

# Plant Salt Stress: Adaptive Responses, Tolerance Mechanism and Bioengineering for Salt Tolerance

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**Abstract** Salinity is an important abiotic environmental stress factor threatening agricultural productivity throughout the world. The detrimental effects of salinity stress are observed at cellular, organ and whole plant level at osmotic phase (early/short-term response) and ionic phase (late/long-term response). High salinity exerts its negative impact on major plant processes such as disrupting the osmotic and ionic equilibrium, protein synthesis, photosynthesis, energy, and lipid metabolism. To adapt and tolerate salt stress, plants have evolved physiological and biochemical mechanisms orchestrated by multiple biochemical pathways of ion homeostasis, osmolytes synthesis, ROS scavenging, and hormonal balance. At the molecular level, such adaptation involves activation of cascade(s) of gene modulations and synthesis of defense metabolites. In recent years, several candidate genes have been identified and employed to facilitate genetic engineering efforts to improve salt tolerance in crop plants. However, there is a further need of improvement for successful release of salt tolerant cultivars at the field level. In this article we present the physiological, biochemical and molecular signatures of plant responses to salinity, and outline their use in genetic engineering to improve salt stress tolerance.

**Keywords** Salinity · Osmotic and ionic stress · Stress tolerance · miRNA · Transgenic plants · Bioengineering

## Introduction

Soil salinity is a major environmental limitation to world agriculture affecting 800 million hectares of land. This estimates over 6 % of the world total land area, affected either by salinity (397 million hectares) or associated forms of sodicity (434 million hectares) (Munns 2005). Under saline conditions, physiological and metabolic

activities of plants are impaired by osmotic stress, ionic stress, nutritional imbalances, or a combination of these factors (Ashraf 2004; Slama et al. 2015). Osmotic imbalance causes water deficit, reduced leaf area expansion and stomatal closure which ultimately lessen the photosynthesis and growth (Roy et al. 2014). The ionic stress causes the excess accumulation of  $\text{Na}^+$  in the older leaves which lead to premature senescence of salt accumulated older leaves (Munns and Tester 2008; Roy et al. 2014). Salinity also affects the major plant processes, such as protein synthesis, energy, and lipid metabolism during the developmental stages of the plant (Tuteja, 2007a). To cope with osmotic and ionic stress, plants have developed different biochemical mechanisms such as osmotic tolerance, ion exclusion and tissue tolerance (Munns and Tester 2008), and synthesis of compatible solutes, alterations in membrane structure, induction of antioxidant defense and hormone homeostasis.

Plant salt stress responses are controlled by multiple genes which contribute to tolerance. (Zhang et al. 2013). Salt stress also induces extensive gene expression alterations in the morphological, physiological, biochemical, cellular and molecular pathways (Chen et al. 2014) and the mechanism is well orchestrated by different genes and transcriptional factors involved in ion transport, senescence-associated response, compatible solute synthesis, antioxidant system, hormonal regulation,  $\text{Ca}^{2+}$  signaling and SOS signaling pathways (Gupta and Huang 2014). A specific gene may be responsible for one or more traits or one trait can be contributed by many genes (Roy et al. 2014). Studies on genetic engineering of metabolic and stress-signalling pathways are of major interest in agricultural research. Significant achievements have been made in developing the stress-tolerant transgenic plants by incorporating genes for osmolytes, ion transporters, transcriptional factors and other signalling molecules (Mantri et al. 2012). In this article, we present an overview of the effects of salinity stress and the major physiological and molecular mechanisms of salt adaptation in plants. In addition, progress made in genetic engineering to improve salt tolerance is also discussed.

## Effect of Salinity Stress on Plants

Plant growth under saline environment is affected at two phases; osmotic phase (early response) and ionic phase (late response) (Munns and Tester 2008) (Fig. 1). The early phase of salt stress is due to the salt outside the root zone whereas salinity stress at the late phase is the result of the toxic effect of salt inside the plant (Munns 2005). Early response results in inhibition of water uptake, root growth, cell elongation, leaf development, reduction of new leaves, and injury to the cells in transpiring leaves etc. Long-term response of salinity stress occurs due to the toxic effects of salt inside the plant, such as sequestration in older leaves which causes premature senescence of leaves, inhibition of photosynthesis and enzyme activities (Munns 2005; De Oliveira et al. 2013; Roy et al. 2014). Stress impact on photosynthesis is either direct via decreased  $\text{CO}_2$  availability caused by diffusion limitations through the stomata and the mesophyll transport of  $\text{CO}_2$  as well as alterations in photosynthetic metabolism, or secondary such as the oxidative stress arising from the superimposition of multiple stresses (Chaves et al. 2009). Leaf senescence is the last step as the nutrients deposited in the leaves are circulated to other developing organs. Saline stress results in early leaf



**Fig. 1** General overview of salt stress effects on plant. Salt stress induced osmotic and ionic stress affects each and every aspect of plant's normal growth and development

senescence, reduced photosynthesis rate and the decline in nutrient accumulation which ultimately results in reduced crop productivity (Fig. 1).

Salinity stress also results in the generation of reactive oxygenic species (ROS) such as superoxide ( $O_2^-$ ), hydrogen peroxide ( $H_2O_2$ ), hydroxyl radical ( $\cdot OH$ ) and singlet oxygen ( $^1O_2$ ) (Jithesh et al. 2006; Luo and Liu 2011). These disrupt the normal metabolism of lipid, protein, and nucleic acid through oxidative damage. The redox state of a cell referred to as the integrated ratio of reduced and oxidized forms of all the redox couples present inside the cell, is controlled by the level of ROS and activities of ROS-producing and ROS-scavenging enzymes. The redox homeostasis is the central regulator of plant growth and development. Since most of the abiotic and biotic stresses cause redox imbalance inside the plants, the functioning of these redox-dependent processes gets disturbed, leading to reduced plant growth and crop yield (Srivastava and Suprasanna 2015). Lipids are the structural constituents of the cellular membrane which play an important role in tolerance to several physiological stressors, biogenesis

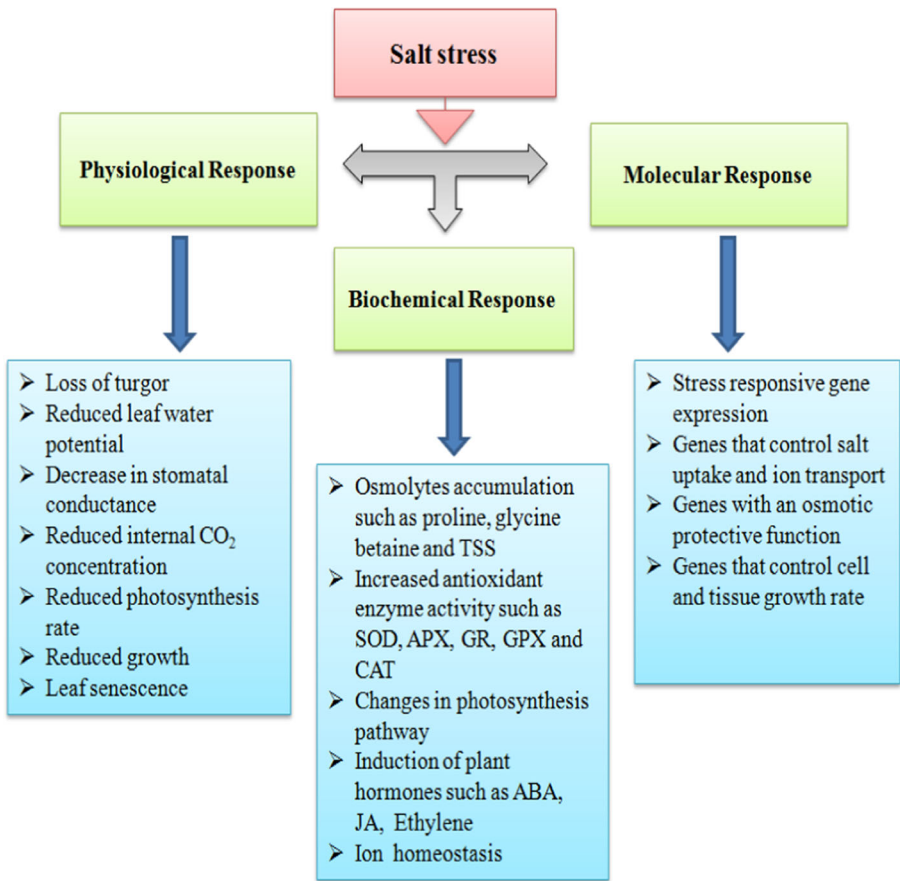
of the photosynthetic apparatus in developing chloroplasts and as the source of storage energy in the form of triacylglycerol (Rahdari and Seyed 2011; Chapman et al. 2012). Salinity stress affects the lipid metabolism strongly by influencing the essential lipid biosynthesis. Exposure of plant to salinity stress reduces the monogalactosyldiglyceride (MGDG) in the leaf which results in the destruction of chloroplast membrane (Rahdari and Seyed 2011). Under salt stress,  $\text{Na}^+$  uptake competes with the  $\text{K}^+$  uptake which results in excess of cytoplasmic  $\text{Na}^+$  sequestration instead of  $\text{Cl}^-$  within the cell. Increased concentration of  $\text{Na}^+$  and  $\text{Cl}^-$  decreases the uptake of  $\text{Ca}^{2+}$ ,  $\text{K}^+$ , and  $\text{Mg}^{2+}$  levels in a number of plants and also affects the ratio of  $\text{K}^+/\text{Na}^+$  ratio (Parida and Das 2005).

## Biochemical and Molecular Mechanisms of Salt Tolerance

Plants are evolved with different biochemical and molecular mechanisms to combat osmotic and ionic stress. These include selective accumulation or exclusion of ions, control of ion uptake by roots and transport into leaves, compartmentalization of ions at the cellular and whole plant levels, synthesis of compatible solutes, changes in photosynthetic pathway, induction of antioxidant enzymes and plant hormones (Fig. 2) (Parida and Das 2005; Roy et al. 2014). At the molecular level, there are several genes associated with different pathways and processes of stress perception, signaling and downstream metabolic conduits (Munns and Tester 2008; Kurusu et al. 2015). The various gene expression modules are responsible for controlling salt uptake, ion transport, osmotic protective function and genes that control cell and tissue growth rate.

### Signal Transduction

Plant adaptation to salinity stress is facilitated through stress perception, signal transduction and metabolic changes (Fig. 3) (Mantri et al. 2012; Kumari et al. 2015). Under saline stress conditions, plants perceive the signals and transmit it to the cellular machinery in order to activate the adaptive response. A general pathway of signal transduction starts with the signal perception (receptors) and consequently, the generation of second messengers like  $\text{Ca}^{2+}$ , inositol phosphate, ROS and phytohormones (Xiong et al. 2002). The signaling cascade involves protein phosphorylation, dephosphorylation, phospholipids metabolism,  $\text{Ca}^{2+}$  sensing (Agarwal et al. 2013). These second messengers regulate the intracellular level of  $\text{Ca}^{2+}$  which initiates the phosphoprotein cascades. These cascades target the proteins carrying the function of cellular protection and also target the transcriptional factors which regulate stress genes (Fig. 4) such as those involved in the synthesis or regulation of molecules such abscisic acid (ABA), ethylene and salicylic acid (SA) which are responsible for second round of the signaling (Xiong et al. 2002; Tuteja, 2007b). Under saline stress, regulation of ion homeostasis is critical for plant salt tolerance. Increase in cytosolic  $\text{Ca}^{2+}$  concentration (Xiong et al. 2002) and subsequent activation of  $\text{Ca}^{2+}$  sensor protein expression and/or activity is seen as a significant response to salt stress (Fig. 4). The amplitude, frequency and duration of the peak of cytosolic  $\text{Ca}^{2+}$  signaling process may vary with stress and type of tissue (Kiegle et al. 2000). Some studies showed the decrease in cytosolic  $\text{Ca}^{2+}$  concentration under salt stress condition whereas many studies revealed the enhanced

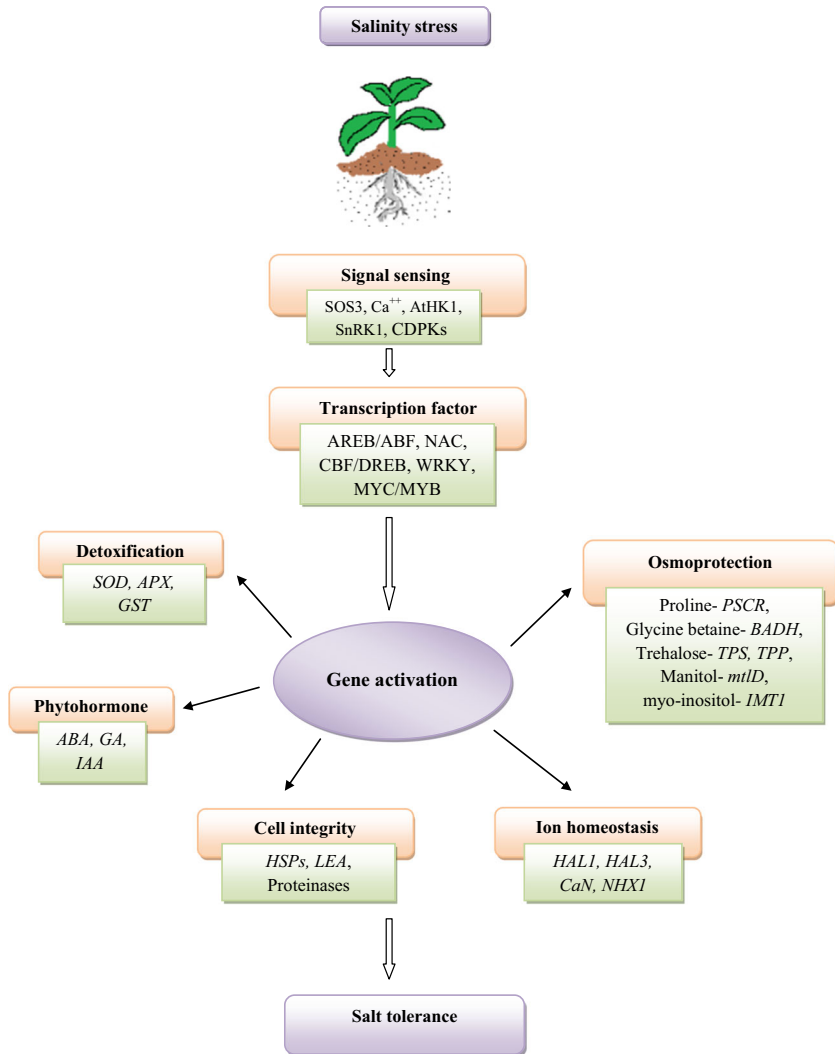


**Fig. 2** Physiological, Biochemical and molecular basis of salt stress tolerance (Modified after De Oliveira et al. 2013)

level of cytosolic  $\text{Ca}^{2+}$  after salt stress (Zhu 2001). From the reported studies it is also apparent that change in cytosolic  $\text{Ca}^{2+}$  concentration is not uniform and it could vary with species and type of cells (Zhu 2001).

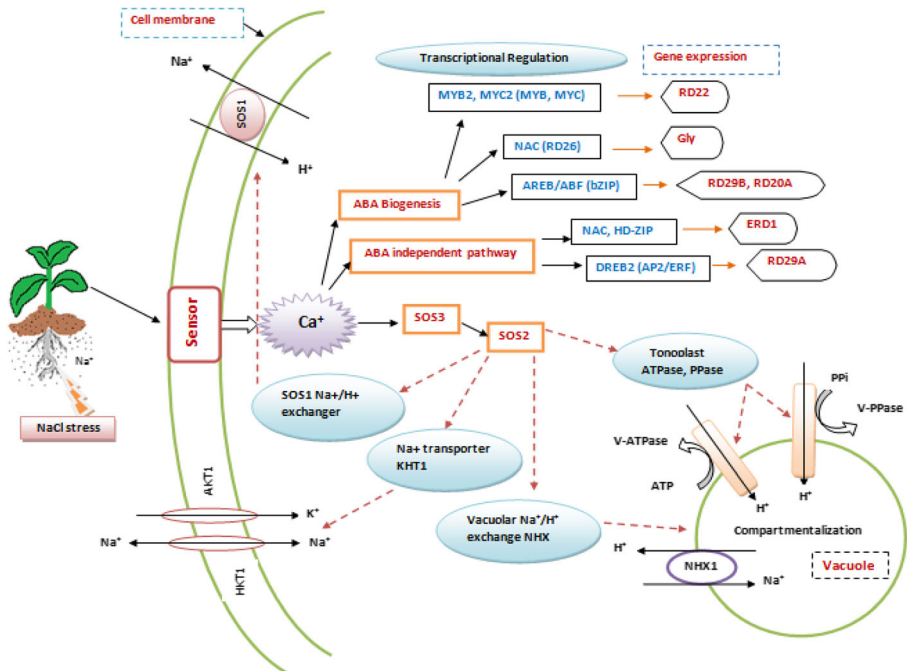
Considerable progress has been made in understanding the signal transduction under  $\text{Na}^+$  toxicity in cells through investigations on the SOS pathway (Zhu 2002). Osmotic and ionic ( $\text{Na}^+$  and/or  $\text{Cl}^-$ ) stress is sensed by the surface of the plasma membrane by a trans-membrane protein or enzymes within the cytosol (Zhu 2003). Increase in cytosolic  $\text{Ca}^{2+}$  in response to salt stress is read by SOS3, a  $\text{Ca}^{2+}$  sensor (Mahajan et al. 2008). The SOS3 protein interacts with an SOS2 protein kinase and forms SOS3-SOS2 complex to activate SOS1 protein, a plasma membrane  $\text{Na}^+/\text{H}^+$  antiporter which results in  $\text{Na}^+$  homeostasis in the cells (Zhu 2003). In the SOS pathway, how  $\text{Na}^+$  toxicity in cell forms is still unclear, however, within a second of the salt stress sensing cytosolic  $\text{Ca}^{2+}$  concentration changes. This change is necessary for the activation of response mechanism through induction or down-regulation of the responsive genes.

In Arabidopsis, CDPKs are involved in abscisic acid (ABA) signaling pathway. The CPK4 and CPK11 play an essential role in ABA-regulated physiological processes



**Fig. 3** A generalized schematic presentation of salinity stress responses of plant (Modified after - Chen et al. 2014; Agarwal et al. 2013). Adaptation to salt stress starts from stress perception of complex stimuli. Signal is perceived by the sensor proteins, these stress signals triggers the downstream signaling processes and gene activation through transcription factors. The mechanisms include cell integrity, phytohormones, antioxidants, synthesis of osmolytes and ion homeostasis. The coordinated action leads to re-establish the cellular homeostasis, protection of functional and structural proteins and membranes, and ultimately the tolerance to salinity stress

such seed germination, seedling growth, stomatal movement and salt tolerance (Zhu et al. 2007). The overexpression of OsDREB1F gene has resulted in increased salt tolerance in both Arabidopsis and rice. The OsDREB1F overexpression resulted in the up-regulation of ABA-independent gene (COR15a and rd29A), and ABA-dependent genes (RAB18 and rd29b) (Wang et al. 2008). The ethylene signal transduction pathway mainly consists of ethylene receptors: negative (CTR1) and positive regulators (EIN2), transcription factors (EIN3/EILs) and ethylene-response factors. Salinity stress



**Fig. 4** A schematic representation Ca<sup>2+</sup> signalling pathway in response to salt stress. Excess of Na<sup>+</sup> are sensed by the sensors at plasma membrane which then induces the increased cytosolic Ca<sup>2+</sup> level. This activates the SOS3-SOS2 protein complex phosphorylates SOS1, the plasma membrane Na<sup>+</sup>/H<sup>+</sup> antiporter which leads to Na<sup>+</sup> ion efflux. SOS2 can regulate NHX1 antiport activity and V-H<sup>+</sup>-ATPase activity. Salt stress causes ABA accumulation which results in different gene expression through ABA-dependent and independent pathways (Modified after Silva and Geros, 2009)

has been shown to induce expression of multiple ethylene signaling genes (Tao et al. 2015). In tobacco, expression of ethylene receptor NTHK1 was increased significantly in response to salt stress (Zhou et al. 2006). Peng et al. (2014) observed that in cotton, ETR1, ETR2, and EIN4 - ethylene receptor genes (CTR1, EIN3, ERF1, and ERF2), ethylene signaling genes and MEKK1-MKK2- MPK4/6 - MAPK cascade genes were up-regulated under short and long duration of salt stress. Furthermore, EBF1/EBF2 degradation was seen due to salt stress which enhanced the accumulation of EIN3 protein (Peng et al. 2014). In Arabidopsis, *etr1-1* gain-of-function mutant showed sensitivity to salt stress, whereas *etr1* loss-of-function mutants displayed enhanced tolerance to high salinity (Zhou et al. 2006). In addition, ethylene signaling components such as ethylene signaling factor CTR1 loss-of function results in significant salinity tolerance by homeostasis of shoot Na<sup>+</sup> and K<sup>+</sup> (Peng et al. 2014).

Multiple signaling messengers like free fatty acids, inositol polyphosphates, DAG-γ-phosphate, diacylglycerol, phosphatidic acid etc. are regulated by phospholipases, lipid kinases, and/or phosphatases (Wang 2004). Cellular concentration of phosphatidic acid (PA) changes rapidly in response to salinity stress and, in rice, elevated levels of phosphatidylinositol bisphosphate (PIP2), phosphatidic acid (PA), and diacylglycerolpyrophosphate (DGPP) were observed under salt stress (Darwish et al. 2009). Mitogen-activated protein kinase (MAPK) cascades have been reported for their role in salt adaptation in rice and Arabidopsis (Ning et al. 2010). MAPK cascade and

cellular lipid transfer processes are linked for the coupling of MAP-type kinases which have role in lipid signaling. In *Arabidopsis thaliana*, phosphorylation of the of plasma membrane  $\text{Na}^+/\text{H}^+$  antiporter SOS1 is mediated by MPK6 under salt stress (Yu et al. 2010). In *Arabidopsis*, mitogen-activated protein kinase (MAPK) signaling pathway consisting of MAPK Kinase1 (MEKK1), MAPK Kinase2 (MKK2) and MAPK4/6 (MPK4/6) is involved in salt tolerance (Teige et al. 2004). In rice, MAPK cascade is associated with the signaling of ROS by the salt-responsive ERF1 (SERF1) transcription factor (Schmidt et al. 2013).

Cellular ROS plays a key role in stress signaling. A study in *Arabidopsis* indicates that the salt and osmotic stress tolerance is associated with the ROS-responsive WRKY15 and mitochondrial retrograde signaling (Vanderauwera et al. 2012). In *Arabidopsis thaliana*, studies also suggested that the HY1 gene encoding heme oxygenases plays an essential role in salt signalling and ROS production. Xie et al. (2011) studied the effect of salt stress on seed germination and root growth of *Arabidopsis* wild-type and four HO mutants *hy1-100*, *ho2*, *ho3*, and *ho4*. The increased expression of RbohD and corresponding ROS production was subsequently responsible for the HY1 expression under mild salt stress.

### Transcriptional Factors

Regulation of gene expression in response to salinity stress is carried out by reprogramming the gene expression governed by transcriptional factors (Chen et al. 2014). Transcription factors such as bZIP, MYC, WRKY, AP2, NAC, C2H2 zinc finger gene, and DREB families are important to control gene expression (Wang et al. 2016). The role of WRKY TFs in response to salt, heat, cold, and osmotic stresses have been extensively reviewed recently (Tripathi et al. 2014; Wang et al. 2016). In *Arabidopsis*, overexpression of WRKY25 and WRKY33 has significantly increased the salt tolerance and ABA sensitivity independent of the SOS-pathway. WRKY25 and WRKY33 play an important regulation of oxidative stress and osmotic stresses (Jiang and Deyholos 2009). Overexpression of *MusaWRKY71* in transgenic banana plants enhanced tolerance towards oxidative and salt stress as indicated by lower membrane damage and better photosynthesis efficiency (Shekhawat and Ganapathi 2013). A number of MYB transcriptional factors have been reported for their role in physiological and biochemical processes such as cell development, cell cycle, hormone synthesis and plant responses to different stresses (Ambawat et al. 2013). In *Arabidopsis*, overexpression of soybean GmMYB76 or GmMYB177 resulted in enhanced tolerance to salt and freezing (Liao et al. 2008). In rice up-regulation of OsMYB2 exhibits enhanced tolerance to salt, cold, and dehydration stresses by the alteration in the expression of numerous stress response genes (Yang et al. 2012). Similarly, another transcriptional factor, bZIP also play a key essential role in developmental processes and respond to high salinity, drought and cold stresses (Jakoby et al. 2002). In salt-sensitive wheat cultivar, expression of bZIP gene was unregulated upon exposure to long-term salinity whereas it was decreased in salt tolerant cultivar (Johnson et al. 2002).

Transcriptional activation of stress-responsive genes is well understood (Shinozaki and Yamaguchi-Shinozaki 2000). The TFs belonging to the ERF/AP2 family which bind to DRE/CRT were isolated and termed as DREB1A/CBF3, DREB1B/CBF1 and DREB1C/CBF2 (Stockinger et al. 1997). DREB1/ CBF overexpression increased



tolerance to salt, drought and freezing (Shinozaki and Yamaguchi-Shinozaki 2000; Kasuga et al. 1999). NAC family of transcriptional factors plays a key role in generating the salt tolerant crops. A stress-responsive SNAC2 gene isolated from upland rice was transferred into japonica rice. The transgenic seedlings showed faster growth, significant shoot length as compared with the wild-type (Hu et al. 2008). From the reported studies it can be concluded that TFs offer a great promise for understanding regulation of various plant processes and can be useful in engineering plant response to different stresses.

## Hormonal Regulation

Phytohormones play an essential role in plant growth and development. To cope with salinity stress during different developmental stages of a plant, phytohormones like Abscisic acid (ABA), Indole Acetic Acid (IAA), Gibberellic Acid, Jasmonic Acid, Salicylic Acid, Brassinosteroids and Ethylene are induced or their levels modulated (Ryu and Cho 2015). Abscisic acid (ABA) plays an important role in salt stress condition because of its rapid accumulation in response to stresses. ABA regulates the plant water status through guard cells and helps to overcome the adverse effect of salinity on growth, photosynthesis and translocation of assimilates (Mahajan and Tuteja 2005). Under salt stress, increased ABA accumulation in the shoot and root was observed which may mitigate the adverse effects of salt stress on photosynthesis, growth, and translocation of assimilates (Cramer and Quarrie 2002; Cabot et al. 2009). The crucial role of ABA in cellular signaling which regulates the salt and water deficit-responsive genes expression has been reported (Zhang et al. 2006a). The ABA-dependent stress signaling activates AREB transcription factors which bind to ABRE element to induce the *RD29B* stress responsive gene. In *Arabidopsis*, ABRE motifs are involved *RD29B* gene expression which encodes a LEA-like protein (Uno et al. 2000). ABA accumulation has been attributed to improving the content of  $K^+$ ,  $Ca^{2+}$ , proline and sugars in the root vacuole in response to salt stress (Chen et al. 2001). Jia et al. (2002) found that ABA accumulation is induced under salt stress differently to that of water deficit, and that root and leaf tissues may have differential ABA inducing mechanisms under salt and water deficit.

Ethylene is the gaseous hormone which regulates the plant growth and development under stress environment, and recently interaction between ethylene signaling pathway and salt stress has been studied in detail (Cao et al. 2008; Ryu and Cho 2015). The over-expressing of *ctr1-1* or *EIN3* showed improved salt tolerance, seed germination rate and seedling growth under salt and osmotic stress (Achard et al. 2006). In *Arabidopsis*, *ETR1* gene expression from the group I subfamily was down-regulated under salt and osmotic stress (Zhao and Schaller 2004). In response to salinity stress, *Cakile maritima*, and *Thellungiella salsuginea* halophytes showed significant accumulation of ACC (Ethylene precursor) in the leaves and roots as compared to the glycophyte *Arabidopsis thaliana* (Ellouzi et al. 2014). Significant ethylene biosynthesis was observed in the salt-tolerant genotype of soybean as compared to salt sensitive genotype in response to salt stress; (Ma et al. 2012).

Recently jasmonic acids (JA) have been studied for their positive role as a regulator of salt tolerance. In *Arabidopsis*, overexpression of wheat *TaAOC1* gene encoding an AOC enzyme elevated the level of JAs which positively regulated the salt tolerance

(Zhao et al. 2014). In tomato, Systemin which acts as a phytohormone promotes JA production and salt tolerance in ABA-dependent manner. Transgenic tomato expressing prosystemin showed improved stomatal conductance and biomass under the saline condition as compared to the wild type (Orsini et al. 2010). Overexpression of several JA and ethylene-inducible genes has been reported for induction of stress tolerance in different species without adverse effects on plant development (Kazan 2015) which could be the starting point for genetic improvement of stress tolerance in plants.

### Modulation in Photosynthesis Pathway

Abiotic stresses severely hamper the photosynthesis mechanism by disrupting the photosystem I and II, carbon fixation, ATP generating system, electron transport, and stomatal conductance (Nouri et al. 2015). Coordinate action of nucleus and chloroplast regulates the photosynthesis related genes and proteins expression. Enhanced expression of chlorophyll a/b binding (CAB) protein, PSI and PSII subunits, ATP synthase and ferredoxin NADPH<sup>+</sup> oxidoreductase were noted in response to stress (Saibo et al. 2009; Nouri et al. 2015). In some plants, Chl b may be converted into Chl a during chlorophyll degradation process, thus resulting in the increased content of Chl a in salt tolerant species (Akram and Ashraf 2011; Ashraf and Harris 2013). Muchate et al. (2016) reported a significantly higher content of chl a, chl b and carotenoids in *Sesuvium* plants exposed to salinity stress (4–12 dS/m electrical conductivity) in comparison to the control. Carotenoids (Car) play an important role as a precursor in signaling during the plant development under abiotic or biotic stress. Carotenoids have potential to enhance nutritional quality and plant yield as well as play an important role in scavenging reactive oxygen species (ROS) (Davison et al. 2002). Zhang et al. (2010) has overexpressed wheat *TaSnRK2.8* gene in Arabidopsis and showed transgenic plants were resistant to drought, salt and cold stresses. Also, under salt stress the efficiency of PSII was less affected and higher chlorophyll content as compared to control plants. In transgenic cotton, overexpression of the Arabidopsis gene *AtNHX1* that encodes a vacuolar Na<sup>+</sup>/H<sup>+</sup> antiporter resulted in higher photosynthetic performance and higher nitrogen assimilation rates as compared with wild-type cotton plants. AtNHX1 overexpression increases vacuolar Na<sup>+</sup> uptake, which enhances the vacuolar solute concentration, ultimately improving the salinity tolerance in transgenic plants.

Although several TFs belonging to different TF families have been studied, very few are known to regulate the photosynthesis related gene expression under salinity stress. These TFs are involved in photosynthesis associated genes expression regulating both the stomatal and non-stomatal response (Saibo et al. 2009; Chaves et al. 2011). Some of the genes associated with photosynthetic pathways are involved in stress recovery whereas in some cases, genes are not altered under stress condition. Overexpression of CBF/DREB1 genes in Arabidopsis resulted in improved survival rate under salt, low temperature and drought conditions (Kasuga et al. 1999). Oh et al. (2007) reported that the CBF/DREB1 gene overexpression is associated with the persistent photosynthesis rate and photochemical efficiency. Hu et al. (2008) reported the overexpression of STRESS-RESPONSIVE NAC1 (SNAC1) transcriptional factors responsible for salt and drought resistance in rice. This may be due to the significant increase in ABA sensitivity and decreased transpiration rate. ABA signalling is associated with the regulation of genes or transcription factors (TFs) which controls the stomatal

movement. The overexpressed AtMYB44 gene, under salt stress down regulated the expression of serine/threonine protein phosphatases 2C (PP2Cs) which leads to enhanced salt tolerance in Arabidopsis plants (Jung et al. 2008). Huang et al. (2009) observed the expression of zinc finger transcriptional factor in rice for the regulation of stomatal aperture in response to salt and drought stress. The transcription rates of Ppchl (encoding a CAM- isozyme- PEPCase) and Gap1 (encoding a CAM isozyme -NAD-dependent glyceraldehyde-3- phosphate dehydrogenase) genes were found increased under saline condition which is mediated by MYB-type transcriptional factor (Schaeffer et al. 1995).

NAC transcription factor ANAC092/ORE1 in the *anac092-1* stay-green mutant of Arabidopsis showed significant chlorophyll content and regulation of leaf senescence in response to salt stress (Balazadeh et al. 2010). Under salt stress condition, deactivation of photosynthesis related enzymes such as Rubisco, sucrosephosphate synthase (SPS), nitrate reductase and fructose-1, 6-bisphosphatase is reported (Chaves et al. 2009). Indirect actions of TFs are also involved in photosynthesis-related genes regulation. Compatible solutes like glycine betaine accumulation is associated with stabilized Rubisco activity under high salinity stress (Sakamoto and Murata 2002). Tao et al. (2013) studied the effect of salt stress on stomatal and metabolic changes in salt sensitive (PI516605) and salt tolerant (BARLP 4317) perennial ryegrass genotypes. Significant leaf water content, Rubisco activity, overexpression of LSU level (Rubisco large subunit) and *rbcl* gene were observed in salt tolerant BARLP 4317 genotype as a mechanism of salt resistance. Under salinity stress, plants acquire an adaptive mechanism by altering the gene expression associated with the chloroplast. Differential expression of proteins was observed in salt sensitive (Pusa Agrani) and salt tolerant (CS-54) *Brassica juncea* genotypes. In the salt tolerant genotype, expression of protein associated with chloroplast results in enhanced expression of photosystems and stabilization of rubisco activity (Yousuf et al. 2016). *Mesembryanthemum crystallinum* plant switches from C3 photosynthesis to CAM (crassulacean acid metabolism) pathway as an adaptive mechanism in response to stress (Cosentino et al. 2013). This is mainly associated with nocturnal CO<sub>2</sub> fixation and diurnal CO<sub>2</sub> assimilation results into improved water use efficiency (WUE) of the plant. Transcriptional regulation is the prime mechanism which is linked with the enhanced CAM-specific gene expression.

### Compatible Solute Accumulation

Most plants increase the cellular concentration of osmotically active compounds under stress conditions. The compatible solutes are hydrophilic and replace water at the surface of proteins, protein complexes, or membranes and do not interfere with normal biochemical mechanism. They alleviate inhibitory effects of high ion concentrations on enzyme activity. Amino acids and some amino acid derivatives, sugars, acyclic and cyclic polyols, fructans, and quaternary amino, ectoine and sulfonium compounds act as compatible solutes (Suprasanna et al. 2005). Compatible solutes play an osmoregulatory function which accumulates in response to osmotic stress whereas some solutes such as trehalose do not respond to osmotic stress, but are protective even at low concentrations and may function as osmoprotectants (Slama et al. 2015). Mannitol at low concentration in chloroplasts helps to reduce damage by generating hydroxyl radicals, and other compatible solutes at high concentrations have found to reduce

the inhibitory effects of ions on enzyme activity. Stabilization of proteins, protein complexes or membranes and oxygen radical scavenging are the main function of compatible solutes.

Accumulation of proline is a common adaptive response to various abiotic stresses (Ramakrishna and Gokare 2011). Several studies demonstrated that proline metabolism has a complex effect on development, stress responses and accumulation of this proline is important for the tolerance to certain adverse environmental conditions (Szabados and Savoure 2009; Suprasanna et al. 2014). Proline is concentrated in the cytosol, chloroplast, cytoplasm, and vacuoles. It plays an important function like osmotic adjustment, detoxification of reactive oxygen species, act as an antioxidant, stabilization of proteins and protein complexes. Lokhande et al. (2010) reported the elevated level of proline and glycine betaine content in *Sesuvium portulacastrum calli* grown at 200 and 400 mM NaCl as compared to the control. Similarly, Muchate et al. (2016) observed the significant higher contents of proline and glycine betaine in plants exposed to salt stress (EC of 16–20 dS/m) in comparison to the control and other salt treatments. Proline and glycine betaine were found in salt-sensitive barley cultivars contributing substantially to cell osmolarity, compensating for reduced cytosolic K<sup>+</sup> (Zhonghua et al. 2007). P5CS and ProDH are rate-limiting enzymes as for as biosynthesis and catabolism of proline is concerned (Slama et al. 2015). Accumulation of proline in response to salt stress is associated with genes P5CS ( $\Delta^1$ - pyrroline-5-carboxylate synthetase) and pyrroline-5-carboxylate reductase (P5CR) (Ashraf and Foolad 2007). Hong et al. (2000) reported the two fold increase in proline concentration with the overexpression of P5CS gene. Besides its role in salt tolerance, proline acts as a signaling or regulatory molecule to maintain the metabolite pools and redox balance, and controls the gene expression (Szabados and Savoure 2009).

Glycine betaine (GB) is found in the chloroplast and plays an important role in osmotic adjustment, protection of thylakoid membrane, protecting chloroplast and photosynthesis system II (PSII) under stress conditions (Ashraf and Harris 2004). Significant glycine betaine accumulation was seen due to the overexpression of BADH gene in rice (Giri 2011). In response to salinity stress, significant accumulation of total soluble sugars has also been noted. The glycophytes exposed to saline condition, show that near about 50 % of total osmotic potential is contributed by the sugars (Ashraf and Harris 2004). Trehalose, a non-reducing disaccharide plays a significant role in metabolic homeostasis. Salt tolerance capacity of the rice cultivars was improved due to the overexpression of OsTPS1 gene encoding trehalose-6-phosphate synthase (Li et al. 2011).

## Antioxidant Defense

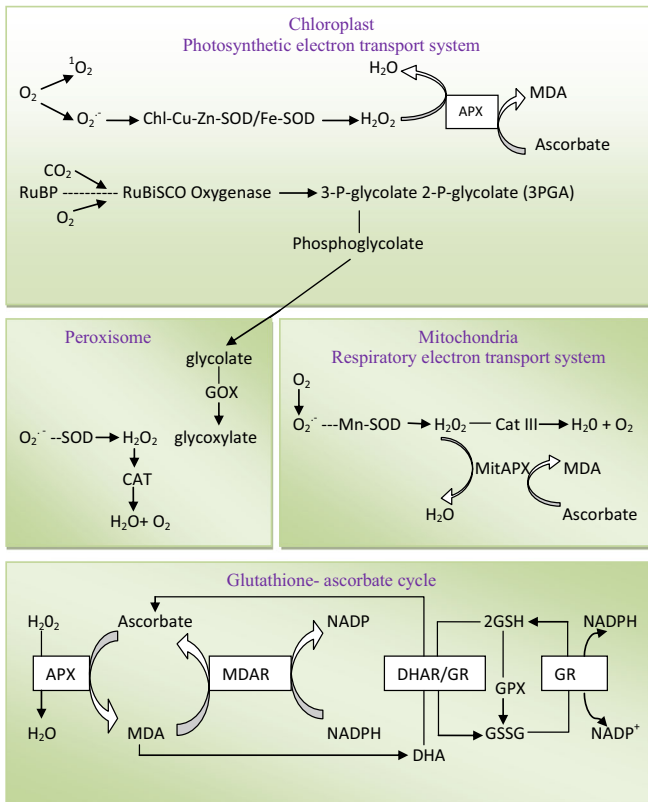
Plants have developed robust ROS scavenging mechanisms, including enzymatic or non-enzymatic defense pathways to counter the deleterious effects of ROS production (Table 1, Fig. 5). Antioxidant enzymes include catalase (CAT), glutathione reductase (GR), superoxide dismutase (SOD), ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), glutathione peroxidase (GPX), guaiacol peroxidase (GOPX) and glutathione-S-transferase (GST). In addition to antioxidant enzymes, non enzymatic antioxidants such as ascorbic acid (ASH), glutathione (GSH), phenolic compounds, alkaloids, non-protein amino acids, alpha- tocopherol and

**Table 1** Reaction catalyzed by major ROS-scavenging antioxidant enzymes

Enzymatic antioxidants	Reactions catalyzed
Superoxide dismutase (SOD) EC 1.15.1.1	$O_2^{\cdot-} + O_2^{\cdot-} + 2H^+ \rightarrow 2H_2O_2 + O_2$
Catalase (CAT) EC 1.11.1.6	$H_2O_2 \rightarrow H_2O + \frac{1}{2} O_2$
Ascorbate peroxidase (APX) EC 1.11.1.11	$H_2O_2 + AA \rightarrow 2H_2O + DHA$
Guaiacol peroxidase (GPX) EC 1.11.1.7	$H_2O_2 + GSH \rightarrow H_2O + GSSG$
Monodehydroascorbate reductase (MDHAR) EC 1.6.5.4	$MDHA + NAD(P)H \rightarrow AA + NAD(P)^+$
Dehydroascorbate reductase (DHAR) EC 1.8.5.1	$DHA + 2GSH \rightarrow AA + GSSG$
Glutathione reductase (GR) EC (1.8.1.7)	$GSSG + NAD(P)H \rightarrow 2GSH + NAD(P)^+$

Modified after: Gill and Tuteja 2010

carotenoids also play an essential role in scavenging free radicals (Jithesh et al. 2006; Gill and Tuteja 2010). Superoxide dismutase (SOD) metabolizes the oxygen ( $O_2$ ) radicals to



**Fig. 5** Schematic representation of antioxidant enzyme activity in response to salinity stress (Modified after Das 2013; Das et al. 2015; Chaudhary et al. 2015). Salinity stress imposes water stress which leads to the stomatal closure resulting in altered  $CO_2/O_2$  ratio. This hampers photosynthetic machinery and leads to the over-reduction of electron transport a response to decreased NADP/NADPH ratio and in turn to increased generation of reactive oxygen species. These ROS are scavenged by enzymatic and non-enzymatic antioxidants

hydrogen peroxide ( $H_2O_2$ ) which protect the plant cell from damage. Superoxide dismutase (SOD) is one of the important antioxidant enzymes essential in defense mechanism against free-radical toxicity by dismutation of superoxide radicals to produce  $H_2O_2$ . Under salt stress and abiotic stresses, catalases scavenging  $H_2O_2$  during  $\beta$ -oxidation of fatty acids and are also involved in photorespiratory functions. On the basis of the metal cofactor, SODs are categorized: Cu/Zn SODs have copper and zinc as their cofactors, Mn-SOD has manganese as their cofactors, Fe SOD has iron as their cofactors found in cytosol, chloroplasts, mitochondria, peroxisomes. Catalase is indispensable for ROS detoxification with the potential to directly convert  $H_2O_2$  into  $H_2O$  and  $O_2$ . CAT has highest turnover rates for all enzymes as one molecule of CAT can convert  $\sim 6$  million molecules of  $H_2O_2$  into  $H_2O$  and  $O_2$  per minute (Gill and Tuteja 2010). An increase in catalase activity has been shown as a measure of antioxidant defense in halophytes (Lokhande et al. 2013). The extent of decrease catalase activity under salinity stress is varied among CAT-1, CAT-2, CAT-3, and CAT-4. 45 % decrease in CAT-2 and 29 % loss in activity was observed for CAT-3 (Jithesh et al. 2006). Ascorbate peroxidases (APX) perform the same function as catalase, however, they catalyze removal of  $H_2O_2$  by using ascorbate as a reductant. It is important in the regulation of the intracellular level of  $H_2O_2$  in higher plants. APX is found in the different cellular compartments such as stroma and thylakoid, microbody including glyoxysomes and peroxisomes, cytosol and mitochondria. They are categorized as stromal APX (sAPX), thylakoid-membrane-bound APX (tAPX), microbody-membrane-bound APX (mAPX), cytosolic APX (cAPX), and mitochondrial-membrane-bound APX (mitAPX). Some researchers reported an increase in APX activity after salinity stress in plants like the pea, cotton, and rice. However in mangroves and other halophytes APX activity is restricted (Jithesh et al. 2006). It has also been reported that the increase in MDAR activity contribute towards increased tolerance against salt and osmotic stresses in transgenic tobacco. Ascorbic acid (ASH) is a water soluble antioxidant occurs in all plant tissues usually higher in photosynthetic cells and meristems (Gill and Tuteja 2010). Glutathione reductase (GR) enzyme catalyzes the NADPH-dependent reduction of oxidized glutathione. GR activity was shown to be high in leaves of salt-stressed plants as compared to control (de Azevedo Neto et al. 2006).

## Ion Homeostasis

Salinity effect is minimized by either restricted entry of salt into the plant or minimizing the  $Na^+$  concentration in the cytoplasm i.e. salt exclusion and or sequestration of  $Na^+$  inside the vacuole. Both glycophytes and halophytes cannot tolerate large amounts of salt in the cytoplasm. High concentration of  $Na^+$  in the soil also causes nutrient deficiency (Munns 2005; Maris and Blumwald 2007; Tuteja 2007a). Plants sense the salinity stress by ionic ( $Na^+$ ) and osmotic stress signals. Excess of  $Na^+$  ions can be sensed at the surface of plasma membrane through transmembrane protein or cell membrane protein or  $Na^+$  sensitive enzymes (Zhu 2003). In addition to this, the plasma membrane  $Na^+/H^+$  antiporter SOS1 has a transmembrane domain and long cytoplasmic tail may act as a  $Na^+$  sensor (Zhu 2003). Under saline stress condition entry of  $Na^+$  through non specific ion channels may cause the membrane depolarization which activates the  $Ca^{2+}$  channels and thus activates salt stress signals (Seki et al. 2002). Salinity stress up-regulates the biosynthesis of ABA (Xiong and Zhu 2003) and

reactive oxygenic species accumulation (Hernandez et al. 2001). Both ABA and reactive oxygenic species regulates the osmotic and ionic homeostasis and also the damage control and repair processes (Chinnusamy et al. 2005). Prevention of excess of  $\text{Na}^+$  entry and/or regulation of  $\text{K}^+$  uptake,  $\text{Na}^+$  efflux from the cell and  $\text{Na}^+$  utilization for osmotic adjustment are the commonly used strategies by plants to maintain the desirable cytosolic  $\text{K}^+/\text{Na}^+$  ratios (Zhu 2002). Under salt stress condition, osmotic adjustment begins either by  $\text{Na}^+$  compartmentalization into the vacuole or by accumulation of compatible solutes. Damage due to the salt stress is prevented by the detoxification of reactive oxygenic species and stress protein belonging to LEA protein family (Zhu 2002). Under salt stress condition  $\text{Na}^+$  enters into the root cell cytosol by cation channels or selective or non selective transporters and enters into the root xylem stream through an apoplastic pathway which varies with species (Chinnusamy et al. 2005). In *Arabidopsis* (Uozumi et al. 2000), it has been reported that high-affinity  $\text{K}^+$  transporters (HKT) act as low-affinity  $\text{Na}^+$  transporters under salinity stress (Gorham et al. 1997). In *Eucalyptus camaldulensis* HKT transporters are more permeable to  $\text{Na}^+$  as compared to the  $\text{K}^+$  when extracellular  $\text{Na}^+$  and  $\text{K}^+$  are of equal concentration (Liu et al. 2001). In transgenic wheat antisense expression of wheat *HKT1* resulted in reduced  $\text{Na}^+$  uptake and improved growth in response to salt stress than that of controlled plants (Laurie et al. 2002). The *AtHKT1;1* gene from *Arabidopsis* was up-regulated in rice root cortex which results in reduced shoot  $\text{Na}^+$  accumulation. Transgenic plant showed improved salt tolerance and also showed up-regulation and down-regulation of membrane transport genes including vacuolar  $\text{H}^+ - \text{pyrophosphatases}$  (Plett et al. 2010). In wheat, HKT transporters play a crucial role in  $\text{Na}^+$  exclusion and higher  $\text{K}^+/\text{Na}^+$  content in leaves under salt stress condition (James et al. 2011). In yeast, HAL1 regulates the  $\text{K}^+$  uptake and HAL3 regulates the  $\text{Na}^+$  efflux. In *Arabidopsis*, HAL3a gene overexpression enhanced transgenic plants tolerance to salinity stress (Espinosa-Ruiz et al. 1999). Similar results were obtained in transgenic tomato, the overexpression of HAL1 gene showed the enhanced level of  $\text{K}^+/\text{Na}^+$  ratio and improved salt tolerance as compared to the control plants. The plant also showed less reduction in fruit yield in comparison with the control plants under 35 mM NaCl stress (Rus et al. 2001). These research outcomes suggest that either suppression of low-affinity  $\text{Na}^+$  transporter activity or its inactivation could significantly improve the salt tolerance of plants. The  $\text{K}^+$  level can be maintained by the activity or expression of potassium-specific transporters or its expression. The activity of potassium-specific-transporters or its expression can maintain cellular potassium levels.

Excess of cytosolic  $\text{Na}^+$  accumulation and  $\text{Na}^+$  transport into the shoots is maintained by the  $\text{Na}^+$  efflux from the root. In *Arabidopsis* sos mutants plasma membrane  $\text{Na}^+/\text{H}^+$  antiporters, SOS1 play an essential role in  $\text{Na}^+$  efflux from the root epidermal cell. SOS carries the function of  $\text{Na}^+/\text{H}^+$  antiporters, on the plasma membrane, efflux of  $\text{Na}^+$  from the root cells and  $\text{Na}^+$  transport from root to shoot (Shi et al. 2000). In transgenic *Arabidopsis* plants, SOS1 overexpression reduces the  $\text{Na}^+$  in the shoot to that of wild-type plants. The transgenic plants exposed to 50–200 mM NaCl stress showed improved growth, ability to bolt, flowering and ultimately the salt tolerance as compared to the control plants (Shi et al. 2000). SOS3-SOS2 kinase complex regulates the  $\text{Na}^+$  efflux through SOS1 under salt stress condition. In transgenic *Arabidopsis* overexpression of an SOS2 overcome

the salt hypersensitivity of *sos2* and *sos3* mutants and improved the tolerance of plants to salt stress (Guo et al. 2004). In addition to this enhanced level of cytosolic  $\text{Ca}^{2+}$  under salt stress results in ABA accumulation which also regulates the SOS pathway (Zhu 2001). In Arabidopsis ABA up-regulate the *AtNHX1* gene which encodes the vacuolar  $\text{Na}^+/\text{H}^+$  exchanger (Shi and Zhu 2002). Under salt stress conditions, *AtNHX1* up-regulation in transgenic tomato showed higher vacuolar  $\text{K}^+$  without influencing vacuolar  $\text{Na}^+$  accumulation (Leidi et al. 2010). It is reviewed that *AtNHX1* is essential for vacuolar  $\text{K}^+$  transport hence stimulating  $\text{K}^+$  uptake by roots subsequently  $\text{K}^+$  ions recycle between cytoplasm and vacuole (Jiang et al. 2010). Plants maintains the osmotic balance by the synthesis of compatible solutes and vacuolar sequestration of  $\text{Na}^+$  ion under salt stress conditions. Sequestration of  $\text{Na}^+$  into the vacuole depends on the  $\text{Na}^+/\text{H}^+$  antiporters activity and phosphatases such as V-type  $\text{H}^+ - \text{ATP}$  and  $\text{H}^+ - \text{PPase}$  (Chinnusamy et al. 2005). In Arabidopsis *AVP1*, a vacuolar H-pyrophosphatase overexpression enhanced the  $\text{Na}^+$  sequestration into the vacuole results in higher relative water content in the leaves. In Arabidopsis  $\text{Na}^+/\text{H}^+$  antiporters *NHX1* gene is up-regulated by salt stress and ABA (Shi and Zhu 2002). In salt stressed transgenic tomato plants, *AtNHX1* overexpression showed salt tolerance and fruit production at 200 mM NaCl stress. Fruits quality and yield of transgenic plants exposed to salt stress was similar to non-treated control plants (Zhang and Blumwald 2001).

## Bioengineering for Improving Salinity Tolerance

In the past two decades, successful studies have demonstrated the potential of genetic engineering approach for developing the salt tolerant plants (Mantri et al. 2012; Cabello et al. 2014). Genetic transformation has been used to generate transgenic plants either by, transferring genes for salt stress related gene(s) to sensitive plants or altering the expression of existing genes (Agarwal et al. 2013). Several studies have validated the role of “single-function” gene(s) as well as transcription factor(s) in response to salt stress. The ‘single-function’ genes include osmolytes, transporters/channel proteins, antioxidative enzymes, lipid biosynthesis genes, polyamines, etc. whereas, regulatory genes constitute bZIP, DREB, MYC/MYB, and NAC factors (Agarwal et al. 2013). Salt tolerance trait is a complex trait and hence efforts to develop transgenic plants with single function or structural genes have made little success. In this regard, regulatory genes to improve stress tolerance can be useful as they are considered as master control modules in plant genomes. Some of the successful examples of plant genetic engineering for improving salinity tolerance are listed in Table 2. Enzymes catalyzing the scavenging of reactive oxygenic species (ROS) play an important role in tissue salt tolerance (Roy et al. 2014). Antioxidative defense in Arabidopsis improved tolerance to salinity and drought stress by the expression of alfalfa helicase MH1 (Luo et al. 2009). In Arabidopsis, higher CAT activity is improved due to the *AtWNK8* expression in root, hypocotyls and stamen. CAT activity in *wnk8* mutants was observed significant under NaCl and sorbitol stress conditions (Zhang et al. 2013). Enhanced APX activity and *OsAPX8* gene expression were



**Table 2** Transgenic plants developed for salt tolerance by over expression of signaling genes

Transferred gene	Gene source	Transformed plant	Plant performance	Reference
CBL4	Maize	Arabidopsis	Salt stress tolerance	Wang et al. (2007)
CBL5	Arabidopsis	Arabidopsis	Salt stress and dehydration tolerance	Cheong et al. (2010)
CIPK6	Chickpea	Tobacco	Salt tolerance	Tripathi et al. (2009)
MAPK5	Rice	Rice	Tolerance to salt ,drought, and cold stresses	Xiong and Yang (2003)
MAPK44	Rice	Rice	Salt stress tolerance	Jeong et al. (2006)
ZmMKK4	Maize	Arabidopsis	Salt stress tolerance	Kong et al. (2011)
AtCPK6	Arabidopsis	Arabidopsis	Salt and drought tolerance	Xu et al. (2010)
NDPK2	Arabidopsis	Arabidopsis	Salt, cold and oxidative stress tolerance	Moon et al. (2003)
SAPK4	Rice	Rice	Salt tolerance	Diedhiou et al. (2008)
ZmSAPK8	Maize	Arabidopsis	Salt tolerance	Ying et al. (2011)
GhMPK2	<i>Cotton</i>	Tobacco	Salt and drought tolerance	Zhang et al. (2011)
TaSnRK2.8	<i>Wheat</i>	Arabidopsis	Salt and drought tolerance	Zhang et al. (2010)
TaCIPK29	Wheat	Tobacco	Salt tolerance	Deng et al. (2013)
BrCIPK1	<i>Rape mustard</i>	Rice	Salt, cold and drought tolerance	Abdula et al. (2016)
SICIPK24	Tomato	Tomato	Salt tolerance	Huertas et al. (2012)
OsMSR2	Rice	Arabidopsis	Salt and drought tolerance	Xu et al., (2011)
MdCIPK6L	Apple	Tomato	Salt, drought and chilling stress	Wang et al. (2012)

reported in roots of rice treated at 150 mM and 200 mM NaCl. However, expression of OsAPX1, OsAPX2, OsAPX3, OsAPX4, OsAPX5, OsAPX6, and OsAPX7 was not observed (Hong et al. 2007).

### Signaling Transduction Related Genes

Considerable progress has been made to decipher the signaling related pathways genes involved in plant salt tolerance, and these have provided great interest for plant genetic engineering. Transfer or overexpression of signaling genes resulted in significant tolerance to salt stress in many plants (Table 1). Different signaling molecules, NO, and H<sub>2</sub>O<sub>2</sub> participate in the ABA-induced stomatal closure, expression of various genes and antioxidant enzyme activity (Zhang et al. 2006a). The enzyme 9-cis-epoxycarotenoid dioxygenase (NCED) is involved in biosynthesis of ABA in higher plants. In transgenic tobacco plants, SgNCED1 overexpression resulted in enhanced ABA accumulation (up to 51–77 %) in leaves which leads to reduced stomatal conductance, transpiration and photosynthesis rate and

increased SOD, CAT and APX activity under salt and drought stresses. Also, The signaling molecules, nitric acid and hydrogen peroxide accumulation was induced in transgenic plants under stress (Zhang et al. 2008). Tripathi et al. (2009) reported that overexpression of mutant Ca-CIPK6 from chickpea increased salt tolerance in transgenic tobacco. Overexpression of AtMPK3 and AtMPK6 activated by AtNDPK2 also resulted in increased salt tolerance which was linked with the reduction in the concentration of ROS in plants (Moon et al. 2003). In rice, SAPK4 overexpression under salt stress significantly improved the seed germination, plant growth, and development. In addition to this plant also showed enhanced photosynthesis and less accumulation of  $\text{Na}^+$  and  $\text{Cl}^-$  (Diedhiou et al. 2008). Activation of mitogen-activated protein (MAP) kinase cascade carries the translation of external stimuli to cellular responses. In transgenic rice plant, MAP kinases overexpression with OsMAPK5 and OsMAPK44 genes showed salt, drought and cold tolerance (Xiong and Yang 2003). It was observed that under salt stress MAP kinase overexpression led to induction of antioxidant genes and it also activated the TFs which play critical role in the control of the downstream genes. In transgenic plants, up-regulation of CBF1, CBF2, CBF3, STZ, and DREB2A transcriptional factors was observed which in turn improved the COR47, RD29A, and P5CS2 genes expression (Kong et al. 2011). From the literature, it is evident that the signaling genes are the key important component in salt tolerance by regulating the genes expression and transcriptional factors involved in salt tolerance.

## Regulatory Genes

Transcription factors (TFs), the master regulators of plant growth and development, are suitable candidates for plant genetic engineering for salt tolerance. These include DREB, NAC, MYB, MYC, Cys2His2 zinc finger, bZIP, AP2/ERF, and WRKY (Wang et al. 2016). Dehydration responsive element binding genes (DREBs) are one of the important transcriptional factors which regulate the stress-induced gene expression. In transgenic Arabidopsis DREB1B/CBF1 or DREB1A/CBF3 expression showed tolerance to salinity stress suggesting that DREBs/CBFs target multiple genes (Kasuga et al. 1999). Overexpression of OsDREB2A in transgenic rice plant improved the tolerance to saline stress conditions (Mallikarjuna et al. 2011). Oh et al. (2005) reported that in transgenic rice, CBF3/DREB1A overexpression under the 35S promoter showed enhanced salinity and drought tolerance without growth inhibition and phenotypic aberrations. Similarly, in transgenic tobacco, AhDREB1 gene overexpression resulted in its putative downstream target genes accumulation. The transgenic tobacco plant also showed better survival under salt stress condition in comparison with wild type of plants (Shen et al. 2003). WRKY proteins under salt stress condition participate in different physiological and developmental processes of plants such as seed size regulation, development of seed coat, leaf development and leaf senescence regulation. Research has been conducted on the role of WRKY genes in different plant species such as soybean, potato, barley, Arabidopsis, poplar, etc. (Chu et al. 2015). WRKY41 (GhWRKY41) was isolated from *Gossypium hirsutum* and transformed into *Nicotiana benthamiana*. In the transgenic plant, GhWRKY41 overexpression

resulted in enhanced tolerance to salt and drought stress which was associated with reduced Malondialdehyde content, regulation of stoma closure, enhanced antioxidant enzyme activity and up-regulation of antioxidant genes (Chu et al. 2015). The NAC family of the transcription factor has an applicable role for the generation of salt tolerant crops. Hu et al. (2008) isolated stress-responsive NAC gene (SNAC2) from upland rice and transferred it into japonica rice. In transgenic japonica rice, overexpression of SANC2 gene results in faster seedling and shoots growth under salt stress condition as compared to the wild type. Xiang et al. (2008) reported the overexpression of OsbZIP23, a member of the basic leucine zipper (bZIP) transcription factor in rice plant resulted in salt and drought tolerance. In Arabidopsis, transfer of wheat TaMYB2A showed tolerance to salt and drought stress conditions (Mao et al. 2011).

### Compatible Organic Solutes

Osmolytes are the low molecular weight organic compounds which protects cell machinery under environmental stress without interfering with cell metabolism (Suprasanna et al. 2016). These include proline, glycine betains, sugars, polyols, polyamines etc. Bioengineering of osmolytes biosynthesis related genes showed enhanced tolerance to abiotic stresses (Suprasanna et al. 2016). In transgenic rice plants, overexpression of P5CS resulted in significant proline accumulation which resulted in salt tolerance (Su and Wu 2004). The over expression of ZFP252 gene in rice resulted in enhancement of free proline and soluble sugars synthesis in response to salt and drought stresses (Xu et al. 2008). Similarly, in another transgenic experiment, rice plants containing chimeric for Ubi1:: TPSP expression significantly improved trehalose accumulation and enhanced tolerance to salt, drought and cold (Jang et al. 2003). In transgenic wheat plants, expression of the mtID improved the synthesis of the small amount of mannitol and tolerance to salinity and water stress (Abebe et al. 2003). Transformation experiments in bread wheat conducted with betA gene showed higher levels of glycine betaine and chlorophyll, lower  $\text{Na}^+/\text{K}^+$  and less cell membrane damage in transgenic seedlings exposed to 200 mM NaCl treatment (He et al. 2010). A CMO gene (AhCMO) from *Atriplex hortensis* introduced into the cotton showed high level of glycine betaine accumulation in the leaves i.e. up to 43 mmol/g FW (Zhang et al. 2009). The increased production of osmoprotectants could very well lead to tolerance to salt tolerance providing a feasible approach to genetically engineer tolerant plants.

### Enhanced Antioxidant Capacitance

Transgenic Arabidopsis plants showed higher salt tolerance by the over expression of a Mn-SOD and significant higher activities of Cu/Zn-SOD, Fe-SOD, CAT and POD as compared to control (Wang et al. 2004). Lu et al. (2007) reported enhanced salt tolerance due to the over-expressing OsAPXa or OsAPXb in transgenic Arabidopsis. However, over expression of OsAPXb was more significant for the APX activity in transgenic plants. Eltayeb et al. (2007) reported the MDAR over expression in transgenic tobacco in response to salt and osmotic stress. In Arabidopsis, alfalfa helicase MH1 expression improved tolerance to salinity and

drought by enhancing the antioxidative defense (Luo et al. 2009). Some of the transgenic plants have been produced by genetic engineering of methylglyoxal (cytotoxic compound) pathway. In many transgenic plants, GlyI and GlyII gene expression for glyoxalase I and glyoxalase II enzymes have been reported for salinity tolerance (Singla-Pareek et al. 2008). In the transgenic rice plants overexpression of the *katE* gene was found to be significant for improved growth under saline condition. The transgenic rice T1 seedlings were soaked in 0, 50, 100, 150, 200, 250, 300, 400, 500 or 600 mM NaCl and examined for growth and CAT activity. The transgenic seedlings able to grow under high salinity (250 mM) and produce seeds in the presence of 100 mM NaCl. It was also reported that CAT activity was 1.5- to 2.5- fold higher in the transgenic rice plants as compared to the non transgenic rice plants (Nagamiya et al. 2007). In the transgenic *Arabidopsis thaliana*, the decrease in lipid peroxidation occurs as a result of PutAPX gene over expression in plants treated with 150 and 175 mM NaCl (Guan et al. 2015). Thus genetic engineering efforts for increased antioxidant capacitance have resulted in transgenic plants for improved stress tolerance as well as higher levels of antioxidants providing better nutritional quality.

### **Ion Transporters**

Salinity tolerance is determined by the ion transport which is central to survival under saline conditions. Net  $\text{Na}^+$  uptake in the roots is mediated by antiporters or ion channel-type transporters (HKT, LCT1 and NSCC). The reduction of  $\text{Na}^+$  uptake in the root cell might be associated with the reduction in activity or numbers of these transporters (Peleg et al. 2011). In transgenic wheat, the reduction of  $\text{Na}^+$  into the root cell was shown to be accomplished by decrease in TaHKT2; 1 expression under saline stress condition (Laurie et al. 2002).  $\text{Na}^+$  efflux from the root cell was mediated by membrane  $\text{Na}^+/\text{H}^+$  antiporters. In the transgenic *Arabidopsis* plants, the overexpression of AtSOS1 significantly improved the ability of plant to grow at high saline condition (Shi et al. 2003). Osmotic balance in the cell is maintained by the accumulation of  $\text{Na}^+$  ions into the vacuole mediated by vacuolar  $\text{Na}^+/\text{H}^+$  antiporter (Peleg et al. 2011). Ohta et al. (2002) reported that orthologue gene (*AgNHX1*) from *Atriplex gmelini* conferred improved salt tolerance in transgenic rice plants. Transformation of AtNHX3 from *Arabidopsis* in sugar beet resulted in significant  $\text{Na}^+$  accumulation in the leaves and also the soluble sugar content in response to salinity stress (Liu et al. 2008a). In transgenic alfalfa, overexpression of vacuolar  $\text{H}^+$  – pyrophosphatase gene, AVP1 from *Arabidopsis* showed increased shoot dry weight under saline stress conditions (Bao et al. 2009). In transgenic tomato, expression of HAL1 gene showed improved fruit production facilitated due to the  $\text{K}^+/\text{Na}^+$  selectivity under salt stress (Rus et al. 2001). The *Arabidopsis*  $\text{Na}^+/\text{H}^+$  antiporter gene was overexpressed in wheat and the transgenic plants were evaluated in salinized field (ECe 10.6 and 13.7  $\text{dS m}^{-1}$ ) conditions. They found that the transgenic plants produced heavier and larger number of grains (Xue et al. 2004). The evaluation of most of the transgenic plants, as mentioned above, is restricted to lab level and green house conditions. It is essential that such success should be translated into field level studies to realize the ultimate aim of growing plants under salinized agricultural lands.

## Hormone Genes

Phytohormones are the regulatory molecules produced by plants in very low concentration. These molecules act as chemical messengers and play key role in signalling mechanism under stress conditions. They include abscisic acid, auxin, cytokinins, ethylene, zeatin, brassinosteroids, gibberellic acid, jasmonic acid, salicylic acid and strigolactone (Wani et al. 2016). Hormones are good targets for manipulation but the maintenance of their level is very important. Their overexpression may result into adverse effects (Peleg et al. 2011). In the transgenic Arabidopsis, overexpression of AtHD2C was associated with enhanced expression of RD29B and RAB18 (ABA-responsive LEA-like genes) which resulted in tolerance of the transgenic plant to salt stress (Sridha and Wu 2006). SgNCED1 gene from *Stylosanthes guianensis* transferred to the tobacco plant was shown with ABA accumulation in the leaf which significantly improved the salt tolerance (Zhang et al. 2008). In transgenic Arabidopsis, AtZEP overexpression conferred salt and drought tolerance with increased plant water content and ABA accumulation. Improved stress resistance was associated with the increased ABA level which resulted in significant expression of ABA-responsive genes (Park et al. 2008). NTHK1 gene of type II ETHY receptor homolog from tobacco was transferred into Arabidopsis results in expression of NTHK1 mRNA and protein. In the transgenic plants increased electrolyte leakage, and reduced root growth was observed under salt stress condition showing the negative effect exerted by ETHY on its receptors (Cao et al. 2007). The transgenic *Triticum aestivum* TaAOC1 gene (encoding an AOC enzyme) expression in Arabidopsis enhanced JA levels and tolerance to salinity stress suggesting that JAs regulate salt tolerance. Furthermore, salt tolerant phenotypes showed TaAOC1 occurrence independently from ABA (Zhao et al. 2014). Overexpression of wheat TaOPR1 gene encoding OPR1 in Arabidopsis promotes the salt tolerance through detoxification of reactive oxygen species. TaAOC1 carrying the salt tolerance which was ABA-independent, in contrast, TaAOC1 promotes ABA-dependent salt tolerance (Dong et al. 2013). Jasmonate enhanced the antioxidant enzyme SOD, POD, CAT, and APX activity in wheat as a salt tolerance response (Qiu et al. 2014). In the transgenic tomato, plant hormone promotes the production of jasmonate and also salt tolerance in an ABA-dependent manner (Orsini et al. 2010).

## MicroRNAs

MicroRNAs are the small 18–22 nucleotide endogenous non coding regulatory RNAs that regulate gene expression at post transcriptional level by mediating mRNA degradation or at translational level by translational repression in sequence specific manner. They are involved in various biological processes like plant growth, development, differentiation, signal transduction and stress response (Macovei et al. 2012). miRNAs play a central role in regulating gene expression in both plant development and stress response pathways (Zhang 2015). Recent reports suggests that miRNAs regulate expression of various genes under abiotic stresses and could be a sound target for genetic improvement of crops against certain abiotic stresses (Zhang 2015; Shriram et al. 2016). There are excellent reviews on the role of miRNA in both biotic and abiotic stress (Sunkar and Zhu 2004; Khraiweh et al. 2012). The *miR319* gene, *Osa-*

*miR319a* from rice was transformed into *Agrostis stolonifera*. Overexpression of *Osa-miR319a* in transgenic plants showed tolerance to salinity and drought stress which were associated with enhanced leaf wax content, water retention and reduced  $\text{Na}^+$  accrual. In transgenic plants gene expression analysis showed that putative *miR319* target genes (*AsPCF5*, *AsPCF6*, *AsPCF8*, and *AsTCP14*) were down-regulated which was associated with enhanced stress tolerance and plant development (Zhou et al. 2013). The transgenic miRNA 402 lines of Arabidopsis showed better seed germination and seedling growth under salt stress which shows miR402 regulates salt stress tolerance in positive manner (Kim et al. 2010). The overexpression of *osa-MIR396c*, *osa-MIR393*, *miRNA417* in Arabidopsis or rice or in both showed negative effects on seed germination and growth under salt stress as compared to wild type plants which shows these miRNAs regulates negatively under salt stress (Gao et al. 2011).

## Genome Editing Tools

Due to extensive genomic research, plethora of information is available about stress tolerance in plants. Role of different genes, small RNAs, proteins under stress conditions is well defined. Applications of these genes in improving salt tolerance and enhancement of crop yield is successful up to some extent (Mickelbart et al. 2015). This has been done using classical biotechnological methods like cloning, over expression, screening, crossing which are laborious and time consuming and also they lacks precision. To overcome these problems novel gene editing tools were introduced. These tools can introduce targeted mutation, insertion or deletion and precise sequence modification in range of organisms. Currently, Zinc finger nuclease (ZFNs), Transcriptional activator-like effector nuclease (TALENs) and Clustered regularly interspaced short palindromic repeat (CRISPR)-Cas 9 (CRISPR- associated nuclease are available for gene editing (Jain 2015). Out of these three techniques, with CRISPR-Cas9 technique nearly any sequence in the genome can be manipulated to understand its function (Kumar and Jain 2015). The ease in designing and efficiency of sgRNAs enabled simultaneous multigene manipulation which can be useful in understanding complex network of genes responsible for stress response in plants. This type of multiplex genome editing has been done in Arabidopsis, Tobacco and Rice (Zhou et al. 2013). Although the CRISPR- Cas9 technique is yet to be implemented in engineering for salt tolerance in plants, in near future it will become a powerful technique in stress improving tolerance in plants.

## Salt-Tolerant Genes from Halophytes for Developing Salt Tolerant Glycophytes

Depending on growth performance of the plants in the saline environment, plants are grouped either as salt-tolerant 'halophytes' or salt-sensitive 'glycophytes'. Halophytic plants can grow in wide variety of saline environment ranging from coastal marshes to inland deserts (Flowers and Colmer 2008). In case of monocot, halophyte, optimum growth is achieved around 50 mM NaCl whereas as for dicots,

better growth is seen around 100–200 mM NaCl (Flowers and Colmer 2008). However in glycophytes, plant growth is affected adversely under saline habitat and even plants do not show survival at 100–200 mM of salt (Flowers and Colmer 2008; Shabala 2013). A number of researchers have studied the salt tolerant responsive gene in various halophytic plants (Xiong and Zhu 2002). In the last few years, the promising approach involved the introduction of salt tolerance responsive genes from halophytes for the development of salt tolerance in glycophytes via genetic engineering methods (Bose et al. 2014). The genes from halophytes have been transferred into different model plants like Arabidopsis, tobacco and few crop species for the improvement in ion homeostasis and salt tolerance levels (Table 3). The majority of the research has been conducted for the improvements in ion homeostasis and salt tolerance in transgenic glycophytes under controlled conditions, however, field-level success is yet to be achieved (Himabindu et al. 2016).

## Future Perspectives

Plant tolerance to salinity involves adaptive responses at cellular, physiological, molecular and whole plant level. Considerable work has been done on the salt tolerant mechanisms controlling ion homeostasis, osmoregulation, detoxification, hormone metabolism and stress signaling (Gupta and Huang 2014). However, in spite of significant research on plant salt-tolerant mechanisms, limited success has been achieved due to the lack of detailed information from genomics, transcriptomic, proteomic studies and more so, from the integrated picture emerging from these disciplines (Cabello et al. 2014). Developing salt-tolerant crops using plant genetic engineering methods has offered great promise. Salt tolerance trait is multigenic in nature but transgenic cultivars developed are in most cases have a single transgene and improvement in salt tolerance is shown in terms of seed germination, plant survival, significant biomass production and osmolytes synthesis when experiments were conducted under controlled conditions (Ashraf and Akram 2009). In spite of the success achieved, the development of transgenic salt tolerant cultivars needs to have a holistic approach with regulation occurring at different metabolic processes. Despite the progress in development of salt tolerance, several questions related to salt tolerance are being addressed using molecular marker development for gene mapping, EST libraries development, complete sequence of plant genomes for Arabidopsis, rice and maize and also the development of several forward genetics tools that can be used in gene function analysis (Colbert et al. 2001). Understanding the mechanism and regulation of gene expression and use of candidate genes, tissue-specific or inducible promoters will pave the way for genetic engineering for developing salt tolerant cultivars.

The advances in high-throughput small RNA sequencing technologies and identification of miRNAs has led to the finding of a new class of regulatory RNAs called isomiRs, which are canonical variants of miRNAs (Sablok et al. 2015). It has been suggested that isomiRs play a role in regulating the miRNAome in stress-induced gene regulation. Advances in genomic and metabolomic analyses using model systems and crop plants should facilitate the resolution of complex networks and unravelling of the additional mechanism(s) of stress tolerance. Plant genetic engineering methods have

**Table 3** Plant genetic transformation for salt tolerance and specific expressed trait conferring salt tolerance

Gene	Gene source	Transgenic plant	Functions/ Response of transgenic plant	Reference
ThSOS1	<i>Thehungiella halophila</i>	Arabidopsis	Silencing of ThNHX1 leads	Oh et al. (2009)
PNHAI	<i>Puccinellia tenuiflora</i>	Arabidopsis	Low Na <sup>+</sup> accumulation, High K <sup>+</sup> level and K <sup>+</sup> /Na <sup>+</sup> ratio, Na <sup>+</sup> extrusion from root.	Wang et al. (2011)
SbSOS1	<i>Salicornia brachiata</i>	Tobacco	salt tolerance, decreased cytosolic Na <sup>+</sup> , reduced oxidative damage	Yadav et al. (2012)
PSOS2	<i>Populus trichocarpa</i>	Hybrid Poplar	Enhanced plasma membrane Na <sup>+</sup> exclusion, scavenging of ROS	Yang et al. (2015)
SsNHX1	<i>Suaeda salsa</i>	Rice	exhibits salt tolerance	Zhao et al. (2006)
AgNHX1	<i>Atriplex gmelini</i>	Rice	Higher vacuolar antiporter activity	Ohta et al. (2002)
AeNHX1	<i>Agropyron elongatum</i>	Arabidopsis and Festuca	improved salt tolerance, osmotic adjustment, photosynthesis	Qiao et al. (2007)
ThNHX1	<i>Thehungiella halophila</i>	Yeast and Arabidopsis	Partially complemented yeast mutant, Arabidopsis exhibits salt tolerance	Wu et al. (2009)
AINHX	<i>Aeluropus littoralis</i>	Tobacco and Soybean	In tobacco K <sup>+</sup> /Na <sup>+</sup> ratio maintained in leaves, more Na <sup>+</sup> accumulates in roots. In Soybean high K <sup>+</sup> and Low Na <sup>+</sup> accumulates in leaves and improved growth	Liu et al. (2014)
SaNHX1	<i>Spartina anglica</i>	Rice	Enhanced salt tolerance	Lan et al. (2011)
HcNHX1	<i>Halostachys caspica</i>	Arabidopsis	salt tolerance and higher Na <sup>+</sup> accumulation in leaves	Guan et al. (2011)
PNHAI and PutNHX	<i>Puccinellia tenuiflora</i>	Rice	Shoots were more tolerant to NaCl	Kobayashi et al. (2012)
SbNHX1	<i>Salicornia brachiata</i>	Tobacco and Jatropha	salt tolerance up to 200 mM NaCl, improved growth	Jha et al. (2011a) ; Jha et al. (2013)
ScNHX1 and ScVP	<i>Suaeda corniculata</i>	Alfalfa	Improved salt and saline alkali tolerance, more Na <sup>+</sup> accumulates in leaves and roots	Liu et al. (2013)
LfNHX1	<i>Leptochloa fusca</i>	Tobacco	exhibits salt tolerance, Better germination and root growth	Rauf et al. (2014)
TsVP	<i>Thehungiella halophila</i>	Yeast, Tobacco and Cotton	suppressed Na <sup>+</sup> hypersensitivity in yeast mutant, improved salt tolerance in tobacco. Improve growth and photosynthetic performance	Gao et al. (2006) and Lv et al. (2008)
SsVP	<i>Suaeda salsa</i>	Arabidopsis	increased activity of V-Pase, improved salt and drought tolerance	Guo et al. (2006)
ScVP	<i>Suaeda corniculata</i>	Suaeda corniculata	more Na <sup>+</sup> accumulation in leaves, improved salt and drought tolerance	Liu et al. (2011)
KfVP1	<i>Kalidium foliatum</i>	Arabidopsis	Improved drought and salt tolerance, Leaves accumulates more Na <sup>+</sup>	Yao et al. (2012)



Table 3 (continued)

Gene	Gene source	Transgenic plant	Functions/ Response of transgenic plant	Reference
ZmVP1	<i>Zoysia matrella</i>	Yeast and Arabidopsis	restore the salt tolerance in salt-sensitive yeast, sodium sequestration in vacuole of Arabidopsis	Chen et al. (2015)
HcVP1 and HcVHA-B	<i>Halostachys caspica</i>	Arabidopsis	increased seed germination and improved growth	Hu et al. (2012)
SaVHAc1	<i>Spartina alterniflora</i>	Rice	improved salt tolerance, up regulated genes involved in cation transport and ABA signalling	Baisakh et al. (2012)
PhaHAK2	<i>Phragmites australis</i> Reed plant	Reed plant	maintenance of high K <sup>+</sup> /Na <sup>+</sup> ratio, salt tolerance	Takahashi et al. (2007)
PutHKT2;1	<i>Puccinellia tenuiflora</i>	Arabidopsis and Yeast	high affinity K <sup>+</sup> /Na <sup>+</sup> symport function in yeast, increased sensitivities to Na <sup>+</sup> , K <sup>+</sup> and Li <sup>+</sup> and in transgenic Arabidopsis	Ardie et al. (2009)
PutAKT1	<i>Puccinellia tenuiflora</i>	Arabidopsis	increased K <sup>+</sup> content, decreased Na <sup>+</sup> content, improved salt tolerance	Ardie et al. (2010)
TsHKT1;2	<i>Theilungia halophila</i>	Yeast mutants and Arabidopsis	Yeast mutants lacking Na <sup>+</sup> , K <sup>+</sup> transporters exhibited strong K <sup>+</sup> transporter activity	Ali et al. (2012)
AhProT1	<i>Atriplex hortensis</i>	Arabidopsis	transgenic plants survive up to 200 mM NaCl with increased biomass	Guo et al. (2002)
PINO1 or PeINO1	<i>Porteresia coarctata</i>	Tobacco, E.coli, S. pombe, rice and mustard	Transgenic tobacco plants were able to grow in 200–300 mM NaCl, conferred salt tolerance	Das-Chatterjee et al. (2006)
SalNO1	<i>Spartina alterniflora</i>	Arabidopsis	transgenic showed salt tolerance during germination and seedling growth and development	Joshi et al. (2013)
SbpAPX	<i>Salicornia brachiata</i>	Peanut & Tobacco	Transgenic showed significant tolerance at 150 mM NaCl, improved growth and germination rate	Singh et al. (2014) and Tiwari et al. (2014)
TaMnSOD	<i>Tamarix androssowii</i>	Poplar	Transgenic lines showed salt tolerance, enhanced SOD activity	Wang et al. (2010)
AmMDAR	<i>Avicennia marina</i>	Tobacco	Transgenic lines showed enhanced APX activity, tolerance to oxidative stress	Kavitha et al. (2010)
SbGSTU	<i>Salicornia brachiata</i>	Tobacco	Transgenic lines showed significant seed germination and growth under salt stress	Jha et al. (2011b)

Modified after: Himabindu et al. (2016)

greatly contributed to the improvement of abiotic stress tolerance, however, overexpression of regulatory elements or effectors are frequently associated with growth limitation and yield penalties due to crosstalk between developmental and stress-response networks (Cabello et al. 2014). In this regard, it is desirable to explore candidate genes that can provide stress tolerance without compromising on growth and yield (Rai et al. 2016). In this study authors reported that by using *Brassica juncea* early responsive to dehydration gene (BjERD4), transgenic Arabidopsis plants showed vigorous plant growth and yield (pod size) and tolerance to salt and osmotic stress, suggesting that such candidate genes can be of interest in developing transgenic crops.

The majority of the experiments on salt tolerance in transgenic plants has been conducted using limited numbers of plants and/or mature plants under controlled laboratory conditions. In some of the cases, the experiments were carried out in greenhouse conditions mimicking conditions that prevail in high-salinity soils (Yamaguchi et al. 2005; Zhang et al. 2011). Hence, it is necessary to evaluate the salt tolerance of the plants under field condition. More essentially salt tolerance needs to be evaluated for yield performance under variable salt levels in field conditions and other environmental factors (Daniells et al. 2001). The conventional breeding programs have not much improved salt tolerance, whereas wide crossing generally reduced yield (Flowers and Flowers 2005). For developing the salt tolerant plants, Turan et al. (2012) suggested that there is a need to consider criteria such as (i) how the transgenic plants response to salt stress conditions in fluctuating natural environment, (ii) response of transgenic plants at different growth stages such at seed germination or reproductive stage, (iii) plant growth, photosynthetic performance and nitrogen use efficiency (NUE) under saline field conditions (iv) transgenic plant yield potential and (v) disease vulnerability and resistance.

## Summary

Plants growth is affected adversely due to the osmotic and ionic stresses induced by salinity. Tolerance to salt stress is a very complex phenomenon which is accomplished by through the adaptive physiological, biochemical and molecular mechanisms. There has been much research on understanding the physiological, biochemical, and genetic basis for salt tolerance. Adaptive mechanisms such as osmotic adjustment, Na<sup>+</sup> exclusion or accumulation, synthesis of compatible solutes, changes in photosynthetic pathways, induction of antioxidant enzyme activity and plant growth regulators play a significant role. Changes in gene expression under stress conditions are evident as markers of associated traits. In recent years, many candidate genes have been identified and used to facilitate the genetic engineering efforts for the development of stress tolerant plants. This includes gene involved in transcription and regulatory factors, osmotic adjustment, phytohormone and oxidative stress related, molecular chaperones, transporters, and various metabolic genes. Salt stress tolerance for most of these genes has been evaluated using the model plants such as Arabidopsis or tobacco and some crop (rice, wheat, barley, maize). Emphasis must be given on the choice of the promoter to drive transgene expression as promoters lead to an abnormal expression of a transgene, possibly causing adverse effects on crop productivity (Roy et al. 2014). Progress has been made towards the understanding the molecular

mechanisms of salt tolerance after the identification of the plasma membrane and vacuolar  $\text{Na}^+/\text{H}^+$  antiporters SOS1 and AtNHX1, the  $\text{Na}^+$  transporter AtHKT1, and the vacuolar  $\text{H}^+$ -PPase AVP1 from Arabidopsis. These transporters play an essential role in cellular mechanisms for salt tolerance and the manipulating these transporters could further useful to improve salt tolerance (Yamaguchi et al. 2005). The available information on salt tolerance mechanisms in halophyte genes provides a framework for future studies in the improvement of salt tolerance in glycophytes. Currently transgenic expression studies in glycophytes are being conducted using the halophyte genes (Table 3). However, extensive transcriptomic, proteomic and metabolomic comparisons are required to identify better candidate genes for crop improvement (Himabindu et al. 2016). Although, it is evident that overexpression of a single gene could also impart salt tolerance to plants but multiple gene insertions are necessary to meet the requirements to obtain insights into these mechanisms (Travella et al. 2005). Research efforts should be directed towards understanding stress perception, signaling, transcriptional factors, metabolic pathways and genes and proteins associated with salt stress responses. Such knowledge generation will be crucial for genetic engineering of salt tolerance in crop plants.

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