Chapter 2
Dynamics of Salt Tolerance: Molecular Perspectives

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Abstract Plant being sessile has to face many environmental stresses and develop physiological and biochemical mechanisms to withstand those stresses. Salinity is one of the major stresses that affects the plant growth and imposes direct impact on productivity and yield. The lack of good-quality irrigation water is forcing farmers to use alternative or degraded waters of irrigation throughout the world. One of the major considerations for using alternative or degraded water is often its high salinity. Salinity affects various physiological and biochemical mechanisms in plants, including germination and growth, photosynthesis, and plant water relations. Plants, when exposed to external stresses, develop mechanisms and responses to defend themselves. Salt tolerance is regulated by a complex network of different component traits. With the availability of the genome sequences of the most crop species, it became feasible to develop the links between physiological performance and underlying biological mechanisms involved in salinity stress. This article describes the effect of salinity on germination, growth, photosynthesis and plant water relations, and the mechanism plant adopts to protect itself, such as ion exclusion from roots, sequestering ions into vacuole, and high tolerance to ion toxicity. Recent advances in research and technology may play critical role in developing new salt-tolerant cultivars that are vigorous and high yielding.

Keywords Salt tolerance · Salt stress · Ion homeostasis · Ion content · Ion transporters · Na^+/H^+ antiporters · Vacuolar compartmentalization

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2.1 Introduction

One of the biggest challenges farmers throughout the globe are facing is the availability of good-quality water for agriculture. Reduced availability of water and increasing demand of water by urban and industrial sectors due to industrialization are enforcing the use of alternative or degraded water for agriculture. One big consideration about using alternative or degraded water is its salt concentration. Salinity is a common problem throughout the world and is among one of the most important abiotic stresses faced by plants. About 20% of the irrigated agricultural land worldwide faces some level of salinity (Flowers and Yeo 1995). With the increasing scarcity of irrigation water, salinity problem is further pronounced. Salinity research is expected to widen in future years. There are many factors that affect soil salinity such as low precipitation, higher surface evaporation, low-quality irrigation, intensive farming, and poor drainage (Tanji 1990; Szabolcs 1994). About 10 million hectares of the land has been estimated to be destroyed by soil salinity each year (Pimentel et al. 2004). It is estimated that 50% of arable land will be affected by salinity by 2050 (Bartels and Sunkar 2005). On one hand, the food demand is growing with the increasing human population; on the other hand, due to increasing soil salinity, the availability of farming land is declining. Thus there is greater need to develop more sustainable crops to increase the productivity by utilizing saline water for irrigation.

Salt stress affects growth and development adversely by inhibiting many metabolic processes and cell expansion and by triggering programmed cell death (Huh et al. 2002). During initial phases of salinity stress, osmotic stress is predominant leading to increased transpiration and loss of absorption capacity of roots (Munns and Tester 2008). Osmotic stress is accompanied by several biochemical and physiological changes including disintegration of membranes, ion imbalance in the cells, decrease photosynthetic activity, and reduced stomatal conductance (Munns 2005; Munns and Tester 2008; Gupta and Huang 2014). Osmotic stress is also an initial step during drought. In response to osmotic stress, plants adjust osmotic pressure inside the cells, which normally happens within the first few hours following the salt stress. Gradually, concentrations of Na^+ and Cl^- increase inside cell cytoplasm resulting into ionic stress(Munns and Tester 2008). Although, ionic stress develops gradually, as it takes time for Na^+ or/and Cl^- concentration to reach the toxic levels, it becomes much more important as compared to the osmotic stress. High concentration of Na^+ not only causes ion imbalance, but it also interferes with K^+ absorption by roots leading to K^+ deficiency, which in turn results in reduced productivity (James et al. 2011).

Most salinity studies conducted in plants do not differentiate between salt stress and salt shock (Shavrukov 2013). When plant is exposed to a single application of high concentration of salt, it leads to plasmolysis resulting into an osmotic shock. Plant tries to cope with the osmotic shock by maintaining ion homeostasis. However, excessive salt concentration in the root cells causes leakage of salt solution in the apoplastic space, which quickly travels to the shoots without much control or regulation. This process leads to early ionic stress in the shoots causing overlap between gene expression relating to osmotic and ionic phases. These experiments identify very few genes associated with ionic phase. On the other hand, gradual increase in salt concentrations results in salt stress, where there is not much overlap between osmotic and ionic responses, and these experiments are able to identify a large number of genes associated with ionic stress (Shavrukov 2013). In response to salt shock, several genes involved in cell turgor maintenance, accumulation of osmolytes, and water balance are induced within the first few hours of the salt treatment (Kawasaki et al. 2001). Several other genes involved in defense-related functions were upregulated in the first 24 hours, likely due to restoration of damaged components of the cell due to osmotic shock (Kawasaki et al. 2001). However, genes involved in ionic response were induced only after a week of salt treatment. In this study, many important genes including ion transporters were not upregulated suggesting that osmotic shock can alter the expression pattern of some stress-related genes which can lead to wrong inference (Shavrukov 2013). The comparison of expression of four superoxide dismutase (SOD) genes in one-step application and stepwise application of NaCl revealed that all four genes were highly upregulated over control in one-step application, whereas in stepwise increase of NaCl, none of the four genes were induced over control (Rubio et al. 2009). These observations suggested that these SOD genes are involved in osmotic shock rather than salt stress itself (Rubio et al. 2009). In field conditions, osmotic shock almost never happens. During spring, salt present on the soil surface moves to subsurface layers, and during summer salt gradually moves back to the upper layers slowly increasing the salt content in the root zone.

Salt tolerance is the ability of plant to survive under higher concentration of soluble salt in their rhizosphere. Salt tolerance is normally measured in terms of sustaining plant vigor and yield under saline conditions as compared to the controlled conditions. There are considerable differences in crop plants for salt tolerance. Barley and alfalfa are considered as moderately tolerant to salinity; on the other hand, *Arabidopsis*, strawberry, and avocadoes are very sensitive (Munns and Tester 2008; Suarez and Grieve 2013; Mauk et al. 2017; Sandhu et al. 2017). For the crops that are sensitive to salt, the specific ion toxicities are the primary cause for the salt stress as in these crops, the salinity damage occurs even in conditions when osmotic pressure is very low (Suarez and Grieve 2013).

In order to solve the complex puzzle of salinity, it is important to understand the genetic determinants that regulate biochemical and physiological responses and manipulate those to developing genetic material tolerant to salinity. The focus of this book chapter is to discuss recent advances in identifying genetic components of the salt tolerance mechanism and understanding their links with the biochemical and physiological responses.

2.2 Effect of Salinity on Plants

The salt stress affects the growth of a plant by limiting germination, leaf expansion, root/shoot growth, photosynthesis, stomatal conductance, and nutrient uptake (Chartzoulakis and Klapaki 2000; Wang and Nii 2000; Stępień and Kłbus 2006; Machado and Serralheiro 2017). All these factors ultimately affect the biomass and yield of the plant.

2.2.1 Germination and Growth

The high salt concentrations affect the plant growth and germination drastically. Salt inhibits the plant growth mainly due to two reasons. Firstly, salt in soil reduces the uptake of water by roots and secondly, salt enters the plant system and accumulates in the cell cytoplasm of different plant tissues, leading to ion toxicity (Munns 1993; Munns and Tester 2008). However, in the plant species that are highly sensitive to salinity, the two-phase effect of salinity is not visible (Munns and Tester 2008).

Salinity affects the plant throughout its life, though this sensitivity varies from one developmental stage to the other (Läuchli and Grattan 2007). Plants are generally tolerant to salt at germination but are sensitive to emergence and early vegetative growth (Lauchli and Epstein 1990; Maas and Grattan 1999). However, as a plant grows and matures, it becomes more tolerant to salt stress. At germination and emergence stage, the salt stress affects the survival rate, but at later stages, salt stress reduces the growth and yield parameters (Machado and Serralheiro 2017).

Seed germination is an important part of the life cycle of a plant. Though the germination stage is considered as the salt-tolerant stage, still the percentage of seed germination, the speed of germination, and the rate of emergence in various crops are decreased with increase in salinity (Carpici et al. 2009; Kaveh et al. 2011; Xu et al. 2011). The possible reason for this reduction is decreased absorption of water by the seed. Saline water has lower osmotic potential that results in reduced imbibition in seeds (Carpici et al. 2009). In addition, salt toxicity disturbs hormonal balance and affects activities of enzymes involved in nucleic acid and protein metabolism (Khan et al. 1994; Dantas et al. 2007).

Germination is known to be more tolerant than growth in most species. In chickpea, some genotypes that were not able to tolerate even 100 mM NaCl during growth could tolerate more than 300 mM NaCl during germination (Flowers et al. 2010). Similarly, seedling germination was significantly reduced at 100 mM of NaCl treatment, while seedling growth expressed as height, leaf area, and dry weight was reduced when salinity exceeded 10 mM NaCl in two greenhouse-grown bell pepper hybrids (Chartzoulakis and Klapaki 2000). On the similar lines, decrease was observed in root length, shoot length, and fresh biomass with increase in salt concentration in black gram (*Phaseolus mungo* L.) (Dash and Panda 2001).

At lower salt concentrations, some crops show improved germination rates. In quinoa, lower concentrations of different salts and sea water dilutions led to increase in germination rate as compared to control, which was attributed to activation of an effective antioxidant mechanism that resulted in improved performance under salt stress (Panuccio et al. 2014). In *Vicia faba*, although at high NaCl concentrations, there was a decrease in plant height, but at low and medium concentrations, increase in plant height was reported (Qados and Amira 2011). Authors attributed improved performance under low and medium salinity levels due to the osmotic adjustment activity leading to improved growth. Similarly, the increases in fresh weight were reported in other crops such as lettuce, cowpea, and pak choi upon low and medium salt treatments (Andriolo et al. 2005; Dantas et al. 2005; Memon et al. 2010). Crops such as spinach and sugar beet are known to utilize both

Na^+ and K^+ for growth and development and hence perform better under moderate levels of salinity (Kronzucker et al. 2013). The enhanced performance under salinity may be due to the use of Na^+ as nutrient by these plants when K^+ is deficient in the growth medium (Kronzucker et al. 2013).

Increased salt concentration in plant tissues can affect enzyme activities and interfere with metabolic and physiological processes. During germination, seeds absorb water that activates metabolic functions needed for germination. Seed porosity is an important parameter that helps in hydration and imbibition of seed during germination. Pectin is an important constituent of cell wall and commonly found in highly methylesterified form and regulates cell wall properties such as elasticity, ion binding, hydration, and porosity (Yan et al. 2018). Knockdown mutants for *pectin methylesterase 31* (*PME31*) resulted in increased sensitivity to salt during germination and led to downregulation of stress-related genes *DREB2A*, *RD29A*, *RD29B*, and *RD26* (Yan et al. 2018). Seeds liberate lots of energy during germination for the growth of the embryo. The increased levels of acid phosphatases were associated with increased levels of phosphate and energy liberation in seeds during germination (Nasri et al. 2016). Some *Arabidopsis* accessions that showed poor germination in 50 mM concentration of NaCl had diminished acid phosphatase activity; on the contrary the accession that showed 100% germination had increased acid phosphatase activity, suggesting that phosphatase may be critical for germination under salt stress conditions (Nasri et al. 2016). As the seeds germinate, endosperm cells enlarge and expand and cell walls in endosperm break. Expansin gene, *AtEXP2*, which is constitutively expressed during seed germination, is believed to be involved in breaking the cell wall in endosperm to make space for radicle emergence (Myburg et al. 2014). AtEXP2 has been shown to be involved in GA-mediated seed germination and provides enhanced tolerance to salt and osmotic stresses (Yan et al. 2014). Similarly, several genes have been characterized that play important role in growth during salinity stress. Transgenic tobacco plants containing the grape *Vv-α-gal/SIP* gene, involved in carbohydrate metabolism, showed enhanced salt tolerance as compared to wild-type (Daldoul et al. 2018). Salt hypersensitive mutant 9 (SAHY9/APUM23 protein) that plays an important role in the regulation of ribosome biogenesis is shown to be involved in growth during salt stress, and the salt hypersensitivity of the mutant is regulated through abscisic acid pathway (Huang et al. 2018). Identifying additional players involved in seed germination and growth under salinity stress may help in understanding metabolic control of salt tolerance in plants.

2.2.2 Effect of Salinity on Photosynthesis and Photosynthetic Pigments

Photosynthesis is a vital process needed by plants to convert solar energy into chemical energy. The decrease in photosynthesis rate under saline conditions is mainly attributable to reduction of water potential of a plant (Behboudian et al. 1986). Due to reduced water potential under saline condition, plant tries to retain water by closing stomata. Though this helps plant in controlling loss of water through transpiration, closing stomata also prevents carbon dioxide intake and significantly affects photosynthetic efficiency (Behboudian et al. 1986). The stomatal conductance decreases considerably during salt stress, limiting the photosynthetic capacity of the plant (Chartzoulakis and Klapaki 2000; Meloni et al. 2003). The other factor affecting photosynthesis is accumulation of Na^+ in older leaves. In plants grown under saline condition, the net photosynthesis decreases in older leaves where Na^+ accumulates, although at whole plant level, there is no significant decrease (Yeo et al. 1985). The net photosynthesis is inversely related to Na^+ accumulation and decreases linearly with increase in Na^+ concentration in the leaves (Yeo et al. 1985). The same trend is observed for Na^+ accumulation and the transpiration rate (Yeo et al. 1985).

Salinity decreases the total chlorophyll and carotenoid contents in general (Munns and Tester 2008; Sandhu et al. 2017). In rice, 200 mM NaCl treatment for 14 days resulted in 41% and 33% reduction in the chlorophyll *a* and *b* contents in the leaves, respectively (Amirjani 2011). The total starch content decreased in the stressed seedlings, but the total reducing and nonreducing sugar contents increased (Amirjani 2011). The rate of net photosynthesis was also reduced in salt-stressed seedlings leading to overall growth reduction (Amirjani 2011). Following the similar pattern as rice, chlorophyll *a* in cucumber was affected to a lesser extent by NaCl as compared to chlorophyll *b* (Stępień and Kłbus 2006; Amirjani 2011). However, the salt stress reduced photosynthesis by both stomata closure and non-stomatal factors (Stępień and Kłbus 2006). The significant decrease in chlorophyll *a*, chlorophyll *b*, total chlorophyll, and carotenoid content was observed after 10 days of treatment with NaCl in *Vicia faba* (Qados and Amira 2011). Similarly, the chlorophyll and total carotenoid contents decreased under salinity treatment in mulberry genotypes (Agastian et al. 2000). At a lower salinity level of 1–2 mS cm^{-1}, the rate of photosynthesis was higher, but it decreased considerably at high salinity levels (Agastian et al. 2000). The growth of *Brassica juncea* was affected under salt stress because of the decreased efficiency of photosystem II (PSII), electron transport rate, and D1 protein (Mittal et al. 2012).

Future studies focusing on understanding the molecular link between salinity and photosynthesis may provide means to identify candidate genes involved in photosynthetic pathways to develop plants with improved salt tolerance.

2.2.3 Water Relation

The direction and the rate of water movement in a plant are regulated by water potential and hydraulic conductivity (Negrao et al. 2017). Salinity in the soil reduces the water uptake by plants due to reduced water potential in the root zone resulting into drought-like conditions (Behboudian et al. 1986; Romero-Aranda et al. 2001). High salinity levels lead to reduction in relative water content in most crops including maize, sorghum, and cucumber (Nagy et al. 1995; Stępień and Kłbus 2006). The leaf water potential, osmotic potential, leaf pressure potential, and relative water content are significantly decreased with increase in salt stress, subsequently affecting many plant processes such as stomatal conductance, transpiration, net photosynthetic rate, and relative water content (Netondo et al. 2004; Munns and Tester 2008; Sinclair et al. 2013). As the high salt concentration in soil reduces water uptake, it also decreases nutrient uptake by plants, leading to lower NO_3^-, K^+, and Mg^{2+} tissue concentrations (Musyimi et al. 2007; Munns and Tester 2008; Machado and Serralheiro 2017; Sandhu et al. 2017). The effect was more in the mature leaves (Musyimi et al. 2007). An increase in water use efficiency (WUE) upon salt stress is commonly observed, as the reduction in water use (due to reduced stomatal conductance) is more than the reduction in carbon gain (Chartzoulakis and Klapaki 2000).

It is an immense challenge for a plant to maintain ion homeostasis under salinity stress, as accumulations of Na^+or Cl^- interfere with plant's ability to store other ions. During salinity stress, as more ions accumulate in the vacuole, plant tries to balance the decreased water potential in vacuole by accumulating compatible solutes such as proline, glutathione, glycine betaine, and polyols in the cytoplasm (Negrao et al. 2017). Exogenous application of glycine betaine is known to enhance salt tolerance by reducing tissue accumulation of Na^+ (Mäkelä et al. 1998). Accumulation of glycine betaine results in increased Na^+ efflux and decreased K^+ efflux from the plant roots (Wei et al. 2017). Glutathione and proline that play role in maintaining cell turgor and redox homeostasis can act conjointly to allow plants to withstand the joint attack of metalloids and salinity (Anjum et al. 2014). Additionally, accumulation of total soluble sugars increases with increased salinity and plays an important role in osmotic adjustment in plants (Moles et al. 2016).

Some genes that play important roles in plant water relations during salinity have been identified. For instance, the stress-induced aquaporin 1 (AQP1) plays a significant role in improving WUE, hydraulic conductance, and yield under salt stress (Sade et al. 2010; Vysotskaya et al. 2010). The overexpression of AQP1 in tobacco increases leaf net photosynthesis, mesophyll CO_2 conductance, and stomatal conductance, whereas its silencing reduces root hydraulic conductivity under salt stress (Sade et al. 2010). Similarly, reduced hydraulic conductivity in salt-tolerant barley plants as compared to the salt-sensitive plants confirmed the role of aquaporin in salt sensitivity (Vysotskaya et al. 2010). The expression of *Arabidopsis HARDY* (*HRD*) gene, an AP2/ERF-like transcription factor, improved the water use efficiency and ratio of biomass to water use in rice (Karaba et al. 2007).

Identification and manipulation of additional genes involved in water/nutrient uptake and effective compartmentalization may provide new means to improve plants to withstand high salinity levels.

2.3 Mechanism of Salt Tolerance

Salt tolerance mechanism is a complex of various different pathways that work in coordination and are interdependent on each other. Plants develop several biochemical and physiological mechanisms to survive in high-salinity environment. Some of the important aspects of salt stress and important mechanisms responsible for salt tolerance are discussed below.

2.3.1 Tissue Na^+ Accumulation as an Indicator of Salt Tolerance

Na^+ plays an important role during salinity stress in several plant species. High salt concentration in soil normally leads to increased Na^+ uptake by plants, causing tissue toxicity. Besides, as several ion transporters transport both Na^+ and K^+ in roots, increased Na^+ concentration in root zone leads to reduced K^+ uptake. In addition, high concentration of Na^+ also leads to K^+ efflux from the root, resulting in lower K^+ concentration, which may be detrimental for the plant. Disruption of K^+ homeostasis leads to reduced photosynthesis, decreased transpiration, and production of reactive oxygen species; all of which result in reduced yield and productivity. Hence, some studies consider K^+:Na^+ as an important parameter for screening lines for salt tolerance (Chen et al. 2007). However, K^+:Na^+ ratio is not always a true indicator of salt tolerance (Genc et al. 2007), perhaps due to the importance of multiple component traits in the salt tolerance mechanism.

In most plant species, the high concentration of Na^+ is toxic to the cell. However, some plant species are able to cope very well with the moderate concentrations of Na^+ or may even benefit from it (Gattward et al. 2012). In particular, when K^+ is deficient in the medium, beneficial effects of Na^+ are pronounced, suggesting that Na^+ does not strictly substitute K^+(Subbarao et al. 2003). Some members of family Amaranthaceae, such as sugar beet and spinach are shown to utilize Na^+ for growth and development (Kronzucker et al. 2013). These species do not need K^+-deficient conditions to see the benefits provided by Na^+, suggesting that Na^+ may play some additive roles besides replacing K^+ (Ors and Suarez 2016). Physicochemical properties of Na^+ are more or less similar to K^+, making it a good replacement for K^+ in maintaining cell osmoticum (Kronzucker et al. 2013). In addition, the cell K^+ also plays important roles in protein synthesis, oxidative phosphorylation, and as an essential ion for many enzyme functions. Na^+, on the other hand, is known to inhibit protein synthesis and oxidative phosphorylation (Greenway and Osmond 1972). Nevertheless, Na^+ can partially assume some of the roles played by K^+ in the activation of enzymes (Kronzucker et al. 2013). Na^+ is known to improve root nitrate uptake and leaf nitrate assimilation, which may contribute toward reported Na^+ benefits in certain plant species (Kronzucker et al. 2013). It has also been established that Na^+-dependent pyruvate transport into chloroplasts is critical for C_4 habit in some C_4 species, which also explains why Na^+ is an important nutrient for some C_4 species (Weber and von Caemmerer 2010).

Many salt tolerance studies conducted in various crop species focused on studying relationship between tissue Na^+ concentration and salt tolerance (McKimmie and Dobrenz 1991; Genc et al. 2007). However, scientific evidence is mounting to show that the tissue Na^+ concentration may not always represent true picture in terms of salt tolerance (Munns and Tester 2008; Sandhu et al. 2017). Recently, in a salinity study in alfalfa, two genotypes that stored least Na^+ in their leaf tissues in salt treatment were among the worst performers in terms of salt tolerance index among 12 genotypes (Sandhu et al. 2017). On the other hand, two best performers were also low Na^+ accumulators under salt stress. These observations indicate that although the tissue Na^+ concentration is important, other component traits of the salt tolerance mechanism also play substantial roles (Sandhu et al. 2017). In strawberries and avocadoes, the tissue Cl^- toxicity played a primary role during salinity stress, and Na^+ concentration in leaves does not relate well with the yield performance (Khayyat et al. 2007; Suarez and Grieve 2013; Mauk et al. 2017).

2.3.2 Na^+ Exclusion from Roots

The mechanism of Na^+ exclusion from roots is considered pivotal in several crop species (Munns and Tester 2008; Liu et al. 2015). The salt overly sensitive (SOS) pathway that is well characterized in *Arabidopsis* is the key for Na^+ exclusion (Qiu et al. 2002). During salt stress, Ca^{2+} signal is sent by the plant that activates the SOS3 protein (Shi et al. 2002; Gupta and Huang 2014). Active SOS3 binds to SOS2 and stimulates its kinase activity, which in turn phosphorylates SOS1 (Shi et al. 2002). SOS1 is a membrane-associated protein that regulates Na^+ efflux from the root. The manipulation of the SOS proteins have been shown to be successful in enhancing salt tolerance in various plant species (Zhang and Blumwald 2001; Shi et al. 2003; Munns and Tester 2008; Gupta and Huang 2014; Liu et al. 2015).

2.3.3 Sequestration of Na^+ into Vacuoles

Even in the presence of an efficient Na^+ exclusion mechanism, high salt concentrations in the soil or irrigation water result in increased Na^+ concentrations in the cytosol. High Na^+ in the cytosol is detrimental to cellular functions. To protect the cytosol from the toxic effects of Na^+, some plants move excessive Na^+ into vacuoles using the Na^+/H^+ exchanger (NHX) proteins. Of the NHX proteins, NHX1 through NHX4 are present in tonoplast and direct Na^+ into vacuole (Barragan et al. 2012). NHX5 and NHX6 target Na^+ into endosomal membranes including vesicles, Golgi, trans-Golgi network, and prevacuolar compartment (Bassil and Blumwald 2014). Several studies targeting the NHX proteins validated important roles of NHX proteins in enhancing salt tolerance in several plant species (Apse et al. 1999; Zhang and Blumwald 2001; Xue et al. 2004; Rajagopal et al. 2007; Bassil et al. 2011; Mishra et al. 2014; Sandhu et al. 2018).

2.3.4 Enhanced Tissue Tolerance to High Na^+ Concentrations

In response to high Na^+ concentration, plants produce organic solutes to maintain ion homeostasis. Some of the common solutes that are studied in response to salinity include proline, glycine betaine, and total soluble sugars. Proline accumulation was far greater in a salt-tolerant genotype of tomato as compared to susceptible one under salt stress (Gharsallah et al. 2016). Proline not only works as an osmolyte, it is an antioxidant and an important component of cell wall (Verbruggen and Hermans 2008; Gharsallah et al. 2016). In addition, proline accumulation in leaves maintains chlorophyll levels and turgor pressure that are critical for photosynthetic activity (Gharsallah et al. 2016; Sandhu et al. 2017). Glycine betaine also plays an important role in ion homeostasis during salt stress (Wei et al. 2017). Treatments involving exogenous application of glycine betaine resulted in enhanced tolerance to salt (Mäkelä et al. 1998), possibly due to its role in reducing K^+ efflux and increasing Na^+ efflux (Wei et al. 2017). It was speculated that increase Na^+ efflux may be regulated through the SOS pathway.

2.3.5 Cl^- Toxicity During Salt Stress

Most salinity studies in plants focus on role of Na^+ in tissue toxicity; however, the role of Cl^- is often disregarded. Now it is well established that for some sensitive plant species, such as avocado, grapes, and strawberries, Cl^- concentration in leaves is better correlated with tissue toxicity (Tregeagle et al. 2010; Suarez and Grieve 2013; Li et al. 2017). Many of the effects of chloride toxicity are hard to differentiate from Na^+ toxicity; however, some effects are unique to Cl^- toxicity (Li et al. 2017).

A number of proteins involved in regulating Cl^- concentration in plants have been characterized in the recent years that include nitrate transporter 1/peptide transporter family proteins (NPFs), homologs of slow anion channel-associated (SLAHs), cation/Cl^- cotransporters (CCCs), cation/H^+ exchangers (CHXs) proteins, aluminum-activated malate transporters (ALMTs), and Cl^- channels (CLCs) (Li et al. 2017). The common mechanism plants use to manage Cl^- concentration in shoot tissue is by abscisic acid (ABA)-mediated Cl^- uploading in the root xylem (Gilliham and Tester 2005). A nitrate transporter 1/peptide transporter family gene, *NPF2.4*, was shown to be involved in catalyzing Cl^- efflux out of cells and regulated xylem uploading (Li et al. 2016a). Knockdown of *AtNPF2.4* resulted in reduction in shoot accumulation of Cl^-, and overexpression of *AtNPF2.4* led to a 23% increase in shoot Cl^-, suggesting an important role of *AtNPF2.4* in regulation of shoot Cl^- concentration during salinity stress (Li et al. 2016a). Similarly, AtSLAH1, a homolog of the slow-type anion channel AtSLAC1, interacts with AtSLAH3 in controlling Cl^- uploading in xylem and regulates root-to-shoot Cl^- transport (Qiu et al. 2016). AtCCC has been shown to be involved in coordinated symport of K^+, Na^+, and Cl^- and played a critical role in plant development (Colmenero-Flores et al. 2007). Under high-salinity environment, the *ccc* mutant plants stored higher Cl^- amounts in shoots and lower Cl^- amounts in roots as compared to the wild-type plants, suggesting its important role in Cl^- homeostasis (Colmenero-Flores et al. 2007).

Some transporters also play role in exclusion of Cl^- from the roots. For instance, NPF2.5 that displayed 83% homology at amino acid level to NPF2.4 was shown to regulate Cl^- efflux from roots, keeping lower concentration of Cl^- in roots, which in turn resulted in lower Cl^- concentration in shoot (Li et al. 2016b).

Sequestration of Cl^- into the vacuole is an efficient mechanism to keep Cl^- concentration low in the cytoplasm. In *Arabidopsis*, two ALMTs, AtALMT9 and AtALT12, are believed to be involved in Cl^- homeostasis by moving Cl^- across vacuolar membrane (Li et al. 2017). AtALMT9 was originally characterized to be involved in the opening and closing of stomata by regulating Cl^- concentration in the vacuole of guard cells (Baetz et al. 2016). AtALMT9 is now believed to regulate Cl^- concentration in roots cells by moving excess Cl^- to the root vacuole (Baetz et al. 2016). A homolog of AtALMT9, AtALT12, is involved in stomatal closure and is a strong candidate that may have a role in long-distance transport of Cl^- (Sasaki et al. 2010). Similarly, CLCs regulate Cl^- homeostasis by sequestering Cl^- into root and leaf vacuoles. Of the two CLCs known to transport Cl^-, AtCLCc directs Cl^- into root vacuoles, and AtCLCg helps in compartmentalization of Cl^- into vacuoles of mesophyll cells (Jossier et al. 2010; Nguyen et al. 2016). Some CHX proteins can regulate both Na^+ and Cl^- concentrations in plants. For instance, GmSALT3/CHX1 that controls Na^+ and Cl^- accumulation in soybean provides yield advantage by maintaining higher seed weight under saline conditions (Liu et al. 2016).

Additional studies focusing on understanding the role of Cl^- transporters in salt tolerance, specifically in crop plants where Cl^- toxicity is the main cause of salt stress, will help in refining tools and techniques to develop genetic material tolerant to salt.

2.4 Conclusions and Future Perspectives

Traditional breeding approaches had limited success in identifying material tolerant to salinity due to the complexity of the salt tolerance mechanism. Besides, selecting plants under salinity based on biomass resulted in selection for plant vigor. Combining vigor-based selections with selections based on component traits of the salt tolerance mechanism such as ion exclusion, sequestering ions into vacuole, and high tolerance to ion toxicity may improve the potential to obtain genotypes that maintain economically feasible yields and have high salt tolerance. In the last decade, significant progress has been made in understanding plant responses to salinity, and the roles of several important players involved have been elucidated. However, still large gaps exist in the comprehensive understanding of the molecular and biochemical responses in crop plants. Future studies should focus on understanding how intra- and intercellular interactions play role during salinity stress response and how they relate to salinity tolerance at whole plant level. Identification and characterization of novel candidate genes and their utilization may help in developing new salt-tolerant genotypes that are vigorous and high yielding.

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