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Abstract

High salt concentration in arable soil/soil salinization poses an increasing agricultural problem all over the world. Such salt accrual is resulting from the sea water mixing with irrigation supply and naturally high content of salts in water used for agricultural irrigation. Further, concerning plants, the stress generated by salt salinization of the soil can be divided into two stages: (i) early osmotic imbalance disturbing water uptake by the plant roots and (ii) the stress created by Na^+ and Cl^- ions that disturb the metabolism together with the productivity of photosynthesis. Plants moderate these stresses by developing certain mechanisms for enhancing water retention (decrease in the loss of water with increase in its uptake) and preventing the detrimental effects of Na^+ ions by their vacuolar entrapment together with exclusion from the tissues of the leaves. However, even after employing the tolerant mechanisms, plants are adversely affected, and this is specially observed as a decrease in crop productivity/yields that ultimately resulting in continuous decrease in arable land resources. Factors of rapid expansion of population size and continuously improving quality of life are exerting a great pressure upon the agriculture resources. Therefore, managing the salt stress in agricultural plants and developing varieties with such mechanisms

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can play a pivotal role in addressing the challenge of rapidly increasing demand of agribusiness. This chapter focuses on the mechanisms of salt tolerance in plants, sensory, and signaling networks associated with salinization, Na^+ transport, gene expression regulatory responses toward salt stress, and the approaches toward designing salt tolerant crops.

Keywords

Salt stress · Crop plants · Salt tolerance · Osmolytes · Gene regulation · Sodium/potassium transfer

11.1 Introduction: Salt-Induced Stress

Plants employ two strategies to cope up with stress created by soil salinization, i.e., sensory mechanism is for recognizing increased osmotic potential and second is for the enhanced presence of Na^+ ions (Fig. 11.1). Responses of the aforementioned two mechanisms are sometimes mutually exclusive of one another, thereby proving the two distinct mechanisms against salt stress. Till date, the precise identification of the key players of these two sensory mechanisms has not been successful. However, there have been some successful attempts in which it has been demonstrated that histidine kinase receptor protein HK1 of *Arabidopsis thaliana* has a functional role in sensing osmotic imbalances (Urao et al. 1999). Some of the physiological responses by plants toward salt-induced osmotic stress are changed in HK1 mutants, while others are unchanged depicting the role of protein entities, excluding HK1, responding to osmotic stress in mutants (Kumar et al. 2013). A quick increase in the Ca^{2+} ion concentration in the cytosol on a brief NaCl /mannitol exposure suggested

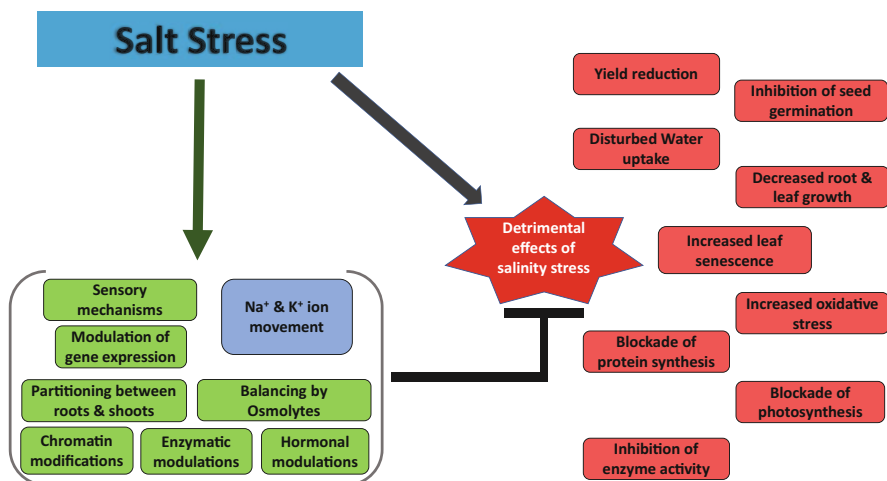


Fig. 11.1 Adverse effects of salt stress and their management in plants

the coupling of the Ca^{2+} ion channel with hyperosmotic sensory apparatus of plants (Knight et al. 1997). The origin of Ca^{2+} ion response in plant's roots and in many types therein possibly implicates sensing by a mechanical ion channel gate structure (Kiegle et al. 2000; Tracy et al. 2008; Kurusu et al. 2013; Marti et al. 2013). Proper cuticle development builds the structural strength of the cellular envelope and supports the mechanical osmo-sensors. Mutations disturbing cuticle development can result in weakened osmotic potential that governed responses and thereby altered water diffusion (Wang et al. 2011b). Consequently, cuticle is a prime factor in altering the mechanical properties of cellular water stress.

Reactive oxygen species (ROS) and annexins are the secondary messengers mediating the salt-induced and ROS-induced responses (Laohavisit et al. 2012, 2013). These secondary messengers work in conjunction with Ca^{2+} ion signaling machinery and get induced by presence of excess salt or osmotic stress (Jiang et al. 2013b). Some kinases can be activated and pass the salt stress/hyperosmotic signal to further activity of proteins through gene expression mechanism (Weinl and Kudla 2009). The Ca^{2+} ions/calmodulin can direct the activation of transcription factors (Weng et al. 2012). Though a quick rise in the Ca^{2+} ion concentration characterizes the osmotic stress response, but other sensory mechanisms that are not governed by Ca^{2+} ions are also found in plants. Identifying the genetic basis of these sensory networks is essential in deciphering the code of plants osmo-sensory response toward increased ionic concentrations and salt stress.

11.2 Modulation of Gene Regulation in Plant Roots Under Salt Stress

The sensory pathways related to increased salt concentrations are linked to several salt tolerance responses by many transcription factors. Groups of transcription family genes are expressed to different degrees when an increased salt concentration is sensed by the salt stress sensory mechanisms (Jiang et al. 2009; Cui et al. 2013). Expression of the genes affecting the salt tolerance response of plants is in turn regulated by these transcription factors. Some of the genes are upregulated or downregulated, depending on their roles in supporting or countering the salt stress, correspondingly. The osmotic constituent of enhanced salinity causes a lowering of the water potential, and therefore, to balance this, uptake of inorganic ions and synthesis of osmolytes must be enhanced. As a result, genes responsible for such responses are upregulated (Geng et al. 2013). Dynamic shift in the synthesis of hormones also plays a role to some extent in the regulation of the transcription of the stress responsive genes (Dinneny et al. 2008; Geng et al. 2013). Initial stress phase is followed first by a short dormant and later growth recovery period. The two phases of dormancy and growth recovery are associated with the corresponding levels of plant hormones. Data mining has shown a secondary signaling network controlling the salt affected plant growth (Kilian et al. 2007; Geng et al. 2013). Majority of the stress-mediated variations are observed after around 3 h of exposure to high salt concentrations (Geng et al. 2013). Cells of the root cortex in the seedlings of

Arabidopsis thaliana show highest amounts of transcriptional activity (Geng et al. 2013). In salt-stressed conditions, lateral growth in roots has been shown to be most significantly caused by the endodermal cellular layers of the roots. Some plant hormones like ABA can affect the directional growth of roots to avoid high salinity areas (Duan et al. 2013). This spatial circumvention to evade high salt microenvironment by directional root growth is defined as halotropism and is found to be mainly caused by another plant hormone auxin under the influence of salt (Galvan-Ampudia et al. 2013). Discovering the molecular pathways underlying halotropism can further clear our understanding regarding plant's capabilities of bearing the high salt load around its roots. Instead of all the findings till now, our understanding of this subject is still incomplete. An interesting study has deciphered that ethylene, along with ABA and auxin, improves the shoot Na^+/K^+ ratio and makes *Arabidopsis* more capable of bearing the salt stress load (Jiang et al. 2013a). Ethylene levels are increased when *ETO1* (ethylene over producer 1) gene is knocked out. The increased ethylene triggers the RBOHF (respiratory burst oxidase homolog F) for ROS synthesis in root stele. Buildup of the ROS inside stele lowers the accumulation of Na^+ in xylem including flow of Na^+ and holding of K^+ in roots. All these changes lead to increased tolerance of salt (Jiang et al. 2013a).

11.3 Role of Na^+ and K^+ Transporters in Salt Stress Tolerance

Control of influx and efflux of ions across plasma membrane has a very significant role in various biotic/abiotic stress tolerant mechanisms. Specifically, the Na^+/K^+ membrane transporters are involved in the resistance against salt stress (Schroeder et al. 2013). The roots contain pathways for the transportation of Na^+ ions across the plasma membrane through their transporters or via the channels for nutrient uptake. In plant leaves, the mutation in some of these transporters and channels can decrease the Na^+ ions intake. Nevertheless, only a few of these channels when present in defective forms can hamper the Na^+ ions uptake into the roots in mutants. Some nonselective cation channels are also found in plant roots to serve as a gateway for Na^+ ions movement (Tapken and Hollmann 2008). Apart from these nonselective channels, specific antiporters for cation/proton exchange are also found in root epidermis for transporting Na^+ ions to stele from cells of the endodermal layer (Hall et al. 2006). The K^+ starvation has been shown to enhance Na^+ ions intake to the roots in *Oryza sativa* through a plant high affinity K^+ ion transporter, OsHKT2;1 (Horie et al. 2007). Another K^+ ion transporter OsHAK5 in *Oryza sativa* is not very permeable for Na^+ ions and provides salt stress bearing ability to tobacco cells when present in overexpressed state (Horie et al. 2011).

Stellar cells transfer the Na^+ ions to the xylem for their further transport to distant plant parts. The cargo of Na^+ ions is set up in the xylem through a control of few K^+ ion channels (de Boer and Wegner 1997). Further removal of Na^+ ions from the xylem is done by separate low affinity transporters (Ren et al. 2005). During salt stress management, ionic movement in plants is done in such a manner that ion homeostasis is maintained. For this K^+ ions are acquired and distributed in a stable

manner with an added advantage of their balancing of Na^+ ions buildup-related toxic effects (Schroeder et al. 1994). The K^+ ion channels that precisely put K^+ ions selectively inside or outside the plant cells have been shown to reduce the levels of toxicity associated with elevated Na^+ ions (Schroeder et al. 1987).

11.4 NHX and SOS-Mediated Maintenance of Low Na^+ Ion Levels in Cytoplasm

Na^+/H^+ exchanger 1 (NHX1) of tonoplast and Salt Overly Sensitive 1 (SOS1/NHX7) antiporters of plasma membrane are the two key factors for lowering the plant cell's Na^+ ion concentrations (Blumwald and Poole 1985; Qiu et al. 2002). The mode of lowering of the Na^+ ion levels by the two mechanisms differs from one another. While NHX detoxifies the Na^+ ions by their sequestration in the vacuoles, the SOS mediates efflux of Na^+ ions from the cell. Plants' enhanced salt tolerance is associated with the overexpression of NHX proteins (Apse et al. 1999). NHX like proteins probably work by causing vacuolar restriction of K^+ ions and maintaining the pH stability (Barragan et al. 2012). Advantageous functioning of NHX type protein AtNHX1 is observed with its overexpression leading to increased vacuolar K^+ ion retention and its transport from roots to shoots in tomato (Leidi et al. 2010). In this manner, overexpressed AtNHX1 reduces Na^+ ion stress by increasing the K^+/Na^+ ratios inside the cell. Mapping of LeNHX3 to a leaf Na^+ ion accrual QTL locus relates the NHX type proteins with Na^+ ion stress management (Villalta et al. 2008). NHX antiporters in vacuoles significantly affect osmolarity balance, cellular growth, and, as a result, development of plant (Bassil et al. 2011). Processing of proteins, vesicle-mediated trafficking, and conveyance of the cargo have potential contribution from the NHX antiporters of the endosomes (Krebs et al. 2010). NHX and other transporters of endosomes have potential roles in salt tolerance through their ability to regulate pH and ionic balance inside organelles of plant cells (Krebs et al. 2010). Involvement of NHX proteins in trafficking of proteins has been shown in double knockout plants for *nhx5* and *nhx6* genes wherein a significant reduction in salt tolerance was observed (Leidi et al. 2010). Moreover, in *Arabidopsis thaliana*, no change in salt tolerance was observed at the cost of H^+ -ATPase inside the vacuoles. The H^+ -ATPase is also known as V-ATPase and its functionality in trans-Golgi network/early endosome (TGN/EE) is required for maintaining salt tolerance (Villalta et al. 2008). Vacuolar upregulated expression of type 1 H^+ -pyrophosphatase AVP1 causes Na^+ ions to be confined inside vacuoles (Undurraga et al. 2012). Practical application of improving salt tolerance under artificially maintained greenhouse environment is successful after overexpression of H^+ -pyrophosphatase in crop plants. This significantly increases the biomass production and grain yields in crops (Schilling et al. 2014).

11.5 Partitioning of Na⁺ Ions from Roots to Shoots in Combating Salt Stress

Plant high affinity potassium transporter (HKT) plays an important role in Na⁺/K⁺ cation transport in plants, and many *HKT* genes have been successfully characterized, e.g., *TaHKT1* (*Triticum aestivum HKT 1*), *AtHKT1* (*Arabidopsis thaliana HKT1*), and *OsHKT1* (*Oryza sativa HKT 1*) (Rubio et al. 1995). HKT transporters are classified into two groups: class I HKT transporters are mostly involved in selective transfer of Na⁺ ions, and the class II HKT transporters mediate the transport of both Na⁺ and K⁺ ions (Rubio et al. 1995; Uozumi et al. 2000). Examples of the functions of HKTs have been provided by the studies showing buildup of Na⁺ ions in leaves on salt stress exposure with associated depletion of Na⁺ ions in roots (Berthomieu et al. 2003). Active role of *OsHKT1;1* and *AtHKT1;1* has been demonstrated in exiting Na⁺ ions out of deeper xylem tissues to prevent toxicity of Na⁺ ions (Davenport et al. 2007; Horie et al. 2011). Plants with overexpressed *AtHKT1;1* have more salt tolerating ability over plants with normal expression of *AtHKT1;1*. Role of *AtHKT1;1* in passive control of Na⁺ ion transfer has already been established by the in vivo patch clamping electrophysiological study on root cells (Xue et al. 2011). Stress generation by high Na⁺ ions in leaves is countered by their removal by *AtHKT1;1*. External rectifier channels for K⁺ ions help to load xylem vessels with potassium ions leading to increase in the ratio of Na⁺ and K⁺ ions. Increased K⁺/Na⁺ ratio counters the salt stress and helps plants to sustain themselves in soils having higher salt concentration. Varied crop plants have common Na⁺ countering mechanisms, e.g., wheat and rice. Results from QTL analysis have revealed that in both wheat and rice plants, the main salt tolerance mechanism is linked to certain common genomic landscapes that are responsible for production of highly effective transporters for Na⁺ ions, e.g., *Nax1* and Na⁺-selective class I HKT transporter (James et al. 2006; Byrt et al. 2007). Some of these QTL identified regions encode the proteins for both Na⁺ ion clearance and HKT transporters to give a protection to leaf sheaths against Na⁺ ions overaccumulation (Huang et al. 2006). When HKT transporter is introduced into a commercial wheat variety from a wild variety, grain yields were significantly increasing instead of the crop plant growing in salt stressed environment (Munns et al. 2012). All these findings confirm the importance of HKT transporters as an effective counter measure against the damaging effects of salt stress in form of high Na⁺ loads.

The mechanisms of maintaining a high K⁺/Na⁺ ion ratio in plant leaves is important for plant's ability to resist the salt stress. Contradictory to the above importance, high Na⁺ ion accumulation is observed in leaves of the plants typically growing in salt rich soils. A weak allele of *AtHKT1;1* is found to be associated with its reduced expression in plant roots (Baxter et al. 2010). This weak allele still confers a slight functionality of removing Na⁺ ions, and thus, toxic concentrations are avoided. Osmotic tuning according to the load of salt stress is not a single factor mechanism. For instance, in plants, where weak allele of *HKT1* is in effect, there may be higher alterations in other salt tolerance pathways like vacuolar sodium ion sequentializations. Thus, knowledge of such cooperative effects that working

together to cope up with salt stress could aid in designing crop varieties with more salt tolerance. Class 2 HKT transporters in *Hordeum vulgare* also depict transport of Na^+ ions, from roots to xylem, even with more intensity when overexpressed and thereby confer higher salt tolerance. Plants employ some regulatory mechanisms pertaining to expression of K^+ ion transporters to modulate their activity, e.g., *AtHKT1;1* of *Arabidopsis thaliana* roots that is depressed by cytokinin negative regulators ARR1 and 2. When salinity is sensed, cytokinin levels fall leading to enhanced expression of *AtHKT1;1* (Mason et al. 2010). ABI4 (ABA-Insensitive 4) is another transcription factor acting negatively for expression of *AtHKT1;1*. ABI4 expression is found to correlate with the expression of *AtHKT1;1* that impart salt tolerance in case when its gene is mutated. When ABI4 is overexpressed, *AtHKT1;1* is lowered in expression, and plants become highly sensitive for the presence of salts (Shkolnik-Inbar et al. 2013). Positive regulators or enhancers of *AtHKT1;1* expression is not discovered yet. A complex network of signals from many *cis*- and *trans*-acting elements control the expression of *AtHKT1;1* in salt induced stress.

11.6 Epigenetic Modifications Induced by Salt Stress

Plants adapt to various environmental conditions by modifications at the level of chromatin. Epigenetic chromatin modifications in plants growing under salinity stress have been demonstrated by their presence in the same generation on exposure to the stress. In an experiment done on *Arabidopsis* plants, two groups were differently and consecutively treated for salt stress. First group was given mild salt treatment in their seedling stage and then grown later in desalted normal conditions. The second group of seedlings was not pretreated with salt and was grown under normal condition. During the stress-free normal condition growth of the seedlings, no differences were observed between the two groups. However, when further salt stress was applied on the two groups of plants, the first group was found to be more tolerant for the salt stress than the second group, which was initially untreated with salt stress. It was speculated that this high salt tolerance was due to the epigenetic histone modifications affecting transcription factor expression (Zhu 2008). Previously stress exposed plants show strongly induced expression of *HKT1* over the unexposed plants. That is why Na^+ ions accumulate differently in the two groups (Sani et al. 2013).

Methylation of target sites at genomic regions is another influential aspect for salt tolerance. The salt stress hypersensitivity is found to be associated with altered *AtHKT1;1* promoter activity (Baek et al. 2011). This alteration was due to unsuccessful methylation at a target site on the promoter. Evidence of salt affected DNA methylation, of transcription factor coding regions along with their promoters, indicated the potential of chromatin modifications in ever-increasing salt tolerance of plants (Song et al. 2012). Under salt stress, temporal variations of gene expression between plants of different genotypes have led to speculations about the involvement of an epigenetic component of chromatin modification and methylation-induced responses (Wang et al. 2011a). However, there is no clear understanding

of the epigenetic influence on salt tolerant mechanisms and its inheritance through generations. Differences in ploidy levels can also affect salt tolerance, particularly the hold up of potassium in *Arabidopsis* (Chao et al. 2013).

11.7 Role of Osmolytes

Lower osmotic potential is maintained in plant cells by accumulation of osmolytes to combat the salt-induced stress. Therefore, to completely decipher the code of salt tolerant mechanisms, it is very important to get an understanding of the mechanisms controlling the activity and presence of osmolytes. Presence of salt stress enhances synthesis of osmolytes, while in the normal conditions when salt stress is not present, osmolytes are catabolized. Proline is one such osmolyte that functions as an important signaling mediator for various cellular activities in the recovery phase (Szabados and Savoure 2010). Blocking proline synthesis in *Arabidopsis* knockout plants caused hypersensitivity to salt and this observation confirms the role of osmolytes in management of salt stress (Szekely et al. 2008). Initially, proline was considered to play a role as an osmolyte only. Later, many other roles of proline were discovered, for instance as ROS, buffering agent, molecular chaperon, and structural component under stress. Another osmolyte, glycine betaine, is produced for osmotic balancing by many plant species (Jain et al. 2021). It is also a protective agent for many enzymes and membrane components. ROS rummaging enzyme activities are maintained by glycine betaine, but proof of its direct role as a ROS scavenger is still lacking (Chen and Murata 2008).

11.8 Other Regulators of Salt Tolerance Response in Plants

The salt response network in plants is constituted by many different genes. The genes guide the salt response network to influence salt tolerance in plants. Therefore, to develop salt tolerant crop varieties, it is very important to have knowledge of these machineries of salt tolerance network (Al-Khayri et al. 2021). In *Arabidopsis*, a serine/threonine protein phosphatase negatively regulates the signaling of ABA, and plant's increased salt tolerance is associated with its decreased transcripts levels. Moreover, expression of the protein phosphatase is downregulated by a transcription factor, *AtMYB20* (Merlot et al. 2001; Cui et al. 2013). Interestingly, aforesaid association is proved from the demonstration of plants with overexpressed *AtMYB20* to be more salt tolerant over wild types and vice versa (Cui et al. 2013). Another protease involved in balancing of Na^+ and salt tolerance is the ubiquitin-specific protease 16 (UBP16). Its expression is induced by the presence of salt, and its presence plays a stabilizing role for the Na^+/H^+ antiporter activity. UBP16 acts in opposition to proteasomal degradation of proteins through deubiquitylation and thus helps in protein stability (Zhou et al. 2012).

11.9 Developing Salt-Tolerant Plant Varieties

Conventional breeding methods are very time consuming, so it is essential to look for alternative and comparatively rapid methods of crop improvements. Genetically diverse germplasm are used to cross the existing varieties, and phenotypes of the offspring generation are looked for improved characteristics. Gene manipulation for salt tolerance has been attempted in *Arabidopsis*, where transcription factor (*bZIP24*) was identified in a comparative study conducted in halo-tolerant plant and *Arabidopsis* (Popova et al. 2008). This transcription factor is responsible for the induction of many stress responsive genes. When *bZIP24* is knocked down by RNAi methods, salt tolerance increases. This was further strengthened by the observation that *bZIP24* targets to depress expression of *AtHKT1;1* (Yang et al. 2009). Thus, halophilic plants can be used as a model for discovery of potential target of salt tolerance regulation. Negative regulators of *AtHKT1;1* have been well studied and serve as targets for gene manipulations for inducing salt tolerance. However, positive regulatory mechanisms of *AtHKT1;1* are not so well identified. Studies in the crop plant species have also revealed insights of salt tolerance networks. In rice, transcription factor *SERF1* (Salt Responsive ERF 1) triggers the ROS-activated MAP kinase cascade in salt-rich conditions (Schmidt et al. 2013). Under high salt concentration, roots show enhanced expression of *SERF1*. When *SERF1* expression is blocked in rice plants, gene expression of salt stress fighting genes is negatively affected. *SERF1* has a definite role in maintaining high Na^+/K^+ ratios in leaves as shown by high salt tolerance in case of its overexpression (Schmidt et al. 2013).

11.10 Conclusion and Future Directions

A comprehensive understanding of the factors and players of salt tolerance mechanisms in plants have been achieved in around the last 20 years. Still, there are many things unclear and need further exploration. More investigations are needed to elucidate the gene expression and regulatory networks of many more Na^+ and K^+ transporters in different plant species. Moreover, the present knowledge about the described mechanisms of salt tolerance must be used to develop newer more capable crop varieties for salt-rich soils currently considered inarable. Modern biotechnological methods need to be used in combination with molecular breeding techniques for developing such varieties. The potential targets for these developments can be the genes, in exclusion or in combination, for ion transporters, ROS scavenging pathways, salt homeostatic systems, and those influencing the plant's salt tolerating ability in any way.

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