changing saline concentrations, thus there are a lot more chances to unravel and identify new genes, transporters, signalling components and antioxidative enzymes which may contribute to salt tolerance if we put more efforts on this very field through modern biotechnological approaches. Those genes which show tolerance to salinity stress in non-halophytic and halophytic plants should be allowed to get overexpressed by fusing the genes with strong promoters and transfer them into salt-sensitive plants through genetic engineering.

Innovative application of molecular tools can provide new approaches to increasing the salt tolerance of crops. This is an exciting and important field that will flourish under close interactions between molecular biologists, geneticists and physiologists and will benefit from timely feedback from plant breeders. Innovative and well-designed molecular strategies can enhance salinity tolerance in salt-sensitive crops. This is an important and emerging field in the area of agronomics and will definitely emerge as greatly beneficial in assisting human life in future hours. Desiring for better results is a congeal hope if scholars and scientists in the field of molecular biology, plant physiology and plant biotechnology would not take on the task coordinately.

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Crops and Methods to Control Soil Salinity

11

Ghulam Mustafa and Mohd Sayeed Akhtar

Abstract

Soil salinity can cause havoc for agroecosystem as it is predicted about 50% of land will be saline in the next 50 years, but the coping techniques may reduce and even mitigate the adverse impact of soil salinity. Saline agriculture is already happening in the world, and hence it can be an opportunity for sustainability of agriculture. There are many crops that perform better in salt-affected areas that farmers need to adapt under saline soil. This is because of the reason that adaptation to soil salinity is highly positive externality that increases the farmer well-being through increased crop production. However, the dilemma is that most of farmers in developing world are unaware to these crop of salt-affected areas and methods of adaptation techniques under salt-affected areas. Thus, the main focus of the present chapter is to give an overview of the past and present studies undergoing on crops and methods to reduce and even mitigate the adverse impact of soil salinity.

Keywords

Adaptation · Soil salinity · Cost-benefit analysis · Externalities · Environmental impact

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11.1 Saline Agriculture: An Opportunity for Saline Soils Use

One of the most influencing problems for the crop production worldwide is soil salinity in arid and semi-arid regions. The emerging regions which are at risk in which soil salinization is increasing are Mediterranean Basin, Australia, Central Asia, Middle East and Northern Africa. Soil salinity has many origins such as natural causes of microscopic salt particles which are carried by the air and wind from the oceans and other is anthropic causes one of most significant is low quality of irrigational water (Epstein et al. 1980). Without using the best quality of irrigational water, the use of salinized land by using salinized water and producing halophytes crop and extending its demand through making halophytes crops a habit of the mankind in their daily diet is the best solution. There is a need of more studies to be conducted on the use of halophytes in saline agriculture and making it a daily diet of the consumers. Also, attention should be given to make it at industrial level. There are some requisites and characteristics for selection of tolerant plants which make the interest as crop in saline agriculture: (a) screen of literature for their natural habitats and (b) determining the salinity threshold after selecting the species (Munns 2002; Koyro et al. 2006). As indicated by Glenn et al. (1999), four basic conditions must be met to make the halophytes as successful irrigated crops:

(i) High yield potential.
(ii) Irrigational needs must not harm and must not increase than conventional crops.
(iii) Halophytes crops must replace the conventional crops
(iv) High salinity agriculture must be applicable to existing infrastructure.

With respect to salt-affected land, inadequate studies have been commenced on the relative evaluation of the economics of “action” and “no action” methods (Qadir et al. 2014). Thus, for more pronounced results, in land degradation, the benefits from inverting the trends could be calculated by the differentiation among the net benefit under “no action” and net benefit under “action” techniques. By excluding total benefits from total costs, the net benefits in both techniques were computed. Under “no action” technique total benefits contained irrigation cost (cost of pumping where appropriate, irrigation water cost and transfer), cost of production and cultivation (fuel and farm equipment used, seedbed and seed provision, herbicides, insecticides and pesticides where required), cost of labour (harvesting and post harvesting administration, sowing and ploughing, field operation and cleaning) and cost of fertilizer (buying, carrying and application of fertilizer). Under “no action” technique, total benefits comprised of on-farm consumption and from the farm produced gross income regarding sale in the market. Similarly, under “action” the same technique is utilized, aside from another component of cost was intensified, that mentioned the cost of inverting land degradation or restoration of land (acquisition and application of soil modification, if desired; subsoiling or deep tilling, physical work cost for instance leveling of land, where desirable; additional water cost utilized for leaching salts from root zone and any other associated costs). This method comprised by project the cost of supplementary inputs used to soften the influence of land degradation on yields of farm. While the economic impacts of saline soil degradation and renovation are grounded on associated costs and benefits as described in the overhead paragraph, there are supplementary benefits originate from services of ecosystem that results from the renovation of degraded lands like aesthetic values and reformation, carbon sequestration and diminution in environmental degradation by the enlargements in soil surface, groundwater quality, structure and health and air quality. Such ecosystem services' assessment is likely to result in satisfactory economic and environmental benefits while for many of the ecosystem services efficient markets are currently nascent or imaginary. Thus, the main focus of the present chapter is to give an overview of the past and present studies undergoing on crops and methods to reduce and even mitigate the adverse impact of soil salinity.

11.2 Methods and Crops to Resolve Soil Salinity

Growth of plants under salt stress is a complicated mechanism and how it is affected by the stress is not fully understood because the response of plants to high salinity is multifaceted and involves diversities in plant metabolism, morphology and physiology (Ali et al. 2012). The effect of salinity on productivity of plants can be reduced by identifying genotype of the plants which have capability to tolerate salinity and incorporation of such traits in the economically beneficial plants. Conventional technique of improvement in the tolerance level in plants is less effective and low level of success due to complexity of physiological and genetic traits. Tolerance at one stage of plant development does not always correlate with tolerance at other stages. Tolerance to salinity condition involves specific stages and development regulated phenomenon, that is, at one stage of development of the plants, tolerance of salinity does not correlate with second stage of development (Foolad 2004). Effective screening method is required for breeding salt tolerance in the plants, the capability to transfer genes and existence of genetic variations in the desired species of plants. In addition to this, several cultivation practices are to be adopted in order to reduce salinity and make plants capable to coupe with worse effects of salinity. Moreover, some proposed practices, for urgent requirements to preserve the environment, like use of chemical fertilizers, freshwater irrigation and application of chemical and leaching salts to deeper soil layers, it is hardly compatible (Cuartero and Fernandez-Munoz 1999).

In order to take protecting measures against further deterioration of the soil, it is important to predict and monitor soil salinity. There are different techniques which are used for predicting the soil salinity in the different countries of the world. For taking proper and timely decision toward modifying the management practices, monitoring and prediction are needed in order to take proper and timely decision. Monitoring salinity means recognizing the areas where salts ponder and noticing temporal and three-dimensional change in its happening. Regular observing of soil salinity is crucial for well-organized soil and water organization and sustainability of agricultural lands. In both arid and semi-arid environment, productive and fertile soils are a scarce resource. Freshwater resources are also scares in these areas which necessitate the use of marginal quality water for agriculture. The main reason of soil salinity is forced increase of agriculture for short-term benefits, overlooking long-term costs for soil services to meet food demand and poor control of soil and water resources. So, there is need to identify the salt-affected land by proper monitoring and mapping. In this situation, the use of traditional method for the monitoring of salt-affected land is inadequate and unfitted and is demanding high costs. For mapping and monitoring, satellite imagery can be influential tool.

For monitoring, mapping and determining soil salinity, two main electrical methods were used. First was aqueous electrical conductivity and second was soil-paste and bulk soil electrical conductivity. On the other hand, RS (remote sampling) and GIS modelling are used for mapping and monitoring the soil salinity. One can be able to predict and forecast the penetrating areas and even improve the number of sampling station with the use of remote sensing data. Early warning system may be attainable, which is extremely important for growing crop efficiency and even for choosing the suitable crop pattern by the use of RS technology. If no one takes the protective measure, the area will convert more saline which will then be more difficult to survive with; salinity mapping should be desired. By tradition, soil salinity monitoring and prediction are often carried out with intensive field work and sampling. Nowadays, most of the studies focused on differentiating salinized soil and non-salinized soil, qualitatively analysing the distribution of soil salinity and monitoring the dynamics of soil salinity. Recently, RS, GIS and modelling have become the favoured technological tool to record soil salinity due to large area analysis which is of greatest importance both from the agriculture and environmental viewpoint. Remote sensing data make it possible to obtain multi-temporal data for varying three-dimensional areas and situation, which is important element to monitor and predict soil salinity.

Agriculturists gave several ideas to tackle the salinity issues which include application of soil amendments, deep tillage, subsoiling, top soil replacement, the cultivation of salt-tolerant species and irrigation management (Sarwar et al. 2011; Ibrahim et al. 2012; Tahir and Sarwar 2013). The standard techniques to tackle with the salinization procedure can be categorized by four initiations:

1. Practice of subsurface trickle irrigation frugality water and consequently less supplementary salts, although the challenge of groundwater impurity due to artificial leaching or natural rain can arise.
2. Improved fertilization augments tolerance to salinity while sensitivity enhances also and through other harmful chemicals such as nitrate contamination rises.
3. Through soil-leaching problem of root zone salinization, where contamination could be detected.
4. Usage of salt-indulgent species. This method will be very suitable for the plants but the problem of soil or groundwater contamination does not solve by it.

Therefore, for sustainablity of agriculture production and to control the salinization process, the most efficient way is to prevent the salinization problems via ecologically clean and safe methods, which are as follows: (i) by deficit irrigation lessening of salt application; (ii) usage of species that can eliminate salt (ions) from the soil; (iii) appliance of least levels of water adequate to acquire a Good Visual Appearance (GVA) of the required yield and the landscape; and (iv) use of species that can face the problem of drought due to less application of water and, therefore, infiltration of salts declines. To solve problem of salinity by using the above-mentioned techniques, researcher should pick the right option according to the research objectives, quality and amount of the accessible water, soil salinity level and time period to clean the soil crops, seeing the environmental and the economical characteristics.

11.2.1 Adding Chemical Amendments

To restore saline soil productivity, chemical amendments are required. The most common amendments are sulphuric acid, sulphur and gypsum, whereas due to corrosive nature of acid it requires special care in application. Through depressing pH, scrapping of salt layer, enough quantity of water, deep ploughing, sanding (i.e. adding sand), subsoiling, land levelling, ESP (exchangeable sodium percentage), SAR (sodium adsorption ratio), drainage and flushing with good quality application of acid become effective in recovering saline soils. It is advised to use sufficient amount of NPK fertilizers, proper crop choice, crop rotation, higher seed rate for higher plant population and avoiding urea and ammonium sulphate for better results in cultivating saline soils. Overall condition of soil can be improved by application of farm yard manure and green manuring through leguminous crops. These not only provide organic matter and other nutrient, but also make the soil porous for aeration and moisture absorption and enhance soil microorganisms. Similarly, substituting calcium with sodium-ions using gypsum assists in modifying the harmful situations.

For extended period of time, it has been stressed that land especially saline soil should not be left empty or uncultivated. If it is left uncultivated, there is a constant upward flux of water which leads to continuous evaporation and salts remain on the surface of land. Thus, growing any kind of vegetation helps to minimize soil salinity. More than 1500 species of plant have been recognized by modern research which contains higher capacity against saline soil and are known as halophytes. For the saline soil, these plants including shrubs and salt tolerant grasses, herbs and trees are the main sources which can be operated in the development of agricultural systems. Moreover, tolerance level of existing crops can be enhanced through molecular biological approaches and conventional plant breeding. For instance, research extended over a decade showed that there are wide varieties of salt-tolerant species that act as “biological pump” and these are very good in saline agriculture. However, their tolerance level varies from region to region.

11.2.2 Growing of Suitable Crops Under Moderately Saline Soil at ECe, 0–15 dSm^{-1}

Soil having 0–15 dSm^{-1} (9600 ppm) Electrical Conductivity (ECe) cause dangerous effect on the plants' growth. But soil with loamy texture and with the 8–10 ft underground water table makes the circumstances less aggressive for sustenance of plants (Alam et al. 2000). Many species could be produced here as mentioned below. Throughout the early establishment stage and for periodical salt leaching from soil skim appropriate quality irrigation water should be employed. Through appropriate management, this could be a cost-effective endeavour: (i) fodder and forage containing berseem, lucerne, guar, dhaincha and *Melilotus* species, i.e. honey clover, sweet clover, white clover and Indian clover; (ii) oilseed like mustard, rape and canola; (iii) sugar cane, wheat, cereals, paddy rice, oat, aegilops, sorghum, triticale, pearl millet, rye, barley and corn; (iv) fruits, such as pomegranate, grape, *Ziziphus* and fig; (v) fibre including cotton, kenaf and sun hemp; and (vi) vegetables, such as red beet, tomato, spinach, sugar beet, carrot, etc.

11.2.3 Growing of Suitable Crops Under Highly Saline Soil at ECe, 15–20 dSm^{-1}

With ECe 15–20 dSm^{-1}, 9600–12,800 ppm means there is higher level of soil salinity and no availability of better quality water for irrigation. In this type of soil, majority of the plants could not grow but only salt-tolerant plants could grow, which are given under, if and only if underground water table is not superficial. By using better quality water for irrigation or regular rain in these areas, the formation and nourishment of different plants will be enhanced: (i) fruits like coconut, wild date and date palm; (ii) woody species as guava, mesquite, jujube, jojoba, mangroves, mustard tree, acacias and *Atriplex* species; (iii) grass as Rhodes grass, Kallar grass, Para grass, orchard grass, tall wheat grass, ryegrass, Bermuda grass and Sudan grass; and (iv) miscellaneous containing aloe, periwinkle, Dodonaea, life plant, purslane, bottle palm, cactus, reed plant, China rose, wild cherry, drumstick tree, wild banana and senna.

11.2.4 Water Management Techniques to Control Soil Salinity

11.2.4.1 Irrigation Frequency

Under saline soil conditions, modifying water management through appropriate irrigation practices is the best way to control salinity and can often lead to increased crop yields. Most of the crops require continuous water supply to grow normally and produce high yields. Therefore, a continuous supply of readily available moisture is necessary. The osmotic pressure or salt concentration in the soil solution is minimal and the soil moisture content is maximum after irrigation: favourable for crop growth. However, osmotic pressure and the concentration of salts in the soil solution increase soon after the soil dries out due to evapotranspirational losses. This process makes the soil water increasingly difficult to be absorbed by the plants. Consequently, crop yield reduces due to high soil moisture stresses if saline soils were watered infrequently. On the other hand, higher soil moisture content prevents the concentration of salts in the soils when salt-affected soils were irrigated frequently. Thus, frequent irrigation minimizes the adverse impacts of salinity in the soil. For these reasons, crops grown under salt-affected area are recommended with frequent irrigation compared to crops grown under non-saline conditions. By this way, plants are not subjected to excessively high soil moisture stresses due to combined influence of low soil water contents and excess salts.

11.2.4.2 Irrigation Method

Adequate soil-water availability is very helpful in salt-affected area through irrigation, but without proper irrigation method, full advantage of irrigation cannot be achieved. Appropriate method of irrigation can significantly play a role in controlling salts in the root zone. Sprinkler irrigation is an ideal method to control the salinity where small quantity of water available as this method ensures the frequent irrigation. When the water application rates are slower than the soil infiltration capacity then leaching of soluble salts is accomplished more efficiently. This water efficiency cannot be achieved through traditional flood irrigations. It has proven in the field survey that sprinkle irrigation requires three times less water compared to traditional flood irrigation method to control salinity issue by the same increment. Sprinkler irrigation will not cause salt leaching and non-uniform water application due to small differences in land levelling. This is another classic advantage of sprinkle irrigation.

Drip or trickle irrigation is another method to control the salinity and particularly suitable for seasonal row or perennial crops. In this irrigation method, water is continuously supplied in the immediate vicinity of plant roots and at a point source. This method keeps a low salt level through continuously high soil moisture in the root zone. Trickle irrigation avoids the salt accumulation at the wetting front because the roots of the growing plants tend to cluster in the high soil moisture zone near the tricklers. Therefore, trickle irrigation found useful when irrigating with high-salinity water. There is great difference of yield under two methods of water application discussed when saline water was used. However, there is no difference in yield under trickle irrigation method when separately used with high-quality water and saline water. Although drip and sprinkler irrigation techniques are highly efficient however, their high initial costs are the major hurdle particularly for small holder farmers. These constraints also prelude these methods in remote areas markets and transport infrastructure are not fully developed.

The location of salts in relation to seed placement or root zone is the main factor to control the salinity. Thus, a soil factor has considerable importance in relation to growth of plants. Methods of irrigation application can be altered to get favourable results regarding salt distribution in relation to growing roots or seed location. For instance, under furrow-type irrigation method, salts tend to gather in the ridges. In this case, salts leach out of the soil under this method and help to build up the ridges.

Hence, furrow plating is useful to get better crops yield under salt-affected areas where soil and farming practices permit. Best example of furrow irrigation modification is to plant in single/double rows or on sloping beds. This is a good way to get better stands under saline situations.

11.2.4.3 Mulching

Salt leaches out to return the soil surface during periods of high evapotranspiration. This happens during periods of fallow and between the two irrigations. When the salinity of groundwater and water-table is high, the process of salinization becomes high. Thus, any practices that flux the water-table downward and/or reduce evaporation from the soil surface will help to control the salinity of root zone. Studies have shown that mulching is very helpful to reduce the salinity (Benz et al. 1967; Sandoval and Benz 1966). They found that not only reduction in soil salinity but also the process increases the wheat yield of 25–50 bushels per hectare in an area where yield were only 62 bushels per hectare. Similarly, Carter and Fanning (1964) reported that greater salt removal and hence higher leaching efficiency are there under periodic sprinkling of mulched soils. They also found that significant reduction in root zone salt concentration of plots where cotton-burr mulch had been applied at the rate of 90 tons per hectare (Fanning and Carter 1963).

11.2.4.4 Other Practices

Every crops' tolerance level toward salinity is different. Tolerance level of crops is not only dependent on crops but also on other factors, such as culture requirements, moisture extraction pattern, rooting depth, optimum growing season and water requirements. Thus, salinity of salt-affected areas may behave differently under different crop rotation in the absence of proper soil and water management practices. Cropping patterns, such as berseem, rice and frequent water requiring crops, reduce salinity effectively when enough irrigation water available. Therefore, knowledge of the expected salt balance of the root zone under various crop rotations will be extremely helpful in planning the best cropping sequences during and after reclamation (Massoud 1976).

Salt contents generated on the raised spots and better leaching in the dips when there are changes in the micro relief in the order of a few centimetres. However, these elevation differences are corrected through proper land shaping before cropping. Salinity also increases during land levelling in the formation of shallow profiles or an exposure of an impervious layer close to surface. Since this practice is carried out at an early stage of field preparation in new surface irrigation projects, therefore it should be carefully executed as a possible cause of salinization. In almost every kind of farming, tillage is necessary part for soil permeability improvements and seed bed preparation. It should be carefully executed otherwise it may cause salinity. It might turn a salty horizon or form a plough layer. By this way, salty horizon may come closer to the soil surface. Proper monitoring of changes in the soil will help the timely adoption of corrective measures for the control of salinity that might otherwise be accentuated.

11.2.5 Wheat Rice Crop Rotation

Generally, many wheat crop varieties are there that act salt tolerance in the soil while on the other hand rice that require a lot of water create soil salinity through irrigation. However, the sensitivity of rice to salinity is decreased in the monsoon season because the amount of salt in the root zones of standing crops decreased. Therefore, wheat rice crop rotation is considered best to salt management. Both crops produce the straw (wheat and rice straw) as residue which can potentially be used to control salinity issues. For instance, rice straw produces in large quantity which is difficult to manage as it is a poor feed for animals. Common trait of removing rice straw is to burn it which is very harmful for the health of soil contents. It causes the minerals losses, such as sulphur ((4–60%), potassium (21%), phosphorus (25%) and nitrogen (80%) (Mandal et al. 2004). It also causes air pollution in the form of CO_2). Globally 4×10^9 Mg of crop residue is produced out of which 75% are produced by cereal crops, like rice, corn, wheat, etc. (Lal 2005). Therefore, proper straw management is necessary.

In today's agriculture, crop residue plays a vital role in maintaining soil fertility as compared to the past when it was only burnt. Using crop residue for soil fertility and to control the salinity is gaining more strength. Mehdi et al. (2011) found that increase the usage of crop residues decreases the use of minerals as well as chemical fertilizer. On the other hand, crop residue is an important factor in soil organic matter which helps to improve the soil nutrients, such as pollution remediation, erosion control, soil reaction, cation exchange capacity, water retention and nutrient availability (Ibrahim et al. 2011, 2012; Cooperband 2002).

Among others, controlling soil salinity issue is best use of crop residues through accumulating the soluble salts in root zone (Sarwar et al. 2008, 2011; Ahn et al. 2010). Further, Sabah et al. noted that EC value of soil amended with maize stalk, rice and rye straw was significantly reduced when compare with control. For instance, rice straw at 5, 10 and 15 Mg per hectare along with proper fertilizer use was beneficial for watermelon growth and hence useful to amend the salt-affected soils (Ahn et al. 2010). The paddy straw in wheat crop was found to increase the yield of crops as well as soil nutrients. The ameliorating effect of crop residue (burnt rice husk, legume residue and rice straw) was studied on growth response of rice crop in acidic soil (Gaind and Nain 2010). Crop residue generally helps to increase the soil organic matter, exchangeable potassium, available phosphorus, total nitrogen and cation exchange capacity of treated plots as compared to control (Sarwar et al. 2008).

11.2.6 Foxtail and Finger Millets

Foxtail millet is an important species of the millet for saline agriculture (Krishnamurthy et al. 2014). It is an annual grass-grown crop for human and bird food. It is widely grown in Australia, Europe, Asia, North America and North Africa. Despite its potential benefits for food security, foxtail millet attracted international research attention due to its high nutritional value, photosynthetic efficiency, health benefits and high drought and salt tolerance. Among these, soil salinity is the main gift of the foxtail millet species. Foxtail millet can be a potential crop for salt-affected soils due to its high level of tolerance to salinity and the salt escape potential due to its short growing duration. It is considered a model crop to study the genomics and biofuel crops among the grasses. It is also considered to be an ideal crop for the changing climate due to low incidence of diseases and pests, nutritional enrichment, high photosynthetic efficiency and short duration of crop. It can mature and yield with a single pre-sowing precipitation. Foxtail millet is very economical from point of view of agricultural inputs and can grow in difficult terrains.

11.2.7 Grafting

Grafting is a useful tool which can provide an alternative to reduce or avoid yield losses that are caused by the salinity stress in high-yielding cucurbit genotypes. Grafting is a well-known tool for the enhancement of the plant characteristics, and investigations have shown that toxicity of metal nutrient have also been limited by grafting (Savvas et al. 2005; Arao et al. 2008; Rouphael et al. 2008; Edelstein et al. 2005). Grafting can serve as an important tool to prevent salt stress by inhibiting Na and Cl uptake that is main source of soil salinity.

11.2.8 Top Soil Replacement

One of the easiest and oldest techniques that enhance the salt tolerance of soil is soil replacement. By replacing poor (saline) soils with organic soils or soft or medium with more proficient materials, for instance, sand, crushed stone or gravel, the substance situation could be enhanced, though some soil when exhausted as a replacement layer are more tough to dense than others (Gaafer et al. 2015). Soil-sustaining capacity could be improved, whereas consolidation resolution reduced by the replacement of soil under shallow foundation. This technique has deep foundation in the form of economic and entails less hindrance to construction over other techniques. Even though it has advantage of soil replacement, soil thickness replacement's determination in several situations is questionable and cantered on experience (Gabr 2012). In a shallow foundation, the area of high stress is 1–1.5, and this area could be replaced with better quality soil (Varghese 2005).

11.3 Trees: A Remedy for Saline Soil

It was found that there was about seven percent (approximately 1000 million hectares) of land area is affected by salinity issues (Dudal and Purnell 1986). Anthropogenic factors are one of the main causes of this salinity. This type of salinity is called secondary salinity. There are numerous ways of secondary salinity but major salinization caused by inappropriate water management, such as by clearing of deep-rooted native vegetation in rainfed areas and by over-irrigation in semi-arid regions. It was estimated that about 74 million hectares are salt-affected due to anthropogenic factors, out of which 43 million hectares is in irrigated (arid and semi-arid regions) land and 31 million hectares is in non-irrigated land (Dregne et al. 1991).

There are two types of genera, namely, halophytes and non-halophytes. Halophyte genera contain species which have involved several salt-tolerant mechanisms. However, some species are non-halophytes but still they can grow well in highly saline soils. On the other hands, some trees, grasses and shrubs are non-halophytes and they do not grow in highly saline soils. They can grow in less salt-affected soils. Non-halophytes rely on avoidance mechanisms that include restricting the entry of salt in the xylem and retaining most of the salt that enters the shoot in old leaves (Yeo 1994).

For large-scale planting to control salinity, a detailed evaluation through selection program is required. This evaluation program includes screening, selection and improvements. This is important to capture naturally occurring generic variability. This evaluation procedure is crucial to increase the salt tolerance of a particular species if it is required for managing salt-affected lands for either productive or amelioration use, especially commercial purposes (Choukr-Allah 1996). There are numerous factors affecting the choice of the species for planting in a particular location. These factors are revegetation, management issues including weediness and silviculture and a wide range of site conditions including the severe sodicity, salinity and waterlogging. Screening procedure varies between species, between sites and within sites. For instance, a "appropriate" species at one place might not be appropriate for another place (Choukr-Allah 1996).

Tree plantings are not only helpful to control the salinity issues but can also play important productive purposes, e.g. carbon storage, firewood, shelter and shade, cut-flower, timber, honey, pulp or sawlogs. Trees significantly reduce the salinity issues when planted on discharge areas by lowering ground water-table level. Numerous trees and shrubs species are there to control the salinity. For instance, planting 200–250 trees per hectare at relatively low stocking may be wise. By this way flushing can be allowed by rainfall. Another advantage of leaving unplanted spaces is to prevent erosion. This can also encourage other ground cover species to prevent salinity (Jackson and Bird 2008).

Although tree plantation helps to reduce salinity when wisely planted but best way to control salinity is to plant salt-tolerant varieties. These kinds of species occur naturally on local areas. Eventually, these species do not generate weed problems, grow in local condition and support biodiversity. These species give better results for salinity issues when planted directly onto saline discharge areas. For instance, study showed that such species can withstand at least 10–20 years in Western Victoria (Jackson and Bird 2008). There are two kinds of salt-tolerant species, e.g. highly tolerant and moderately tolerant species. Former is better for salinity and waterlogging issues and can be planted onto saline discharge areas while later can sustain lower slopes surrounding discharge areas. Both species can be grown through direct seedling or natural regeneration. These kinds of species can easily and cheaply handle. These natural species include genera such as *Atriplex*, *Tamarix* and *Prosopis*. They occur naturally on salt-affected soil and/or occur naturally near/or within inland or coastal sites when groundwater or soil are saline.

11.3.1 Economic Advantages

The economics of farm forestry will be determined by such factors as the domestic and international market value of the tree products generated, distance from processing plants and/or ports annual increment. On the other hand, grasses and shrubs can be used for less severe salt-affected areas, while their use can be determined by prevailing climatic and edaphic conditions of the area. For instance, shrubs could be used primarily for fodder and forage under good to moderate climatic conditions. Branches of shrub species can also be used for other productive purposes, such as carvings, furniture and match sticks. Reclamation and amelioration of problem soils can be done through shrubs and grasses by lowering shallow waterlogging.

11.3.2 Ways of Planting Trees

There are numerous methods of tree, shrubs and grasses planting to control salinity.

11.3.2.1 Direct Seedling

Usually direct seedling for tree plantations is not reliable; however, salt-tolerant shrubs can be used through direct seedling and have been successfully direct seeded. Generally, direct seedling is not preferred for shrubs and grasses like tree cases because large quantity of seed material is needed to cover a relatively reasonable-sized area.

11.3.2.2 Nursery-Grown Seedling

Nursery-grown seedling is preferred for seeding of trees, shrubs and grasses into salt-affected lands. This is easy to handle and care. Most advance technique such as genetic modified seed can also be prepared through this way.

11.3.2.3 Mallen Niche Seeder

Mallen Niche Seeder has been used for seedling especially in Australia. This is the mechanized seed-sowing method. This can increase the economic feasibility of seedling. While the use of the pre-conditioning treatments (e.g. exposure to increase salt level or to waterlogging) in the nursery may confer advantage upon planting trees, no significant gains in survival and growth have yet been reported. For instance, Inoculation of *A. amplicaps* with salt-tolerant *Rhizobium* increases seedling growth in the glasshouse and of *Casuarina cunninghamiana* increases growth in the field. Tree performance can show significant improvement through several cultural operations and land preparation techniques. These include (i) application of organic and inorganic fertilizer, (ii) angering or ripping of planting holes, (iii) use of mounds in waterlogging-affected area and (iv) addition of mulch.

11.3.3 Caveats

Tree plantation helps to reduce salinity but in the long term it may cause soil salinity. In the long run, salt can accumulate in the root zone as a result of water use by roots. For instance, dense planting on discharge site can increase salt accumulation. This salt gathering also depends on rainfall, site characteristics, evaporation and transportation by trees, local and regional hydrogeology. For instance, it was found that soil salinity increases to age 13–15 years in Southwest Victoria (Jackson and Bird 2008).

Salinity issues found where groundwater was already saline and near or above-ground level in piezometers. Nevertheless, substantial increases in soil salinity potentially leave discharge sites further degraded and could affect the long-term productivity, health or survival of trees. Perennial plants, such as *Atriplex* species, have a deep-rooted nature. This deep-rooted habit assists with proper water management. This is the classic way to control salinity. Eventually, establishment of perennial plants on salt-affected area has the advantage of reducing annual maintenance costs. Therefore, extremely salt-affected and bare land needs to be revegetated with halophytic plants, such as *Atriplex* species. As the conditions become less severe, it is possible to plant non-halophytic trees, shrubs and grasses.

Salt-tolerant trees can withstand for longer period of times as compared to conventional varieties. But, their tolerance level varies in their tolerance of waterlogging. Very few can survive long periods of “wet feet” in their first and second years. Therefore, plantation on mounds improves early survival rates to a greater extend. Summer or autumn is the best season for mounding. This allows leaching of salt before spring planting to improve survival. For instance, trials in the Southwest Victoria indicate that an early tree survival rate is 87% and 40% on mounds at two extremely wet sites (Jackson and Bird 2008).

11.4 Conclusions and Future Prospects

Salinity can cause huge losses to the agriculture which then can affect the human life through less availability of foods. However, saline agriculture can be an opportunity to increase the food availability. But, dilemma is that most of the farmers are not aware to crops and methods for areas affected by salinity. In this chapter, different crops are identified that can grow better in the salinity-affected areas. Further, different techniques are also identified that can lower or even mitigate the adverse impact of salinity. Moreover, different species of trees are also given in this chapter that are not only helpful for salinity but also good for environment. Policy recommendation is that knowledge about methods to resolve salinity and awareness about crops suitable for saline soils should be spread.

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Ameliorating Plant Salt Stress Through Bacterial Inoculation: Prospects and Challenges

12

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Abstract

Soil salinity stress has dramatically upset the fertile lands and subsequently applies a vast effect on the agricultural crop production. The salt stress has contrary influences for the plant enhancement and finally resulting in reduction of crop yields. The plants hold specific mechanism for salt stress extenuation like stimulate hormones, ionic exchange, several enzymes, and initiation of plant signaling for their metabolic and genetic edges that reduce the salt stress. In addition to the plant intrinsic appliances, several plant growth-promoting bacteria have specific mechanism which performs significant part for the stress tolerance and crop growth promotion. These microbes enhance plants to produce various plant growth hormones including auxin, cytokinine, and gibberellin as well as instable toxic compounds. Furthermore, microbes could perform a vital role in relation to their properties like saline tolerant, genetic diversity, fusion of soil solutes and hormone production, biocontrol ability, and plant interface. Consequently, exploiting the above discussed unique properties of microbes can be a cost effective strategy in reducing salinity stress that could be utilized and implemented for salinity amelioration.

Keywords

Crop production · Mechanism · Plant growth-promoting rhizobacteria · Salinity · Stress

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12.1 Introduction

The soil saline (salinity) is one of the most overwhelming environmental stresses which impact large-scale reduction in terms of crop production and its quality (Shahbaz and Ashraf 2013). A salt-affected soil is generally interfered to the normal plant growth due to its excessive salt concentration around the plant root area (EC > 4 dSm^{-1}) with high exchangeable sodium (>15%). Salt-affected soils are classified into saline, saline-sodic, and sodic, dependent in salt amounts, variety of salts, sodium percentage, and soil alkalinity. In the world, approximately 20% of cultivated land and 33% of irrigated lands are salt-affected (Jamil et al. 2011). The incidence of salt intrusion in agricultural land can be dominated due to change in climate, increase of groundwater and low-quality irrigation water, and enormous introduction of irrigation related with intensive farming. Extreme soil salinity lessens the productivity of several crops, including most of the vegetables, which are mainly delicate during planting (Machado and Serralheiro 2017).

Soil salinization happens with the accumulation of soluble salts of sodium, magnesium, and calcium in soil to the extent that soil fertility is severely reduced and impacts agricultural production, environmental health, and finally country economics. Primarily, salinity influences the metabolic rate of the soil microbes with reduction of crop production; nevertheless, in the case of innovative phases, it eliminates all foliage, subsequently transforming fertile and prolific land into infertile lands (Shrivastava and Kumar 2015). Salt stress, being one of the most prominent abiotic stresses, can get the physical and chemical variations into crops. It is, therefore, required that the mega plants mitigate and overcome this big stress challenge and, furthermore, utilize, to handle these challenges, biotechnological tools, such as gene transcription and alteration in the proteins (Hakeem et al. 2013).

Currently, the total salt-affected soil on the surface of the earth is approximately 954 million ha. Such kinds of salt-related soils are spread worldwide. Over about eighty (80) million ha of the land are in Africa; almost 147 million ha in Central, North, and South America; 357 million ha in Australia; and more than fifty (50) million ha in Europe (Table 12.1). Likewise, a large mass of approximately 320 million ha of land in South and Southeast Asia is under the stress of salt. Out of 79.7 million ha area of Pakistan, 22 million ha is agricultural land. It is estimated that out

Table 12.1 Salt-affected soils in the world

Regions	Salt-affected soils (million ha)
Africa	80
Central, North, and South America	147
Australia	357
Europe	50
South and Southeast Asia	320
Total	954

Source: Modified from Alam et al. (2000)

of 22 million ha, 6.28 million ha (28.54%) is salt affected. Among these, about 1.89 million ha are with salt, 0.28 million ha and 3.05 million ha are saline-sodic soils (Alam et al. 2000). The FAO estimates that approximately 50 million ha of irrigated soil in the world show about 50% of salt saturation problems and ten million ha are uncontrolled because of these issues annually (FAO 2018) and agricultural land more than 20% and more than 50% of the crop land in the world is salt affected (Flowers and Yes 1995).

Salinity relates with the soil and water quality apprehension, particularly in dry and semiarid areas required for water increases daily for irrigation of agricultural crops. Dry and semiarid areas are the regions with less evapotranspiration (<1250 mm), and high Na ions persist in the soil rhizosphere due to water scarcity. Mainly salt-affected soils pertain to calcite and calcium salts with tremendously low solubility. Sodium and saline-sodic soils succeed in almost 75 countries all over the world, and their area is slowly and gradually enhancing across the agricultural countries (Qadir et al. 2005).

In addition, the salt-affected areas increase about 10% per year due to different causes, namely, low rainfall, maximum land surface evaporation, indigenous rocks, and applying salt water and other deprived cultural practices. It is projected that half of the cultivable land will be degraded due to salt-affected till 2050 (Jamil et al. 2011). Soil salinity is expected to have a large impact on agriculture in the upcoming time. Cumulative increase in nationalization is related with growing salinity in water possessions (Ibekwe et al. 2010). Several plants do not express their original genetic prospective for their growth, development, and yields in salt stress that decrease directly economic and commercial values of the lands. The salt-affected soil is categorized into three types (saline, sodic, and salt-sodic soils). There are many reasons for salt-affected land such as climate with more evapotranspiration than average precipitation; poor quality of irrigation water, i.e., brackish groundwater; parent material of soil which contains salts; uneven distribution of rainfall; physiological inequality solute accumulate in the upper places; and poor management skills. Soils cause serious obstacles to successful agricultural production. This chapter discusses briefly about the problems of soil salinity in agriculture and its possible mechanisms to reduce stress in plants by plant growth-promoting rhizobacteria (PGPR).

12.2 Salt Stress Problems

The soil salt stress is one of the most destructive abiotic stresses that interrupt the plant from germination up to plant maturity. This issue becomes high especially in the arid areas which have yearly low rainfall and are prone to high evapotranspiration. The land under salinity stress is rapidly increasing on a daily basis, and it is considered that about half of the fertile land would become saline by the year 2050. The causes of salinity are highly diverse and depend on a large number of factors: amount, intensity, and duration of salinity and crop growth stages. There are several causes due to soil salinity is occurred. The main causes which are mostly enhancing this problem are discussed as follows:

12.2.1 Causes of Soil Salinity

Soil salinity is a multifactorial phenomenon, i.e., caused by various factors or combinations of factors. Salinization is a complex process that may change in various ways in different areas, often caused to a significant extent by the overexploitation of natural resources, in particular, unsustainable and inappropriate methods of agriculture and land use, and incorrectly managed water resources, the consequences of which become serious by climate change with extreme droughts and other contests (FAO 2018). The salinity depend on many factors such as climate with more evapotranspiration than average precipitation, poor quality irrigation water including brackish groundwater, parent material, uneven distribution of rainfall, physiological unbalances, poor management skills, and seepage from water channels and canals. The two main processes, i.e., natural and anthropogenic, occur for the soil salinity (Shrivastava and Kumar 2015).

12.2.1.1 Natural Process

The accumulation of salts in the soil can take place through natural processes, such as physical or chemical, and transportation of base materials, geological deposits, or underground waters. It could occur because of parent material, for example, carbonate minerals or feldspar as well as the underlying sources of water that play an important role in soil salinization. The rise in sea level also causes leakage to areas below the sea level. In droughts, saline soils are formed due to lack of rainfall to evapotranspirate and reduce soil fertility. In addition, air winds in the coastal areas blow significant amounts of the salts (Shrivastava and Kumar 2015).

12.2.1.2 Anthropogenic Process

Measures undertaken by the human being may result in salinization by use of salt-rich irrigation water due to misuse of the underground waters or other inappropriate irrigation methods and poor drainage conditions that lead to the use of seawater. The use of excessive water for irrigation in the dry climate causes heavy soil and salt accumulation, as it is not by raining. This process occurs at high irrigation rates and sowing areas associated with the clay texture tissue of the soil. Failure to do enough without drainage has become a serious cause of land salinization. Irrigated land prevents the salinity of the salts brought to irrigation water (Shrivastava and Kumar 2015).

12.2.2 Adversative Influences of Soil Salinity

Soil salinity directly influences plant's growth and seed germination, vegetation and water, and uptake of plant nutrient (Akbarimoghaddam et al. 2011). Soil salinity affects plant development such as seed and plant germination, plant vegetation, and reproductive development. Soil salinity levies ionic toxicity, osmotic stress, soil elemental transparency, and oxidative pressure on the plants, thus restricting soil absorption. Ground purity reduces the plant solution significantly because of P ions with precipitation of Ca ions (Bano and Fatima 2009). The soil nutrients, such as Na, Cl, and B, have definite toxicity impact on the plants. The extreme addition of Na in cell walls can move to quick osmotic stress and death of the plant cells (Munns 2002). Most of the plants are susceptible to these elements which could be influenced moderately with low salt amounts in the case of the soil which comprises adequate quantities of soil toxic elements. Further, several salts are also essential for plants, but their accessibility levels in the soil could interrupt the plant nutritional equilibrium for the nutrient uptake. The soil salinity influences photosynthesis predominantly due to a lessening in leaf area, contents of chlorophyll, and leaf stomatal conductance which may lead to make slighter decline in the efficacy of photosynthesis (Netondo et al. 2004). Salt contents in soil unfavorably affect the reproductive development, extending proliferation, refining programmed cell death in some tissue types, ovule abortion, and sterile embryos. In addition, salty growing medium affects several opposing effects on vegetation by low osmotic potential of soil solution, specific ionic influences, and nutritional differences with many joint factors (Ashraf et al. 2004).

12.3 Role of Microbes in Ameliorating Salt Stress

Salt-stressed soil mostly suppresses the plant development (Paul, 2012). The rhizosphere in natural climate is abundant with endocellular and intracellular microbes, and these microbes directly influence the plant rhizospheric zone (Gray and Smith 2005). Rhizospheric microbes, prominently beneficial bacteria and fungi, could be able to develop the plant efficiency in stress conditions and, subsequently, increase the direct and indirect revenues (Dimkpa et al. 2009). Some of PGPR can straightly stimulate plant growth and development by supplying nitrogen, phytohormones, iron confiscated through bacterial siderophore production, and soluble phosphates (Hayat et al. 2010). The secondary mechanisms are defending the plants from soil pathogens affected by pathogenic fungal spores (Lutgtenberg and Kamilova 2009).

Microbes can play an important role in ameliorating salt stress through exploiting their unique properties like salt tolerance, genetical diversity, synthesis of solvents, synthesis of phytohormone production (Naher et al. 2013 and Panhwar et al. 2014), biocontrol ability, and their interface with the plants. These microorganisms perform a significant function with exclusive characteristics like salt-tolerant conditions, genetic diversity, fusion of solutes, growth hormone production, biocontrol ability, and contact with the crops (Shrivastava and Kumar 2015). Identification of new halophilic and halo-tolerant bacteria that promote vegetation could be utilized to salt-sensitive plants. Extensive research has been done on various halophilic and halo-tolerant microbial strains to explore the plant growth-promoting actions (Orhan 2016). Taking into account the importance of plant beneficial microbes for developing tolerance against the salt stress in plants, various aspects and mechanisms of bacteria have been demonstrated that perform major part in promoting yield of many crops. The plants that colonize salt-tolerant growth-promoting bacteria could be used as reduction in cost and used as an economical device for the salt stress and plant growth enhancement in salinity-affected areas (Numan et al. 2018).

Various approaches have been designed to reduce the toxic effects of salinity problems including plant genetic manipulation introduced with the use of PGPR (Dimkpa et al. 2009). The microorganism functions in promoting crop growth, nutrients, and control of several diseases have been well established. The beneficial microbes colonize in the rhizosphere/endorhizosphere of many plants and develop plant growth by various direct and indirect mechanisms (Ramadoss et al. 2013). Earlier studies specify that the use of PGPR has developed a capable substitute to lessen the salinity stress in the soil (Yao et al. 2010) and the microbial mechanism (Fig. 12.1) and perform a vital part for managing biotic and abiotic stresses which is highly significant (Prasad et al. 2015).

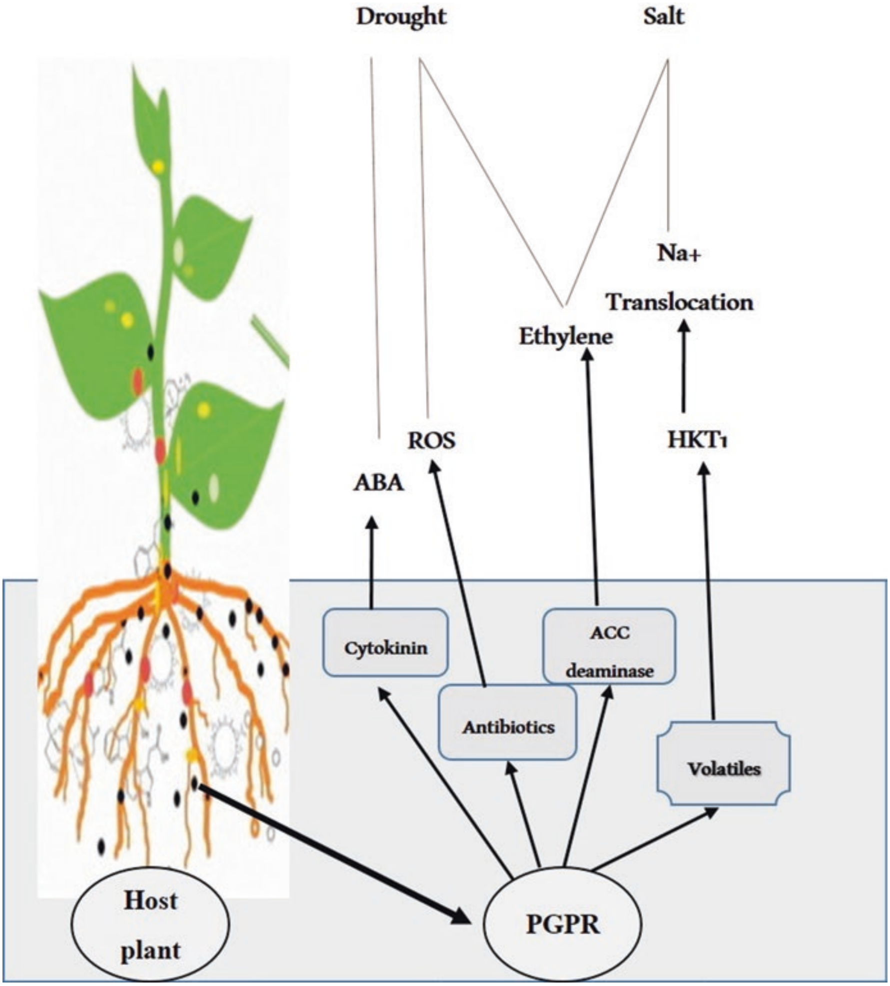

Fig. 12.1 Mechanism of PGPR mediated in relation to salt tolerance. (Adapted from Prasad et al. 2015)

Table 12.2 Role of plant growth-promoting bacteria in salinity stress alleviation in plants

PGPRs	Crop plant	Effect	References
Achromobacter piechaudii	Tomato	Reduced levels of ethylene and improved plant growth	Mayak et al. (2004)
Azospirillum sp.	Maize	Restricted Na^+ uptake and increased $^{K+}$ and Ca^{2+} uptake along with increased nitrate reductase and nitrogenase activity	Hamdia et al. (2004)
Pseudomonas syringae, P. fluorescens, Enterobacter aerogenes	Maize	ACC deaminase activity	Nadeem et al. (2007)
P. mendocina	Lettuce	ACC deaminase activity and enhanced uptake of essential nutrients	Kohler et al. (2010)
P. pseudoalcaligenes, Bacillus pumilus	Rice	Increased concentration of glycine betaine (compatible solute)	Jha et al. (2013)
P. putida	Cotton	Increase the absorption of the Mg^{2+}, K^+ and Ca^{2+} and decrease the uptake of the Na^+ from the soil	Yao et al. (2010)
P. putida, E. cloacae, Serratia ficaria and *P. fluorescens*	Wheat	Enhanced germination percentage, germination rate, and index and improved the nutrient status of the wheat plants	Nadeem et al. (2013)
Acinetobacter spp. and *Pseudomonas* sp.	Barley and Oats	Production of ACC deaminase and IAA	Chang et al. (2014)
Rhizobium sp. and *Pseudomonas* sp.	Mung bean	IAA production and ACC deaminase activity	Ahmad et al. (2013)

The microbes collected from various salt-affected soils have stress tolerance ability due to the presence of plant growth-promoting bacterial properties and have direct relation with seed germination. Due to inoculation of these microbes, plants display more root and shoot lengths, biomass, and biochemical properties like chlorophyll, carotenoids, and proteins (Tiwari et al. 2011). In recent times, microbes related to various genera, such as *Rhizobium*, *Bacillus*, *Pseudomonas*, *Pantoea*, *Paenibacillus*, *Burkholderia*, *Chromobacterium*, *Azospirillum*, *Microbacterium*, *Methylobacterium*, *Variovorax*, *and Enterobacter*, which confer tolerant on the host plants in various abiotic stresses has been reported (Grover et al. 2011). The inoculation of these beneficial microbes could lessen salt stress of crops, thus opening the way of a latest way for the microbial application (Table 12.2).

12.4 Alleviation of Salt Stress in Crops Using PGPR

12.4.1 PGPRs Effective Candidates for Developing Soil Aggregates and Amelioration Salt Stress

Salinity influences the growth enhancement and yield of various crops, and the stability of soil aggregates have significant characteristics that control the plant growth in semiarid environments, consequently improving soil structure stability which has a great importance for crop production. The involvement of microbial activity either as free-living or allied with plant roots, and their activity to soil cumulative stability, has proven to be extremely beneficial for the delivery of salt soil suitable for agriculture (Paul 2013). In addition to developing own stress-tolerant mechanisms, PGPRs could provide tolerance against plant abiotic stresses. Interface of PGPR with various crops in salt circumstances reduces the amount of poor growth and hence supports to endure plants and recover presentation in unfavorable situations (Dimkpa et al. 2009). Some of the PGPR can straightly enhance plant growth and development by giving plant enhancement factors including nitrogen, phytohormones, and siderophore production and P solubilization (Hayat et al. 2010; Panhwar et al. 2014). Further PGPR protect the plants against several soilborne diseases which may cause very harmful pathogenic fungal diseases (Lugtenberg and Kamilova 2009).

12.4.2 PGPR-Arbitrated Plant Root Proliferation and Plant Vigorousness

The PGPR colonize in plant rhizosphere and enhance the growth of the plants in different ways. Paul and Sarma (2006) significantly improved the PGPR strains, *P. fluorescens*, root biomass of black pepper. Similarly, tomato-colonizing PGPR strains showed root improvement in plants (Table 12.3). Rhizobacteria-arbitrated root explosion is well proven that has been deployed successfully under salt stress (Diby et al. 2005a). Thus, a productive approach to lessen the adverse effect of salt stresses in the plants can be screening of various PGPRs present in stressed plant atmosphere. Inoculation of various plant species with PGPRs also resulted in the root proliferation and growth of plants. This can lead to increased tolerance for

Table 12.3 Effect of IRB isolates on the number of roots pieces on tomato stem (Adapted from Paul and Sharma (2006)

PGPRs	No. of root pieces	Rhizobacterial population on roots (1×10^6 cfu/g fresh weight)
PGPR-IRB1	30	2.8
PGPR-IRB2	34	2.3
PGPR-IRB3	44	1.6
PGPR-IRB4	48	2.9
PGPR-IRB5	70	2.6
PGPR-IRB6	76	2.1
PGPR-IRB7	97	2.4
PGPR-IRB8	80	2.4
PGPR-IRB9	116	2.5
PGPR-IRB10	109	1.9

abiotic stress. Furthermore, Paul and Nair (2008) studied that the root colonization ability of the salt-tolerant *Pseudomonas* strain is not vulnerable by salinity in soil. Plant root growth promotion leads to a greater root area and may consequently have an encouraging impact on the procurement of soil water and nutrient uptake (Diby et al. 2005b). Higher hydration caused by PGPR strains can be attributed to enhanced water use efficiency (Saravanakumar and Samiyappan 2007).

Moreover, microbial excited stress tolerant in several plants by various mechanisms suggested based on the earlier studies which have been done. The production of phytohormones (indole acetic acid, gibberellins) and other non-identified substances produced by PGPR leads to enhanced plant roots, root area, and number of root points for increasing nutrient uptake, hence resulting to develop plant vigorousness under salt stress circumstances (Egamberdieva and Kucharova 2009). PGPR strains have been reported to increase the growth of various crops such as tomato, pepper, canola, bean, and lettuce salad (Barassi et al. 2006). Increased proline production, laterally with reduced electrolyte leakage, sustaining comparative water contents in leaves and uptake of ions, resulted in salt tolerance in *Zea mays* inoculated with *Rhizobium* and *Pseudomonas* (Bano and Fatima 2009). Rhizobacteria live on different locations visible to regular stress condition which will perhaps be more adaptable and could perform in a better way for plant growth especially in stress conditions. For instance, Yao et al. (2010) reported that *P. putida* inoculation in cotton crop promoted growth and germination in salt stress conditions.

12.4.3 Phytohormones Production by Bacterial Strain

Most of the beneficial microbes like PGPR have the ability to produce phytohormones which promotes plant growth and roots which may perform a physiological function in the plants. Production of IAA, gibberellins, and other plant growth regulators produced by PGPR is considered to enhance the root length, root area, and root surface, important to increase nutrient uptake, which increases plant growth in stress conditions (Egamberdieva 2012). IAA production is a comparatively collective feature of the PGPR, and such bacterial strains are supposed to counter salt quality in plants. Morphologically, root changes are repeatedly observed during the inoculation of *Azospirillum* and are recognized to the crop production substances, IAA, cytokinin, and gibberellin, that is particularly the most important characteristic (Panhwar et al. 2014; Naher et al. 2016). Sadeghi et al. (2012) showed that *Streptomyces* inoculation has increased vegetation in wheat crop and synthesized IAA during the salt accumulation. Similarly, several scientists presented the IAA production capability of several rhizobacterial isolates (Table 12.4).

Table 12.4 Production of indole acetic acid by selected PGPR strains isolated from various crops

Isolates	Crops	References
Azotobacter sp.	Sesbania	Ahmad et al. (2005)
Alcalygens sp. IRB5	Tomato	Paul and Sarma (2006)
Azotobacter sp. IRB6	Tomato	Paul and Sarma (2006)
Pseudomonas putida IRB4	Tomato	Paul and Sarma (2006)
Burkholderia sp. IRB7	Tomato, Pepper	Paul and Sarma (2006)
Bacillus lichiniformis IRB9	Tomato, pepper	Paul and Sarma (2006)
Bacillus cereus RC18	Wheat, Spinach	Cakmakci et al. (2007)
Pseudomonas tolaasii	Brassica	Chandra et al. (2007)
Bacillus spp. (PSB9)	Aerobic rice	Panhwar et al. (2011)
Bacillus spp. (PSB16)	Aerobic rice	Panhwar et al. (2011)
Burkholderiathailandensis	Wetland Rice	Panhwar et al. (2014)
Sphingomonaspituitosa	Wetland Rice	Panhwar et al. (2014)
Bacillus sp. (strain Sb13)	Wetland rice	Naher et al. (2016)
Burkholderia sp. (Sb16)	Wetland Rice	Naher et al. (2016)

12.4.4 Salt Stress Reduction by Induced Systemic Tolerance

The induced systemic tolerance (IST) has been projected for microbial-induced physical and chemical variations that lead to increased tolerance to abiotic stress. These microbes control the plant pathogens directly/indirectly by the absorption of nutrients phytohormone production, enzymatic reduction of plant ethylene levels, and siderophore production (Kohler et al. 2010). It has been reported that the infection of arbuscular mycorrhiza improves vegetation in salt stress (Cho et al. 2012). Kohler et al. (2010) confirmed the beneficial effects of PGPR (*Pseudomonas* sp.) strains for the stabilization of soil aggregates. Further, *P. alcaligenes*, *Bacillus polymyxa*, and *Mycobacterium phlei* were capable in high temperatures and high salt concentrations, hence giving them probable reasonable edge to endure in dry and saline soil (Egamberdiyeva et al. 2007).

The soil microbes remain in near relation with the plants and perform important functions in stress relief in crops grown on salt grounds because of their sole characteristics of salinity tolerance: their interface with plants (Fig. 12.2). In addition, some plant species also have such type of resistance against certain soil stresses (Aishah et al. 2016). The inoculation of these microbes in saline soil can alleviate stress and consequently initiate ability and capability in sustainable agriculture. Many earlier reports demonstrate the assumption of the PGPR for positive effects in agriculture sector in relation to sustaining crop productivity with promising results. The beneficial microbes offer exceptional models for explaining the salt stress-tolerant mechanism and its accessibility and response which can subsequently be adapted to crop-induced stresses in crop plants (Grover et al. 2011).

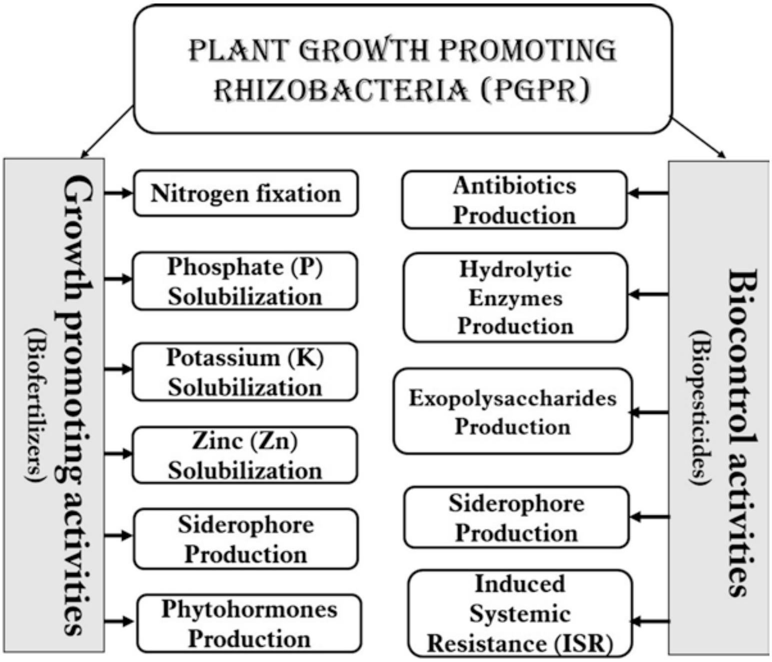

Fig. 12.2 Functions of PGPR for plant growth enhancement under stress and nonstress conditions. (Adapted from Vaishnav et al. 2016)

12.5 Mechanisms of Salt Escape by Microbes

Concentrated saline solutions, such as salts, coastal beach wines, or artificial salt deposits, are occupied by some of efficient prokaryotic microorganisms. Worldwide salt deposits demonstrate that marine saltwater evaporation and the improvement of saline habitations have been a continuing procedure for many years and provide enough time for the development of particular halophilic bacteria and archaea. Halophiles requiring high concentration (0.5 M NaCl) for their proper growth (Reed 1986) implemented two varied mechanisms of osmoregulatory solvents to handle ionic gift and substantial water stress. Two principles of mechanics have developed on Earth to lessen the chemical potential of cell water, which enables an osmotic variation of microorganisms such as salt in cytoplasm mechanism and organic osmolytic mechanism.

12.5.1 Salt in Cytoplasm Mechanism

Salt in cytoplasm mechanism was first revealed in halobacteria and is considered the classic arctic strategy of osmo-adaptation. Fermenter bacteria; asetogenic anaerobes, like *Haloanaerobium*, *Halobacteroides*, *Sporohalobacter*, and *Acetohalobium*;

and sulfate defenders are prominent to serve this plan (Oren et al. 1997). In spite of the profusion of NaCl in the characteristic of haloarchaeal atmosphere, halophiles archaea contain cytoplasm comparatively Na-free. Instead, K gathers in the cell by energy radiant on K uptake system including Cl^- and K^+ which could occur in molar solutions in cytoplasm. Since the K^+ solution in the cell is hundred fold higher than in the contiguous area, part of the protons that causes energy must be implemented to sustain the ionic gradient. However, mechanism in halophilic-anaerobic bacteria is considered differently. Whereas, it is proved that the organisms utilize as low as likely for the preservation of ion radicals. Measurement of the ion configuration of exponential growth cells of *Haloanaerobium praevalens* shows that K is the dominant cation; nevertheless, Na^+ limits are also comparatively high. Cells ingoing the standing phase eventually replace K^+ for Na^+ (Oren et al. 1997). Analysis of *Haloanaerobium and Acetoethylicum* even suggests that Na^+ can be the major cation in constant cells similarly in exponentially growing cells.

12.5.2 Organic Osmolytic Mechanism

Organic osmolytic mechanism is extensive between the bacteria and eukarya and is available in various algae (Robertson et al. 1992). In reply to an osmotic stress, these microorganisms chiefly store organic compounds such as sugar, phenolics, and amino acids (derivatives) by absorption from the nearby environment. These nonionic, extremely water-soluble components do not interrupt metabolism although at great cytoplasmic solutions and are therefore appropriate with the name of companionable solutes. Halophilic bacterial cells that use responsive solvents can preserve fundamentally the similar enzymatic machinery as non-halophilic, but a few adjustments in their inner proteins are marginally more expensive than the cytoplasmic proteins in *Escherichia coli* (Paul and Nair 2008). Halophiles using the organic osmolytic mechanism are highly flexible than organisms that use “salt in cytoplasm strategy” due to its broad salt tolerance, it is able to grow in low salt environments.

The disclosure of microorganisms to maximum osmolality environments causes the floodwater to flow rapidly in the osmotic tendency of cell, thereby causing a decrease in turpentine and cytoplasmic dehydration. The soil microbes have made different adaptations to prevent water flow. Cytoplasma has high ion strength to maintain osmotic equilibrium by preserving the environment-like cytoplasmic salt concentration. The first reaction to osmotic changes and perseverance of water begins with collecting solutions that are suitable for K^+ in cells. Organic osmolytes comprise sugars and amino acids with varied derivatives. Organic solvents can be synthesized either de novo or if absorbed by organisms (Paul and Nair 2008). *Pseudomonas* remains in a state of stress due to the production of exopolysaccharides, which prevents the hydraulic stress and the change in water potential by regulating the water retention and distribution of carbon sources in the microbial environment (Sandhya et al. 2009).

12.6 Predictions and Challenges

One of the most vital limitations on agricultural production in the world is abiotic stress, including salt stress. Currently, a number of researchers have shown the herbicidal microbial interactions that can contribute to the development of tolerant mechanisms in saline soil thus, need to understand the details of various molecular and biochemical mechanisms. However, microorganisms in the salt region or rhizosphere of halophytic plants can avail a respected source for improving plant salt tolerance.

Plant-associated microbes can perform an important role in resisting abiotic stress. These microorganisms may contain rhizoplants, rhizospheres and endophytes, and symbiotic fungal mushrooms. Development of stress-tolerant plants by changing genetic engineering and plant breeding is important, but it is a long and costly process. To eliminate stress in plants, microbial inoculation can provide a choice for friendly environment that can be achieved in a limited time. Microbes can protect wheat and other crops from damaging salt deterioration effects and stresses. The microbes may seem tolerant to salt stress by modulating the factors of at least partial transcription. The transcription factors activate reactions that are compatible with activating the expression of genes encoding ion channels and transducers to reduce accumulation of toxic ions in cytosol. Similarly, genes participate in the synthesis of osmoprotectants and antioxidant protection against plant stresses.

However, further confirmation is necessary to validate the meticulous part of these transcription factors, along with other alleged mechanisms that participate in PGPR-mediated salt quality tolerance in the plants. An unfathomable consideration for the applied problems, limitations, and bounds of prokaryotic survival under extreme situations can be established from the study of halophilic bacteria and archaea. By making available current leaders, joint future research in this area is needed, especially on field assessment and inoculation of possible efficient microorganisms as biopharmaceuticals in stressed soils.

12.7 Conclusions and Future Prospects

The salt-stressed areas are rapidly increasing every year around the-globe and a major public concern nowadays. Saline-soils drown the plant growth and yield. Salinity-also affects the sustainability of beneficial-microorganisms which are being related with plant rhizosphere. Demand for good, ecologically viable and environmentally sound methods is increasing in salt farming (worldwide). The introduction of these microbes, including PGPR, is a significant substitute to certain traditional farming methods which are being widely used. PGPR living together with plant roots proposed to improve plant vegetation and stress relief by various ways. PGPR with salt-tolerant ability have specific mechanisms for osmotolerance, which can deliver a significant benefit to plants grown in saline soil in relation to better plant growth and yield. PGPR improves vegetation through various mechanisms such as chelation of toxic elements, fixation of symbiotic N_2, solubilization of mineral P and other important nutrients, plant hormone production, and biocontrol of plant pathogens. Thus, the applications of microbes (PGPR) increase the efficiency of nutrition and enhance product growth without causing extreme increases in soil salinity.

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13

Proline Accumulation and Oxidative Stress: Diverse Roles and Mechanism of Tolerance and Adaptation Under Salinity Stress

Amrina Shafi, Insha Zahoor, and Umar Mushtaq

Abstract

Osmotic stress caused by salinity is one of the major abiotic factors limiting crop productivity because it affects almost all plant functions. The complex regulatory processes of plant salt adaptation involve control of water flux and cellular osmotic adjustment via biosynthesis of osmoprotectants such as proline. "Proline," a low-molecular compatible solute, accumulation leads to a decrease in cell osmotic potential which permits osmotic adjustment and stabilization and protection of membranes from damaging effects of salt/osmotic stresses. Free proline has been suggested as a metabolic measure of stress and is suggested to play an important role as an organic osmolyte and may be part of a general adaptation to water stress through water retention and prevention of dehydration. Proline has a diverse role in plant system, such as it can protect proteins and enzymes by stabilizing their structures and preventing aggregation during refolding and serve as a sink for the nitrogen from nitrogenous compounds derived from the net loss of protein, and lastly it may represent merely a manifestation of the damaging effects of stress. Cell membrane stability under salt stress is considered to be a major component in water stress tolerance, and proline might perform a protective function by scavenging reactive oxygen species (ROS). Therefore, the present chapter focuses on the recent advances in understanding proline signalling networks of biotechnological relevance in plant salt tolerance and adaptation.

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Abiotic stress tolerance · Cell membrane · Osmotic stress · Proline homeostasis · Salinity stress

13.1 Introduction

High salinity is an important abiotic stress that reduces crop productivity in arid and semiarid regions of the world (Foolad 2007), which has induced surface soil salinization to various degrees (Munns 2005). Nearly about 800 million ha of land and 32 million ha of agricultural land are salt-affected (FAO 2016). Growth, survival and yield of plants have been significantly reduced due to salinity which affects both physiology and biochemistry of plants (Singh et al. 2014; Munns and Tester 2008). Improving salt tolerance of crop plants has the potential to make marginal areas agriculturally productive (Foolad 2007). To improve tolerance and adaptation of plants, it is important to understand the physiological, biochemical and molecular mechanisms evolved by plants to survive with salt stress. During salt stress, several physiological modifications (Fig. 13.1) are initiated such as disruption of membrane stability, impediment of water uptake, nutrient imbalance, low photosynthetic activity, enhanced antioxidant enzymes activity, and generation of ROS via Fenton reaction which will interact with essential macromolecules, leading to cellular damage (Munns and Tester 2008; Yildiz and Terz 2013). These physiological disorders arise during salt stress in plants due to entry and accumulation of both Na^+ and Cl^- into the cells (Fig. 13.1), leading to severe ion imbalance, known as hyperionic stress (Hasegawa et al. 2000). High salt concentrations in soil inhibit plant growth (Fig. 13.1) in three ways: (i) alteration in osmotic pressure of plant cells to prevent plant cell dehydration, (ii) exchange of nutrition ions such as K^+ and Ca^{2+}, and (iii) direct lethal effects of high Na^+ and Cl^- levels on enzymatic and membranous systems.

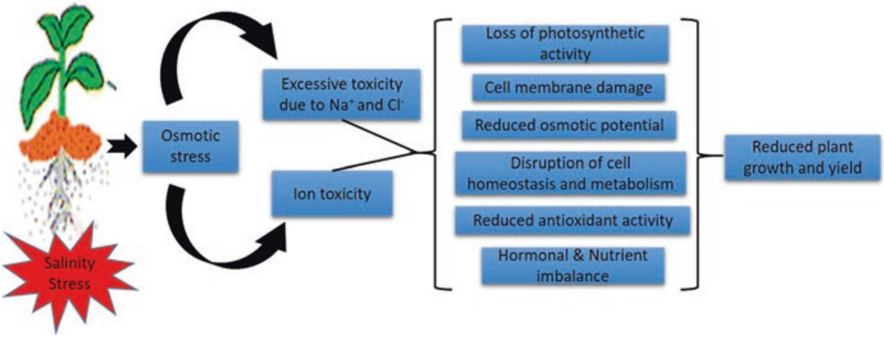

Fig. 13.1 An overview of salinity stress

In order to maintain homeostasis under salt stress conditions, plants have evolved multifaceted physiological and molecular mechanisms to tolerate and protect themselves from these adversative situations (Yamaguchi-Shinozaki and Shinoaki 1993). Complex molecular responses including compatible solutes accumulation, stress proteins production and the expression of different sets of genes are part of the plant signalling and defence system against salinity (Hasegawa et al. 2000; Sairam and Tyagi 2004). General metabolic adaptation, which enables plants to cope with water or osmotic stress, involves the increased synthesis of osmoprotectants. Accumulation of metabolites that act as compatible solutes such as proline, glycinebetaine, etc. is one of the primary strategies to mitigate the effect of salinity stress (Hasegawa et al. 2000; Munns and Tester 2008) followed by exclusion of sodium (Roy et al. 2014). Compatible osmolytes, once accumulated, are thought to reduce the cellular water potential below external values, driving or preserving water in the cell, thus buffering the immediate effect of water shortages (Apse and Blumwald 2002) and may help to maintain the relatively high water content necessary for plant growth and cellular functions.

Proline is a water-soluble amino acid and beneficial solute that accumulate in the plant under different kinds of stresses, and the capacity of proline accumulation depends upon the level of stress, and its concentration varies from one species to another (Hayat et al. 2012; Verbruggen and Hermans 2008). Now it is an established fact that the proline accumulation within plants under adverse or stress conditions act as an osmoregulatory compound and play a significant role in developing stress tolerance (Hare and Cress 1997). *De novo* synthesis and reduction in proline oxidation seemed to be the most predominant mechanism of proline accumulation. Proline accumulation is used as a selection parameter for salt stress tolerance, and its accumulation is caused either by the induction of expression of proline biosynthesis genes or by the repression degradation pathway genes (Marco et al. 2015). Proline accumulation has been demonstrated to be correlated with salinity tolerance in plants (Yancy et al. 1982). Plants under various forms of stress, the concentration increases up to 80% of the amino acid pool (Matysik et al. 2002), a leaf of salt-stressed sorghum (de Lacerda et al. 2001) and soybean (Çelik and Atak 2012). The role of proline and its metabolism under stress conditions have received considerable attention in many plants, and now it is generally accepted that proline has multifunctional roles (Fig. 13.2) such as:

- An osmoprotectant and a protective agent for cytosolic enzymes and cellular organelles (Rejeb et al. 2014).
- Proline serves as a sink for energy to regulate redox potentials and contributes to stabilizing subcellular structures (e.g. membranes and proteins), scavenging reactive oxygen species (ROS), modulating cell redox homeostasis and supplying energy (Smirnoff and Cumbes 1989).
- As a solute that protects macromolecules against denaturation as a means of reducing acidity in the cell (Venkamp et al. 1989).
- Proline has also been considered as a carbon and nitrogen source for rapid recovery from stress and growth (Singh et al. 1973).

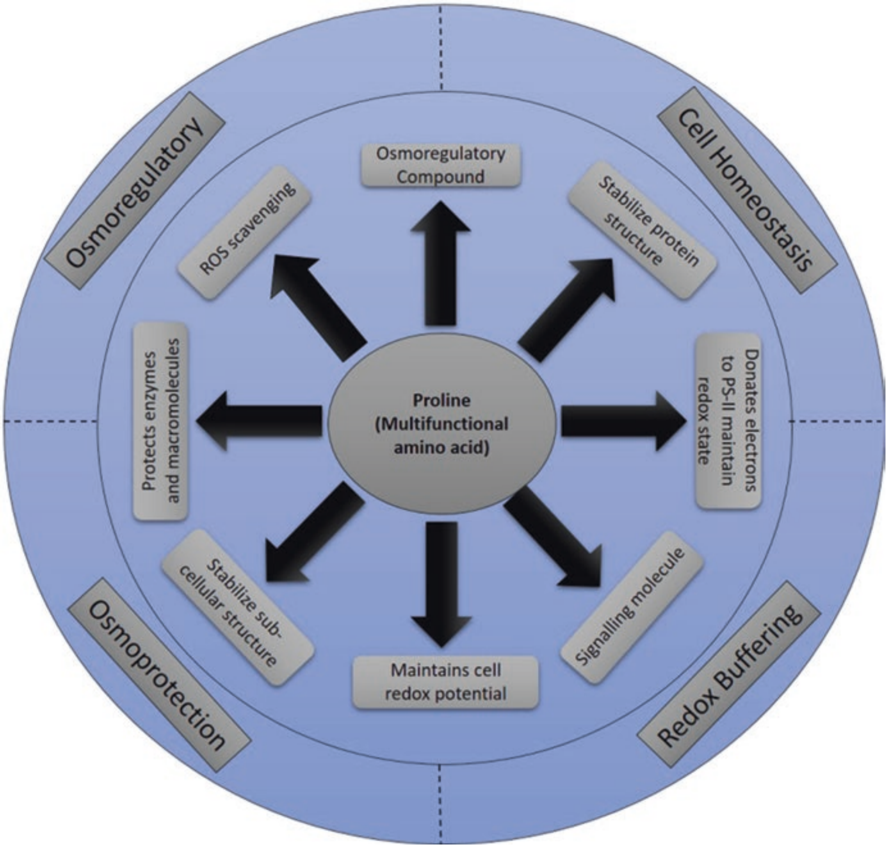

Fig. 13.2 Potential roles of proline during abiotic stress

- Proline counteracts the adverse effects of various stresses on plants by affecting uptake and accumulation of inorganic nutrients and reducing cellular damages and increasing antioxidant defence systems (Hoque et al. 2008).
- Proline has also a potential role in functioning as a signalling molecule to interact with other metabolic pathways under stress conditions, supplying energy and functioning as a signal (Kishor et al. 2005a, b; Verbruggen and Hermans 2008; Szabados and Savouré 2010).
- It may also function as a protein-compatible hydrotrope alleviating cytoplasmic acidosis and maintaining appropriate NADP$^+$/NADPH ratios compatible with metabolism (Hare and Cress 1997). Moreover, Pro is thought to be macromolecules or a component of signal transduction pathways that regulate stress-responsive genes (Szabados and Savouré 2010; Székely et al. 2008). An additional benefit of replenishment of NADP$^+$ supply by proline biosynthesis may be to support redox cycling.

Proline is also water soluble which also justify its accumulation when water-deficit conditions arise and its accumulation has been proposed not only due to salinity-induced signals but also due to its basic chemical properties. Multifaceted activities of proline have been stated by many indicating not only counteraction of stress but also cellular redox buffering (Lehmann et al. 2010; Szabados and Savoure 2010). Thus, due to its intrinsic properties as well as specific aspects of proline metabolism, proline upregulation has attained prominence in plant stress biology. As salinity is a constraint limiting plant growth and productivity of crops throughout the world, understanding the mechanism underlying plant response to salinity which will provide new insights into the improvement of salt-tolerant crops is of importance. So, it is of great importance to understand and utilize the regulatory mechanism of proline metabolism to improve the stress resistance of plants. Based on these facts, the present chapter focus on how proline biosynthesis and transport under salt stress conditions play a multifunctional role in terms of osmotic adjustment, membrane stability, protein protection, ROS scavenging, photosynthesis, seed germination, etc. Therefore, the present chapter focuses on the recent advances in understanding proline signalling networks of biotechnological relevance in plant salt tolerance and adaptation.

13.2 Proline Biosynthesis: Anabolism and Catabolism

“Proline”, a multifunctional amino acid (Fig. 13.2) and also a signalling molecule, acts as a plant growth regulator by triggering cascade signalling processes (Szabados and Savouré 2009). Proline is preferred as a common osmolyte in plants and gets upregulated against different stresses (Yildiz and Terz 2013; Szabados and Savouré 2009). Compartmentalization of proline metabolism implies that extensive intracellular proline transport must occur between the cytosol, chloroplasts and mitochondria (Fig. 13.3). There are two alternative routes in proline biosynthesis in higher plants: the L-ornithine and the L-glutamate pathways. Glutamate and ornithine pathways synthesize necessary proline in plants in cytoplasm and chloroplast whereas its degeneration occurs in mitochondria (Szabados and Savouré 2010). Proline synthesis in higher plants is directly linked to a cellular process involving the electron-transport chain via two different pathways (Fig. 13.3).

- The Glu pathway normally lies in the cytosol and chloroplasts (Verbruggen and Hermans 2008). Bifunctional enzyme 1Δ-pyrroline-5-carboxylate synthase (P5CS) catalyses the generation of glutamate-γ-semialdehyde (GSA) from glutamate costing ATP and NADPH and GSA (Fig. 13.3), thus if generated, will be spontaneously converted to 1-pyrroline-5-carboxylate (P5C). P5C is then reduced to proline by 1-pyrroline-5-carboxylate reductase (P5CR). P5CS1 and P5CS2 are the two isoforms of P5CS of which P5CS1 is localized in the chloroplast and is active in stress-induced proline synthesis and P5CS2 in the cytosol (Szekely et al. 2008).

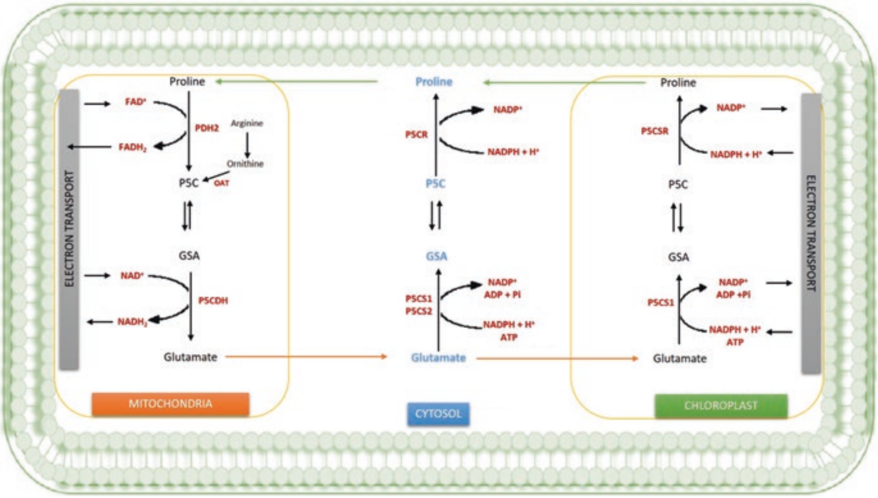

Fig. 13.3 Proline metabolism in higher plants. Proline biosynthesis occurs in the cytosol and is mediated by P5CS and P5CR enzymes. Under salt stress conditions, P5CS1 accumulates in the chloroplasts, leading to enhanced proline biosynthesis in the chloroplast. Proline degradation occurs in the mitochondria, where proline is oxidized to P5C and glutamate through sequential action of PDH and P5CDH. The ornithine pathway uses arginine and produces P5C and glutamate in mitochondria. Mitochondrial P5C can be recycled to proline in the cytosol by P5CR

- The Orn pathway occurs in the mitochondria (Fig. 13.3). In the ornithine pathway of proline synthesis, ornithine-δ-aminotransferase (δ-OAT) converts ornithine into GSA. Ornithine-δ-aminotransferase forms GSA and glutamate by transferring the δ-amino group of Ornithine to α-ketoglutarate. Spontaneously due to the cyclic equilibrium with P5C, GSA will be converted to P5C which will be transported to the cytosol where P5CR mediates the synthesis of proline (Verbruggen and Hermans 2008). Since ornithine act as a precursor for the formation of GSA or P5C, this pathway is named as the ornithine pathway (Delauney et al. 1993). Ornithine levels will be maintained by arginine biosynthesis cycle. In the mitochondria, glutamate dehydrogenase (GDH) also generates glutamate from ammonium and α-ketoglutarate which can also deaminate glutamate for the production of α-ketoglutarate.

The catabolism of proline commences with the oxidation of proline to P5C by proline dehydrogenase (PDH), using FAD as a cofactor. P5C is sequentially converted to glutamate by pyrroline-5-carboxylate dehydrogenase (P5CDH) using NAD^+ (Fig. 13.3). Proline accumulation is mediated by its increased synthesis and reduced oxidation. Among the above-mentioned enzymes involved in proline metabolism, P5CS is generally considered to be the key enzyme for proline synthesis, while PDH plays a key role in degradation. At times interconversion of proline and glutamate termed as proline cycle (Verslues and Sharma 2010). Intracellular proline levels are determined by biosynthesis, catabolism and transport between cells and different cellular compartments. Computer predictions suggest a mainly cytosolic localization of the biosynthetic enzymes (P5CS1, P5CS2 and P5CR), whereas a mitochondrial localization is predicted for the enzymes involved in proline catabolism, such as PDH1, PDH2, P5CDH and OAT. Although signal peptides could not be identified within the primary structure of P5CS1, P5CS2 and P5CR enzymes, the PDH1, P5CDH and OAT proteins have well recognizable mitochondrial targeting signals. Proline oxidase is the main regulatory enzyme responsible for the accumulation of osmolytes; it converts proline into glutamate. The biosynthetic pathway from glutamate involves an extremely high rate of consumption of reductants and also proline degradation is capable of high-energy output. Proline accumulation appears to be a worthy means of storing energy since the oxidation of one molecule of proline can yield 30 ATP equivalents.

13.3 Potential Functions of Proline Transporters Under Osmotic Stress Conditions

It has been well established that under various stress conditions, transport of nitrogenous compounds is altered and specific amino acids, such as proline, accumulate (Breitkreuz and Shelp 1995). Under the assumption that proline accumulation is a cell-autonomous effect and a capacity of all cell types, researchers so far have concentrated on biosynthesis rather than translocation. Inter- and intracellular transport of proline is critical for cellular homeostasis. Although intracellular transporters have yet to be identified, patterns of intercellular transporter transcription have been derived for a number of plant species (Lehmann et al. 2010). Physiological data suggest that proline uptake into mitochondria is an active process, hinting at the existence of specific amino acid transporters (Yu et al. 1983). Plasma membrane proline transporters, identified in several plants, mediate proline transport between cells and organs, but are not involved in organellar transport (Grallath et al. 2005). Two proline carriers have recently been identified in the mitochondria of durum wheat (*Triticum durum*): a proline uniporter, which facilitates proline transport into the mitochondrial matrix, and a proline/glutamate antiporter, involved in the Pro/Glu shuttle between the mitochondrial matrix and the cytosol (Di Martino et al. 2003). Basic amino acid transporters (BAC) can deliver arginine and ornithine through the mitochondrial membrane (Palmieri et al. 2006). Up to now, some proline transporters have been isolated and characterized in many plants such as *Arabidopsis thaliana* (Grallath et al. 2005), *Lycopersicon esculentum* (Schwacke et al. 1999) and *Chrysanthemum lavandulifolium* (Zhang et al. 2014), but research about their roles in proline transport is still scarce and superficial. In addition, because of the subcellular compartmentation of proline metabolism in plants, the dynamic transport process of proline is also vital for the protective role of proline. Although some specific proline transporters have been isolated and characterized, there are only a few reports showing a direct role of ProTs in proline transport in plants (Kishor et al. 2005a, b; Lehmann et al. 2010).

The intracellular localization, substrate selectivity and affinity of three *Arabidopsis thaliana* AtProTs (belonging to a branch of amino acid transporter family 1) *AtProT1*, *AtProT2* and *AtProT3* are all localized at the plasma membrane: *AtProT1* is transcribed in the phloem, *AtProT2* in the root epidermis and cortex, and *AtProT3* in the leaf epidermis (Grallath et al. 2005). While the tomato gene *LeProT1* is transcribed uniquely in the pollen grain (Schwacke et al. 1999), transcription of the barley gene *HvProT* is root tip-specific and results in an enhanced local accumulation of proline which encourages root elongation under salt stress (Ueda et al. 2008). Analysis of the behaviour of heterologous expressors of *HvProT* in *A. thaliana* indicates that when *ProT*genes are knocked out, then either other amino acid transporters act to compensate or proline metabolism is altered (Ueda et al. 2008). The elevated transcript abundance of *ProDH* in these knock-out lines seems to disrupt ProT activity and activate proline degradation (Ueda et al. 2008). Although the cellular concentration of proline is regulated by increasing its synthesis and decreasing its degradation, long-distance proline transporters seem to contribute heavily to the enlargement of the proline sink in reproductive tissues (Schwacke et al. 1999; Grallath et al. 2005). Besides the *ProT* genes, gene-encoding amino acid/auxin permease (AAP) and the amino acid/polyamine/choline families also mediate proline uptake across the plasma membrane (Rentsch et al. 2007). The selective transport of proline by ProTs and their expression analysis during the plant's exposure to salinity stress suggest that the ProTs represent an important component of proline homeostasis both under stressful and non-stressful.

13.4 Proline Biosynthesis Under Salinity Stress

Proline accumulation is a common physiological response and an adaptive mechanism of higher plants to cope with salinity and osmotic stress (Ashraf and Foolad 2007). Under stress condition, the standard model is that upregulated proline synthesis and downregulated proline degradation lead to proline accumulation (Fig. 13.3) and, thereby, stress tolerance (Nanjo et al. 2003). Moreover, proline accumulation under stress conditions may be caused by induction of proline biosynthesis enzymes, reduction the rate of proline oxidation conversion to glutamate, decline utilization of proline in proteins synthesis and enhancing proteins turnover (Tammam et al. 2008). Gene-encoding proline biosynthesis enzymes have recently been cloned from higher plants (Delauney and Verma 1990). Proline biosynthesis is controlled by the activity of two P5CS genes in plants, encoding one housekeeping and one stress-specific P5CS isoform (Zhang et al. 1995). Some studies demonstrate that, in stress condition, most of the proline accumulated in plants is the result of enhanced synthesis from glutamate (Delauney and Verma 1993; Hare and Cress 1997), which is essentially regulated at the transcription level of Δ1-pyrroline-5-carboxylate synthase 1 (P5CS1) encoding the rate-limiting enzyme of the pathway. Studying the effects of salt stress on enzyme activities involved in proline metabolism could provide valuable information on the physiological significance of its accumulation.

- PDH1 expression is very low under normal condition but it is activated by 24-hr salt treatment and PDH2 expression is generally low but it is induced by salt stress (Funck et al. 2010).
- In *Arabidopsis thaliana*, salt stress differently activate the expression of two related P5CS genes: *AtP5CS1 (At2g39800*) and *AtP5CS2 (At3g55610*). *AtP5CS1* is responsible mainly for Pro accumulation during salt stress (Strizhov et al. 1997). *AtP5CS2* transcription is slightly elevated by salt stress (Strizhov et al. 1997).
- Proline catabolism is controlled by ProDH (*At3g30775*), whose transcription is activated by enhanced proline levels and is repressed under osmotic stress conditions (Verbruggen et al. 1996).
- Another key enzyme gene for proline synthesis through Orn pathway, *KvOAT* expression increased with increasing salt concentrations or prolonged salt stress. Thus, for proline biosynthesis, the proline-regulated expression of *KvP5CS1* played a more important role than *KvOAT* for proline accumulation in leaves under salt stress (Lehmann et al. 2010; Szabados and Savouré 2010).
- It has recently been reported that pretreatment of Arabidopsis with menadione sodium bisulphite could induce more rapid and higher proline accumulation upon subsequent salt stress, which was associated with a hypomethylation state at the promoter region of P5CS1 and proline dehydrogenase 1 (PDH1) (Zhang et al. 2013).
- In rice, the progenies of osmotically stressed plants were found to acquire altered DNA demethylation in P5CS1 and ornithine-δ-aminotransferase (δ-OAT), but not in pyrroline-5-carboxylate reductase (P5CR), correlated with the inheritable upregulation of P5CS1 and δ-OAT and elevated proline accumulation (Jiménez-Arias et al. 2015).
- In pea *(Pisum sativum*) mesophyll protoplasts, P5CR activity was localized in chloroplasts, suggesting that P5CR accumulates in plastids under high osmotic conditions (Rayapati et al. 1989).
- Housekeeping proline biosynthesis probably occurs in the cytosol, and in *Arabidopsis,* it is controlled by the P5CS2 gene (Székely et al. 2008). During osmotic stress, proline biosynthesis is augmented in the chloroplasts, which is controlled by the stress-induced P5CS1 gene in Arabidopsis (Savouré et al. 1995; Strizhov et al. 1997). Therefore, proline can be synthesized in different subcellular compartments, depending on the environmental conditions.
- In rice, OsP5CS upregulated for 10 h after the treatment of 250 mM NaCl and kept the trend for 24 h. In a recent study, the activity of OsP5CS enzyme was reported to increase by about 19%, subjected to 425 mM NaCl and led to proline accumulation (Bagdi and Shaw 2013).
- In alfalfa, MsP5CS1 and MsP5CS2 cDNAs were isolated and their expression under 90 mM NaCl was studied in roots of 6-day-old alfalfa seedlings (Strizhov et al. 1997). In the ectopic expression of moth bean P5CS in wheat, the transgenic lines could tolerate the salinity up to the concentration of 200 mM.

13.5 Enhanced Proline Accumulation Is Linked to Disturbed Homeostasis Under Salt-Stress Exposure

One of the most important mechanisms by higher plants under salt stress is the accumulation of compatible solutes such as proline, which helps to maintain the osmotic pressure and function as a stress resistor (Fig. 13.2). Proline accumulation generally observed in the cytoplasm with higher concentrations and chloroplast stroma whereas compatible solutes like sugars accumulated in vacuoles (Voetberg and Sharp 1991). After the plant is exposed to salt stress in *Arabidopsis*, it accounted for 20% of the amino acid pool (Verbruggen et al. 1996). In most plant species studied, proline accumulation during stress is the result of reciprocal action of increased biosynthesis and inhibited degradation (Kishor et al. 2005a, b). Increased proline accumulation under NaCl stress may be due to the breakdown of a proline-rich protein or de novo synthesis of proline (Tewari and Singh 1991). Proline accumulation under salt stress can be explained by the higher inhibitory rate of proline dehydrogenase and proline oxidase (Misra and Gupta 2005). The biosynthesis and degradation of proline and its accumulation in plants are regulated by different abiotic stresses, and salinity has a great concern (Yang et al. 2000). Proline provides tolerance against different abiotic stresses by increasing their endogenous level and their intermediate enzymes in plants.

- Several reports show a significant role of proline in osmotic adjustment, protecting cell structure and its function in plants in salt-tolerant and salt-sensitive cultivars of many crops (Turan et al. 2007).
- In response to salinity stress, proline accumulation normally occurs in the cytosol where it contributes substantially to the cytoplasmic osmotic adjustment, e.g. *Distichlis spicata* cells treated with 200 mM NaCl, the cytosolic proline concentration was estimated to be more than 230 mM (Ketchum et al. 1991). In the apical region of maize roots growing at a water potential of −1.6 MPa, proline concentration reached approximately 120 mM and accounted for up to 50% of the osmotic adjustment (Voetberg and Sharp 1991).
- Dracup (1991) mentioned that proline levels increase with increasing salt concentrations. This is consistent with the experiments of Lutts et al. (1999) in rice cultivars that are sensitive to salt stress. Similar results were obtained in experiments made with *Sapindus trifoliatus* L. somatic embryo using different NaCl concentrations.
- The extent of proline accumulation varies in different plant species: the proline content in leaves increased with increasing NaCl concentrations and prolonged stress time. The similar finding was reported in green gram (Misra and Gupta 2005), canola (Xue et al. 2009) and *Jerusalem artichoke* (Huang et al. 2013).
- In case of a salt-sensitive variety of barley, accumulation of osmolytes proline along with NaCl-induced K^+ efflux was found an effective indicator of salt stress tolerance (Di Martino et al. 2003). Petrusa and Winicov (1997) demonstrated that salt-tolerant alfalfa plants rapidly doubled their proline content in roots, whereas in salt-sensitive plants the increase was slow.

- A comparative study between sensitive and tolerance genotype reported that the proline content increased under salt stress conditions, while an average increase of proline was five-fold in root and 20-fold in leaf (Munns 2002). By comparing different cultivars of sunflower in response to salt stress, Shahbaz et al. (2011) found that salt-tolerant cultivars exhibited greater proline accumulation.
- A significantly higher accumulation of proline associated with osmotic adjustment and protection of membrane stability has been reported in salt-stressed alfalfa (Amooaghaie 2011), soybean (Weisany et al. 2012), and barley (Turkyilmaz et al. 2014).
- Proline accumulation in response to salinity stress has been observed in several monocot species, such as sorghum (De Lacerda et al. 2003) and sugarcane (García and Medina 2003). Sugarcane has been found to accumulate proline in leaves and roots when subjected to 100 mM NaCl treatment for 60 days (García and Medina 2003).
- Several techniques to enhance the endogenous proline accumulation for salt defence mechanism exist, such as exogenous application (Hoque et al. 2007), biosynthesis gene(s) overexpression (Han and Hwang, 2003) and degradation gene(s) knockout (Nanjo et al. 1999a). Exogenous application of proline may be a good approach to decrease the undesirable effects of salinity stress on plants.

13.6 Proline and Its Role in Osmoregulation and ROS Detoxification

ROS abundance is controlled by a combination of enzymatic and non-enzymatic means, and in plants subjected to stress, proline seems to scavenge singlet oxygen and free radicals including hydroxyl ions and hence stabilize proteins, DNA, as well as a membrane (Matysik et al. 2002). Proline is known to be an osmoregulatory solute in plants under hyperosmotic stress (Delauney and Verma 1993). It is also involved in cell osmoregulation and protection of proteins during dehydration, and it can act as an enzymatic regulator under stress conditions (Rontain et al. 2002). The activity of osmolytes like proline and glycine betaine have been observed to be having a considerable effect on radicals generated during salinity stress. In addition, proline can act as a ROS scavenger and has been shown to enhance both the expression of antioxidant enzyme-coding genes and their activity (Hoque et al. 2007). Okuma et al. (2004) suggested that proline acts as a free radical scavenger that alleviates salt stress and reduces the oxidation of lipid membranes. Therefore, proline provides protection against NaCl-induced oxidative damage by decreasing the levels of ROS accumulation and lipid peroxidation, as well as improving membrane integrity. As an alternative to direct ROS scavenging feature, proline can protect and stabilize ROS scavenging enzymes and activate alternative detoxification pathways. Several studies have attributed an antioxidant feature to proline, suggesting ROS scavenging activity and proline acting as a singlet oxygen quencher (Smirnoff and Cumbes 1989; Matysik et al. 2002). Some of the recent work which supports the role of proline in osmoregulation is as follows:

- In salt-stressed tobacco cells, proline increased the activities of methylglyoxal detoxification enzymes, enhanced peroxidase, glutathione-S-transferase, superoxide dismutase and catalase activities, and increased the glutathione redox state (Székely et al. 2008; Hoque et al. 2008).
- Free radical levels were reduced in transgenic algae and tobacco plants engineered for a hyperaccumulation of proline by P5CS overexpression and acceleration of the proline biosynthetic pathway (Hong et al. 2000).
- Compromised proline accumulation in p5cs1 insertion mutants led to the accumulation of ROS and enhanced oxidative damage (Siripornadulsil et al. 2002), supporting the role of proline in ROS scavenging.
- In the desert plant Pancratium maritimum, catalase and peroxidase were found to be stabilized by proline during salt stress (Khedr et al. 2003).
- The salt-hypersensitive p5cs1 *Arabidopsis* mutant shows reduced activities of key antioxidant enzymes of the glutathione-ascorbate cycle, leading to hyperaccumulation of H_2O_2, enhanced lipid peroxidation and chlorophyll damage (Székely et al. 2008).
- In wheat seedlings (Naidu et al. 1991) and in durum wheat, a positive correlation was observed between proline levels and osmotic potential, and it was concluded that proline is an osmoregulatory component.
- In grapevine, proline ameliorates the enzymatic inactivation of ascorbate peroxidase (APX) and peroxidase, while it reduces the activity of superoxide dismutase (SOD) and catalase (CAT; Ozden et al. 2009).
- Proline can enhance the primary photochemical activity of isolated *Brassica juncea* thylakoid membranes by limiting photoinhibition (Alia and Saradhi 1991). The damaging effects of singlet oxygen and hydroxyl radicals on photosystem II (PSII) can be reduced by proline in isolated thylakoid membranes (PSII) (Alia and Mohanty 1997).
- Proline may also function as a protein-compatible hydrotrope and as a hydroxyl radical scavenger (Smirnoff and Cumbes 1989).
- Transgenic *Arabidopsis* and potato plants overexpressing antioxidants genes (SOD and APX) also showed enhanced accumulation of proline under salinity stress (Shafi et al. 2014, 2015a, b, 2017).

Thus, perhaps proline protects cells against osmotic stress by increasing their antioxidant enzyme capacity. Instead, the antioxidant capacity of proline may be related to the scavenging of hydroxyl radicals (OH) radicals (Smirnoff and Cumbes 1989). Unravelling the underlying mechanics of OH radical quenching by proline remains a priority area of research in the context of proline's function as an antioxidant. In addition, the transient accumulation of proline might serve as a safety valve to calibrate cellular redox state during stress (Kuznetsov and Shevyakova 1997). In plants, proline also scavenges singlet oxygen and free radicals that induce damages and performs an important role in the protection of proteins against denaturation

(Alia and Saradhi 1991). It can be concluded that proline accumulation and antioxidant activities may cooperate with each other for more tolerance to salt stress.

13.7 A Transgenic Approach to Improve Salinity Stress Tolerance by Overexpression of Proline Biosynthesis Genes

Metabolic engineering involves directed improvement of cellular properties through the modification of specific biochemical reactions or the introduction of new ones, with the use of recombinant DNA technology (Stephanopoulos 1999). Several roles have been proposed for the massive stress-induced accumulation of proline in plants, including osmotic adjustment, membrane protection, free radical scavenging and redox buffering (Verbruggen and Hermans 2008; Kishor et al. 2005a, b). These physiological roles of proline in the context of various abiotic stresses have been experimentally explored by overexpressing or suppressing a number of synthesis and catabolism pathway genes (Strizhov et al. 1997). Osmoprotectant synthesis in naturally stress-tolerant species is highly regulated by stress. In addition to the use of stress-inducible promoters for engineering osmoprotectant synthesis pathways, genes involved in stress signal sensing are additionally useful for engineering stress-tolerant plants (Table 13.1). One option is the enhancement of proline biosynthesis via increased expression of rate-limiting genes in transgenic plants which could be done either via repeatedly engineering the gene or by crossing and selecting transgenic plants engineered for different traits (Table 13.1). Elevated levels of proline, caused by overexpression of P5CS, can confer enhanced tolerance to salt stress in plants (Han and Hwang 2003). The gene products involved in ion homeostasis have been identified by the use of yeast model systems and by analysing mutants altered for salt sensitivity (Liu et al. 2000). The overexpression of key proline synthesis pathway genes results in modifications to the shoot/root biomass ratio, the inflorescence architecture and economic yield under stressful conditions (Kishor et al. 1995), while knockout transgenic lines have highlighted its importance for development (Székely et al. 2008).

Proline regulates expression of a number of genes related to antioxidant enzymes under salt stress. Among different genes, one of the Δ1-pyrroline-5-carboxylate synthase genes is responsible for upregulating the stress-induced proline accumulation under salinity stress (Kim and Nam 2013). Furthermore, proline is known to induce expression of salt stress-responsive genes, which possess proline-responsive elements in their promoters (Satoh et al. 2002). Some of the salt stress-induced genes such as *RD29A* encode putative protective proteins (Yamaguchi-Shinozaki and Shinozaki 1993), whereas others, such as *AtMYB* and *AtPLC*, encode proteins that share sequence identities to transcription factors and signalling components (Urao et al. 1993).

Table 13.1 Transgenic plants with engineered proline biosynthesis genes

Transgenic plants	Genes engineered	Response	References
Tobacco	Co-expressed *OsP5CS1* and *OsP5CS2* genes from rice	High proline content and the increased root biomass under stress conditions.	Kishor et al. (2005a, b)
Tobacco	Overexpressing Vigna P5CS	Increased concentration of proline and resistance to salinity stresses	Kishor et al. (1995) and Hong et al. (2000)
Rice, wheat and carrot	Overexpressing P5CS gene	Plants showed resistance to salinity stress	Zhu et al. (1998), Sawahel and Hassan (2002) and Han and Hwang (2003)
Arabidopsis	*P5CS* mutant lines and expressed P5CS antisense	ROS scavenging enzymes demonstrated significantly lower activities. Indicating that either proline protects the enzymes of the glutathione-ascorbate cycle or increases their activities	Nanjo et al. (1999a,b)
Potato	*Arabidopsis P5CS* gene transferred into potato	High proline content compared to those at control under high salt stress (100 mM NaCl) and exhibited improved salinity tolerance	Hmida-Sayari et al. (2005)
Petunias and pigeon pea (*Cajanus cajan*)	Petunia was modified with pyrroline-5-carboxylate synthase genes (*AtP5CS* from *A. thaliana* or *OsP5CS* from *Oryza sativa* L.)	More proline production which resulted in water stress tolerance for a period of 14 days	Surekha et al. (2014)
Rice and wheat	Overexpressing *P5CS* gene	Salt stress tolerance due to proline accumulation	Zhu et al. (1998)
Arabidopsis	*P5CS* gene promoter	Enhanced proline accumulation and stress tolerance	Zhang et al. (1997)
Rice	*P5CS* gene isolated from rice and then introduced in the same plant	Improved growth and stress tolerance	Hur et al. (2004)
Indica rice	Overexpressing *P5CSF129A* gene	Enhanced proline accumulation and salt stress tolerance	Rathinasabapathi (2000)
Pigeon pea	Mutagenized with the alternate version (*P5CSF129A*) of wild *P5CS* gene from *Vigna aconitifolia*	Four times higher proline content with more chlorophyll, better growth and relative water content and reduced levels of lipid peroxidation	Surekha et al. (2014)

(continued)

Table 13.1 (continued)

Transgenic plants	Genes engineered	Response	References
Arabidopsis	Antisense proline dehydrogenase cDNA	Overexpresses proline and showed tolerance to salinity (600 mmol NaCl)	Nanjo et al. (2003)
Brassica napus	Induced expression of BnP5CS1and BnP5CS2	BnPDH expression was inhibited under salt stress, ABA treatment and dehydration, prior to proline accumulation	Xue et al. (2009)
Arabidopsis	PDH inhibition by knockout or antisense repression	Marginal increases in pro levels under normal conditions	Mani et al. (2002); Nanjo et al. (2003)
Arabidopsis	PDH and P5CDH downregulation and P5CS upregulation	Improved proline metabolism and accumulation under salinity stress	Verslues and Sharma (2010)

13.8 Role of Proline Metabolism in the Plant for Interaction and Development of Stress Tolerance

An increase in proline content is a positive indicator of salinity tolerance, and proline accumulation in response to environmental stresses has been considered by a number of investigators as an adaptive trait concerned with stress tolerance (Rhodes and Hanson 1993). It is generally assumed that proline is acting as a compatible solute in osmotic adjustment (Fig. 13.4), acts as an enzyme protectant, stabilizes membranes and cellular structures during hostile conditions, detoxifies free radicals by forming long-lived adducts with them and affects solubility of various proteins by interacting with their hydrophobic residues (Delauney and Verma 1993; Hare and Cress 1997). Proline accumulation and stress tolerance correlation have been reported in different studies, and it has been observed that proline concentrations are higher in stress-tolerant plants than in stress-sensitive plants (Misra and Gupta 2005). A positive correlation between abiotic stress tolerance and free proline accumulation has been reported (Martinez et al. 2003). There are many transgenic plants with high-stress tolerance generated that involve multiple physiological and biochemical mechanisms and numerous genes. Transgenic plants with commercial value should at the same time retain relatively high productivity and other traits important for agriculture. Moreover, genetic modification combined with marker-assisted breeding programs with stress-related genes and QTLs, and ultimately, the different strategies should be integrated, and genes representing distinctive approaches should be combined to substantially increase plant stress tolerance. Thus, proline content in plant tissues was used as a marker-assisted selection molecule in some breeding programs that endeavoured to improve salinity tolerance in plants (Ashraf and Foolad 2013). However, proline accumulation can influence

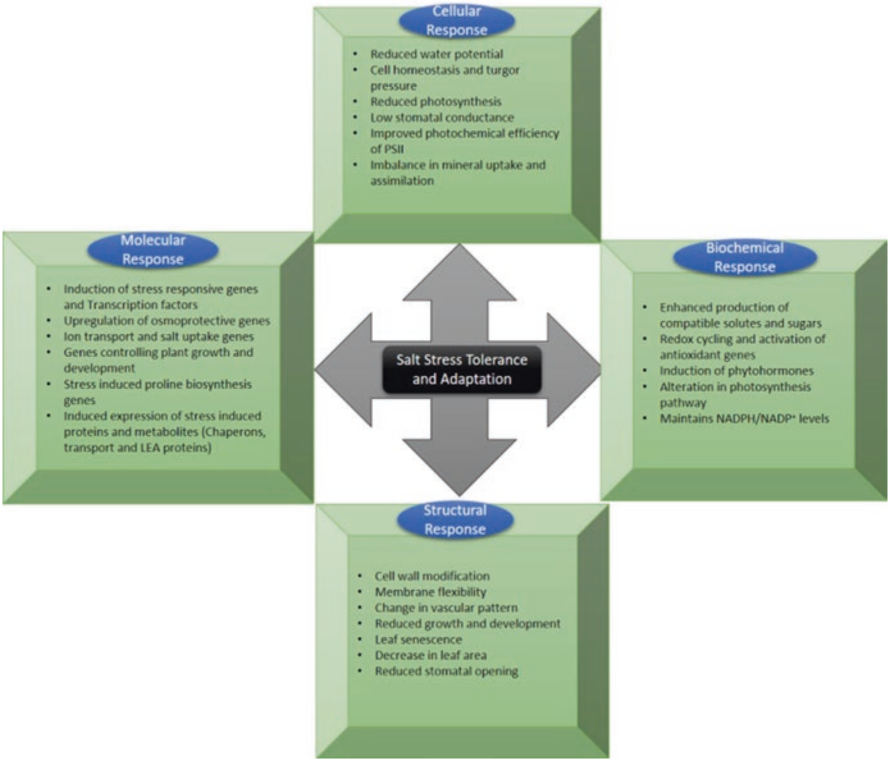

Fig. 13.4 Adaptive and tolerance mechanism under salt stress

stress tolerance in multiple ways (Fig. 13.4), and proline accumulation has been correlated to salt stress tolerance in plants.

- In *Capsicum chinense* Jacq. varieties, Rex (tolerant) and Chichen-Itza (sensitive) mechanism of salt tolerance was observed more intolerant variety due to the accumulation of proline under salt stress (Bojórquez-Quintal et al. 2014).
- Increased proline content and improved tolerance to abiotic stresses, in salt-stressed calli of *Suaeda nudiflora* as a halophyte plant, suggested that proline protects the callus cells from membrane damage caused by free radicals during salt stress (Cherian and Reddy 2003).
- Proline accumulation and aroma generation in the aromatic rice have been reported by a number of authors, e.g. Yoshihashi et al. (2002) demonstrated that the accumulation of proline as osmoregulator is positively correlated with aroma generation in seeds of KDML105 rice grown in the regions of dry and increased salt conditions. Suprasanna et al. (1998) reported that the supplementation of L-proline into the culture medium can yield an increase in aroma production in callus cultures of aromatic rice PB1 and basmati. Therefore, proline content is

often used as a marker-assisted breeding tool aimed at improving salinity tolerance.
- Several comprehensive studies using transgenic plants or mutants demonstrate that proline metabolism has a complex effect on development and stress responses, and that proline accumulation is important for the tolerance of certain adverse environmental conditions (Hare and Cress 1997; Kishor et al. 2005a, b; Verbruggen and Hermans 2008; Székely et al. 2008).
- The intensity of side effect of salinity may vary depending upon the amount of salt deposition in the soil and tolerance level of a particular crop (Munns 2002).

Various attempts were taken by the worker to find out some important selection criteria for salinity tolerance during the early stage of sowing especially up to seedling establishment, but initial screening or evaluation has no much importance in respect to yield under salinity stress condition (Munns 2002). With the advancement of molecular techniques, data has clearly indicated that proline metabolism is actively controlled by stress-induced signals, and the usage of reverse genetics (Székely et al. 2008) has made possible more direct tests of the requirement of proline accumulation for stress resistance. The main perplexing question is how proline contributes to plant stress resistance, the metabolic modulation that allows elevated levels of proline to accumulate, and whether modification of proline metabolism is beneficial for biotechnological improvement of plants.

13.9 Implications of Crosstalk Between Phytohormones and Sugars on Proline Homeostasis Under Salinity

Phytohormones and sugars influenced the proline metabolism differently; the highest proline accumulation was observed in the case of abscisic acid (ABA) treatments. Recent studies proposed that phytohormones play a role in regulating and controlling proline metabolism during salinity tolerance (Iqbal et al. 2014). How various hormones interact with one another to regulate *P5CS* and *ProDH* expression and thereby influence the accumulation/degradation of proline during development is an important aspect for understanding proline homeostasis.

- Several studies supported the effect of ABA on the expression of P5CS and P5CR during abiotic stress (Hare et al. 1999).
- Treatment with ABA, a stress-inducing hormone, caused an increase in proline in plant tissues (Yang et al. 2000). There is crosstalk between the mechanisms that respond to high salinity conditions in plants, where ABA plays a central role.
- At the molecular level, ABA induces proline synthesis in some plant species, and thereby enhanced salt tolerance (Yang et al. 2000). In *Arabidopsis*, ABA is required to activate the two *P5CS* genes, which control the level of proline synthesized in the cell (Strizhov et al. 1997). These observations imply that the activation of the proline synthesis pathway is tightly linked to ABA-mediated signal transduction.

- Data mining suggests that *ProDH* is regulated to some extent by a network of group-S basic leucine zipper transcription factor proteins associated with ABA-independent signal transduction (Weltmeier et al. 2006). Thus, de novo production of ABA enhances salinity tolerance in some plant species.
- Increased proline contents were detected not only upon stress conditions but also after ABA treatments (Szalai et al. 2017).
- The facts that proline accumulation is mediated by both ABA-dependent and ABA-independent signalling pathways and that ABA modulates proline synthesis both on the transcriptional level through induction of gene expression of Δ1-pyrroline-5-carboxylate synthase (*P5CS*) and on a post-transcriptional level by stabilizing the *P5CS* transcript are well-studied (Hare et al. 1999). It appears that water stress triggers the production of ABA which, in turn, induces various genes.
- Auxin also acts as a regulator of both *P5CS* and *OsOAT* (You et al. 2012). Mutations in *AtABI1* and *AtAXR2*, which affect, respectively, ABA and auxin signal transduction, decrease the abundance of both *AtP5CS1* and *AtP5CS2* transcript during salinity stress.
- Nitric oxide and H_2O_2 both mediate ABA signalling and activate *P5CS1* (Neill et al. 2008), although the former also downregulates *ProDH1*. The accumulation of Pro in the course of osmotic stress is partially due to ABA andH_2O_2 signalling (Rejeb et al. 2014).
- Salicylic acid supports proline accumulation via an enhancement in P5CR activity (Misra and Saxena 2009).
- Proline accumulation under osmotic stress is accompanied by a rise in the concentration of soluble sugars. Exogenous application of glucose and sucrose have been observed to increase concentrations of proline (Larher et al. 1993).
- The connection exists between polyamine metabolism and abscisic acid signalling that leads to the controlled regulation and maintenance of polyamine and proline levels under osmotic stress conditions in wheat seedlings (Pal et al. 2018).
- Level of sugars increased during photosynthesis promote accumulation of proline (Pesci 1993).
- In several other studies, the role of sucrose in negative regulation of proline dehydrogenase encoding genes has been demonstrated (Weltmeier et al. 2006). Enhanced proline accumulation as a response to external sugars due to their higher concentrations highlights their correlation.

Thus, either directly or indirectly, osmolytes like proline along with soluble solutes like carbohydrates orphytohormones like ABA, salicylic acid is involved in combating negative effects of salinity stress (Gil et al. 2011). Thus, the participation of various hormones and sugars serves to regulate proline synthesis and degradation, and thereby controls proline homeostasis.

13.10 Role of Proline in Protein Protection

Proline is considered as an inert compatible osmolyte that protects subcellular structures and macromolecules under osmotic stress (Kishor et al. 2005a, b). Proline has been shown to function as a molecular chaperone able to protect protein integrity and enhance the activities of different enzymes. The decreased protein content in plants under salt stress may reflect the inhibition of protein synthesis, increased degradation or the inhibition of amino acid incorporation into proteins, resulting in the accumulation of free amino acids (Piza et al. 2003). However, proline accumulation can influence stress tolerance in multiple ways (Fig. 13.3). Proline has been shown to function as a molecular chaperone able to protect protein integrity and enhance the activities of different enzymes. Examples of such roles include the prevention of protein aggregation, stabilization of M4 lactate dehydrogenase during extreme temperatures and protection of nitrate reductase during osmotic stress (El-Enany 1995). The damaging effects of singlet oxygen and hydroxyl radicals on photosystem II (PSII) can be reduced by proline in isolated thylakoid membranes (PSII) (Alia and Mohanty 1997). In salt-stressed tobacco cells, proline increased the activities of methylglyoxal detoxification enzymes, enhanced peroxidase, glutathione-S-transferase, superoxide dismutase and catalase activities and increased the glutathione redox state (Hoque et al. 2008). In the desert plant *Pancratium maritimum*, catalase and peroxidase were found to be stabilized by proline during salt stress.

Proline plays an important role in protein accumulation and in cell adaptation to salinity stress (El-Enany 1995); thus, accumulation of proline in the plant may be related to osmotic and saline stress tolerance. According to Amin et al. (2009), increase in the amount of proline, protein and sugars accumulation in the plants would lead to the resistance against losing water, protect turgor, reduce the membrane damage and accelerate the growth of plants in stress conditions. The higher accumulation of proline under stress conditions was attributed to increased activities of proline biosynthesis enzymes such as ornithine aminotransferase and pyrroline-5-carboxylate reductase, as well as due to inhibition of proline degradation enzymes, proline oxidase and proline dehydrogenase (Kishor et al. 2005a, b). Proline alleviates cytoplasmic acidosis by acting as a protein-compatible hydrotrope, which also maintains the necessary ratios of $NADP^+$/NADPH as per the requirements of metabolism (Strizhov et al. 1997; Hare and Cress 1997). Recent studies also suggested that the protective role of proline consists in protecting the protein turnover machinery against stress damage and upregulating stress-protective proteins (Khedr et al. 2003). Proline may also serve as an organic nitrogen reservoir ready to be used after stress relief to sustain both amino acid and protein synthesis (Sairam and Tygai 2004). The increase in the proline content under stress condition may be due to the breakdown of a proline-rich protein or *de novo* synthesis of proline (Tewari and Singh 1991). It could also be due to the prevention of feedback inhibition of the biosynthetic enzyme caused by sequestering proline away from its site of synthesis or by relaxed feedback inhibition of the regulatory step enzyme or by decreased activity of enzymes involved in degradation of proline such as proline dehydrogenase and proline oxidase (Girija et al. 2002). Thus, apart from its major role of stress osmolytes solute, proline plays versatile functions in plants and is one of the building blocks of protein structure under environmental stress conditions.

13.11 Role of Proline in Seed Germination

Seed germination is an important stage of plant development and high salt levels had detrimental effects on germination percentage. Several physiological and biochemical changes take place during seed germination, like protein synthesis from stored and newly synthesized mRNA, and reserve mobilization (Bewley 1997). Germination can be influenced by many abiotic factors that restrict or inhibit it; salinity is one of the major abiotic stresses affecting seed germination, plant growth and reproduction (Zhu 2002). Proline accumulation is a common response to abiotic stress, but elevated concentrations have also been noted during pollen and seed maturation. Thus, to cope with abiotic stress, it is important to develop strategies to increase the proline sink in the reproductive tissue. A fourfold increase in proline concentration and enhanced proteolytic activity of proline-rich storage and cell wall proteins in the germinating seed together provide a plentiful source of proline. It also underpins the importance of the expansion of the proline sink during the transition from vegetative to reproductive growth and the initiation of seed development (Kishor et al. 2014). Proline synthesis plays an important role in promoting germination: an increase in free proline was observed prior to radicle emergence in *Arabidopsis* seeds, and feedback inhibition of proline synthesis by exogenous proline decreased germination rate (Hare et al. 2003). Kubala et al. (2015) showed the role of priming-induced modulation of activities of concerned genes and proline turnover enzymes, and its interplay with higher levels of hydrogen peroxide, in enhancing the seed germination capability under various stress. Proline deficiency in plants causes a defect in the growth and development of flowers and seeds of Arabidopsis thaliana (Lehmann et al. 2010). Also, proline metabolism and catabolism, which help to maintain the redox balance of the cell, are required for efficient flowering and seedling of plants (Hare and Cress 1997). It probably affects flowers and seeds by transporting carbon and nitrogen and reducing agents to them (Verbruggen and Hermans 2008). Overexpression of moth bean P5CSF129A in tobacco increased the proline level twice as did P5CS of moth bean, and consequently, the transgenic lines with P5CSF129A showed much higher germination and much lower free radicals (Zhu et al. 1998). Xue et al. (2009) have indicated that stress-induced accumulation of proline in rapeseed results from the activated biosynthesis and also the inhibited proline degradation.

Exogenous application of proline during germination has indeed been reported to improve seed germination and seedling growth under salt stress conditions in several species of canola (*B. napus*) (Athar et al. 2009). Therefore, the salinity of soil plays a very wide role for better crop growth and development from the initial stage of seed germination to the yield of the crop. In *Arabidopsis*, both *P5CS1* and *P5CS2* are strongly transcribed throughout embryogenesis (Szekely et al. 2008). In the *p5cs2* mutant, embryo abortion is common, ascribed to the premature desiccation of the embryos, which tend to develop more slowly than do those that develop from a heterozygous mutant (Szekely et al. 2008). The accumulation of proline during seed maturation is correlated with the triggering of desiccation tolerance. A lack of proline and oligosaccharide accumulation has been identified as underlying the desiccation sensitivity of *Machilus thunbergii* seeds (Lin and Chen 1995). In addition to its importance in the embryo, proline also plays a role in the endosperm. In rice, the compound 2-acetyl-1-pyrroline (2AP) gives basmati and jasmine varieties their distinctive fragrance (Buttery et al. 1983). The endosperm storage proteins of most of the small-grain cereals are also particularly rich in proline. Thus, proline makes a contribution both to the quality of cereal grain storage proteins and to the aroma of the rice grain.

13.12 Osmolyte Accumulation in Halophytes

Halophyte relatives of *Arabidopsis*, such as *Thellungiella halophile* and *Lepidium crassifolium*, have elevated proline levels under unstressed conditions and accumulate proline to a higher level than does *Arabidopsis* when exposed to high salinity (Tipirdamaz et al. 2006). The capacity for proline hyperaccumulation therefore accompanies the extremophile character of certain plant species and is likely that it contributes to their stress tolerance; however, it is not an absolute requirement for adaptation to extreme environmental

- In *Thellungiella*, high proline accumulation results from enhanced P5CS and reduced PDH expression levels (Kant et al. 2006).
- High proline levels can improve the salt tolerance of the halophyte plant *Pancratium maritimum*, by stabilizing detoxifying enzymes and protein turnover machinery and stimulating the accumulation of stress-protective proteins (Khedr et al. 2003).
- In *Silene vulgaris*, constitutive proline content was higher in metal-tolerant ecotypes, whereas metal-induced proline accumulation was higher in a non-tolerant ecotype (Schat et al. 1997).
- In *Atriplex halimus*, proline accumulated higher concentrations than glycine betaine reflecting the growth conditions of this species (Tipirdamaz et al. 2006).
- In *Distichlis spicata* even though proline is predominant, its contribution to the osmotic potential is low (Fan et al. 1993).
- In *Sporobolus virginicus*, high NaCl appears to promote proline accumulation (Marcum and Murdoch, 1992). Similarly, high proline accumulation has also been observed in *S. portulacastrum* plants exposed to salinity (Ghnaya et al. 2007).
- Synthesis and accumulation of proline also occur in cell suspension cultures of halophytes, e.g. increased proline accumulation in response to NaCl stress was found in suspension cultures of *Mesembryanthemum crystallinum* (Thomas and Bohnert 1993).

- Its accumulation at whole plant level under salt stress in halophytes has been reported by many workers in coastal plants like Stewart and Lee (1974) in *Triglochin maritima* and *Armeria maritima*.
- In the halophyte species *Limonium latifolium*, proline was sequestered to vacuoles in non-stressed plants, whereas in salt-stressed plants, a high proline content was detected in the cytosol, suggesting the importance of de novo proline biosynthesis as well as transport for proline accumulation (Gagneul et al. 2007).

13.13 Proline Accumulation in Trees and CAM Plants

Proline accumulation under salt stress confirmed the idea of Ashraf and Foolad (2007), who has stated that proline is known to accumulate in large quantities in higher plants in response to environmental stresses.

- Proline accumulation in the olive tree constitutes an adaptive mechanism to harmful stress, and to maintain the ionic balance in the vacuoles, the salt-stressed olive trees tend to accumulate proline in its cytoplasm to a high level to improve water uptake to actively growing tissues by osmotic adjustment effects (Hasegawa et al. 2000).
- The capacity for osmotic adjustment, via the accumulation of proline, during stress imposition has been found in leaves of grapevine (Shultz and Matthews 1993) and in apple trees (Lakso et al. 1984).
- Furthermore, Ain-Lhout et al. (2001) have shown that proline content in two Mediterranean shrubs (*Halimium halimifolium* L. and *Pistacia lentiscus* L.), during increasing water deficit, was twice the amount found in an olive tree.
- Proline accumulation was also reported in date palms when grown under high salinity, where proline may function as an extra player leading to resistance or may represent a common tolerance mechanism salt stress (Yaish 2015).

CAM (Crassulacean acid metabolism) plants have the capacity to synthesize osmolytes like sugars, glycinebetaine and proline under water-stressed conditions, allowing osmotic adjustment in these plants (Kerepesi and Galiba 2000). *Aloe barbadensis* accumulated 75% more proline when stressed than control plants (Delatorre-Herrera et al. 2010). *Mesembryanthemum crystallinum* is a halophyte and also a CAM plant, where proline has been observed to be accumulating even at severe salinity concentrations like 400 mM (Thomas and Bohnert 1993). Proline accumulation has preceded in these plants prior to the shift from C3 mode to CAM mode under the light, explaining its dual role as osmoregulator to counteract salt stress and as a substrate for dark respiration (Sanada et al. 1995). Proline also functions as an antioxidant under salinity stress; *Ananas comosus* accumulated proline even at 200 mM salinity stress conditions along with the accumulation of organic acid malate (Hasan and Abdullah 2007). Accumulation of proline substantiated by upregulation of P5CS was observed in *Opuntia streptacantha* under salinity stress (Silva-Ortega et al. 2008).

13.14 Proline Role in Mitochondrial Functions

Proline can act as a signalling molecule to modulate mitochondrial functions, influence cell proliferation or cell death and trigger specific gene expression, which can be essential for plant recovery from stress (Szabados and Savouré 2009). In the mitochondria, proline has distinct protective functions such as:

- Proline protects complex II of the mitochondrial ETC during salt stress and therefore stabilized mitochondrial respiration. The recently discovered P5C proline cycle can deliver electrons to mitochondrial electron transport without producing glutamate and, under certain conditions, can generate more ROS in the mitochondria.
- After stress, proline pools supply a reducing potential for mitochondria through the oxidation of proline by PDH and P5CDH, provide electrons for the respiratory chain and therefore contribute to energy supply for resumed growth (Kishor et al. 2005a, b).
- Rapid breakdown of proline upon relief of stress may provide sufficient reducing agents that support mitochondrial oxidative phosphorylation and generation of ATP for recovery from stress and repairing of stress-induced damages (Hare and Cress 1997; Hare et al. 1999).
- Proline besides balancing the osmotic strength of cytosol plays an important role in cell signalling, protein translation and in scavenging free radical (Szabados and Savouré 2009).
- Proline catabolism in the mitochondria is connected to oxidative respiration and administers energy to resume growth after stress. Moreover, proline oxidation can regulate mitochondrial ROS levels and influence programmed cell death. Besides its stabilizing effect on cellular structure, proline can also detoxify free radicals by forming adducts with them (Smirnoff and Cumbes 1989).

13.15 Proline Roles in Photosynthesis

During salt stress, plants show high chlorophyll degradation symptoms like chlorosis, a common morphological and physiological symptom. To overcome adverse effects of salinity proline accumulation occurs preferentially in leaves to preserve chlorophyll content and maintain turgor pressure, which protects the photosynthetic activity under stress conditions (Silva-Ortega et al. 2008). In addition to the predictable role of proline in osmoregulation (Yancey 2005), they are also proposed to act as low-molecular-weight chaperones, stabilizing the photosystem II complex, protecting the structure of enzymes and proteins, maintaining membrane integrity and scavenging ROS. The enhanced rate of proline biosynthesis in the chloroplasts can contribute to the stabilization of redox balance and maintenance of cellular homeostasis by dissipating the excess of reducing potential when electron transport is saturated during adverse conditions. Some of the reports of the probable role of proline in mediating photosynthesis are:

- Huang et al. (2013) reported higher proline accumulation in leaves as compared to root samples in artichoke, indicating its role in maintaining chlorophyll level and cell turgor to protect photosynthetic activity under salt stress.
- Proline has been regarded as an antioxidant and ROS scavenger with abilities to quench radicals like singlet oxygen (Smirnoff and Cumbes 1989; Matysik et al. 2002). Proline alleviates salt stress-induced enhancement in oxygenase as well as carboxylase activities of Rubisco.
- Accumulation of P5CS1 and P5CR in chloroplasts during salt stress suggests that, under adverse conditions, glutamate-derived proline biosynthesis increases in plastids, where photosynthesis occurs.
- During stress conditions, the rate of the Calvin cycle is diminished, which prevents oxidation of NADPH and restoration of $NADP^+$. When combined with high light, electron flow in the electron transport chain is suppressed by the insufficient electron acceptor $NADP^+$ pool, leading to singlet oxygen production in the PSI reaction centre and accumulation of ROS (Chaves et al. 2009).
- An enhanced rate of proline biosynthesis in chloroplasts during stress can maintain the low $NADPH:NADP^+$ ratio, contribute to sustaining the electron flow between photosynthetic excitation centres, stabilize the redox balance and reduce photoinhibition and damage of the photosynthetic apparatus (Hare and Cress 1997).
- Reduced rate of CO_2 assimilation under adverse environmental conditions results in exposure of chloroplasts to excess excitation energy and increases the rate of ROS formation (Smirnoff 1993).
- Its role is known to diminish ROS and prevent programmed cell death. In isolated thylakoid membranes of PS II, proline reduces the damaging effects of hydroxyl radicals and singlet oxygen (Alia and Mohanty 1997).
- It has been shown that proline enhanced the photochemical electron transport activities of isolated membranes of *Brassica juncea* by arresting photoinhibitory damage via its ability to scavenge free radicals (Alia and Saradhi 1991).

13.16 Conclusions and Future Prospects

According to the climatic models, global warming will lead to the sea-level rise which will enhance seawater intrusion into coastal marshes and will increase water salinization. Water salinization affects the physiological and biochemical processes leading to productivity and huge losses of agriculture productivity worldwide. Thus, plant biologists aimed at overcoming severe environmental stresses need to be quickly and fully implemented. Together with conventional plant physiology, genetics and biochemical approaches to studying plant responses to abiotic stresses have begun to bear fruit recently. Relevant information on biochemical indicators at the cellular level may serve as selection criteria for tolerance of salts in agricultural crops. The accumulation of compatible solutes is often regarded as a basic strategy for the protection and survival of plants under abiotic stress conditions, including both salinity and oxidative stress. Proline accumulation is a prominent metabolic adaptation of higher plants during various environmental stresses and has been recognized as a multifunctional molecule, which acts as an active osmolyte, a metal chelator, an antioxidant and a signalling molecule. Proline may alleviate the negative impact of salt by decreasing osmotic stress that consequently maintain the membrane integrity and its function. Proline homeostasis appears to be critical during both growth and development, and its imbalance leads to various abnormalities. Improvement of salt tolerance of crop plants via engineering proline metabolism is an existing possibility and should be explored more extensively. The fact that proline can act as a signalling molecule and influence defence pathways and regulate complex metabolic and developmental processes offers additional opportunities for plant improvement. The exogenous application of proline could offer a simple and economical approach for farmers to reduce the crop loss risk in salt-contaminated land. However, further studies are needed at physiological and molecular levels to gain deeper insight in understanding the interaction of NaCl-induced oxidative stress and alleviation mechanism of exogenous proline in crops.

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