Engineering Salinity Tolerance in Plants: Progress and Prospects

Shabir H. Wani[1*], Vinay Kumar[2,3], Tushar Khare[2], Rajasheker Guddimalli[4], Maheshwari Parveda[4], Katalin Solymosi[5], Penna Suprasanna[6], Kavi Kishor PB[4]

[1]Mountain Research Centre for Field Crops, Khudwani, Anantnag-192 101, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, J&K, India, ORCiD: 0000-0002-7456-4090

[2]Department of Biotechnology, Modern College, Savitribai Phule Pune University, Ganeshkhind, Pune 411 016, India,

[3]Department of Environmental Science, Savitribai Phule Pune University, Ganeshkhind, Pune 411 016, India ORCiD: 0000-0002-9569-2411

[4]Department of Genetics, Osmania University, Hyderabad 500 007, India

[5]Department of Plant Anatomy, Institute of Biology, ELTE - Eötvös Loránd University, Budapest 1053, Hungary, ORCiD: 0000-0001-5246-2547

[6]Nuclear Agriculture and Biotechnology Division, Bhabha Atomic Research Centre, Trombay, Mumbai 400 085, India, ORCiD: 0000-0002-6572-6190

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*Corresponding author:
Shabir Hussain Wani
[1]Mountain Research Centre for Field Crops, Khudwani, Anantnag-192 101, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, J&K, India, ORCiD: 0000-0002-7456-4090
shabirhussainwani@gmail.com
Phone: +917006907220

Abstract

Soil salinity exerts significant constraints on global crop production, posing a serious challenge for plant breeders and biotechnologists. The classical transgenic approach for enhancing salinity tolerance in plants revolves by boosting endogenous defence mechanisms, often via a single gene approach, and usually involves the enhanced synthesis of compatible osmolytes, antioxidants, polyamines, maintenance of hormone homeostasis, modification of transporters and/or regulatory proteins, including transcription factors (TFs) and alternative splicing events. Occasionally, genetic manipulation of regulatory proteins or phytohormone levels confers salinity-tolerance, but all these may cause undesired reduction in plant growth and/or yields. In this review, we present and evaluate novel and cutting-edge approaches for engineering salt tolerance in crop plants. First, we cover recent findings regarding the importance of regulatory proteins and transporters, and how they can be used to enhance salt tolerance in crop plants. We also evaluate the importance of halobiomes as a reservoir of genes that can be used for engineering salt-tolerance in glycophytic crops. Additionally, the role of microRNAs as critical post-transcriptional regulators in plant adaptive responses to salt stress are reviewed and their use for engineering salt-tolerant crop plants is critically assessed. The potentials of alternative splicing mechanisms and targeted gene-editing technologies in understanding plant salt-stress responses and developing salt-tolerant crop plants is also discussed.

Keywords

CRISPR/Cas9, halobiome, ion transporters, microRNAs, regulatory elements, salinity stress, transcription factors

1 Introduction

Extreme soil salinity is one of the most destructive abiotic stresses for global agriculture as it can lead to the degradation of arable soils, particularly those that are heavily irrigated (Akram et al. 2017; Kumar et al. 2017a). Over 800 million ha of land are estimated to be affected by salinity worldwide and 32 million ha of dryland agriculture is thought to be salt-affected (FAO 2015). Salinity stress drastically reduces agricultural productivity via adverse impacts on seed germination, plant growth and development, plant vigour and crop yields (Cheeseman 2015). The reduced plant growth caused by high soil salinity is due to salinity-induced water stress, oxidative stress, nutritional imbalances, ion toxicity, membrane disorganization, reduced cell division and expansion, disruption of key metabolic processes, and genotoxicity. The goal of most studies dealing with salinity tolerance is to boost the capacity of crop plants to maintain growth and productivity when cultivated on saline soils (Cao et al. 2018; Kumar et al. 2018). As salinization of cultivated land is an increasing global problem, understanding the biological impacts of salt exposure on plants and the development of salt-tolerant crop plants is urgently required (Munns et al. 2012; Latef et al. 2017; Nguyen et al. 2017). Of the various approaches that have been and are being utilized to enable crop production in salinity affected areas, crop improvement by breeding is perhaps one of the best strategies to accomplish this goal. The use of conventional plant breeding methods resulted in significant yield improvement in some crops when grown on saline soils and this resulted in the development of salinity-tolerant crops. However, conventional plant breeding approaches are most often lengthy, laborious and are dependent on access to germplasm with sufficient genetic variability (Wani et al. 2016). For some crops, genetic engineering strategies provide a viable alternative to conventional plant breeding and are now becoming more widely used throughout the world to produce salt-tolerant cultivars.

In the past few decades, plant genetic engineering approaches for the production of salt-tolerant plants revolved around manipulating single genes from a diverse range of metabolic pathways, including compatible-solute synthesis, ion-homeostasis and antioxidant synthesis/metabolism, as well as signaling/regulatory elements including transcription factors (TFs) (Cabello et al. 2014; Zhang et al. 2016). Although these single-gene manipulation strategies achieved some success, both strategies have disadvantages and limitations. Single-gene manipulation does not seem to be ideal because salinity tolerance is a complex trait probably influenced by several genes and factors concomitantly. On the other hand, modifying signaling and regulatory pathways requires precise and detailed knowledge on their functioning, and often plants with improved tolerance do not perform well under unstressed conditions. In contrast to drought, soil salinity is a stressor that is constantly present in the soil, however, the intensity of the stress as well as the response of the plants at different developmental stages or organs also greatly varies. Therefore, identifying novel approaches to produce salt-tolerant plants, such as co-expression of multiple-genes, epigenetic control of gene-expression and targeting post-transcriptional modifications (small/micro-RNAs) are required (Shriram et al. 2016; Kumar et al. 2017b). Although many reviews were published focusing on transgenic strategies that can potentially be used to produce salt-tolerant crop plants, in this review, we discuss and critically evaluate from a practical perspective both developments in conventional plant breeding and novel genetic engineering methods that can be used to develop salt-tolerant crops. Besides evaluating the potential genes from various metabolic pathways that confer salt-tolerance in both model and crop plants, we also discuss how genetic modification of signalling/regulatory elements (including TFs), epigenetic control of gene-expression, post-transcriptional modifications (small/micro-RNAs), and genome editing technologies help to produce plants with targeted genetic engineering (CRISPR-Cas).

2 Engineering regulatory elements for increased salt tolerance

As plants live in highly variable environments, they have evolved mechanisms that enable them to rapidly respond to changing environmental conditions including soil salinity. It is necessary to understand these mechanisms at the whole-plant, organ, tissue, cellular, physiological and molecular levels. When plants encounter adverse conditions such as salinity, a variety of genes with various functions can be upregulated or downregulated, and any resultant changes in developmental and/or physiological processes can contribute to stress tolerance (Tran and Mochida 2010; Mochida et al. 2011). While some of these upregulated or downregulated genes encode proteins that play critical roles in stress associated growth or metabolic changes, many other genes encode regulatory proteins such as TFs, which control salt-sensing and signal transduction pathways and the expression of a range of salinity stress-responsive genes (Gupta and Huang 2014; Li and Tran 2017; Mann et al. 2019). TFs play a vital role in connecting the salt-sensory pathways to the various genes required for plant salt-tolerance (Nishiyama et al.

2012). These genes play critical roles in regulating plant stress responses, to various abiotic stress factors including salinity (Joshi et al. 2016; Wang et al. 2016a). Several studies reported the development of transgenic plants with modified TF expression that improved salt-tolerance (Joshi et al. 2016). Also, the TF families ERF/AP2, bZIP, MYB, MYC, NAC, WRKY, and zinc-finger proteins were shown to have regulatory roles associated with plant stress-responses (Kazan, 2015; Sun et al. 2016).

As demonstrated in many studies of various plant species, stress-responsive TFs function in conjunction with the promoter regions to regulate the expression of salt-stress responsive genes involved in salt-tolerance. For example, transgenic *Oryza sativa* lines over-expressing OsDREB2A were relatively tolerant to salinity compared to their wild-type counterparts (Mallikarjuna et al. 2011). Expression of the rice TF SALT-RESPONSIVE ERF1 (*SERF1*), resulted in root-specific stimulation in response to salt and hydrogen peroxide (H_2O_2) treatments (Schmidt et al. 2013). In the absence of SERF1, disruption of a salinity-induced MAPK cascade involved in acquisition of salt-tolerance occurs. It was shown that SERF1 binds to the promoters of *MAP3K6, MAPK5, DREB2A*, and *ZFP179* genes, and constitutive overexpression of *SERF1* enhances salinity tolerance in rice plants (Schmidt et al. 2013). In addition, transgenic *Arabidopsis* plants expressing TF gene *OsAP21* and *SbAP37* exhibited better growth than wild-type plants under salt/drought/temperature stress conditions (Jin et al. 2013; Maheshwari et al. 2017). In a similar study, the *HhBREB2* gene belonging to AP2/EREBP TF family was isolated from *Halimodendron halodendron.* This gene was placed into the A-5 cluster of the DREB subfamily, on the basis of its similarity to the AP2/ERF domain. Overexpression of the *HhBREB2* gene in *Arabidopsis* led to increased salt and drought tolerance in the transgenic plants, thus providing evidence that *HhBREB2* is an important TF involved in the regulation of salinity associated signalling in plants (Ma et al. 2015b).

MYB-type TFs play diverse roles in plant development and plant responses to abiotic stress. Transgenic rice plants overexpressing *OsMYB2*, an R2R3-type MYB gene, were more tolerant to salt, cold, and dehydration than wild-type plants (Yang et al. 2012). *OsMYB2*-overexpressing plants accumulated greater amounts of soluble sugars and proline, and exhibited enhanced upregulation of genes encoding proline synthesis and transporter proteins than wild-type plants. Greater upregulation of stress-related genes such as *OsLEA3, OsRab16A* and *OsDREB2A*, were noticed in *OsMYB2*-overexpressing plants. Besides, overexpression of

OsMYB48-1, a novel MYB-related TF in rice plants, enhanced the tolerance to drought stress imposed by mannitol and PEG, and to salinity stress (Xiong et al. 2014). It was shown that the ARS1 gene, which encodes an R1-MYB type TF, had increased expression in the leaves of salt stressed tomato plants and that the role of ARS1 was related to reduced transpirational water loss in plants under salinity stress (Campos et al. 2016). Another family of TFs shown to be involved in salt-tolerance are the NAC proteins (*NAM*, no apical meristem; *ATAF*, Arabidopsis transcription activation factor and CUC, Cup-shaped cotyledon). This TF mega-family was one of the first plant-specific TF families to be identified and in addition to their involvement in the regulation of plant development, NAC proteins are believed to be involved in various abiotic stress responses, including salinity and drought (Tran et al. 2010; Rahman et al. 2016). The role of the rice *SNAC1* gene was investigated using transgenic wheat where the *SNAC1* gene was placed under the control of a maize ubiquitin promoter (Saad et al. 2013). It was concluded that drought and salt-tolerance were enhanced in these transgenic wheat plants (Saad et al. 2013). A NAC gene from wheat was also characterized for a possible role in drought, salinity and freezing tolerance, where a *TaNAC67-GFP* gene fusion was introduced into *Arabidopsis* under the control of *CaMV*-35S promoter and transgenic plants were examined at both physiological and morphological levels under various abiotic stresses (Mao et al. 2014). These transgenics showed improved tolerance to drought, salt and freezing stresses (Mao et al. 2014). In another study, TaNAC29 isolated from wheat was introduced into *Arabidopsis* using the *Agrobacterium tumefaciens*-mediated floral dip method (Huang et al. 2015). The greenhouse-grown transgenics showed enhanced tolerance to salt and drought stresses (Huang et al. 2015). Likewise, SNAC1 gene from rice was transferred using *A. tumefaciens* into *Boehmeria nivea* and the resulting transgenics displayed increased tolerance towards salt and drought stresses, both at the seedling and fiber maturation stages (An et al. 2015). A classic stress-responsive NAC TF CarNAC4 from chickpea was characterized and its function was studied in transgenic *Arabidopsis*. Transgenics exhibited enhanced tolerance to drought and salinity, by stimulating other stress-responsive genes including *RD29A*, *ERD10*, *COR15A, COR47*, *KIN1* and *DREB2A* (Yu et al. 2016), thus confirming the role of *CarNAC4* as a TF involved in the regulation of salt and drought stresses. Thus, the above studies confirmed the importance of *NAC* genes in salinity and drought stress tolerance. However, genetic engineering of stress related regulatory networks can result in salt and drought tolerance in crop plants, but this approach has the potential to cause pleiotropic effects and reduce yields in plants grown in the absence of stress or eventually in the presence of other (e. g. biotic) stresses. Moreover, these methods have the disadvantage of not yet available for several important crops including wheat (*Triticum aestivum*). To overcome any potentially negative impacts associated with engineering, TFs alongside the use of conditional or tissue-specific promoters should be considered (Cabello et al. 2014). In addition, methodological developments have to be achieved in case of genetically and molecularly more challenging crops.

3 Manipulating ion transport and transporters

Salt stress impacts plant growth and crop productivity by causing hyperosmotic stress, resulting in decreased water potential which limits water uptake and thus cell expansion, and on a longer time-scale by hyperionic stress, i.e. toxicity due to excess Na^+ and Cl^- ion concentrations (Maathuis et al. 2014). While Cl^- is an essential micronutrient, it can be toxic to plants at higher concentrations (Khare et al. 2015). Information on Cl^- transport and genetic engineering specifically for Cl^- tolerance is inadequate and so is not discussed below. Although the mechanisms involved in plant salt-tolerance are not yet fully understood, it is clear that the control of water movement through aquaporin channels (Martinez-Ballesta and Carvajal 2014), and ion transport and transporters play crucial roles in plant salt-tolerance and that the mechanisms that regulate water and ion transport may be suitable targets for the production of salt tolerant crops (Rahman et al. 2017). Na^+ and Cl^- ions are first taken up by the outer root cells, then transported to the root xylem and finally from the root to the shoot, where they may be stored in vacuoles or in the apoplastic space, or possibly recirculated back to the root system or to older leaves that are less active (Figure 1). In some salt tolerant plant species, Na^+ and Cl^- ions can also be excreted via special structures, which take the form of glands or bladders (Shabala et al. 2014). Salt-tolerance mechanisms also include mitigation of the effects of ion-induced stress by the production of osmoprotectants and compatible solutes (glycine betaine, mannitol, ononitol, trehalose, polyamines), proteins (stress and heat shock proteins, late embryogenesis abundant proteins, signalization and hormone modulating factors), antioxidants and antioxidant enzymes that prevent oxidative damage (Gupta and Huang 2014). However, production of the above can have high energy costs and thus reduce the crop yields. Key factors associated with the prevention of ion toxicity under salt stress are (i) restriction of Na^+ and Cl^- uptake, (ii) restriction of Na^+ and Cl^- transport (iii) maintenance of beneficial ion homeostasis e.g. that of K^+, which is an essential nutrient for plant growth and development. For example, a high K^+/Na^+ ratio can be maintained by inhibition of NaCl-induced K^+ efflux from the cytoplasm (Kumar and Khare 2016).

Although the biochemical and biotechnological targeting of the Na^+ uptake system of plants at the root level (i.e. soil-epidermis interface) would seem very promising, there are only few attempts related to it. This is probably due to (i) the complexity of Na^+ uptake involving several not well characterized components like non-selective cation channels (*NSCC*) and high-affinity potassium transporters (*HKT*) and (ii) its interference with the uptake of other essential cations, especially K^+ (e.g. at *AKT1, AtHAK5* transporters). The passive influx of Na^+ into the cytoplasm cannot be fully prevented under salt stress; therefore, other salt tolerance mechanisms evolved in plants to decrease ion toxicity within the cells. These include its (i) active export (futile cycling) from root cells by plasma membrane (PM) Na^+/H^+ antiporters (e.g. *SOS1*, *NHA1* or *NHXLP*), or its (ii) exclusion from the cytoplasm to other intracellular compartments (like vacuoles, plastids or endosomes) with the help of NHX-type vacuolar $Na^+(K^+)/H^+$ antiporters both at the root and the shoot (especially leaf) level or both mechanisms (Tables 1 and 2). The SOS system also has important roles in the complex regulatory networks activated under stress (Ji et al. 2013). PM located H^+-ATPases (e.g. *AHA1*/2/3 - **Figure 1**) and H^+-pyrophosphatases (Wang et al. 2016b) are important to regulate ion uptake at the root level or exclusion in leaf mesophyll cells. In many species salt tolerance mechanisms also include long-distance transport processes or especially their inhibition (**Figure 1**).

This review focuses on recent studies that involve the genetic modification of plants with single or multiple ion transport components to improve salt tolerance (**Figure 1; Tables 1** and **2**). For Na^+ and Cl^- transport and transporters in plants following reviews give excellent information (Hasegawa 2013; Maathuis et al. 2014; Nieves-Cordones et al. 2016; Almeida et al. 2017). Most of the transporters have tissue- and organ-dependent expression patterns in many species. Several homologs of them (e.g. *NHX* and *HKT*) are present and differently expressed in various intracellular compartments, and the expression of different transport components is often mutually regulated in a complex manner (Plett et al. 2010; Yadav et al. 2012; Gouiaa and Khoudi, 2015; Ma et al. 2017; Hamamoto et al. 2015; Almeida et al. 2017) and also depending on the developmental stage and the strength of the stress (Zhang et al. 2017). Therefore, the exact impact of genetic engineering of transport components needs to be carefully assessed and analyzed for each species and in the complex context of plant nutrition.

3.1 Membrane transport and salt stress tolerance

PM proton (H^+)-ATPases, vacuolar membrane H^+-ATPases, PM and vacuolar membrane H^+-pyrophosphatases (H^+-PPases) constitute proton pumps in plants. Besides proton pumps, Na^+ and K^+ transporters also play a vital role during salt stress tolerance. Their functions and the transgenics developed utilizing these genes are briefly described below.

3.1.1 Proton pumps and salt stress tolerance

Plant H^+ pumps play primary roles for the transport of ions and solutes across cell membranes. In plants, three major H^+ transport proteins [PM H^+-ATPase, vacuolar H^+-ATPase and PM and vacuolar H^+-pyrophosphatases (later called VPPases)] were detected and found to be associated with salt stress tolerance. It is known that H^+-pumps produce an electrochemical potential gradient which is the motive force that is essential for root nutrient uptake, stomatal aperture, phloem loading and cell growth (Blumwald et al. 2000; Gaxiola et al. 2007; Mansour 2014).

3.1.1.1 Plasma membrane H^+-ATPase

PM H^+-ATPases or P-type ATPases (P-ATPases) are encoded by a large gene family. These transporters couple ATP hydrolysis with H^+ transport at the membrane level (Gaxiola et al. 2007, Fuglsang et al. 2010). Mansour (2014) noticed that high activity of P-ATPase under salt stress repolarizes the NaCl-induced depolarization of PM and is associated with salt stress tolerance. Indeed, higher P-ATPase enzyme activity and salt stress tolerance was noticed in halophytes (Mansour 2014). Sun et al. (2009) pointed out P-type ATPases reduce Na^+ influx and K^+ efflux and maintain proper K^+/Na^+ levels and is also associated with the movement of stomatal aperture (Zhang et al. 2001; Gaxiola et al. 2007). Bose et al. (2015) found out that PM H^+-ATPase activity is higher in halophytes in comparison with glycophytes under salt stress conditions indicating its role in salinity tolerance. Vitart et al. (2001) observed a reduction in growth in a PM H^+-ATPase *aha4* mutant *Arabidopsis* when exposed to salt stress. Gevaudant et al. (2007) demonstrated increased salt tolerance in transgenic tobacco plants expressing a PM H^+-ATPase, lacking the auto-inhibitory domain. However, the ubiquitous and not tissue specific expression of the active proton pump only increased salt tolerance during germination and early growth of seedlings, and caused altered development of the adult plants (Gevaudant et al. 2007) and cell expansion in another experiment (Niczyj et al. 2016), and thus does not represent a promising tool to develop salt tolerant crops. A PM H^+-ATPase4 gene (*PMA4*) isolated from *Nicotiana plumbaginifolia* when overexpressed in tobacco showed no difference in growth under normal conditions, but, impaired sucrose translocation, stomatal opening, plant growth and male fertility were noticed when the endogenous and the transgene PMA4 were co-suppressed (Zhao et al. 2000). Zhang et al. (2014) overexpressed the PM H^+-ATPase of the salt-tolerant *Chloris virgata* (*ChvPMA* and *ChvPMAΔC*) in genetically modified yeast and found enhanced resistance to salt and lower pH conditions. Moreover, the yeast overexpressing *ChvPMAΔC* displayed better growth than *ChvPMA* at an external pH 4.0 in the presence of NaCl. Transgenic *A. thaliana* containing the transgene *ChvPMAΔC* exhibited better root growth than plants containing *ChvPMA* in the presence of NaCl stress (Zhang et al. 2014). Recently, Fan et al. (2018) overexpressed a PM H^+-ATPase (*SpAHA1*) isolated from the halophyte *Sesuvium portulacastrum* in *Arabidopsis thaliana* that conferred salt tolerance by improving seed germination ratio, root growth, and biomass of transgenics. In addition to improved ion homeostasis, transgenic plants displayed lower oxidative stress (Fan et al. 2018). The above studies indicate the important yet not fully understood roles of PM H^+-ATPase in imparting salt stress tolerance.

3.1.1.2 Vacuolar membrane H^+-ATPase (V-ATPase)

It is a multisubunit enzyme complex (encoded by many genes), composed of two subcomplexes (V1 and V0) that help in H^+ translocation (Gaxiola et al. 2007). Jiang et al. (2010) and Bassil and Blumwald (2014) noted that V-ATPase provides the driving force for the vacuolar compartmentalization of Na^+. V-ATPase is highly abundant in the tonoplast and its activity is modulated in order to cope with environmental changes (Ratajczak 2000). Enhanced V-ATPase activity as well as upregulation of transcript levels of some subunits was reported under salt stress by Kirsch et al. (1996) and Silva and Geros (2009). Klychnikov et al. (2007) found out that 14-3-3 proteins (activators of PM ATPases) interact with some of the subunits (VHA-A) in a phosphorylation dependent way in *Hordeum vulgare* for the regulation of V-ATPase activity. Dietz et al. (2001) showed the functional significance of V-ATPase in the ability of plants to show resistance to abiotic stress. Golldack and Dietz (2000), Baisakh et al. (2008) demonstrated upregulation of V-ATPase during early stages of salt stress indicating its involvement in the process. In response to salt stress, *VHA-A* transcript was upregulated in tobacco (Narasimhan et al. 1991), sugarbeet (Lehr et al. 1999) and wheat (Golldack et al. 2001). Higher transcript levels of *VHA-B* were noticed under salt stress in *Mesembryanthemum crystallinum* (Golldack and Dietz 2000), and wheat (Wang et al. 2011). Likewise, *VHA-C* gene was found triggered by salt stress in *M. crystallinum* (Kluge et al. 2003), and *Pennisetum glaucum* (Tyagi et al. 2005). However, studies on the role of orthologous expression of genes encoding V-ATPase subunits on the ability of plants to withstand salt stress are scarce though RNAi mutants were studied by Padmanaban et al. (2004). Gaxiola et al. (2001) pointed out that overexpression of *VHA* would not be easy since it consists of multisubunit complex that needs to be expressed at the correct level. Baisakh et al. (2012) showed enhanced salt stress tolerance of rice transgenics expressing a vacuolar H^+-ATPase subunit c1 (*SaVHAc1*) gene from the halophyte grass *Spartina alternifolia*. They noticed high accumulation of Na^+ levels in roots and leaves, and yet the plants were not affected by the toxic Na^+ ions. This could be due to the sequestration of Na^+ ions at the tonoplast by Na^+-H^+-antiporter that was energized by a proton motive force created by the overexpression of the above gene as pointed out by Apse et al. (1999). Schumacher et al. (1999) demonstrated that V-ATPase is involved in plant development and signaling as evident from the V-ATPase mutant, det3, which reduced subunit C transcript. Xu et al. (2011), Wang et al. (2011) and Zhang et al. (2014b) overexpressed subunit *C1* of VHA from *Limonium bicolor*, subunits *B* and *E* of VHA from *Triticum aestivum*, respectively, and found improved salt tolerance in tobacco (Xu et al. 2011) and *Arabidopsis* (Wang et al. 2011; Zhang et al. 2014b). It is clear from the studies of Baisakh et al. (2012) that *SaVHAc1* (isolated from *Spartina alterniflora*) is involved in cell expansion and maintenance of net photosynthesis (i.e. higher chlorophyll content in transgenics in comparison with wild-type plants) with higher root and leaf growths and yields under salt stress. Zhang et al. (2013) showed that RNAi-directed downregulation of vacuolar H^+-ATPase subunit-*α* results in enhanced stomatal aperture and density in rice. He et al. (2014) reported high expression levels of *A*, *C*, *D*, *F* and *α*-subunits under salt stress conditions. They also overexpressed wheat V-H^+-ATPase subunit genes that imparted salt tolerance significantly to the transgenic *Arabidopsis thaliana* plants. Further, Dong et al. (2015) found that ectopic expression of subunit *A* of *MdVHA-A* (vacuolar H^+-ATPase subunit A) isolated from *Malus domestica* enhanced the salt tolerance in tobacco. Wang et al. (2016a) showed that overexpression of a vacuolar-type HC-ATPase C subunit gene from *Iris lactea* (*IrlVHA*-c), enhanced the salt tolerance in tobacco. Liu et al. (2018) demonstrated that the gene encoding subunit A of the vacuolar H^+-ATPase from cotton (*GhVHA-A*) plays an important role in conferring tolerance to water deficit. The above studies also infer that the genes isolated from halophytes could be effectively utilized for alleviating salt stress in the crop plants.

3.1.1.3 PM and vacuolar membrane bound H^+PPases

In plants, pyrophosphate (PPi) is produced as a by-product during the activation or polymerization steps of many biosynthetic pathways. It is hydrolyzed by soluble pyrophosphatase (H^+-PPase) enzyme in the plastids (Weiner et al. 1987). On the other hand, cytosol of higher plants contains very little PPi. Zhen et al. (1997) found out that cytosolic PPi acts as an energy source for energizing the vacuolar membrane via PPi-dependent proton pump. H^+-PPases are hydrophobic single subunit proteins unlike that of PM and V-ATPases. They generate H^+ gradient across the vacuole, Golgi and PM using the energy generated during hydrolysis of PPi (Gaxiola et al. 2007). While Silva et al. (2009) showed that V-H^+-PPase activity decreases when plants are exposed to salt stress, enhanced activity was also recorded in several taxa (Parks et al. 2002; Queirós et al. 2009). H^+-PPases are of two types (type I and type II). For their activity, type I H^+-PPases require cytosolic K^+ and are sensitive to inhibition by Ca^{2+}. On the other hand, type II H^+-PPases are insensitive to K^+ ions but Ca^{2+} sensitive. Type I H^+-PPases acidify the vacuole (Gaxiola et al. 2007) and are also noticed in the PM (Alexandersson et al. 2004). Maeshima (2000) showed that vacuolar H^+-PPase activity is upregulated under salt stress conditions. Gene that encodes vacuolar H^+-pyrophosphatase (*VPPase*) was cloned both from dicots (*Arabidopsis*) as well as monocots (*Sorghum bicolor*) and overexpressed in diverse plants like *Arabidopsis*, finger millet etc. (Guo et al. 2006; Anjaneyulu et al. 2014; Pizzio et al. 2015). Such transgenics displayed both salt and drought stress tolerance (Sarafian et al. 1992, Gaxiola et al. 2001, Guo et al. 2006, Pizzio et al. 2015). The tolerance to salt and water stresses in these transgenic plants is due to increased solute uptake into vacuoles as evident from the measurements of Na^+ and K^+ ions (Anjaneyulu et al. 2014). The above studies infer that VPPase is an important gene involved in H^+ translocation and abiotic stress tolerance. The regulation of V-H^+-ATPase as well as V-H^+-PPase activities by salt stress is reported, but correlative pattern is missing for activation or deactivation of these proton pumps in response to salt stress and water deficit. It appears that P-ATPases, V-ATPases and H^+-PPases are regulated by a common mechanism and 14-3-3 proteins are known to regulate many membranes localized proteins such as ion pumps (Bunney et al. 2002). Taken together, all the three ion pumps seem to play a pivotal role during salt stress in higher plants, but their engineering is challenging (especially in case of the multisubunit V-ATPase). Also, their use to improve crop yields under glasshouse or field conditions remains questionable.

3.2 Na^+-H^+-antiporters (NHX), sodium-proton exchangers (NHE), and salt stress

Transport of Na^+ into the plant cells is mediated by non-selective cation channels (*NSCCs*) that do not have selectivity for cations. Demidchik and Maathuis (2007) pointed out that these are permeable to many monovalent cations at the PM tonoplast level. *NSCCs* are of many types such as depolarization-activated (voltage-dependent), hyper-polarization-activated (voltage-independent), reactive oxygen species (ROS)-activated *NSCCs*, amino acid-activated *NSCCs*, cyclic nucleotide-gated (CNG) *NSCCs*, etc. Apse and Blumwald (2007) and Zhang et al. (2010) suggest that Na^+ influx across the PM occurs via *NSCC*/*VIC* in root cortical cells.

NHX transporters are modulated by and hence associated with salt stress, long-distance transport of Na^+ from root to shoot, protein targeting and trafficking and even functioning of stomata as pointed out by Bassil et al. (2011a, 2011b), Barragan et al. (2012), and Wu et al. (2016). *AtNHX1* is the first reported plant transporter that mediates Na^+ transport into vacuoles (Apse et al., 1999). Further, *AtNHX1* or salt overly sensitive pathway (SOS) gene *AtSOS1* overexpression in tomato resulted in better salt tolerance (Apse et al. 1999, Shi et al., 2003). At the same, it was shown that salt or Na^+ does not accumulate in the fruit, but accumulates only in the foliage of tomato (Zhang and Blumwald, 2001; Shi et al., 2003). Liu and Zhu (1998) noticed that a calcium sensor homolog is required for salt tolerance. Later, Liu et al. (2000) demonstrated that in *Arabidopsis thaliana*, *SOS2* gene encodes a protein kinase required for salt stress tolerance. Subsequent studies revealed that the regulation of Na^+/H^+ exchange in *A. thaliana* is carried out by the SOS pathway (Qiu et al. 2004). Besides *NHX* family members, *NHE*-type protein family members (both PM and organellar membrane-bound) were identified in plants that may perform regulation of intracellular pH and cell volume (Counillon and Pouyssegur 2000). The roles of all NHX members (8 in most eukaryotes) during salt stress were amply demonstrated in many transgenics (Apse et al. 1999; Apse and Blumwald 2007; Shi et al. 2008; Kronzucker and Britto 2011; Bassil et al. 2011a; Yadav et al. 2012). But the numbers of *NHE* members that exist in higher plants except in *S. bicolor* (9), the functions of *NHE* family members (during salt stress), and their tissue specific expressions are not completely known though redundant functions are suggested. Recently, Kumari et al. (2018) showed that their tissue specific expressions under salt and drought stress conditions in *Sorghum bicolor*, suggesting their tissue specific role during abiotic stress. Further, *NHX*-like proteins were detected earlier, and one such protein bound to the PM was isolated from *S. bicolor* and overexpressed in tomato (Kumari et al. 2017). Transgenic tomato plants displayed better salt stress tolerance in comparison with wild-type plants. But it is obscure how many such *NHX*-like proteins exist in higher plants, where they are localized intracellularly and whether they have any role in development and abiotic stress tolerance.

3.1.3 Transport of Na^+ in chloroplasts and salt stress tolerance

In C_4 plants, Na^+ is transported into the chloroplasts for the synthesis of phosphoenolpyruvate (PEP) for photosynthetic CO_2 fixation. In C_4 plants, a Na^+/pyruvate symporter BASS2 (bile acid/sodium symporter family protein 2), helps in co-transport of Na^+ and pyruvate (Figure 1). Na^+ is exported out by a sodium hydrogen antiporter *NHD1* in such plants (Furumoto et al. 2011). On the other hand, in C_3 plants, Na^+ accumulation in chloroplasts inhibits photosynthesis and thus may be detrimental to their survival. Müller et al. (2014) used knock out mutants (NHD1) of *A. thaliana* and demonstrated that such plants are not able to export Na^+, photosynthesis is impaired in them and they are unable to tolerate NaCl stress. This indicates that Na^+ homeostasis in chloroplasts may be important for plant survival as well as to their photosynthetic activity and thus productivity and yield.

It appears that halophytes overcome stomatal limitation by switching to CO_2 concentrating mechanism under saline conditions. Number of chloroplasts also increases per cell in halophytes and salt entry into chloroplast stroma appears necessary for the formation of grana and PSII (Bose et al. 2017). Such a phenomenon has not yet been reported in glycophytes so far. Halophytes accumulate more Cl^- ions and use it in functional roles but not glycophytes. Several studies characterized a total of 53 salt-responsive genes encoding chloroplast localized proteins. This implies that multiple pathways like thylakoid membrane organization, activity of PS II, assimilation of CO_2, photorespiration, ROS scavenging, osmotic and ion homeostasis and ABA biosynthesis and signaling are triggered in chloroplasts in response to salt stress (Suo et al. 2017).

Tonoplast localized K^+ channel (*TPK1*) was found crucial for maintaining intracellular K^+/Na^+ ratio in *Arabidopsis*. Latz et al. (2013) showed that salt stress triggers phosphorylation of the *Arabidopsis* vacuolar K^+ channel *TPK1* by calcium-dependent protein kinase (*CDPK*) and modulates cytosolic K^+ influx under NaCl stress conditions. Carraretto et al. (2016) demonstrated that TPK3 is localized to the chloroplast membranes and mediates K^+ homeostasis, an important event for plant fitness. It is essential for us now to understand the intricate mechanisms how these transporters move both cations and anions such as Na^+, K^+ and Cl^- across the chloroplast envelope membrane and thylakoids (reviewed in Szabó and Spetea 2017) and their precise regulation of PSI and PSII activities under salt stress conditions.

3.1.4 Potassium transporters and salt stress

K^+ is an essential nutrient for plant metabolism, therefore, the detrimental effects of salt stress are often associated with disturbances in the K^+ uptake and thus intracellular K^+ homeostasis or the K^+/Na^+ ratio (Amtmann and Beilby 2010, Srivastava et al. 2020). PM located H^+ pumps are involved in creating a pH gradient and thus contribute to the maintenance of membrane potential providing the driving force for essential ion (K^+) uptake through voltage-gated channels and also for the exclusion of Na^+ in exchange for H^+ on plasma membrane located Na^+/H^+ exchangers such as *SOS1* (Bose et al. 2015). On the other hand, voltage-independent channels with increased K^+/Na^+ selectivity and thus reduced Na^+ uptake may contribute to improved salt tolerance as shown in the case of the ‘salt excluder’ *Thellungiella salsuginea* (previously *T. halophila*) when compared with *Arabidopsis*, its glycophyte relative (Volkov et al. 2004; Volkov and Amtmann 2007, Amtmann and Beilby 2010). Biochemical and biotechnological targeting of these channels to restrict Na^+ uptake is also beneficial for the K^+/Na^+ homeostasis as it does not impact the negative membrane potential and thus the selective uptake of K^+ via inward rectifying K^+ channels (Amtmann and Beilby 2010). Influx of Na^+ (or K^+) into the root cells may also occur on some members of the high-affinity potassium (K^+) transporter (*HKT*) family (Kronzucker and Britto 2011). Data on cultivars with different salinity tolerance have shown that genetic modification (i.e. partial deletion or point mutations, etc.) of *HKT1* (now termed *HKT2*;1) may contribute to improved K^+/Na^+ selectivity, altered activity and thus increased salt tolerance (Cotsaftis et al. 2012).

Transgenic expression of a selective, K^+-inward-rectifying channel (KIRC) of the halophyte *Puccinellia tenuis* (*PutAKT1*) in *Arabidopsis* resulted in decreased shoot and root Na^+ content and thus enhanced salt tolerance (Ardie et al. 2010), while under salt stress the level of *AKT1* expression was in general downregulated especially in salt excluder cultivars (Golldack et al. 2003, Fuchs et al. 2005). These and other data (Wang et al. 2007) indicate that some halophytes can tolerate salt by being able to maintain high K^+ uptake and simultaneously restrict Na^+ influx to the root cells (Ardie et al. 2010).

Taken together, a high number of ion transport components (alone or simultaneously with other components) have been genetically modified to improve salt tolerance (**Tables 1, 2**). However, no GM crop has been commercialized on the basis of these data (Fita et al. 2015). This is due to the fact, that complex interactions, mutual and tissue- or cell-specific regulations and competitions of the uptake systems of essential ions need to be considered and may all need to be targeted individually or simultaneously but specifically for enhanced salt tolerance. Therefore, due to the complex nature of plant ion homeostasis and regulatory networks that operate it, the development of transgenics or crop plants with better tolerance is quite challenging and not very promising at the moment in this field.

4 RNA interference (RNAi) technologies for the production of salt-tolerant plants

RNAi is a biological process by which small RNA (sRNA) molecules stop the translation of targeted mRNAs and thereby inhibit or alter gene expression. RNAi is regarded as an important mechanism involved in the post-transcriptional regulation of gene expression in plants (Shriram et al. 2016; Kumar et al. 2017b). Plants have evolved a complex set of interfering non-coding RNA (ncRNA) species, including the microRNAs (miRNAs) and small interfering RNAs (siRNAs), to silence targeted genes very precisely and without affecting the expression of other genes. In this review, only miRNAs are covered. These small ncRNAs are the products of dsRNAs cleaved by DICER or DCL enzyme-mediated systems. ncRNAs when coupled with RISC and argonaute proteins lead to RNAi (Saurabh et al. 2014). RNA mediated gene silencing plays pivotal roles in orchestrating the expression, stability and inheritance of plant genomes, as well as in defenses against biotic and abiotic stressors. Numerous investigations demonstrated that RNAi can function as a master regulator of the regulatory networks associated with the expression of stress related genes (Sunkar et al. 2012; Lotfi et al. 2017). Selected salt responsive plant miRNAs, their targets and the traits regulated by the respective miRNAs are presented in **Figure 2**.

RNAi is emerging as a powerful tool for targeted gene silencing or as a means to alter gene expression for the improvement of crop plants. Owing to the development of other genomic tools such as deep sequencing and degradome analysis, coupled with computational tools, an increasing number miRNAs involved in growth/developmental processes, and stress-responses in plants are being identified and analysed for their functionality. Major databases and computational tools to identify miRNAs and predict their target genes are presented in **Table 3**. Approaches to regulate the expression of genes by the overexpression or knock-down of specific miRNAs offer a novel and potentially effective approach to engineer salinity tolerance into crop plants are gaining momentum (Zhang 2015; Khare et al. 2018). Recent studies have confirmed that manipulating miRNA gene(s) significantly improves crop plant tolerance to environmental stresses (Leng et al. 2017; Yang et al. 2017). Over-expressing or repressing stress responsive-miRNAs or their targets, miRNA-resistant target genes and miRNAs all have potential for increasing the stress tolerance of crop plants. Overexpression of the *gma-miR172* transcript improved salt tolerance of *Arabidopsis* plants, with higher germination and cotyledon greening rates, and longer roots (Li et al. 2015). Transgenic plants showed 73-78% germination rate, with 72-77% seedlings turning green, at 150 mM NaCl. In contrast, wild-type plants showed only 63% germination and 57.6% greening rates, at the same NaCl level (Li et al. 2015) and it has been demonstrated that salt stress inhibits chlorophyll biosynthesis (Abdelkader et al. 2007). Pan et al. (2016) reported roles for *miR172a* in enhanced salinity tolerance and long-distance stress signalling. They investigated the growth of *miR172a* hairy root lines and *miR172a* deficient lines under 200 mM NaCl salt stress. Overexpression of *osa-miR528* in transgenic *Agrostis stolonifera* resulted in shortened internodes, a higher number of tillers, better water retention, membrane integrity, and K^+ homeostasis, improved salt tolerance and higher antioxidant enzyme activities compared to wild-type plants (Yuan et al. 2015). Furthermore, ectopic expression of *miR398* genes was associated with oxidative stress tolerance in grapevine (Leng et al. 2017). In contrast, Yang et al. (2017) showed that the overexpression of *osa-miR171c* reduced the salt tolerance of transgenic rice. These authors concluded that *osa-miR171c* was involved in the regulation of stomatal development, several physiological changes, the expression of stress-related genes and ABA-dependent signalling pathways, and that it was by regulating these processes that *osa-miR171c* influenced the salt tolerance of rice (Yang et al. 2017). The above studies demonstrate the potential of miRNA-based manipulations to enhance plant salt-tolerance and the fact that genes encoding miRNAs represent potential targets for engineering salt tolerance in glycophytic crop plants. However, further research is required to field evaluate these transgenics for their yield potential and also to better understand species-specific differences in miRNA-transformed transgenic plants.

5. QTLomics of salt tolerance

Identification and analysis of quantitative trait loci (QTL) has progressed phenomenally towards marker development and crop breeding applications (Ismail and Horie 2017; Cobb et al. 2019). It has become feasible to characterize at the molecular level the genes involved in novel traits associated with abiotic stress tolerance, especially salt tolerance. This has become possible owing to the extensive physiological and molecular studies on tolerance to various abiotic (ionic and/or osmotic stresses) and information gateway on stress specific as well as shared stress adaptation mechanisms. Successful efforts have been made for the identification of important, novel QTLs in rice, cotton, soybean and other crops. A significant finding has been the identification of salt-tolerance associated QTLs, especially seedling stage Saltol QTL which is associated with Na^+/K^+ homeostasis under salt stress (Ren et al. 2005; Thompson et al. 2010). Another QTL which is contained within Saltol is the SKC1 associated with shoot K^+ concentration (Ren et al. 2005). In rice, a QTL, Saltol, was inrrogressed into an elite salinity sensitive rice cv Pusa Basmati 1121 for achieving seedling-stage salt stress tolerance. The newly bred line showed superior agronomic performance and better seedling stage tolerance to salinity (Babu et al. 2017a). Several rice breeding programs across the globe have successfully adopted this QTL for marker assisted breeding programs aimed at developing tolerant lines.

QTLs other than Saltol from Hasawi rice genotype have also been used for adoption into breeding programs to derive rice inbred lines which led to the construction of a genetic map and the identification of twenty new QTLs for salt tolerance (Bizimana et al. 2017). In addition to rice, QTLs have also been analyzed for breeding salt tolerance in other crops. SSR markers have been used to scan crop genomes to identify markers associated with salt tolerance in cotton

(Zhao et al. 2016) and cucumber (Kere et al. 2017). In maize, Luo et al. (2017) mapped the QTLs for salt tolerance using a doubled haploid population and high-density SNP markers. The authors isolated major QTLs for salt tolerance and identified two candidate genes involved in ion homeostasis. In soybean, Guan et al. (2014) used fine-mapping method in land races and wild germplasm to isolate *GmSALT3*, a salt tolerance–associated gene. This gene was shown to be localized to the endoplasmic reticulum and encoded a cation/H^+ exchanger family transporter. There has been steady progress in the information on crop genome sequence and accessibility to genome datasets and this is expected to lead the molecular breeding of salt tolerance (Morton et al. 2019). Crop breeding for salt tolerance can be primarily achieved through combining mapping based genomic and phenotypic data, meta analysis of QTLs and detection of QTLs for different tissue and stage specific tolerance traits. Extensive genome-wide diversity investigations need be conducted to explore allelic diversity at Saltol and other major traits of interest to guide marker assisted salt tolerance breeding (Ismail and Horie 2017).

6 Alternative splicing and salt stress tolerance

Multiple mature mRNAs may arise from one gene because of alternate splice site options. This has been documented since long, but their role during stress tolerance was not known until recently. A wide array of mature RNAs may arise due to intron retention, exon skipping, and also alternative 5′ or 3′ splicing sites (Syed et al. 2012; Reddy et al. 2013). As pointed out by Gracheva et al. (2011), alternatively spliced transcripts can yield proteins with different structures or metabolic functions. Further, their cellular locations may also alter as demonstrated by Kriechbaumer et al. (2012). Surprisingly, such alternatively spliced variants compete with normal variants and therefore protein function interference has been noticed in a dominant negative manner (Seo et al. 2011, Pose et al. 2013). Thus, alternative splicing can increase both transcriptome and proteome diversity.

A large body of emerging evidences suggest that there is a dynamic regulation of pre-mRNA splicing which has been correlated with stress tolerance. A plethora of splicing factors have been noticed in higher plants. Notably, in *A. thaliana*, Sm-like conserved protein 5 (LSm5) has been recognized, which promotes inaccurate selection of splice sites in the genome of plants (Cui et al. 2014). Another splicing factor *PRP31* has been found later which is involved in transcriptional gene silencing and stress response in *A. thaliana* (Du et al. 2015). They reported that under cold stress, *PRP31* is critical for pre-mRNA splicing. This splicing factor ensures modulation of cold-responsive gene expression in *A. thaliana*. The experiments conducted by Du et al. (2015) infer that splicing machinery has a wide array of functions to perform such as pre-mRNA splicing, gene regulation, transcriptional gene silencing and also abiotic stress response in higher plants. Thus, alternative splicing has been playing crucial roles at post-transcriptional level during abiotic stress tolerance. Another component of spliceosome called Ski-interacting protein (SKIP/SNW1) functions not only as a splicing factor but also as a transcriptional co-activator for induced genes (Chen et al. 2011; Wang et al. 2012; Feng et al. 2015). Interestingly, a DEAD box helicase has been found essential for pre-mRNA splicing, cold-responsive gene regulation, and cold tolerance in *A. thaliana* (Guan et al. 2013). Feng et al. (2015) found that SKIP controls alternative splicing under salt-stress environment by interfering with the cleavage of 5′ and 3′ splice donor and acceptor sites during stress conditions. How the mRNA splicing machinery modulates salt stress responses in plants has been answered only partially.

Not only salt stress, but also other stresses like temperature stress can produce splice variants. Alternative splicing has been found to incorporate premature termination codons (PTCs) into transcripts. Such transcripts are usually degraded, else produce truncated proteins in plants (Kalyna et al. 2012; Ottens and Gehring 2016). Dehydration responsive element-binding (DREB) protein 2B was found regulated under drought stress conditions by alternative splicing (Matsukura et al. 2010). One of the isoforms, *OsDREB2B1*, which retains an exon functions under normal conditions. The second isoform *OsDREB2B2* produces a functional protein. This functional protein is triggered under both drought and high temperature stresses and also promotes the expression of several genes involved in stress tolerance (Matsukura et al. 2010). Further, Sugio et al. (2009), Staiger and Brown (2013), and Cheng et al. (2015), demonstrated that in *Arabidopsis* and rice the heat shock transcription factor A2 (*HSFA2*) helps the introduction of a PTC into the splicing isoform *HSFA2-II* and creates truncated protein under normal conditions. Contrarily, the alternative splice isoform *HSFA2-I*, instead encoded a protein with transcription activation activity and was also triggered by heat stress. In *A. thaliana*, and rice this splice isoform activated heat stress-responsive genes under high temperature conditions (Cheng et al. 2015, Sugio et al. 2009). High temperature stress imposed in grapevines by Jiang et al. (2017) resulted in the alternative splicing of 70% of the genes. Among the various splice events, intron retention was frequent. Not surprisingly, intron retention events were high at higher temperatures (40 °C and 45 °C) than at lower (35 °C), indicating that intron retention is a key posttranscriptional regulation event. Simultaneously, the levels of RNA-binding proteins were also increased with an increase in temperature. Their investigations revealed that a multiprotein bridging factor1c and *HSFA2* were associated with heat tolerance by modulating alternative splicing and translational levels. The findings of Gu et al. (2018) highlighted the critical role being played by another spliceosomal protein U1A which is involved in alternative splicing as well as a regulatory role in hostile environments. Thus, alternative splicing, a universal phenomenon appears to play many critical roles during abiotic stress conditions. However, it is vital to find out the components that transduce the signal to splice the pre-mRNA molecules and we need to improve our understanding on how these are involved in salt tolerance or how they could be used to produce plants with improved tolerance and acceptable yields under field conditions.

7 Exploring halobiomes as a pool of genes for the production of salt-tolerant plants

The halobiome can be referred to as a group of halophilic and/or halotolerant bacteria, algae, fungi and plants (halophytes) that are able to withstand hypersaline environments. Hence, the halobiome can be regarded as a potential reservoir of genes associated with salt-tolerance, which could be used to engineer salt-tolerant crop plants. Salt-tolerance in halophytes involves structural and developmental adaptations that include succulence, leaf shedding and the presence of salt secreting assemblies such as salt glands or salt hairs (Shabala et al. 2014). Many halophytes achieve tolerance using cellular mechanisms that include reduced Na^+ influx, vacuolar compartmentalization of it or elimination of Na^+ ions through PM anti-porters (Flowers and Colmer 2015). While all plants have cellular mechanisms that can potentially confer a degree of salt-tolerance, in halophytes these mechanisms are expressed at higher levels than in glycophytes.

Hence, quantitative not necessarily qualitative difference in the expression of key genes or intrinsically more active proteins involved in salt tolerance is often the basis for better survival in halophytes (Himabindu et al. 2016). Overexpression of salt tolerance genes such as *SOS, NHX, HKT* etc., isolated from glycophytes or their halophytic gene homologs, showed that the genes from halophytes generally provide a better salinity tolerance than those isolated from glycophytes (Volkov 2015). Selection of effective promoters and regulatory sequences are the key to achieve good levels of transgene expression and recent studies show that the promoter regions and the *cis*-regulatory elements of various stress-inducible genes found in halophytes are strongly expressed under saline conditions. For example, promoter regions of the *SlBADH* and *SlPEAMT* genes of *Suaeda liaotungensis* (Zhang et al. 2008; Li et al. 2016), the *TsVP1* from *Thellungiella halophila* (Sun et al. 2010), and *SbGSTU* gene from *Salicornia brachiata* (Tiwari et al. 2016) showed the ability to cause a multi-fold upregulation of their associated genes in plants under salt stress. In addition, genes from halophytes including TFs (*MYB, NAC, DREB*) associated with enzymatic or non-enzymatic antioxidants (BADH, APX, SOD etc.) and antiporter genes (*NHX, HKT, SOS*) were successfully used to enhance salt-tolerance in glycophytic plants. **Table 4** provides a summary of halophyte genes used in transgenesis-experiments.

In general, halophytes are plants used for isolating genes implicated in salt tolerance, however, some other salt tolerant non-plant organisms are also explored for this purpose. Genes encoding enzymes involved in the production of some osmoprotectants, as well as antioxidative enzymes and some ribosomal proteins from non-plant halobionts were successfully transferred into plants, resulting in salt-tolerance (Table 5). Three genes, *ectA* (2,4-diaminobutyric acid acetyltransferase), *ectB* (2,4-diaminobutyric acid aminotransferase) and *ectC* (L-ectoine synthase), isolated from the halophilic bacterium *Halomonas elongata*, were expressed in transgenic plants (Moghaieb et al. 2011). The resultant transgenic lines exhibited improved ectoine synthesis and accumulation, and the plants grew better under saline conditions (100-300 mM NaCl) with improved biomass production and photosynthetic rates, and reduced lipid peroxidation. Also, the genes encoding glycine sarcosine methyltransferase (*GSMT*) and sarcosine dimethylglycine methyltransferase (*SDMT*), involved in the synthesis of glycine betaine (GB), were isolated from the halotolerant cyanobacterium *Aphanothece halophytica* and expressed in rice (*Oryza sativa*) plants (Niu et al. 2014). These rice transgenics accumulated high levels of GB, and performed better under saline conditions in comparison with wild-type plants. Ribosomal proteins from the extremely halophilic fungus *Aspergillus glaucus* were also shown to alleviate salt stress when expressed in *Arabidopsis thaliana* and *Nicotiana tabacum* (Liu et al. 2014; Liang et al. 2015).

In addition to the successful application of transgenic technologies, the information generated by whole genomes and transcriptome analysis of organisms from the halobiome could be of great value for crop improvement. The genomes of several halotolerant organisms were sequenced including the halophytes like *Thellungiella salsuginea* (Wu et al. 2012), *T. parvula* (Dassanayake et al. 2011), the halophilic methanogenic archaea *Methanohalophilus mahii* SLP^T (Spring et al. 2010), the halophilic archaeon *Halococcus hamelinensis* (Burns et al. 2012) and the halophilic bacterium *Halomonas elongate* (Lafi et al. 2016), and these genomes could provide candidate genes for future transformation studies in the future. However, it is important to note that the transformation of crop plants using a single gene approach might not provide total tolerance with high yield potential, as abiotic stress tolerance is often controlled by multiple genes (Abe et al. 2012). While omics and transgenic approaches were demonstrated to mitigate the negative effects of salinity, introducing halotolerant bacterium into salt contaminated fields was proved to be beneficial for the growth of *Pisum sativum* (Ali et al. 2015), *Lycopersicon esculentum* (Fan et al. 2016), *Arachis hypogaea* (Sharma et al. 2016), *Chenopodium quinoa* (Yang et al. 2016) and *Triticum aestivum* (Raheem and Ali 2015). Therefore, exploring halobiomes to identify and isolate genes that confer salt-tolerance could be a promising approach to enable crop plants to be grown in saline soils, but also simpler (but maybe more expensive and laborious) agricultural methods like introduction of special soil bacteria and fungi may be also interesting (Aroca and Ruiz-Lozano 2012; Shrivastava and Kumar 2015).

8 Plant genome editing for producing salt-tolerant plants

Genome editing (GE) methods have enabled specific and predictable modifications to be tailored into crop genomes and these techniques are now revolutionizing plant breeding and crop improvement efforts (Puchta 2017; van der Wiel et al. 2017). GE methods utilize customized nucleases to introduce mutations (insertions or deletions) or precisely change gene sequences (Jain, 2015). Several GE methods, e.g. zinc finger nucleases (*ZFNs*), transcriptional activator-like effector nucleases (*TALENs*) and clustered regularly interspaced short palindromic repeat (CRISPR)-Cas9 (CRISPR-associated nuclease 9) are now readily available (Voytas 2013; Kumar and Jain 2015). These sequence-specific nucleases induce double-strand breaks (DSBs) in DNA at specific sites in the genome which are subsequently followed by DNA repair processes (non-homologous end joining or homology-directed repair) and result in genome modifications such as targeted mutagenesis, gene insertion, or gene replacement. While the non-homologous end joining mode creates insertions or deletions, the homology-directed mode can achieve precise modifications such as gene replacement or gene insertion.

The GE technology was successfully used on a variety of important crop plants (Puchta, 2017). However, there have been few studies using GE to develop salt-tolerant plants. Due to the complexity of the salt tolerance trait and the need to manipulate multiple genes, often in multiple pathways, it is essential to introduce site-directed changes with no apparent off-target effects in order to minimize unintended yield penalties (Osakabe et al. 2016; Shi et al. 2017). The *ARGOS8* genomic sequence was edited using the CRISPR-Cas method and resultant maize lines exhibited ubiquitous and higher levels of expression in most tissues and growth phases. The authors suggested that this precise means of modifying specific genes and altering their expression could be used to develop tolerant plants with high grain yields (Shi et al. 2017). Lou et al. (2017) reported the generation of mutations in the gene for ABA signalling 'osmotic stress/ABA-activated protein kinase 2' (*SAPK2*) to study its functional role, and observed that sapk2 mutants exhibited an ABA-insensitive phenotype. Their study suggests that this gene could be useful to improve salt and associated osmotic stress tolerance. GE can also be used to study the effects of gene modifications on plant functionality and aid in designing plants with specific modifications tailored to the trait of interest.

Genes associated with salt-tolerance could be specifically targeted using the CRISPR-Cas9 system as this approach was used to confer tolerance to other abiotic stresses as detailed below. Osakabe et al. (2016) used truncated gRNAs (*tru-gRNAs*) and the CRISPR-Cas9 system for accurate site-modification, while Merlot et al. (2007) induced mutation in an abiotic stress tolerance gene encoding *OST2 (AHA1)* in *Arabidopsis*. Two dominant mutations in the *ost2* locus resulted in the constitutive activity of guard cell proton pumps and inhibited stomatal responses to ABA (Merlot et al. 2007). In addition, it was found that the use of *tru-gRNA* guided Cas9, driven by a germline specific promoter, increased heritability in successive generations, and the mutant plants exhibited altered stomatal response and hence this approach could be used as a means to produce crops that can tolerate drought and/or salinity.

Salt-tolerance involves the controlled expression of multiple genes and the coordination of regulatory, signalling and metabolic pathways. Moreover, functional redundancy among the genetic pathways controlling salinity and other stress response pathways makes it difficult to achieve total knockout of functionality. In this regard, it was advocated that multiplex genome editing (Zhou et al. 2014; Ma et al. 2015) can be employed to deduce the functions of multiple functionally redundant genes involved in the same biological process, such as abiotic stress responses (Jain 2015). To this end, multiple gRNAs could be used to accomplish the targeted mutagenesis for traits like salt tolerance, homology-directed repair (HDR) may be an efficient method to induce specific change(s) in salt responsive pathways. HDR-mediated gene targeting can also be used for pyramiding of several genes involved in a stress response pathway or regulatory network (Jain 2015). For multiplex gene editing in rice, Wang et al. (2018) developed a simplified single transcriptional unit (*SSTU*) CRISPR system using FnCpf1, LbCpf1 or Cas9 to target eight genes in the Late Embryogenesis Abundant (LEA) family, and generated a higher proportion of heterozygous and chimeric mutants induced by Cpf1 albeit with lower editing efficiency. Though phenotypic nature of mutations has not been shown in this work, this study can be helpful for multiplex genome editing in plants for stress tolerance.

It is now becoming evident through several reports of genome-wide association studies that single base-pair changes constitute much of the variation in elite traits (Zhang et al. 2018). This has formed a basis for base editing as a novel strategy for inducing point mutations in crops. The recent advancement in the CRISPR/Cas9-mediated genome-editing is the base-editing technology obviating the need for a DNA repair template (Komor et al. 2016). The methods use different enzyme resource such as Cas9 nickase or dead Cas9 which can be conjoined with base-converting enzymes (Hess et al. 2017). The cytosine (C) to uracil (U) conversion is mediated by cytidine aminases, and this could result in C•G which could ultimately become T•A substitution (Gaudelli et al. 2017). The G•C substitutions are created from the conversion of adenine (A) to inosine (I) by adenine deaminases which is treated by polymerases as G, and hence the A•T to G•C substitution. Presently the base editors for adenine and cytosine are reported to bring about transition mutations of all the four bases in plants (Kang et al. 2018, Li et al. 2018a, Hua et al. 2019). The cytidine deaminase-based gene editing also has shown additional advantages of inducing nonsense mutations to disorder and knockout gene functions (Billon et al. 2017). Towards this, CRISPR/Cpf1 system is a promising tool for gene editing especially AT-rich regions (Begemann et al., 2017; Tang et al., 2017; Wang et al., 2017; Li et al., 2018b). The Cpf1 nucleases from *Francisella novicida* (FnCpf1) and *Lachnospiraceae* bacterium ND2006 (LbCpf1) have shown good potential for introducing accurate gene insertions and indel mutations at the target site in the rice genome (Begemann et al. 2017). Both Cpf1 produced up to

8% high frequency of targeted insertions as compared to most often *Cas8* mediated genome editing methods. With such an unprecedented arsenal of gene and base editing tools and precision, plant gene engineering could undoubtedly lead to modifying genes involved in the responses of plants to different stresses including production of salt-tolerant crop plants with better yields.

9 Conclusion and future outlook

It is important to emphasize that studies investigating the TFs or other factors associated with salinity stress should be carefully designed and evaluated in order not to describe and target less relevant factors associated with the salt shock response (i.e. osmotic shock caused by exposure to a single, high concentration of salt, which only occurs in nature due to rare climatic events such as after a tsunami). Of greater importance are the salt-tolerance related processes that occur in plants that are grown in fields where salt concentrations are gradually increasing over time (Shavrukov 2013). The genes and TFs affected by salt shock versus gradual increases in field salt levels may greatly vary and the latter seems to be agronomically relevant with respect to the breeding of salt-tolerant crops. Similarly, breeding practices using selection for salt stress tolerance at seedling stage (and under laboratory conditions) may be less relevant from the agricultural point of view. Therefore, field trials using proper controls are needed to reliably evaluate yields of different cultivars and thus to successfully improve crop productivity (Roy et al. 2014).

Land plants have evolved many different salt-tolerance mechanisms and thus different plant species have different transporters, gene expression patterns and mechanisms to regulate ion fluxes. Unfortunately, our understanding of the ion transporters that are involved in the uptake, exclusion, sequestration and transport of salt and thus salt-tolerance is still limited for most of the crops. There are also many uncertainties, nomenclatural problems, contradictions based on hastily made generalized statements, and yet unanswered questions with respect to salt-tolerance. It appears that there are no general rules in salt tolerance mechanisms, or at least that salt-tolerance mechanisms are likely to vary greatly from plant species to species (Nieves-Cordones et al. 2016), cultivar to cultivar, population to population, and individual to individual, and between organs, tissues and cells and also depending upon alternative splicing events. The experimental and/or field treatments used and plant growth conditions can also have greater impact how plants respond to salt. Furthermore, it has to be considered that the same ion transporter can have different effects on, for instance, shoot Na^+ accumulation when modified (down- or upregulated) in different cell types (e.g. root epidermal cells vs. xylem cells) and therefore the expression of ion transporter/transport components may need to be regulated at the cell specific level (e.g. Møller et al. 2009). Also, minor differences in transcription or protein/gene structure of a transporter can have great impact on a plant's ability to control ion fluxes and thus tolerate salt stress (e.g. Cotsaftis et al. 2012). This outlines the need for further functional analyses and characterization of various ion transporters from various organisms, an improved understanding of their regulation and cell- or tissue-specific expression patterns, as well as the need to characterize halobionts as potential sources of genes or TFs providing improved salt tolerance in glycophytes (Volkov 2015; Assaha et al. 2017; Mishra and Tanna 2017). The real impact of such approach is demonstrated by the fact that alfalfa plants overexpressing *Salsola soda NHX* gene could grow in up to 400 mM NaCl over 50 days (Li et al. 2011). Improved and enhanced ion sequestration in the vacuole under salt stress decreases the solute potential of the cells and thus alleviates the osmotic component of salt stress by facilitating the water uptake (Lv et al. 2008). Therefore, in spite of excellent promising studies and the accumulation of an avalanche of information, engineering ion transport components for improved salt tolerance is a complex and demanding task, especially if the aim is to develop crop plants that are both salt-tolerant and possess agronomically important traits (i.e. yield) when grown under commercially relevant field conditions. This is not an easy task even with newest genetic engineering tools or using transcription factors, because alterations in ion homeostasis often result in yield burden. Not surprisingly, to the best of our knowledge, no GM crops with increased salinity tolerance have been commercialized (Fita et al. 2015). Our recent searches in major relevant public databases (USDA APHIS - https:// www. aphis. usda.gov / aphis / ourfocus /biotechnology/ permits- notifications- petitions; ISAAA - http:// www.isaaa.org /gm approval database/) resulted in no records for 'salt stress' in ISAAA and a few records in APHIS which requested permission for field trials of some potentially salt tolerant GM crops (e.g. soybean, Chinese silvergrass), but no data on potentially commercialized and cultivated salt tolerant GM crops have been found. However, certain field-based studies where overexpression of gene(s) has improved the salinity tolerance and growth performance of transgenic plants in saline fields (Paspula et al. 2011; Schilling et al. 2014) should be looked upon as lead for developing salt-tolerant crops at commercial levels. In addition to a combination of genetic engineering and conventional breeding tools, other practices like for instance the domestication or increased use of halophyte plants (e.g. quinoa) as crops on saline soils (Fita et al. 2015) look promising.

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Conflict of Interest

Authors declare that there is no conflict of interest

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Table 1. Single ion transporter genes (from glycophyte sources) recently genetically engineered to improve salt tolerance.

Source organism	Gene	Transgenic host (additional information)	Reference
Plasma membrane bound high-affinity potassium (K$^+$) transporter (HKT)			
Arabidopsis thaliana	*AtHKT1;1*	*Arabidopsis thaliana* (specific expression in root cortex and epidermis cells)	Plett et al. (2010)
		Oryza sativa (specific expression in root cortex and epidermis cells)	Plett et al. (2010)
Hordeum vulgare	*HvHKT2;1*	*Hordeum vulgare* (overexpression)	Mian et al. (2011)
Triticum turgidum ssp. *Durum* (*original source: T. monococcum*)	*TmHKT7* a.k.a. *TmHKT1;4-A2*	*Triticum aestivum*	James et al. (2011)
	TmHKT8 a.k.a. *TmHKT1;5-A*	*Triticum aestivum*	James et al. (2011)
Oryza sativa cv. *Indica*	*OsHKT1;1* and its promoter	*Oryza sativa* cv. *japonica*	Campbell et al. (2017)
Glycine max	*GmHKT1;4*	*Nicotiana tabacum*	Chen et al. (2014)
Triticum monococcum	*TmHKT1;5-A*	*Triticum turgidum* ssp. *durum*	Munns et al. (2012)
Plasma membrane bound Na$^+$ insensitive K$^+$ transporter			
Oryza sativa cv. Nipponbare	*OsHAK5*	*Nicotiana tabacum* cv. BY2 cells	Horie et al. (2011)
Plasma membrane Na$^+$/H$^+$ antiporter system			
Arabidopsis thaliana	*AtSOS1*	*Arabidopsis thaliana* (overexpression)	Yang et al. (2009)
		Nicotiana tabacum cv. *Xanthi-nc*	Yue et al. (2012)
Artemisia japonica	*AjSOS1*	*Chrysanthemum morifolium*	Gao et al. (2016)
		Arabidopsis thaliana	Gao et al. (2016)
Chrysanthemum crissum	*CcSOS1*	*Chrysanthemum morifolium*	An et al. (2014a); Gao et al. (2016)
		Arabidopsis thaliana	Gao et al. (2016)
Chrysanthemum morifolium	*CmSOS1*	*Chrysanthemum morifolium* (overexpression)	Gao et al. (2016)
		Arabidopsis thaliana	Gao et al. (2016)
Crossostephium chinense	*CrcSOS1*	*Chrysanthemum morifolium*	Gao et al. (2016)
		Arabidopsis thaliana	Gao et al. (2016)
Gossypium hirsutum	*GhSOS1*	*Arabidopsis thaliana*	Chen et al. (2017)
Sorghum bicolor	*SbNHXLP*	*Solanum lycopersicum*	Kumari et al. (2017)
Plasma membrane Na$^+$ pumping ATPase			
Physcomitrella patens	*PpENA1*	*Oryza sativa*	Jacobs et al. (2011)
NHX-type vacuolar Na$^+$(K$^+$)/H$^+$ antiporter			
Arachis hypogaea	*AsNHX1*	*Nicotiana tabacum*	Zhang et al. (2017)
Arabidopsis thaliana	*AtNHX1*	*Actinidia deliciosa*	Tian et al. (2011)

		Arabidopsis thaliana (overexpression)	Liu et al. (2010)
		Arachis hypogaea	Asif et al. (2011)
	AtNHX3	*Beta vulgaris*	Liu et al. (2008)
Hordeum vulgare	*HvNHX2*	*Solanum tuberosum*	Bayat et al. (2010)
Malus domestica	*MdNHX1*	*Malus domestica* (overexpression in rootstocks of grafted plants or in calli, respectively)	Li et al. (2010), (2013); Sun et al. (2017)
		Arabidopsis thaliana	Sun et al. (2017)
Solanum torvum	*StNHX1*	*Glycine max*	Chen et al. (2014)
Triticum aestivum	*TaNHX3*	*Nicotiana tabacum*	Lu et al. (2014)
Vigna radiata	*VrNHX1*	*Arabidopsis thaliana*	Mishra et al. (2014)
NHX-type endosomal K^+/H^+ antiporter			
Solanum lycopersicum	*LeNHX2*	*Arabidopsis thaliana*	Rodriguez-Rosales et al. (2008)
		Solanum lycopersicum (overexpression)	Huertás et al. (2013)
Triticum aestivum	*TaNHX2*	*Glycine max*	Cao et al. (2011)
		Solanum lycopersicum	Yarra et al. (2012)
		Medicago sativa	Zhang et al. (2012)
Vacuolar H^+-ATPase subunits			
Iris lactea	*IrlVHA-c* (C subunit)	*Nicotiana tabacum*	Wang et al. (2016)
Limonium bicolor	*LbVHA-c1* (C1 subunit)	*Nicotiana tabacum*	Xu et al. (2011)
Malus domestica	*MdVHA-A*	*Nicotiana tabacum*	Dong et al. (2015)
Triticum aestivum	*TaVB* (B subunit)	*Arabidopsis thaliana*	Wang et al. (2011)
	W36 (putative E subunit)	*Arabidopsis thaliana*	Zhang et al. (2014c)
	Several subunit genes	*Arabidopsis thaliana*	He et al. (2014)
Vacuolar H^+-pyrophosphatase			
Arabidopsis thaliana	*AVP1*	*Agrostis stolonifera* L.	Li et al. (2010)
		Arabidopsis thaliana (overexpression)	Undurraga et al. (2012)
		Arachis hypogaea	Qin et al. (2013)
		Gossypium hirsutum	Pasapula et al. (2011)
		Hordeum vulgare	Schilling et al. (2014)
		Saccharum officinarum	Kumar et al. (2014)
Populus trichocarpa	*PtVP1.1*	*Populus davidiana* × *P. bolleana*	Yang et al. (2015)
Sorghum bicolor	*SbVPPase*	*Eleusine coracana*	Anjaneyulu et al. (2014)

Table 2. Two or more ion transporter genes simultaneously genetically engineered to improve salt tolerance.

Targets/Genes	**Source organism(s) (genes)**	**Transgenic host**	**Reference**
Plasma membrane Na$^+$/H$^+$ antiporter and NHX-type vacuolar Na$^+$/H$^+$ antiporter	*Arabidopsis thaliana* (co-overexpression of *AtSOS1* and *AtNHX1*)	*Arabidopsis thaliana*	Pehlivan et al. (2016)
Plasma membrane Na$^+$/H$^+$ antiporter 'system'	*Arabidopsis thaliana* (*AtSOS1+AtSOS2+AtSOS3*)	*Festuca arundinacea*	Ma et al. (2014)
Plasma membrane Na$^+$/H$^+$ antiporter and a MYC-like bHLH transcriptional activator	- *Chrysanthemum crassum* (*CcSOS1*) - and *Chrysanthemum dichrum* (*CdICE1*)	*Chrysanthemum morifolium* 'Jinba'	Song et al. (2014)
NHX-type vacuolar Na$^+$/H$^+$ antiporter and vacuolar H$^+$-pyrophosphatase	*Arabidopsis thaliana* (*AtNHX1* and *AVP1*)	*Gossypium hirsutum*	Shen et al. (2015)
	Pennisetum glaucum (*PgNHX1*) and *Arabidopsis thaliana* (*AVP1*)	*Solanum lycopersicum*	Bhaskaran and Savithramma (2011)
	Oryza sativa (*OsNHX1* and *OsVP1*)	*Oryza sativa* (overexpression)	Liu et al. (2010)
	Suaeda salsa (*SsNHX1*) and *Arabidopsis* (*AVP1*)	*Oryza sativa*	Zhao et al. (2006)
	Suaeda corniculata (*ScNHX1* and *ScVP*)	*Medicago sativa*	Liu et al. (2013)
	Triticum aestivum (*TNHX1* and *TVP1*)	*Arabidopsis thaliana*	Brini et al. (2007)
		Solanum lycopersicum	Khoudi et al. (2009)
	Triticum aestivum (*TNHXS1* and *TVP1*)	*Nicotiana tabacum*	Gouiaa et al. (2012)
		Solanum lycopersicum	Gouiaa and Khoudi (2015)
	Zygophyllum xanthoxylum (*ZxNHX* and *ZxVP1-1*)	*Beta vulgaris*	Wu et al. (2015a)
		Lotus corniculatus	Bao et al. (2014)
		Medicago sativa	Bao et al. (2016)

Table 3. List of major databases/repositories and tools developed in recent years and available for prediction or identification of non-coding RNAs (including miRNAs) and their targets

Database/ Tool	**Description**	**Web link**	**Reference**
miRBase	A biological database which acts as an archive of miRNA sequences and annotations from more than 220 organisms including plants	http://www.mirbase.ogr/	Kozomara and Griffiths-Jones (2014)
RNAcentral	A ncRNA sequence database coordinated by European Bioinformatics Institute and associated with 25 different databases	http://rnacentral.org/	RNAcentral Consortium (2017)
PMTED	A target expression prediction database exclusively for plant miRNAs	http:// pmted.agrinome.org/	Sun et al. (2013)
miRPlant	A program with tools to predict novel plant miRNAs	https://sourceforge.net/projects/mirplant/	An et al. (2014)
miRDeep-P	A computational tool for analyzing the miRNA transcriptome in plants.	https://sourceforge.net/projects/mirdp/	Yang and Li (2011)
P-SAMS	A plant sRNA maker site especially for amiRNAs and syn-tasiRNA	http://p-sams.carringtonlab.org	Fahlgren et al. (2016)
PmiRKB	Plant miRNA knowledge base	http://bis.zju.edu.cn/pmirkb/	Meng et al. (2010)
NONCODE	An integrated knowledge database dedicated to ncRNAs from various organisms including *Arabidopsis*	http://www.noncode.org/	Zhao et al. (2016)
PNRD	A plant ncRNA database with 25739 entries of 11 ncRNA-types from 150 plant species	http://structuralbiology.cau.edu.cn/PNRD/index.php	Yi et al. (2015)
plantDARIO	Web-based tools and platform for quantitative and qualitative analyses of RNA-seq data in plants	http://snostrip.bioinf.uni-leipzig.de/index.py	Patra et al. (2014)
miRge	Tool for processing sRNA-seq data to determine miRNA entropy	http://atlas.pathology.jhu.edu/baras/miRge.html.	Baras et al. (2015)
PmiRExAt	A database resource that provides plant miRNA expression profile and query tool for 1859 wheat, 2330 rice and 283 maize miRNAs	http://pmirexat.nabi.res.in	Gurjar et al. (2016)
miRPursuit	A pipeline to provide running end-to-end analyses of high-throughput sRNA-seq data in non-/model plants from known and novel sequences	https://readthedocs.org/projects/mirpursuit/	Chaves et al. (2017)
mirEX 2.0	A platform to explore plant miRNA expression data based on qRTPCR and NGS	http://www.combio.pl/mirex	Zielezinski et al. (2015)
miRA	A tool useful for identifying miRNA precursors in plants	https://github.com/mhuttner/miRA	Evers et al. (2015)
miRandb	miRNA algorithmic network database, a meta-database offering online services for miRNA research	http://mirandd.ir	Aghaee-Bakhtiari et al. (2017)

Table 4. Halophytes as sources of genes for enhanced salt-tolerance in glycophytes.

Donor halophyte	**Gene**	**Description**	**Transformed glycophyte**	**Reference**
Aeluropus littoralis	AlNHX1	Vacuolar Na$^+$/H$^+$ antiporter	*Nicotiana tabacum*	Zhang et al. (2008)
Ammopiptanthus mongolicus	AmVP1	Vacuolar H$^+$-pump	*Arabidopsis thaliana*	Wei et al. (2012)
Atriplex dimorphostegia	AdNHX1	Vacuolar Na$^+$/H$^+$ antiporter	*Oryza sativa*	Li et al. (2008)
Atriplex gmelini	AgNHX1	Vacuolar Na$^+$/H$^+$ antiporter	*Oryza sativa*	Ohta et al. (2002)
Atriplex hortensis	AhBADH	Synthesis of glycine betaine	*Nicotiana tabacum*	Jia et al. (2002)
Chenopodium glaucum	CgNHX1	Vacuolar Na$^+$/H$^+$ antiporter	*Oryza sativa*	Li et al. (2008)
Chloris virgata	*CvPMAΔC*	Plasma membrane H$^+$-ATPase	*Arabidopsis thaliana*	Zhang et al. (2014a)
Halostachys caspica	HcNHX1	Vacuolar Na$^+$/H$^+$ antiporter	*Arabidopsis thaliana*	Guan et al. (2011)
	HcVHA-B	Vacuolar H$^+$-ATPase, subunit B	*Arabidopsis thaliana*	Hu et al. (2012)
	HcVP1	Vacuolar H$^+$-pump	*Arabidopsis thaliana*	Hu et al. (2012)
Kalidium foliatum	KfVP1	Vacuolar H$^+$-pump	*Arabidopsis thaliana*	Yao et al. (2012)
Pennisetum glaucum	PgNHX1	Vacuolar Na$^+$/H$^+$ antiporter	*Brassica juncea* *Oryza sativa*	Rajagopal et al. (2007) Verma et al. (2007)
Puccinellia tenuifolia	PutAKT1	Plasma membrane located K$^+$ inward rectifying channel (KIRC)	*Arabidopsis thaliana*	Ardie et al. (2010)
	PtNHA1	Plasma membrane Na$^+$/H$^+$ antiporter	*Arabidopsis thaliana*	Wang et al. (2011)
	PutNHX	Vacuolar Na$^+$/H$^+$ antiporter	*Oryza sativa*	Kobayashi et al. (2012)
Salicornia brachiata	SbMYB15	R2R3-type transcription factor (TF)	*Nicotiana tabacum*	Shukla et al. (2015)
	SbASR1	Abscisic acid stress ripening-1	*Arachis hypogea*	Tiwari et al. (2015)
	SbNHX1	Vacuolar Na$^+$/H$^+$ antiporter	*Jatropha curcas* *Ricinus communis* *Cuminum cyminum*	Jha et al. (2013) Patel et al. (2015) Pandey et al. (2016)
	SbpAPX	Peroxisomal ascorbate peroxidase	*Nicotiana tabacum*	Singh et al. (2014)
	SbpAPX	Peroxisomal ascorbate peroxidase	*Arachis hypogea*	Singh et al. (2014)
	SbSDR1	Salt and drought responsive gene	*Nicotiana tabacum*	Singh et al. (2016)
	SbSOS1	Plasma membrane Na$^+$/H$^+$ antiporter	*Nicotiana tabacum*	Yadav et al. (2012)

Salicornia europaea	SeNHX1	Vacuolar Na$^+$/H$^+$ antiporter	*Medicago sativa*	Zhang et al. (2014c)
Salix matsudana	SmQR	Quinone reductase	*Arabidopsis thaliana*	Song et al. (2016)
Salsola soda	SsNHX1	Vacuolar Na$^+$/H$^+$ antiporter	*Medicago sativa*	Li et al. (2011)
Sesuvium portulacastrum	SpAQP1	Aquaporin gene	*Nicotiana tabacum*	Chang et al. (2016)
	SpAHA1	Plasma membrane H$^+$-ATPase	*Arabidopsis thaliana*	Fan et al. (2018)
Spartina alterniflora	SaVHAc1	Vacuolar H$^+$-ATPase subunit c1	*Oryza sativa*	Baisakh et al. (2012)
Suaeda liaotungensis	SlBADH	Betaine aldehyde dehydrogenase	*Zea mays*	Wu et al. (2008)
Suaeda corniculata	SlNAC	NAC transcription factor	*Arabidopsis thaliana*	Li et al. (2014)
	ScBADH	Betaine aldehyde dehydrogenase	*Arabidopsis thaliana*	Wang et al. (2016)
	ScVP	Vacuolar H$^+$-pump	*Arabidopsis thaliana*	Liu et al. (2011)
Suaeda salsa	SsCAX1	Vacuolar H$^+$/Ca^{2+} transporter	*Arabidopsis thaliana*	Han et al. (2012)
	SsDREB	Dehydration-responsive element-binding (DREB) transcription factor	*Nicotiana tabacum*	Zhang et al. (2015)
	Ss.sAPX	Stroma ascorbate peroxidase	*Arabidopsis thaliana*	Li et al. (2012)
Tamarix androssowii	TaMnSOD	Manganese superoxide dismutase	*Populus davidiana* x *P. bolleana*	Wang et al. (2010)
Thellungiella salsuginea	TsNHX1	Vacuolar Na$^+$/H$^+$ antiporter	*Arabidopsis thaliana*	Wu et al. (2009)
	TsLEA1	Late embryogenesis abundant (LEA)	*Arabidopsis thaliana*	Zhang et al. (2012)
	TsTIP1	Tonoplast aquaporin gene	*Arabidopsis thaliana*	Wang et al. (2014)
	ThSOS1	Plasma membrane Na$^+$/H$^+$ antiporter	*Arabidopsis thaliana*	Oh et al. (2009)
Zoysia matrella	ZmVP1	Vacuolar H$^+$-pyrophosphatase (VP)	*Arabidopsis thaliana*	Chen et al. (2015)

Table 5. Additional sources of genes (from halobionts) for enhanced salt-tolerance in glycophytes.

Donor type	**Organism**	**Gene**	**Transformed glycophyte**	**Improved character/s**	**References**
Halotolerant cyanobacterium	*Aphanothece halophytica*	ApGSMT and ApDMT	*Oryza sativa* L., cv. Nipponbare	Enhancement in glycine betaine synthesis, improved growth and survival rate during stress as well as recovery stage	Niu et al. (2014)
Halophilic bacterium	*Halomonas elongata*	HeectA, HeectB and HeectC	*Lycopersicon esculentum* cv. 'Momotaro' and cv. 'Saturn'	Increased accumulation of ectoine, improved dry weight values and photosynthetic rate, decreased accumulation of MDA in leaves	Moghaieb et al. (2011)
Halophilic archaeon	*Natrinema altunense*	NaMnSOD	*Oryza sativa* L., cv. Nipponbare	Increased SOD and CAT activities, reduced superoxide radical and hydrogen peroxide levels, reduced ion leakage and MDA content	Chen et al. (2013)
Halophilic fungi	*Aspergillus glaucus*	AgRPS3aE	*Arabidopsis thaliana* (Columbia-0) *Nicotiana tabacum* cv. SR-1	Increased leaf area and root length	Liang et al. (2015)
	Aspergillus glaucus	AgRPL44	*Nicotiana tabacum*	Increased root length, fresh weight	Liu et al. (2014)
Halotolerant algae	*Dunaliella salina* strain Y6	Ds-A3-3 and Ds-26-16	*Nicotiana tabacum*	Increased leaf area, root length, enhanced level of SOS gene expression	Gong et al. (2014)
	Chlamydomonas strain W80	GPX-like protein cDNAs (GPX and GPX)	*Nicotiana tabacum* cv. Xanthi	Increased tolerance against oxidative and salt stress with reduced lipid peroxidation and increased photosynthetic and antioxidative system	Yoshimura et al. (2003)

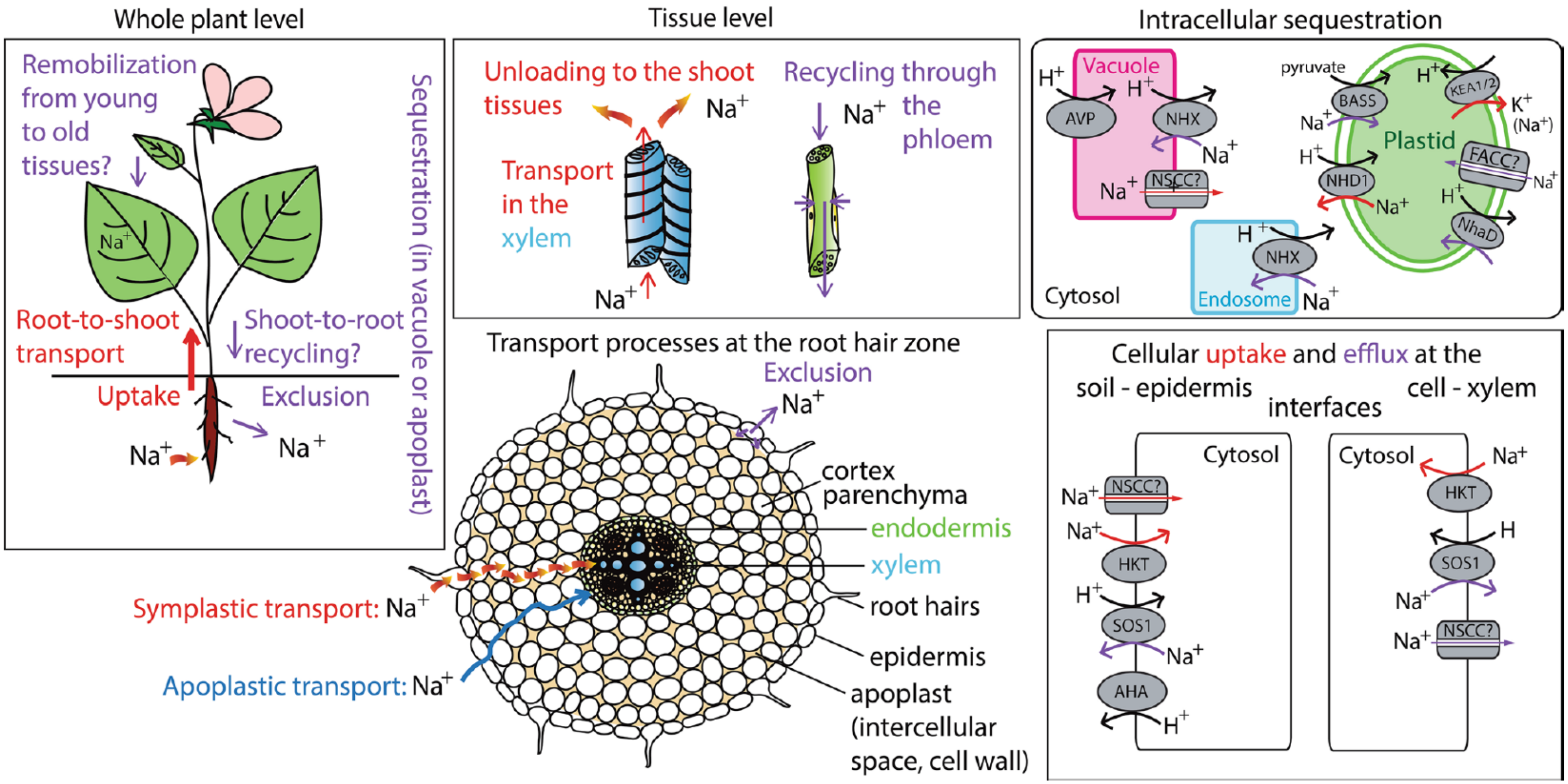

Figure 1. Summary of Na$^+$ fluxes and transport in plants. Represented processes include those at the whole plant level, in various tissues and tissues interfaces, as well as within cells. Ions, e.g. Na$^+$, from the soil can enter the root cortex via apoplastic transport through cell wall spaces and intercellular cavities. Except for the branching zone (sites of lateral root formation) and the meristematic and elongation zones, the endodermis forms a barrier that stops the apoplastic flow of Na$^+$ and forces all ions to move through the symplast into the xylem. With respect to root hairs, ions can enter the cytoplasm through specific channels and transporters, and are then transported via the symplast to the central vascular cylinder. Once loaded into the xylem, Na$^+$ is transported to the shoot, where it is unloaded from the xylem into the shoot tissues and apoplast. It is still a matter of debated whether Na$^+$ recycling can occur through the phloem back down to the root. Specific transport mechanisms are probably involved in excluding Na$^+$ flow from the xylem towards back into cortex and from epidermis cells back into the soil. Redistribution of Na$^+$ from young tissues and organs towards older 'sinks', organs that may be sacrificed, is another possibility that has been suggested as a salt tolerance mechanism. Processes or components are unproven at present are indicated by question marks.

Figure 2. Selected salt responsive plant miRNAs, their targets and the traits regulated by these miRNAs (summary diagram showing information obtained from important crops including *Oryza sativa, Zea mays, Triticum aestivum, Hordeum vulgare, Glycine max, Solanum tuberosum, and Solanum lycopersicum*).