Chapter 8
Genomic Roadmaps for Augmenting Salinity Stress Tolerance in Crop Plants

P. Suprasanna, S. A. Ghuge, V. Y. Patade, S. J. Mirajkar, and G. C. Nikalje

Abstract Serious antagonistic impacts of saline environment on plant growth, development, and yield are well established. In this regard, researchers and breeders have been utilizing many conventional as well as modern approaches to aid the process of developing salt-tolerant crops. Biotechnological tools have made the task of engineering salinity tolerance in plants easier. Currently, two major annexes are effectively employed to develop salt-tolerant crops, first, investigation of genetic variation via marker-assisted selection (MAS) and second the transgenic technology. Sustenance of plants under dynamically growth-limiting saline environment depends on alterations and/or switching between multiple biochemical pathways involved in response. A number of key regulatory genes have been successfully identified and characterized in this context which can be explored to serve the purpose of alleviation in salt-tolerant nature of plants. Several genomics-abetted approaches have been reported aiming toward improvement in growth and yield of crops under saline environment. Present chapter focuses on genomic roadmaps for augmentation of crop salt tolerance by various methods including MAS, transgenic breeding, manipulations in small non-coding RNAs, and genome editing. These approaches utilize key players involved in salinity-mediated plant defense mechanisms, such as ion transporters,

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osmolytes, antioxidants, transcription factors, signaling proteins, and microRNA. The chapter attempts to summarize the effective targets and exploration of these key entities to raise salt-tolerant plants through various genomics-related tools.

Keywords Marker assisted selection · Ion transporters · Osmolytes · Antioxidants · Transcription factors · microRNA · Transgenics

Abbreviations

AFLP	Amplified fragment length polymorphisms
AOX	Alternate oxidase
APX	Ascorbate peroxidase
AtNHX1	Na+/H+ antiporter
CaM	calmodulin
CAT	Catalase
CBL	Calcineurin B-like proteins
CDPKs	Calcium-dependent protein kinases
CML	CaM-related proteins
GPX	Glutathione peroxidase
ILs	Introgression lines
MAS	Marker-assisted selection
MQTL	Meta-QTL
mt1D	Mannitol-1-phosphate dehydrogenase
P5CS	delta1-pyrroline-5-carboxylate synthetase
QTL	Quantitative trait loci
RAPD	Random amplified polymorphic DNA
RFLP	Restriction fragment length polymorphisms
RNAi	RNA interference
SNPs	Single nucleotide polymorphisms
SOD	Superoxide dismutase
SOS	Salt overly sensitive
SSR	Simple sequence repeats
STMS	Sequence-tagged microsatellite site
TFs	Transcription factors
TPSP	Trehalose-6-phosphate synthase/phosphatase

8.1 Introduction

Among the abiotic stresses, salinity stress is one of the most important environmental factors which considerably affect plant growth and productivity. Salinity affects about one third of the world's irrigated land (Munns and Tester 2008), and it negatively influences water and nutrient homeostasis within living tissues. The deleterious effects on agricultural crops primarily include growth reduction and yield loss. In this context, both the conventional and modern crop improvement approaches are employed to facilitate development of novel genetic resources for use in direct or indirect breeding for improving salinity tolerance in crop plants. Currently, a wide range of mutational, biotechnological, and genomics-assisted tools are available which are more or less focused on gene discovery and boosting up the process of novel gene introduction or modification (Nongpiur et al. 2016).

Apparently, two main approaches are used to improve and impart salinity tolerance in crop plants. The first is through exploring natural genetic variation, either through selection under stress conditions or through quantitative trait loci (QTL) followed by marker-assisted selection (MAS). The other one is through transgenic technology by modifying the expression of endogenous genes or introducing novel genes (of plant or non-plant origin) to impart stress tolerance. Crop improvement via conventional breeding approaches has yielded limited success due to complexity of the trait since the process is time and labor intensive and requires well-characterized germplasm. In this regard, genetic engineering methods have become useful to develop transgenic crops tolerant to abiotic stresses (Yamaguchi and Blumwald 2005). The primary step before proceeding to make transgenics is the identification of functional and regulator genes serving to control different metabolic pathways, including ion homeostasis, antioxidant defense system, osmolyte synthesis, and other signaling pathways.

Salt stress increases ion toxicity and also affects uptake and movement of other essential nutrients such as potassium in the cell. This may occur either in a monophasic or biphasic manner depending on the duration and extent of exposure to saline conditions. A short exposure usually leads to osmotic or oxidative stress which would be followed by ionic stress upon long-term exposure (Munns and Tester 2008). To sustain under such dynamic growth-limiting situations, plants need to incur switching between multiple biochemical pathways that are much more complex when combined with other biotic and abiotic stresses. A general view of plant responses to salinity stress is presented in Fig. 8.1.

Significant progress has been made in the identification of genes involved in plant salt-stress responses (Hanin et al. 2016). Till date, a number of key genes involved in salinity tolerance have been isolated, characterized, and validated by using different transgenic methods. The candidate genes for salt tolerance are categorized into genes with functional and regulatory role (Shinozaki et al. 2003). The first group includes those involved in osmolyte biosynthesis, ion transporters, water channels, antioxidant systems, sugars, polyamines, heat shock proteins, and late embryogenesis abundant proteins. The second group are involved in the regulation of transcriptional and posttranscriptional machinery besides genes of signaling pathways. Some of these are transcription factors (TFs), protein kinases and phosphatases. In addition, there are several other strategies for attaining abiotic stress tolerance which are being tested for salt tolerance such as, using the stress-inducible promoters to avoid the pleiotropic effects (Checker et al. 2012), employing the pro-

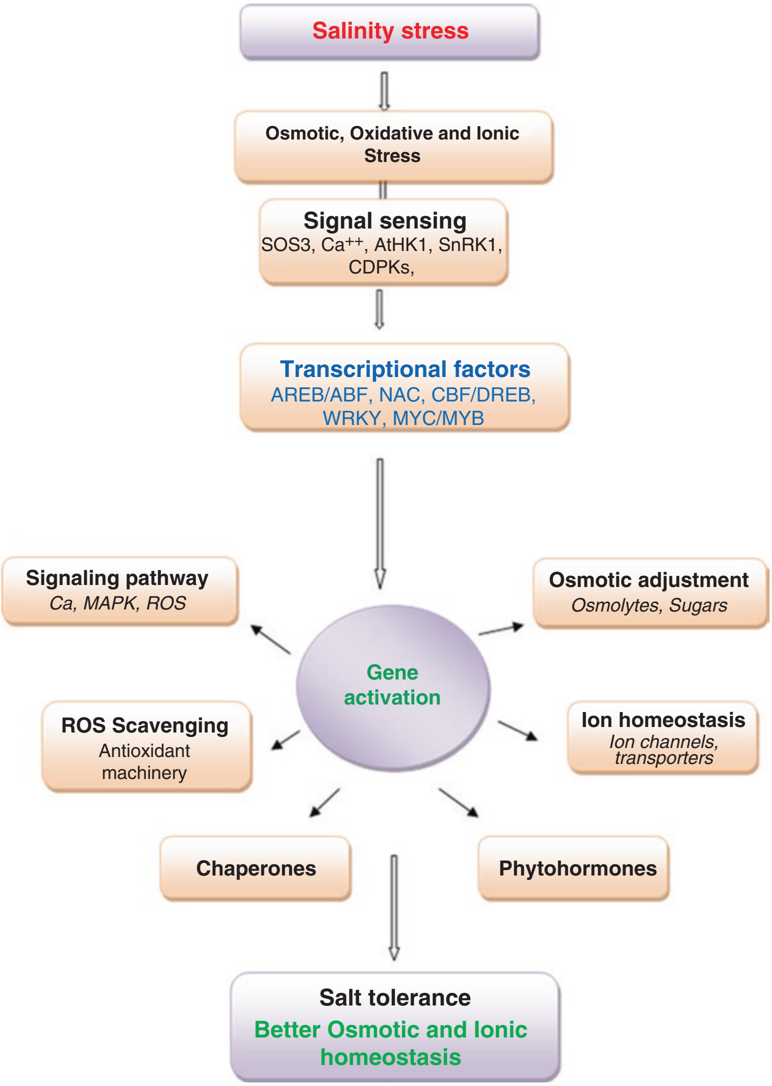

Fig. 8.1 Mechanistic view of plant responses to salinity stress

tein post-translational modifications such as ubiquitination (Lyzenga and Stone 2012; Guo et al. 2008) and the use of halophyte gene resources.

Several state-of-the-art genomics-assisted approaches (Fig. 8.2), such as transgenic overexpression, RNAi, microRNA, genome editing, and genome-wide association studies, are being used for improving salt tolerance in crop plants (Mickelbart et al. 2015; Nongpiur et al. 2016). Overexpression of these genes has been shown as a successful strategy to improve plant tolerance to different abiotic stresses including salinity (Türkan and Demiral 2009; Cominelli et al. 2013; Hanin et al. 2016). In

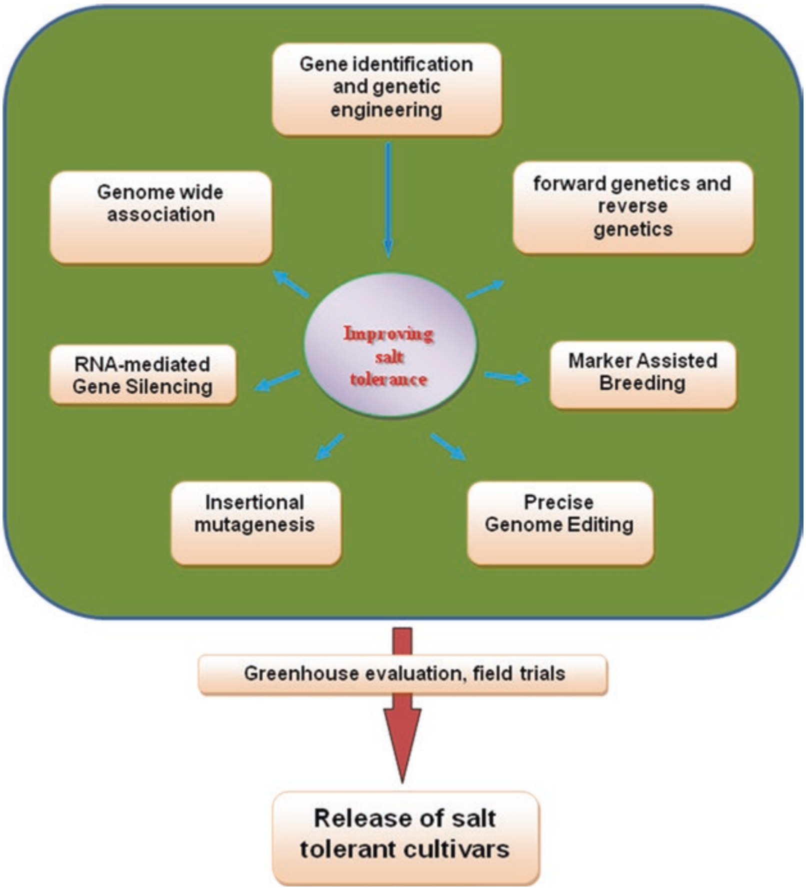

Fig. 8.2 Genomics-based roadmaps for improving plant salt tolerance

this article, we present an overview of the different genomics-based molecular genetic approaches (marker-assisted selection and transgenic breeding) that have contributed to improve the salt tolerance in crop plants.

8.2 Marker-Assisted Selection for Enhancement of Crop Salinity Stress Tolerance

Despite of availability of broad genetic resources, the slow progress in the genetic improvement for salt tolerance through conventional breeding is attributed to the complex nature of the trait accompanied with the high environmental influence and requirement of huge experimental fields (Flowers and Yeo 1997; Munns 2002; Thomson et al. 2010; Munns and Tester 2008). Further, phenotypic screening for salinity tolerance in the large sample population has remained a big challenge through the laborious conventional techniques (Mantri et al. 2014).

Marker-assisted selection (MAS) is a new precision breeding tool that allows the indirect and accurate selection for a desired trait from breeding population based on tightly linked molecular markers, viz., restriction fragment length polymorphisms (RFLP), amplified fragment length polymorphisms (AFLP), random amplified polymorphic DNA (RAPD), simple sequence repeats (SSR) or microsatellites, sequence-tagged microsatellite site (STMS), single nucleotide polymorphisms (SNPs), etc. It enables rapid and accurate screening for complex and polygenic traits which are difficult to score phenotypically through mapping or tagging of the trait linked quantitative trait loci (QTL). The successful applications of molecular marker-assisted breeding have proved its enhanced efficiency and accuracy in improved biotic and abiotic stress tolerance in rice and several other important crops (Singh et al. 2012; Ellur et al. 2016; Babu et al. 2017a, b). MAS offers advantage over the other genetic improvement tools as having relaxed biosafety regulations at development, field testing, commercial release, and import/export of the developed improved genotypes as well as their wider public acceptance.

Among field crops, rice being an important global staple food crop, considerable progress has been made for molecular breeding for improvement in tolerance to abiotic stresses such as salinity stress (Table 8.1). Through rigorous research on molecular breeding programs, the molecular marker maps for important agricultural crops have been constructed with varying density among the species. For development of molecular breeding tools, sources for abiotic stress tolerance have been identified through rigorous screening of genotypes in various crops. Ravikiran et al. (2017) identified two rice genotypes, CST 7–1 and Arvattelu as source for seedling-stage salinity tolerance, based on screening of 192 diverse genotypes under salinity stress (EC ~ 12 dS m^{-1}) using morphophysiological markers. Screening of the genotypes with SSR markers associated with Saltol region on chromosome 1 revealed RM 493 and RM 10793 as good candidates for marker-assisted selection of seedling-stage salinity tolerance. Linh et al. (2012) reported improved salt tolerance in high-yielding Bac Thom 7 rice cultivar through introgression of the Saltol QTL from donor parent FL478. The microsatellite markers, viz., RM493 and RM3412b tightly linked to the Saltol QTL, were used for foreground selection. The selected backcross lines displayed salt tolerance with agronomic performance similar to that of the original Bac Thom 7. The marker-assisted selection enabled rapid and efficient background (for the recurrent parent genome) and foreground (for target locus Saltol) selections in early generations with minimum linkage drag.

Another study by Babu et al. (2017a) reported use of marker-assisted backcrossing to transfer seedling-stage salt-stress tolerance by transferring a QTL, Saltol, into an elite salinity-sensitive rice cultivar Pusa Basmati 1121. RM 3412 STMS marker linked tightly to the QTL was used for indirect foreground selection. Back cross (BC) lines homozygous for the QTL were advanced to develop four improved near isogenic lines (NILs) of PB1121 with the salt tolerance. The field evaluation confirmed effect of QTL integration into the improved NILs in terms of greater salt

Table 8.1 Successful examples of breeding for salinity stress tolerance through marker-assisted selection

Sr. No.	Crop species	Type of DNA marker	Donor source	QTL/Allele	Mapping population	Tolerance achieved	References
1	Rice	SSR	FL478	qSaltol	Back cross	Improved salt tolerance in high-yielding cultivar bac Thom 7	Linh et al. (2012)
2	Rice	SSR	FL478	qSaltol	Back cross	Improved salt tolerance in BRRI dhan49	Hoque et al. (2015)
3	Soybean	SSR	Tolerant wild accession JWS156–1	*Ncl*	Back cross	Improved salt tolerance in salt-sensitive soybean cultivar Jackson (PI 548657)	Do et al. (2016)
4	Rice	Sequence-tagged microsatellite site (STMS)	FL478	qSaltol	NIL	Salt tolerance in sensitive Pusa Basmati 1121	Babu et al. (2017a)
5	Rice	SNP	Hasawi	qSESI12.1 and qSESF12.1	RIL	Seedling-stage salt tolerance in RIL with IR29	Bizimana et al. (2017)
6	Rice	SNP	DJ15	qST1.2 and qST6	RIL/NIL	Seedling salt tolerance enhanced in sensitive japonica rice variety Koshihikari	Quan et al. (2018)

tolerance at seedling stage and similar or better performance for other agronomic traits than the recurrent parent.

Recently, De Leon et al. (2017) utilized SSR and SNP markers to characterize introgression lines (ILs) of a high salinity-tolerant donor line Pokkali in an elite highly salt-sensitive rice cultivar Bengal and to further identify QTLs for traits contributing to salinity stress tolerance. As expected, because of abundance, more number of QTLs were detected using SNP markers than the SSR. The study emphasized marker-assisted breeding through introgression of salt injury score (SIS) QTLs, in addition to other major QTLs Saltol or qSKC1, for improved salinity tolerance. The identified tolerant ILs can be used as donor breeding lines for selective transfer of salinity tolerance without any linkage drag of undesirable traits from Pokkali to other recipient-sensitive varieties as well as for mapping and further positional cloning of the genes responsible for the trait.

Babu et al. (2017b) identified seedling-stage salt-tolerant *indica* landraces (Badami, Shah Pasand and Pechi Badam), *Oryza rufipogon* accessions (NKSWR2 and NKSWR17) and one each of Basmati rice (second Basmati) and *japonica* cultivars (Tompha Khau) based on phenotypic screening under hydroponics. The salt tolerance level was similar to that of high salt-tolerant genotypes Pokkali and FL478. Molecular diversity study of the diverse rice genotypes using polymorphic SSR markers linked with *Saltol* QTL revealed weak linkage disequilibrium-LD, suggesting its low usefulness in MAS, if the target foreground markers chosen are wide apart. LD mapping identified markers (RM10927, RM10871) linked with QTLs associated with salt tolerance traits. The study also identified *Saltol* marker, RM27, positioned on chromosome 10, associated with root Na/K ratio.

Efforts are also being made to identify novels QTLs for salinity tolerance from different sources in rice and other crops. The enhanced salt tolerance can be achieved through pyramiding of different novel QTLs in one genetic background through MAS. Bizimana et al. (2017) used Hasawi rice genotype, which conferred seedling-stage salinity tolerance due to novel QTLs other than Saltol, as a source to develop 300 recombinant inbred lines with high-yielding salt-sensitive cultivar-IR29. Further for identification of QTLs linked to salinity tolerance, a genetic linkage map was constructed using 194 polymorphic SNP markers. The study reported identification of 20 new QTLs on different chromosomes for salt tolerance through composite interval mapping.

In addition to rice, efforts are also being made for analysis of QTLs for breeding salt tolerance in other crops. In cotton, Zhao et al. (2016) identified salt-tolerant and salt-sensitive upland cotton cultivars through screening based on seedling emergence rates in response to 0.3% salt-NaCl. Seventy-four SSR markers were used to scan the genomes of these diverse cultivars, and eight markers associated with salt tolerance were identified through association analysis for further application in marker-assisted breeding. Similarly, Kere et al. (2017) screened salt-sensitive-11439S and salt-tolerant-11411S inbred parental lines with SSR markers to identify the QTLs for application in MAS for breeding salinity tolerance in cucumber. The analysis confirmed significant association of SSR markers with salt tolerance traits such as survival rate, relative leaf numbers, and percent green leaves, and salinity tolerance was evaluated by visual scoring. Recently, Luo et al. (2017) made efforts to map the critical QTLs contributing to salt tolerance in field-grown mature maize plants using a permanent doubled-haploid (DH) population and high-density SNP markers. Major QTLs responsible for salt tolerance and two candidate genes involving in ion homeostasis were mapped on chromosome 1. The mapped QTLs can be used in breeding salt-tolerant maize varieties through MAS.

Physiological and molecular studies on tolerance to various abiotic (ionic and/or osmotic stresses), viz., salinity, drought, etc., have revealed stress-specific as well as shared stress adaptation mechanisms, highlighting the complexity of stress response and adaptation in plants. In view of this, meta-QTL (MQTL) for tolerance to abiotic stresses including drought, salinity, and water logging through meta-analysis in barley has been recently projected (Zhang et al. 2017). The study conducted meta-analysis to detect and map the major QTL for drought, salinity, and water logging tolerance from different mapping populations on the barley physical map. Fine-mapped QTL for the stress tolerance were validated on MQTLs for further successful MAS in barley breeding.

8.3 Transgenic Breeding: Functional Genes Conferring Salinity Tolerance

8.3.1 Ion Transporters

In general, plants cannot withstand high salt concentration although the plant species differ in their mode of responses to the external salt exposure. Several distinct responses are generated in the plants to avoid high-salinity-induced harmful effects. One of the most distinguishing responses is avoiding salinity stress by compartmentation and the exclusion of detrimental ions like Na^+ and Cl^- from tissues that are very sensitive like the mesophyll and their relocation into the apoplast or vacuole (Sperling et al. 2014). Confinement of harmful ions within a root or apoplastic zone and maintainance of high K^+/Na^+ ratio are the major tolerance strategies for salt tolerance (Shabala and Cuin 2008).

Ion transporters are key players in maintaining ion homeostasis and in salt detoxification processes (Serrano et al. 1999; Hasegawa 2013). Various salts are present in soil out of which sodium chloride (NaCl) is the most significant. The Na^+/H^+ antiporter predominantly transports Na^+ ion from cytoplasm to the vacuole. Therefore, overexpression of genes that are involved in Na^+ transport was studied to a great extent with considerable success. Vacuolar Na^+/H^+ antiporter (*AtNHX1*) from the *Arabidopsis* was among the first and most studied gene. In *Arabidopsis* salt tolerance was conferred by overexpressing vacuolar Na^+/H^+ antiporter (Apse et al. 1999). Following with this initial success, many events were reported where transgenic plants exhibited higher potential for vacuolar sequestration of Na^+ that subsequently avoid its harmful buildup into the cytoplasm. For example, overexpression of *AtNHX1* and other NHX proteins from various hosts in many other plant species like tomato, *B. napus*, wheat, and cotton has been shown to increase salt tolerance (Zhang and Blumwald 2001; Zhang et al. 2001; Xue et al. 2004; He et al. 2005; Munns and Tester 2008). Vacuolar-type H^+-ATPase and the vacuolar pyrophosphatase are the two types of H^+ pumps that are present in vacuolar membrane (Dietz et al. 2001; Otoch et al. 2001; Wang et al. 2001). Overexpression of genes from wheat (*Triticum aestivum*) *TaNHX1* and H^+-pyrophosphatase (*TVP1*) resulted in improved salinity stress tolerance in *Arabidopsis* (Brini et al. 2007a). Similarly improved tolerance to salt stress was found in tobacco (Gouiaa et al. 2012) and tomato (Gouiaa and Khoudi 2015) by overexpression of Na(+)/H(+) antiporter H(+)- pyrophosphatase. On the other hand, the *HKT* gene family has a major role in preventing Na^+ ion toxicity in shoots by root-to-shoot partitioning of Na^+. The significant role of *HKT*s in Na^+ transport in plants makes them promising candidates to enhance salinity tolerance. The identification of the wheat *HKT1* (*TaHKT2;1*) gene

(Schachtman and Schroeder 1994; Rubio et al. 1995) has steered the study of many HKT genes from several other crops (Horie et al. 2009). Moller et al. (2009) shown that targeted overexpression of *AtHKT1;1* in the stele enhances salt tolerance in *A. thaliana*. Lately, Do et al. (2016) showed a close syndicate between the higher expression of the *Ncl* gene (homologous to the Na^+/H^+ antiporter gene family) in the root, the lesser buildup of Na^+, K^+, and Cl^- in the shoot under salt stress. Overexpression of *Ncl* into a Japanese soybean cultivar Kariyutaka resulted in enhanced salt tolerance (Do et al. 2016).

Growing evidence was found on the role of salt overly sensitive (*SOS*) stress signaling pathway in ion homeostasis and salinity tolerance (Sanders 2000; Hasegawa et al. 2000). Most of the *SOS* signaling pathway was reported to be involved in exportation of Na^+ out of the cell. The *SOS* signaling pathway includes three main proteins, namely, SOS1, SOS2, and SOS3. Plasma membrane-localized Na^+/H^+ antiporter (SOS1) is also known as NHX7 (Qiu et al. 2002). SOS2 (serine/threonine kinase) activated by salt stress elicited Ca^+ signals, while SOS3 protein is a myristoylated Ca^+ binding protein (Liu et al. 2000; Ishitani et al. 2000). *Arabidopsis* plants overexpressing genes for *SOS1*, *SOS3*, *AtNHX1* + *SOS3*, *SOS2* + *SOS3*, or *SOS1* + *SOS2* + *SOS3* resulted in improved tolerance to salt stress (Yang et al. 2009). Similarly, Kumar et al. (2009) have shown that salinity stress tolerance in *Brassica* is correlated with transcript abundance of the genes related in *SOS* pathway.

8.3.2 Osmolytes

Osmolytes are small organic compounds having a protective role. Osmolytes are important for two functional roles: osmotic adjustment at high concentrations; and it plays unknown protective role at lower concentrations. Under salt-stress conditions, plant cell accumulates various osmolytes along with Na^+ exclusion from the cytoplasm, to counter the osmotic pressure of harmful ions in vacuoles. Osmolytes like proline, glycine betaine, and sucrose accumulating upon salt stress in many plant species including halophytes are well studied and characterized (Flowers et al. 1977). Table 8.2 presents some of the successful examples of transgenic plants developed using different osmolyte genes.

Hu et al. (2015) found experimental evidences for the accumulation of sugars and amino acids. Particularly, sucrose and trehalose sugar and amino acids like proline, valine, glutamate, asparagine, glutamine, phenylalanine, and lysine accumulated under salt-stress conditions. Also, sugars like sucrose and pinitol are accumulated more in leaves, while starch accumulated in roots under salinity stress conditions. It has been found that these sugars (pinitol and sucrose) and starch can also increase in nodules under salt stress (Bertrand et al. 2015). Similarly, Boriboonkaset et al. (2013) found enrichment of soluble starch and soluble sugar in flag leaf of Pokkali genotype (salt tolerant) of rice which may have alternative role in osmotic adjustment in salt defense mechanism. In tomato plants, jasmonic acid and nitric oxide when applied exogenously, either individually or in combination,

Table 8.2 Example of functional genes used in the improvement of salt-stress tolerance of crop plants

Possible role	Gene(s)	Donor	Transgenic plant	References
Proline biosynthesis	*P5CS*	*Arabidopsis*	Tobacco	Kishor et al. (1995)
Proline biosynthesis	*P5CS*	*Vigna aconitifolia*	Tobacco	Hong et al. (2000)
Proline biosynthesis	*P5CS*	Moth bean	Rice	Su and Wu (2004)
Proline biosynthesis	*P5CS*	*Phaseolus vulgaris*	*Arabidopsis*	Chen et al. (2013)
Proline biosynthesis	*P5CSF129A*	*Sorghum bicolor*	Sorghum	Reddy et al. (2015)
Glycine betaine Biosynthesis	*codA*	*E. coli*	Rice	Sakamoto et al. (1998)
Mannitol biosynthesis	*mt1D*	*E. coli*	Tobacco	Tarczynski et al. (1992)
Mannitol biosynthesis	*mt1*	*E. coli*	Wheat	Abebe et al. (2003)
Vacuolar sequestration of Na + and K+	*TNHX1 and H(+)-*	*Triticum*	*Arabidopsis*	Brini et al. (2007a)
	PPase TVP1	*Triticum aestivum*	Tobacco	Gouiaa et al. (2012)
Vacuolar sequestration of Na + and K +?	*Na+/H+ antiporter* *AtNHX1*	*Arabidopsis*	Tomato	Zhang and Blumwald (2001)
			Brassica napus	Zhang et al. (2001)
			Wheat	Xue et al. (2004)
			Cotton	He et al. (2005)
Vacuolar	*H + −pyrophosphatase*		Cotton	Pasapula et al. (2011)
Membrane-bound proton pump	*(AVP1) AtNHX + AVP1*	*Arabidopsis*	Barley	Schilling et al. (2013)
			Cotton	Shen et al. (2014)
			Tomato	Gouiaa and Khoudi (2015)
Homologous to NHX gene family	*Ncl*	*Glycine max*	Soybean	Do et al. (2016)
Enhanced salinity tolerance	*AtNHX1 and SOS*	*Arabidopsis*	*Arabidopsis*	Yang et al. (2009)
Tolerance against salt and chilling stress	*Glutathione S- transferase(GST)* *Glutathione peroxidase (GPX)*	Tobacco	Tobacco	Roxas et al. (1997)
ROS-scavenging	*Ascorbate peroxidase (AtAPX)*	*Arabidopsis*	Tobacco	Badawi et al. (2004)
Higher activity of SOD	*Cytosolic copper/zinc superoxide dismutase (CuZnSOD)*	*Avicennia marina*	Rice	Prashanth et al. (2008)
SOD and APX	*Cu/Zn sod (cytsod)*	*Spinacia oleracea*	Toabcco	Faize et al. (2011)
Activity	*Cytosolic apx1 (cytapx)*	*Pisum sativum*		
Osmoprotection	*Late embryogenesis abundant protein (HVA7)*	*Hordeum vulgare*	Rice	Xu et al. (1996)

(continued)

Table 8.2 (continued)

Possible role	Gene(s)	Donor	Transgenic plant	References
Ononitol production	*imt1*	*M. crystallinum*	Tobacco	Sheveleva et al. (1997)
Spermine and spermidine decarboxylase	*S-adenosyl methionine*	Tritordeum	Rice	Roy and Wu (2002)
(SAMDC) production				
Salt and osmotic	*Dehydrin (DHN-5)*	*Triticum*	*Arabidopsis*	Brini et al. (2007b)
Stress tolerance				
In ABA biosynthesis and xanthophyll cycle; enhanced salt tolerance	*Zeaxanthin epoxidase (AtZEP)*	*Arabidopsis*	*Arabidopsis*	Park et al. (2008)
Increased proline	*Osmotin*	Tobacco	Tomato	Goel et al. (2010)
C-tocopherol production; Enhanced salt tolerance	*γ-Tocopherol methyl transferase (γ -TMT)*	*Arabidopsis*	*Brassica juncea*	Yusuf et al. (2010)
Maintaining chlorophyll in salt stress	*Xyloglucan endotrans-glucosylase/hydrolase (CaXTH3)*	Hot pepper	Tomato	Choi et al. (2011)
Increase in germination, chlorophyll and osmotic constituents like sugars	*Dehydration-responsive RD22*	*Vitis vinifera*	Tobacco	Jamoussi et al. (2014)

helped to boost the proline, flavonoid, and glycine betaine synthesis under NaCl salt treatments (Ahmad et al. 2018).

Engineering plants by overexpressing the osmolytes was considered as one of the ways to enhance salt tolerance in plants. In *Arabidopsis*, knockout of the *P5CS1*, a key gene in proline biosynthesis, which encodes a *delta1-pyrroline-5-carboxylate synthetase* (*P5CS*), impairs proline synthesis resulting in salt hypersensitivity (Székely et al. 2008). *P5CS* transformed in tobacco and rice has shown increased proline production, linked with increased salt-stress tolerance (Kishor et al. 1995; Su and Wu 2004). Also, transgenic rice expressing the moth bean *P5CS* gene showed enhanced tolerance to higher dose of NaCl (Su and Wu 2004). Recently, mutated *P5CS* (*P5CSF129A*) gene was overexpressed in *Sorghum* and found that transgenic plants accumulated more proline and showed salt-stress tolerance. Moreover overproduction of proline through transfer of a *P5CSF129A* gene conferred protection of photosynthetic and antioxidant enzyme activities (Reddy et al. 2015). Glycine betaine is yet another important osmolyte that helps to balance the osmotic potential of intracellular ions under salinity. Under high salinity, glycine betaine accumulation increased in lamina leaves and bladder hairs of *Atriplex gmelini* (Tsutsumi et al. 2015). Overexpressing c*holine oxidase* in rice plant showed increased levels of glycine betaine and improved tolerance to salt and cold stress (Sakamoto et al. 1998). It was found that transgenic *Arabidopsis* and tobacco plants transformed with bacterial *mtlD* gene which encodes for *mannitol-1-phosphate dehydrogenase* conferred salt tolerance and thereby maintained normal growth and development under high salt-stress growth conditions (Binzel et al. 1998; Thomas et al. 1995). Ectopic expression of bacterial gene *mannitol-1-phosphate dehydrogenase* (*mt1D*), an enzyme involved in mannitol biosynthesis, in tobacco successfully enhanced salt tolerance (Tarczynski et al. 1992). Genes for trehalose biosynthesis have also been employed in improving salt tolerance by developing transgenic plants for overproduction of trehalose (Penna 2003; Turan et al. 2012). Garg et al. (2002) demonstrated tolerance to salt and drought stress in rice by using tissue-specific or stress-inducible expression of a bifunctional trehalose-6-phosphate synthase/phosphatase (*TPSP*) fusion gene (comprising the *E. coli* trehalose biosynthetic genes). Li et al. (2011) reported that transgenic plants overexpressing rice trehalose-6-phosphate synthase (*OsTPS1*) showed improved salinity tolerance without much alteration in plant phenotype. It is also suggested that stress-inducible solute accumulation by using stress-specific or stress-inducible promoters may be better to achieve salt-specific expression of genes for osmotic adjustment.

8.3.3 Antioxidants and Protective Proteins

Abiotic stress causes the accumulation of reactive oxygen species (ROS) that can damage sensitive plant tissues during high salt stress by disturbing cell wall, enzymes, and membrane functions. Antioxidant enzymes and nonenzymatic compounds play a crucial role in detoxifying salinity stress-induced ROS. Salt-stress tolerance is positively correlated with antioxidant enzyme activity and with the accumulation of nonenzymatic antioxidant compounds (Gupta et al. 2005). Antioxidants include superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), ascorbate peroxidase (APX), glutathione reductase (GR), etc. Hence, overexpressing ROS-scavenging enzymes is shown to induce salinity tolerance in plants (Roxas et al. 1997; Badawi et al. 2004; Miller et al. 2008). Overexpression of ascorbate peroxidase (APX) in tobacco chloroplasts enhances the tolerance to salinity and drought stress (Badawi et al. 2004). The alternate oxidase (AOX) pathway plays a role under stress conditions. Smith et al. (2009) constitutively overexpressed an AOX1a gene in *Arabidopsis* plants and demonstrated superior salt tolerance than wild-type plants suggesting that genes of the AOX pathway can be useful to improve tolerance to stressful environmental conditions including salinity.

Other proteins like polyamines, osmotin, and LEA proteins mitigate salt stress by protecting macromolecules like nucleic acids, proteins, and carbohydrates from damages caused by ion toxicity and by drought conditions. Polyamines play a critical role in salinity and other abiotic stress tolerance by increasing level of polyamines which shows positive correlation of increased level of polyamines with stress tolerance in plants (Yang et al. 2007; Groppa and Benavides 2008; Gupta et al. 2013). Overproduction of spermidine and spermine in rice enhances salt tolerance (Roy and Wu 2002). Xu et al. (1996) found that *HVA7*, a LEA from barley, when transferred to rice, confers tolerance to drought and salinity stress. Dehydrins, another

LEA protein, were shown to enhance plant tolerance to various stresses (Hanin et al. 2011). Brini et al. (2007b) found positive correlation between wheat *dehydrin DHN-5* and salt tolerance. The study showed that the expression of the wheat *dehydrin DHN-5* in *Arabidopsis* led to an increase in tolerance to salt and osmotic stresses. Some of the transgenics where functional genes overexpressed to impart salt tolerance in different plant species have been shown in Table 8.2.

8.4 Transgenic Breeding: Regulatory Genes Controlling Salinity Stress Responses

8.4.1 Transcription Factors

Plant response to salt stress is a complex process and involves a vast array of genes working in different or overlapping regulatory pathways. As stress response is complex process and regulated by multi-genes, it is very challenging to achieve success in improving plant stress tolerance with the single functional gene approach (Mittler and Blumwald 2010; Varshney et al. 2011). Thus, instead of manipulating single functional gene, engineering regulatory genes or master regulators can be potential strategy for controlling stress responses. Transcription factors (TFs) and signaling proteins are master regulators of many genes involved in stress responses; hence, they are possible candidates for genetic engineering to obtain salinity-tolerant crops (Table 8.3). Transcription factors from various families like *AP2/ERF*, *NAC*, *MYB*, *MYC*, *DREB*, *Cys2/His2 zinc finger*, *bZIP*, and *WRKY* have been reported to be involved salt-stress tolerance (Golldack et al. 2011, 2014).

NAC TF family found to be involved in abiotic stress responses along with other important functions in plants (Nakashima et al. 2012). Hu et al. (2008) reported that overexpression of *SNAC1* and *SNAC2* genes from *NAC* TF family helps the survival of transgenic *Oryza sativa* plants under high-salinity conditions. Similarly, overexpression of TF gene *OsNAC04* leads to drought and salinity stress tolerance in *O. sativa* (Zheng et al. 2009). The AP2/ERF another family of plant-specific TFs which is known to play key role against various abiotic stresses (Mizoi et al. 2012). The transgenic plants for gene *GmDREB2* from soybean showed enhanced salinity and drought tolerance (Chen et al. 2007). Overexpression *Oryza sativa MYB2* (TF from MYB family) exhibited salt tolerance by variation in expression levels of various stress responsive genes (Yang et al. 2012).

Several groups reported a key role of *WRKY* TFs in responses to various abiotic stresses including salinity stress (Banerjee and Roychoudhury 2015). Li et al. (2013) reported *ZmWRKY33* (WRKY TF family) enhanced tolerance to salinity stress in *Arabidopsis* while overexpressing *GmWRKY54* exhibited salt tolerance, probably through the regulation of another TF *DREB2A and STZ/Zat10* (Zhou et al. 2008). *bZIP* is also another family of TFs having an important role in response to various abiotic stresses including salinity stress (Jakoby et al. 2002). Wang et al. (2010) demonstrated that overexpression of *ThbZIP1* gene of *Tamarix hispida* from TF *bZIP*

Table 8.3 Examples of regulatory genes leading to improvement of salt-stress tolerance of crop plants

Enhanced tolerance	Gene(s)- (TF family)	Donor	Transgenic plant	References
Enhanced drought and salinity tolerance	*OsDREB2A - (AP2/ERFBP)*	*Oryza sativa*	Rice	Mallikarjuna et al. (2011)
Enhanced salinity tolerance	*StDREB1 - AP2/ERFBP*	*Solanum tuberosum*	Potato	Bouaziz et al. (2013)
Enhanced salinity tolerance	*GmERF7-(AP2/ERFBP)*	*Glycine max*	Tobacco	Zhai et al. (2013)
Enhanced drought and salinity tolerance	*TaERF3 -(AP2/ERFBP)*	*Triticum aestivum*	Wheat	Rong et al. (2014)
Enhanced drought and salinity tolerance	*EaDREB2 -(AP2/ERFBP)*	*Erianthus arundinaceus*	Sugarcane	Augustine et al. (2015)
Enhanced drought and salinity tolerance	*SsDREB - (AP2/ERFBP)*	*Suaeda salsa*	Tobacco	Zhang et al. (2015)
Enhanced drought and salinity tolerance	*TaPIMP1-(MYB)*	*Triticum aestivum*	Tobacco	Liu et al. (2011)
Enhanced drought, cold, salinity tolerance	*OsMYB2-(MYB)*	*Oryza sativa*	Rice	Yang et al. (2012)
Enhanced drought, cold, salinity tolerance	*MdSIMYB1-(MYB)*	*Malus* × *domestica*	Apple	Wang et al. (2014)
Enhanced NaCl, ABA, mannitol tolerance	*SbMYB2-(MYB)* *SbMYB7-(MYB)*	*Scutellaria baicalensis*	Tobacco	Qi et al. (2015)
Enhanced salinity tolerance	*OsMYB91-(MYB)*	*Oryza sativa*	Rice	Zhu et al. (2015)
Enhanced drought, cold, salinity tolerance	*SlAREB1 -(bZIP)*	*Solanum lycopersicum*	Tomato	Orellana et al. (2010)
Enhanced drought, cold, salinity tolerance	*ThbZIP1-(bZIP)*	*Tamarix hispida*	Tobacco	Wang et al. (2010)
Enhanced salinity tolerance	*LrbZIP -(bZIP)*	*Nelumbo nucifera*	Tobacco	Cheng et al. (2013)
Enhanced salinity and drought tolerance	*OsbZIP71-(bZIP)*	*Oryza sativa*	Rice	Liu C. et al. (2014)
Enhanced salinity tolerance	*GhWRKY39-(WRKY)*	*Gossypium hirsutum*	Tobacco	Shi et al. (2014)
Enhanced salinity and drought tolerance	*TaWRKY10-(WRKY)*	*Triticum aestivum*	Tobacco	Wang et al. (2013)
Enhanced salinity and drought tolerance	*ZmWRKY58-(WRKY)*	*Zea may*	Rice	Cai et al. (2014)
Enhanced salinity and drought tolerance	*MtWRKY76-(WRKY)*	*Medicago truncatula*	*Medicago truncatula*	Liu et al. (2016)
Enhanced salinity and drought tolerance	*OsNAC04-(NAC)*	*Oryza sativa*	Rice	Zheng et al. (2009)
Enhanced salinity and drought tolerance	*OsNAP -(NAC)*	*Oryza sativa*	Rice	Chen et al. (2014)
Enhanced salinity and drought tolerance	*ONAC022 -(NAC)*	*Oryza sativa*	Rice	Hong et al. (2016)

(continued)

Table 8.3 (continued)

Enhanced tolerance	Gene(s)- (TF family)	Donor	Transgenic plant	References
Enhanced salinity tolerance	*ShCML44*	Rice	Rice	Xu et al. (2013)
Enhanced salinity, cold, and drought tolerance	*ShCML44*	Wild tomato	Tomato	Munir et al. (2016)
Enhanced salinity, cold, and drought tolerance	*OsCDPK7(CDPK)*	*Oryza sativa*	Rice	Saijo et al. (2000)
Enhanced salinity and drought tolerance	*OsCPK4(CDPK)*	*Oryza sativa*	Rice	Campo et al. (2014)
Enhanced salinity tolerance	*CalcineurinA subunit*	Mouse	Rice	Ma et al. (2005)
Enhanced salinity tolerance	*ZmMKK4*	*Zea mays*	*Arabidopsis*	Kong et al. (2011)
Enhanced salinity and drought tolerance	*GhMPK2*	*Gossypium hirsutum*	Tobacco	Zhang et al. (2011)
Enhanced salinity, cold, and drought tolerance	*OsMAPK5*	Rice	Rice	Xiong et al. (2003)
Enhanced salinity tolerance	*OsMKK6*	Rice	Rice	Kumar and Sinha (2013)
Enhanced salinity and drought tolerance	*MKK5*	*Arabidopsis*	*Arabidopsis*	Xing et al. (2015)
Enhanced salinity tolerance	*PtMAPKK4*	*Populus trichocarpa*	Tobacco	Yang et al. (2017)
Enhanced salinity and drought tolerance	*OsSIK1*	Rice	Rice	Ouyang et al. (2010)
Enhanced salinity tolerance	*PtSnRK2*	Poplar	*Arabidopsis*	Song et al. (2016)

Modified after Wang et al. (2016)

contributes to salinity tolerance by enhancing the activity of antioxidant enzymes such as peroxidase and superoxide dismutase and by accumulating compatible osmolytes like soluble sugars and soluble proteins. *SlAREB1* and *SlAREB2* are two members of *bZIP* TF in *Solanum lycopersicum*. Transgenic tomato overexpressing *SlAREB1* plants showed improved salinity and drought tolerance (Orellana et al. 2010).

8.4.2 Signaling Proteins

In addition to TFs, genetic engineering of signaling proteins has also become one of the feasible approaches. Some of the examples of transgenics where regulatory genes are overexpressed to impart salt tolerance are presented in Table 8.3. Several studies have reported that abiotic stresses (cold, high salt, and drought) trigger rapid increase in plant cells calcium (Ca^{2+}) levels (Sanders et al. 2002). Calcium signaling is often coupled with protein phosphorylation and dephosphorylation mediated by protein kinases and phosphatases, respectively. Changes in cellular Ca^{2+} level are being mediated by different Ca^{2+} binding proteins like calmodulin (CaM) and CaM-related proteins (CML), calcium-dependent protein kinases (CDPKs), and calcineurin B-like proteins (CBL) (Bouché et al. 2005). CDPK is one of the best studied protein kinases in the Ca^{2+} signaling pathway. Another family of protein kinases that function in stress tolerance is mitogen-activated protein kinases (MAPKs) (Zhang and Klessig 2001).

Overexpression of *ShCML44*, cold-responsive calmodulin-like gene in tomato, showed enhanced tolerance to salinity stress with higher germination rate and better growth of seedling (Munir et al. 2016). Similarly, transgenic rice overexpressing *OsMSR2*, a novel calmodulin-like gene, enhanced salinity tolerance with altered expression pattern of genes related to stress (Xu et al. 2013). Transgenic rice plant expressing calcineurin A subunit from mouse exhibited a higher level of salinity stress tolerance; also it has been observed that Na^+ content is higher in roots of untransformed wild-type plants than that of transgenic roots (Ma et al. 2005).

The *OsCPK4* gene is a member of calcium-dependent protein kinases in rice. Recently Campo et al. (2014) showed that transgenic rice plants overexpressing of *OsCPK4* significantly enhances salt and drought tolerance. Mitogen-activated protein kinase (MAPK) cascades also play crucial regulatory roles in various stress responses other than plant development processes. Zhang et al. (2011) reported ectopic expression of cotton *GhMPK2* in *Nicotiana tabacum* and found elevated levels of proline and induced expression of several genes related to stress, and as a result transgenic *Nicotiana tabacum* exhibited enhanced drought and salt tolerance. Similarly, overexpression *MAPK* from rice (*OsMAPK5*) exhibited increased kinase activity along with increased tolerance for salinity and other abiotic stresses like drought and cold (Xiong and Yang 2003). Overexpression of mitogen-activated protein kinase kinase 5 (*MKK5*) in *Arabidopsis* wild-type plants improved their tolerance level against various salt treatments (Xing et al. 2015). In another study overexpression of mitogen-activated protein kinase kinase 4 from *Populus trichocarpa* (*PtMAPKK4*) shows improved salt tolerance in tobacco. Specifically, under salt-stress condition, *PtMAPKK4* overexpressing lines showed improved germination and growth and development (Yang et al. 2017). However, some MAPKs can also have contrary effects particularly in case of rice; overexpression of *OsMAPK33* caused increased sensitivity to salinity and drought stress compared to wild-type plants (Lee et al. 2011). Receptor-like kinases (RLKs), another type of kinase, also have an important role in stress responses. Overexpressing *OsSIK1* (*OsSIK1-ox*), one of the putative RLKs, showed greater tolerance to salt stress as compared to control plants, gene-silenced plants by RNA interference (RNAi), knockout mutants *sik1* in rice (Ouyang et al. 2010). Sucrose non-fermenting 1 (SNF1)-related protein kinases (SnRKs) is one type of well-characterized protein kinase involved in stress responses (Halford and Hey 2009). In one study *PtSnRK2.5* and *PtSnRK2.7*genes (SnRKs from Poplar) heterologously overexpressed in *Arabidopsis* and found that overexpression of *PtSnRK2* leads to enhanced tolerance level for salt stress.

8.4.3 Manipulating the miRNAs

In the past decade, miRNAs have become major players in the regulation of plant response to environmental abiotic stresses (Zhang 2015, Shriram et al. 2016). There has been great interest in exploring regulatory roles of microRNAs against different stresses including salt for their exploitation in genetic engineering for higher stress tolerance, biomass, and yield (Zhang and Wang 2016, Patel et al. 2018). Transgenic plants overexpressing miR319 showed significantly higher plant tolerance to drought and salinity stress in creeping bentgrass (*Agrostis stolonifera*) (Zhou et al. 2013). In another study, expression of miR408 was shown to improve higher tolerance to salinity, cold, and oxidative stress in *Arabidopsis* seedlings (Ma et al. 2015). A rice microRNA *osa-miR393* was overexpressed in *Arabidopsis* plant resulting in enhanced salt tolerance (Gao et al. 2011a; b). In several other studies, novel microRNAs have been identified suggesting that these well-characterized candidates could become targets for plant genetic engineering investigations as successful in silico predictions could result in finding the target genes involved in pathways of signaling, ion homeostasis besides sustained plant growth under salt stress.

8.5 Halophyte Genes for Improving Salt-Stress Tolerance of Crops

In plants, gene expression and regulation decides the fate of plants from growth and development to stress tolerance. Modification/ manipulation in the regulation of these entities can dramatically change the fate of plant’s life. In this sense, stress tolerance of plants can be improved by manipulating particular genes. In terms of stress tolerance, it is proved that the tolerant and sensitive plants possess same set of genes, but their efficient regulation or subtle changes in gene sequence can make one plant sensitive and other plant tolerant to the same environmental condition. This phenomenon is also true for salt-sensitive glycophytes and salt-tolerant halophytic plants. The halophytes are naturally tolerant to high salinity. Their genetic analysis revealed that differences in promoter activities and gene duplication in halophytes as compared to their glycophytic relative is responsible for their high salt tolerance (Nikalje et al. 2017). For example, the *NHX8* showed stress-induced expression in *Arabidopsis* while in *Thellungiella*, it showed constitutive expression. However *Arabidopsis* possess a single copy of *CBL10*, while *T. parvula* contain three copies; such changes make *Thellungiella* more salt tolerant. In addition, the efficient post-translational modifications are highly efficient in halophytes (Bose et al. 2015), and the halophytic gene sequences are more complex with presence of extra transposons and intergenic sequences (Rui et al. 2007). Therefore, for genetic improvement of crops, it may be important to choose genes from halophytic origin. Overexpression of *NHX1* gene of *Aeluropus littoralis* in soybean resulted in less sodium accumulation in aerial parts than underground parts, increased potassium ion content under salt stress and increased salt tolerance up to 150 mM NaCl (Liu et al. 2014). Shabala and Potossin (2014) opined that retention of potassium ions under salt stress is a key factor for salt tolerance in plants and specialty of halophytes. Further, Bose et al. (2015) have confirmed this by showing that maintenance of negative water potential because of high H^+ ATPase activity is important for halotropism. Similarly different halophytic antiporters were overexpressed in rice, and the transgenic plants showed high salt tolerance. *PtNHA1* and *PtNHX* from *Puccinellia tenuiflora* were transformed into rice, and the resulting transgenic rice showed improved tolerance to NaCl and $NaHCO_3$. Transgenic rice harboring *AgNHX1* from *Atriplex gmelinii* increased vacuolar antiporter activity by almost eightfold and improved its tolerance up to 300 mM NaCl (Ohta et al. 2002).

8.6 New Research on the Salt Pan

Genome editing tools have opened up new avenues for specific and targeted modifications in the crop plants (de Wiel et al. 2017). The method enables the introduction of targeted precise genomic changes using customized nucleases (Jain 2015). Genes associated with salt tolerance such as those involved in signaling, ion homeostasis, osmolyte synthesis, and transporters can be the suitable candidates for editing based manipulation. The plasma membrane ATPase plays a critical role in the regulation of ion homeostasis under salt stress and hence has been used as the target gene in a recent study. Osakabe et al. (2016) induced mutation of an abiotic stress tolerance gene encoding OPEN STOMATA 2 (OST2) (AHA1) – a major plasma membrane H + -ATPase via the precise site modification by using truncated gRNAs (tru-gRNAs) in the CRISPR-Cas9 system (Table 8.3).

High-throughput screening methods have advanced our knowledge about the genomes and phenomes. Plant stress biology research depends on robust screening methods for contrasting salt-stress-responsive phenotypes at different levels of tissue, organ, and whole-plant level. This branch of research, plant phenomics, is now being applied to facilitate efficient and reliable evaluation of stress (and salt) tolerant lines. Several such platforms for phenotyping are now available. Some of these include the High Resolution Plant Phenomics Centre (http://www.plantphenomics.org.au/HRPPC), Plant AccelatorTM (http://www.plantaccelerator.org.au/), Jülich Plant Phenotyping Centre- JPPC (http://www.fz-juelich.de/ibg/ibg-2/EN/_organisation/JPPC/JPPC_node.html) and Deep Plant Phenomics (Ubbens and Stavness 2017). Campbell et al. (2015) have developed a novel approach to analyze the dynamic plant responses to salt stress and studied the genetic basis of salt stress associated, genetically determined changes using a longitudinal genome-wide association model. This study highlights the use of image-based phenomics platforms combined with genome-wide association studies (GWAS) for dissecting the plant stress responses and should enable to establish liaison between expressed phenotypes with related genomic regions and environmental conditions. Further research into plant genetic manipulation via precise genetic tools will benefit from efficient phenotyping screens and high-throughput analysis tools.

8.7 Conclusions

Increasing salinity severely affects crop productivity and is becoming threat to world agriculture. The development of several genomics-assisted approaches including genetically modified plants has been advocated to circumvent this problem. Toward this goal, several stress-responsive genes have been identified and successfully introduced into other crops to create transgenic crops with enhanced stress tolerance. The most impressive results were obtained when manipulating transcription and signaling factors, as they control a broad range of downstream events, which results in superior tolerance to multiple stresses. However, challenges still lie ahead before successfully improving crop yield under saline conditions as most methods have been limited by the problem of yield penalty. Salinity tolerance involves a complex of responses at molecular, cellular, metabolic, physiological, and whole-plant levels. The marker-assisted selection as the molecular breeding method has begun to deliver its expected benefits in commercial breeding programs for salinity stress tolerance. For this, in addition to the key loci identified for salt tolerance traits majorly in rice, emphasis should also be given on identification and validation of other new loci in rice and other crops and their pyramiding in elite genetic background for enhanced salt tolerance through molecular marker-assisted breeding. Generation of salinity-tolerant transgenic varieties should necessarily involve gene stacking where multiple genes need to be overexpressed using advanced genetic engineering tools. Furthermore, the critical step is the field trials required to evaluate the transgenic plants, especially focusing on their growth and tolerance in the whole life period. New and novel information is generated through omics methods such as metabolomics and proteomics, and it is expected to develop more understanding of the salt-stress responses. It is also equally important that further understanding how plants perceive stress signals (salt sensors, osmosensors), transmit, and trigger a cascade of genetic mechanisms is necessary to develop crop plants that can tolerate extreme environments. With the current renewed interest in stress genomics, fast-forward approaches of phenomics, allele mining, and stress-metabolite profiling, it is expected to gain thorough understanding of salt-adaptive diversity for use in crop breeding for salt tolerance. Continued research should be aimed at development of salt-tolerant crop germplasm to expand the utilization of saline soils for enhancing agricultural productivity and environmental sustainability.

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