Review

Salt-tolerant genes from halophytes are potential key players of salt tolerance in glycophytes

Yeduguri Himabindu[a,b], Thammineni Chakradhar[c], Madhava C. Reddy[b], Andrey Kanygin[d], Kevin E. Redding[d], Thummala Chandrasekhar[a,d,*]

[a] Department of Environmental Science, Yogi Vemana University, Kadapa 516003, Andhra Pradesh, India
[b] Department of Biotechnology and Bioinformatics, Yogi Vemana University, Kadapa 516003, Andhra Pradesh, India
[c] Dryland Cereals, International Crops Research Institute for the Semi Arid Tropics (ICRISAT), Hyderabad 502324, Telangana, India
[d] Department of Chemistry and Biochemistry, Arizona State University, Tempe 85287, Arizona, USA

ABSTRACT

Crop productivity strongly depends on several biotic and abiotic factors. Salinity is one of the most important abiotic factors, besides drought, extreme temperatures, light and metal stress. The enhanced burden of secondary salinization induced through anthropogenic activities increases pressure on glycophytic crop plants. The recent isolation and characterization of salt tolerance genes encoding signaling components from halophytes, which naturally grow in high salinity, has provided tools for the development of transgenic crop plants with improved salt tolerance and economically beneficial traits. In addition understanding of the differences between glycophytes and halophytes with respect to levels of salinity tolerance is also one of the prerequisite to achieve this goal. Based on the recent developments in mechanisms of salt tolerance in halophytes, we will explore the potential of introducing salt tolerance by choosing the available genes from both dicotyledonous and monocotyledonous halophytes, including the salt overly sensitive system (SOS)-related cation/proton antiporters of plasma (NHX/SOS1) and vacuolar membranes (NHX), energy-related pumps, such as plasma membrane and vacuolar H^+ adenosine triphosphatase (PM & V-H^+ATPase), vacuolar H^+ pyrophosphatases (V-H^+PPase) and potassium transporter genes. Various halophyte genes responsible for other processes, such as crosstalk signaling, osmotic solutes production and reactive oxygen species (ROS) suppression, which also enhance salt tolerance will be described. In addition, the transgenic overexpression of halophytic genes in crops (rice, peanut, finger millet, soybean, tomato, alfalfa, jatropha, etc.) will be discussed as a successful mechanism for the induction of salt tolerance. Moreover, the advances in genetic engineering technology for the production of genetically modified crops to achieve the improved salinity tolerance under field conditions will also be discussed.

© 2015 Elsevier B.V. All rights reserved.

Contents

* Corresponding author. Present address: Department of Chemistry and Biochemistry, Arizona State University, Tempe 85287, AZ, USA.
E-mail addresses: tcsbiotech@gmail.com, cthummal@asu.edu (T. Chandrasekhar).

1. Introduction

Plants have been divided into salt-tolerant 'halophytes' and salt-sensitive 'glycophytes', depending on growth performance of these organisms in saline habitats. Glycophytes display severely subdued growth and even death in the presence of 100–200 mM of salt, whereas halophytes specifically belonging to the dicotyledonous halophyte group can survive in more than 200 mM (Flowers and Colmer, 2008; Shabala, 2013). High salinity is one of the most severe abiotic environmental factors, and at a global scale, no other substance is as deleterious as salt in restricting plant growth and development. Thus, salt tolerance in glycophytic crop species has become a major issue in the last several years (Zhu, 2000; Slama et al., 2015). The primary salinization of water occurs through natural processes leading to increased salt input, decreased freshwater input, or increased freshwater extraction. However, secondary salinization through human activities poses a much greater and ever-increasing problem. As soil salinity increases, the extraction of water from the soil becomes increasingly difficult for plants. In addition, salinity has often been associated with the decreased yield and stability of some crop species (Hasegawa et al., 2000; Rengasamy, 2006). Surprisingly, an estimated one to two percent reduction in the world's irrigated area is observed yearly as an effect of salinization, indicating the severity and importance of secondary salinization. This obstacle must be addressed for sustainable agriculture development (Tuteja, 2007). A promising approach involves the introduction of halophytic genes via genetic engineering methods to produce salt-tolerant glycophytic crop varieties (Munns and Tester, 2008; Bose et al., 2015).

The priority on fascinating halophytes and their salt tolerance genes primarily reflects the mechanisms by which these plants tolerate waters and soils that have a high content of salt. Generally, most halophytic cells contain more than 500 mM Na^+ and Cl^-, except for extreme halophytes, such as *Tecticornia*, which contains approximately 2000 mM intracellular Na^+, to maintain positive turgor pressure for osmotic adjustments (Flowers et al., 2015). To survive in high salinity conditions, the osmotic movement of water in and out of the cytoplasm of the cells of halophytes occurs to prevent desiccation. In addition to the adjustment of the water potentials, the enhanced exclusion and sequestration of excess salts, formation of salt glands and bladders to accumulate additional salts, maintenance of net K^+ to Na^+ selectivity, synthesis of organic solutes, scavenging of ROS and maintenance of water use efficiency (H_2O loss per net CO_2 uptake) also contribute to effective salt tolerance in these plants (Ozgur et al., 2013; Shabala et al., 2014; Shabala and Pottosin, 2014). Hence, gene homologs from halophytes would be particularly interesting, as these genes might be functionally more efficient than those from glycophytes (Tables 1–3).

In contrast, there is evidence that the glycophytic genes also confer salt tolerance when expressed in different model species, including crop species (Zhang and Blumwald, 2001; He et al., 2005; Hou and Bartels, 2015). However, the origin of the salt tolerance of halophytes has evolved through the accumulation of adaptive mutations leading to physiological and biochemical modifications required to thrive in high salinity (Rozema and Flowers, 2005; Bromham, 2014). In certain cases, orthologs from halophytes might

be functionally more superior for growth in saline-rich areas than their counterparts in glycophytes, exhibiting tolerance level up to 500 mM (Song and Wang, 2014). In addition, halophytes also maintain bioenergetic processes (photosynthesis and respiration) with less utilization of produced energy for stress adjustment and saving higher energy, while glycophytes do not (Kosova et al., 2013a,b; Bose et al., 2014a,b; Srivastava et al., 2015). The modified lipid composition of the tonoplast layer of halophytes contributes to low sodium ion leakage back into the cytosol as a valid contribution to the salt tolerance observed in these plants (Leach et al., 1990; Glenn et al., 1999). Recently, Aquino et al. (2011) argued that the accumulation of sulfated polysaccharides (SP) in salt-tolerant species is associated with salt tolerance, and glycophytes do not contain these polysaccharides. In addition these authors suggested that negatively charged cell wall polysaccharides might play a role in high salt stress tolerance, and therefore halophytic plants exhibit adaptation to high salt environments, a trait likely conserved during plant evolution. Differences in metal uptake between glycophytes and halophytes are also important concerns for variations in tolerance (Jordan et al., 2002; Anschutz et al., 2014). Progress in the isolation and characterization of halophytic orthologs and the identification of the roles for these genes in salinity tolerance has been achieved; few transgenic glycophytic crop plants have been produced through the introduction of halophyte genes (see below). Overall, the proper selection of candidate genes requires thorough knowledge of the molecular mechanisms of halophytes. Although the current understanding of these mechanisms recently developed, herein we summarized what is a known, identifying gaps in the knowledge, and proposed potential halophytic genes for the introduction of increased salt tolerance.

2. Unique salt tolerance mechanisms in halophytes

The widespread occurrence of halophytes among higher plants of different phylogenetic clades indicates the polyphyletic origin of salt tolerance and explains the diversity in growth patterns compared with glycophytes. Thus, it is likely that there is no single salt tolerance mechanism in these organisms. In most studies, salt tolerance genes from halophytes showed homology with those of glycophytes, but the salt tolerance mechanisms in halophytes might differ in various qualitative and quantitative respects (Rozema and Schat, 2013; Cabot et al., 2014; Bose et al., 2015). Most of the information concerning salt tolerance mechanisms in halophytes gathered recently, indicating that they might involve a range of adaptations, such as osmotic tolerance, ion exclusion or inclusion and tissue tolerance, proved their dominance in exhibiting salt tolerance when compared to glycophytes (Radyukina et al., 2007; Ellouzi et al., 2011; Bose et al., 2014a,b; Shabala and Pottosin, 2014).

Full genome, transcriptome and proteome comparisons between related glycophytes and halophytes might be adequate strategies to test this hypothesis. Based on recent expression studies, including transcriptome and proteome analyses of the responses of various glycophytic and halophytic plant species to salinity, revealed differences between these plants (Jithesh et al., 2006a,b; Wang et al., 2008; Barkla et al., 2012; Dinakar and Bartels, 2013; Yang et al., 2013; Very et al., 2014). It is likely that

Table 1
List of the transporters, antiporters and assisted genes from halophytes involved in salt tolerance mechanism.

S. No	Gene	Isolated from	Expressed in	Observations/remarks	Reference
1	*PeSOS1*	*Populus euphratica*	*E.coli* (EP432 strain)	Partially suppressed the salt sensitive phenotype	Wu et al. (2007)
2	*CnSOS1A* and *CnSOS1B*	*Cymodocea nodosa*	Yeast defective in Na^+ efflux and *E.coli* K^+ uptake mutant	The truncated protein of CnSOS1A suppressed the yeast defect of Na^+ efflux mutant and role of K^+ uptake in *E.coli*	Garciadeblas et al. (2007)
3	*ThSOS1*	*Thellungiella halophila*	Yeast defective in Na^+ efflux transporter and *Arabidopsis*	Partially complemented Na^+/Li^+ sensitive phenotype of yeast mutant and increased salt tolerance in *Arabidopsis*. Silencing of *ThNHX1* leads to loss of halophytism	Nah et al. (2009) and Oh et al. (2009)
4	*CqSOS1A* and *CqSOS1B*	*Chenopodium quinoa*	*C.quinoa* and *Arabidopsis*	Down regulated in salt stress and need to know more information	Maughan et al. (2009)
5	*PtNHA1* or *PtSOS1*	*Puccinellia tenuiflora*	*Arabidopsis*	Accumulation of Na^+ was lower where as the K^+ level was higher in transgenic plants. The ratio of K^+/Na^+ was higher. Na^+ extrusion from root. Transgenic plants also enhanced activity of ROS scavenging enzymes	Wang et al. (2011) and Kobayashi et al. (2012)
6	*LgSOS1*	*Limonium gmelinii*	Homology studies	Homology studies with other species revealed it is SOS1	Ling et al. (2009)
7	*McSOS1* & Other antiporters	*Mesembryanthemum crystallinum*	Yeast, *E.coli* and *M. crystallinum*	Yeast complement assay proved the salt tolerance	Cosentino et al. (2010)
8	*SbSOS1*	*Salicornia brachiata*	Tobacco	*SbSOS1* conferred salt tolerance by decreased cytosolic Na^+ and also reduced oxidative damage	Yadav et al. (2012)
9	*KvSOS1*	*Kosteletzkya virginica*	Homology studies	Bioinformatics studies revealed that homology with other plasma membrane SOS1	Wang et al. (2014a)
10	*PtSOS2*	*Populus trichocarpa*	Hybrid Poplar	Enhanced plasma membrane sodium exclusion activity and more scavenging of ROS	Yang et al. (2015)
11	*AgNHX1*	*Atriplex gmelini*	Yeast and Rice	*AgNHX1* showed partial complementation of yeast vacuolar antiporter function. The vacuolar antiporter activity was eight times higher in transgenic rice	Hamada et al. (2001) and Ohta et al. (2002)
12	*SsNHX1*	*Suaeda salsa*	Rice	Transgenic rice exhibited tolerance in salt stress	Zhao et al. (2006)
13	*AeNHX1*	*Agropyron elongatum*	Yeast, *Arabidopsis* and *Festuca*	In yeast mutant, *AeNHX1* showed complementation of antiporter function. In Arabidopsis and festuca it improved salt tolerance, osmotic adjustment and also photosynthesis.	Qiao et al. (2007)
14	*SeNHX1*	*Salicornia europaea*	Tobacco and Alfalfa	Along with *BADH* gene produced osmolyte betaine and transgenic plants showed more accumulation of Na^+. Exhibited salt tolerance and proline accumulation in alfalfa. Involved in disease resistance too.	Zhou et al. (2008), Zhang et al. (2014) and Chen et al. (2015a)
15	*ThNHX1*	*Thellungiella halophila*	Yeast and *Arabidopsis*	Partially complemented yeast mutant and transgenic *Arabidopsis* displayed salt tolerance	Wu et al. (2009)
16	*AlNHX*	*Aeluropus littoralis*	Tobacco and Soybean	Transgenic tobacco plants accumulated more Na^+ in roots and a high K^+/Na^+ ratio was maintained in leaves. At 150mM NaCl, transgenic soybean exhibited lower Na^+ and higher K^+ level in leaves and improved growth	Zhang et al. (2008) and Liu et al. (2014)
17	*PeNHX1–6*	*Populus euphratica*	Yeast and Onion	*PeNHX1–6* partially compensated the function of Na^+/H^+ antiporter in yeast mutant and displayed salt tolerance. *PeNHX3* localized at tonoplast in onion	Ye et al. (2009) and Wang et al. (2014b)
18	*SaNHX1*	*Spartina anglica*	Rice	Enhanced salt tolerance in transgenic rice	Lan et al. (2011)
19	*HcNHX1*	*Halostachys caspica*	*Arabidopsis*	Transgenic plants exhibited more Na^+ in leaves for salt tolerance	Guan et al. (2011)
20	*SsNHX1*	*Salsola soda*	Alfalfa	Enhanced tolerance up to 400mM NaCl for 50 days. The toxic effects of Na^+ in the cell were reduced by transporting Na^+ into vacuole.	Li et al. (2011)
21	*PtNHA1* and *PutNHX*	*Puccinellia tenuiflora*	Rice	Shoots were more tolerant to NaCl and roots showed tolerance to Na_2CO_3	Kobayashi et al. (2012)
22	*KcNHX1/ KcNHX2*	*Karelinia caspica*	*Karelinia caspica*	Silencing of *KcNHX1* by RNA interference led to decreased salt tolerance.*KcNHX2* displayed minor role in salt tolerance	Liu et al. (2012)
23	*SbNHX1*	*Salicornia brachiata*	Tobacco and Jatropha	Transgenic lines exhibited salt tolerance to 200 mM NaCl and different growth parameters suggested enhanced salt tolerance	Jha et al. (2011a) and Jha et al. (2013)
24	*ScNHX1* and *ScVP*	*Suaeda corniculata*	Alfalfa	Improved salt and saline alkali tolerance to 300 or 200mM NaCl with 100mM NaHCO3. Transgenic plants accumulated more Na+ in leaves and roots	Liu et al. (2013)
25	*LfNHX1*	*Leptochloa fusca*	Tobacco	Transgenic plants exhibited tolerance to salt and drought stress. Better germination and root growth was observed at different concentrations of herbicide and NaCl	Rauf et al. (2014)
26	*ZHA1*	*Zostera marina*	Homology studies	ZHA1 was found to be similar to plasma membrane H^+-ATPase of tobacco (PMA4)	Fukuhara et al. (1996)
27	*TsVP*	*Thellungiella halophila*	Yeast, Tobacco and Cotton	*TsVP* suppressed Na^+ hypersensitivity in yeast mutant and improved salt tolerance in tobacco. Transgenic lines showed 60% more dry weight than wild type at 300mM NaClTransgenic lines were tolerant to 150 and 200mM NaCl and showed improved growth and photosynthetic performance	Gao et al. (2006) and Lv et al. (2008)
28	*SsVP*	*Suaeda salsa*	*Arabidopsis*	Transgenic lines showed increased activity of V-PPase at 200mM NaCl as well as improved drought and salt tolerance	Guo et al. (2006)
29	*ScVP*	*Suaeda corniculata*	*Arabidopsis*	Transgenic plants accumulated more Na^+ in leaves and roots and also showed improved salt,and drought tolerance	Liu et al. (2011)
30	*KfVP1*	*Kalidium foliatum*	*Arabidopsis*	*KfVP1* Improved drought and salt tolerance. Leaves of transgenic plants accumulated more Na^+	Yao et al. (2012)
31	*ZmVP1*	*Zoysia matrella*	*Yeast* and *Arabidopsis*		Chen et al. (2015b)

Table 1 (Continued)

S. No	Gene	Isolated from	Expressed in	Observations/remarks	Reference
				ZmVP1 restore the salt tolerance in salt-sensitive yeast and overexpression studies showed the sodium sequesteration in vacuole of transgenic *Arabidopsis*	
32	*ThVHAc1*	*Tamarix hispida*	Yeast	Resistant to different abiotic stresses	Gao et al. (2011)
33	*HcVP1 and HcVHA-B*	*Halostachys caspica*	*Arabidopsis*	Transgenic plants showed increased seed germination and improved growth in presence of NaCl. More Na$^+$ was accumulated in the leaves of transgenic plants	Hu et al. (2012)
34	*SaVHAc1*	*Spartina alterniflora*	Rice	*SaVHAc1* improved salt tolerance and also up regulated genes involved in cation transport and ABA signaling	Baisakh et al. (2012)
35	*McHKT1*	*Mesembryanthemum crystallinum*	Yeast	Involved initially in K$^+$ transport in yeast system	Su et al. (2001, 2003)
36	*CnHAK1 and CnHAK2*	*Cymodocea nodosa*	*E. coli* and Yeast mutants	K$^+$ influx in *E.coli* mutant not in yeast mutant	Garciadeblas et al. (2002)
37	*PhaHAK2*	*Phragmites australis*	Reed plant	Continuous K$^+$ uptake and maintenance of high K$^+$/Na$^+$ ratio under salt stress for tolerance	Takahashi et al. (2007)
38	*McHAK1-4*	*Mesembryanthemum crystallinum*	Yeast mutants	Complemented yeast mutant defective in low- and high-affinity K$^+$ uptake and proved the salinity tolerance	Su et al. (2002)
39	*PutHKT2;1*	*Puccinellia tenuiflora*	*Arabidopsis* and Yeast	*PutHKT2;1* displayed high affinity K$^+$-Na$^+$ symport function in yeast and increased sensitivities to Na$^+$, K$^+$ and Li$^+$ and in transgenic *Arabidopsis*.	Ardie et al. (2009)
40	*PutAKT1*	*Puccinellia tenuiflora*	*Arabidopsis*	Improved salt tolerance and also showed increased K$^+$ content and decreased Na$^+$ content in transgenic plants	Ardie et al. (2010)
41	*KPutB1 & KOB1*	*Puccinellia tenuiflora* and *Rice*	Yeast and *Arabidopsis*	increased shoot K$^+$ and decreased root Na$^+$	Ardie et al. (2011)
42	*TsHKT1;2*	*Thellungiella halophila*	Yeast mutants and *Arabidopsis*	Yeast mutants lacking Na$^+$ or K$^+$ transporters exhibited strong K$^+$ transporter activity	Ali et al. (2012, 2013)
43	*SsHKT1;1*	*Suaeda salsa*	Yeast mutant strain CY162 and *Arabidopsis*	Expression suppressed a K$^+$ transport-defective phenotype of the yeast. Potassium selective in transgenic *Arabidopsis* and exhibited salt tolerance	Shao et al. (2008, 2014)

Refer for other potassium transporters such as AlHAK (Su et al., 2007) and AlHKT2;1 (Sanadhya et al., 2015) in 4.1.2 Section.
Similar gene details were kept in the same column though characterized in different year.

glycophytes and halophytes might possess the same set of genes, but exhibit differential expression and in most of the instances, difference in post translational regulation between halophytes and glycophytes causes the formation of salt-intolerant or salt-tolerant phenotypes (Edelist et al., 2009; Bonales-Alatorre et al., 2013a,b). Very recently Bose et al. (2015) proved the importance of post-translational modifications in halophytes for enhanced salinity tolerance by comparing the H$^+$-ATPase transcript levels in both halophytes and glycophytes. H$^+$-ATPase also provides the driving force for the potassium retention apart from sodium exclusion through NHX exchangers. H$^+$-ATPase transcript levels were increased much stronger in *Arabidopsis* when compared with halophyte *C. quinoa* and in contrast H$^+$-pump activity was much less indicating the vital role of post-translational modifications in halophytes. Number of potential differences between halophytes and glycophyters are depicted in Table 3. Also this information explains the variations in genome, transcriptome and proteome levels including structural and functional variations. In addition, the observed differences in expression might reflect differences in habitat apart from differences in salt responses *per se*. For example, the differential expression/function of SOS1 and HKT (high affinity potassium transporters) depends on habitat, revealing the complexity of salinity tolerance (Maathuis et al., 2014; Su et al., 2015; Katschnig et al., 2015). In addition, the functional priority of vacuolar antiporters in K$^+$ homeostasis instead of sodium sequestration also suggests the intricacy of halophyte salt tolerance mechanisms (Kobayashi et al., 2012; Very et al., 2014; Shabala and Pottosin, 2014). Liu et al. (2014) demonstrated that transgenic soybean overexpressing *Aeluropus littoralis* NHX1 displayed less sequestration of Na$^+$ into shoot vacuoles, and the accumulation of potassium in both the shoots and roots was observed. Probably this may be attributed to sodium leakage back to the cytoplasm by the function of fast-activating (FV) and slow-activating (SV) channels in vacuolar membranes (Bonales-Alatorre et al., 2013a,b; Pottosin et al., 2014). In addition, halophytes do not require efficient antioxidant activity in ROS mediated salinity tolerance and have efficient sodium exclusion methods when compared to glycophytes (Bose et al., 2014a,b). Infact halophyte does not allow excessive ROS production indicates its behaviour to deal high salinity conditions (for details see Section 4.1.4). Moreover, halophytes might have evolved specific mechanisms for survival under high salinity conditions that might not be present in glycophytes. However, additional studies are needed to clarify the salt tolerance mechanisms in halophytes.

3. Possible mechanisms of salt tolerance in halophytes

Salt tolerance in plants is a complex network, based on the interactions of multiple physiological responses involving several genes and gene products. In high-salt environments, plants initially suffer from osmotic stress, reflecting water deficit, and subsequently experience ion-specific stress resulting from altered ion concentrations both in halophytes and glycophytes (Blumwald et al., 2000; Shabala and Mackay, 2011). Exceeding critical cytoplasmic Na$^+$ and Cl$^+$ concentrations also causes damage in halophytes too (specifically monocotyledons), reflecting a reduction of the net photosynthesis rate through stomatal closure. Thus, as mentioned above, halophytic plants have developed efficient survival methods based on recent molecular evidences for these strategies (Velarde-Buendía et al., 2012; Kosova et al., 2013a,b; Ozgur et al., 2013; Shabala, 2013; Slama et al., 2015). Several halophyte genes associated with cation/proton antiporters on the plasma membrane (*SOS1*), vacuolar membrane (*NHX*), plasma membrane and vacuolar H$^+$-ATPases and vacuolar H$^+$-pyrophosphatase, potassium transporters and other genes involved in salt tolerance mechanisms, apart from defined pathways, i.e., crosstalk, osmolytes, antioxidant enzymes, salt glands and bladders etc., are discussed in this review (Fig. 1 and Tables 1 and 2). Fig. 1 is a simplified schematic representation of the predicted salt tolerance mechanisms in halophytes, focusing on some available genes and related components from halophytes.

Table 2
List of the miscellaneous genes from halophytes involved in salt tolerance mechanism.

S. No	Gene	Isolated From	Expressed in	Observations/Remarks	Reference
1	AhProT1	Atriplex hortensis	Arabidopsis	Proline supply and transgenic plants could survive up to 200 mM NaCl in MS media with increased biomass	Guo et al. (2002)
2	NtP5CS	Nitraria tangutorum	E.coli	Proline supply andrecombinant E.coli cells exhibited better performance under abiotic stresses including salinity	Zheng et al. (2014)
3	Badh	Atriplex hortensis, Suaeda maritime and Suaeda liaotungensis	Different model plants including rice and tomato	Another osmolyte glycinebetaine production and transgenics survived upto 200mM NaCl	Guo et al. (1997), Sahu and Shaw (2009) and Wang et al. (2013a)
4	PINO1orPcINO1	Porteresia coarctata	Tobacco, E.coli, S. pombe, rice and mustard	Transgenic tobacco plants were able to grow in 200–300 mM NaCl PcINO1 conferred salt tolerance to evolutionary diverse organisms from prokaryotes to eukaryotes	Das-Chatterjee et al. (2006)
5	SaINO1	Spartina alterniflora	Arabidopsis	Salt stress tolerance (150mM NaCl) was improved in transgenic Arabidopsis during germination and seedling growth and development. Also the transgenic plants were less sensitive to photo-inhibition	Joshi et al. (2013)
6	SbpAPX	Salicornia brachiata	Peanut &Tobacco	Transgenic plants showed significant tolerance at 150 mM NaCl. Improved drought tolerance along with salt tolerance. The vegetative growth as well as the germination rate was enhanced in transgenic plants.	Singh et al. (2014) and Tiwari et al. (2014)
7	Sod1	Avicennia marina	Rice var. Pusa Basmati-1	Transgenic plants could tolerate up to 150mM NaCl for 8 days and also exhibited tolerance to drought	Prashanth et al. (2008)
8	TaMnSOD	Tamarix androssowii	Poplar	Transgenic lines showed salt tolerance and enhanced activity of SOD whereas the MDA and REC were decreased significantly	Wang et al. (2010)
9	ThMSD	Eutrema halophilum	E.coli and Arabidopsis	Transgenic lines exhibited higher germination at 100 and 150mM NaCl	Xu et al. (2014)
10	SbMT-2	Salicornia brachiata	Tobacco	Function in ROS scavenging and salt tolerance	Chaturvedi et al. (2014)
11	AmMDAR	Avicennia marina	Tobacco	Transgenic tobacco lines showed increased APX activity and decreased levels of MDA a product of lipid peroxidation and thus showed enhanced tolerance to oxidative stress	Kavitha et al. (2010)
12	SbGSTU	Salicornia brachiata	Tobacco	Transgenic lines showed better seed germination and growth under salt stress	Jha et al. (2011b)
13	AhDREB, AtDREB & SbDREB2A	Atriplex hortensis, Atriplex halimus and Salicornia brachiata	Tobacco	SbDREB2A conferred salt as well as drought tolerance. Transgenic plants accumulated less Na$^+$ and higher K$^+$	Shen et al. (2003), Khedr et al. (2011) and Gupta et al. (2014)
14	SbSI-2	Salicornia brachiata	E.coli and Tobacco	SbSI-2 conferred desiccation and salt tolerance to E.coli, salt and osmotic tolerance to tobacco. Transgenic tobacco lines showed expression of ROS scavenging genes and other stress related genes	Yadav et al. (2014)
15	SlNAC1 & SlNAC2	Suaeda liaotungensis	Arabidopsis	Enhanced salt, drought and cold stress tolerance	Yang et al. (2014), Li et al. (2014b)
16	SlPAPA1	Suaeda liaotungensis	Arabidopsis	Functions as a transcriptional activator and improved drought tolerance and salt tolerance	Li et al. (2014a)
17	AlSAP	Aeluropus littoralis	Tobacco	Up regulation of stress related genes was found in transgenic lines. They also exhibited improved tolerance to abiotic stresses like drought, heat and freezing apart from salt tolerance	Saad et al. (2010)
18	SbASR-1	Salicornia brachiata	Tobacco	Transgenic seeds showed better seed germination and seedling growth under salt stress condition. The content of Na$^+$ was found to be less in the leaves of transgenic lines	Jha et al. (2012)
19	SlASR	Suaeda liaotungensis	Arabidopsis	Improved abiotic stress tolerance to drought, and low temperature along with NaCl tolerance	Hu et al. (2014)
20	TsLEA1	Thellungiella salsuginea	Yeast and Arabidopsis	Exhibited salt tolerance in both the species	Zhang et al. (2012)
21	TaLEA	Tamarix androssowii	Poplar	Transgenic lines showed significant decrease in MDA content and relative electrolyte leakage.Improved salt and drought resistance	Gao et al. (2013)
22	SaARF	Spartina alterniflora	Arabidopsis	Under salt stress, the transgenic lines showed enhanced seed germination and seedling growth. Antioxidant genes AtSOD1 and AtCAT were activated and the transgenic plants were also tolerant to drought	Karan and Subudhi (2014)
23	AlRab7	Aeluropus lagopoides	E.coli	Excludes Na$^+$ through salt glands and demonstrated that this protein is involved in vesicle fusion with the vacuole and salt tolerance	Rajan et al. (2014)
24	SaSce9	Spartina alterniflora	Arabidopsis	Improved salinity drought tolerance of transgenic plants. They also showed increased expression of stress responsive genes AtSOD, AtCAT, AtNHX1, AtSOS1, AtP5CS, AtRD22	Karan and Subudhi (2012)
25	SbUSP	Salicornia brachiata	E.coli	Demonstrated enhanced salt tolerance along with other abiotic stress tolerance	Udawat et al. (2014)
26	SaRBP1	Suaeda asparagoides	Arabidopsis	The transgenic lines exhibited tolerance to salt and mannitol. They produced more number of lateral roots, longer primary roots and more fresh weight than controls	Ayarpadikannan et al. (2012)
27	ThCYP1	Thellungiella halophila	Fission yeast and Tobacco	Enhanced salt tolerance in yeast and tobacco. ThCYP1 is highly expressed in roots and the ThCYP1proteins are enriched in the nucleus	Chen et al. (2007)
28	PcSrp	Porteresia coarctata	Yeast and Finger millet	Transgenic plants could survive up to 250mM NaCl and also accumulated higher Na$^+$and K$^+$ in the roots under stress conditions	Mahalakshmi et al. (2006)
29	Pr$_{AlSAP}$	Aeluropus littoralis	Tobacco	PrAlSAP is an age-dependent, abiotic stress inducible, organ specific and tissue specific promoter for expression	Saad et al. (2011)

Similar gene details were kept in the same column though characterized in different year.

The reduction of excess Na^+ and retention of K^+ ions in the cytosol is one of the ways for plant salt tolerance. The earlier incident accomplished via two mechanisms: (1) the removal of additional Na^+ from cells through the SOS1 or Na^+/H^+ antiporter in the plasma membrane or (2) the sequestration of excess Na^+ in vacuoles through the NHX or Na^+/H^+ antiporter in the vacuolar membrane (Blumwald and Poole, 1985; Zhu, 2003; Bassil et al., 2012). Later it was also proved that these vacuolar antiporters were involved in sequestering of K^+ into vacuoles (Rodriguez-Rosales et al., 2008; Leidi et al., 2010; Barragán et al., 2012). Both the types of antiporters are ubiquitous membrane proteins that catalyze the electroneutral exchange of Na^+ or for H^+ across the membrane, thereby playing important roles in cellular Na^+/K^+ and pH homeostasis. The *Arabidopsis thaliana* genome contains eight *NHX* paralogs with gene products localized to the plasma membrane (*NHX7/SOS1* and *NHX8*), endosecretory system (*NHX5* and *NHX6*) and vacuole (*NHX1*, *NHX2*, *NHX3* and *NHX4*) (McCubbin et al., 2014). Aside from salt tolerance, these proteins are also involved in basic cellular functions, such as ion and pH homeostasis, membrane trafficking, protein processing, cell expansion, and stomatal conductance, among others (Reguera et al., 2013).

Calcium-permeable nonselective cation channels (NSCC) including cyclic nucleotide-gated channel (CNGC) and glutamate-like receptor (GLR) with unknown other factors are responsible for sodium entry directly or indirectly in to the cell. Depending upon the plant species within seconds to hours of salt treatments, the influx of sodium through non-selective cation channels in cytoplasm stimulates depolarization of the plasma membrane, activating potassium (K^+) outward-rectifying channels (KOR) and decreasing net passive potassium uptake through inward-rectifying K^+ channels ((KIR) (Demidchik et al., 2003; Shabala and Cuin, 2008; Demidchik, 2014)). Role of osmo-sensing mechanisms i.e. accumulation of cGMP suppresses influx of Na^+ by deactivating NSCC and allow the apoplastic Ca^{2+} into the cytosol through CNGC. Enhanced cytosolic Ca^{2+} occurs within seconds and activates slow-activating vacuolar two pore channel1 (SV/TPC1) to release vacuolar Ca^{2+}. Thus the enhanced concentration of cytosolic Ca^{2+} typically indicates salt stress (with various other stresses) in plants. Consequently activation of cytosolic calcium induces the calmodulin (CaM)-dependent kinases, which leads to activation of other plasma membrane H^+-ATPases, restoring membrane voltage and inhibiting depolarization-activated NSCCs. Enhanced levels of calcium also leads to production of ROS which in turn depends on the activation of respiratory burst oxidase homolog (Rboh), which is controlled by ethylene, Abscisic acid (ABA)-dependent protein kinase (sucrose non-fermenting like kinase 2.6) and phospatidic acid (PA) binding. Both ROS and Ca^{2+} execute the release of ABA which in turn initiates the transcription process (Fig. 1).

The binding of elevated Ca^{2+} with calcineurin B-like 4 (CBL4/SOS3) protein activates and binds serine/threonine protein kinase (CIPK24/SOS2) (Liu and Zhu, 1998; Halfter et al., 2000; Zhu, 2003; Guo et al., 2004). The complex of SOS3/CBL4 and SOS2/CIPK phosphorylates the C-terminus of SOS1, thereby increasing the activity of this protein, leading to Na^+ efflux with the help of proton pumping plasma membrane ATPases ((PM-ATPase) (Shi et al., 2000; Qiu et al., 2002; Martinez-Atienza et al., 2007; Quintero et al., 2011)). Among the five *SOS* genes identified, only three genes (*SOS1, SOS2* and *SOS3*) are involved in the salt signaling pathway for salt tolerance in *Arabidopsis* (Shi et al., 2000; Qiu et al., 2002; Zhu, 2003; Munns and Tester, 2008; Flowers and Colmer, 2008; Wang et al., 2009). The existence of *SOS4* in halophytes remains unclear. In halophytes, a number of *SOS1* genes have been isolated and characterized from different species (Table 1). SOS1 expression has been identified in the epidermal cells of roots and in cells surrounding the vasculature of roots and shoots (Maathuis et al.,

2014). SOS1 is not only involved in the removal of Na^+ from cytoplasm, but it also functions in the long distance transport of Na^+ from the roots to the shoots, i.e., xylem loading under low or moderate salt conditions and xylem removal under high salt conditions (Shi et al., 2002). In addition, salt-tolerant species have efficient mechanisms when compared to salt sensitive plants for Na^+ exclusion from the cytosol through SOS1 and this is achieved by combined action of several complementary mechanisms (Bose et al., 2014a,b). For example, *Salicornia dolichostachya*, a salt-accumulating halophyte, shows high levels of SOS1 expression compared with its glycophyte relative *Salicornia oleracea*, and increased SOS1 expression has been implicated in xylem loading and unloading apart from sodium exclusion. In addition, the complete suppression of HKT1;1-mediated Na^+ retrieval from the xylem was also observed (Katschnig et al., 2015). This example suggests that halophytes use different strategies to cope with salt, and these strategies often depend on the saline environment. The overexpression of the halophyte SOS2 ortholog in poplar (*Populus trichocarpa*) results in salt tolerance, and these transgenic plants exhibit enhanced plasma membrane Na^+/H^+ exchange (Yang et al., 2015), adding support to the hypothesis that the interaction of SOS2 with SOS1 is stimulatory. Currently, there is little information on the characterization *SOS3* orthologs in halophytes. As mentioned above, SOS1, SOS2 and SOS3 mechanisms have not been well described in halophytes, and future studies might provide additional details concerning this system ((Fig. 1) (Mansour, 2014; Slama et al., 2015)). Based on available information, we proposed the formation of a SOS3/SOS2 complex, although the transport processes remain obscure (Taji et al., 2010). 14-3-3 proteins are general regulatory factors expressed in most of the eukaryotic cells. These proteins have capacity to bind with functionally diverse signaling proteins, including phosphatases, kinases and transmembrane receptors and play important roles in a wide range of vital regulatory processes, such as cell cycle control, mitogenic signal transduction and apoptotic cell death (Fu et al., 2000). Recently Zhou et al. (2014a) showed that these 14-3-3 proteins inhibit salt tolerant SOS pathway by interacting with SOS2 and repress its kinase activity. Authors also tested and confirmed the inhibition by using different phosphorylated site mutants in SOS2. By overexpressing 14-3-3 we have a chance to know the impacts of different salinity tolerance mechanism apart from SOS pathway.

The vacuolar NHX proteins are a group of integral membrane antiporters that catalyze the exchange of cations across tonoplast membranes under the proton electrochemical gradient generated through vacuolar H^+-ATPase and H^+-PPase ((Fig. 1) (Blumwald et al., 2000; Yao et al., 2012; Wang et al., 2013b)). *NHX1-4* of *Arabidopsis* are involved in K^+/Na^+ exchange for H^+ in the vacuole (Bassil et al., 2012; Barragán et al., 2012; McCubbin et al., 2014). Several studies in *E. coli*, yeast, plants and animals strongly suggest that NHX-type antiporters act as proton leaks to fine-tune the luminal pH of specific intracellular compartments (Reguera et al., 2014). Apart from sodium sequestration, the vacuolar NHX antiporters primarily mediate cytosolic K^+ uptake into the plant vacuole, demonstrated in studies of *nhx1/nhx2* knockouts (Bassil et al., 2011; Barragán et al., 2012). Similarly, *AlNHX1* overexpression in transgenic soybean resulted in less Na^+ in the shoots and more K^+ in both the roots and shoots, indicating a role for *NHX1* in K^+ homeostasis, instead of excess Na^+ sequestration (Liu et al., 2014). Kobayashi et al. (2012) showed that transgenic rice expressing *Puccinellia tenuiflora NHX* exhibited higher K^+ contents in the shoots under NaCl treatments. Information on halophyte vacuolar genes is available in both mono and dicotyledonary species in Table 1 and also hereafter, the dicotyledonous halophyte is referred to as 'DH' and the monocotyledonous halophyte is referred to as 'MH' in this review. We propose that most of the vacuolar antiporters are more suitable and promising genes for genetic

Table 3
Differences between halophytes and glycophytes in salinity tolerance.

Factor	Glycophyte	Halophyte	Differences observed[a]	Reference
Genome Analysis				
Genes involved in salinity	Glycophyte	Halophyte	There are homology differences between same orthologs in glycophytes and halophytes are common and some of the differences are mentioned in the main text.	Zhu et al. (1998), Flowers and Colmer (2008), Kosova et al. (2013a, b) and Bose et al. (2014a,b)
SOS 1	*Arabidopsis thaliana*	*Thellungiella halophila*	Presence of five Long Terminal Repeat retrotransposons, MuDR DNA transposons and intergenic sequences in *T.halophila*	Nah et al. (2009)
SOS 1	*Arabidopsis thaliana*	*Thellungiella halophila*	*ThSOS1S* a splice variant of *T.halophila* contains transmembrane domain but lacks c terminal hydrophilic domain and functions as Na$^+$/H$^+$ antiporter	Taji et al. (2010)
NHX1	*Arabidopsis thaliana*, *Oryza sativa*	*Aeluropus littoralis*	Ten putative hydrophobic regions in AlNHX1 differs from nine transmembrane domains of OsNHX1 and AtNHX1	Zhang et al. (2008)
NHX3	*Arabidopsis thaliana*	*Populus euphratica*	Domain switch analysis revealed the presence of TM11 a transmembrane domain in PeNHX3. The TM11 domain controls the Li+ transport activity of PeNHX3	Wang et al. (2014b)
HKT	*Arabidopsis thaliana*, Rice	*Mesembryanthemum crystallinum*, *Thellungiella halophila*, *Suaeda salsa*	Different HKT genes involved in salinity tolerance also differed genetically between halophytes and glycophytes	Very et al. (2014) and Demidchik (2014)
INO1	*Oryza sativa*	*Porteresia coarctata*	A stretch of 37 amino acids between Trp- 174 and Ser-210 is the salt tolerance determining domain in PINO1	Majee et al. (2004)
AOC	*Lycopersicon*	*Bruguiera sexangula*	*B. sexangula* AOC homolog mangrin differs from AOC of *Lycopersicon* and *Arabidopsis* by a stretch of unusual 70 aminoacids. The sequence is essential for salt tolerance phenotype of the halophyte	Yamada et al. (2002)
	Arabidopsis thaliana	*Thellungiella parvula*	*T.parvula* genome showed a higher copy number of genes involved in ion transport than *A.thaliana*. In contrast *T.parvula* shows low copy number of genes involved in signal transduction	Dassanayake et al. (2011)
	Arabidopsis thaliana	*Thellungiella halophila*	Alterations in genomic DNA(chromosome inversion, sequence loss and gene translocation) contributes to the genomic variation between *T.halophila* and *A.thaliana*	Deng et al. (2009)
Structural Analysis- Genes responsible for special structures such as salt glands and bladders				
Salt gland and bladder genes	Mostly absent in glycophytes	*Plumbaginaceae*, *Frankeniaceae* and *Chenopodiaceae* families in halophytes	Presence of genes involved in building the glands and bladders for salt secretion in halophytes which is absent in glycophytes. Twenty six special genes are involved in slat gland formation in *Limonium bicolor*.	Liphschitz and Waisel (1974), Semenova et al. (2010) and Yuan et al. (2015)
Tonoplast structure	Not in glycophytes	*Suaeda maritima*	Variations in lipid composition of the tonoplast of the halophyte *Suaeda maritime* and also proved difference in compartmentalization of salts	Leach et al. (1990)
Sulfated polysaccharides	Rice	*Ruppia maritima*	Accumulation of sulfated polysaccharides (SP) in salt-tolerant *Ruppia maritima* species and is absent in rice plant, which is also key for salinity tolerance.	Aquino et al. (2011)
Transcriptome Analysis				
	Glycophyte	Halophyte	Variations in transcript levels related to ion-transport, photosynthesis, respiration and other metabolic activities under salinity conditions are mentioned in the main text.	Ozgur et al. (2013), Kosova et al. (2013a,b) and Bose et al. (2014a,b)
INPS1	*Arabidopsis thaliana*	*Mesembryanthemum crystallinum*	A five fold upregulation of *inps1* and 10 fold increase of accumulation of free myo-inositol was observed in halophyte where as the *inps1* was not induced in the glycophyte.	Ishitani et al. (1996)
ALDH	*Arabidopsis thaliana*	*Eutrema parvulum* and *Eutrema salsugineum*	Different expression profiles of *ALDH7B4* and *ALDH10A8* in *A. thaliana* and *E.salsugineum* was observed.	Hou and Bartels (2015)
	Arabidopsis thaliana	*Thellungiella halophila*	Redox homeostasis genes such as GPX and GST and G protein genes were upregulated in both species where as some redox homeostasis genes AtTRX4, ATGSTU20 were only incduced in *T. halophila* and the transcript intensity of GPX (ATGPX4) and a GST (AtGSTU26) was also increased in *T.halophila*. *T.halophila* adapted a strategy of utilizing conserved resources and energy to survive under stress conditions	Gong et al. (2005)
	Helianthus annus H. petiolaris	*Helianthus paradoxus*	Constitutive expression of genes related to potassium and calcium transport (*KT1, KT2, ECA1*) in halophyte *H.paradoxus*	Edelist et al. (2009)
SOS1	*Arabidopsis thaliana*	*Thellungiella halophila*, *Thellungiella parvula*	Both the halophytic species showed basal and salt induced expression of *SOS1*. Moreover pyrimidine rich 5′ untranslated proximal region of *SOS1* is conserved in halophytes where as it is missing in *A.thaliana*	Oh et al. (2010)
	Arabidopsis thaliana	*Thellungiella halophila*	A large number of stress inducible genes including *Fe-SOD, P5CS, PDF1.2, AtNCED*, P-protein, *b-glucosidase,* and *SOS1* genes were expressed in *T.halophila* in the absence of stress. Proline accumulated at higher levels in salt cress under unstressed conditions due to higher expression of *P5CS*	Taji et al. (2004)
Vacuolar antiporter	*Spinacia oleracea*	*Salicornia dolichostachya*	The activities of V-H$^+$-ATPase, V-H$^+$-PPase and Na$^+$/H$^+$ exchanger was induced in *S.oleracea* whereas the activities are constitutively high in *S.dolichostachya* but not induced	Katschnig et al. (2014)
H$^+$-ATPase	*Arabidopsis thaliana*	*Atriplex lentiformis*, *Chenopodium quinoa*	H$^+$-ATPase transcripts are high in *Arabidopsis*, but salinity tolerance is more in *C.quinoa*	Bose et al. (2015)

Table 3 (Continued)

Factor	Glycophyte	Halophyte	Differences observed[a]	Reference
SOS1 and *HKT1;1*	*Spinacia oleracea*	*Salicornia dolichostachya*	Highly constitutive expression of *SOS1* involved in Na$^+$ xylem loading as well as complete supression of *HKT1;1* involved in Na$^+$ xylem retrieval was observed in salt accumulating halophyte *S. dolichostachya*	Katschnig et al. (2015)
SOS1 and *PDH*	*Arabidopsis thaliana*	*Thellungiella halophila*	Shoot *SOS1* is highly induced by salt stress whereas the root *SOS1* is constitutively expressed under unstressed conditions. Higher proline content under unstressed conditions and lower PDH (Proline Dehydrogenase) activity was observed in *T.halophila* compared to *A.thaliana*	Kant et al. (2006) and Taji et al. (2004)
Proteome Analysis				
	Glycophyte	Halophyte	Variations in protein homology related to ion-transport, photosynthesis, respiration and other metabolic activities under salinity conditions are mentioned in the main text.	
Photosynthetic proteins	*Triticum aestivum*	*T. aestivum/Thinopyrum ponticum hybrid* (SR3)	Five fold increase in CP24 precursor involved in stabilization of PSII was observed in SR3. The abundance of DWARF3 in SR3 contributes to its potential recovery from stress	Peng et al. (2009)
	Oryza sativa	*Porteresia coarctata*	Increased abundance of proteins involved in osmolyte synthesis, cell wall synthesis, chaperon function, stabilization of PSII (CP47) as well as RubisCo larger subunit and RubisCo activase was found in *P.coarctata* under salt stress conditions	Sengupta and Majumder (2009, 2010)
	Arabidopsis thaliana	*Thellungiella halophila*	Most of the differentially expressed proteins were involved in photosynthesis, energy metabolism and stress response. It seems that *T.halophila* could maintain sufficient photosynthetic activity and produce energy to cope with stress	Pang et al. (2010)
Ribosomal proteins	*Arabidopsis thaliana*	*Thellungiella halophila*	Ribosomal proteins are varied within these two species. S7, S24, S15a proteins are up regulated in salt stress in *T.halophila*	Pang et al. (2010)
Proteosomal proteins	*Arabidopsis thaliana*	*Thellungiella halophila*	*T.halophila* Alpha type 6 and Beta type 1 are increased in salinity conditions	Pang et al. (2010)
Functional Analysis				
A. Ion transport activity				
Metal uptake	Glycophyte	*Halophyte*	Variation in chelator facilitated metal uptake by halophyte and glycophyte	Jordan et al. (2002)
Ion transport	*Arabidopsis thaliana*	*Thellungiella halophila*	Better K$^+$ assimilation in *T.halophila* and higher K$^+$/Na$^+$ ratios leading to salt tolerance. *T.halophila* accumulates less Na$^+$ and retains more K$^+$. (*Some of the differences in ion transport evidences are there in main text*)	Volkov et al. (2003), Volkov and Amtmann (2006) and Ghars et al. (2008)
B. Metabolism				
SQDG	*Arabidopsis thaliana*	*Aster tripolium* and *Sesuvium portulacastrum*	Increased activity of SQDG sulphoquinovosyldiacylglycerol involved in stabilization of ATPase complexes and PSII was observed in halophytes *A tripolium* and *S.portulacastrum* under salt stress	Ramani et al. (2004)
P5CS	*Arabidopsis thaliana*	*Nitraria tangutorum*	NtP5CS could improve the salt tolerance in E.coli more efficiently than AtP5CS	Zheng et al. (2014)
C. Photosynthesis				
Protection of photo-systems	Glycophyte Rice	Halophyte *Porterecia coarctata*	Under salinity conditions halophytes protects the photosystems and maintain net photosynthesis	Sengupta and Majumder (2009)
Carbon assimilation	Glycophyte	Halophyte	Halophytes also maintain different carbon assimilation under stress conditions. Change the pathway from C3 to C4 or CAM	Sunagawa et al. (2010)
Calvin cycle	*Arabidopsis thaliana*	*Thellungiella halophila*	Calvin cycle enzymes increased in *Thellungiella halophila* under high salt conditions	Pang et al. (2010)
	Arabidopsis thaliana	*Thellungiella halophila*	Plastid terminal oxidase (PTOX) acts as an alternative sink for PSII electron flow in *T.halophila*	Stepien and Johnson (2009)
D. Respiration				
ROS production in respiration process	Glycophyte	Halophyte	Difference in ROS production during respiration process between glycophyte and halophyte occurs.	Bose et al. (2014a,b)
FBP aldolase function	*Arabidopsis thaliana*	*Thellungiella halophila*	Enhanced levels of FBP alodolase was observed in halophytes under high salt conditions	Pang et al. (2010)
E. ROS production and antioxidant response				
ROS production	Glycophyte-*Arabidopsis* Pea	Halophyte *Cakile maritime* Barley	Variation in ROS production such as hydrogen peroxide observed in many halophytic species Difference between pea and barley in controlling hydrogen peroxide production	Ellouzi et al. (2011) and Bose et al. (2014a,b)
	Brassica juncea	*Sesuvium portulacastrum*	Ratios of NADP/NADPH, NAD/NADH and ATP/ADP suggests the minimal oxidative damage and better utilization of energy in *S. portulacastrum*.	Srivastava et al. (2015)
	Plantago media	*Plantago maritima*	Increased activity of SOD, CAT, GR enzymes resulting in lower levels of H$_2$O$_2$ production and lipid peroxidation was observed in *P.maritima*	Sekmen et al. (2007)
	Lycopersicon esculentum	*Lycopersicon pennellii*	Increased lipid peroxidation accompanied with two fold increase in H$_2$O$_2$ and decreased activity of antioxidant enzymes was observed in salt stressed roots of glycophyte	Shalata et al. (2001)
		Thellungiella halophila		Radyukina et al. (2007)

Table 3 (Continued)

Factor	Glycophyte	Halophyte	Differences observed[a]	Reference
	Plantago major		*T.halophila* differs form glycophyte *P.major* by its salt accumulating ability	
	A. thaliana	*Cakile maritime*	After 4hr treatment of salt stress, H_2O_2 levels were significantly increased in both species but the levels were declined in halophyte but remained high in glycophyte.	Ellouzi et al. (2011)
	Pisum sativum	*Rhizophora stylosa* and *Rhizophora mangle*	MDAR activity was similar in all species. GR activity was lower and APX activity was 40 % higher in halophytes and SOD activity was 40 times more in halophytes	Cheeseman et al. (1997)
	Hordeum vulgare	*Hordeum marinum*	The activity of antioxidant enzymes was increased in halophyte. New isoenzymes APX5 and GR5 were identified in the halophyte	Seckin et al. (2010)
	Lycopersicon esculentum	*Lycopersicon pennellii*	Increased activity of SOD, CAT, MDAR, DHAR and GPX was observed in halophyte under salt stress	Mittova et al. (2003)
	Arabidopsis thaliana	*Thellungiella halophila*	The catalase 3 isoform and three APX isoforms were abundant in halophyte	Wang et al. (2004)
F. Energy consumption during stress conditions				
Energy Saving	Pea	Barley	Less ATP consumed with higher energy saving by salt-tolerant barley when compared to pea plant	Bose et al. (2014b)

[a] Differences in levels of salt tolerance between halophytes and glycophytes mentioned in the main text.

transformation because the entire process will take place in the cell itself, unlike SOS1, which showed different functions depends on external saline environment. Future studies might unveil the formation of CBL10 and CIPK24 complexes and the phosphorylation of the C-terminus of NHX particularly in halophytes, leading to sodium compartmentalization in vacuoles. As mentioned above sodium sequesteration into vacuoles is key mechanism for salt tolerance in old leaves of halophytes instead young leaves used salt glands or bladders for excess salt exclusion. Hence it is important to mention the activities of sodium permeable tonoplast fast-activating (FV) and slow-activating (SV) channels which regulates sodium leaking from vacuole into cytosol. Once if both the channels are down-regulated or blocked by choline, then the inhibition of sodium leakage ultimately leads to higher levels of salt tolerance (Bonales-Alatorre et al., 2013b; Pottosin et al., 2014). Most of the recent studies on both the channels revealed the importance of sodium sequestration into vacuole for efficient salinity tolerance (Bonales-Alatorre et al., 2013a,b). Hence both FV and SV genes are also useful candidate genes for efficient salinity tolerance studies in salt-sensitive glycophytic crops.

As described above, the removal and sequestration of Na^+, retension and uptake of K^+ from the cytoplasm are the mechanisms that plants have evolved to reduce excess Na^+ or maintain the K^+ content through proton pumping ATPases, such as plasma membrane (PM) and vacuolar (V) H^+ ATPase and vacuolar (V) H^+ pyrophosphatase (Table 1). The proton-pumping ATPase (H^+-ATPase) of the plant plasma membrane or vacuole generates the proton motive force across the membrane necessary to activate most of the ion and metabolite transport (Fig. 1). The role of the proton motive force (PMF) in Na^+/K^+ exclusion and pumping into vacuoles has been discussed in different living systems (Braun et al., 1986; Rea et al., 1987; Apse et al., 1999; Blumwald et al., 2000). However, although few reports on both plasma membrane and vacuolar H^+ ATPases and vacuolar H^+ pyrophosphatases are available in the halophyte system, these proteins might indicate the presence of a PMF that motivates and maintains homeostasis and prevents the deleterious effects of excess Na^+ in the cytosol (Niu et al., 1993; Yao et al., 2012; Wang et al., 2013b; Bose et al., 2015). In addition, these H^+ pumps function jointly with cation/proton antiporters to mediate a number of different cellular functions, such as fine-tuning endosomal pH, vesicular trafficking, etc. (Bassil et al., 2012). Fukuhara et al. (1996) demonstrated that the plasma membrane H^+-ATPase, *ZHA1* from the

monocotyledonous halophyte *Zostera marina* was expressed more in mature leaves, which are exposed to sea water and exhibited higher salinity tolerance compared with younger leaves (Table 1).

Most salinity studies have primarily focused on sodium ions compared with potassium ions. In fact the potassium homeostasis is necessary to mitigate the detrimental effects by excess sodium and is major inorganic cation in the cell cytoplasm. Potassium plays a key role in anion neutralization/control of cell membrane polarization, osmoregulation, also essential for the activity of many enzymes in several metabolic pathways and involved in salinity tolerance (Very et al., 2014). Due to the limited availability of K^+ in soils, most of the plants living in the natural environment often suffer from K^+ deficiency and exhibits intolerance to high salinity. But salt-tolerant species have the superior ability of tolerance to high salinity majorly due to post-translational modifications of particular traits and efficient adaptive defence system when compared to salt-sensitive species (Demidchik, 2014; Shabala and Pottosin, 2014). Potassium uptake and efflux and long distance relocation are very important and are mediated by different potassium selective and non-selective channels and transporters through passive (difference in electrochemical potentials across the membrane) or active (transporters or exchangers consume metabolic energy i.e. ATP) methods (Fig. 1).

There are three families involved in potassium transport in plants, namely Shaker K^+ channel, HAK (High Affinity K)/KUP (K UP take)/KT (K Transporter) and HKT (High affinity K Transporter) transporters and mostly active at the plasma membrane (Wang and Wu, 2013, Very et al., 2014; Shabala and Pottosin, 2014). Shaker family presents in bacteria, fungi, animals and plants and in contrast both HAK/KUP/KT and HKT/Trk/Ktr families are absent in animals. Shaker family particularly in *Arabidopsis* is again subdivided into five groups. Group 1 and 2 includes five inwardly rectifying channels i.e. AKT1 and AKT6 (*Arabidopsis* K^+ Transporter 1 and 6), SPIK (Shaker Pollen Inward K^+ Channel), KAT1 and KAT2 (K^+ Channel *Arabidopsis Thaliana* 1 and 2), Group 3 and 4 comprise single member each namely AKT2 and AtKC1 (*Arabidopsis* Potassium Channel α subunit). AKT2 mediates weakly inwardly rectifying currents and AtKC1 is a silent regulator. Group 5 contains two outwardly rectifying channels namely GORK (guard cell outward rectifying K^+ channel) and SKOR (stelar K^+ outward rectifying channel). During salt stress, stomatal closure is directly driven by the release of potassium through depolarization activated GORK in epidermal cells, which is regulated by ABA
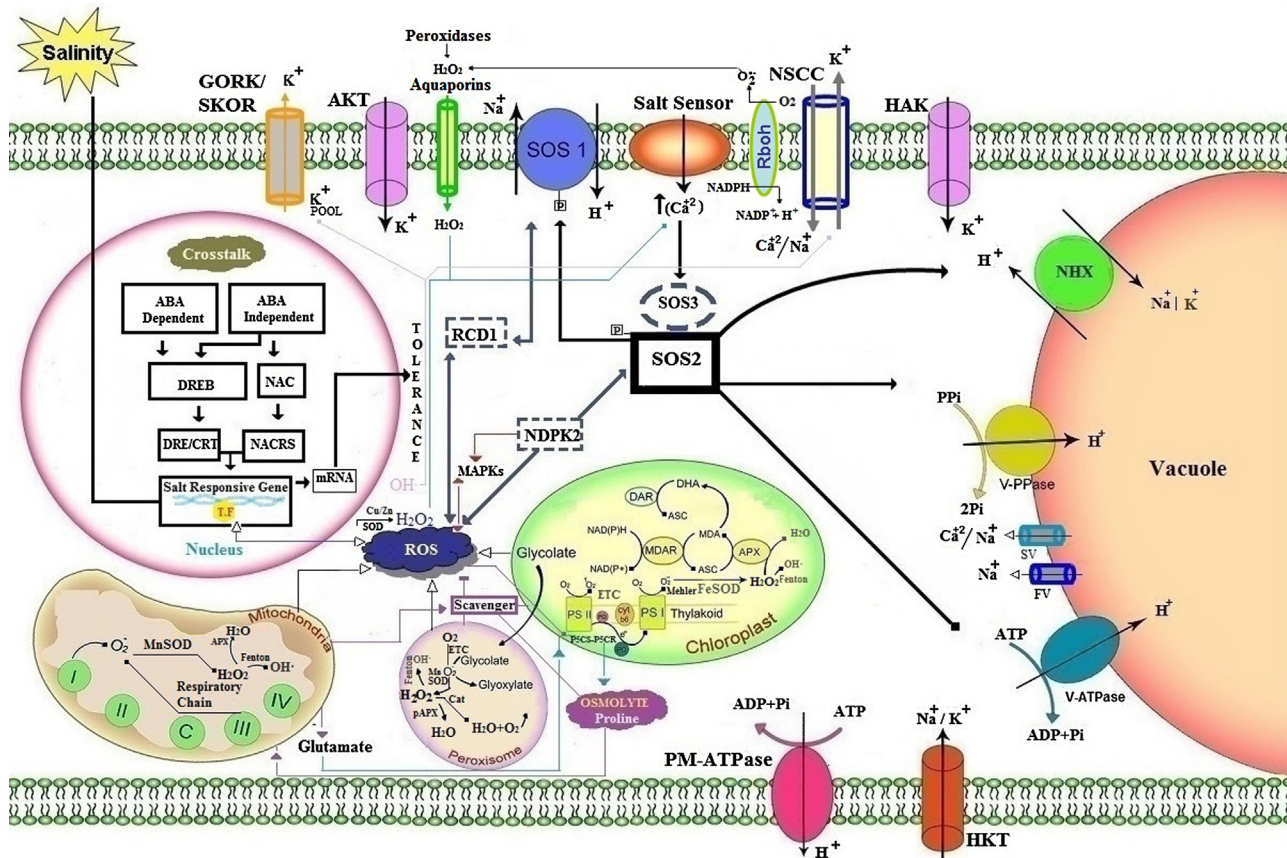
Fig. 1. Schematic representation of salt tolerance mechanism in a classical halophyte cell.
SOS—salt overly sensitive, NHX—sodium/proton antiporters, PM-ATPase—plasma membrane ATPase, V-ATPase—vacuolar H ATPase, V-PPase—vacuolar pyrophosphatase, HKT—high affinity potassium transporter, HAK—high affinity K⁺ transporter, AKT—*Arabidopsis* K⁺ Transporter, ROS—reactive oxygen species, RCD—radical induced cell death1, NDPK2—nucleoside diphosphate kinase 2, MAPKs—mitogen-activated protein kinases, APX—ascorbate peroxidase, SOD—superoxide dismutase, MDAR—mono dehydro ascorbate reductase, MDA—monodehydroascorbate, ASA—ascorbate, DHA—dehydroascorbate, DAR—dehydroascorbate reductase, NADP—nicotinamide adenine dinucleotide phosphate, ATP—adenosine tryphosphate, ADP—adenosine diphosphate, PPiinorganic phosphate, PSI—photosystem 1, PSII—photosystem II, Cytb6—cytochrome b6, PC—plasto cyanin, PQ—plasto quinone, ABA—abscisic acid, DREB—dehydration responsive element binding, NAC ((no apical meristem (NAM) Arabidopsis thaliana transcription activation factor (ATAF1/2) and cup-shaped cotyledon (CUC2)), DRE/CRT—dehydration responsive element/C-repeat, NACRS—NAC recognition site, mRNA—messenger RNA, Rboh—respiratory burst oxidase homolog, NSCC—non selective cation channels, GORK—guard cell outward rectifying K⁺ channel, SKOR—stelar K⁺ outward rectifying channel, T.F—transcription factor, SV—slow activating channel, FV—fast activating channel, Genes in incomplete boxes indicates not isolated/studied in halophytes.

and other stress factors. SKOR is responsible for potassium efflux from xylem parenchyma cells to xylem vessesls. HAK/KUP/KT group is also categorized into four clusters based on the sequence homology (Rubio et al., 2000; Anschutz et al., 2014). Cluster I includes AtHAK5 and OsHAK1 which mediates K⁺ uptake in roots. Cluster II showed the difference in transport properties and there is no much information on cluster III and IV. HKT transporters also subdivided into two subfamilies and are categorized into K⁺ or Na⁺ uniporters and K⁺-Na⁺ symporters with varying permeabilities. Subfamily 1 is probably represented in most of the higher plants and is a Na⁺ selective transporter, where as subfamily 2 identified in monocotyledons and is K⁺ selective transporter. Rice subfamily 2 members OsHKT2;1 and OsHK2;2 are known to be K⁺-Na⁺ symporters. Recently Munns et al. (2012) demonstrated that the over-expression of *TmHKT1;5-A* from wheat relative *Triticum monococcum* improved the salt tolerance and yield in durum wheat by significantly reducing transport of sodium into aerial parts, including leaves. Notably, most of the information on potassium transporters and the role of these proteins in tolerance, particularly in halophytes were recently revealed (Very et al.,

2014). The AKT-, HAK- and HKT-type transporters from halophytes have been studied for application aspects (Fig. 1 and Table 1).

In addition to effective salt exclusion and inclusion methods mentioned above, many halophytes carry salt excretory structures such as glands and bladders which shift ions to leaf surfaces, where solutes crystallize and are eventually blown away (Vassilyev and Stepanova, 1990; Shabala, 2013; Yuan et al., 2015). There are number of families including *Plumbaginaceae*, *Frankeniaceae*, *Chenopodiaceae* possess the salt glands as well as bladders in different plant parts mostly the leaf surface (Flowers and Colmer, 2008). Salt glands are highly specialised organs that consist of several cells designed to excrete salt. These are composed of a secretary cell (SC), accessory cell (AC), collection cell (CC), inner cup and outer cup cells (IC and OC) covered by a cuticle layer separating it from other epidermal and mesophyll cells. Recently Yuan et al. (2015) found that twenty six genes are responsible for salt gland differentiation in the halophyte *Limonium bicolor* and also identified five leaf developmental stages by comparing the transcriptome analysis. The density and salt secretion of salt gland is controlled by numerous factors.

More than half of the halophytes consists epidermal bladders and sequester excess salts from metabolically active cells (Flowers and Colmer, 2008; Shabala et al., 2014). Salt bladders are modified epidermal root hair and their size is large enough to sequester 1000 times more Na$^+$ than vacuoles (Shabala and Mackay, 2011). Salt bladders are represented by different forms in plants like trichomes, glandular hairs and surface glands. Although some information is available on the development of such structures, there is no much information on their salt loading mechanism. Cells of epidermis initially developed into trichome structures which consists inferior (I) and superior (S) cells. Superior cells grow exterior and form trichome initial (TI) which again divided into apical (A) and basal (B) cells. Basal cell formed the stalk cell (SC) and apical cells enlarged in size and formed the globular shaped bladders with extremely large central vacuole inside. Shabala et al. (2014) confidently argued that the importance of salt bladders in removing excessive salts from salt-sensitive crop speices is one of the best opportunities. Despite limited knowledge on molecular mechanism underlying sodium exclusion through these organs, the most practical approach might be modifying the size and shape of trichomes (Shabala, 2013) and the trichome promoters have already been used for targeted expression of genes (Pattanaik et al., 2014; Zhou et al., 2014b; Le et al., 2014). Recently Oh et al. (2015) reported cell type-specific responses to salinity by analyzing the epidermal bladder cell transriptomes in *Mesembryanthemum crystallinum*. McEBC transcriptome carries a unique profile that represents cell type-specific roles in the plant stress adapatation mechanism. Seven percent of the EBC reference transcriptome exhibited significantly different expression between salt-treated and control plants and also observed the changes in ion transport, energy generation, production of osmolytes, stress signaling and organelle functions in response to high salinity. Overall application of these candidate genes may be helpful to produce salt-tolerant traits.

The genes involved in salt tolerance mechanisms, except for the defined pathways as described above, are grouped under miscellaneous (Table 2). In addition to the above mentioned factors, the genes involved in the synthesis of compatible solutes or osmoprotectants such as proline, glycine-betaine, inositol etc., suggest another interesting tolerance mechanism to protect cells from salinity damage (Das-Chatterjee et al., 2006; Zheng et al., 2014). Salinity tolerance through osmolytes is an energy consuming process and this leads to ultimately yield penalty for crop species. Crosstalk genes and signaling transcription factors such as drought related DREB (drought responsive element binding factor) isolated under different abiotic stresses might also improve high salinity tolerance, reflecting structural and functional similarities between these genes (Khedr et al., 2011). Similarly Wang et al. (2015) suggested that OsHKT1;1 has a role in salinity tolerance by controlling sodium flow into shoots and preventing sodium toxicity which is regulated by the MYB (OsMYBc) transcription factor.

Stress-induced reactive oxygen species and the management of oxidative stress through specific genes/factors have also been implicated in stress tolerance (Tiwari et al., 2014). Under many stress conditions, plant cells generate ROS, such as superoxide ($O_2 \cdot ^-$), singlet oxygen ($^1O_2$), hydroxide radicals ($OH\cdot$), and hydrogen peroxide (H_2O_2). These ROS species arise in the chloroplast (via PSII over excitation, Mehler reaction on the PSI acceptor side, singlet oxygen dismutation and Fenton–Haber Weiss reactions), mitochondria (via complexes I and III, singlet oxygen dismutation and Fenton–Haber Weiss reactions) and peroxisomes (via photorespiratory reactions and Fenton–Haber Weiss reactions). ROS is also produced by apoplastic NADPH oxidases (Rboh), including cell wall-associated peroxidases (POX) and oxalate oxidases (Kawano, 2003; Zhang et al., 2009; Ozgur

et al., 2013). Hydrogen peroxide is the most stable of the ROS species and is diffusible to nearby cells through aquaporins. ROS scavenging (detoxification) is achieved through an efficient antioxidative system comprising enzymatic antioxidants including superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), peroxidase (POX) as well redox regulatory enzymes such as monodehydroascorbate reductase (MDAR), glutathione reductase (GR), glutathione peroxidase (GPX), glutathione S- transferases (GST) etc. Furthermore ROS homeostasis is achieved through ascorbate, glutathione, proline, glycine-betaine, carotenoids, polyamines, polyols, polyphenols, ferritins, tocopherols, sulphated polysaccharides and phenolics which serve as potent non-enzymatic antioxidants (Bose et al., 2014a,b). In addition, recent studies on novel factors associated with different cellular functions, such as vesicular trafficking and various growth factors, have provided clues for salt tolerance mechanism (Chen et al., 2007; Karan and Subudhi, 2014). In this review, we examined how tolerance is modulated through the above-mentioned genes and their associated signaling partners based on the available information from halophyte genes. However, this analysis does not comprehensively cover all of the relevant information, as the information on halophyte salt signaling mechanisms is limited.

4. Salt tolerance studies using halophyte genes in different systems

Halophytic genes similar to glycophytic genes involved in salt tolerance mechanisms have been extensively studied in various model species, including *E. coli*, yeast, *Arabidopsis*, tobacco and few crop species (Fig. 2). Improvements in ion homeostasis and salt tolerance levels have been revealed through the different experimental approaches described below. However, additional overexpression studies with glycophytic crop plants are needed to achieve field-level success.

4.1. Salt tolerance studies using halophyte genes in bacteria, yeast and model plants

Most of the biological experiments were initially handled with transformable species, such as *E. coli*, yeast and model plants, such as *Arabidopsis* and tobacco, to evaluate the effects of genes via gene expression and phenotypic characterization (Ottow et al., 2005). In general, most of the glycophytes depend on plasma membrane
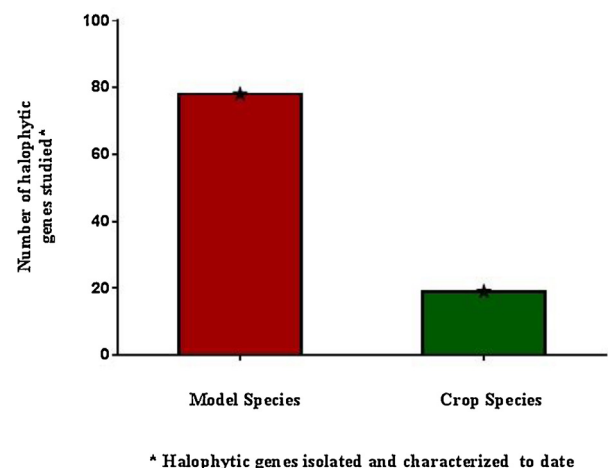

* Halophytic genes isolated and characterized to date

Fig. 2. Estimation of various halophyte genes studied in model and crop species*.

Na$^+$/H$^+$ antiporters (SOS1) and halophytes depend on vacuolar Na$^+$/H$^+$ antiporters (NHX) to avoid excess cytoplasmic sodium ion toxicity (Fig. 1). For easy to understand the salt tolerance mechanism in halophytes we separated this review into different sections by citing upto date identified genes/factors related to sodium and potassium regulation, osmotic regulation, regulation through antioxidant mechanism, signal transduction and transcriptional factors and finally thorough salt glands and bladders.

4.1.1. Sodium tolerance/regulation for salt tolerance

We started reviewing the cation/proton antiporters on plasma membrane and vacuole generally called as NHX, which are the most important components in salt tolerance mechanism, where Na$^+$ excludes from the cytosol to maintain ion homeostasis. Recently Wu et al. (2007) isolated the Na$^+$/H$^+$ antiporter, PeSOS1 from the dicotyledonous halophyte *Populus euphratica*, a highly salt-tolerant tree species that can bear up to 450 mM NaCl. *PeSOS1* showed 64% identity with *AtSOS1*, and functional analyses performed using the salt-sensitive *E. coli* EP432 strain, lacking Na$^+$/H$^+$ antiporter activity and the expression of PeSOS1, suppressed this defect, indicating a role of this gene as a plasma membrane SOS1 antiporter (Table 1). Monocotyledonous halophyte *Cymodocea nodosa* antiporters, namely CnSOS1A and CnSOS1B, were isolated, and the truncated sequences displayed high degree of similarity with the SOS1 antiporters of *Arabidopsis*, rice, wheat and tomato (Garciadeblas et al., 2007). To understand the function of the proteins encoded by *CnSOS1*, cDNAs were expressed in a *S. cerevisiae* mutant defective for the Na$^+$ efflux system. The full-length polypeptide was less active in suppressing this defect than the truncated protein, indicating that SOS2 and SOS3 are not necessary for the functional expression of C-terminally truncated CnSOS1 protein in yeast. Interestingly, CnSOS1B was not effective in this assay, indicating that this protein might perform a slightly different function (for more information see below the potassium regulation). The model halophyte *Thellungiella halophila* SOS1 (ThSOS1) and *M. crystallinum* SOS1 (McSOS1), belonging to the DH group, similarly suppressed the salt-sensitive phenotype of yeast AXT3K (Δena1-4 Δnha1 Δnhx1) mutants lacking major Na$^+$ transporters (Nah et al., 2009; Cosentino et al., 2010). ThSOS1 also exhibited increased salt tolerance in transgenic *Arabidopsis* through the removal of excess cytoplasmic sodium out of the cell, and the loss of halophytism with the expression of mutant *thsos*1 was observed in 4 plant lines (Oh et al., 2009). Maughan et al. (2009) demonstrated that the *CqSOS1A* and *CqSOS1B* genes from DH *Chenopodium quinoa* are down-regulated under salt stress. More work will be required to understand the function of these genes in salt tolerance, if any (Table 1). In general, a higher K$^+$/Na$^+$ ratio is more important for many species than simply maintaining a low concentration of cytoplasmic Na$^+$. Similar observations have been reported for *Arabidopsis* overexpressing the MH *P. tenuiflora* SOS1 (*PtNHA1*), and genetically modified (GM) plants accumulated less Na$^+$ and more K$^+$ compared with wild-type plants under 150 mM NaCl stress (Wang et al., 2011). This might be due to efficient role of PtNHA1 in sodium exclusion and coordinated physiological activities between the K$^+$/Na$^+$ exchangers for potassium retention under high salinity. Under salt stress conditions, the activities of two ROS detoxifying enzymes APX and CAT were higher than those in control plants, indicating that SOS1 overexpression also initiates superabundant ROS scavenging. The *SOS1* from the extreme DH *Salicornia brachiata* (*SbSOS1*) was hyper inducible in the roots, even at low salt concentrations, and this protein was upregulated through salt stress, extruding excessive Na$^+$ into apoplasts (Yadav et al., 2012). GM tobacco lines overexpressing *SbSOS1* displayed lower Na$^+$ in root and leaf tissues but accumulated more Na$^+$ in stem and xylem sap compared with wild-type plants, indicating a

role for this protein in Na$^+$ loading to xylem from root to leaf tissues, consistent with the hypothesis of Olias et al. (2009), apart from sodium exclusion. Furthermore, the relative water content (RWC) is the most appropriate measure of plant water status in terms of the physiological consequence of cellular water deficit, and transgenic plants exhibited more RWC during salinity tolerance. Increased proline, soluble sugars and amino acid contents were observed under high salt conditions (100 and 200 mM NaCl) compared with wild-type plants. Accumulation of less ROS also indicates a reduced potential for oxidative damage resulting from salt stress. This is also might be attributed to SOS1 interaction with RCD1 (radical induced cell death 1), a regulator of oxidative stress responses, via the predicted cytoplasmic tail to regulate the expression of ROS-scavenging genes according to Katiyar-Agarwal et al. (2006) (Fig. 1). As per recent reports, halophytes accumulate less ROS because of efficient sodium exclusion strategies and no need of high antioxidant activity (Bose et al., 2014a,b). In contrast, most of the previous works demonstrated that the halophytes possess the higher levels of anti-oxidantal acitivity (for details please see Section 4.1.4, ROS mediated salt tolerance). The increased K$^+$ and Ca^{2+} content in transgenic root tissue compared with wild-type plants under salt stress indicated that superior behaviour of SbSOS1 might also indirectly affect potassium and calcium transport (Yadav et al., 2012).

To avoid the effects of Na$^+$ toxicity, plants have developed mechanisms to compartmentalize excess cations into vacuoles using vacuolar antiporters (NHX), V-type H$^+$-ATPase and H$^+$-pyrophosphatases (Fig. 1). We also propose that vacuolar antiporters are the best candidate genes for overexpression and salt tolerance studies, due to complications of multiple roles for SOS1 depending on the salinity conditions and the presence of K$^+$ leaks. The overexpression of vacuolar *AgNHX1* (from DH *Atriplex gmelinii*), *AeNHX1* (from MH *Agropyron elongatum*) and six other vacuolar antiporter genes *PeNHX1-6* (from *P. euphratica*) in a yeast mutants with a defective vacuolar antiporter system resulted in the full or partial complementation of the defect (Hamada et al., 2001; Qiao et al., 2007; Ye et al., 2009). Furthermore, transgenic *Arabidopsis* overexpressing *AeNHX1* exhibited 200 mM NaCl tolerance and improved osmotic adjustments and photosynthesis rates, which might contribute to the normal development of transgenic plants under salt stress. The vacuolar antiporter from the dicotyledonous halophyte *Salicornia europaea* (*SeNHX1*) was coexpressed in tobacco plants with betaine aldehyde dehydrogenase (*BADH*), which is involved in the production of the osmoprotectant glycine betaine. GM plants displayed the increased accumulation of betaine and sodium compared with their wild-type counterparts and demonstrated salt tolerance under 200 mM NaCl stress conditions (Zhou et al., 2008). This study provided one of the best examples of using two candidate genes in the same construct to achieve increased impact in salinity tolerance. Recently, Chen et al. (2015a) reported the extended role of SeNHX1 in pathogen resistance, apart from salt tolerance, through the elimination of ROS after pathogen attack in transgenic tobacco. Also transgenic lines resulted higher biomass and K$^+$/Na$^+$ ratio at post germination or early seedling stage under NaCl treatment. The monocotyledonary halophyte *A. littoralis* vacuolar antiporter gene, *AlNHX* was cloned and overexpressed in tobacco plants. GM tobacco plants displayed rooting in medium containing 250 mM NaCl. T$_1$ transgenic lines survived up to 400 mM NaCl for one month through the accumulation of Na$^+$ in root vacuoles along with relative high K$^+$/Na$^+$ ratio in the leaves (Zhang et al., 2008). As a monocotyledonous gene, the role of *AlNHX* is particularly exceptional, and these results suggest the priority of halophytic genes over glycophyte genes. The sodium sequesteration in vacuole is the most important concern specifically in salt

treatments without leaking back to cytosol. This may also be attributed to tonoplast fast-activating and slow-activating channels activity i.e. avoidance of sodium leaking back from vacuoles for enhanced tolerance (Bonales-Alatorre et al., 2013a,b). Similarly, the overexpression of vacuolar NHX from *T. halophila* and *Halostachys caspica* conferred improved salt and osmotic tolerance in *Arabidopsis* and transgenic lines exhibiting more Na$^+$ in the roots and leaves, likely reflecting the increased sequestration of Na$^+$ in vacuoles (Wu et al., 2009; Guan et al., 2011). The *ThNHX1* silencing increased salt and osmotic sensitivity in transgenic plants compared with control plants. Recently, Jha et al. (2011a) generated transgenic tobacco plants overexpressing *NHX1* from *S. brachiata* (*SbNHX1*), which belongs to class-1 type NHX antiporters of halophytes. Both L77 and L88 transgenic lines showed salt tolerance up to 300 mM NaCl without affecting net photosynthetic rates. Studies concerning the overexpression of *NHX1* from the monocotyledon *Leptochloa fusca* (*LfNHX1*) increased salt tolerance in tobacco, evidenced through different growth parameters (Rauf et al., 2014). In addition, tobacco transgenic lines exhibited an increased net photosynthetic rate and higher relative water contents, stomatal conductance and cell membrane stability index compared with non-transgenic plants. In addition, the transgenic lines PB3 and PB6 also exhibited drought tolerance and survived without water supply for seven days, indicating superior osmotic adjustment in this halophytic trait. These candidate genes will likely be extremely useful for multiple stress tolerance studies (Table 1). Till date there is no report on SV and FV channel overexpression studies in any crop species.

The removal and sequestration of Na$^+$ into the vacuole are the primary mechanisms in plants for the reduction of excess Na$^+$ in the cytosol using proton pumping ATPases and pyrophosphatases (Braun et al., 1986; Rea et al., 1987; Blumwald et al., 2000). The proton-pumping ATPase (H$^+$-ATPase) of the plant plasma membrane or tonoplast generates the proton motive force across the membrane necessary to activate the transport of most ions and metabolites (Fig. 1). Vacuolar H$^+$-pyrophosphatase (Ppase) is an electrogenic H$^+$ pump that acidifies vacuoles in plants cells through the hydrolysis of inorganic phosphate (PP$_i$) to drive proton transport from the cytosol into vacuoles (Maeshima, 2000). A vacuolar H$^+$ Ppase gene (*TsVP*) was cloned from the halophyte *T. halophila*, a salt-tolerant plant that survives in the presence of 500 mM NaCl (Gao et al., 2006). The expression of *TsVP* in the salt-sensitive *ena1* mutant of yeast suppressed Na$^+$ hypersensitivity and restored function. Transgenic tobacco plants with *TsVP* accumulated more solutes in the leaves, and the biomass in transgenic leaf tissue was 60% greater than that in the leaves of control plants under 300 mM NaCl salt stress. The monodehydroascorbate (MDA) content and cell membrane damage were also less in transgenic lines, further demonstrating salt tolerance (Table 1). *Suaeda salsa* bears coastal sea water and salinity fluctuations resulting from water evaporation and tidal inundation. This plant does not have salt glands or salt bladders in the leaves, thus excess Na$^+$ is compartmentalized into the vacuole. Guo et al. (2006) isolated *SsVP* and overexpressed this gene in *Arabidopsis*, showing upregulation at 24–72 h after 200 mM NaCl treatment. The Na$^+$ contents were higher in transgenic plants than in wild-type plants, reflecting the activity of secondary vacuolar transporters and exhibiting both salt and drought tolerance. Similarly, transgenic yeast expressing the vacuolar membrane H$^+$-ATPase C subunit gene, *ThVHAc1* from *Tamarix hispida* displayed increased tolerance to salinity and other abiotic stresses, such as drought, extreme temperature and heavy metal (Gao et al., 2011). Transgenic *Arabidopsis* lines overexpressing the vacuolar pyrophosphatases from *Suaeda corniculata* (*ScVP*) and *Kalidium foliatum* (*KfVP1*) belonging to the DH group exhibited salt tolerance through

the accumulation of Na$^+$ in the leaves (Liu et al., 2011; Yao et al., 2012). The seeds of the dicotyledonous halophyte *H. caspica* germinate in the presence of 700 mM NaCl, and this plant naturally grows in soils with high salt concentrations (100 g/kg dry soil). The full-length *HcVP1* and *HcVHA-B* genes encoding vacuolar H$^+$ Ppase and the B subunit of H$^+$ ATPase, respectively, were isolated and characterized (Zhao et al., 2005). The expression levels of *HcVP1* and *HcVHA-B* transcripts were low in the absence of NaCl and significantly high with increasing salt concentrations up to 500 mM NaCl. The overexpression of the genes *HcVP1* and *HcVHA-B* in *Arabidopsis* improved salt tolerance through the increased accumulation of Na$^+$ in vacuoles (Hu et al., 2012). Recently Chen et al. (2015b) reported that the improved salt-tolerant ability in salt-sensitive yeast strain and also in *Arabidopsis* by inserting the *Zoysia matrella* vacuolar pyrophosphatase (*ZmVP1*). Authors also noticed that the higer levels of expression of different stress related genes such as *AtNHX1, AtLEA, ATP5CS, AtMn-SOD* and *AtAPX1* which is an indication of higher levels of activities such as sodium sequesteration into vacuole, assimilation of potassium, osmotic regulation and antioxidant activities of transgenic plants. As mentioned above, high affinity potassium transporters divided into Na$^+$ uniporters or Na$^+$-K$^+$ symporters (Fig. 1). The expression of *P. tenuiflora* HKT2;1 (*PutHKT2;1*) resulted in high-affinity K$^+$-Na$^+$ symport function in yeast and increased sensitivity to Na$^+$, K$^+$, and Li$^+$ in transgenic *Arabidopsis* involving Na$^+$ uptake at higher external K$^+$ concentrations (Ardie et al., 2009).

4.1.2. Potassium regulation for salt tolerance

Most living organisms must maintain certain levels of potassium to counterbalance excess sodium salts (Su et al., 2001; Shabala and Pottosin, 2014). Generally salinity leads to reduced water availability, defects in cell metabolism and oxidative stress damage by ROS production due to salt stress. To mitigate such detrimental effects, plants have evolved with sophisticated system of passive and active transporters for potassium homeostasis. Halophytes species possess much better ability to maintain higher tissue potassium under high salinity to balance the sequestered sodium ions in vacuoles (Shabala and Mackay, 2011). Potassium influx, efflux and retention studies through NSCC, NHX exchanger group broadly studied, but little information is available on overexpression studies in glycophytic crop speices using similar halophytic orthologous genes (Fig. 1 and Table 1). Moreover recent developments in information on potassium homeostasis and to eliminate the overlaping with sodium mechanism, we considered potassium regulation separately in this section. Involvment of vacuolar NHX exchangers in regulation of potassium from cytosol to vacuole was reported recently (Rodriguez-Rosales et al., 2008; Leidi et al., 2010; Barragán et al., 2012). Surprisingly the expression of truncated forms of either *CnSOS1A* or *CnSOS1B* of *C. nodosa* (also *AtSOS1*) using an arabinose responsive promoter in the *E. coli* K$^+$ uptake mutant resulted in K$^+$ uptake. This finding might be attributed to the indirect effects of SOS1 on any other potassium uptake transporters under salt stress conditions or another reason is passive transport (Garciadeblas et al., 2007). Generally depolirization of the plasmamembrane, activation of cation channels including NSCC are responsible for potassium efflux in high salinityc conditions. Apart from NHX group based potassium homeostasis activities, Garciadeblas et al. (2002) isolated the *CnHAK1* and *CnHAK2* genes of the KUP/HAK/KT potassium transporter family from seagrass *C. nodosa*. The overexpression of *CnHAK1*, but not *CnHAK2*, induces K$^+$ or Rb$^+$ influx in *E. coli* mutants [*TKW4205* (*thi rha lacZ nagA recA Sr::Tn10 delta kdpABC5 trkA405 Kup1*), deficient in the three K$^+$ uptake systems, Kdp1, TrkA and Kup] depending on the ion concentration gradient; however,

this same function was not exhibited in the yeast mutant [*Wdelta 3* (*Mat* a *ade2 ura3 trp1 trk1delta::LEU2 trk2delta::HIS3*) deficient in the TRK1 and TRK2 K⁺ uptake systems]. In contrast *CnHAK1* mediates potassium efflux when external solution contains low potassium levels indicates it as low affinity potassium transporter. Similarly, the expression of *McHAK1 to 4* (except *McHAK3*) from *M. crystallinum* complemented yeast mutants defective in potassium uptake (Su et al., 2002). Authors also found the tissue specific expression i.e. McHAK1 and McHAK4 transcripts abundant in stems, leaves and roots and McHAK2 was mostly present in stems and McHAK3 in root tissues. Moreover the potassium levels in shoot and root ability to retain potassium strongly correlated with salinity tolerance in different plants species including salt-tolerant barley (Garthwaite et al., 2005; Cuin et al., 2008). Another potassium transporter *AlHAK* from *A. littoralis* also exhibited the high affinity potassium uptake in yeast mutant defective in K⁺ uptake (Su et al., 2007). Another group potassium transporter AKT from *P. tenuiflora* (*PutAKT1*) was upregulated under both excess and deficit potassium conditions. Interestingly, *PutAKT1* was not down-regulated under excess sodium conditions indicating that this gene was potassium specific and displayed improved salt tolerance through increased K⁺ uptake when overexpressed in *Arabidopsis* (Ardie et al., 2010). Generally halophytes have superior ability to retain K⁺ when compared with glycophytes, and exhibited their ability to maintain highly negative membrane potential due to higher H⁺-ATPase activity (Bose et al., 2015).

HKT transporters can be classified into two functional subgroups: Na⁺ uniporters or Na⁺-K⁺ symporters (Horie et al., 2009; Yao et al., 2010; Ali et al., 2012). An important *McHKT1* isolated from *M. crystallinum* was involved in K⁺ transport function in the yeast mutant deficient in potassium uptake (Su et al., 2003). Upon salt stress, enhanced amount of McHKT1 transcript was observed during the first 6–10 h in this halophyte and later decline rapidly to wild-type levels with indication of initial influx of sodium. *TsHKT1;2* isolated from *Thellungiella salsuginea* and *SsHKT1:1* from *S. salsa* displayed selectivity for K⁺ over Na⁺ and the maintenance of K⁺ in yeast mutants lacking Na⁺ or K⁺ transporters under high salinity conditions. Moreover, K⁺ accumulation in the shoots and salt tolerance were observed in transgenic *Arabidopsis* overexpressing *SsHKT1;1* (Ali et al., 2012; Ali et al., 2013; Shao et al., 2014). High cytosolic potassium determines the cell fate and if cell loses excess potassium then cell shrinkage, damage of metabolic activities and ultimately leads to programmed cell death (PCD). So potassium retention status is very important for salt tolerance which is one of the assets of halophytes (Shabala and Pottosin, 2014). The ectopic expression of the K⁺ Channel β subunits from *P. tenuiflora* (*KPutB1*) and rice (*KOB1*) improved salt tolerance in yeast through alterations of K⁺ homeostasis and in transgenic *Arabidopsis* through increased shoot K⁺ and decreased root Na⁺ (Ardie et al., 2011). Recently, Sanadhya et al. (2015) reported that the potassium transporter *AlHKT2;1* from the monocotyledon *Aeluropus lagopoides* uptakes K⁺ and maintains a high K⁺/Na⁺ ratio under salt stress in yeast. These results also indicates apart from potassium retention, K⁺/Na⁺ ratio is key determinant for salinity tolerance due to physical and chemical similarities between these two and both have controls on metabolic pathways through inducing certain enzymatic activities. Say for instance the activities of starch synthase and pyruvate kinases are high in the presence of potassium than in the presence of sodium (Oria-Hernandez et al., 2005). Till date there is no report on overexpression studies of GORK which involved in ROS mediated potassium efflux in glycophytic crop plants. Taken together, these expression data suggest that the careful selection of candidate genes is a critical step prior to the initiation of a specific genetic engineering project.

Other genes involved in salt tolerance mechanisms, apart from defined pathways, are grouped under miscellaneous (Table 2). Furthermore, halophytic genes involved in crosstalk, the production of osmoprotectants and prevention of oxidative damage through ROS to directly or indirectly combat excess salt are also of interest in the context of improving salinity tolerance.

4.1.3. Osmolyte mediated salt tolerance

In general, most halophytes tolerate high-salt conditions through the accumulation of highly soluble compounds called osmoprotectants (or osmolytes), such as proline, glycine betaine, inositol, etc., to maintain osmotic pressure in the cytoplasm and stabilize proteins and membranes. Most of the metabolic pathways are involved in this process to achieve salinity tolerance. Proline is an important and widespread osmoprotectant that also serves as an important reservoir for carbon and nitrogen. Proline protects plants in response to salt stress through the regulation of usable nitrogen accumulation, which mitigates the disruptive effects of NaCl (Fig. 1 and Table 2). In addition, proline scavengers control excess ROS levels in the cytoplasm. The overexpression of the proline transporter gene from dicotyledonous halophyte *Atriplex hortensis, AhProT1* in *Arabidopsis* and GM plants conferred salinity protection under conditions as high as 200 mM NaCl, with increased biomass, whereas wild-type plants grew slowly at 100 mM NaCl, died at 150 mM NaCl and perished at 175 or 200 mM NaCl (Guo et al., 2002). Increased salt tolerance in GM plants reflects the accumulation of proline in root tips, likely credited to *AhProT1*. This might be attributed that the application of proline also decreased hydroxyl-radical induced K⁺ efflux as per Cuin and Shabala (2007).

Glutamine and ornithine are two abundant precursors for proline synthesis in two different pathways (Zheng et al., 2014). The glutamate pathway of proline biosynthesis is mediated through the enzymes pyrroline-5-carboxylate synthase (P5CS) and pyrroline-5-carboxylate reductase (P5CR), acting in concert to convert glutamate to proline. Salinity stress induces the expression of P5CS, P5CR and the Orn pathway, thereby inducing proline dehydrogenase (proline degradation pathway) and the accumulation of proline in the cytosol (Fig. 1). *E. coli*, expressing *P5CS* isolated from the dicotyledonous halophyte *Nitraria tangutorum* (*NtP5CS*), exhibited better performance under abiotic stresses, including salinity, compared with *AtP5CS*. Phylogenetic and subcellular localization analyses of NtP5CS-GFP protein showed that NtP5CS was more active, reflecting differences in protein structure between these two species. This finding provides further evidence of differential tolerance between halophytes and glycophytes (Zheng et al., 2014). Inositol is another important osmolyte in several biological systems, including flora. The MIPS (L-*myo*-inositol 1-phosphate synthase) enzyme and structural gene *INO1* (inositol-3-phosphate synthase) was isolated from the monocotyledon *Porteresia coarctata* (*PcINO1*), and the expression of this gene in *E. coli* improved salt tolerance (Das-Chatterjee et al., 2006). Similarly, the expression of *INO1* from the monocotyledon *Spartina alterniflora* (*SaINO1*), which might result in increased inositol production, exhibits increased tolerance to salinity (up to 150 mM NaCl) in GM *Arabidopsis*, not only during germination but also during subsequent plant growth and development. These transgenic plants were also less sensitive to photo-inhibition compared with wild-type plants under salt stress, although this mechanism was obscure, indicating multiple roles (Joshi et al., 2013). The osmoprotectant glycine betaine, a bipolar quaternary ammonium compound, is synthesized through the two-step oxidation of choline in chloroplasts. The first step is catalyzed through choline monooxygenase (CMO) and the second step is catalyzed through betaine aldehyde dehydrogenase in the chloroplasts. The Badh gene from different halophyte sources

has been isolated and expressed in different plants, showing tolerance to high salt conditions (Guo et al., 1997; Sahu and Shaw, 2009; Wang et al., 2013a; see Table 2). Recently Pottosin et al. (2014) revealed the importance of choline (precursor for osmoprotectant glycine betaine) in blocking the functions of slow-activating channels, which leads to inhibition of sodium leakage from vacuole and maintain high salinity tolerance. Late embryogenesis abundant (LEA) proteins are one of the most important stress-associated gene families, and an abscisic acid-stress-ripening (*ASR*) gene from this family was isolated from *S. brachiata* and overexpressed in tobacco. Glycine-rich SbASR1 contains 63 percent disorder-promoting amino acid residues, and transgenic plants displayed improved adaptation to high-salt stress (De Jonge et al., 2000; Jha et al., 2012). The expression of TsLEA1 from *Thellungiella salsuginea* and TaLEA from *Tamarix androssowii* in yeast mutants improved salt tolerance ((Table 2) Zhang et al., 2012; Gao et al., 2013)). Similarly, another nuclear localized *Suaeda liaotungensis ASR* gene exhibited salt tolerance in transgenic *Arabidopsis* (Hu et al., 2014). Consumption of energy and involvement of yield penalty in this method, probably may not be appropriate for plant breeders to achieve salt tolerance unless if it is inevitable.

4.1.4. ROS mediated salt tolerance

The complex roles of ROS generated in salinity stress in halophytes are not fully understood, mainly due to their short life span, but this gap has been gradually filled over the last decade or so (Shabala, 2013). Moreover, ROS are also know to function as signaling components, apart from their major role in inducing cellular damage by degradation of membranes, oxidation of proteins inactivation of enzymes and altering the gene expression (Fig. 1). Recent studies on interactions between ROS along with other pathways involved in salt tolerance in halophytes highlight major differences with glycophytes (Table 3, Ozgur et al., 2013). Before that Katiyar-Agarwal et al. (2006) proved direct evidence for crosstalk between ion homeostasis and oxidative stress detoxification pathways. These authors showed that apart from the functional long cytoplasmic tail, the 10–12 transmembrane domains of SOS1 might act as a sodium sensor that interacts with RCD1, a regulator of oxidative stress (Fig. 1). This study also elucidated the role of SOS1 in oxidative stress tolerance. In addition, Chung et al. (2008) described SOS1 transcript stability mediated through ROS in *Arabidopsis*

and discussed the role of SOS1 in oxidative stress tolerance. In another study Verslues et al. (2007) demonstrated the interaction of SOS2 with nucleoside diphosphate kinase2 (NDPK2) and catalases, suggesting a link between hydrogen peroxidase signaling and salt stress. NDPK2 regulates mitogen-activated protein kinases (MAPKs) involved in oxidative stress responses. MAPKs are activated by H_2O_2 and has role in changes in the protein phophorylation state in signaling process

Hydrogen peroxide, the most abundant and stable ROS, is produced through dismutation of superoxide using SOD and via cell membrane NADPH-dependent oxidases (e.g. respiratory burst oxidase homologues, Rboh) and cell wall-attached peroxidases (Fig. 1). The balance between SODs and peroxide-scavenging enzymes in cells is considered to be crucial in determining salinity tolerance and cell fate. Based on metal cofactors and locations, SODs are classified into Mn-SODs, Cu/Zn-SODs and Fe-SODs (Modarresi et al., 2012). The Mn-SOD from *T. halophila* (*ThMSD*) was expressed in *E. coli*, conferring resistance to salt stress. The overexpression of *Arabidopsis* with the same gene exhibited salinity tolerance and higher germination rates at 100 and 150 mM NaCl compared with wild-type plants (Xu et al., 2014). The maintenance of a high antioxidant capacity and ROS-scavenging has been associated with augmented tolerance is one of the concept. Recently Bose et al. (2014a,b) argued that halophytes do not require efficient antioxidant activity, because this group possesses efficient sodium exclusion strategies from cytosol. Moreover they also argued that halophyte does not allow excessive ROS production. Ellouzi et al. (2011) also proved this hypothesis by estimating hydrogen peroxide in *Cakile maritima* (halophyte) and *Arabidopsis thaliana* (glycophyte). The results revealed that the initial accumulation of ROS was maximum in both halophyte and glycophyte in salt treatment and after four hours there was a rapid decline in accumulation of ROS in halophyte *C. maritima,* indicating the halophytes may not require a high level of antioxidant activity (Fig. 3). Like Ca^{2+}, hydrogen peroxide is a ubiquitous second messenger and able to respond to different physiological reactions. The role of H_2O_2 on signaling mechanism influence the transcription factors (DREB, NAC, bZIP, MYB, ZAT, WRKY etc.), different micro RNAs and the crosstalk pathways with auxin, salicylic acid, ABA enable to switch the tolerance to different stresses. In addition, production of hydrogen peroxide requires Ca^{2+} influx, which activates NADPH oxidases located at the plasma membrane (Fig. 1). Bose et al. (2014a,b)
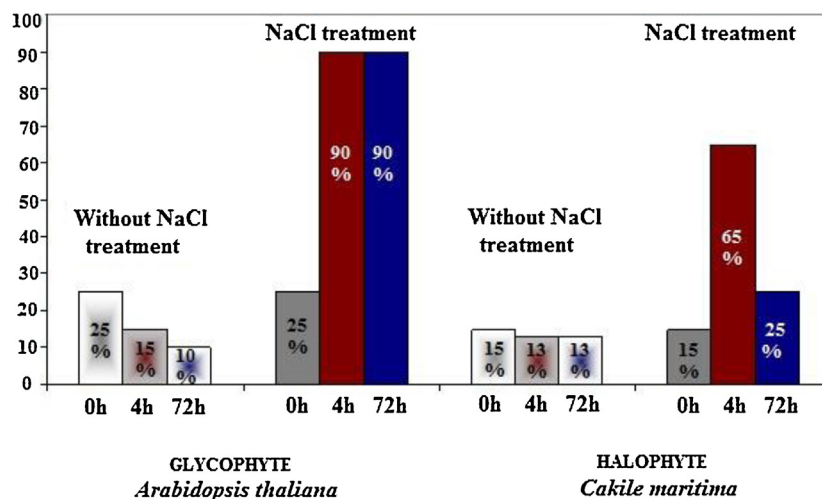

Fig. 3. Estimation of hydrogen peroxide (ROS) in glycophyte (*Arabidopsis thaliana*) and halophyte (*Cakile maritima*) through DAB staining*.
(*Data from Ellouzi et al., 2011)

introduced the concept of "H_2O_2 signatures" for initiation of ROS in priming halophytes to elevated NaCl levels. In this conception, initial higher levels of superoxide dismutase are required for rapid induction of H_2O_2 to trigger the salinity adaptive responses and after that the other enzymatic antioxidants (e.g. catalase, ascorbate peroxidase and peroxidase) decreases the level of H_2O_2 once the signal has been processed and resulted hydroxyl radicals scavenged by non-enzymatic antioxidants and ultimately protect the cellular damage. The role of singlet oxygen, which is produced by reaction of O_2 with triplet states in antennae and reaction center pigments, has been considered limited because of its short half-life (Bose et al., 2014a,b). Based on the above concept the levels of ROS in halophytes are less when compared to glycophytes at later stages. This concept may clearly indicate the superior ability of halophytes in salinity tolerance by involving ROS in signaling to elevate excess sodium for better tolerance. Bose et al. (2014a,b) proved that the superior tolerance of barley for salinity when compared to salt-sensitive pea plants through efficient control of xylem sodium loading and hydrogen peroxide accumulation as well reduced sensitivity of NSCC to H_2O_2 and less energy (ATP) utilization to maintain membrane potential under high salt conditions.

Class I ascorbate peroxidase reduces H_2O_2 to H_2O using ascorbate as the electron donor. APX from the succulent *S. brachiata* (*SbpAPX*) is restricted to the peroxisome, with predicted localization in the peroxisomal membrane (Tiwari et al., 2014). The expression of *SbpAPX* is upregulated through most abiotic stress conditions and transgenic tobacco lines overexpressing *SbpAPX* exhibited improved salt tolerance (Table 2). This may be credited to release of hydroxyl radicals via APX activity also leads to potassium efflux through GORK or NSCC (Fig. 1). Monodehydroascorbate reductase is a flavin adenine dinucleotide (FAD) enzyme involved in ascorbate regeneration, using nicotinamide adenine dinucleotide phosphate (NADP) as an electron donor to reduce monodehydroascorbate to ascorbate in several plant cell compartments, such as chloroplasts, mitochondria, cytosol, glyoxysomes and leaf peroxisomes. The upregulation of MDAR has been reported in response to various environmental stresses, and this receptor is one of the most important antioxidant systems for protection against damage through ROS in plant cell. Transgenic tobacco overexpressing dicotyledonous *Avicennia marina* MDAR exhibited more efficiency in converting monodehydroascorbate to ascorbate, and improving the level of ascorbate ensures sufficient APX availability for H_2O_2 detoxification, thereby improving oxidative response activity compared with wild-type plants; transgenic lines also showed improved survival under salt stress conditions (Kavitha et al., 2010). Glutathione S transferases (GSTs) are ubiquitous multifunctional proteins involved in the protection of plants from ROS, and these enzymes are expressed during normal and abiotic stress conditions. A potential defense system in halophytes has been developed based on different GSTs to sustain constantly changing weather and other environmental conditions. Cytosolic Tau class GST proteins are strictly plant-specific and have been well characterized for their role in the detoxification of herbicides in plants. The expression of *GST* gene isolated from *S. brachiata* in tobacco resulted in higher seed germination and survival in high salinity (Jha et al., 2011b). A group of polypeptides, called metallothioneins (MT), which bind to heavy metals and play a role in the ion homeostasis of essential metals and detoxification of non-essential metals, i.e., ROS scavenging, have also been observed as adequate candidate genes for salinity tolerance (Chaturvedi et al., 2014). Tobacco plants overexpressing *S. brachiata* MT-2 exhibited ROS scavenging and salt tolerance (Table 2).

4.1.5. Crosstalk genes mediated salt tolerance

Together, drought and salinity affect more than 10% of the arable land and lead to more than 50% decline in average yields of major crops worldwide (Tuteja, 2007). The increased crosstalk between salinity and drought, including other abiotic stresses, has been demonstrated at various occasions (Akhtar et al., 2012). Although salt and drought stresses have similar effects on water potential, salinity has additional cytotoxic effects within the cell in the form of excess sodium. The loss of water potential as a result of drought, high salt or dehydration reduces transpiration and negatively impacts numerous biological processes, including solute transport, photosynthetic carbon fixation respiration and defense mechanisms (Shen et al., 2003; Khedr et al., 2011). Dehydration responsive element binding factors play a major role in the expression of many stress-inducible genes mostly in an abscisic acid-independent manner through interactions with the dehydration-responsive element/C-repeat (DRE/CRT) cis-element present in the promoter region of different abiotic stress-responsive genes, confirming a critical role for DREB in improving abiotic stress tolerance (Fig. 1 and Table 2). Agarwal et al. (2007) demonstrated that the phosphorylated DREB2A protein from *Pennisetum glaucum* did not show binding with drought-responsive elements (DREs), instead binding was restored with dephosphorylated protein. In another study, the middle region of DREB2A functions as a negative regulatory domain (NRD), and the removal of this region resulted in the constitutive activation of the DREB transcription factor and provided tolerance under various abiotic stresses (Sakuma et al., 2006). The transgenic expression of *S. brachiata* DREB2A in tobacco induced low Na^+ and high K^+ levels in foliar tissues conferring improved salt tolerance (Gupta et al., 2014). The accumulation of higher levels of proline and low amounts of MDA and hydrogen peroxide (H_2O_2) under stress conditions were also observed. An intronless *SbSI2* (*S. brachiata salt-inducible-2*) has been cloned, and in silico analysis revealed that SbSI2 protein contains nuclear localization signals. The overexpression of this protein in tobacco lines resulted in the upregulation of DREB2 domains, which further enhanced the expression of abiotic stress-responsive genes. In addition, transgenic lines showed salt and osmotic tolerance with improved growth parameters, such as relative water content, higher K^+/Na^+ ratio, high chlorophyll, increased osmolyte levels and low Na^+ and ROS compared with control plants. SbSI2 protein also exhibited salinity tolerance in *E. coli* (Yadav et al., 2014).

NACs (no apical meristem (NAM) *Arabidopsis thaliana* transcription activation factor (ATAF1/2) and cup-shaped cotyledon (CUC2)) are the largest transcription factor family in plants, and determining the roles for individual NAC proteins has become a daunting task. However, some NAC groups are involved in different abiotic stress tolerance mechanisms (Fig. 1). *SlNAC1* and 2 have been isolated from the dicotyledon *S. liaotungensis* and expressed in *Arabidopsis* (Li et al., 2014b; Yang et al., 2014). GM *Arabidopsis* lines overexpressing both genes displayed improved tolerance to drought, salinity and cold stresses; specifically, longer root lengths were observed (low root inhibition) under high salt conditions. Another large family of zinc finger HIT proteins PAPA-1 (Pim-1-associated protein-1 (PAP-1)-associated protein-1-like (PAPA-1-like)) are generally involved in inducing growth and cell cycle arrests at the G1 phase in different models. Li et al. (2014a) reported that the *SlPAPA1* from *S. liaotungensis* plays a role in abiotic stress tolerance. Enhanced drought and salt stress tolerance was observed in transgenic *Arabidopsis* through increased germination and survival and lower root inhibition rates (Table 2). Saad et al., 2010, 2011, 2012 isolated a novel *SAP* gene (stress associated protein) from *A. littoralis* encoding A20/ANI zinc finger proteins, originally involved in animal immune and plant stress responses. However, augmented Na^+ accumulation in senescent basal leaves

and low rates of water loss were observed in overexpressed tobacco lines, elucidating the role of *AlSAP* in salt tolerance mechanism. Several proteins involved in protein folding or degradation have also been observed. The stress inducible SUMO (small ubiquitin-like modifier)-conjugating enzyme SaSce9 from *S. alterniflora* exhibited tolerance to salinity in transgenic *Arabidopsis* (Karan and Subudhi, 2012).

Mahalakshmi et al. (2006) recently isolated the *PcSrp* gene encoding a serine-rich protein from *P. coarctata*, which grows in coastal brackish water in northeastern India. The presence of characteristic SPSPSPSS and SSSSS amino acid stretches and transmembrane domains indicates that this gene encodes a cell wall membrane protein conferring salt tolerance in yeast. Fructose-1,6-biphosphate aldolase (FBA), with a central role in gluconeogenesis, glycolysis and the Calvin cycle, might also affect salt tolerance. Wei et al. (2009) reported that the *Sesuvium portulacastrum FBA* gene influences salt resistance in *E. coli*. Moreover, various factors, including vesicular trafficking regulated through adenosine diphosphate ribosylation factor1, SaARF1, from *S. alterniflora,* rat brain7 belonging to the small GTPase family protein, AlRab7 from *A. lagopoides*, the cyclophilin protein, ThCYP1 from *T. halophila*, the universal stress protein, SbUSP from *S. brachiata* and the RNA-binding protein, SaRBP1, from *Suaeda asparagoides* also exhibited tolerance to salinity and other abiotic stresses in *E. coli,* yeast and model plants, although little molecular evidence has been shown ((Chen et al., 2007; Ayarpadikannan et al., 2012; Udawat et al., 2014; Karan and Subudhi, 2014; Rajan et al., 2014) (Table 2)).

4.2. Salt tolerance studies using halophyte genes in different crop plants

Transgenic studies have focused on phenotypic traits for salt tolerance and overall yield. Herein, we discuss the transgenic expression of halophytic genes associated with salt tolerance mechanism in selected crops (Tables 1 and 2). Rice (*Oryza sativa*) is the most important staple food crop for more than half of the population worldwide and provides 90% of the calories from carbohydrates and 10% of the calories from protein. Several rice varieties have been used for both food and industrial purposes (e.g., bran for oil and hull for fuel and brewing). Although decreased rice yields, reflecting abiotic stresses, including salinity, have been observed in many reports, Kobayashi et al. (2012) produced genetically engineered rice with antiporters, such as SOS1 (*PtNHA1*) and NHX (*PutNHX*) from *P. tenuiflora*, suggesting the increased tolerance of shoots to NaCl and roots to NaHCO$_3$, likely reflecting the extrusion of Na$^+$, without limiting the entrance of Na$^+$ at the root. GM lines also maintained lower Na$^+$ and higher K$^+$ content in the shoots under NaCl treatments. In another GM rice line, the expression of vacuolar antiporter from *A. gmelinii* (*AgNHX1*) resulted in an eight-fold increase of vacuolar antiporter activity and exhibited tolerance up to 300 mM NaCl (Ohta et al., 2002). Generally, the activity of the halophyte vacuolar Na$^+$/H$^+$ antiporter is more reliable and therefore increased Na$^+$ pumping into vacuoles, thus imparting sodium tolerance in this GM rice. Similarly, genetically modified rice overexpressing the *S. salsa* vacuolar antiporter (*SsNHX1*) and monocotyledon *Spartina anglica* vacuolar antiporter (*SaNHX1*) independently exhibited enhanced salt tolerance up to 300 and 150 mM NaCl compared with controls (Zhao et al., 2006; Lan et al., 2011). The expression of vacuolar ATPase subunit c1 (*SaVHAc1*) from *S. alterniflora* in rice improved salt tolerance up to 200 mM NaCl and caused the upregulation of genes involved in cation transport and ABA signaling mechanisms (Baisakh et al., 2012). The independent overexpression of osmoprotectant genes, i.e., betaine aldehyde dehydrogenase from *Atriplex hortensis* (*AhBADH*)

and inositol synthase from *P. coarctata* (*PcINO1*) in rice, demonstrated enhanced levels of salinity tolerance (Guo et al., 1997; Das-Chatterjee et al., 2006). The photosynthetic efficiency of the GM plants grown in increasing salt indicated the substantial protection of photosystems, particularly PSII, in *PcINO1* transgenic plants and suggested a role for inositol in protecting chloroplasts from damage. Rice (*Indica, pusa basmati*) overexpressing the *SOD1* gene encoding a cytosolic Cu/Zn SOD from *Avicennia marina* (Prashanth et al., 2008) showed increased tolerance to methyl viologen-mediated oxidative stress and exhibited improved tolerance to various abiotic stresses, including high salinity, and increased grain yield was also observed.

Finger millet (*Eleusine coracana*) is a cereal grass primarily cultivated for grain and is considered as a helpful famine crop that is easily stored. This cereal crop is also a staple food initially cultivated in African and South Asian countries. This grain is readily digestible, highly nutritious and versatile. Similar to other cereal grains, finger millet is an energy feed valuable for high carbohydrate content. This crop has also been used to produce liquor and beer, generating the by-products used for livestock feeding. Finger millet is also useful for protection against soil erosion. This crop also suffers from several abiotic stresses. *P. coarctata* serine rich protein gene (*PcSrp*) has been introduced into finger millet under the control of the rice *Actin-1* promoter. GM lines showed significant salinity tolerance levels up to 250 mM NaCl through the increased accumulation of Na$^+$ in the roots than shoots, likely reflecting the restriction of Na$^+$ from the transpiration stream and the recirculation of Na$^+$ back into the roots, thereby preventing Na$^+$ accumulation in aerial plant parts (Mahalakshmi et al., 2006). The authors also observed an increase in root and shoot length, fresh weight and fertile seed production in transgenic lines.

Soybean (*Glycine max*) is a leguminous crop important for edible seeds and used as soy meal, flour, edible oil and biodiesel. Soybean is one of the most health-promoting cheap foods, with excellent protein content (including all 8 essential amino acids), high levels of essential fatty acids and numerous vitamins and minerals. Soybeans have considerable amounts of α-linolenic acid, omega-6 fatty acid and isoflavones (genistein and daidzein), thereby reducing the risk of developing breast, cervical, ovarian, lung and colon cancer. Soy proteins also helpful in lowering cholesterol levels and decrease the occurrence of diseases associated with high blood pressure. Recently, Liu et al. (2014) produced GM soybeans overexpressing vacuolar NHX1 from *A. littoralis,* resulting in salt tolerance up to 150 mM NaCl and less Na$^+$ accumulation in the shoots than in the roots. The K$^+$ content in both the shoots and roots of GM plants was significantly higher under salt stress, indicating that the transgenic plants maintains the K$^+$ retention and this is an indication of superior ability of halophytes (Demidchik, 2014; Shabala and Pottosin, 2014). Recently Bose et al. (2015) proved this hypothesis in halophytes and this may be credited to their ability to maintain highly negative membrane potential due to higher H$^+$-ATPase activity. Groundnut or peanut (*Arachis hypogaea*) is an important legume oil crops with seeds that are a rich source of carbohydrates, protein and vitamins A and B. Peanut oil contains arachidic and lignoceric acids, oleic and linoleic acid glycerides and residual oil cake used as a fertilizer. As a legume crop, peanuts improve soil fertility and have also been valued as a rotation crop because these plants do not require much water. Recently, Singh et al. (2014) expressed *S. brachiata* ascorbate peroxidase (*SbpAPX*) in peanut, thereby enhancing chlorophyll and water content under salt stress. Electrolyte leakage was also decreased in the transgenic lines, tolerating up to 150 mM NaCl and probably enhanced hydroxyl radicals mediated potassium efflux also a possible explanation for this tolerance. Mustard (*Brassica juncea*) is widely grown as a vegetable, and the seeds are used as condiments and for the production of essential oils. These oils are

edible, and the seed residue is used as cattle feed and fertilizer. Mustard has also been widely applied in folk medicine. Yield loss due to salinity has been frequently observed. Das-Chatterjee et al. (2006) overexpressed the gene INO1 (inositol-3-phosphate synthase) from P. coarctata (PcINO1) in mustard plants, and the GM lines showed salinity tolerance through the enhanced production of the osmolyte inositol and this is energy consumable process. Tomato (Solanum lycopersicum) is a vegetable and edible fruit commonly consumed worldwide. Tomato comprises thousands of cultivars with varying fruit sizes, colors, shapes and secondary metabolites. Tomatoes are also a rich source of lycopene, which might have beneficial health effects. The betaine aldehyde dehydrogenase gene was cloned from S. liaotungensis and over-expressed in tomato using CaMV35S and BADH P5 promoters (Wang et al., 2013a). GM tomato with P5 promoter exhibited enhanced tolerance to high salinity (200 mM NaCl) indicating the importance of promoters.

Alfalfa (Medicago sativa) is a perennial legume widely grown across the globe as a forage crop to feed cattle, reflecting the high protein content and highly digestible fiber of this plant. Humans also consume alfalfa sprouts as rich sources of vitamins. Alfalfa has been used in medicine and possesses numerous health benefits. Remarkable decreases in alfalfa yield have been caused through salinity for several years. Zhang et al. (2014) expressed vacuolar NHX1 from S. europaea in alfalfa plants, resulting in improved salt tolerance. These authors also observed increased proline content in transgenic plants and exhibited higher SOD, POD and CAT activities than in wild-type plants to scavenge the excess ROS. GM alfalfa plants expressing the SsNHX1 gene from the dicotyledonous euhalophyte Salsola soda under the control of the rd29A stress-inducible promoter tolerated p to 400 mM NaCl (Li et al., 2011). The coexpression of both the vacuolar ScNHX1 antiporter and vacuolar H-Ppase ScVP from S. corniculata (generated through cross-pollination) in alfalfa enhanced salt tolerance up to 300 mM NaCl (Liu et al., 2013). The introduction of multiple halophytic genes has great promise. Fescue (Festuca) is a type of grass widely used for fodder, which improves the digestion of animals and is a rich source of proteins, vitamins and minerals. Fescue is also grown as an ornamental plant for decorative landscape purposes, and this grass is often used on golf courses. Genetically engineered fescue, overexpressing the root-specific vacuolar AeNHX1 from Agropyron elongatum, exhibited increased salt tolerance, i.e., up to 300 mM NaCl (Qiao et al., 2007).

Cotton (Gossypium hirsutum) is a major fiber crop native to tropical and subtropical regions and is important to the economies of many developing countries. Cotton not only provides fiber for the textile industry but also plays a role in the feed and oil industries, as cotton seeds are rich in oil and protein. Previous studies have reported yield degradation resulting from several abiotic factors, including salinity. The vacuolar H^+-Ppase gene from T. halophila (TsVP) was overexpressed in cotton (cultivar Luyuan890), thereby enhancing performance under saline conditions, in terms of shoot and root growth and photosynthetic capacity (Lv et al., 2008). Increased Na^+, Cl^- and soluble sugar accumulation has also been observed in these GM plant lines, likely reflecting the increased activity of vacuolar secondary transporters. The same TsVP gene improved drought resistance in monocot maize and cotton and phosphate tolerance in maize (Lv et al., 2009).

The production of biofuels from plant biomass is rapidly becoming an important economic activity. Jatropha (Jatropha curcas) is a second generation biofuel resource with high oil content. This crop is also gaining economic importance, reflecting industrial and medicinal values. Jatropha oil and aqueous Jatropha oil extracts has potential pesticidal properties used to control the pests of cotton (e.g., cotton bollworm), potato and corn. Jha et al. (2013) expressed the vacuolar antiporter from S. brachiata (SbNHX1) in Jatropha and confirmed salt tolerance up to 200 mM NaCl through the estimation of different growth parameters, such as increased sodium, and chlorophyll, reduced rates of electrolyte leakage and less malondialdehyde in the leaves, indicative of reduced oxidative damage in these transgenic plants. These authors also reported that the JL8 line of transgenic Jatropha contains three transgene copies and showed higher expression than JL2 and JL19 lines, which contain single gene copies, and both JL2 and JL8 exhibited increased levels of salinity tolerance. These studies indicated that transgenic lines of either single or multiple copies improved the function. These salt-tolerant varieties have been useful in coastal regions for establishment of biofuel industries.

As mentioned in Fig. 2, extensive research in crop plants instead of model species might unveil elaborate information on salinity tolerance mechanisms in halophytes in the near future. Some halophytic genes, such as Limonium gmelinii SOS1, Kosteletzkya virginica SOS1, Karelinia caspica NHX1 and 2, Phragmites australis HAK2, Tamarix androssowii MnSOD and different NHX's from M.

■ Genes isolated from number of dicotyledonous halophytes (DH group)

■ Genes isolated from number of monocotyledonous halophytes (MH group)

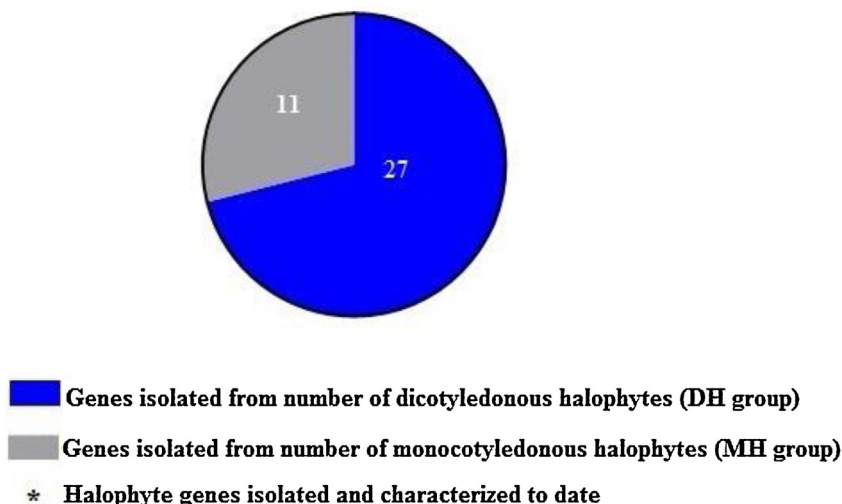＊ Halophyte genes isolated and characterized to date

Fig. 4. Estimation of genes studied from both dicotyledonous and monocotyledonous halophytes*.

crystallinum, have only been isolated, characterized and overexpressed in halophytes, revealing a role for these genes in salt tolerance mechanisms ((Ling et al., 2009; Wang et al., 2010, 2014a; Liu et al., 2012; Takahashi et al., 2007; Cosentino et al., 2010) (Tables 1 and 2)).

5. Need for improvements in genetic engineering technology

The selection of specific halophyte genes is an important concern because dicotyledonous are generally more tolerant than monocotyledons, with the exceptions of *AlNHX,* which exhibited tolerance against 400 mM NaCl in T_1 transgenic tobacco lines (Zhang et al., 2008; Flowers and Colmer, 2008). Coincidently a majority of salt tolerance studies were only conducted with genes isolated from dicotyledonous halophytes (Fig. 4). Most of the studies reviewed herein have relied on the use of strong promoters. Promoters affording strong constitutive expression have been widely used in many plant studies, including those on environmental stress tolerance. For example, *CaMV 35S*, *rice actin-1*, *rbcs* and *maize ubiquitin* genes have conferred specifically both biotic and abiotic stress tolerance. However, future studies on crop plants might require more finesse. High constitutive expression of a gene can be detrimental to the host plant, resulting in negative effects, such as sterility, abnormal morphology, gene silencing, yield penalty, etc. (Kurek et al., 2002; Kanneganti and Gupta, 2008). This problem can be solved using tissue specific or inducible promoters to restrict gene expression to a specific tissue, at a particular stage in development or in response to a specific type of stress. The sequence upstream of the *AlSAP* ORF from the C4 halophyte grass *A. littoralis* is designated Pr_{AlSAP} (Saad et al., 2011). Pr_{AlSAP} is an abiotic stress responsive, age dependent and tissue-specific promoter, making this promoter an ideal candidate for many agricultural applications. Chief among these would be those in which the expression of a gene is needed only in the vegetative part of the plants. We also anticipate increased success in the introduction of new genes into plant genomes. Transgenic plants frequently contain undesirable changes generally referred to as somaclonal variations, in addition to the introduced transgene, whatever it might be. Thus, the use of the proper explant for genetic transformation is also a considerable prerequisite (Skirvin et al., 1994). In general, variations are less likely observed from direct regeneration explants, such as meristematic tissues, contain shoot axillary buds and shoot tips and direct embryos. In contrast, callus cultures exhibit increased variations through indirect regeneration (Bregitzer et al., 2002). It is evident from several reports that the overexpression of a single gene could also impart salt tolerance to plants (mentioned above), but multiple gene insertions through site-specific recombination cloning (a.k.a. Gateway technology) is a method to insert more than one gene into a chromosomal locus. For example, the introduction of a complete set of *SOS1, SOS2* and other pumping genes using this technique is a promising methodology to meet the requirements to obtain insights into these mechanisms. Recent technologies, zinc-finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs), including the emergence of clustered regulatory interspaced short palindromic repeat (CRISPR), might also be helpful to unveil the molecular mechanisms of salt tolerance in halophytes. Considering the advantages of single or multiple copies, the inheritance of transgenes is difficult to predict and transgene silencing is possible at both the transcriptional and post transcriptional levels (Pawlowski and Somers, 1996; Koprek et al., 2001; Travella et al., 2005). These findings are compulsory to evaluate the biosafety or environmental risk assessment of GM crops (Bae et al., 2008). Our group is involved in biosafety tests using GM crops at field levels, and currently we are pursuing the phenotypic evaluation of transgenic *Chrysanthemum* overexpression with salt tolerance genes encoding the *P. coarctata* serine-rich protein *PcSrp*.

6. Conclusions

Recent knowledge on halophyte genes and their roles in superior salt tolerance mechanisms urges us to write this review to create salt-tolerant glycophytes using biotechnological approaches. It is likely that salt stress initially triggers salt sensors and SOS systems, involving multiple signaling partners, and the individual or combined effects of these genes might induce salt tolerance through changes in ion homeostasis (both influx and efflux), the formation of osmoprotectants, activation of crosstalk genes, induction of antioxidants and the development of salt gland or bladders (Cheeseman, 2014; Shabala et al., 2014; Slama et al., 2015). The schematic representation of salt tolerance mechanisms based on the available information in halophyte genes provides a framework for future studies in improvement of salt tolerance (Fig. 1). Currently, all overexpression studies to improve salt tolerance have been based on the assumption of a glycophytic pathway for salt tolerance and recent works using genes from halophytes will certainly helpful. We also discussed the unique salt tolerance mechanism in halophytes and their dominance in salt tolerance compared with glycophytes. In addition, there is high probability of additional genes or additional regulation in halophytes, thereby increasing salt tolerance needs to sortout in future works. Glycophytes and halophytes probably possess the same set of most of the genes, but differ in the environment-dependent regulation of these genes (please check Table 3 for differences between halophytes and glycophytes in salt tolerance). Post-translational modifications are extremely important to mention in halophytic phenotype characterization. Still extensive transcriptomic, proteomic and metabolomic comparisons are required to identify good candidate genes for crop improvement.

In previous studies, we primarily focused on glycophytic salt stress genes and their tolerance mechanisms (Hussain et al., 2008). But here we focused on halophytic genes, as a 'model' to enhance salt tolerance in glycophytic crop species and are also useful for the detailed understanding of the physiological, biochemical and molecular basis of salinity tolerance mechanisms. However, not all attempts at the overexpression of salt-tolerant genes have displayed expected salt tolerance and desirable phenotypic changes. Other factors, such as transformation with more than one gene, the selection of the promoter and explant for genetic transformation, transgene copy number, sub-cellular localization and expression profiles have also been considered for the levels of tolerance. The biosafety or environmental risk assessment of GM crops at field levels are also an important concerns. We concluded that genetic engineering using halophyte genes might offer an excellent platform for developing glycophytic crops with improved salinity tolerance.

References

Agarwal, P., Agarwal, P.K., Nair, S., Sopory, S.K., Reddy, M.K., 2007. Stress inducible DREB2A transcription factor from *Pennisetum glaucum* is a phosphoprotein and its phosphorylation negatively regulates its DNA-binding activity. Mol. Genet. Gen. 277, 189–198. doi:http://dx.doi.org/10.1007/s00438-006-0183-z.

Akhtar, M., Jaiswal, A., Taj, G., Jaiswal, J.P., Qureshi, M.I., Singh, N.K., 2012. DREB1/CBF transcription factors: their structure, function and role in abiotic stress tolerance in plants. J. Genet. 91, 385–395. doi:http://dx.doi.org/10.1007/s12041-012-0201-3.

Ali, A., Park, C.H., Aman, R., Ali, Z., Yun, D.J., 2013. Role of HKT1 in *Thellungiella salsuginea*, a model extremophile plant. Plant Signal. Behav. 8 doi:http://dx.doi.org/10.4161/psb.25196 pii: e25196.

Ali, Z., Park, H.C., Ali, A., Oh, D.H., Aman, R., Kropornicka, A., Hong, H., Choi, W., Chung, W.S., Kim, W.Y., Bressan, R.A., Bohnert, H.J., Lee, S.Y., Yun, D.J., 2012. TsHKT1;2, a HKT1 homolog from the extremophile *Arabidopsis* relative *Thellungiella salsuginea*, shows K(+) specificity in the presence of NaCl. Plant Physiol. 158, 1463–1474. doi:http://dx.doi.org/10.1104/pp.111.193110.

Anschutz, U., Becker, D., Shabala, S., 2014. Going beyond nutrition: regulation of potassium homoeostasis as a common denominator of plant adaptive responses to environment. J. Plant Physiol. 171, 670–687. doi:http://dx.doi.org/10.1016/j.jplph.2014.01.009.

Apse, M.P., Aharon, G.S., Snedden, W.A., Blumwald, E., 1999. Salt tolerance conferred by overexpression of a vacuolar Na⁺/H⁺ antiporter in *Arabidopsis*. Science 285, 1256–1258. doi:http://dx.doi.org/10.1126/science.285.5431.1256.

Aquino, R.S., Grativol, C., Mourao, P.A., 2011. Rising from the sea: correlations between sulfated polysaccharides and salinity in plants. PLoS One 28 (6), 4: e18862. doi:http://dx.doi.org/10.1371/journal.pone.0018862.

Ardie, S.W., Xie, L., Takahashi, R., Liu, S., Takano, T., 2009. Cloning of a high-affinity K⁺ transporter gene *PutHKT2;1* from *Puccinellia tenuiflora* and its functional comparison with *OsHKT2;1* from rice in yeast and *Arabidopsis*. J. Exp. Bot. 60, 3491–3502. doi:http://dx.doi.org/10.1093/jxb/erp184.

Ardie, S.W., Liu, S., Takano, T., 2010. Expression of the AKT1-type K⁺ channel gene from Puccinellia tenuiflora, PutAKT1, enhances salt tolerance in *Arabidopsis*. Plant Cell Rep. 29, 865–874. doi:http://dx.doi.org/10.1007/s00299-010-0872-2.

Ardie, S.W., Nishiguchi, S., Liu, S., Takano, T., 2011. Ectopic expression of the K⁺ channel β subunits from *Puccinellia tenuiflora* (*KPutB1*) and rice (*KOB1*) alters K⁺ homeostasis of yeast and *Arabidopsis*. Mol. Biotechnol. 48, 76–86. doi:http://dx.doi.org/10.1007/s12033-010-9349-3.

Ayarpadikannan, S., Chung, E., Cho, C.W., So, H.A., Kim, S.O., Jeon, J.M., Kwak, M.H., Lee, S.W., Lee, J.H., 2012. Exploration for the salt stress tolerance genes from a salt-treated halophyte, *Suaeda asparagoides*. Plant Cell Rep. 31, 35–48. doi:http://dx.doi.org/10.1007/s00299-011-1137-4.

Bae, T.W., Vanjildorjn, E., Song, S.Y., Nishiguchi, S., Yang, S.S., Song, I.J., Chandrasekhar, T., Kang, T.W., Kim, J.L., Koh, Y.J., Park, S.Y., Lee, J., Lee, Y.E., Ryu, K. H., Riu, K.Z., Song, P.S., Lee, H.Y., 2008. Environmental risk assessment of genetically engineered herbicide-tolerant *Zoysia japonica*. Journal of Env. Quality 37, 207–218. doi:http://dx.doi.org/10.2134/jeq2007.0128.

Baisakh, N., RamanaRao, M.V., Rajasekaran, K., Subudhi, P., Janda, J., Galbraith, D., Vanier, C., Pereira, A., 2012. Enhanced salt stress tolerance of rice plants expressing a vacuolar H⁺-ATPase subunit c1 (*SaVHAc1*) gene from the halophyte grass *Spartina alterniflora Löisel*. Plant Biotech. J. 10, 453–464. doi:http://dx.doi.org/10.1111/j.1467-7652.2012.00678.x.

Barkla, B.J., Vera-Estrella, R., Pantoja, O., 2012. Protein profiling of epidermal bladder cells from the halophyte *Mesembryanthemum crystallinum*. Proteomics 12, 2862–2865. doi:http://dx.doi.org/10.1002/pmic.201200152.

Barragán, V., Leidi, E.O., Andrés, Z., Rubio, L., Luca, D.A., Fernández, J.A., Cubero, B., Pardo, J.M., 2012. Ion exchangers NHX1 and NHX2 mediate active potassium uptake into vacuoles to regulate cell turgor and stomatal function in *Arabidopsis*. Plant Cell 24, 1127–1142. doi:http://dx.doi.org/10.1105/tpc.111.095273.

Bassil, E., Tajima, H., Liang, Y.C., Ohto, M.A., Ushijima, K., Nakano, R., Esumi, T., Coku, A., Belmonte, M., Blumwald, E., 2011. The *Arabidopsis* Na⁺/H⁺ antiporters NHX1 and NHX2 control vacuolar pH and K⁺ homeostasis to regulate growth, flower development, and reproduction. Plant Cell 23, 3482–3497. doi:http://dx.doi.org/10.1105/tpc.111.089581.

Bassil, E., Coku, A., Blumwald, E., 2012. Cellular ion homeostasis: emerging roles of intracellular NHX Na⁺/H⁺ antiporters in plant growth and development. J Exp Bot 63, 5727–5740. doi:http://dx.doi.org/10.1093/jxb/ers250.

Blumwald, E., Aharon, G.S., Apse, M.P., 2000. Sodium transport in plant cells. BBA Biomem 1465, 140–151. doi:http://dx.doi.org/10.s0005-2736(00) 00135-8.

Blumwald, E., Poole, R.J., 1985. Na⁽⁺⁾/H⁽⁺⁾ antiport in isolated tonoplast vesicles from storage tissue of *Beta vulgaris*. Plant Physiol 78, 163–167. doi:http://dx.doi.org/10.1104/pp.78.1.163.

Bonales-Alatorre, E., Pottosin, I., Shabala, L., Chen, Z.H., Zeng, F.R., Jacobsen, S.E., Shabala, S., 2013a. Differential activity of plasma and vacuolar membrane transporters contributes to genotypic differences in salinity tolerance in a halophyte species, *Chenopodium quinoa*. Int. J. Mol. Sci. 14, 9267–9285. doi:http://dx.doi.org/10.3390/ijms14059267.

Bonales-Alatorre, E., Shabala, S., Chen, Z.H., Pottosin, I., 2013b. Reduced tonoplast fast-activating and slow-activating channel activity is essential for conferring salinity tolerance in a facultative halophyte, quinoa. Plant Physiol. 162, 940–952. doi:http://dx.doi.org/10.1104/pp.113.216572.

Bose, J., Rodrigo-Moreno, A., Shabala, S., 2014a. ROS homeostasis in halophytes in the context of salinity stress tolerance. J. Exp. Bot. 66, 1241–1257. doi:http://dx.doi.org/10.1093/jxb/ert430.

Bose, J., Shabala, L., Pottosin, I., Zeng, F., Velarde-Buendía, A.M., Massart, A., Poschenrieder, C., Hariadi, Y., Shabala, S., 2014b. Kinetics of xylem loading, membrane potential maintenance, and sensitivity of K(+)-permeable channels to reactive oxygen species: physiological traits that differentiate salinity tolerance between pea and barley. Plant Cell Environ. 37, 589–600. doi:http://dx.doi.org/10.1111/pce.12180.

Bose, J., Rodrigo-Moreno, A., Lai, D., Xie, Y., Shen, W., Shabala, S., 2015. Rapid regulation of the plasm membrane H+-ATPase activity is essential to salinity tolerance in two halophyte species, *Atriplex lentiformis* and *Chenopodium quinoa*. Ann. Bot. 115, 481–494. doi:http://dx.doi.org/10.1093/aob/mcu219.

Braun, Y., Hassidim, M., Lerner, H.R., Reinhold, L., 1986. Studies on H-translocating ATPases in plants of varying resistance to salinity: I. salinity during growth modulates the proton pump in the halophyte *Atriplex nummularia*. Plant Physiol. 81, 1050–1056. doi:http://dx.doi.org/10.1104/pp.81.4.1050.

Bregitzer, P., Zhang, S., Cho, M.-J., Lemaux, P.G., 2002. Reduced somaclonal variation in barley is associated with culturing highly differentiated, meristematic tissues. Crop Sci. 42, 1303–1308. doi:http://dx.doi.org/10.2135/cropsci2002.1303.

Bromham, L., 2014. Macroevolutionary patterns of salt tolerance in angiosperms. Ann. Bot. 1–9 doi:http://dx.doi.org/10.1093/aob/mcu229.

Cabot, C., Sibole, J.V., Barcelo, J., Poschenrieder, C., 2014. Lessons from crop plants struggling with salinity. Plant Sci. 226, 2–13. doi:http://dx.doi.org/10.1016/j.plantsci.2014.013.

Chaturvedi, A.K., Patel, M.K., Mishra, A., Tiwari, V., Jha, B., 2014. The *SbMT-2* gene from a halophyte confers abiotic stress tolerance and modulates ROS scavenging in transgenic tobacco. PLoS One 9 (10) doi:http://dx.doi.org/10.1371/journal.pone.0111379.

Cheeseman, J., Herendeen, L., Cheeseman, A., Clough, B., 1997. Photosynthesis and photoprotection in mangroves under field conditions. Plant Cell Environ 20, 579–588. doi:http://dx.doi.org/10.1111/j.1365-3040.1997.00096.x.

Cheeseman, J.M., 2014. The evolution of halophytes, glycophytes and crops, and its implications for food security under saline conditions. New Phytol 1–14. doi:http://dx.doi.org/10.1111/nph.13217137.2010.03208.x.

Chen, A.P., Wang, G.L., Qu, Z.L., Lu, C.X., Liu, N., Wang, F., Xia, G.X., 2007. Ectopic expression of *ThCYP1*, a stress-responsive cyclophilin gene from *Thellungiella halophila*, confers salt tolerance in fission yeast and tobacco cells. Plant Cell Rep. 26, 237–245. doi:http://dx.doi.org/10.1007/s00299-006-0238-y.

Chen, X., Bao, H., Jia, W., Li, Y., 2015a. Overexpression of *SeNHX1* improves both salt tolerance and disease resistance in tobacco. Plant Signal Behav. 3;10 (4), e993240. doi:http://dx.doi.org/10.4161/15592324.2014.993240.

Chen, Y., Zong, J., Tan, Z., Li, L., Hu, B., Chen, C., Chen, J., Liu, J., 2015b. Systematic mining of salt-tolerant genes in halophyte-Zoysia matrella through cDNA expression library screening. Plant Physiol. Biochem. 89, 44–52. doi:http://dx.doi.org/10.1016/j.plaphy.2015.02.007.

Chung, J.-S., Zhu, J.-K., Bressan, R.A., Hasegawa, P.M., Shi, H., 2008. Reactive oxygen species mediate Na⁺-induced *SOS1* mRNA stability in *Arabidopsis*. Plant J 53, 554–565. doi:http://dx.doi.org/10.1111/j.1365-313X.2007.03364.x.

Cosentino, C., Fischer-Schliebs, E., Bertl, A., Thiel, G., Homann, U., 2010. Na⁺/H⁺ antiporters are differentially regulated in response to NaCl stress in leaves and roots of *Mesembryanthemum crystallinum*. New Phytol . 186, 669–680. doi:http://dx.doi.org/10.1111/j.1469-8.

Cuin, T.A., Betts, S.A., Chalmandrier, R., Shabala, S., 2008. A root's ability to retain K⁺ correlates with salt tolerance in wheat. J Exp Bot 59, 2697–2706. doi:http://dx.doi.org/10.1093/jxb/ern128.

Cuin, T.A., Shabala, S., 2007. Compatible solutes reduce ROS-induced potassium efflux in *Arabidopsis* roots. Plant Cell Environ. 30, 875–885. doi:http://dx.doi.org/10.1111/j.1365-3040.2007.01674.x.

Das-Chatterjee, A., Goswami, L., Maitra, S., Dastidar, K.G., Ray, S., Majumder, A.L., 2006. Introgression of a novel salt-tolerant L-myo-inositol 1-phosphate synthase from *Porteresia coarctata* (Roxb.) Tateoka (*PcINO1*) confers salt tolerance to evolutionary diverse organisms. FEBS Lett. 580, 3980–3988. doi:http://dx.doi.org/10.1016/j.febslet.2006.03.033.

Dassanayake, M., Oh, D.H., Haas, J.S., Hernandez, A., Hong, H., Ali, S., Yun, D.J., Bressan, R.A., Zhu, J.K., Bohnert, H.J., Cheeseman, J.M., 2011. The genome of the extremophile crucifer *Thellungiella parvula*. Nat. Genet. 43, 913–918. doi:http://dx.doi.org/10.1038/ng.889.

De Jonge, H.R., Hogema, B., Tilly, B.C., 2000. Protein *N*-myristoylation: critical role in apoptosis and salt tolerance. Science 63, 1–4. doi:http://dx.doi.org/10.1126/stke.2000.63.pe1.

Demidchik, V., Shabala, S.N., Coutts, K.B., Tester, M.A., Davies, J.M., 2003. Free oxygen radicals regulate plasma membrane Ca2⁺ and K⁺-permeable channels in plant root cells. J. Cell Sci. 116, 81–88. doi:http://dx.doi.org/10.1242/jcs.00201.

Demidchik, V., 2014. Mechanisms and physiological roles of K⁺ efflux from root cells. J. Plant Physiol. 171, 696–707. doi:http://dx.doi.org/10.1016/j.jplph.2014.01.015.

Deng, Z., Li, Y., Xia, R., Wang, W., Huang, X., Zhang, L., Zhang, S., Yang, C., Zhang, Y., Chen, M., Xie, Q., 2009. Structural analysis of 83-kb genomic DNA from *Thellungiella halophila*: sequence features and microcolinearity between salt cress and *Arabidopsis thaliana*. Genomics 94, 324–332. doi:http://dx.doi.org/10.1016/j.ygeno.

Dinakar, C., Bartels, D., 2013. Desiccation tolerance in resurrection plants: new insights from transcriptome, proteome and metabolome analysis. Front. Plant Sci. 28 (482) doi:http://dx.doi.org/10.3389/fpls.2013.00482.

Edelist, C., Raffoux, X., Falque, M., Dillmann, C., Sicard, D., Rieseberg, L.H., Karrenberg, S., 2009. Differential expression of candidate salt-tolerance genes in the halophyte *Helianthus paradoxus* and its glycophyte progenitors *H. annuus*

and *H. petiolaris* (Asteraceae). Am. J. Bot. 96, 1830–1838. doi:http://dx.doi.org/10.3732/ajb.0900067.

Ellouzi, H., Ben Hamed, K., Cela, J., Munne-Bosch, S., Abdelly, C., 2011. Early effects of salt stress on the physiological and oxidative status of *Cakile maritima* (halophyte) and *Arabidopsis thaliana* (glycophyte). Physiol. Plant 142, 128–143. doi:http://dx.doi.org/10.1111/j.1399-3054.2011.01450.x.

Flowers, T.J., Colmer, T.D., 2008. Salinity tolerance in halophytes. New Phytol. 179, 945–963. doi:http://dx.doi.org/10.1111/j.1469-8137.2008.02531.x.

Flowers, T.J., Munns, R., Colmer, T.D., 2015. Sodium chloride toxicity and the cellular basis of salt tolerance in halophytes. Ann. Bot. 115, 419–431. doi:http://dx.doi.org/10.1093/aob/mcu217.

Fu, H., Subramanian, R.R., Masters, S.C., 2000. 14-3-3 proteins: structure, function, and regulation. Annu. Rev. Pharmacol. Toxicol. 40, 617–647. doi:http://dx.doi.org/10.1146/annurev.pharmtox.40.1.617.

Fukuhara, T., Pak, J.Y., Ohwaki, Y., Tsujimura, H., Nitta, T., 1996. Tissue-specific expression of the gene for a putative plasma membrane H(+)-ATPase in a seagrass. Plant Physiol. 110, 35–42. doi:http://dx.doi.org/10.1104/pp.110.1.1.35.

Gao, F., Gao, Q., Duan, X., Yue, G., Yang, A., Zhang, J., 2006. Cloning of an H⁺-PPase gene from *Thellungiella halophila* and its heterologous expression to improve tobacco salt tolerance. J. Exp. Bot. 57, 3259–3270. doi:http://dx.doi.org/10.1093/jxb/erl090.

Gao, C., Wang, Y., Jiang, B., Liu, G., Yu, L., Wei, Z., Yang, C., 2011. A novel vacuolar membrane H⁺-ATPase c subunit gene (*ThVHAc1*) from *Tamarix hispida* confers tolerance to several abiotic stresses in *Saccharomyces cerevisiae*. Mol. Biol. Rep. 38, 957–963. doi:http://dx.doi.org/10.1007/s11033-010-0189-9.

Gao, W., Bai, S., Li, Q., Gao, C., Liu, G., Li, G., Tan, F., 2013. Overexpression of *TaLEA* Gene from *Tamarix androssowii* improves salt and drought tolerance in transgenic poplar (*Populus simonii, P. nigra*). PLoS One 8, 1–7. doi:http://dx.doi.org/10.1371/journal.pone.0067462.

Garciadeblas, B., Benitom, B., Rodriguez-Navarro, A., 2002. Molecular cloning and functional expression in bacteria of the potassium transporters CnHAK1 and CnHAK2 of the seagrass *Cymodocea nodosa*. Plant Mol. Biol. 50, 623–633. doi:http://dx.doi.org/10.1023/A.1019951023362.

Garciadeblas, B., Haro, R., Benito, B., 2007. Cloning of two SOS1 transporters from the seagrass *Cymodocea nodosa*. SOS1 transporters from *Cymodocea* and *Arabidopsis* mediate potassium uptake in bacteria. Plant Mol. Biol. 63, 479–490. doi:http://dx.doi.org/10.1007/s11103-006-9102-2.

Garthwaite, A.J., von Bothmer, R., Colmer, T.D., 2005. Salt tolerance in wild *Hordeum* species is associated with restricted entry of Na⁺ and Cl⁻ into the shoots. J. Exp. Bot. 56, 2365–2378.

Ghars, M.A., Parre, E., Debez, A., Bordenave, M., Richard, L., Leport, L., Bouchereau, A., Savoure, A., Abdelly, C., 2008. Comparative salt tolerance analysis between *Arabidopsis thaliana* and *Thellungiella halophila*, with special emphasis on K⁺/Na⁺ selectivity and proline accumulation. J. Plant. Physiol. 165, 588–599. doi:http://dx.doi.org/10.1016/j.jplph.2007.05.014.

Glenn, E., Brown, J.J., Blumwald, E., 1999. Salt-tolerant mechanisms and crop potential of halophytes. Crit. Rev. Plant Sci. 18, 227–255. doi:http://dx.doi.org/10.1080/07352689991309207.

Gong, Q., Li, P., Ma, S., Indu Rupassara, S., Bohnert, H.J., 2005. Salinity stress adaptation competence in the extremophile *Thellungiella halophila* in comparison with its relative *Arabidopsis thaliana*. Plant J. 44, 826–839.

Guan, B., Hu, Y., Zeng, Y., Wang, Y., Zhang, F., 2011. Molecular characterization and functional analysis of a vacuolar Na⁺/H⁺ antiporter gene (HcNHX1) from *Halostachys caspica*. Mol. Biol. Rep. 38, 1889–1899. doi:http://dx.doi.org/10.1007/s11033-010-0307-8.

Guo, S., Yin, H., Zhang, X., Zhao, F., Li, P., Chen, S., Zhao, Y., Zhang, H., 2006. Molecular cloning and characterization of a vacuolar H⁺-pyrophos-phatase gene, *SsVP*, from the halophyte *Suaeda salsa* and its overexpression increases salt and drought tolerance of *Arabidopsis*. Plant Mol Biol 60, 41–50. doi:http://dx.doi.org/10.1007/s11103-005-2417-6.

Guo, Y., Zhang, L., Xiao, G., Cao, S., Gu, D., Tian, W., Chen, S., 1997. Expression of betaine aldehyde dehydrogenase gene and salinity tolerance in rice transgenic plants. Sci. China. C Life. Sci. 40, 496–501. doi:http://dx.doi.org/10.1007/bf03183588.

Guo, Y., Qiu, Q.S., Quintero, F.J., Pardo, J.M., Ohta, M., Zhang, C., Schumaker, K.S., Zhu, J.K., 2004. Transgenic evaluation of activated mutant alleles of SOS2 reveals a critical requirement for its kinase activity and C-terminal regulatory domain for salt tolerance in *Arabidopsis thaliana*. Plant Cell 16, 435–449. doi:http://dx.doi.org/10.1105/tpc.019174.

Guo, Y.S., Wan-Ke, Z., Dong-Qing, Y., Bao-Xing, D., Jin-Song, Z., Shou-Yi, C., 2002. Overexpression of proline transporter gene isolated from halophyte confers salt tolerance in *Arabidopsis*. Acta Bota. Sinica 44, 956–962.

Gupta, K., Jha, B., Agarwal, P., 2014. A dehydration-responsive element binding (*DREB*) transcription actor from the succulent halophyte *Salicornia brachiata* enhances abiotic stress tolerance in transgenic tobacco. Mar. Biotech. 16, 657–673. doi:http://dx.doi.org/10.1007/s10126-014-9582-z.

Halfter, U., Ishitani, M., Zhu, J.K., 2000. The *Arabidopsis* SOS2 protein kinase physically interacts with and is activated by the calcium-binding protein SOS3. Proc. Natl. Acad. Sci. U. S. A. 97, 3735–3740. doi:http://dx.doi.org/10.1073/pnas.97.7.3735.

Hamada, A., Shono, M., Xia, T., Ohta, M., Hayashi, Y., Tanaka, A., Hayakawa, T., 2001. Isolation and characterization of a Na⁺/H⁺ antiporter gene from the halophyte *Atriplex gmelini*. Plant Mol. Biol. 46, 35–42. doi:http://dx.doi.org/10.1023/A.1010603222673.

Hasegawa, P.M., Bressan, R.A., Zhu, J.K., Bohnert, H.J., 2000. Plant cellular and molecular responses to high salinity. Ann. Rev. Plant Phys. Plant Mol. Biol. 51, 463–499. doi:http://dx.doi.org/10.1146/annurev.arplant.51.1.463.

He, C., Yan, J., Shen, G., Fu, L., Holaday, A.S., Auld, D., Blumwald, E., Zhang, H., 2005. Expression of an *Arabidopsis* vacuolar sodium/proton antiporter gene in cotton improves photosynthetic performance under salt conditions and increases fiber yield in the field. Plant Cell Physiol. 46, 1848–1854. doi:http://dx.doi.org/10.1093/pcp/pci201.

Horie, T., Hauser, F., Schroeder, J.I., 2009. HKT transporter-mediated salinity resistance mechanisms in *Arabidopsis* and monocot crop plants. Trends Plant Sci. 14, 660–668. doi:http://dx.doi.org/10.1016/j.tplants.2009.08.009.

Hou, Q., Bartels, D., 2015. Comparative study of the aldehyde dehydrogenase (ALDH) gene superfamily in the glycophyte *Arabidopsis thaliana* and *Eutrema* halophytes. Ann. Bot. 115, 465–479. doi:http://dx.doi.org/10.1093/aob/mcu152.

Hu, Y.X., Yang, X., Li, X.L., Yu, X.D., Li, Q.L., 2014. The SlASR gene cloned from the extreme halophyte *Suaeda liaotungensis K.* enhances abiotic stress tolerance in transgenic *Arabidopsis thaliana*. Gene 549, 243–251. doi:http://dx.doi.org/10.1016/j.gene.2014.07.071.

Hu, Y.Z., Zeng, Y.L., Guan, B., Zhang, F.C., 2012. Overexpression of a vacuolar H⁺-pyrophosphatase and a B subunit of H⁺-ATPase cloned from the halophyte *Halostachys caspica* improves salt tolerance in *Arabidopsis thaliana*. Plant Cell Tiss. Org. Cult. 108, 63–71. doi:http://dx.doi.org/10.1007/s11240-011-0013-9.

Hussain, T.M., Chandrasekhar, T., Hazara, M., Sultan, Z., Saleh, B.K., Gopal, G.R., 2008. Recent advances in salt stress biology-a review. Biotech. Mol. Boil. Rev. 3, 8–13. doi:http://dx.doi.org/10.5897/BMBR.

Ishitani, M., Majumder, A.L., Bornhouser, A., Michalowski, C.B., Jensen, R.G., Bohnert, H.J., 1996. Coordinate transcriptional induction of *myo*-inositol metabolism during environmental stress. Plant J. 9, 537–548.

Jha, A., Joshi, M., Yadav, N., Agarwal, P., Jha, B., 2011a. Cloning and characterization of the *Salicornia brachiata* Na⁺/H⁺ antiporter gene SbNHX1 and its expression by abiotic stress. Mol. Bio. Rep. 38, 1965–1973. doi:http://dx.doi.org/10.1007/s11033-010-0318-5.

Jha, B., Sharma, A., Mishra, A., 2011b. Expression of SbGSTU (tau class glutathione S-transferase) gene isolated from *Salicornia brachiata* in tobacco for salt tolerance. Mol. Bio. Rep. 38, 4823–4832. doi:http://dx.doi.org/10.1007/s11033-010-0625-x.

Jha, B., Lal, S., Tiwari, V., Yadav, S., Agarwal, P., 2012. The SbASR-1 gene cloned from an extreme halophyte *Salicornia brachiata* enhances salt tolerance in transgenic tobacco. Mar. Biotech. 14, 782–792. doi:http://dx.doi.org/10.1007/s10126-012-9442-7.

Jha, B., Mishra, A., Jha, A., Joshi, M., 2013. Developing transgenic *Jatropha* using the SbNHX1 gene from an extreme halophyte for cultivation in saline wasteland. PLoS One 8, e71136. http://dx.doi.org/10.1371/journal.pone.0071136.

Jithesh, M., Prashanth, S., Sivaprakash, K., Parida, A., 2006a. Monitoring expression profiles of antioxidant genes to salinity, iron, oxidative, light and hyperosmotic stresses in the highly salt tolerant grey mangrove, *Avicennia marina* (Forsk.) Vierh. by mRNA analysis. Plant Cell Rep. 25, 865–876. doi:http://dx.doi.org/10.1007/s00299-006-0127-4.

Jithesh, M.N., Prashanth, S.R., Sivaprakash, K.R., Parida, A.K., 2006b. Antioxidative response mechanisms in halophytes: their role in stress defence. J. Genet. 85, 237–254.

Jordan, F.L., Robin-Abbot, M., Majer, R.M., Glenn, E.P., 2002. A comparison of chelator-facilitated metal uptake by a halophyte and a glycophyte. Environ. Toxicol. Chem. 21, 2698–2704. doi:http://dx.doi.org/10.1002/etc.5620211224.

Joshi, R., Ramanarao, M.V., Baisakh, N., 2013. *Arabidopsis* plants constitutively overexpressing a myo-inositol 1-phosphate synthase gene (SaINO1) from the halophyte smooth cordgrass exhibits enhanced level of tolerance to salt stress. Plant Physiol. Biochem. 65, 61–66. doi:http://dx.doi.org/10.1016/j.plaphy.2013.01.009.

Kanneganti, V., Gupta, A., 2008. Over expression of OsiSAP8, a member of stress associated protein (SAP) gene family of rice confers tolerance to salt, drought and cold stress in transgenic tobacco and rice. Plant Mol. Biol. 66, 445–462. doi:http://dx.doi.org/10.1007/s11103-007-9284-2.

Kant, S., Kant, P., Raveh, E., Barak, S., 2006. Evidence that differential gene expression between the halophyte, *Thellungiella halophila*, and *Arabidopsis thaliana* is responsible for higher levels of the compatible osmolyte proline and tight control of Na⁺ uptake in *T. halophila*. Plant Cell Environ. 29, 1220–1234. doi:http://dx.doi.org/10.1111/j.1365-3040.2006.01502.x.

Katschnig, D., Jaarsma, R., Almeida,P, Rozema, J., Schat, H., 2014. Differences in proton pumping and Na/H exchange at the leaf cell tonoplast between a halophyte and a glycophyte. AoB Plants 6, plu023. doi:http://dx.doi.org/10.1093/aobpla/plu023.

Katschnig, D., Bliekb, T., Rozemaa, J., Schat, H., 2015. Constitutive high-level SOS1 expression and absence of HKT1;1 expression in the salt-accumulating halophyte *Salicornia dolichostachya*. Plant Sci. 234, 144–154. doi:http://dx.doi.org/10.1016/j.plantsci.2015.02.011.

Katiyar-Agarwal, S., Zhu, J., Kim, K., Agarwal, M., Fu, X., Huang, A., Zhu, J.-K., 2006. The plasma membrane Na⁺/H⁺ antiporter SOS1 interacts with RCD1 and functions in oxidative stress tolerance in *Arabidopsis*. Proc. Natl. Acad. Sci. 5, 18816–18821. doi:http://dx.doi.org/10.1073/pnas.0604711103.

Karan, R., Subudhi, P., 2012. A stress inducible SUMO conjugating enzyme gene (SaSce9) from a grass halophyte *Spartina alterniflora* enhances salinity and drought stress tolerance in *Arabidopsis*. BMC Plant Biol. 12, 1–14. doi:http://dx.doi.org/10.1186/1471-2229-12-187.

Karan, R., Subudhi, P., 2014. Overexpression of an adenosine diphosphate-ribosylation factor gene from the halophytic grass *Spartina alterniflora* confers

salinity and drought tolerance in transgenic *Arabidopsis*. Plant Cell Rep. 33, 373–384. doi:http://dx.doi.org/10.1007/s00299-013-1537-8.

Kavitha, K., George, S., Venkataraman, G., Parida, A., 2010. A salt-inducible chloroplastic monodehydroascorbate reductase from halophyte *Avicennia marina* confers salt stress tolerance on transgenic plants. Biochimie 92, 1321–1329. doi:http://dx.doi.org/10.1016/j.biochi.2010.06.009.

Khedr, A.H.A., Serag, M.S., Nemat-Alla, M.M., Abo-Elnaga, A.Z., Nada, R.M., Quick, W.P., Abogadallah, G.M., 2011. A *DREB* gene from the xerohalophyte *Atriplex halimus* is induced by osmotic but not ionic stress and shows distinct differences from glycophytic homologues. Plant Cell Tis. Org. Cult. 106, 191–206. doi:http://dx.doi.org/10.1007/s11240-010-9906-2.

Kobayashi, S., Abe, N., Yoshida, K.T., Liu, S., Takano, T., 2012. Molecular cloning and characterization of plasma membrane and vacuolar type Na$^+$/K$^+$ antiporters of an alkaline salt tolerant monocot *Puccinellia tenuiflora*. J. Plant Res. 125, 587–594. doi:http://dx.doi.org/10.1007/s10265-012-0475-9.

Koprek, T., Rangel, S., McElroy, D., Louwerse, J.D., Williams-Carrier, R.E., Lemaux, P.G., 2001. Transposon-mediated single-copy gene delivery leads to increased transgene expression stability in barley. Plant Physiol. 125, 1354–1362. doi:http://dx.doi.org/10.1104/pp.125.3.1354.

Kosova, K., Vitamvas, P., Urban, M.O., Prasil, I.T., 2013a. Plant proteome responses to salinity stress comparison of glycophytes and halophytes. Funct. Plant Biol. 40, 775–786. doi:http://dx.doi.org/10.1071/FP12375.

Kosova, K., Prail, I.T., Vitamvas, P., 2013b. Protein contribution to plant salinity response and tolerance acquisition. Int. J. Mol. Sci. 14, 6757–6789. doi:http://dx.doi.org/10.3390/ijms14046757.

Kawano, T., 2003. Roles of the reactive oxygen species-generating peroxidase reactions in plant defense and growth induction. Plant Cell Rep. 21, 829–837.

Kurek, I., Stöger, E., Dulberger, R., Christou, P., Breiman, A., 2002. Overexpression of the wheat FK506-binding Protein 73 (FKBP73) and the heat-induced wheat FKBP77 in transgenic heat reveals different functions of the two isoforms. Trans. Res. 11, 373–379. doi:http://dx.doi.org/10.1023/A.1016374128479.

Lan, T., Duan, Y., Wang, B., Zhou, Y., Wu, W., 2011. Molecular cloning and functional characterization of a Na$^+$/H$^+$ antiporter gene from halophyte *Spartina anglica*. Turk. J. 35, 535–543. doi:http://dx.doi.org/10.3906/tar-1003-2.

Le, J., Liu, X.G., Yang, K.Z., Chen, X.L., Zou, J.J., Wang, H.Z., Wang, M., Vanneste, S., Morita, M., Tasaka, M., Ding, Z.J., Friml, J., Beeckman, T., Sack, F., 2014. Auxin transport and activity regulate stomatal patterning and development. Nat. Commun. 5 (3090) doi:http://dx.doi.org/10.1038/ncomms4090.

Leach, R.P., Wheeler, K.P., Flowers, T.J., Yeo, A.R., 1990. Molecular markers for ion compartmentation in cells of higher plants. II. Lipid composition of the tonoplast of the halophyte *Suaeda maritima* (L.) Dum. J. Exp. Bot. 41, 1089–1994. doi:http://dx.doi.org/10.1093/jxb/41.9.1089.

Leidi, E.O., Barragán, V., Rubio, L., El-Hamdaoui, A., Ruiz, M.T., Cubero, B., Fernández, J.A., Bressan, R.A., Hasegawa, P.M., Quintero, F.J., Pardo, J.M., 2010. The AtNHX1 exchanger mediates potassium compartmentation in vacuoles of transgenic tomato. Plant J. 61, 495–506. doi:http://dx.doi.org/10.1111/j.1365-313X.2009.04073.

Li, W., Wang, D., Jin, T., Chang, Q., Yin, D., Xu, S., Liu, B., Liu, L., 2011. The vacuolar Na$^+$/H$^+$ antiporter gene SsNHX1 from the halophyte Salsola soda confers salt tolerance in transgenic alfalfa (*Medicago sativa* L.). Plant Mol. Biol. Rep. 29, 229–278. doi:http://dx.doi.org/10.1007/s11105-010-0224-y.

Li, X.L., Hu, Y.X., Yang, X., Yu, X.D., Li, Q.L., 2014a. A novel zinc-finger HIT protein with an additional PAPA-1-like region from *Suaeda liaotungensis* K. enhanced transgenic *Arabidopsis* drought and salt stresses tolerance. Mol. Biotechnol. 56, 1089–1099. doi:http://dx.doi.org/10.1007/s12033-014-9789-2.

Li, X.L., Yang, X., Hu, Y.X., Yu, X.D., Li, Q.L., 2014b. A novel NAC transcription factor from *Suaeda liaotungensis* K. enhanced transgenic *Arabidopsis* drought, salt, and cold stress tolerance. Plant Cell Rep. 33, 767–778. doi:http://dx.doi.org/10.1007/s00299-014-1602-y.

Ling, L.Z., Jian-bo, Z., Lian-pu, C., 2009. Cloning and sequence analysis of a Na$^+$/H$^+$ antiporter gene in halophyte *Limonium gmelinii*. Acta Horticul. Sinica 36, 1353–1358.

Liphschitz, N., Waisel, Y., 1974. Existence of salt glands in various genera of the *Gramineae*. New Phytol. 73, 507–513. doi:http://dx.doi.org/10.1111/j.1469-8137.1974.tb02129.

Liu, J., Zhang, S., Dong, L., Chu, J., 2014. Incorporation of Na$^+$/H$^+$ antiporter gene from *Aeluropus littoralis* confers salt tolerance in soybean (*Glycine max* L.). Indian J. Biochem. Biophy. 51, 58–65 doi: 24791418.

Liu, J., Zhu, J.K., 1998. A calcium sensor homolog required for plant salt tolerance. Science 280, 1943–1945. doi:http://dx.doi.org/10.1126/science.280.5371.1943.

Liu, L., Fan, X.D., Wang, F.W., Wang, N., Dong, Y.Y., Liu, X.M., Yang, J., Wang, Y.F., Li, H.Y., 2013. Coexpression of *ScNHX1* and *ScVP* in transgenic hybrids improves salt and saline–alkali tolerance in alfalfa (*Medicago sativa* L.). J. Plant Growth Reg. 32, 1–8. doi:http://dx.doi.org/10.1007/s00344-012-9270-z.

Liu, L., Wang, Y., Wang, N., Dong, Y.Y., Fan, X.D., Liu, X.M., Yang, J., Li, H.Y., 2011. Cloning of a vacuolar H$^+$-pyrophosphatase gene from the halophyte *Suaeda corniculata* whose heterologous overexpression improves salt, saline–alkali and drought tolerance in *Arabidopsis*. J. Integr. Plant Biol. 53, 731–742. doi:http://dx.doi.org/10.1111/j.1744-7909.2011.01066.x.

Liu, L., Zeng, Y., Pan, X., Zhang, F., 2012. Isolation, molecular characterization, and functional analysis of the vacuolar Na$^+$/H$^+$ antiporter genes from the halophyte *Karelinia caspica*. Mol. Bio. Rep. 39, 7193–7202. doi:http://dx.doi.org/10.1007/s11033-012-1551-x.

Lv, S.L., Lian, L.J., Tao, P.L., Li, Z.X., Zhang, K.W., Zhang, J.R., 2009. Overexpression of *Thellungiella halophila* H(+)-PPase (TsVP) in cotton enhances drought stress

resistance of plants. Planta 229, 899–910. doi:http://dx.doi.org/10.1007/s00425-008-0880-4.

Lv, S.L., Zhang, K., Gao, Q., Lian, L., Song, Y., Zhang, J., 2008. Overexpression of an H$^+$-PPase gene from *Thellungiella halophila* in cotton enhances salt tolerance and improves growth and photosynthetic performance. Plant Cell Physiol. 49, 1150–1164. doi:http://dx.doi.org/10.1093/pcp/pcn090.

Maathuis, F.J.M., Ahmad, I., Patishtan, J., 2014. Regulation of Na$^+$ fluxes in plants. Front. Plant Sci. 16 (5), 46. doi:http://dx.doi.org/10.3389/fpls.2014.00467.

Maeshima, M., 2000. Vacuolar H$^+$-pyrophosphatase BBA. Biomembrane 1465, 37–51. doi:http://dx.doi.org/10.1016/S0005-2736(00)00130-9.

Mahalakshmi, S., Christopher, G.S.B., Reddy, T.P., Rao, K.V., Reddy, V.D., 2006. Isolation of a cDNA clone (*PcSrp*) encoding serine-rich-protein from *Porteresia coarctata T.* and its expression in yeast and finger millet (*Eleusine coracana* L.) affording salt tolerance. Planta 224, 347–359. doi:http://dx.doi.org/10.1007/s00425-005-0218-4.

Majee, M., Maitra, S., Dastidar, K.G., Pattnaik, S., Chatterjee, A., Hait, N.C., Das, K.P., Majumder, A.L., 2004. A novel salt-tolerant l-*myo*-inositol-1-phosphate synthase from *Porteresia coarctata* (Roxb.) 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. doi:http://dx.doi.org/10.1074/jbc.M310138200.

Mansour, M.M., 2014. The plasma membrane transport systems and adaptation to salinity. J. Plant Physiol. 171, 1787–1800. doi:http://dx.doi.org/10.1016/j.jplph.2014.08.016.

Martinez-Atienza, J., Jiang, X., Garciadeblas, B., Mendoza, I., Zhu, J.K., Pardo, J.M., Quintero, F.J., 2007. Conservation of the salt overly sensitive pathway in rice. Plant Physiol. 143, 1001–1012. doi:http://dx.doi.org/10.1104/pp.106.

Maughan, P.J., Turner, T.B., Coleman, C.E., Elzinga, D.B., Jellen, E.N., Morales, J.A., Udall, J.A., Fairbanks, D.J., Bonifacio, A., 2009. Characterization of *Salt Overly Sensitive 1* (*SOS1*) gene homoeologs in quinoa (*Chenopodium quinoa Willd.*). Genome 52, 647–657. doi:http://dx.doi.org/10.1139/G09-041.

McCubbin, T., Bassil, E., Zhang, S., Blumwald, E., 2014. Vacuolar Na$^+$/H$^+$ NHX-type antiporters are required for cellular K$^+$ homeostasis, microtubule organization and directional root growth. Plants 3, 409–426. doi:http://dx.doi.org/10.3390/plants3030409.

Mittova, V., Tal, M., Volokita, M., Guy, M., 2003. Up-regulation of the leaf mitochondrial and peroxisomal antioxidative systems in response to salt-induced oxidative stress in the wild salt-tolerant tomato species *Lycopersicon pennellii*. Plant Cell Environ. 26, 845–856. doi:http://dx.doi.org/10.1046/j.1365-3040.2003.01016.x.

Modarresi, M., Nematzadeh, G.A., Moradian, F., Alavi, S.M., 2012. Identification and cloning of the Cu/Zn superoxide dismutase gene from halophyte plant *Aeluropus littoralis*. Genetika 48 (1), 30–134. doi:http://dx.doi.org/10.1134/s1022795411100127.

Munns, R., Tester, M., 2008. Mechanisms of salinity tolerance. Ann. Rev. Plant Biol. 59, 651–681. doi:http://dx.doi.org/10.1146/annurev.arplant.59.032607.092911.

Munns, R., James, R.A., Xu, B., Athman, A., Conn, S.J., Jordans, C., Byrt, C.S., Hare, R.A., Tyerman, S.D., Tester, M., Plett, D., Gilliham, M., 2012. Wheat grain yield on saline soils is improved by an ancestral Na transporter gene. Nat. Biotechnol. 30, 360–364. doi:http://dx.doi.org/10.1038/nbt.2120.

Nah, G., Pagliarulo, C.L., Mohr, P.G., Luo, M., Sisneros, N., Yu, Y., Collura, K., Currie, J., Goicoechea, J.L., Wing, R.A., Schumaker, K.S., 2009. Comparative sequence analysis of the *salt overly sensitive1* orthologous region in *Thellungiella halophila* and *Arabidopsis thaliana*. Genomics 94, 196–203. doi:http://dx.doi.org/10.1016/j.ygeno.2009.05.007.

Niu, X., Zhu, J.K., Narasimhan, M.L., Bressan, R.A., Hasegawa, P.M., 1993. Plasma-membrane H(+)-ATPase gene expression is regulated by NaCl in cells of the halophyte *Atriplex nummularia* L. Planta 190, 433–438. doi:http://dx.doi.org/10.1007/BF00224780.

Oh, D.H., Barkla, B.J., Vera-Estrella, R., Pantoja, O., Lee, S.Y., Bohnert, H.J., Dassanayake, M., 2015. Cell type-specific responses to salinity – the epidermal bladder cell transcriptome of *Mesembryanthemum crystallinum*. New Phytol. 207, 627–644. doi:http://dx.doi.org/10.1111/nph.13414.

Oh, D.H., Leidi, E., Zhang, Q., Hwang, S.M., Li, Y., Quintero, F.J., Jiang, X., D'Urzo, M.P., Lee, S.Y., Zhao, Y., Bahk, J.D., Bressan, R.A., Yun, D.J., Pardo, J.M., Bohnert, H.J., 2009. Loss of halophytism by interference with SOS1 expression. Plant Physiol. 151, 210–222. doi:http://dx.doi.org/10.1104/pp.109.137802.

Oh, D.H., Dassanayake, M., Haas, J.S., Kropornika, A., Wright, C., d'Urzo, M.P., Hong, H., Ali, S., Hernandez, A., Lambert, G.M., Inan, G., Galbraith, D.W., Bressan, R.A., Yun, D.J., Zhu, J.K., Cheeseman, J.M., Bohnert, H.J., 2010. Genome structures and halophyte-specific gene expression of the extremophile *Thellungiella parvula* in comparison with *Thellungiella salsuginea* (*Thellungiella halophila*) and *Arabidopsis*. Plant Physiol. 154, 1040–1052. doi:http://dx.doi.org/10.1104/pp.110.163923.

Ohta, M., Hayashi, Y., Nakashima, A., Hamada, A., Tanaka, A., Nakamura, T., Hayakawa, T., 2002. Introduction of a Na$^+$/H$^+$ antiporter gene from *Atriplex gmelini* confers salt tolerance to rice. FEBS Lett. 532, 279–282. doi:http://dx.doi.org/10.1016/S0014-5793(02)03679-7.

Olias, R., Eljakaoui, Z., Li, J., De, Morales, P.A., Marín-Manzano, M.C., Pardo, J.M., Belver, A., 2009. The plasma membrane Na$^+$/H$^+$ antiporter SOS1 is essential for salt tolerance in tomato and affects the partitioning of Na$^+$ between plant organs. Plant Cell Environ. 32, 904–916. doi:http://dx.doi.org/10.1111/j.1365-3040.2009.01971.x.

Ottow, E.A., Polle, A., Brosche, M., Kangasjärvi, J., Dibrov, P., Zorb, C., Teichmann, T., 2005. Molecular characterization of PeNhaD1: the first member of the NhaD

Na$^+$/H$^+$ antiporter family of plant origin. Plant Mol. Biol. 58, 75–88. doi:http://dx.doi.org/10.1007/s11103-005-4525-8.

Oria-Hernandez, J., Cabrera, N., Pérez-Montfort, R., Ramirez-Silva, L., 2005. Pyruvate kinase revisited: the activating effect of K$^+$. J. Biol. Chem. 280, 37924–37929.

Ozgur, R., Uzilday, B., Sekmen, A.H., Turkan, I., 2013. Reactive oxygen species regulation and antioxidant defence in halophytes. Funct. Plant Biol. 40, 8–9 doi:10/1071/FP12389.

Pang, Q., Chen, S., Dai, S., Chen, Y., Wang, Y., Yan, X., 2010. Comparative proteomics of salt tolerance in *Arabidopsis thaliana* and *Thellungiella halophila*. J. Proteome Res. 9, 2584–2599. doi:http://dx.doi.org/10.1021/pr100034f.

Pattanaik, S., Patra, B., Singh, S.K., Yuan, L., 2014. An overview of the gene regulatory network controlling trichome development in the model plant, *Arabidopsis*. Front. Plant Sci. 5 (259) doi:http://dx.doi.org/10.3389/fpls.2014.00259.

Pawlowski, W.P., Somers, D.A., 1996. Transgene inheritance in plants genetically engineered by microprojectile bombardment. Mol. Biotechnol. 6, 17–30. doi:http://dx.doi.org/10.1007/bf02762320.

Peng, Z., Wang, M., Li, F., Lv, H., Li, C., Xia, G., 2009. A proteomic study of the response to salinity and drought stress in an introgression strain of bread wheat. Mol. Cell Proteomics 8, 2676–2686. doi:http://dx.doi.org/10.1074/mcp.m900052-mcp200.

Pottosin, I., Bonales-Alatorre, E., Shabala, S., 2014. Choline but not its derivative betaine blocks slow vacuolar channels in the halophyte *Chenopodium quinoa*: implications for salinity stress responses. FEBS Lett. 588, 3918–3923. doi:http://dx.doi.org/10.1016/j.febslet.2014.09.003.

Prashanth, S.R., Sadhasivam, V., Parida, A., 2008. Over expression of cytosolic copper/zinc superoxide dismutase from a mangrove plant *Avicennia marina* in indica Rice var Pusa Basmati-1 confers abiotic stress tolerance. Trans. Res. 17, 281–291. doi:http://dx.doi.org/10.1007/s11248-007-9099-6.

Qiao, W.H., Zhao, X.Y., Li, W., Luo, Y., Zhang, X.S., 2007. Overexpression of AeNHX1, a root-specific vacuolar Na$^+$/H$^+$ antiporter from *Agropyron elongatum*, confers salt tolerance to *Arabidopsis* and *Festuca* plants. Plant Cell Rep. 26, 1663–1672. doi:http://dx.doi.org/10.1007/s00299-007-0354-3.

Qiu, Q.S., Guo, Y., Dietrich, M.A., Schumaker, K.S., Zhu, J.K., 2002. Regulation of SOS1, a plasma membrane Na$^+$/H$^+$ exchanger in *Arabidopsis thaliana*, by SOS2 and SOS3. Proc. Natl. Acad. Sci. U. S. A. 99, 8436–8441. doi:http://dx.doi.org/10.1073/pnas.122224699.

Quintero, F.J., Martinez-Atienza, J., Villalta, I., Jiang, X., Kim, W.Y., Ali, Z., Fujii, H., Mendoza, I., Yun, D.J., Zhu, J.K., Pardo, J.M., 2011. Activation of the plasma membrane Na$^+$/H$^+$ antiporter Salt-Overly-Sensitive 1 (SOS1) by phosphorylation of an auto-inhibitory C-terminal domain. Proc. Natl. Acad. Sci. U. S. A. 108, 2611–2616. doi:http://dx.doi.org/10.1073/pnas.1018921108.

Radyukina, N.L., Kartashov, A.V., Ivanov, Y.V., Shevyakova, N.I., Kuznetsov, V.V., 2007. Functioning of defense systems in halophytes and glycophytes under progressing salinity. Russ. J. Plant Physiol. 54, 806–815. doi:http://dx.doi.org/10.1134/s1021443707060131.

Rajan, N., Agarwal, P., Patel, K., Sanadhya, P., Khedia, J., Agarwal, P.K., 2014. Molecular characterization and identification of target protein of an important vesicle trafficking gene *AlRab7* from a salt excreting halophyte *Aeluropus lagopoides*. DNA Cell Biol. 34, 83–91. doi:http://dx.doi.org/10.1089/dna.2014.2592.

Ramani, B., Zorn, H., Papenbrock, J., 2004. Quantification and fatty acid profiles of sulfolipids in two halophytes and a glycophyte grown under different salt concentrations. Z. Naturforsch. C 59, 835–842.

Rauf, M., Shahzad, K., Ali, R., Ahmad, M., Habib, I., Mansoor, S., Berkowitz, G., Saeed, N., 2014. Cloning and characterization of Na$^+$/H$^+$ antiporter (LfNHX1) gene from a halophyte grass *Leptochloa fusca* for drought and salt tolerance. Mol. Biol. Rep. 41, 1669–1682. doi:http://dx.doi.org/10.1007/s11033-013-3015-3.

Rea, P.A., Griffith, C.J., Sanders, D., 1987. Purification of the *N,N′*-dicyclohexylcarbodiimide-binding proteolipid of a higher plant tonoplast H$^+$-ATPase. J. Biol. Chem. 262, 14745–14752.

Reguera, M., Peleg, Z., Abdel-Tawab, Y.M., Tumimbang, E.B., Delatorre, C.A., Blumwald, E., 2013. Stress-induced cytokinin synthesis increases drought tolerance through the coordinated regulation of carbon and nitrogen assimilation in rice. Plant Physiol. 163, 1609–1622. doi:http://dx.doi.org/10.1104/pp.113.227702.

Reguera, M., Bassil, E., Blumwald, E., 2014. Intracellular NHX-type cation/H$^+$ antiporters in plants. Mol. Plant 7, 261–263. doi:http://dx.doi.org/10.1093/mp/sst091.

Rengasamy, P., 2006. World salinization with emphasis on Australia. J. Exp. Bot. 57, 1017–1023. doi:http://dx.doi.org/10.1093/jxb/erj108.

Rodriguez-Rosales, M.P., Jiang, X., Gálvez, F.J., Aranda, M.N., Cubero, B., Venema, K., 2008. Overexpression of the tomato K$^+$/H$^+$ antiporter LeNHX2 confers salt tolerance by improving potassium compartmentalization. New Phytol. 179, 366–377.

Rozema, J., Flowers, T., 2005. Crops for a salinized world. Science 322, 1478–1480. doi:http://dx.doi.org/10.1126/science.1168572.

Rozema, J., Schat, H., 2013. Salt tolerance of halophytes, research questions reviewed in the perspective of saline agriculture. Env. Exp. Bot. 92, 83–95. doi:http://dx.doi.org/10.1016/j.envexpbot.2012.08.004.

Rubio, F., Santa-Maria, G.E., Rodriguez-Navarro, A., 2000. Cloning of *Arabidopsis* and barley cDNAs encoding HAK potassium transporters in root and shoot cells. Physiol. Plant 109, 34–43. doi:http://dx.doi.org/10.1034/j.1399-3054.2000.100106.x.

Saad, B.R., Zouari, N., Ramdhan, B.W., Azaza, J., Meynard, D., Guiderdoni, E., Hassairi, A., 2010. Improved drought and salt stress tolerance in transgenic tobacco overexpressing a novel A20/AN1 zinc-finger *AlSAP* gene isolated from the halophyte grass *Aeluropus littoralis*. Plant Mol. Biol. 72, 171–190. doi:http://dx.doi.org/10.1007/s11103-009-9560-4.

Saad, R.B., Romdhan, W.B., Zouari, N., Azaza, J., Mieulet, D., Verdeil, J.L., Guiderdoni, E., Hassairi, A., 2011. Promoter of the *AlSAP* gene from the halophyte grass *Aeluropus littoralis* directs developmental-regulated, stress-inducible, and organ-specific gene expression in transgenic tobacco. Trans. Res. 20, 1003–1018. doi:http://dx.doi.org/10.1007/s11248-010-9474-6.

Saad, B.R., Fabre, D., Mieulet, D., Meynard, D., Dingkuhn, M., Al-Doss, A., Guiderdoni, E., Hassairi, A., 2012. Expression of the *Aeluropus littoralis AlSAP* gene in rice confers broad tolerance to abiotic stresses through maintenance of photosynthesis. Plant Cell Environ. 35, 626–643. doi:http://dx.doi.org/10.1111/j.1365-3040.2011.02441.x.

Sahu, B.B., Shaw, B.P., 2009. Isolation, identification and expression analysis of salt induced genes in *Suaeda maritima*, a natural halophyte, using PCR-based suppression subtractive hybridization. BMC Plant Biol. 9, 69. doi:http://dx.doi.org/10.1186/1471-2229-9-69.

Sakuma, Y., Maruyama, K., Osakabe, Y., Qin, F., Seki, M., Shinozaki, K., Yamaguchi-Shinozaki, K., 2006. Functional analysis of an *Arabidopsis* transcription factor, DREB2A, involved in drought responsive gene expression. Plant Cell 18, 1292–1309. doi:http://dx.doi.org/10.1105/tpc.105.035881.

Sanadhya, P., Agarwal, P., Khedia, J., Agarwal, P.K., 2015. A low-affinity K$^+$ transporter *AlHKT2;1* from recretohalophyte *Aeluropus lagopoides* confers salt tolerance in yeast. Mol. Biotechnol. 57, 489–498. doi:http://dx.doi.org/10.1007/s12033-015-9842-9.

Seckin, B., Turkan, I., Sekmen, A.H., Ozfidan, C., 2010. The role of antioxidant defense systems at differential salt tolerance of *Hordeum marinum* Huds. (sea barleygrass) and *Hordeum vulgare* L. (cultivated barley). Environ. Exp. Bot. 69, 76–85. doi:http://dx.doi.org/10.1016/j.envexpbot.2010.02.013.

Sekmen, A.H., Türkan, I., Takio, S., 2007. Differential responses of antioxidative enzymes and lipid peroxidation to salt stress in salt-tolerant *Plantago maritima* and salt-sensitive *Plantago media*. Physiol. Plant. 131, 399–411. doi:http://dx.doi.org/10.1111/j.1399-3054.2007.00970.x.

Semenova, G.A., Fomina, I.R., Biel, K.Y., 2010. Structural features of the salt glands of the leaf of *Distichlis spicata* 'Yensen 4a' (Poaceae). Protoplasma 240, 75–82. doi:http://dx.doi.org/10.1007/s00709-009-0092-1.

Sengupta, S., Majumder, A.L., 2009. Insight into the salt tolerance factors of a wild halophytic rice, *Porteresia coarctata*: a physiological and proteomic approach. Planta 229, 911–929. doi:http://dx.doi.org/10.1007/s00425-008-0878-y.

Sengupta, S., Majumder, A.L., 2010. *Porteresia coarctata* (Roxb.) Tateoka, a wild rice: a potential model for studying salt-stress biology in rice. Plant Cell Environ. 33, 526–542. doi:http://dx.doi.org/10.1111/j.1365-3040.2009.02054.x.

Shabala, S., 2013. Learning from halophytes: physiological basis and strategies to improve abiotic stress tolerance in crops. Ann. Bot. 112, 1209–1221. doi:http://dx.doi.org/10.1093/aob/mct205.

Shabala, S., Bose, J., Hedrich, R., 2014. Salt bladders: do they matter? Trends. Plant Sci. 19, 687–691. doi:http://dx.doi.org/10.1016/j.tplants.2014.09.001.

Shabala, S., Cuin, T.A., 2008. Potassium transport and plant salt tolerance. Physiol. Plant 133, 651–669.

Shabala, S., Mackay, A., 2011. Ion transport in halophytes. Adv. Bot. Res. 57, 151–199. doi:http://dx.doi.org/10.1016/B978-0-12-387692-8.00005-9.

Shabala, S., Pottosin, I., 2014. Regulation of potassium transport in plants under hostile conditions: implications for abiotic and biotic stress tolerance. Physiol. Plant 151, 257–279. doi:http://dx.doi.org/10.1111/ppl.12165.

Shalata, A., Mittova, V., Volokita, M., Guy, M., Tal, M., 2001. Response of the cultivated tomato and its wild salt-tolerant relative *Lycopersicon pennellii* to salt-dependent oxidative stress: the root antioxidative system. Physiol. Plant 112, 487–494. http://dx.doi.org/10.1034/j.1399-3054.2001.1120405.x.

Shao, Q., Zhao, C., Han, N., Wang, B.S., 2008. Cloning and expression pattern of *SsHKT1* encoding a putative cation transporter from halophyte *Suaeda salsa*. DNA Seq. 19, 106–114. doi:http://dx.doi.org/10.1080/10425170701447465.

Shao, Q., Han, N., Ding, T., Zhou, F., Wang, B., 2014. *SsHKT1;1* is a potassium transporter of the C$_3$ halophyte *Suaeda salsa* that is involved in salt tolerance. Funct. Plant Biol. 41, 790–802. doi:http://dx.doi.org/10.1071/FP13265.

Shen, Y.G., Zhang, W.K., Yan, D.Q., Du, B.X., Zhang, J.S., Liu, Q., Chen, S.Y., 2003. Characterization of a DRE-binding transcription factor from a halophyte *Atriplex hortensis*. Theor. Appl. Genet. 107, 155–161. doi:http://dx.doi.org/10.1007/s00122-003-1226-z.

Shi, H., Ishitani, M., Kim, C., Zhu, J.K., 2000. The *Arabidopsis thaliana* salt tolerance gene SOS1 encodes a putative Na$^+$/H$^+$ antiporter. Proc. Natl. Acad. Sci. U. S. A. 97, 6896–6901. doi:http://dx.doi.org/10.1073/pnas.120170197.

Shi, H.Z., Quintero, F.J., Pardo, J.M., Zhu, J.K., 2002. The putative plasma membrane Na$^+$/H$^+$ antiporter SOS1 controls long-distance Na$^+$ transport in plants. Plant Cell 14, 465–477. doi:http://dx.doi.org/10.1105/tpc.010371.

Singh, N., Mishra, A., Jha, B., 2014. Ectopic over-expression of peroxisomal ascorbate peroxidase (*SbpAPX*) gene confers salt stress tolerance in transgenic peanut (*Arachis hypogaea*). Gene 547, 119–125. http://dx.doi.org/10.1016/j.gene.2014.06.037.

Skirvin, R.M., McPheeters, K.D., Norton, M., 1994. Sources and frequency of somaclonal variations. Hort. Sci. 29, 1232–1237.

Slama, I., Abdelly, C., Bouchereau, A., Flowers, T., Savoure, A., 2015. Diversity, distribution and roles of osmoprotective compounds accumulated in halophytes under abiotic stress. Ann. Bot. 239. doi:http://dx.doi.org/10.1093/aob/mcu239 pii: mcu.

Song, J., Wang, B., 2014. Using euhalophytes to understand salt tolerance and to develop saline agriculture: *Suaeda salsa* as a promising model. Ann. Bot. doi:http://dx.doi.org/10.1093/aob/mcu194 pii: mcu194.

Srivastava, A.K., Srivastava, S., Lokhande, V.H., D'souza, F.S., Suprasanna, P., 2015. Salt stress reveals differentiaL antioxidant and energetic responses in glycophyte (*Brassica juncea* L.) and halophyte (*Sesuvium portulacastrum* L.). Front. Environ. Sci. 3 (19) doi:http://dx.doi.org/10.3389/fenvs.2015.00019.

Stepien, P., Johnson, G.N., 2009. Contrasting responses of photosynthesis to salt stress in the glycophyte *Arabidopsis* and the halophyte *Thellungiella*: role of the plastid terminal oxidase as an alternative electron sink. Plant Physiol. 149, 1154–1165. doi:http://dx.doi.org/10.1104/pp.108.132407.

Sunagawa, H., Cushman,J, C., Agarie, S., 2010. Crassulacean acid metabolism may alleviate production of reactive oxygen species in a facultative CAM plant, the common ice plant *Mesembryanthemum crystallinum* L. Plant Prod. Sci. 13, 256–260. doi:http://dx.doi.org/10.1626/pps.13.256.

Su, H., Golldack, D., Zhao, C., Bohnert, H.J., 2002. The expression of HAK-type K(+) transporters is regulated in response to salinity stress in common ice plant. Plant Physiol. 129, 1482–1493. doi:http://dx.doi.org/10.1104/pp.001149.

Su, H., Golldack, D., Katsuhara, M., Zhao, C., Bohnert, H.J., 2001. Expression and stress-dependent induction of potassium channel transcripts in the common ice plant. Plant Physiol. 125, 604–614. doi:http://dx.doi.org/10.1104/pp.001149.

Su, H., Balderas, E., Vera-Estrella, R., Golldack, D., Quigley, F., Zhao, C., Pantoja, O., Bohnert, H.J., 2003. Expression of the cation transporter McHKT1 in a halophyte. Plant Mol. Biol. 52, 967–980. doi:http://dx.doi.org/10.1023/A.1025445612244.

Su, Q., Feng, S., An, L., Zhang, G., 2007. Cloning and functional expression in *Saccharomyces cereviae* of a K⁺ transporter, AlHAK, from the graminaceous halophyte *Aeluropus littoralis*. Biotech. Lett. 29, 1959–1963. doi:http://dx.doi.org/10.1007/s10529-007-9484-5.

Su, Y., Lou, W., Lin, W., Ma, L., Kabir, M.H., 2015. Model of cation transportation mediated by high-affinity potassium transporters (HKTs) in higher plants. Biol. Proced. 17, 1. doi:http://dx.doi.org/10.1186/s12575-014-0013-3.

Taji, T., Komatsu, K., Katori, T., Kawasaki, Y., Sakata, Y., Tanaka, S., Kobayashi, M., Toyoda, A., Seki, M., Shinozaki, K., 2010. Comparative genomic analysis of 1047 completely sequenced cDNAs from an *Arabidopsis*-related model halophyte, *Thellungiella halophila*. BMC Plant Biol. 10, 261. doi:http://dx.doi.org/10.1186/1471-2229-10-261.

Taji, T., Seki, M., Satou, M., Sakurai, T., Kobayashi, M., Ishiyama, K., Narusaka, Y., Narusaka, M., Zhu, J.K., Shinozaki, K., 2004. Comparative genomics in salt tolerance between *Arabidopsis* and *Arabidopsis*-related halophyte salt cress using *Arabidopsis* microarray. Plant Physiol. 135, 1697–1709. doi:http://dx.doi.org/10.1104/pp.104.039909.

Takahashi, R., Nishio, T., Ichizen, N., Takano, T., 2007. Cloning and functional analysis of the K⁺ transporter, PhaHAK2, from salt-sensitive and salt-tolerant reed plants. Biotech. Lett. 29, 501–506. doi:http://dx.doi.org/10.1007/s10529-006-9246-9.

Tiwari, V., Chaturvedi, A.K., Mishra, A., Jha, B., 2014. The transcriptional regulatory mechanism of the peroxisomal ascorbate peroxidase (*pAPX*) gene cloned from an extreme halophyte, *Salicornia brachiata*. Plant Cell Physiol 55, 201–217. doi:http://dx.doi.org/10.1093/pcp/pct172.

Travella, S., Ross, S.M., Harden, J., Everett, C., Snape, J.W., Harwood, W.A., 2005. A comparison of transgenic barley lines produced by particle bombardment and *Agrobacterium*-mediated techniques. Plant Cell Rep. 23, 780–789. doi:http://dx.doi.org/10.1007/s00299-004-0892-x.

Tuteja, N., 2007. Mechanisms of high salinity tolerance in plants. Methods Enzymol. 428, 419–438. doi:http://dx.doi.org/10.1016/S0076-6879(07)28024-3.

Udawat, P., Mishra, A., Jha, B., 2014. Heterologous expression of an uncharacterized universal stress protein gene (*SbUSP*) from the extreme halophyte, *Salicornia brachiata*, which confers salt and osmotic tolerance to *E. coli*. Gene 536, 163–170. doi:http://dx.doi.org/10.1016/j.gene.2013.11.020.

Vassilyev, A.E., Stepanova, A.A., 1990. The ultrastructure of ion-secreting and non-secreting salt gland *Limonium platyphyllum*. J. Exp. Bot. 41 (1), 41–46. doi:http://dx.doi.org/10.1093/jxb/41.1.41.

Velarde-Buendía, A.M., Shabala, S., Cvikrova, M., Dobrovinskaya, O., Pottosin, I., 2012. Salt-sensitive and salt-tolerant barley varieties differ in the extent of potentiation of the ROS-induced K(+) efflux by polyamines. Plant Physiol. Biochem. 61, 18–23. doi:http://dx.doi.org/10.1016/j.plaphy.2012.09.002.

Verslues, P.E., Batelli, H., Grillo, S., Agius, F., Kim, Y.-S., Zhu, J., Agarwal, M., Katiyar-Agarwal, S., Zhu, J.-K., 2007. Interaction of SOS2 with nucleoside diphosphate kinase 2 and catalases reveals a point of connection between salt stress and H₂O₂ signaling in *Arabidopsis thaliana*. Mol. Cell Biol. 27, 7771–7780. doi:http://dx.doi.org/10.1128/MCB.00429-07.

Very, A.A., Nieves-Cordones, M., Daly, M., Khan, I., Fizames, C., Sentenac, H., 2014. Molecular biology of K⁺ transport across the plant cell membrane: what do we learn from comparison between plant species. J. Plant Physiol. 15 (171), 748–769. doi:http://dx.doi.org/10.1016/j.jplph.2014.01.011.

Volkov, V., Wang, B., Dominy, P.J., Fricke, W., Amtmann, A., 2003. *Thellungiella halophila*, a salt-tolerant relative of *Arabidopsis thaliana*, possesses effective mechanisms to discriminate between potassium and sodium. Plant Cell Environ. 27, 1–14. doi:http://dx.doi.org/10.1046/j.0016-8025.2003.01116.

Volkov, V., Amtmann, A., 2006. *Thellungiella halophila*, a salt-tolerant relative of *Arabidopsis thaliana*, has specific root ion-channel features supporting K⁺/Na⁺ homeostasis under salinity stress. Plant J. 48, 342–353. doi:http://dx.doi.org/10.1111/j.1365-313X.2006.02876.

Wang, C.M., Zhang, J.L., Liu, X.S., Li, Z., Wu, G.Q., Cai, J.Y., Wang, S.M., 2009. *Puccinellia tenuiflora* maintains a low Na⁺ level under salinity by limiting unidirectional Na⁺ influx resulting in a high selectivity for K⁺ over Na⁺. Plant Cell Environ. 32, 486–496. doi:http://dx.doi.org/10.1111/j.1365-3040.2009.01942.x.

Wang, J.Y., Lai, L.D., Tong, S.M., Li, Q.L., 2013a. Constitutive and salt-inducible expression of *SlBADH* gene in transgenic tomato (*Solanum lycopersicum* L. cv. Micro-Tom) enhances salt tolerance. Biochem. Biophys. Res. Commun. 432, 262–267. doi:http://dx.doi.org/10.1016/j.bbrc.2013.02.001.

Wang, Y., Jin, S., Wang, M., Zhu, L., Zhang, X., 2013b. Isolation and characterization of a conserved domain in the eremophil H⁺-PPase family. PLoS One 8 (7), e70099. doi:http://dx.doi.org/10.1371/journal.pone.0070099.

Wang, H., Tang, X., Shao, C., Shao, H., Wang, H., 2014a. Molecular cloning and bioinformatics analysis of a new plasma membrane Na⁺/H⁺ antiporter gene from the halophyte *Kosteletzkya virginica*. Sci. World J. 141675, 1–7. doi:http://dx.doi.org/10.1155/2014/141675.

Wang, L., Feng, X., Zhao, H., Wang, L., An, L., Qiu, Q.S., 2014b. Functional analysis of the Na⁺, K⁺/H⁺ antiporter PeNHX3 from the tree halophyte *Populus euphratica* in yeast by model-guided mutagenesis. PLoS One 9 (8), e104147. doi:http://dx.doi.org/10.1371/journal.pone.0104147.

Wang, M.-C., Peng, Z.-Y., Li, C.-L., Li, F., Liu, C., Xia, G.-M., 2008. Proteomic analysis on a high salt tolerance introgression strain of *Triticum aestivum/Thinopyrum ponticum*. Proteomics 8, 1470–1489. doi:http://dx.doi.org/10.1002/pmic.200700569.

Wang, R., Jing, W., Xiao, L., Jin, Y., Shen, L., Zhang, W., 2015. The rice high-affinity potassium transporter1;1 is involved in salt tolerance and regulated by an MYB-Type transcription factor. Plant Physiol. 168, 1076–1090. doi:http://dx.doi.org/10.1104/pp.15.00298.

Wang, X., Yang, R., Wang, B., Liu, G., Yang, C., Cheng, Y., 2011. Functional characterization of a plasma membrane Na⁺/H⁺ antiporter from alkali grass (*Puccinellia tenuiflora*). Mol Biol Rep 38, 4122–4813. doi:http://dx.doi.org/10.1007/s11033-010-0624-y.

Wang, Y.C., Gu, G.Z., Li, H.Y., Wu, Y.J., Wang, C., Liu, G.F., Yang, C.P., 2010. Enhanced salt tolerance of transgenic poplar plants expressing a manganese superoxide dismutase from *Tamarix androssowii*. Mol. Biol. Rep. 37, 1119–1124. doi:http://dx.doi.org/10.1007/s11033-009-9884-9.

Wang, Y., Wu, W.H., 2013. Potassium transport and signaling in higher plants. Ann. Rev. Plant Biol. 64, 451–476. doi:http://dx.doi.org/10.1146/annurev-arplant-050312-120153.

Wang, Z-l., Li, P.-H., Fredricksen, M., Gong, Z.-Z., Kim, C., Zhang, C., Bohnert, H.J., Zhu, J.-K., Bressan, R.A., Hasegawa, P.M., 2004. Expressed sequence tags from *Thellungiella halophila*, a new model to study plant salt-tolerance. Plant Sci. 166, 609–616. doi:http://dx.doi.org/10.1016/j.plantsci.2003.10.030.

Wei, F., Zhang, Z., Zhang, Y., 2009. Cloning and molecular characterization of fructose-1,6-bisphosphate aldolase gene regulated by high-salinity and drought in *Sesuvium portulacastrum*. Plant Cell Rep 28, 975–984. doi:http://dx.doi.org/10.1007/s00299-009-0702-6.

Wu, C., Gao, X., Kong, X., Zhao, Y., Zhang, H., 2009. Molecular cloning and functional analysis of a Na⁺/H⁺ antiporter gene ThNHX1 from a halophytic plant *Thellungiella halophila*. Plant Mol. Biol. Rep. 27, 1–12. doi:http://dx.doi.org/10.1007/s11105-008-0048-1.

Wu, Y., Ding, N., Zhao, X., Zhao, M., Chang, Z., Liu, J., Zhang, L., 2007. Molecular characterization of PeSOS1: the putative Na(+)/H (+) antiporter of *Populus euphratica*. Plant Mol. Biol. 65, 1–11. doi:http://dx.doi.org/10.1007/s11103-007-9170-y.

Xu, X.J., Zhou, Y.Z., Ren, D.T., Bu, H.H., Feng, J.C., Wang, G.Y., 2014. Cloning and characterization of gene encoding a Mn-containing superoxide dismutase in *Eutrema halophilum*. Biol. Plant. 58, 105–113. doi:http://dx.doi.org/10.1007/s10535-013-0363-8.

Yadav, N., Shukla, P., Jha, A., Agarwal, P., Jha, B., 2012. The *SbSOS1* gene from the extreme halophyte *Salicornia brachiata* enhances Na⁺ loading in xylem and confers salt tolerance in transgenic tobacco. BMC Plant Biol. 12, 1–18. doi:http://dx.doi.org/10.1186/1471-2229-12-188.

Yadav, N.S., Singh, V.K., Singh, D., Jha, B., 2014. A novel gene *SbSI-2* encoding nuclear protein from a halophyte confers abiotic stress and tolerance in *E. coli* and tobacco. PLoS One 9, e101926. doi:http://dx.doi.org/10.1371/journal.pone.0101926.

Yamada, A., Saitoh, T., Mimura, T., Ozeki, Y., 2002. Expression of mangrove allene oxide cyclase enhances salt tolerance in *E. coli*, yeast, and tobacco cells. Plant Cell Physiol. 43, 903–910.

Yang, R., Jarvis, D.E., Chen, H., Beilstein, M.A., Grimwood, J., Jenkins, J., Shu, S., Prochnik, S., Xin, M., Ma, C., Schmutz, J., Wing, R.A., Mitchell-Olds, T., Schumaker, K.S., Wang, X., 2013. The reference genome of the halophytic plant *Eutrema salsugineum*. Front. Plant Sci. 21, 4–46. doi:http://dx.doi.org/10.3389/fpls.2013.00046.

Yang, X., Hu, Y.X., Li, X.L., Yu, X.D., Li, Q.L., 2014. Molecular characterization and functional analysis of *SlNAC2* in *Suaeda liaotungensis* K. Gene 543, 190–197. doi:http://dx.doi.org/10.1016/j.gene.2014.04.025.

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 Biotech. J. 1–12. doi:http://dx.doi.org/10.1111/pbi.12335.

Yao, M., Zeng, Y., Liu, L., Huang, Y., Zhao, E., Zhang, F., 2012. Overexpression of the halophyte *Kalidium foliatum* H⁺-pyrophosphatase gene confers salt and drought tolerance in *Arabidopsis thaliana*. Mol. Biol. Rep. 39, 7989–7996. doi:http://dx.doi.org/10.1007/s11033-012-1645-5.

Yao, S., Lan, H., Zhang, F., 2010. Variation of seed heteromorphism in *Chenopodium album* and the effect of salinity stress on the descendants. Ann. Bot. 105, 1015–1025. doi:http://dx.doi.org/10.1093/aob/mcq060.

Ye, C.Y., Zhang, H.C., Chen, J.H., Xia, X.L., Yin, W.L., 2009. Molecular characterization of putative vacuolar NHX-type Na(+)/H(+) exchanger genes from the salt-

resistant tree *Populus euphratica*. Physiol. Plant 137, 166–174. doi:http://dx.doi.org/10.1111/j.1399-3054.2009.01269.x.

Yuan, F., Lyu, M.J., Leng, B.Y., Zheng, G.Y., Feng, Z.T., Li, P.H., Zhu, X.G., Wang, B.S., 2015. Comparative transcriptome analysis of developmental stages of the *Limonium bicolor* leaf generates insights into salt gland differentiation. Plant Cell Environ. 38, 1637–1657. doi:http://dx.doi.org/10.1111/pce.12514.

Zhang, G.H., Su, Q., An, L.J., Wu, S., 2008. Characterization and expression of a vacuolar Na(+)/H(+) antiporter gene from the monocot halophyte *Aeluropus littoralis*. Plant Physiol. Biochem. 46, 117–126. doi:http://dx.doi.org/10.1016/j.plaphy.2007.10.022.

Zhang, H.-X., Blumwald, E., 2001. Transgenic salt-tolerant tomato plants accumulate salt in foliage but not in fruit. Nat. Biotech. 19, 765–768. doi:http://dx.doi.org/10.1038/90824.

Zhang, L.Q., Niu, Y.D., Huridu, H., Hao, J.F., Qi, Z., Hasi, A., 2014. *Salicornia europaea* L. Na$^+$/H$^+$ antiporter gene improves salt tolerance in transgenic alfalfa (*Medicago sativa* L.). Genet. Mol. Res. 13, 5350–5360. doi:http://dx.doi.org/10.4238/2014.

Zhang, Y., Li, Y., Lai, J., Zhang, H., Liu, Y., Liang, L., Xie, Q., 2012. Ectopic expression of a LEA protein gene *TsLEA1* from *Thellungiella salsuginea* confers salt-tolerance in yeast and *Arabidopsis*. Mol. Biol. Rep. 39, 4627–4633. doi:http://dx.doi.org/10.1007/s11033-011-1254-8.

Zhang, Y., Zhu, H., Zhang, Q., Li, M., Yan, M., Wang, R., Wang, L., Welti, R., Zhang, W., Wang, X., 2009. Phospholipase dalpha1 and phosphatidic acid regulate NADPH oxidase activity and production of reactive oxygen species in ABA-mediated stomatal closure in *Arabidopsis*. Plant Cell 21, 2357–2377. doi:http://dx.doi.org/10.1105/tpc.108.062992.

Zhao, F., Wang, Z., Zhang, Q., Zhao, Y., Zhang, H., 2006. Analysis of the physiological mechanism of salt-tolerant transgenic rice carrying a vacuolar Na$^+$/H$^+$

antiporter gene from *Suaeda salsa*. J. Plant Res. 119, 95–104. doi:http://dx.doi.org/10.1007/s10265-005-0250-2.

Zhao, K.F., Fan, H., Song, J., Sun, M.X., Wang, B.Z., Zhang, S.Q., Ungar, I.A., 2005. Two Na$^+$ and Cl$^-$ hyperaccumulators of the *Chenopodiaceae*. J. Int. Plant Biol. 47, 311–318. doi:http://dx.doi.org/10.1111/j.1744-7909.2005.00057.x.

Zheng, L., Dang, Z., Li, H., Zhang, H., Wu, S., Wang, Y., 2014. Isolation and characterization of a Δ1-pyrroline-5-carboxylate synthetase (*NtP5CS*) from *Nitraria tangutorum* Bobr. and functional comparison with its *Arabidopsis* homologue. Mol. Biol. Rep. 41, 563–572. doi:http://dx.doi.org/10.1007/s11033-013-2893-8.

Zhou, H., Lin, H., Chen, S., Becker, K., Yang, Y., Zhao, J., Kudla, J., Schumaker, K.S., Guo, Y., 2014a. Inhibition of the *Arabidopsis* salt overly sensitive pathway by 14-3-3 proteins. Plant Cell 26, 1166–1182. doi:http://dx.doi.org/10.1105/tpc.113.117069.

Zhou, L., Zheng, K., Wang, X., Tian, H., Wang, X., Wang, S., 2014b. Control of trichome formation in *Arabidopsis* by poplar single-repeat R3 MYB transcription factors. Front. Plant Sci. 5 (262) doi:http://dx.doi.org/10.3389/fpls.2014.00262.

Zhou, S., Chen, X., Zhang, I., 2008. Improved salt tolerance in tobacco plants by co-transformation of a betaine synthesis gene *BADH* and a vacuolar Na$^+$/H$^+$ antiporter gene *SeNHX1*. Biotech. Lett. 30, 369–376. doi:http://dx.doi.org/10.1007/s10529-007-9548-6.

Zhu, J.K., 2000. Genetic analysis of plant salt tolerance using *Arabidopsis*. Plant Physiol. 124, 941–948. doi:http://dx.doi.org/10.1104/pp.124.3.941.

Zhu, J.K., Liu, J., Xiong, L., 1998. Genetic analysis of salt tolerance in *Arabidopsis* evidence for a critical role of potassium nutrition. Plant Cell 10, 1181–1191 doi:10.11o5/tpc/10.7.1181.

Zhu, J.K., 2003. Regulation of ion homeostasis under salt stress. Curr. Opin. Plant Biol. 6, 441–445. doi:http://dx.doi.org/10.1016/s1369-5266(03) 00085-2.