Advances in Functional Genomics in Investigating Salinity Tolerance in Plants

Joydeep Banerjee, Arpita Das, Maryam Vahedi, and Saikat Gantait

1 Introduction

Soil salinity has diverse effects on the morphological, physiological, and biochemical characteristics of plants, which results in reduction in yield (Chinnusamy et al. 2005). Salt stress modifies the dimension of morphological parameters that includes root length, leaf area, plant heights, root and branch length, stem diameter, and number of branches and nodes (Yu et al. 2015). Salinity induces morphological and anatomical changes that results in reduction in the dry weight of leaves and roots, root length, root volume, average root diameter, chlorophyll and net photosynthesis, and stomatal conductance (Zhang et al. 2014a; Yan et al. 2015). Photosynthesis is the most important process that is influenced by salt stress by decrease in CO_2 availability, which is induced by diffusion limitations through the stomata and the

J. Banerjee
Department of Agricultural and Food Engineering, Indian Institute of Technology Kharagpur, Kharagpur, Paschim Midnapore, West Bengal, India

A. Das
Department of Genetics and Plant Breeding, Faculty of Agriculture, Bidhan Chandra Krishi Viswavidyalaya, Nadia, West Bengal, India

Regional Research Sub-Station, New Alluvial Zone, Bidhan Chandra Krishi Viswavidyalaya, Nadia, West Bengal, India

M. Vahedi
Department of Horticultural Science, Faculty of Agricultural Sciences and Engineering, College of Agriculture and Natural Resources, University of Tehran, Karaj, Iran

S. Gantait (✉)
Department of Genetics and Plant Breeding, Faculty of Agriculture, Bidhan Chandra Krishi Viswavidyalaya, Nadia, West Bengal, India

Crop Research Unit, Bidhan Chandra Krishi Viswavidyalaya, Nadia, West Bengal, India

S. H. Wani (ed.), *Recent Approaches in Omics for Plant Resilience to Climate Change*, https://doi.org/10.1007/978-3-030-21687-0_8

mesophyll (Chaves et al. 2009). Various responses of chlorophyll content have been found in plants (Sudhir and Murthy 2004). Salt stress enhances the accumulation of NaCl in chloroplasts of plants that triggers reduction during photosynthetic electron transport activities in photosynthesis (Sudhir and Murthy 2004). Salt stress inhibits photosynthesis because of water scarcity and lowered carbon substrate (Chaves et al. 2009).

Salinity reduces the growth of plant by three major inhibitory effects namely (1) osmotic effect, (2) ion toxicity, and (3) nutritional imbalance (Ali et al. 2004). Salinity influences seed germination through osmotic effects in beans and wheat (Almansouri et al. 2001; Kaymakanova 2009). In addition, salinity decreases soil water potential due to osmotic stress, it induces ion imbalance in cells due to lower concentrations of K^+, Ca^{2+}, and NO_3^-, and it also causes ion (Na^+ and/or Cl^-) toxicity (Tavakkoli et al. 2011). High concentrations of soil Cl^- is more toxic in reducing growth and yield as compared to Na^+ (Tavakkoli et al. 2010).

Salinity stress causes oxidative stress that further triggers production of toxic reactive oxygen species (ROS). Salinity influences the activity of antioxidant enzymes such as superoxide dismutase (SOD), peroxidase (POD), ascorbate peroxidase (APX), glutathione reductase (GR), guaiacol peroxidase (GPX), dehydroascorbate reductase (DHAR), monodehydroascorbate reductase (MDAR), glutathione *S*-transferase (GST), and catalase (CAT). Non-enzymatic antioxidants include phenolics, flavonoids, and tocopherols, which are in active state and scavenge high ROS levels (AbdElgawad et al. 2016). Accumulation of proline in salt-stressed plants decreases osmotic potential in vacuoles in order to maintain chlorophyll level, protein, membrane and subcellular structure, and cell turgor (Cicek and Cakirlar 2002). It plays vital role in scavenging ROS products in salt tolerance (Gharsallah et al. 2016). Glycine betaine (GB) is another osmotic adjustment agent that decreases the ROS concentration and lipid peroxidation in higher plants (Banu et al. 2009). Polyamines also modulate ion channels and activate antioxidant enzymes for osmotic adjustment (Zapata et al. 2017).

Salinity alters the levels of plant hormones such as abscisic acid (ABA), cytokinins, ethylene, and jasmonates that further play a key role in alleviating NaCl-induced salt stress on plant growth and development (Parida and Das 2005). Salinity influences stomatal conductance at the initial stage; afterward the ABA is produced (Munns and Tester 2008). ABA generally increases H^+-pumping under salinity condition in rice (Pons et al. 2013). Cytokinins are antagonists of ABA and have opposite effects in several developmental processes, mainly stomatal opening (Kaya et al. 2009). Ethylene is known as a stress-hormone; ethylene signaling modulates salt response at different levels, including membrane receptors, components in cytoplasm, and nuclear transcription factors (TFs) in the pathway (Cao et al. 2008). The drought stress can be induced by salinity and it influences on redox regulation in plant cells (Uddin et al. 2016). The redox system is important for keeping the cellular homeostasis in environmental stresses (Trachootham et al. 2008). In addition, the late embryogenesis abundant (LEA) proteins are produced by plants to protect themselves from the damage caused by environmental stresses (especially the salt stress) (Bhardwaj et al. 2013).

2 Functional Genomics Approaches for Salt Tolerance

A number of genes were found to be involved in salt tolerance across diverse plant genera. Through functional genomics, transcriptomics, and proteomics approaches the functional significance of some of the major genes have been discovered, which plays a pivotal role in salt tolerance of plants. A number of transgenic approaches have been adopted to over-express genes that are involved in salt-tolerant mechanisms and in addition to that down-regulation of some important genes in transgenic plants also demonstrated salt tolerance. This part of the chapter mostly describes several transgenic methods developed either through over-expression strategies (for enhancing osmoprotectant accumulation, over-expression of TFs, antioxidants and genes involved in ion transport, and heterologous expression of some important proteins involved in salt stress mechanisms) or, using gene-silencing approach for enhanced salt tolerance.

2.1 Over-Expression Strategies for Salt Tolerance in Plants

Over-expression of several genes involved in production of compatible solutes was found to exhibit improved salt tolerance. Those compatible solutes are small, electrically uncharged molecules playing important roles in plant protection as well as membrane and protein stabilization under abiotic stress conditions without hampering normal growth and development (Yancey 1994). Several researches conducted to overcome salt stress using compatible solutes or osmoprotectants and that can be categorized into three groups like over-accumulation of amino acid (e.g., proline), over-production of polyols or sugars (e.g., mannitol, sorbitol, trehalose, etc.), and over-accumulation of quaternary amines (e.g., GB and polyamines).

Several researches were conducted on over-expression of candidate genes for elevated proline accumulation and subsequently improved salt tolerance. A gene, *P5CS* (Δ^1-Pyrroline-5-Carboxylate Synthetase) was found to code a bifunctional enzyme that is responsible for proline synthesis in plants. Over-expression of *P5CS* gene from moth bean (*Vigna aconitifolia*) in transgenic tobacco (*Nicotiana tabacum*) revealed improved salt tolerance possibly due to hyper-accumulation of proline in transgenic plants (Kishor et al. 1995) whereas; over-expression of a mutant *P5CS* (*P5CSF129A*) gene from *V. aconitifolia* in tobacco also documented increased tolerance to salt stress (Hong et al. 2000). Later on, *P5CS* gene was heterologously expressed in different crops like rice, wheat, carrot, etc. by various researchers (Zhu et al. 1998; Sawahel and Hassan 2002; Han and Hwang 2003). Another group isolated *P5CS* gene from *Arabidopsis thaliana* and subsequently over-expressed in potato (*Solanum tuberosum*) and the resultant transgenic potato showed improved tolerance to salinity stress without hampering the tuber yield (Hmida-Sayari et al. 2005). Further study identified two P5CS orthologs (*OsP5CS1* and *OsP5CS2*) from rice and co-expression of those two genes in tobacco depicted

enhanced abiotic stress (including salt stress) tolerance due to over-accumulation of proline in transgenic plants (Zhang et al. 2014b).

A number of researches were conducted to over-accumulate various polyols or sugars in transgenic plants through metabolic engineering. Initially, a mannitol-1-phosphate dehydrogenase (*mtlD*) gene from *Escherichia coli* was transgenically expressed in tobacco and it resulted over accumulation of mannitol and subsequently demonstrated salt tolerance up to 250 mol m^{-3} NaCl (Tarczynski et al. 1993). Further researches transgenically expressed *mtlD* gene in other crops (Chinnusamy et al. 2005). Likewise, another gene sorbitol-6-phosphate dehydrogenase (*Stpd1*) was used by Gao et al. (2001) to accumulate sorbitol in transgenic plant that conferred salt tolerance. Majee et al. (2004) isolated L-myo-inositol-1-phosphate synthase (*MIPS*) gene from halophytic wild rice (*Porteresia coarctata*) and subsequently that gene was heterologously expressed in transgenic tobacco plants. Transgenic tobacco demonstrated tolerance up to 200–300 mM NaCl stress. Trehalose is a non-reducing sugar molecule, which was found to possess osmoprotectant property. Preliminary studies were conducted to isolate trehalose-6-phosphate synthase (*otsA*) and trehalose-6-phosphate phosphatase (*otsB*) genes from *E. coli* and over-expression of those genes in rice as a fusion gene (Garg et al. 2002). Resultant transgenic rice plants showed enhanced trehalose accumulation and thusimproved salt tolerance. Subsequently, Ge et al. (2008) isolated trehalose-6-phosphate phosphatase (*OsTPP1*) gene from rice and over-expressed in rice background. The transgenic plants conferred salt as well as abiotic stress tolerance. Thereafter, another rice gene named trehalose-6-phosphate synthase (*OsTPS1*) was over-expressed in rice and the resultant plants showed over-accumulation of proline as well as trehalose and subsequently tolerance to salt stress (Li et al. 2011). Thus, over-expression of several polyol/sugar accumulating genes was found to show improved salt tolerance in transgenic plants.

The third category of osmoprotectant is quaternary amines and most of researches were conducted to over-accumulate GB compound belonging to this group. Upon exposure to abiotic stresses, GB content is increased in certain plants and that could be used as an osmoprotectant (Khan et al. 2015). Hence, several approaches have been taken to over-accumulate GB content in economically important crops. Initially, two group of scientists heterologously expressed choline oxidase (*codA*) gene, which is responsible for GB synthesis, from *Arthrobacter globiformis* into *Synechococcus* sp. PCC 7942 (Deshnium et al. 1995; Nomura et al. 1995) that was unable to produce GB in natural conditions, but transgenically expressed *codA* gene accumulated GB in that species and subsequently the resultant strain became more salt tolerant. In addition to over-expression in cyanobacteria, several scientists over-expressed *codA* gene in tobacco, *Arabidopsis*, rice and other plants (Hayashi et al. 1997; Jing et al. 2013; Chinnusamy et al. 2005). Another choline oxidase gene (*COX*) was isolated from *Arthrobacter pascens* and used for the generation of salt-tolerant transgenic plants (Sakamoto and Murata 2001; Su et al. 2006). Further researches were conducted to over-accumulate GB in transgenic tobacco plants by over-expressing Betaine-aldehyde dehydrogenase (*BADH*) gene from spinach (*Spinacia oleracea*) and the generated transgenic plants acquired enhanced salt tolerance compared to the untransformed control plants (Yang et al. 2008).

2.2 Transcription Factors

Genetic engineering through over-expression of different TFs was found to have significant salt tolerance in different plant species. Over-expression of a dehydration-responsive element-binding (DREB) protein from *A. thaliana* named *AtDREB1A*, in transgenic tobacco, showed enhanced salt tolerance under controlled condition (Cong et al. 2008) whereas; over-expression of a *Glycine max* DREB gene (*GmDREB1*) in transgenic wheat depicted enhanced salt tolerance compared to wild-type plants under natural field condition (Jiang et al. 2014). Along with DREB, NAC TFs were found to be involved in salt tolerance in different plants. Over-expression of different NAC TFs namely SNAC2, ONAC045, and ONAC022 in transgenic rice documented improved salt tolerance (Hu et al. 2008; Zheng et al. 2009; Hong et al. 2016). *Caragana intermedia* is an extremely salt as well as drought-tolerant desert leguminous shrub. Over-expression of two NAC TFs (CiNAC3 and CiNAC4) from *C. intermedia* into *A. thaliana* caused elevated salt tolerance compared to wild-type plants (Han et al. 2015). Another group of TF (MYB) was also found to be associated with salt stress in different plant species (Roy 2016). An approach taken to functionally characterize OsMYB2, a R2R3 type MYB TF from rice, depicted that over-expression of OsMYB2 improved salt tolerance in transgenic rice plant (Yang et al. 2012). Another study revealed that over-expression of OsMYB48-1 in rice lead to enhanced tolerance to salt as well as drought-stress under simulated condition (Xiong et al. 2014). Although over-expression of a number of TFs documented their importance in salt tolerance, how those TFs regulate the downstream salt stress signaling cascade is still unclear.

2.3 Antioxidants and Detoxification Genes

Antioxidants plays crucial role to cope up the plants from the excessive generated ROS during salt stress as well as other stresses. Plants possess several enzymatic antioxidants like SOD, catalase, and peroxidase along with some non-enzymatic antioxidants including phenol, ascorbic acid, thiol compounds which play crucial role in detoxifying ROS. Over-expression of a cDNA encoding both GST and glutathione peroxidase activity in transgenic tobacco showed faster growth compared to the control tobacco plants under salt-stressed condition (Roxas et al. 1997). MDAR enzyme plays major role for synthesis of ascorbate, an important antioxidant available in plant. Over-expression of *AtMDAR1* gene from *A. thaliana* into tobacco plants (Eltayeb et al. 2007) caused enhanced tolerance of the transgenic tobacco plants to salt stress. In another experiment a catalase gene (*katE*) from *E. coli* was over-expressed in tobacco and upon exposure to salt stress, transgenic plants showed significantly lesser photoinhibition compared to the wild-type plants (Al-Taweel et al. 2007). Very recently a gene named as *Delila* (*Del*), was isolated from snapdragon (*Antirrhinum majus*) and characterized through heterologous expression in

tobacco (Naing et al. 2017). Over-expression of *Del* gene in transgenic tobacco revealed enhanced anthocyanin accumulation as well as elevated antioxidant activity and additionally the transgenic tobacco plants showed improved salt tolerance. Thus, it can be concluded that over-production of several antioxidant enzymes or their combinatory effects might have positive involvement in salt tolerance.

2.4 Genetic Engineering for Ion Transporters

Several transporter proteins were found to possess pivotal role in maintaining osmotic regulation as well as ion homeostasis and recombinant expression of those proteins that depicted improved salt tolerance (Blumwald 2000). Over-expression of a Na^+/H^+ antiporters (*AtNHX1*) in *Arabidopsis* conferred enhanced salt tolerance (up to 200 mM NaCl) as compared to the control plant (Apse et al. 1999). Further studies revealed that over-expression of a vacuolar Na^+/H^+ antiporter in transgenic tomato could successfully produce fruit even at 200 mM NaCl (Zhang and Blumwald 2001). Roy et al. (2014) nicely reviewed several transgenic researches dealing with transporters or proton pumps especially involved in salt tolerance. Likewise, high affinity potassium transporters (*HKT*) were also found to play significant role in salt tolerance (Hauser and Horie 2010). Another study documented that over-expression of a barley transporter (HvHKT2; 1) in barley could reinforce the salt-accumulating mechanism in the transgenic plant (Mian et al. 2011). Salt overlay sensitive gene (*SOS1*) was found to be responsible for producing a membrane protein homologous to Na^+/H^+ transporter (Wu et al. 1996). Over-expression of *AtSOS1* from *Arabidopsis* in transgenic tobacco revealed significant salt tolerance in transgenic plants compared to the untransformed ones (Yue et al. 2012). In a different experiment, another SOS gene (*PtSOS2*) from poplar (*Populus trichocarpa*) was over-expressed in poplar tree and it was found that compared to the untransformed one, in the transgenic tree Na^+/H^+ exchange ratio as well as Na^+ efflux in the plasma membrane were significantly increased along with better ROS scavenging mechanism under salt stress condition (Yang et al. 2015). In this way the functional genomics study of several transporter proteins clearly demonstrated their involvement in salt tolerance.

2.5 Over-Expression of Proteins Having Physiological Significance (LEA, PR, Germin)

LEA proteins were mostly found to be expressed in the seed tissues of the plant and those are expressed at the late stages of the embryo development (Roberts et al. 1993). Over-expression of a barley LEA protein (*HVA1*) in transgenic rice depicted enhanced salt tolerance in transgenic plants (Xu et al. 1996). Later on another LEA protein from wheat (*DHN-5*) was heterologously expressed in *Arabidopsis* and the generated transgenic plants showed enhanced Na^+ and K^+ accumulation as well as

salt tolerance (Brini et al. 2007). Along with the proteins directly involved in salt tolerance or salt stress signaling, how LEA proteins are involved in controlling salt stress in plants has been nicely presented by Bhardwaj et al. (2013).

In addition to that along with LEA proteins, several pathogenesis related (PR) proteins were found to be associated with salt stress. Over-expression of a PR protein (PR-5) from *Arabidopsis* to *S. tuberosum* documented salt tolerance in transgenic plants (Evers et al. 1999). Similarly, heterologous expression of another PR protein (RSOsPR10) in bentgrass showed improved salt tolerance in transgenic plant (Takeuchi et al. 2016). Germin and germin-like proteins (GLPs) are somewhat similar to PR proteins and those proteins are involved in biotic as well as abiotic stress tolerance (Barman and Banerjee 2015). Over-expression of a germin gene possessing oxalate oxidase activity in *S. tuberosum* was found to show enhanced tolerance to salt stress (Turhan 2005).

2.6 Gene Silencing Approaches for Salt Tolerance in Plants

Although over-expression strategies were found to establish several salt-tolerant transgenic lines, there are instances where gene-silencing approaches have also been utilized for metabolic engineering to achieve salt tolerance in plants. Proline dehydrogenase is an enzyme that is responsible for catalyzing proline, an important osmoprotectant. Gene-silencing of proline dehydrogenase gene (*AtProDH*) through antisense RNA technology in *Arabidopsis* conferred constitutive accumulation of proline and improved tolerance to NaCl stress (600 mM NaCl) compared to untransformed as well as vector control plants (Nanjo et al. 1999). Unlike germin of wheat, a rice GPL was found to be negatively involved in salt tolerance. A recent study documented that RNAi-mediated gene-silencing of rice GPL 1 (*OsGLP1*) in rice showed enhanced salt tolerance in transgenic rice at early stages of growth and development as well as in rice calli (Banerjee et al. 2017).

It is clear that since long time several transgenic approaches have been taken either to over-express or down-regulate different target genes to confer salt tolerance. Further research is needed to get robust stress tolerant lines in farmers' field for the future because salt stress is an ever-increasing abiotic stress across the world.

3 Expression Studies Under Salt Stress (Some Case Studies)

The effects of salinity on gene expression have been evaluated in a variety of plants, profiling the global genes help us to understand this complex mechanism. Up-regulation of the *SOS1*, *SOS2*, *SOS3*, *HKT1*, *AKT1*, *NHX1*, *P5CS1*, *HSP90.7*, *HSP81.2*, *HSP71.1*, *HSPC025*, *OTS1*, *SGF29*, and *SAL1* genes cause tolerance in alfalfa genotypes (Sandhu et al. 2017). The *S*alt *O*verly *S*ensitive (SOS) signaling pathway mediates cellular signaling under salt stress, to maintain ion homeostasis

(Ji et al. 2013). It includes SOS3, SOS2, and SOS1. SOS3 is a Ca^{2+} sensor belonging to calcineurin B-like (CBL) protein family, SOS2 act as a serine–threonine protein kinase, belonging to the sucrose non-fermenting 1-related kinase 3 (SnRK3) family and SOS1 is characterized as a plasma membrane Na^+/H^+ antiporter (Sathee et al. 2015). *LEA* genes are a large gene family in plants that are mainly expressed in seeds of rice during salt stress (Gao and Lan 2016). The first category of salt-responsive genes is attributed to plant hormone and calcium signaling pathways and the second category are TFs. TFs including basic leucine zipper (bZIP), WRKY, APETALA2/ETHYLENE RESPONSE FACTOR (AP2/ERF), MYB, basic helix–loop–helix (bHLH), and NAC families will be active in response to salinity (Deinlein et al. 2014). The third category is linked to ion metabolism and transfer (Wang et al. 2018). Energy production and ion homeostasis associated proteins are produced under salt stress (Wang et al. 2009). Ion transporters, protective proteins involved in photosynthesis (D2 protein) and protective compounds (lignin) are activated during salinity (Kosová et al. 2013).

4 miRNA-Mediated Salt-Stress Regulation

Abiotic stress generated by mineral salts is one of the major natural limiting factors affecting crop growth and thus productivity. Salinity stress is a complex phenomena owing the plant species subjected to three types of stress conditions—water stress generated by the osmoticum, mineral toxicity due to the presence of salt and disturbances in the mineral nutrition of the plant. In order to cope up with the detrimental effects of salt stress, plants have developed various elaborate mechanisms to exclude excess salt from their cells or to tolerate salt within the cells (Munns and Tester 2008). Management practices like reclamation of salt, use of salt-tolerant genotypes, method and time of planting can be tailored to mitigate the problem to a considerable extent. In recent years, research works have been initiated to untangle the physiology and molecular mechanism behind the stress tolerance and also to identify the stress responsive proteins and their respective genetic network. Plant resistance to salt stress is controlled by various stress responsive gene complexes (Zhu 2003; De Costa et al. 2007). Precise expression of the genes and their accurate regulation, which is attained by multiple mechanisms at different levels such as transcriptional, post-transcriptional, and post-translational regulations (Mirlohi and He 2016) facilitate plant species to alleviate the problem of salt stress. In the post-transcriptional regulation recent trends are focusing on the role of small non-coding RNAs (sRNAs) categorically microRNAs (miRNAs) in the salinity and other abiotic stress tolerance. Post-transcriptional regulation of genes through small RNAs is known as RNA interference or RNA silencing. MicroRNAs (miRNAs) are short (21–24 nt) RNA molecules that control gene expression at the post-transcriptional level by cleavage of mRNA targets or by inhibition of their translation (Llave et al. 2002; Palatnik et al. 2003; Li et al. 2013). All the plant miRNA genes (MIRs) are originated from the RNA polymerase II (RNAPII) followed by splicing and

tailoring of the long primary RNA transcripts for production of mature and functional miRNA. The processing to pre-miRNA(s) occurs in the nucleus by Dicer Like-1 (DCL1), which then makes a cut of pre-miRNA(s) to liberate the miRNA together with its reverse complement, forming the miRNA–miRNA∗ (or miRNA5p–miRNA3p) duplex (Dolata et al. 2016). The site of this biogenesis process is the cell nucleus involving various protein complexes of RNase III type endoribonuclease family. Like other RNA polymerase II transcripts, the 5′ end of the miRNA primary precursor, is also protected by a specific cap structure that is recognized and bound by the nuclear cap-binding protein complex (CBC) consisting of two subunits: CBP20 and CBP80 (Hugouvieux et al. 2001; Kmieciak et al. 2002; Daszkowska-Golec et al. 2013). Similarly, the 3′ end contains a poly (A) tail after undergoing processing through polyadenylation machinery. After completion of processing the duplex, the miRNA unwound by a helicase in the cytoplasm to release the mature miRNA. Recent genetic and biochemical studies unravel the role of miRNA in the post-transcriptional down-regulation of gene expression through annealing to reverse complementary sequences owing to breakage or translational suppression of the target mRNAs (Fig. 1).

Moreover, these miRNAs encourage DNA methylation thus facilitating gene expression at transcription level. Recent studies reported that during stress, the miRNA could alter the expression of different stress responsive genes thus playing a vital role in plant resistance mechanism. The identification of plant miRNA families (miR156, miR159, miR164, miR171, etc.) began in the year 2000 (Llave et al. 2002;

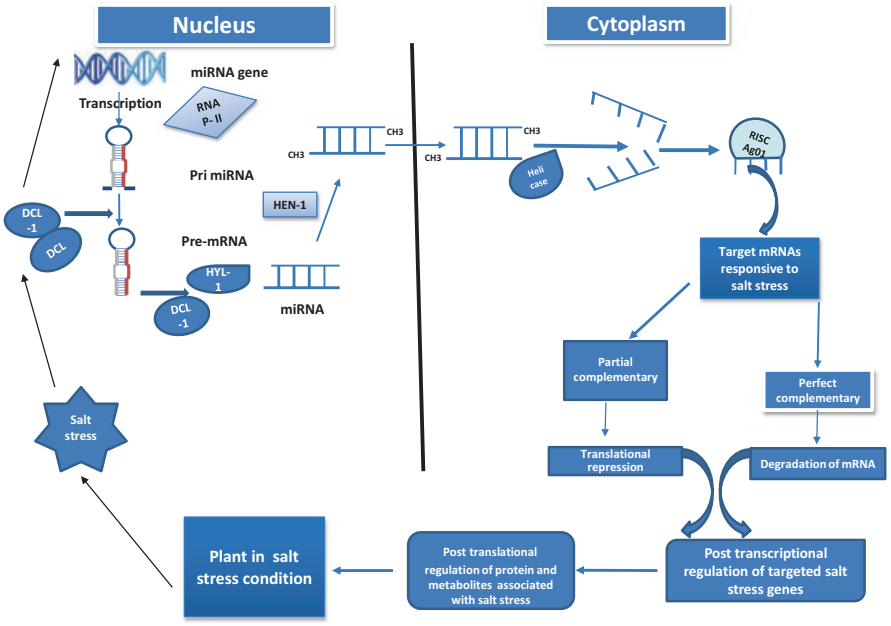

Fig. 1 Schematic illustration of post-transcriptional regulation of miRNA-mediated salt-stress-regulated genes (Source: Mangrauthia et al. 2013; modified by Authors)

Reinhart et al. 2002). Due to difficulties in cloning of miRNA followed by sequencing and prediction using various bioinformatics prediction tools the identification was not satisfactory through classical approaches. The progress of miRNA investigation was accelerated by the development of the next generation sequencing techniques (also called deep sequencing) and complex computational algorithms (Lai et al. 2003; Rajagopalan et al. 2006; Fahlgren et al. 2007; Jagadeeswaran et al. 2010; Rosewick et al. 2013). The first abiotic stress-related miRNAs in plants was reported by Sunkar and Zhu (2004). Later beside the genes several differentially regulated miRNAs have been identified in different plant species like *Arabidopsis*, *Glycine max*, *Glycine soja*, *Gossypium hirsutum*, *Medicago truncatula*, *Nicotiana tabacum*, *Oryza sativa*, *Panicum virgatum*, *Phaseolus vulgaris*, *Populus euphratica*, *Saccharum officinarum*, *Physcomitrella patens*, *Sorghum bicolour*, *Triticum aestivum*, and *Zea mays* under salt-stressed condition. The behaviour of several miRNAs under salt stress is similar to that reported in other species, suggesting a common regulatory mechanism operating widely across species. At present, 8496 miRs from 73 plant species are listed (Kozomara and Griffiths-Jones 2014). It was found that in response to salt stress, miR156, miR158, miR159, mi RNA160, miRNA164, mi RNA165, miRNA166, miR167, miR168, miR169, miR171, miRNA 172, miRNA1b, miRNA319, miR394, miR395, miR396, miR397, miR399 were regulated both in up- and down-regulatory pattern targeting squamosa promoter binding protein, cationic amino acid transporter, SPL TF, MYB TF, auxin response factor, DCL1, NAC family genes, SBP like TFs, APETALA2 like factor, Scarecrow like TF in different crop species like *A. thaliana* (Liu et al. 2008), *Zea mays* (Ding et al. 2009), *Vigna unguiculata* (Paul et al. 2011), *Saccharum* spp. (Bottino et al. 2013), and *Triticum aestivum* (Wang et al. 2014). However, the expression of miR160, miR393, miR4, miR402, miR417, miR482, miR1447, miR1507, and miR2118 were increased in response to salt stress in different crop species targeting F-box protein, protein kinase, APS-reductase, NBS-LRR resistance gene in *Arabidopsis* (Sunkar and Zhu 2004), *P. euphratica* (Qin et al. 2011) while the miR398 was down-regulated in *Arabidopsis*, thus establishing a role for miRNAs in the adaptive response to salt stress (Liu et al. 2008). A better understanding of miRNA-mediated gene regulation under salt stress will certainly help in elucidating the complex network of regulatory molecules, genes, proteins, and metabolites. Most miRNAs regulated the expression of multiple target genes belonging to the same gene family in plants. In response to salt stress, miR156 was found in up-regulated pattern in *A. thaliana* (Liu et al. 2008), *P. euphratica* (Qin et al. 2011), *Gossypium raimondii* (Xie et al. 2014), *P. virgatum* (Sun et al. 2012), and *V. unguiculata* (Paul et al. 2011) targeting squamosa promoter binding-like protein, cationic amino acid transporter, SPL binding protein, etc. However, down-regulation of the same group of miRNA was observed in *Zea mays* (Ding et al. 2009; Kong et al. 2010) regulating the target gene SPL-like TFs in relation to salt stress. The miRNA 158 was associated with salt stress in *A. thaliana* targeting the F-box family protein and pentatricopeptide repeat (PPR) containing protein (Liu et al. 2008). In case of miRNA159 down-regulation were observed in *A. thaliana* (Chen et al. 2012), *Nicotiana tabacum* (Frazier et al. 2011) targeting the MYB TF gene whereas up-regulation were observed *in P. virgatum*.

MiR160 targeted the auxin responsive factor in up-regulated fashion in case of *Vigna*, *Triticum*, and *Gossypium*. With the advancement of genomic techniques, a better understanding of the miRNA-mediated gene regulation targeting the expression of multiple regulatory genes and associated proteins under salt stress can be possible. This can provide a new arena to improve the resistance mechanism in plant under salt stress condition either through conventionally or through transgenic mechanism. It has been found that transgenic rice and *Arabidopsis* constitutively over-expressing osa-MIR396c-reduced salt and alkali stress tolerance compared to that of wild-type plants (Gao et al. 2010). Utilizing various computational and experimental approaches this untapped area can get new dimension regarding discovery of miRNA associated with regulating the expression of salt-stress-related genes. In future this will direct to find out new components regarding salt stress mechanism in plant.

5 Genome Editing for Salt Tolerance

Recently, the clustered regularly interspersed short palindromic repeats (CRISPR)/Cas9 technology has been proved as an efficient nuclease-based technology for genome editing which is the best alternative strategy for genetic engineering without having the conflict related with *Agrobacterium*-mediated or direct gene uptake strategies. This approach opens up new vista for editing each member of a gene family targeting multiple gene of interest without modifying the expression of other genes. The CRISPR are loci with variable short spacers interspersed by short repeats, later transcribed into non-coding RNAs (ncRNA). This ncRNA then forms a complex with the Cas and guides the complex to slice complementary target DNA. This system acting on very small desirable target sequence of 1–20 nt of the target gene need to be altered. The principle of this system is based on the function of an endonuclease, Cas9 protein, having two functional domains—RuvC-like domain and the HNH nuclease domain (Cong et al. 2013). This endonuclease enzyme is driven by a synthetic single guide RNA for recognition of its target sequences and ultimately produces double stranded breaks in the target sequences which consequently trigger DNA repairing mechanism and generate various site-specific genetic alterations through non-homologous end joining (NHEJ) or through homology-directed repair (HDR). These genetic alterations cause aberrations either through insertion or deletion and consequently generate frame-shift mutation thus regulating the expression of the functional domain of the target gene (Gratz et al. 2013; Zhou et al. 2014) (Fig. 2).

The CRISPR/Cas9-based genome editing system is easy to opt and cost-effective therefore having immense potential in future gene editing programme. Sometimes, instead of nuclease-based mechanism this system relies on the principle of nickase activity. In that case, the nuclease activity for one strand has been removed, leaving a nickase activity (Cong et al. 2013). Other recent developments include the use of a disarmed nuclease, lacking nuclease activity, which will competitively bind a

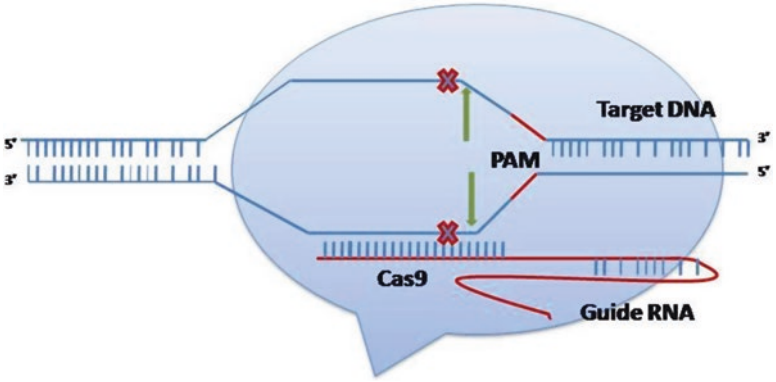

Fig. 2 Schematic illustration of CRISPR/Cas9 technology a new vista for genome editing

defined site to block the access of other molecules such as TFs and down-regulate or turn off the expression of a gene (Gilbert et al. 2013). Similarly, a CRISPR/Cas9 complex can retain transcriptional activators. Further bacterial genera have comparable genes to Cas9, some of which are now being analyzed and developed (Schiml et al. 2014; Zetsche et al. 2015), and further advances are expected to arise from all the variations on this theme (Schaeffer and Nakata 2015). CRISPR/Cas9 is a new emerging concept and has been considered safe for genome editing focusing on biotic and abiotic stress tolerance and creating new metabolites. Recently this system has been utilized in rice for achieving salt stress tolerance following *Arabidopsis*. The Δ 1-pyrroline-5-carboxylate synthetase (P5CS) is the rate-limiting enzyme in proline (Pro) synthesis and is involved in drought and salt-stress-tolerance in plants. In rice, an OsP5CS gene was isolated from a stress-treated commercial rice variety and based on the DNA isolated sequence, four gRNA (guide RNA) constructs were designed for CRISPR-Cas9 editing of the OsP5CS (*Outermost Cell-specific Gene5*) isolated from the commercial rice variety in order to increase the proline accumulation in cells (Jiang et al. 2013). This study laid the foundation for the development of high yielding rice varieties resistant to drought and salinity using genome editing technology. In the near future, this genome editing technology can be proved as a valuable tool in discovering the functions of signaling pathway components. The number of examples is increasing rapidly, and transmission to the next generation has been shown in several cases.

6 Conclusion

A number of studies were conducted on over-expression of candidate genes responsible for subsequently improved tolerance against salinity. Apart from the over-expression approaches that created various salt-tolerant transgenic lines, there were successful gene-silencing methods, utilized for metabolic engineering to attain salt tolerance in plants. Yet, there are ample scopes on use of over-expression ion channel,

antiporter, etc. from stress tolerant plants to salt sensitive crop plants. Nevertheless, a thorough screening is needed to identify if any toxic compound is being produced in the transgenic plants. Functional genomics or biotechnology has a great potential to combat such an ever-increasing salt stress problem to save agriculture.

Acknowledgments Authors acknowledge the library assistance from the Bidhan Chandra Krishi Viswavidyalaya, Mohanpur and Indian Institute of Technology Kharagpur, West Bengal, India. We further are thankful to the reviewer(s)/editor(s) of this chapter for their comments and suggestions on the manuscript.

References

AbdElgawad H, Zinta G, Hegab MM, Pandey R, Asard H, Abuelsoud W (2016) High salinity induces different oxidative stress and antioxidant responses in maize seedlings organs. Front Plant Sci 7:276

Al-Taweel K, Iwaki T, Yabuta Y, Shigeoka S, Murata N, Wadano A (2007) A bacterial transgene for catalase protects translation of D1 protein during exposure of salt-stressed tobacco leaves to strong light. Plant Physiol 145:258–265

Ali Y, Aslam Z, Ashraf MY, Tahir GR (2004) Effect of salinity on chlorophyll concentration, leaf area, yield and yield components of rice genotypes grown under saline environment. Int J Environ Sci Technol 1:221–225

Almansouri M, Kinet JM, Lutts S (2001) Effect of salt and osmotic stresses on germination in durum wheat (*Triticum durum* Desf.). Plant Soil 231:243–254

Apse MP, Aharon GS, Snedden WS, Blumwald E (1999) Salt tolerance conferred by overexpression of a vacuolar Na^+/H^+ antiport in *Arabidopsis*. Science 285:1256–1258

Banerjee J, Gantait S, Maiti MK (2017) Physiological role of rice germin-like protein 1 (OsGLP1) at early stages of growth and development in indica rice cultivar under salt stress condition. Plant Cell Tiss Org Cult 131:127–137

Banu MNA, Hoque MA, Watanabe-Sugimoto M, Matsuoka K, Nakamura Y, Shimoishi Y, Murata Y (2009) Proline and glycinebetaine induce antioxidant defense gene expression and suppress cell death in cultured tobacco cells under salt stress. J Plant Physiol 166:146–156

Barman AR, Banerjee J (2015) Versatility of germin-like proteins in their sequences, expressions, and functions. Funct Integr Genomics 15:533–548

Bhardwaj R, Sharma I, Kanwar M, Sharma R, Handa N, Kaur H, Kapoor D, Poonam RB (2013) LEA proteins in salt stress tolerance. In: Ahmad P, Azooz MM, Prasad MNV (eds) Salt stress in plants. Springer, New York, pp 79–112

Blumwald E (2000) Sodium transport and salt tolerance in plants. Curr Opin Cell Biol 12:431–434

Bottino MC, Rosario S, Grativol C, Thiebaut F, Rojas CA, Farrineli L, Hemerly AS, Ferreira PCG (2013) High-throughput sequencing of small RNA transcriptome reveals salt stress regulated microRNAs in sugarcane. PLoS One 8:e59423

Brini F, Hanin M, Lumbreras V, Amara I, Khoudi H, Hassairi A, Pagès M, Masmoudi K (2007) Overexpression of wheat dehydrin DHN-5 enhances tolerance to salt and osmotic stress in *Arabidopsis thaliana*. Plant Cell Rep 26:2017–2026

Cao YR, Chen SY, Zhang JS (2008) Ethylene signaling regulates salt stress response: an overview. Plant Signal Behav 3:761–763

Chaves MM, Flexas J, Pinheiro C (2009) Photosynthesis under drought and salt stress: regulation mechanisms from whole plant to cell. Ann Bot 103:551–560

Chen L, Wang T, Zhao M, Tian Q, Zhang WH (2012) Identification of aluminum-responsive microRNAs in *Medicago truncatula* by genome-wide high-throughput sequencing. Planta 235:375–386

Chinnusamy V, Jagendorf A, Zhu JK (2005) Understanding and improving salt tolerance in plants. Crop Sci 45:437–448

Cicek N, Cakirlar H (2002) The effect of salinity on some physiological parameters in two maize cultivars. Bulg J Plant Physiol 28:66–74

Cong L, Ran FA, Cox D, Lin S, Barretto R, Habib N, Hsu PD, Wu X, Jiang W, Marraffini LA, Zhang F (2013) Multiplex genome engineering using CRISPR/Cas systems. Science 339:819–823

Cong L, Zheng H-C, Zhang Y-X, Chai T-Y (2008) *Arabidopsis DREB1A* confers high salinity tolerance and regulates the expression of GA dioxygenases in Tobacco. Plant Sci 174:156–164

Daszkowska-Golec A, Wojnar W, Rosikiewicz M, Szarejko I, Maluszynski M, Szweykowska-Kulinska Z, Jarmolowski A (2013) *Arabidopsis* suppressor mutant of abh1 shows a new face of the already known players: ABH1 (CBP80) and ABI4-in response to ABA and abiotic stresses during seed germination. Plant Mol Biol 81:189–209

De Costa W, Zorb C, Hautung W, Schubert S (2007) Salt resistance is determined by osmotic adjustment and abscisic acid in newly developed maize hybrids in the first phase of salt stress. Physiol Plant 131:311–321

Deinlein U, Stephan AB, Horie T, Luo W, Xu G, Schroeder JI (2014) Plant salt-tolerance mechanisms. Trends Plant Sci 19:371–379

Deshnium P, Los DA, Hayashi H, Mustardy L, Murata N (1995) Transformation of Synechococcus with a gene for choline oxidase enhances tolerance to salt stress. Plant Mol Biol 29:897–907

Ding D, Zhang L, Wang H, Liu Z, Zhang Z, Zheng Y (2009) Differential expression of miRNAs in response to salt stress in maize roots. Ann Bot 103:29–38

Dolata J, Bajczyk M, Bielewicz D, Niedojadlo K, Niedojadlo J, Pietrykowska H, Walczak W, Szweykowska-Kulinska Z, Jarmolowski A (2016) Salt stress reveals a new role for ARGONAUTE1 in miRNA biogenesis at the transcriptional and posttranscriptional levels. Plant Physiol 172:297–312

Eltayeb AE, Kawano N, Badawi GH, Kaminaka H, Sanekata T, Shibahara T, Inanaga S, Tanaka K (2007) Overexpression of monodehydroascorbate reductase in transgenic tobacco confers enhanced tolerance to ozone, salt and polyethylene glycol stresses. Planta 225:1255–1264

Evers D, Overney S, Simon P, Greppin H, Hausman JF (1999) Salt tolerance of *Solanum tuberosum* L overexpressing an heterologous osmotin-like protein. Biol Plant 42:105–112

Fahlgren N, Howell MD, Kasschau KD, Chapman EJ, Sullivan CM, Cumbie JS, Givan SA, Law TF, Grant SR, Dangl JL, Carrington JC (2007) High-throughput sequencing of *Arabidopsis* microRNAs: evidence for frequent birth and death of MIRNA genes. PLoS One 2:e219

Frazier TP, Sun G, Burklew CE, Zhang B (2011) Salt and drought stresses induce the aberrant expression of microRNA genes in tobacco. Mol Biotechnol 49:159–165

Gao J, Lan T (2016) Functional characterization of the late embryogenesis abundant (LEA) protein gene family from *Pinus tabuliformis* (Pinaceae) in *Escherichia coli*. Sci Rep 6:19467

Gao M, Tao R, Miura K, Dandekar AM, Sugiura A (2001) Transformation of Japanese persimmon (*Diospyros kaki* Thunb.) with apple cDNA encoding NADP-dependent sorbitol-6-phosphate dehydrogenase. Plant Sci 160:837–845

Gao P, Bai X, Yang L, Lv D, Li Y, Cai H, Ji W, Guo D, Zhu Y (2010) Over-expression of *osa-MIR396c* decreases salt and alkali stress tolerance. Planta 231:991–1001

Garg AK, Kim JK, Owens TG, Ranwala AP, Choi YD, Kochian LV, Wu RJ (2002) Trehalose accumulation in rice plants confers high tolerance levels to different abiotic stresses. Proc Natl Acad Sci U S A 99:15898–15903

Ge LF, Chao DY, Shi M, Zhu MZ, Gao JP, Lin HX (2008) Overexpression of the trehalose-6-phosphate phosphatase gene *OsTPP1* confers stress tolerance in rice and results in the activation of stress responsive genes. Planta 228:191–201

Gharsallah C, Fakhfakh H, Grubb D, Gorsane F (2016) Effect of salt stress on ion concentration, proline content, antioxidant enzyme activities and gene expression in tomato cultivars. AoB Plants 8:plw055

Gilbert LA, Larson MH, Morsut L, Liu Z, Brar GA, Torres SE, Stern-Ginossar N, Brandman O, Whitehead EH, Doudna JA, Lim WA (2013) CRISPR-mediated modular RNA-guided regulation of transcription in eukaryotes. Cell 154:442–451

Gratz SJ, Cummings AM, Nguyen JN, Hamm DC, Donohue LK, Harrison MM, Wildonger J, O'Connor-Giles KM (2013) Genome engineering of Drosophila with the CRISPR RNA-guided Cas9 nuclease. Genetics 194:1029–1035

Han KH, Hwang CH (2003) Salt tolerance enhanced by transformation of a P5CS gene in carrot. J Plant Biotechnol 5:149–153

Han X, Feng Z, Xing D, Yang Q, Wang R, Qi L, Li G (2015) Two NAC transcription factors from *Caragana intermedia* altered salt tolerance of the transgenic *Arabidopsis*. BMC Plant Biol 15:208

Hauser F, Horie T (2010) A conserved primary salt tolerance mechanism mediated by HKT transporters: a mechanism for sodium exclusion and maintenance of high K^+/Na^+ ratio in leaves during salinity stress. Plant Cell Environ 33:552–565

Hayashi H, Alia Mustardy L, Deshnium P, Ida M, Murata N (1997) Transformation of *Arabidopsis thaliana* with the *codA* gene for choline oxidase; accumulation of glycinebetaine and enhanced tolerance to salt and cold stress. Plant J 12:133–142

Hmida-Sayari A, Gargouri-Bouzid R, Bidani A, Jaoua L, Savouré A, Jaoua S (2005) Overexpression of Δ1-pyrroline-5-carboxylate synthetase increases proline production and confers salt tolerance in transgenic potato plants. Plant Sci 169:746–752

Hong Y, Zhang H, Huang L, Li D, Song F (2016) Overexpression of a stress-responsive NAC transcription factor gene *ONAC022* improves drought and salt tolerance in rice. Front Plant Sci 7:4

Hong Z, Lakkineni K, Zhang K, Verma DPS (2000) Removal of feedback inhibition of D1-pyrroline-5-carboxylate synthetase results in increased proline accumulation and protection of plants from osmotic stress. Plant Physiol 122:1129–1136

Hu HH, You J, Fang YJ, Zhu XY, Qi ZY, Xiong LZ (2008) Characterization of transcription factor gene *SNAC2* conferring cold and salt tolerance in rice. Plant Mol Biol 67:169–181

Hugouvieux V, Kwak JM, Schroeder JI (2001) An mRNA cap binding protein, ABH1, modulates early abscisic acid signal transduction in *Arabidopsis*. Cell 106:477–487

Jagadeeswaran G, Zheng Y, Sumathipala N, Jiang H, Arrese EL, Soulages JL, Zhang W, Sunkar R (2010) Deep sequencing of small RNA libraries reveals dynamic regulation of conserved and novel microRNAs and microRNA-stars during silkworm development. BMC Genomics 11:52

Ji H, Pardo JM, Batelli G, Van Oosten MJ, Bressan RA, Li X (2013) The Salt Overly Sensitive (SOS) pathway: established and emerging roles. Mol Plant 6:275–286

Jiang Q, Hu Z, Zhang H, Ma Y (2014) Overexpression of *GmDREB1* improves salt tolerance in transgenic wheat and leaf protein response to high salinity. Crop J 2:120–131

Jiang W, Zhou H, Honghao B, Fromm M, Yang B, Weeks DP (2013) Demonstration of CRISPR/Cas9/sgRNA-mediated targeted gene modification in *Arabidopsis*, tobacco, sorghum and rice. Nucleic Acids Res 41:e188

Jing J, Li H, He G, Yin Y, Liu M, Liu B, Qiao G, Lin S, Xie L, Zhuo R (2013) Over-expression of the *codA* gene by *Rd29A* promoter improves salt tolerance in *Nicotiana tabacum*. Pak J Bot 45:821–827

Kaya C, Tuna AL, Yokaş I (2009) The role of plant hormones in plants under salinity stress. In: Salinity and water stress. Springer, Dordrecht, pp 45–50

Kaymakanova M (2009) Effect of salinity on germination and seed physiology in bean (*Phaseolus vulgaris* L.). Biotechnol Biotechnol Equip 23:326–329

Khan MS, Ahmada D, Khan MA (2015) Utilization of genes encoding osmoprotectants in transgenic plants for enhanced abiotic stress tolerance. Electron J Biotechnol 18:257–266

Kishor KPB, Hong Z, Miao GH, Hu CAA, Verma DPS (1995) Overexpression of D1-pyrroline-5-carboxylate synthetase increase proline production and confers osmotolerance in transgenic plants. Plant Physiol 108:1387–1394

Kmieciak M, Simpson CG, Lewandowska D, Brown JW, Jarmolowski A (2002) Cloning and characterization of two subunits of *Arabidopsis thaliana* nuclear cap-binding complex. Gene 283:171–183

Kong Y, Elling A, Chen B, Deng X (2010) Differential expression of microRNAs in maize inbred and hybrid lines during salt and drought stress. Am J Plant Sci 1:69–76

Kosová K, Prášil IT, Vítámvás P (2013) Protein contribution to plant salinity response and tolerance acquisition. Int J Mol Sci 14:6757–6789

Kozomara A, Griffiths-Jones S (2014) miRBase: annotating high confidence microRNAs using deep-sequencing data. Nucleic Acids Res 42:D68–D73

Lai EC, Tomancak P, Williams RW, Rubin GM (2003) Computational identification of Drosophila microRNA genes. Genome Biol 4:R42

Li HW, Zang BS, Deng XW, Wang XP (2011) Overexpression of the trehalose-6-phosphate synthase gene *OsTPS1* enhances abiotic stress tolerance in rice. Planta 234:1007–1018

Li S, Liu L, Zhuang X, Yu Y, Liu X, Cui X, Ji L, Pan Z, Cao X, Mo B, Zhang F, Raikhel N (2013) MicroRNAs inhibit the translation of target mRNAs on the endoplasmic reticulum in *Arabidopsis*. Cell 153:562–574

Liu HH, Tian X, Li YJ, Wu CA, Zheng CC (2008) Microarray-based analysis of stress-regulated microRNAs in *Arabidopsis thaliana*. RNA 14:836–843

Llave C, Kasschau KD, Rector MA, Carrington JC (2002) Endogenous and silencing-associated small RNAs in plants. Plant Cell 14:1605–1619

Majee M, Maitra S, Ghosh Dastidar K, Pattnaik S, Chatterjee A, Hait N, Das KP, Majumder AL (2004) A novel salt-tolerant L-*myo*-inositol 1-phosphate synthase from *Porteresia coarctata* Tateoka, a halophytic wild rice: molecular cloning, bacterial overexpression, characterization and functional introgression into tobacco conferring salt-tolerance phenotype. J Biol Chem 279:28539–28552

Mangrauthia SK, Agarwal S, Sailaja B, Madhav MS, Voleti SR (2013) MicroRNAs and their role in salt stress response in plants. In: Salt stress in plants. Springer, New York, pp 15–46

Mian A, Oomen RJ, Isayenkov S, Sentenac H, Maathuis FJ, Véry AA (2011) Over-expression of an Na^+-and K^+-permeable HKT transporter in barley improves salt tolerance. Plant J 68:468–479

Mirlohi S, He Y (2016) Small RNAs in plant response to abiotic stress. In: Shankar AK, Shankar C (eds) Biotic and biotic stress in plants - recent advances and future perspectives. Intech, Croatia, pp 63–80

Munns R, Tester M (2008) Mechanisms of salinity tolerance. Annu Rev Plant Biol 59:651–681

Naing AH, Park KI, Ai TN, Chung MY, Han JS, Kang YW, Lim KB, Kim CK (2017) Overexpression of snapdragon Delila (*Del*) gene in tobacco enhances anthocyanin accumulation and abiotic stress tolerance. BMC Plant Biol 17:65

Nanjo T, Kobayashia M, Yoshibab Y, Kakubaric Y, Yamaguchi-Shinozaki K, Shinozaki K (1999) Antisense suppression of proline degradation improves tolerance to freezing and salinity in *Arabidopsis thaliana*. FEBS Lett 461:205–210

Nomura M, Ishitani M, Takabe T, Tai AK, Takabe T (1995) Synechococcus sp. PCC 7942 transformed with Escherichia coli bet genes produces glycine betaine from choline and acquires resistance to salt stress. Plant Physiol 107:703–708

Palatnik JF, Allen E, Wu X, Schommer C, Schwab R, Carrington JC, Weigel D (2003) Control of leaf morphogenesis by microRNAs. Nature 425:257–263

Parida AK, Das AB (2005) Salt tolerance and salinity effects on plants: a review. Ecotoxicol Environ Saf 60:324–349

Paul S, Kundu A, Pal A (2011) Identification and validation of conserved microRNAs along with their differential expression in roots of *Vigna unguiculata* grown under salt stress. Plant Cell Tiss Org Cult 105:233–242

Pons R, Cornejo MJ, Sanz A (2013) Is ABA involved in tolerance responses to salinity by affecting cytoplasm ion homeostasis in rice cell lines? Plant Physiol Biochem 62:88–94

Qin Y, Duan Z, Xia X, Yin W (2011) Expression profiles of precursor and mature microRNAs under dehydration and high salinity shock in *Populus euphratica*. Plant Cell Rep 30:1893–1907

Rajagopalan R, Vaucheret H, Trejo J, Bartel DP (2006) A diverse and evolutionarily fluid set of microRNAs in *Arabidopsis thaliana*. Genes Dev 20:3407–3425

Reinhart BJ, Weinstein EG, Rhoades MW, Bartel B, Bartel DP (2002) MicroRNAs in plants. Genes Dev 16:1616–2166

Roberts JK, DeSimone NA, Lingle WL, Dure L III (1993) Cellular concentrations and uniformity of cell-type accumulation of two lea proteins in cotton embryos. Plant Cell 5:769–780

Rosewick N, Momont M, Durkin K, Takeda H, Caiment F, Cleuter Y, Vernin C, Mortreux F, Wattel E, Burny A, Georges M, Van den Broeke A (2013) Deep sequencing reveals abundant non-canonical retroviral microRNAs in B-cell leukemia/lymphoma. Proc Natl Acad Sci U S A 110:2306–2311

Roxas VP, Smith RK Jr, Allen ER, Allen RD (1997) Overexpression of glutathione S-transferase/glutathione peroxidase enhances the growth of transgenic tobacco seedlings during stress. Nat Biotechnol 15:988–991

Roy S (2016) Function of MYB domain transcription factors in abiotic stress and epigenetic control of stress response in plant genome. Plant Signal Behav 11:e1117723

Roy SJ, Negrão S, Tester M (2014) Salt resistant crop plants. Curr Opin Biotechnol 26:115–124

Sakamoto A, Murata N (2001) The use of bacterial choline oxidase, a glycinebetaine-synthesizing enzyme, to create stress resistant transgenic plants. Plant Physiol 125:180–188

Sandhu D, Cornacchione MV, Ferreira JF, Suarez DL (2017) Variable salinity responses of 12 alfalfa genotypes and comparative expression analyses of salt-response genes. Sci Rep 7:42958

Sathee L, Sairam RK, Chinnusamy V, Jha SK (2015) Differential transcript abundance of salt overly sensitive (SOS) pathway genes is a determinant of salinity stress tolerance of wheat. Acta Physiol Plant 37:169

Sawahel WA, Hassan AH (2002) Generation of transgenic wheat plants producing high levels of the osmoprotectant proline. Biotechnol Lett 24:721–725

Schaeffer SM, Nakata PA (2015) CRISPR/Cas9-mediated genome editing and gene replacement in plants: transitioning from lab to field. Plant Sci 240:130–142

Schiml S, Fauser F, Puchta H (2014) The CRISPR/Cas system can be used as nuclease for in planta gene targeting and as paired nickases for directed mutagenesis in *Arabidopsis* resulting in heritable progeny. Plant J 80:1139–1150

Su J, Hirji R, Zhang L, He C, Selvaraj G, Wu R (2006) Evaluation of the stress-inducible production of choline oxidase in transgenic rice as a strategy for producing the stressprotectant glycine betaine. J Exp Bot 57:1129–1135

Sudhir P, Murthy SDS (2004) Effects of salt stress on basic processes of photosynthesis. Photosynthetica 42:481–486

Sun G, Stewart CN, Xiao P, Zhang B (2012) MicroRNA expression analysis in the cellulosic biofuel crop switchgrass (*Panicum virgatum*) under abiotic stress. PLoS One 7:e32017

Sunkar R, Zhu JK (2004) Novel and stress-regulated microRNAs and other small RNAs from *Arabidopsis*. Plant Cell 16:2001–2019

Takeuchi K, Takeuchi K, Hasegawa H, Gyohda A, Komatsu S, Okamoto T, Okada K, Terakawa T, Koshiba T (2016) Overexpression of *RSOsPR10*, a root-specific rice PR10 gene, confers tolerance against drought stress in rice and drought and salt stresses in bentgrass. Plant Cell Tiss Org Cult 127:35–46

Tarczynski MC, Jensen RG, Bohnert HJ (1993) Stress protection of transgenic tobacco by production of the osmolyte mannitol. Science 259:508–510

Tavakkoli E, Fatehi F, Coventry S, Rengasamy P, McDonald GK (2011) Additive effects of Na^+ and Cl^- ions on barley growth under salinity stress. J Exp Bot 62:2189–2203

Tavakkoli E, Rengasamy P, McDonald GK (2010) High concentrations of Na^+ and Cl^- ions in soil solution have simultaneous detrimental effects on growth of faba bean under salinity stress. J Exp Bot 61:4449–4459

Trachootham D, Lu W, Ogasawara MA, Valle NRD, Huang P (2008) Redox regulation of cell survival. Antioxid Redox Signal 10:1343–1374

Turhan H (2005) Salinity response of transgenic potato genotypes expressing the oxalate oxidase gene. Turk J Agric For 29:187–195

Uddin MN, Hossain MA, Burritt DJ (2016) Salinity and drought stress: similarities and differences in oxidative responses and cellular redox regulation. In: Ahmad P (ed) Water stress and crop plants: a sustainable approach, vol 1. Wiley, Chichester, UK, pp 86–101

Wang B, Sun YF, Song N, Wei JP, Wang XJ, Feng H, Yin ZY, Kang ZS (2014) MicroRNAs involving in cold, wounding and salt stresses in *Triticum aestivum* L. Plant Physiol Biochem 80:90–96

Wang J, Zhu J, Zhang Y, Fan F, Li W, Wang F, Zhong W, Wang C, Yang J (2018) Comparative transcriptome analysis reveals molecular response to salinity stress of salt-tolerant and sensitive genotypes of indica rice at seedling stage. Sci Rep 8:2085

Wang X, Fan P, Song H, Chen X, Li X, Li Y (2009) Comparative proteomic analysis of differentially expressed proteins in shoots of *Salicornia europaea* under different salinity. J Proteome Res 8:3331–3345

Wu SJ, Ding L, Zhu JK (1996) SOS1, a genetic locus essential for salt tolerance and potassium acquisition. Plant Cell 8:617–627

Xie F, Wang Q, Sun R, Zhang B (2014) Deep sequencing reveals important roles of microRNAs in response to drought and salinity stress in cotton. J Exp Bot 66:789–804

Xiong H, Li J, Liu P, Duan J, Zhao Y, Guo X, Li Y, Zhang H, Ali J, Li Z (2014) Overexpression of *OsMYB48-1*, a novel MYB-related transcription factor, enhances drought and salinity tolerance in rice. PLoS One 9:e92913

Xu D, Duan X, Wang B, Hong B, Ho THD, Wu R (1996) Expression of a late embryogenesis abundant protein gene HVA1, from barley confers tolerance to water deficit and salt stress in transgenic rice. Plant Physiol 110:249–257

Yan N, Marschner P, Cao W, Zuo C, Qin W (2015) Influence of salinity and water content on soil microorganisms. Int Soil Water Conserv Res 3:316–323

Yancey PH (1994) Compatible and counteracting solutes. In: Strange SK (ed) Cellular and molecular physiology of cell volume regulation. CRC Press, Boca Raton, FL, pp 81–109

Yang A, Xiaoyan DX, Zhang WH (2012) A R2R3-type MYB gene, *OsMYB2*, is involved in salt, cold, and dehydration tolerance in rice. J Exp Bot 63:2541–2556

Yang X, Liang Z, Wen X, Lu C (2008) Genetic engineering of the biosynthesis of glycinebetaine leads to increased tolerance of photosynthesis to salt stress in transgenic tobacco plants. Plant Mol Biol 66:73–86

Yang Y, Tang R-J, Jiang C-M, Li B, Kang T, Liu H, Zhao N, Ma X-J, Yang L, Chen S-L, Zhang H-X (2015) Overexpression of the *PtSOS2* gene improves tolerance to salt stress in transgenic poplar plants. Plant Biotechnol J 13:962–973

Yu X, Liang C, Chen J, Qi X, Liu Y, Li W (2015) The effects of salinity stress on morphological characteristics, mineral nutrient accumulation and essential oil yield and composition in *Mentha canadensis* L. Sci Hortic 197:579–583

Yue Y, Zhang M, Zhang J, Duan L, Li Z (2012) SOS1 gene overexpression increased salt tolerance in transgenic tobacco by maintaining a higher $K^{(+)}/Na^{(+)}$ ratio. J Plant Physiol 169:255–261

Zapata PJ, Serrano M, García-Legaz MF, Pretel MT, Botella MA (2017) Short term effect of salt shock on ethylene and polyamines depends on plant salt sensitivity. Front Plant Sci 8:855

Zetsche B, Gootenberg JS, Abudayyeh OO, Slaymaker IM, Makarova KS, Essletzbichler P, Volz SE, Joung J, van der Oost J, Regev A, Koonin EV (2015) Cpf1 is a single RNA-guided endonuclease of a class 2 CRISPR-Cas system. Cell 163:759–771

Zhang HX, Blumwald E (2001) Transgenic salt-tolerant tomato plants accumulate salt in foliage but not in fruit. Nat Biotechnol 19:765–768

Zhang L, Ma H, Chen T, Pen J, Yu S, Zhao X (2014a) Morphological and physiological responses of cotton (*Gossypium hirsutum* L.) plants to salinity. PLoS One 9:e112807

Zhang X, Tang W, Liu J, Liu Y (2014b) Co-expression of rice *OsP5CS1* and *OsP5CS2* genes in transgenic tobacco resulted in elevated proline biosynthesis and enhanced abiotic stress tolerance. Chin J Appl Environ Biol 2014:717–722

Zheng XN, Zhen B, Lu GJ, Han B (2009) Overexpression of a NAC transcription factor enhances rice drought and salt tolerance. Biochem Biophys Res Commun 379:985–989

Zhou H, Liu B, Weeks DP, Spalding MH, Yang B (2014) Large chromosomal deletions and heritable small genetic changes induced by CRISPR/Cas9 in rice. Nucleic Acids Res 42:10903–10914

Zhu B, Su J, Chang M, Verma DPS, Fan YL, Wu R (1998) Overexpression of a Δ1-pyrroline-5-carboxylate synthase gene and analysis of tolerance to water and salt stress in transgenic rice. Plant Sci 139:41–48

Zhu JK (2003) Regulation of ion homeostasis under salt stress. Curr Opin Plant Biol 6:441–445