Chapter 1
Salinity Responses and Adaptive Mechanisms in Halophytes and Their Exploitation for Producing Salinity Tolerant Crops

Karim Ben Hamed, Amira Dabbous, Hassan El Shaer, and Chedly Abdely

Abstract The increasing salinization of cultivated lands and associated annual losses in agricultural production require a better understanding of key physiological mechanisms conferring salinity tolerance in crops. The effective way of gaining such knowledge comes from studying halophytes. Halophytes have the advantages of tolerating and even benefitting from salt concentrations that kill most crop species. This review summarized the main strategies of resistance of halophytes to salt stress and the specificities in their responses that distinguish them from glycophytes. Many studies showed that the superior salinity tolerance in halophytes is mainly attributed to a set of complementary and well-orchestrated mechanisms for ion, osmotic and reactive oxygen species (ROS) homeostasis. We also gave special attention to the acclimation in halophytes that allows plants to improve stress tolerance to salt at a later period of plant growth. Armed with such information on halophytes, it will be possible to produce salt tolerant crops through genetic modification, priming and effective breeding strategies.

Keywords Halophytes · Signalling · Salinity · Priming · Osmolytes · ROS homeostasis · Acclimation · Crops · Vacuolar compartmentation · Gene expression · H_2O_2

Abbreviations

CAT	Catalases
HKT	High potassium transporter

K. Ben Hamed (✉) · A. Dabbous · C. Abdely
Laboratory of Extremophile Plants, Centre of Biotechnology of Borj Cedria,
Hammam Lif, Tunisia
e-mail: karimbenhamed2016@gmail.com

H. El Shaer
Desert Research Center, Cairo, Egypt

V. Kumar et al. (eds.), *Salinity Responses and Tolerance in Plants, Volume 2*,
https://doi.org/10.1007/978-3-319-90318-7_1

NHX	Na/H+ exchanger
P5CS	Pyrroline-5-carboxylate synthetase
PDH	Proline dehydrogenase
PLC	Phospholipase C
PLD	Phospholipase D
POD	Peroxidases
ROS	Reactive oxygen species
SOD	Superoxide dismutase
SOS	Salt overly sensitive
TF	Transcription factor
VHA	Vacuolar H^+ ATPase

1.1 Breeding for Salinity Tolerance: Current Stand

Worldwide, salt stress is one of the main environmental constraints that decrease plant growth and crop productivity. Alarmingly, salinity is increasing particularly in the most populous and least developed countries, in Central Asia and Africa, where salinization affects up to 50% of irrigated areas. Every minute, 3 ha are lost for agriculture due to inappropriate irrigation practices and between 10 and 20 M ha of irrigated land deteriorates to zero productivity each year (Panta et al. 2014). Most crop plants like cereals, forages and legumes are very sensitive to the presence of sodium in the soil. Interestingly some plants called halophytes have developed original adaptation to cope with the presence of salt.

The biological approach that consists in improving the performance of cultivated plants has been proposed as one of the effective means to overcome salinity issue. For example, the overexpression of genes encoding sodium and potassium transporters like Na/H antiporter or HKT1 genes (Zhang and Blumwald 2001; Ruiz 2002; Shi et al. 2003; Apse and Blumwald 2002; Munns et al. 2012), improved the growth of some salt sensitive species like rice, tomato, wheat rapeseed and Arabidopsis under salt stress. Nevertheless, the progress towards improving the salt tolerance of these crops remains disappointingly slow. Thus, in spite of the fact that it is not a single crop that can be used seawater salinity (Flowers 2004). The main reason for this is the multigenic nature of the salt response, which also involves simultaneous changes at different levels (whole plant, tissues, cells and even organelles). On the other hand, when a gene is over-expressed for example, the effects of the modification will be attenuated by dilution at whole plant level. Thus, the analysis of several bibliographic data shows that the improvement of tolerance to salinity is only possible through coupling of genetic approach and functional genomics, biochemical and physiological approaches of biophysics, ecophysiology taking into account the interaction of the genome with its environment. The main aim is to look for mechanisms which extend beyond the cellular scale and which are part of the whole plant, which amounts to adopting an approach of integrative biology.

Moreover, *Thellungiella salsuginea* is a halophyte from the eastern coast of China, very close to *Arabidopsis thaliana* since the degree of homology of the sequences between the two species is about 90%. *Thellungiella* becomes the halophyte model for functional genomics. Curiously, very few genes are induced by salt in this plant. On the other hand, many genes are constitutively expressed at a level higher than that of *Arabidopsis* under salinity. Some of these genes are involved in Na^+ excretion, antioxidant systems, osmolyte biosynthesis, protection against desiccation etc. (Taji et al. 2004; Gong et al. 2005).

From the comparison of *Arabidopsis* and *Thellungiella* emerges the hypothesis that halophyte is more salt tolerance is not associated with the acquisition of new genes but with a change in the regulation of many pre-existing genes, thus the interest of the investigation and the comparative analysis of these regulatory pathways in plants with the same ability to tolerate salt stress.

In this chapter, we have summarized the main strategies of salt resistance in halophytes and focus on the special hallmarks that are better activated or pre-activated to elicit quick and better responses in halophytes than in glycophytes, resulting in adaptation to saline environment. We continue with some proper approaches that used halophyte specificities to develop salt tolerant crops.

1.2 Strategies of Salt Resistance in Halophytes

In salty environments, halophytes are experiencing three types of constraints, (1) Osmotic constraint: the salt causes a lowering of the water potential of the medium thus disrupting the water supply to the plant. (2) Ion stress associated with toxic accumulation of Na^+ and Cl^- in tissues. (3) Nutritional stress resulting from the competition of Na^+ with the other essential cations K^+, Mg^{2+}, Ca^{2+} and Cl^- with NO_3^-, $H_2PO_4^-$ and SO_4^{2-}. These three stresses have a common denominator is that they may be responsible for an accumulation of reactive oxygen species (ROS). Halophytes, plants living in a salt environment, had to develop adaptive mechanisms for these stresses. These adaptations are located at different levels of the plant, in organs, cells, membranes and at the molecular level (Pitman and Laüchli 2002). Two main strategies are adopted by halophytes (Fig. 1.1), depending on whether they are dicotyledons or monocotyledons, the former adopt an osmotic regulation strategy (osmotic or tolerance strategy) and the latter adopt an avoidance strategy. These strategies could not be mutually exclusive, and a particular plant may use several of these different strategies depending on particular circumstances (Souid et al. 2016; Shabala and McKay 2011) Fig. 1.2.

1.2.1 Tolerance Strategy or Osmotic Strategy

This strategy is based on a high ability to absorb and transport Na^+ and Cl^- from the roots to the aerial parts before their accumulation in the vacuoles of the halophytes (Flowers 1985). This salt accumulation may be beneficial to these plants

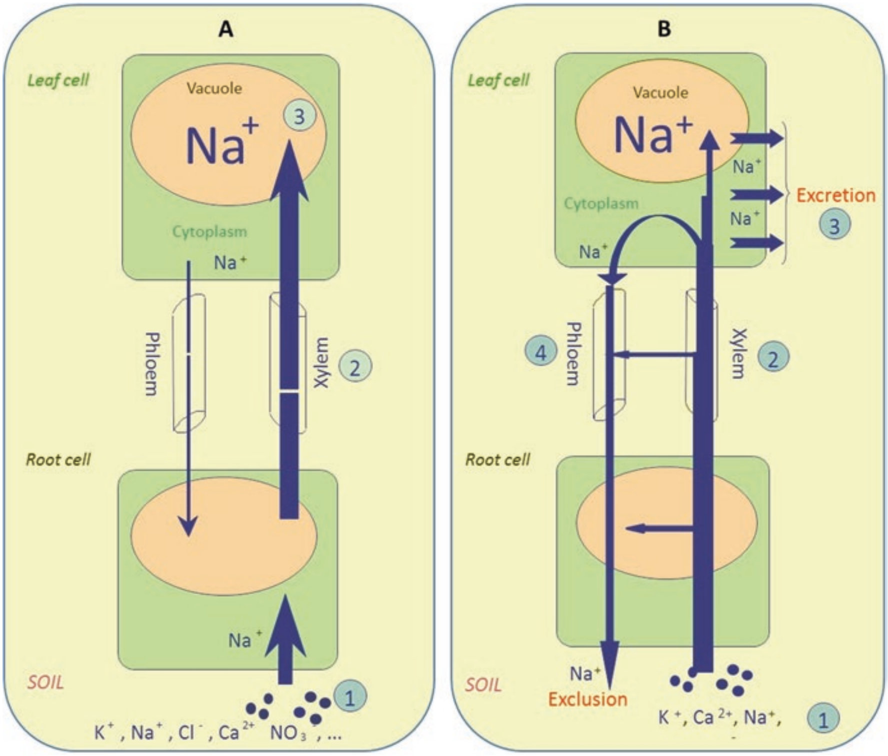

Fig. 1.1 Strategies of salt resistance in halophytes. (**a**) Salt tolerance (**b**) Salt avoidance

compared to glycophytes since it could lead to a lowering of the water potential of the cells, which could increase their water supply and reduce sweating. In most dicotyledonous halophytes, “inclusive” behavior is effective because it is associated with high growth rates and sequestration of large quantities of inorganic ions in the vacuole (Flowers and Colmer 2008). Most halophytes used Na^+ and Cl^- for osmotic adjustment, some others used K^+ and SO_4^{2-}. However, levels of accumulation depend on species and environmental conditions. The accumulation of Na^+ and Cl^-in the vacuole also increases stem or leaf succulence. The succulence in leaves or stems characteristic of numerous halophytes, is associated with an increase in cell size, a decrease in surface area per tissue volume and a high water content per unit surface area (Short and Colmer 1999). The increased succulence being correlated with the water status of the leaves could be interpreted as an adaptation in terms of conservation of internal water and dilution of accumulated salts (Debez et al. 2004).

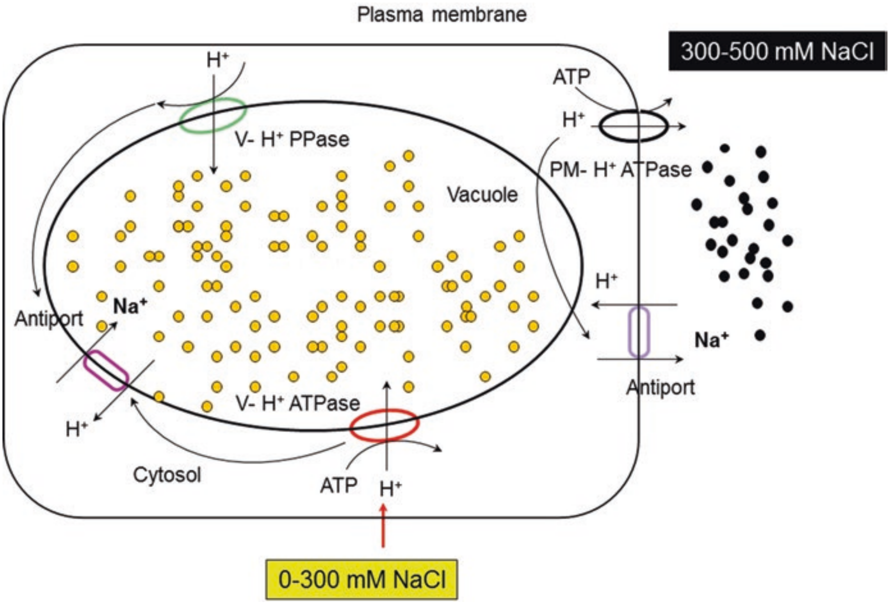

Fig. 1.2 Complementary mechanisms for ion homeostasis in the halophyte *Cakile maritima* involving V H+-ATPase (at moderate salinity, up to 300 mM NaCl) and PM H+ ATPase (at high salinity, 300–500 mM NaCl)

1.2.2 Avoidance Strategy

The avoidance of salt requires certain physiological and structural adaptations. There are two main saline avoidance methods for halophytes. (1) Root exclusion: Na^+ is transported in the xylem, transported to the leaves by transpiration, and then partially recirculated by the phloem to the roots and excreted in the medium (Bradley and Morris 1991; Berthomieu et al. 2003). (2) Excretion: Some halophytes are capable to deposit high (molar) amounts of NaCl in specialized external (epidermal) structures like salt glands and salt bladders (Dassanayake and Larkin 2017; Shabala et al. 2014). About 50% of all halophyte plants contain salt bladders. With the diameter of the bladder being often 10 times bigger than epidermal cells, each epidermal bladder cell has about 1000 times larger volume, and hence, could sequester 1000-fold more Na^+ compared with leaf cell vacuoles (Shabala et al. 2014). The mechanism of excretion has been described particularly in the case of salt glands. Thus, the movement of the liquid from the basal cell to the apical cell is done through the connections of the plasmodesm between the two cells. A solution enriched with salt is stored in the collecting chamber between the wall of the apical cell and the cuticle which has peeled off. The latter stretches by bringing the saline solution to the surface of the leaf via small open spaces of the cuticle (Gorham 1996).

1.3 Learning from Signaling Pathways in Halophytes in Response to Salinity

The general consensus is that there is nothing really unique to halophytes, neither in their anatomical features, nor in their physiological mechanisms. The major difference to glycophytes is how efficiently these mechanisms are controlled in these two plant groups (Shabala and Mackay 2011; Shabala 2013). The key to improving salinity stress tolerance in crops may lie in understanding how salt stress-responsive mechanisms are regulated in halophytes.

1.3.1 Regulation of Ion Homeostasis by SOS Signaling Pathway in Halophytes

Several studies have established the Salt Overly Sensitive (SOS) pathway as the canonical model for the mechanism responsible for salt tolerance. The SOS pathway involves interplay among Na^+-H^+ antiporters for transporting sodium, and the activation of the kinase that phosphorylates the transporter (Ji et al. 2013). Among them, SOS1, a plasma membrane Na^+-H^+ antiporter, has been shown to be a critical component for maintaining salt homeostasis by pumping sodium out of cells upon activation. Considering the characterization of sodium extrusion based on SOS1 in the highly sodium-sensitive, glycophytic Arabidopsis, it remained to be studied whether the SOS pathway, and particularly SOS1, had any function and relevance for the sodium tolerance of halophytic species. The function of SOS1 in *Thellungiella salsuginea* has now been characterized (Bressan et al. 2001; Amtmann 2009) with special emphasis on intracellular processes that might be altered by the inhibition of SOS1 expression. The study identified SOS1 as an intrinsic part of the halophytic nature of this species because the down-regulation of SOS1 transcript expression converted *Thellungiella* into a glycophytic species (Oh et al. 2009a, b). A computational analysis of the primary and secondary structures of halophytes and glycophytes showed that no major differences in SOS1 between both groups (Kim and Bressan 2016). The major difference in Na^+ tolerance between *A. thaliana* and *T. salsuginea* was due to a much higher Na^+ influx in *A. thaliana*. This observation is consistent with others' findings that SOS1 is required for Na^+ tolerance but overexpression of SOS1 from either *A. thaliana* or *T. salsuginea* does not result in a major improvement in Na^+ tolerance (Shi et al. 2003).

1.3.2 Vacuolar Compartmentalization System

The vital control of intracellular sodium concentration for plants coping with salinity was achieved by intracellular accumulation and compartmentalization of Na^+ predominantly in vacuoles, and thereby the cytoplasm keeps tolerable concentrations

(Shabala 2013; Flowers and Colmer 2008).The sequestration of Na^+ in the vacuole is possible thanks to Na^+/H^+ antiporters located in the tonoplast. The great benefit of the halophytes is that they could have a constitutive expression of this tonoplast antiporter and can stimulate their activity under a salt stress (Zhang et al. 2008). The activity of NHX Na^+/H^+ antiporters which use the pH gradient generated by vacuolar V H^+-ATPases to actively transport Na^+ against its electrochemical gradient toward the vacuole (Zhu 2001; Hasegawa 2013). The combined stimulation of these both transporters seems to be important in the adaptation of halophytes to salt stress (Qiu et al. 2007). The compartmentalization of Na^+ in the vacuole contributes not only to ion homeostasis and cell turgor, it also protects the metabolic enzymes from salt toxicity (Aharon et al. 2003). The accumulation of Na^+ and Cl^- in the vacuole also increases stem or leaf succulence. The succulence in leaves or stems characteristic of numerous halophytes, is associated with an increase in cell size, a decrease in surface area per tissue volume and a high water content per unit surface area (Short and Colmer 1999). In *C. maritima* the Na^+ concentration of the leaves increased with increasing NaCl concentration in the medium (Debez et al. 2004) and no salt-related toxicity symptoms were noted. Moreover high leaf Na^+ concentration was associated with increased leaf thickness and succulence (Debez et al. 2006) suggesting the existence of mechanisms for compartmentalization of Na^+ in these organs (Fig. 1.2). The increased succulence of the leaves could be interpreted as an adaptation in terms of conservation of internal water and dilution of accumulated salts (Koyro and Lieth 2008). Moreover, the increased activity of V H^+-ATPases since 300 mM NaCl in *C. maritima* leaves has been reported (Debez et al. 2006). This activity could provide the necessary proton driving force triggering sodium transport towards the vacuole (Fig. 1.2).

1.3.3 Osmolyte Biosynthesis Pathways: Opposite Regulation in Halophytes and Glycophytes

In halophytes, the accumulation of glycine betaine and proline two important organic solutes is involved in osmotic adjustment in response to salt stress (Flowers and Colmer 2008). Halophytes are more efficient in controlling the catabolism of the proline and greatly accumulate it in salt stressed cells than glycophytes (Slama et al. 2015). The leaves and roots of *C. maritima* present a proline pool similar to *A. thaliana* in control situation but after 72 h of salt stress, the leaves of *C. maritima* accumulate twofold more proline than *A. thaliana* and threefold in the roots (Ellouzi et al. 2014). They have also the ability to accumulate 18-fold more betaine than the glycophytes (Jdey et al. 2014). Thus, *C. maritima* could use organic osmolytes in addition to Na^+ for osmotic adjustment. It is further noteworthy that glycine-betaine as proline accumulation could also play a role in ROS homeostasis in halophytes (Katschnig et al. 2013) and serve in preservation of the structural and functional integrity at the cellular level (Jdey et al. 2014).The beneficial effect of proline accumulation on salt tolerance has been demonstrated in a range of halophyte species (Szabados and Savoure 2010; Slama et al. 2015). *Thellungiella salsuginea* and

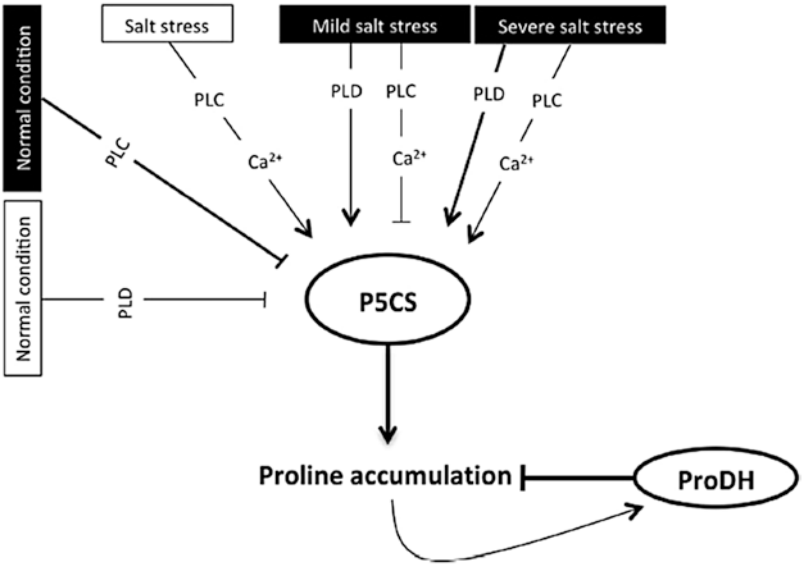

Fig. 1.3 Regulation of proline metabolism in plants. Most data were obtained using *Arabidopsis thaliana* and *Thellungiella salsuginea*. Circles designate enzymes and rectangles the environmental context; open and black boxes correspond to the signalling pathways demonstrated in A. thaliana and T. salsuginea, respectively. Abbreviations: *P5CS* pyrroline-5-carboxylate synthetase, *ProDH* proline dehydrogenase, *PLC* phospholipase C, PLD, phospholipase D, *ROS* reactive oxygen species. (Modified from Ghars et al. 2012)

Lepidium crassifolium, two halophytic wild relatives of *Arabidopsis*, accumulated more proline under control and salt-stressed conditions (Murakeozy et al. 2003; Taji et al. 2004; Ghars et al. 2008). Proline hyperaccumulation in *T. salsuginea* was shown to be a result of enhanced proline synthesis via pyrroline-5-carboxylate synthetase (P5CS) and reduced proline catabolism by proline dehydrogenase PDH (Taji et al. 2004; Kant et al. 2006). Interestingly, in *Arabidopsis*, phospholipase D (PLD) functions as a negative regulator of proline accumulation under control conditions (Thiery et al. 2004) whereas phospholipase C (PLC) acts as a positive regulator of proline accumulation during salt stress (Parre et al. 2007). However, this regulation is opposite in *Thellungiella*, where PLD functions as a positive regulator and PLC acts as a negative regulator (Ghars et al. 2012). This opposite regulatory function exerted by *T. salsuginea* on proline accumulation (Fig. 1.3) suggests that halophytes have a ‘stress-anticipatory preparedness’ strategy (Taji et al. 2004).

1.3.4 ROS Homeostasis

Reactive oxygen species (ROS) are important in the cell for all organisms in their signaling pathway. But, large accumulation of ROS in the cell triggered an oxidative stress. This stress is a source of damages as lipid peroxidation, DNA damage, and protein denaturation for examples. The sensitivity to ROS seems to be different between the glycophytes and halophytes. Actually, halophytes endure a higher level of ROS since high salinity can lead to the formation of ROS. Scavenging of ROS is thus critical in plant salt tolerance, and accordingly halophytes are equipped with powerful antioxidant systems, including enzymatic and non-enzymatic components to regulate the level of ROS in the cell (Bose et al. 2014). Halophytes have the capacity to use the superoxide dismutase (SOD) at its better yield (Jithesh et al. 2006; Ozgur et al. 2013). This enzyme converts the $O_2^{\cdot-}$ in H_2O_2, a more stable ROS that can be next transformed into H_2O_2 thanks to the catalase (CAT) and the peroxidase (POX).

The kinetics of H_2O_2 signalling appears to be much faster in halophytes than in glycophytes (Bose et al. 2014). For example, the salt-induced H_2O_2 accumulation peaked at 4 h upon salt stress onset in the leaves of a halophyte *Cakile maritime* and declined rapidly afterwards. In the glycophyte *A. thaliana*, however, H_2O_2 continued to accumulate even after 72 h after salt application (Ellouzi et al. 2011) (Fig. 1.4). Similarly, salt stress-induced H_2O_2 production was higher in the leaves of a halophyte (*Populus euphratica*) in comparison with the leaves of a glycophyte (*P. popularis*) after 24 h of salinity treatment (Sun et al. 2010), suggesting that elevated H_2O_2 levels upon stress exposure are *essential* to confer salt stress signalling and adaptation to stress in halophytes. Enhanced H_2O_2 production in the chloroplasts of *Thellungiella salsuginea* is pivotal for the "salt stress preparedness" mechanism in this halophyte species (Wiciarz et al. 2015), consistent with this notion. While (transiently) elevated H_2O_2 appears to be essential for salt-stress signaling in halophytes, interaction between H_2O_2 and transition metals (Fe^{2+} or Cu^{2+}) present in cell walls may result in the generation of highly reactive hydroxyl radicals ($OH^{\cdot}$) via the Fenton reaction. Hydroxyl radicals damage cellular structures, decrease cytosolic K^+/Na^+ ratio by directly activating a

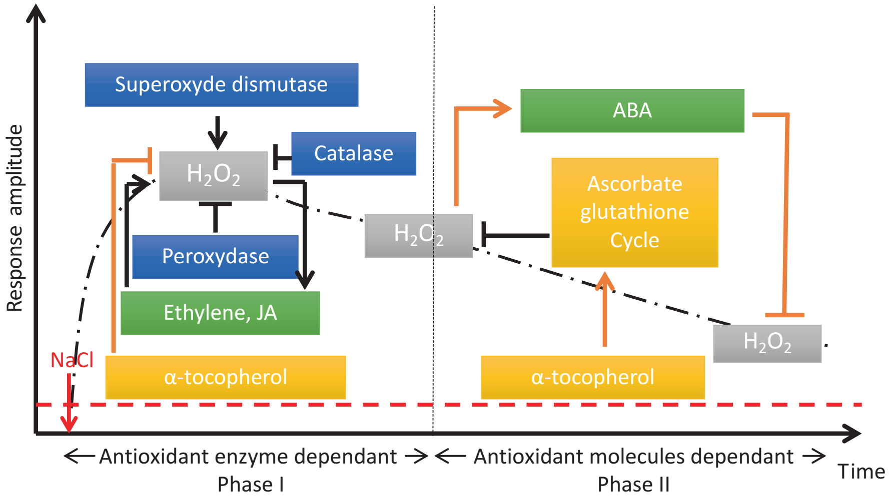

Fig. 1.4 Early oxidative burst and coordination of the antioxidant response of *C. maritima* to salt stress. Red dotted line: response in absence of salt. *ABA* abscissic acid, *JA* jasmonic acid

range of Na^+, K^+ and Ca^{2+}-permeable cation channels and eventually cause cell death (Demidchik et al. 2010). The abundance of the metal binding proteins (e.g. ferritin) in specific tissues or cellular compartments controls the concentration of the transition metal ions available for the Fenton reaction, hence regulates the H_2O_2-mediated signal propagation in plants. Salt stress increases ferritin deposits in the leaves of the halophytic *Mesembryanthemum crystallinum* (Paramonova et al. 2004). Similarly, a green micro-alga (*Dunaliella salina*) that grows only under highly saline conditions accumulates large quantities of a triplicated transferrin-like protein (Liska et al. 2004). Furthermore, there is a transient increase in the transcripts of ferritin gene (*Fer1*) in the leaves of a mangrove *Avicennia marina* 12 h after salt stress (Jithesh et al. 2006). These observations suggest that halophytes can indeed regulate the propagation of H_2O_2-mediated signalling by increasing the abundance of metal binding proteins in all the tissue/cellular organelles where H_2O_2 accumulates, preventing it from being converted into hydroxyl radicals.

1.4 Learning from the Acclimation of Halophytes to Their Natural Biotopes

In saline environments, salinity is heterogenous spatially and temporally. For example, halophytes naturally adapted to seasonal salinity fluctuations or unpredictable changes in salinity, owing to their ability to keep a stress imprint or ‘memory’ that improved plant responses when challenged by future salt stress events (Ben Hamed et al. 2013). Our recent studies on the halophyte *Cakile maritima*, native to Tunisian coasts, suggest a relatively long-term stress memory in plants pre-exposed to salinity which resulted in a lower oxidative stress when subsequently exposed to salinity (Ellouzi et al. 2014). This suggests that among the set of defence mechanisms triggered by stress pre-exposure there are imprints which persist for several weeks after relief of a first stress that allowed stress pre-exposed plants either to prevent and/or scavenge reactive oxygen species more efficiently than non-pre-exposed plants. As possible mechanisms for a stress imprint, Bruce et al. (2007) suggested the accumulation of transcription factors or signalling proteins, epigenetic changes including chemical changes at the DNA (DNA methylation and acetylation), histone modification or accumulation of small RNAs.

1.5 How Can We Exploit Regulation Pathways and Acclimation in Halophytes to Produce Salt Tolerant Crops?

There are several possible practical ways of increasing the salt tolerance in crop species. These include: (1) over-expression of genes from halophytes and (2) priming involving prior exposure to a biotic or abiotic stress factor making a plant more resistant to future exposure. Priming can also be achieved by applying natural or synthetic compounds which act as signalling transducers, 'activating' the plant's defense system. It appears that each of these approaches targets intracellular K^+/Na^+ and ROS homeostasis and ion transport across cell membranes, improving the cytosolic K^+/Na^+ ratio and assisting cell osmotic adjustment (Fig. 1.4).

1.5.1 Over-Expression of Genes from Halophytes in Salt Sensitive Species

The recent isolation and characterization of salt tolerance genes encoding signalling components from halophytes has provided tools for the development of transgenic crop plants with improved salt tolerance and economically beneficial traits (Fig. 1.5). These genes encode protein implicated in Na^+ sequestration (H^+ ATPase, NHX transporters) and exclusion (SOS1), synthesis of osmolytes, ROS detoxification, signal perception and regulating factors and other unknown functions (Himabindu et al. 2016).

Halophytic genes involved in salt tolerance mechanisms have been extensively studied in various model species (approximately 80 genes), including E. coli, yeast, Arabidopsis, tobacco and few crop species (only 20 genes) (Himabindu et al. 2016). Few reports used these genes from monocot halophytes for crop improvement, in comparison to the studies that used dicot halophytes genes (Himabindu et al. 2016). For example, vacuolar H^+-ATPases encoding genes from dicots halophytes were proved to be potential key players of salt tolerance in glycophytes. We recently showed that this gene (*Lm*VHA-E1) is up-regulated in the halophyte *Lobularia maritima* under different stress conditions (Dabbous et al. 2017), similar to what was reported by *Mesembryanthemum crystallinum* (Kluge et al. 2009), *Suaeda salsa* (Ratajczak 2000; Wang et al. 2001) and *Tamarix hispida* (Gao et al. 2011). The overexpression of this gene in *A. thaliana* led to improved stress tolerance to

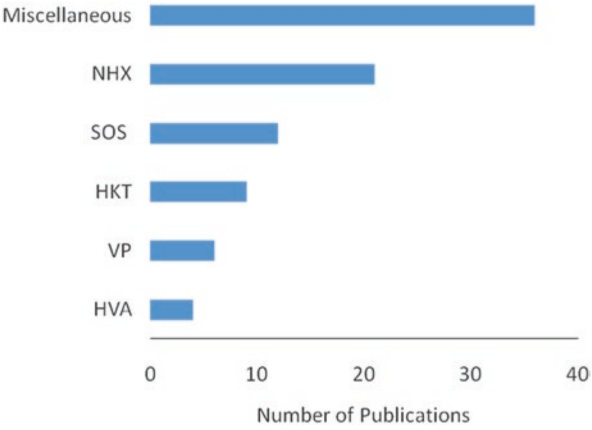

Fig. 1.5 Salt tolerance studies using halophyte genes in different crop plants

salinity and osmotic stress in transgenic plants, mainly associated with reduced relative water loss and oxidative damages and increased levels of sodium possibly due to higher H+-ATPase activity compared to the wild-type plants (WT). Accordingly, the higher levels of expression of different stress related genes such as AtNHX1 AtP5CS, AtCAT, AtSOD, AtPOD and AtLEA, indicated higher levels of activities in sodium sequestration into vacuoles, in osmotic regulation and in ROS scavenging of transgenic plants. Altogether, our results suggest an essential role for V-H^+-ATPase subunit E1 in improving the tolerance of plants not only to to salinity but also osmotic stress. In another study, Hu et al. (2012) found that tomato plants overexpressing an MdVHA-B from apple (*Malus domestica*) exhibited improved drought-stress tolerance. These results indicate that enhanced vacuolar H+ pumping in transgenic plants could be an effective approach to improve the drought tolerance of crops. In addition to their role in ion transport, V-H^+-ATPases energize the transport of solutes such as amino acids (proline), betaine, polyols and sugars, across the tonoplast (Chen and Murata 2002). There is also evidence that the V-ATPase subunits may have additional functions independent of proton pumping, thus adding another layer of complexity to the biology of V-ATPases. For example, VHA-B1 has been identified in nuclear complexes with hexokinase, and a series of phenotypes of a vha-B1 mutant indicate that this subunit is involved in glucose signalling (Cho et al. 2006). According to our results, *Lm*VHA-E1 improved the tolerance of *A. thaliana* to mannitol-induced osmotic stress probably through an increased accumulation of compatible solutes like proline, as indicated by an increased expression of P5CS gene in transgenic lines. Further experiments are needed if a clear conclusion on the relationship between V-H^+-ATPases and osmotic stress tolerance is to be drawn. It is evident from our study and several other reports that the over-expression of a single gene could impart salt tolerance to plants. But, it is well-known that salinity tolerance is a multi-gene trait and transformation with more than one gene will be necessary to meet the requirements to obtain insights into the mechanisms of tolerance improvement.

1.5.2 H_2O_2 Priming

Several recent studies on plants have demonstrated that pre- treatment with exogenous H_2O_+ can induce salt tolerance (Table 1.1). These findings demonstrate that H_2O_2 priming can induce tolerance to salinity in plants by modulating physiological and metabolic processes such as photosynthesis, proline accumulation and ROS detoxification, and that this ultimately leads to better growth and development. Importantly, ROS metabolism also plays a pivotal role in the development of stress and cross stress tolerance.

H_2O_2 priming represents a fruitful area of future research, which should help plant scientists explore the molecular mechanisms associated with abiotic stress tolerance and promote a more environmental friendly sustainable agriculture (Hossain et al. 2015). As shown in previous section, halophytes have mechanisms

Table 1.1 List of some successful studies showing the ameliorative effect of H_2O_2 priming on the response of crop species to salinity

Plant	Method of application	Response to salt stress after pre-treatment	Reference
Rice	Low concentrations (<10 μM) of H_2O_2 or NO	Greener leaves and a higher photosynthetic activity increases in ROS scavenging enzyme activities and increased expression of genes encoding pyrroline-5-carboxylatesynthase, sucrose- phosphate synthase, and the small heat shock protein 26	Uchida et al. (2002)
Maize	1 μM $H_2O_2$for 2 days, 25 mM for 1, 5 and 10 days	By enhancing antioxidant metabolism and reducing lipid peroxidation in both leaves and roots	Azevedo-Neto et al. (2005)
Triticum aestivum	Seeds were soaked in H_2O_2 (1–120 μM,8 h) and subsequently grown in saline conditions (150 mM NaCl)	Lower H2O2 in seedlings from primed seeds, better photosynthesis, maintained turgor, under salt stress	Wahid et al. (2007)
Hordeum vulgare	Seedlings pre-treated with H_2O_2 (1 and 5 mM), 150 mM NaCl for 4 and 7 d	Lower Cl^- leaf content, Higher rates of CO_2 fixation and lower malondialdehyde (MDA) and H_2O_2 contents under salt stress	Fedina et al. (2009)
Wheat	H_2O_2 0.05 μM	Enhanced GSH content and increased the activities of APX, CAT, SOD, and POD	Li et al. (2011)
Avena sativa	H_2O_2 0.5 mM	Up-regulation of the activities of CAT and SOD	Hossain et al. (2015)
Maize	Foliar H_2O_2 priming	Less H_2O_2 accumulation and maintenance of the leaf RWC and chlorophyll contents	Gondim et al.(2012, 2013)
Triticum aestivum	H_2O_2: 50, 100 μM	Reductions in both Na^+ and Cl^- ion levels and an increase in proline content and in N assimilation. Improved water relations, increased levels of photosynthetic pigments and greater growth rates	Ashfaque et al. (2014)
Panax ginseng	100 μM H_2O_2, for 2 days	Better seedling growth, and chlorophyll and carotenoid contents, lower oxidative stress increased activities of APX, CAT, and guaiacol peroxidase,	Sathiyaraj et al. (2014)

to utilize ROS, especially H_2O_2, for signaling purposes that confer acclamatory stress tolerance through the modulation of osmotic adjustment, ROS detoxification, photosynthetic C fixation and hormonal regulation (Ben Hamed et al. 2016). A large number of studies have suggested that H_2O_2 treatment is capable of inducing salt stress tolerance through the induction of a small oxidative burst. This burst subsequently activates a ROS-dependent signalling network, thereby enhancing the accumulation of latent defense proteins, such as ROS-scavenging enzymes and transcription factors (TFs), resulting in a primed state and an enhanced stress response (Borges et al. 2014). Many researchers have suggested a central role for H_2O_2 in intracellular and systemic signaling routes that increase tolerance and acclimation to abiotic stresses. Recent findings have shown that effective ROS signaling may require an increased flux of thiol-dependent antioxidants. With respect to signal transduction, ROS can interact with other signaling pathways, such as the activation of NADPH oxidase dependent or monomeric G protein; lipid-derived signals; induction of MAPK; redox sensitive TFs; regulation of Ca^{2+}; and plant hormone signal transduction. An understanding of the H_2O_2 physiology of plants, particularly H_2O_2 sensing and the identification of the components of H_2O_2 signaling network and H_2O_2 cross-talk with other growth factors, is of practical importance if we aim at improving the performance of crop plants growing under salt stress conditions.

1.5.3 Salinity Pre-treatment

Plants exposure to low level salinity activates an array of processes leading to an improvement of plant stress tolerance. This has already been demonstrated for different crop species such as soybean, rice, sorghum, pea and maize (Amzallag et al. 1990; Umezawa et al. 2000; Djanaguiraman et al. 2006, Pandolfi et al. 2012, 2016). For example, soybean pretreated for 23 days showed a higher survival rate under severe stress conditions (Umezawa et al., 2000); in rice, 1 week of pretreatment decreased leaf area and total dry matter production, but improved growth rate and shoot and root length after 1 week of severe salt treatment (Djanaguiraman et al. 2006); and in sorghum pre-treated plants maintained the same growth rate before and after the exposure to high level of salt, and they could stand a concentration much higher than non acclimated plants (Amzallag et al. 1990). In *Pisum staivum* and *Zea mays*, 7 days were enough to activate an array of processes leading to an improvement of plant stress tolerance (Pandolfi et al. 2012, 2016).

In many studies, acclimation to salinity was reported mainly to be related to ion-specific rather than osmotic component of salt stress. For example, in *Pisum sativum*, although acclimation took place primary in the root tissues, the control of xylem ion loading and efficient Na^+ sequestration in mesophyll cells are found to be important components of this process (Pandolfi et al. 2012). In maize, acclimation to salinity is not attributed to better ability of roots to exclude Na^+, given the lack of any significant difference in net Na^+ fluxes between acclimated and non-acclimated roots (Pandolfi et al. 2016). These findings are in a full agreement that acclimated plants accumulated more Na^+ in the shoot compared with non-acclimated ones, although further investigations are needed in order to unravel a clear picture of the ionic component of the acclimation mechanisms at the molecular level. Reported results allow us to suggest that the contribution of the root SOS1 plasma membrane transporters in this process is relatively minor, and instead points out at the important role of vacuolar compartmentation of Na^+ as a component of acclimation mechanism.

1.6 Conclusions and Perspectives

There is an urgent need to obtain high salt tolerant crops, because salinity is continuously spreading, productivity of conventional crop plants is significantly decreasing and world population is growing. This challenge can be achieved through the exploitation of halophytes, either through the engineering of their salt tolerance or miming acclamatory processes in salt sensitive crops. Most studies of halophytes concentrate on changes in plant physiology and gene expression to understand the mechanisms of salt tolerance. However adaptation to salinity is also connected with complex ecological processes within the rhizosphere induced by microorganisms (procaryotes and fungi) inhabiting roots and leaves of halophytes. It is therefore necessary to also learn from such microorganisms in order to increase the salt tolerance in crop species.

References

Aharon R, Shahak Y, Wininger S, Bendov R, Kapulnik Y, Galili G (2003) Over expression of a plasma membrane aquaporin in transgenic tobacco improves plant vigor under favorable growth conditions but not under drought or salt stress. Plant Cell 15:439–447

Amtmann A (2009) Learning from evolution: *Thellungiella* generates new knowledge on essential and critical components of abiotic stress tolerance in plants. Mol Plant 2:3–12

Amzallag N, Lerner HR, Poljakoff Mayber A (1990) Induction of increased salt tolerance in *Sorghum bicolor* by NaCl pretreatment. J Exp Bot 41:29–34

Apse MP, Blumwald E (2002) Engineering salt tolerance in plants. Nat Biotech 21:81–85

Ashfaque F, Khan MIR, Khan NA (2014) Exogenously applied H_2O_2 promotes proline accumulation, water relations, photosynthetic efficiency and growth of wheat (*Triticum aestivum* L.) under salt stress. Annu Res Rev Biol l4:105–120

Azevedo-Neto AD, Prisco JT, Eneas-Filho J, Medeiros JVR, Gomes-Filho E (2005) Hydrogen peroxide pre-treatment induces salt stress acclimation in maize plants. J Plant Physiol 162:1114–1122

Ben Hamed K, Ellouzi H, Talbi OZ, Hessini K, Slama I, Ghnaya T, Abdelly C (2013) Physiological response of halophytes to multiple stresses. Funct Plant Biol 40:883–896

Ben Hamed K, Ben Hamad I, Bouteau F, Abdelly C (2016) Insights into the ecology and the salt tolerance of the halophyte *Cakile maritima* using mutidisciplinary approaches. In: Khan MA, Ozturk M, Gul B, Ahmed MZ (eds) Halophytes for food security in dry lands. Elsevier, Oxford, pp 197–211

Berthomieu P, Conéjéro G, Nublat A, Brackenbury WJ, Lambert C, Savio C, Uozumi N, Oiki S, Yamada K, Cellier F et al (2003) Functional analysis of AtHKT1 in *Arabidopsis* shows that Na^+ recirculation by the phloem is crucial for salt tolerance. EMBO J 22:2004–2014

Borges AA, Jiménez-Arias D, Expósito-Rodríguez M, Sandalio LM, Pérez JA (2014) Priming crops against biotic and abiotic stresses: MSB as a tool for studying mechanisms. Front Plant Sci 5:642. https://doi.org/10.3389/fpls.2014.00642

Bose J, Rodrigo-Moreno A, Shabala S (2014) ROS homeostasis in halophytes in the context of salinity stress tolerance. J Exp Bot 65:1241–1257

Bradley PM, Morris JT (1991) Relative importance of ion exclusion, secretion and accumulation in *Spartina alterniflora* Loisel. J Exp Bot 42:1525–1532

Bressan RA, Zhang C, Zhang H, Hasegawa PM, Bohnert HJ, Zhu JK (2001) Learning from the *Arabidopsis* experience. The next gene search paradigm. Plant Physiol 127:1354–1360

Bruce TJA, Matthes M, Napier JA, Pickett JA (2007) Stressful ‘memories’ of plants: evidence and possible mechanisms. Plant Sci 173:603–608

Chen THH, Murata N (2002) Enhancement of tolerance of abiotic stress by metabolic engineering of betaines and other compatible solutes. Curr Opin Plant Biol 5:250–257

Dabbous A, Ben Saad R, Brini F, Farhat-Khemekhem A, Zorrig A, Abdely C, Ben Hamed K (2017) Over-expression of a subunit E1 of a vacuolar H^+-ATPase gene (*Lm* VHA-E1) cloned from the halophyte *Lobularia maritima* improves the tolerance of *Arabidopsis thaliana* to salt and osmotic stresses. Environ Exp Bot 127:128–141

Dassanayake M, Larkin JC (2017) Making plants break a sweat: the structure, function, and evolution of plant salt glands. Front Plant Sci 8:406. https://doi.org/10.3389/fpls.2017.00406

Debez A, BenHamed K, Grignon C, Abdelly C (2004) Salinity effects on germination, growth, and seed production of the halophyte *Cakile maritima*. Plant Soil 262:179–189

Debez A, Saadaoui D, Ramani B, Ouerghi Z, Koyro HW, Huchzermeyer B, Abdelly C (2006) Leaf H+-ATPase activity and photosynthetic capacity of Cakile maritima under increasing salinity. Environ Exp Bot 57:285–295

Demidchik V et al (2010) Arabidopsis root K^+-efflux conductance activated by hydroxyl radicals: single-channel properties, genetic basis and involvement in stress-induced cell death. J Cell Sci 123:1468–1479

Djanaguiraman M, Sheeba JA, Shanker AK, Devi DD, Bangarusamy U (2006) Rice can acclimate to lethal level of salinity by pretreatment with sublethal level of salinity through osmotic adjustment. Plant Soil 284:363–373

Ellouzi H, Ben Hamed K, Cela J, Munné-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

Ellouzi H, Ben Hamed K, Hernández I, Cela J, Müller M, Magné C, Abdelly C, Munné-Bosch S (2014) A comparative study of the early osmotic, ionic, redox and hormonal signalling response in leaves and roots of two halophytes and a glycophyte to salinity. Planta 240:1299–1317

Fedina IS, Nedeva D, Çiçek N (2009) Pre-treatment with H2O2 induces salt tolerance in barley seedlings. Biol Plant 53:321–324

Flowers TJ (1985) Physiology of halophytes. Plant Soil 89:41–56

Flowers TJ (2004) Improving crop salt tolerance. J Exp Bot 396:307–319

Flowers TJ, Colmer TD (2008) Salinity tolerance in halophytes. New Phytol 179:945–963

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

Ghars MA, Parre E, Debez A, Bordenave M, Richard L, Leport L, Bouchereau A, Savouré 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

Ghars MA, Richard L, Lefebvre-De Vos D, Leprince AS, Parre E, Bordenave M, Abdelly C, Savouré A (2012) Phospholipases C and D modulate proline accumulation in *Thellungiella halophila/salsuginea* differently according to the severity of salt or hyperosmotic stress. Plant Cell Physiol 53:183–192

Gondim FA, Gomes-Filho E, Costa JH, Alencar NLM, Priso JT (2012) Catalase plays a key role in salt stress acclimation induced by hydrogen peroxide pre treatment in maize. J Plant Physiol Biochem 56:62–71

Gondim FA, Miranda RS, Gomes-Filho E, Prisco JT (2013) Enhanced salt tolerance in maize plants induced by H_2O_2 leaf spraying is associated with improved gas exchange rather than with non-enzymatic antioxidant system. Theor Exp Plant Physiol 25:251–260

Gong Q, Li P, Ma S, Rupassara SI, Bohnert H (2005) Salinity stress adaptation competence in the extremophile *T. halophila* in comparison with its relative *A. thaliana*. Plant J 31:826–839

Gorham J (1996) Mechanisms of salt tolerance of halophytes. In: Choukrallah R et al (eds) Halophytes and biosaline agriculture. Marcel Dekker, Inc, New York, pp 31–53

Hasegawa PM (2013) Sodium (Na$^+$) homeostasis and salt tolerance of plants. Environ Exp Bot 92:19–31

Himabindu Y, Chakradhar T, ReddyMC KA, Redding KE, Chandrasekhar T (2016) Salt-tolerant genes from halophytes are potential key players of salt tolerance in glycophytes. Environ Exp Bot 124:39–63

Hossain MA, Bhattacharjee S, Armin S-M, Qian P, Xin W, Li H-Y, Burritt DJ, Fujita M, Tran L-SP (2015) Hydrogen peroxide priming modulates abiotic oxidative stress tolerance: insights from3 ROS detoxification and scavenging. Front Plant Sci 6:420. https://doi.org/10.3389/fpls.2015.00420

Hu DG, Wang SH, Luo H, Ma QJ, Yao YX, You CX, Hao YJ (2012) Overexpression of MdVHA-B, a V–ATPase gene from apple, confers tolerance to drought in transgenic tomato. Sci Hortic 145:94–101

Jdey A, Slama I, Rouached A, Abdelly C (2014) Growth, Na$^+$, K$^+$, osmolyte accumulation and lipid membrane peroxidation of two provenances of *Cakile maritima* during water deficit stress and subsequent recovery. Flora 209:54–62

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

Jithesh MN, Prashanth SR, Sivaprakash KR, Parida AK (2006) Antioxidative response mechanisms in halophytes: their role in stress defence. J Genet 85:237–254

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

Katschnig D, Broekman R, Rozema J (2013) Salt tolerance in the halophyte *salicorniadolichostachya* moss: growth, morphology and physiology. Environ Exp Bot 92:32–42

Kim C, Bressan R (2016) A computational analysis of salt overly sensitive 1 homologs in halophytes and glycophytes. PeerJ PrePrints 4:e1668v1 https://doi.org/10.7287/peerj.preprints.1668v1

Kluge C, Lamkemeyer P, Tavakoli N, Golldack D, Kandlbinder A, Dietz KJ (2009) cDNA cloning of 12 subunits of the V-type ATPase from Mesembryanthemum crystallinum and their expression under stress. Mol Membr Biol 20:171–183

Koyro HW, Lieth H (2008) Global water crisis: the potential of cash crop halophytes to reduce the dilemma. In: Lieth H, Sucre MG, Herzog B (eds) Mangroves and halophytes: restoration and utilisation. Tasks for vegetation sciences, vol 43. Springer, Dordrecht, pp 7–19

Li JT, Qiu ZB, Zhang XW, Wang LS (2011) Exogenous hydrogen peroxide can enhance tolerance of wheat seedlings to salt stress. Acta Physiol Plant 33:835–842

Liska AJ, Shevchenko A, Pick U, Katz A (2004) Enhanced photosynthesis and redox energy production contribute to salinity tolerance in *Dunaliella* as revealed by homology-based proteomics. Plant Physiol 136:2806–2817

Munns R, James RA, Xu B, Athman A, Conn SJ, Jordans C, Byrt CS, Hare RA, Tyerman SD, 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

Murakeözy EP, Nagy Z, Duhazé C, Bouchereau A, Tuba Z (2003) Seasonal changes in the levels of compatible osmolytes in three halophytic species of inland saline vegetation in Hungary. J Plant Physiol 160:395–401

Oh DH, Leidi E, Zhang Q, Hwang SM, Li Y, Quintero FJ et al (2009a) Loss of halophytism by interference with SOS1 expression. Plant Physiol 151:210–222

Oh DO, Zahir A, Yun DJ, Bressan RA, Bohnert HJ (2009b) SOS1 and halophytism. Plant Signal Behav 4(11):1081–1083

Ozgur R, Uzilday B, Sekmen AH, Turkan I (2013) Reactive oxygen species regulation and antioxidant defence in halophytes. Funct Plant Biol 40:832–847

Pandolfi C, Mancuso S, Shabala S (2012) Physiology of acclimation to salinity stress in pea (*Pisum sativum*). Environ Exp Bot 84:44–51

Pandolfi C, Azzarello E, Mancuso S, Shabala S (2016) Acclimation improves salt stress tolerance in Zea mays plants. J Plant Physiol 201:1–8

Panta S, Flowers T, Lane P, Doyle R, Haros G, Shabala S (2014) Halophyte agriculture: success stories. Environ Exp Bot 107:71–83

Paramonova NV, Shevyakova NI, Kuznetsov VV (2004) Ultrastructure of chloroplasts and their storage inclusions in the primary leaves of *Mesembryanthemum crystallinum* affected by putrescine and NaCl. Russ J Plant Physiol 51:86–96

Parre E, Ghars MA, Leprince AS, Thiery L, Lefebvre D, Bordenave M, Luc R, Mazars C, Abdelly C, Savouré A (2007) Calcium signaling via phospholipase C is essential for proline accumulation upon ionic but not nonionic hyperosmotic stresses in Arabidopsis. Plant Physiol 144:503–512

Pitman MG, Läuchli A (2002) Global impact of salinity and agricultural ecosystems. In: Läuchli A, Lüttge U (eds) Salinity: environment – plants – molecules. Springer Netherlands, Dordrecht, pp 3–20

Qiu NW, Chen M, Guo JR, Bao HY, Ma XL, Wang BS (2007) Coordinate up-regulation of V-H^+-ATPase and vacuolar Na+/H+ antiporter as a response to NaCl treatment in a C-3 halophyte *Suaeda salsa*. Plant Sci 172:1218–1225

Ratajczak R (2000) Structure, function and regulation of the plant vacuolar H+ translocating ATPase. Biochim Biophys Acta 1465:17–36

Ruiz JM (2002) Engineering salt tolerance in crop plants. Curr Opin Biotechnol 13:146–150

Sathiyaraj G, Srinivasan S, Kim YJ, Lee OR, Balusamy SDR, Khorolaragchaa A et al (2014) Acclimation of hydrogen peroxide enhances salt tolerance by activating defense-related proteins in *Panax ginseng* CA. Meyer. Mol Biol Rep 41:3761–3771

Shabala S (2013) Learning from halophytes: physiological basis and strategies to improve abiotic stress tolerance in crops. Ann Bot 112:1209–1221

Shabala S, Mackay A (2011) Ion transport in halophytes. Adv Bot Res 57:151–199

Shabala S, Bose J, Hedrich R (2014) Salt bladders: do they matter? Trends Plant Sci 19:687–691

Shi H, Lee BH, Wu SJ, Zhu JK (2003) Overexpression of a plasma membrane Na^+/H^+ antiporter gene improves salt tolerance in *Arabidopsis thaliana*. Nat Biotechnol 21:81–85

Short DC, Colmer TD (1999) Salt tolerance in the halophyte *Halosarcia pergranulata* subsp. *Pergranulata*. Ann Bot 83:207–213

Slama I, Abdelly C, Bouchereau A, Flowers T, Savouré A (2015) Diversity, distribution and roles of osmoprotective compounds accumulated in halophytes under abiotic stress. Ann Bot 115:433–447

Souid A, Gabriele M, Longo V, Pucci L, Bellani L, Smaoui A, Abdelly C, Ben Hamed K (2016) Salt tolerance of the halophyte *Limonium delicatulum* is more associated with antioxidant enzyme activities than phenolic compounds. Funct Plant Biol 43:607–619

Sun J, Wang MJ, Ding MQ, Deng SR, Liu MQ, Lu CF, Zhou XY, Shen X, Zheng XJ, Zhang ZK, Song J, Hu ZM, Xu Y, Chen SL (2010) H_2O_2 and cytosolic Ca^{2+} signals triggered by the PM H^+-coupled transport system mediate K^+/Na^+ homeostasis in NaCl-stressed *Populus euphratica* cells. Plant Cell Environ 33:943–958

Szabados L, Savouré A (2010) Proline: a mulitfunctional amino acid. Trends Plant Sci 15:89–97

Taji T, Seki M, Satou M, Sakurai T, Kobayashi M, Ishiyama K, Narusaka Y, Narusaka M, Zhu JK, Shinozaki K (2004) Comparative genomics in salt tolerance between *Arabidopsis* and *Arabdopsis*-related halophyte salt cress using *Arabidopsis* microarray. Plant Physiol 135:1697–1709

Thiery L, Leprince A-S, Lefebvre D, Ghars MA, Debarbieux E, Savouré A (2004) Phospholipase D is a negative regulator of proline biosynthesis in *Arabidopsis thaliana*. J Biol Chem 279:14812–14818

Uchida A, Jagendorf AT, Hibino T, Takabe T, Takabe T (2002) Effects of hydrogen peroxide and nitric oxide on both salt and heat stress tolerance in rice. Plant Sci1 63:515–523

Umezawa T, Shimizu K, Kato M, Ueda T (2000) Enhancement of salt tolerance in soybean with NaCl pretreatment. Physiol Plant 110:59–63

Wahid A, Perveen M, Gelani S, Basra SMA (2007) Pre treatment of seed with H_2O_2 improves salt tolerance of wheat seedlings by alleviation of oxidative damage and expression of stress proteins. J Plant Physiol1 64:283–294

Wang BS, Lüttge U, Ratajczak R (2001) Effects of salt treatment and osmotic stress on V-ATPase and V-PPase in leaves of the halophyte Suaeda salsa. J Exp Bot 52:2355–2365

Wiciarz M, Gubernator B, Kruk J, Niewiadomska E (2015) Enhanced chloroplastic generation of H_2O_2 in stress-resistant *Thellungiella salsuginea* in comparison to *Arabidopsis thaliana*. Physiol Plant 153:467–476

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

Zhang GH, Su Q, An LJ, 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

Zhu JK (2001) Plant salt tolerance. Trends Plant Sci 6:66–71