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REVIEW

Salt-Stress Signaling

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Salinity stress has a major impact on plant growth and development. Increasing concentrations of salt in farm soils means that researchers must develop tolerant crops if the global food supply is to be sustained. Salt adaptation involves a complex network of different mechanisms whose responses to high salinity are regulated in an integrated fashion. The salt-stress signaling cascade(s) that activates these mechanisms starts by perceiving the saline environment. However, little is known about the components involved in either the perception or signaling of this stress. The mechanisms that are activated under such conditions include those responsible for ion homeostasis and osmotic adjustment. Here, we review the current understanding of those molecular mechanisms used by plants to respond and adapt to salt stress. Particular attention is paid to the information yielded by genetic analyses of the yeast model *Saccharomyces cerevisiae* and the higher-plant model system of *Arabidopsis*.

Keywords: Arabidopsis, ion homeostasis, osmotic stress, salt stress, signaling, yeast

SALT STRESS

High levels of salt in the soil can severely limit plant growth and productivity. Reports by the FAO (2005) indicate that 2% of agricultural land is salt-affected. This is also true for 20% of all irrigated acres, a land base that comprises just 15% of the total cultivated area but produces one-third of the world's food. The problem of soil salinity is increasing (Chinnusamy et al., 2005). Plants fall into one of two groups depending on their ability to tolerate high salt concentrations. Halophytes grow well in environments with elevated contents of NaCl (usually, although other salts may be found as well). For example, these species survive when cultured with NaCl concentrations as high as 500 mM, which is double the amount found in seawater (Inan et al., 2004). In contrast, the salt-sensitive, glycophyte group comprises most other plants, including the vast majority of crop species, and members cannot grow in the presence of high salt concentrations. Because of this great threat to the global food supply (Flowers, 2004), agronomic and horticultural species must be identified that show increased salt tolerance in order to sustain the increased production that will be required in many regions of the world. Moreover, while enhancing salt tolerance in crops will directly improve their yields in soils suffering from primary salinity, maximizing such tolerance in perennial species used for fodder or fuel will also help reduce the spread of secondary salinity (Munna, 2005).

High levels of salt induce both hyperionic and hyperosmotic stress (Fig. 1), and can result in plant damage due to membrane disorganization, the generation of reactive oxygen species, metabolic toxicity, inhibited photosynthesis, and the attenuation of nutrient acquisition (Hasegawa et al., 2000; Parida et al., 2002). This stress is usually caused by elevated Na^+ and Cl^- concentrations in the soil. In addition, such an osmotic imbalance interferes with the ability of plants to absorb water, leading to physiological consequences similar to those induced by dehydration stress. Understanding the molecular basis for salt-stress signaling and tolerance mechanisms is essential if we are to breed for and genetically engineer salt tolerance in crop plants. Because the signaling mechanism is also a fundamental aspect of basic plant biology, further insights into it will improve our knowledge in many subjects, ranging from gene regulation and signal-transduction to ion-transport and mineral nutrition. This review outlines the recent advances made in research on salt-stress signaling that have been achieved through molecular genetics analyses of yeast and *Arabidopsis*.

ION HOMEOSTASIS

Homeostasis of intracellular ion concentrations is crucial to the proper functioning of living cells. Consequently, ion flux is stringently regulated so that the levels of toxic ions are kept low while essential ions are accumulated. In environments of high salt (usually NaCl), the ionic steady states of Na^+, Cl^-, K^+, and even Ca^{2+} are disturbed (Flowers et al., 1977; Hasegawa et al., 2000). For example, the potassium level is perturbed because external Na^+ inhibits the influx of K^+ (Fig. 1), thereby preventing the cell from acquiring this fundamental nutrient. High NaCl contents also induce the cytosolic accumulation of Ca^{2+}, which subsequently triggers adaptive stress responses. Ion homeostasis is dependent on transmembrane transport proteins that mediate ion flux. These transport proteins include H^+-translocating ATPases and pyrophosphatases, Ca^{2+}-ATPases, other active transporters, and channels (Niu et al., 1995; Sze et al., 1999). Most were found to be transport proteins based on structure/function information or by functional complementation of transport-deficient yeast mutants. Many of the transport determinants that mediate ion homeostasis in yeast are very similar to those that function in plants (Dreyer et al., 1999).

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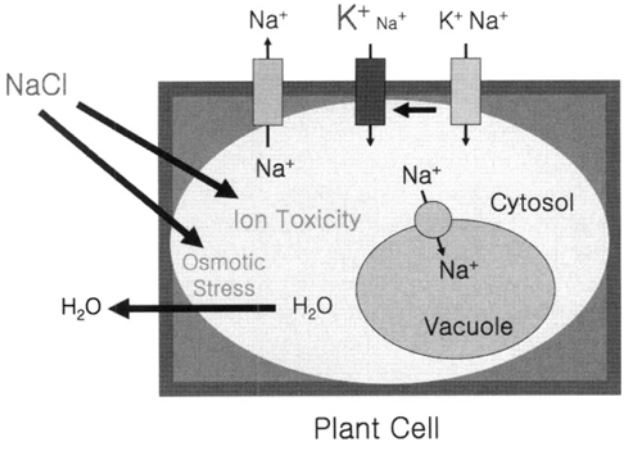

Figure 1. Plant adaptation to high salinity. When they are exposed to high NaCl concentrations, their apoplastic levels of Na^+ and Cl^- alter aqueous and ionic thermodynamic equilibria, resulting in hyperosmotic stress, ionic imbalance, and toxicity. Survival and growth is dependent on plant capacity to re-establish, expeditiously, their cellular osmotic and ionic homeostasis, i.e., ability to adapt to stress environment. Tolerance to NaCl stress is mediated by processes that 1) restrict Na^+ uptake across plasma membrane (presumably due to combination of reduced influx and increased efflux), 2) facilitate Na^+ and Cl^- sequestration into vacuole, and 3) regulate compatible osmolyte production and accumulation. Coordinated control of these processes is essential for osmotic adjustment and ion homeostasis required in salt adaptation.

Na^+ homeostasis

The intracellular Na^+ and Cl^- concentrations in plant cells are regulated by Na^+/H^+ antiport proteins that are located not only on the plasma membrane but also on the tonoplast (vacuole membrane) (Dietz et al., 2001). These proteins are energized by H^+ electrochemical potential gradients that are established across both limiting membranes. In particular, the tonoplast Na^+/H^+ transporter proteins cause Na^+ and Cl^- ions to be sequestered in the vacuole, where they are the primary solutes that affect its osmotic adjustment (Blumwald and Poole, 1985). Compartmentation of Na^+ in the vacuole requires energy-dependent transport, and then alters vacuolar alkalization (Apse et al., 1999). Plant cDNAs encoding NHE-like proteins have been isolated that can functionally complement a yeast mutant that lacks the endomembrane Na^+/H^+ transporter NHX1 (Shi and Zhu, 2002). These proteins include two *Arabidopsis NHXs*, *AtNHX1* and *AtNHX2*. Their expression is induced by salt stress and abscisic acid (ABA) (Yokoi et al., 2002). AtNHX1 promoter-GUS analysis has also revealed that AtNHX1 is strongly expressed in the guard cells and root hair cells (Shi and Zhu, 2002). Together these observations suggest that AtNHX1 functions in storing Na^+ in the enlarged vacuoles of root hair cells. In addition, the ability of NaCl to up-regulate *AtNHX1* transcript levels is reduced in *abi* mutants but not in *sos* mutants. This implies that salt stress up-regulates *AtNHX1* transcription and that this up-regulation is partly dependent on ABA biosynthesis and signaling.

As mentioned, the activity of Na^+/H^+ transporter proteins on the plasma membrane and the tonoplast, which determines the Na^+ concentrations in the cytosol and vacuole, respectively, is regulated by H^+ electrochemical potential gradients that are established across these limiting membranes. Those gradients are generated by electrogenic H^+ pumps. The tonoplast pump, called vacuolar-type H^+-ATPase (V-ATPase) (Dietz et al., 2001), is indispensable for plant growth in a normal environment due to its roles in energizing and maintaining solute homeostasis. Under stress conditions, such as high salinity, V-ATPase activity is up-regulated, so that it energizes the tonoplast and enhances the sequestering of ions in the vacuole (Wang et al., 2001).

K^+ homeostasis

Na^+ is toxic to plant cells because the transporter that takes up K^+ into the root also shows affinity for Na^+ (Fig. 1). K^+ is an essential co-factor of many cellular enzymes, unlike Na^+. High extracellular NaCl concentrations therefore lower the K^+/Na^+ ratio in the cytosol, which interferes with the functioning of K^+-dependent enzymes. Notably, while halophytes grown in high NaCl concentrations have elevated levels of cytosolic Na^+, their cytosolic K^+/Na^+ ratios are larger than those of glycophytes grown under similar conditions (Flowers et al., 1977). This is because the affinity of the K^+ transporter in halophyte cells changes during salt stress, such that its affinity for K^+ exceeds its affinity for Na^+ (Fig. 1). Therefore, this promotes the accumulation of K^+ and decreases the cytosolic Na^+ concentrations (Anderson et al., 1992; Hirsch et al., 1998).

Ca^{2+} homeostasis

The observation that externally supplied Ca^{2+} reduces the toxic effects of NaCl has led to the theory that Ca^{2+} ions may play a role in salt adaptation. Presumably, externally supplied Ca^{2+} favors the uptake of K^+ over Na^+. In addition, high salinity increases the transport of Ca^{2+} from the apoplast and intracellular compartments to the cytosol, thereby elevating cytosolic Ca^{2+} concentrations; this indicates that Ca^{2+} acts intracellularly (Ono et al., 1999; Shi et al., 2000). These increased intracellular Ca^{2+} levels in response to salt stress have been reported from several studies. First, when ACA4, which encodes a vacuolar Ca^{2+}-ATPase in *Arabidopsis*, is placed into a yeast strain that cannot transport Ca^{2+}, the yeast becomes more tolerant of high levels of extracellular NaCl (Geisler et al., 2000). Second, transgenic tobacco plants that over-express the Ca^{2+}/H^+ antiporter AtCAX1 are hypersensitive to salt (Hirschi, 2004). Third, when *Arabidopsis* over-express the ionotropic glutamate receptor AtGluR2, which decreases the efficiency of Ca^{2+} utilization, those plants become hypersensitive to Na^+ and K^+ ionic stresses (Kim et al., 2001). It is now understood that Ca^{2+} heightens the K^+/Na^+ selectivity of root K^+ transport systems during NaCl stress (Cramer et al., 1987), thereby maintaining a minimal threshold level of K^+. It is likely that Ca^{2+} achieves this in plant cells by a mechanism similar to the one found in yeast (Liu and Zhu, 1997). Thus, NaCl stress stimulates the influx of Ca^{2+} from the apoplast and vacuoles, thereby transiently increasing cytosolic Ca^{2+} levels. The Ca^{2+} ions then act as a secondary messenger to regulate K^+/Na^+ selectivity and, therefore, enhance K^+ influx (Xiong and Zhu., 2002) However, the receptor that recognizes NaCl and initiates this signaling cascade has not yet been identified.

OSMOLYTE BIOSYNTHESIS

Generally, changes in the external osmotic potential of cells lead them to accumulate metabolites. Those with osmolyte functions (i.e., they can act as compatible solutes) include sugars (sucrose and fructose), sugar alcohols (glycerol and methylated inositols), complex sugars (trehalose, raffinose, and fructans), ions (K^+), and charged metabolites [glycine betaine, dimethyl sulfonium propionate (DMSP), proline, and ectoine].

Role of osmolytes in vivo

Osmolyte accumulation appears to aid the osmotic adjustment in cells by lowering their cytosolic osmotic potential. This accumulation does not inhibit ordinary metabolic reactions. Because compatible solutes are typically hydrophilic, it is believed that, under osmolytic stress, they replace the water at the surfaces of proteins, protein complexes, or membranes, thereby acting as osmoprotectants and low-molecular-weight chaperones (Hasegawa et al., 2000). At high concentrations, compatible solutes can reduce the inhibitory effects of ions on enzyme activity, increase the thermal stability of enzymes, and prevent the dissociation of enzyme complexes, such as the oxygen-evolving complex of Photosystem II (Hayashi et al., 1997). For example, glycine betaine protects thylakoids and plasma membranes against freezing damage or heat destabilization, even at low concentrations (Ono et al., 1999). Thus, osmolytes have the following characteristics: they do not inhibit primary metabolic pathways, even when present at high concentrations; they maintain enzymatic activity; and they moderate changes in the pH and charge balance of the cell (Zhu, 2002).

Metabolic pathways of osmolytes

Typically, the steps leading to osmolyte synthesis are linked to primary metabolic pathways that show high flux rates. Examples are the biosynthetic pathways that generate proline, glycine betaine, D-pinitol, and ectoine. These pathways are involved in amino acid biosynthesis from glutamic acid (proline) or aspartate (ectoine), choline metabolism (glycine betaine), and *myo*-inositol synthesis (pinitol). Some osmolyte biosynthetic enzymes are stress-inducible. For example, glycine betaine is produced in higher plants from choline *via* the stress-inducible enzymes choline monooxygenase and betainealdehyde dehydrogenase (Leung and Giraudat, 1998; Ono et al., 1999).

SALT-STRESS SIGNALING IN YEAST

The yeast *Saccharomyces cerevisiae* is a ubiquitous unicellular eukaryotic microorganism (Mustacchi et al., 2006). It is frequently used as a model system because, like with plants, it has a cell wall and vacuole, and disruption of its genes is easily achieved through homologous recombination. Therefore, a salt-sensitive phenotype of yeast cells has also been used to identify genes from the higher plant *Arabidopsis thaliana* that complement this phenotype (i.e., Functional Complementation). These genes include those encoding ion transporters and other molecules that promote salt tolerance (Xiong and Zhu, 2002; Yokoi et al., 2002; Shin et al., 2004).

Studies with *S. cerevisiae* have led to the discovery of two important pathways that lead to salt-stress tolerance. One is the high-osmolarity glycerol (HOG1) pathway, which is a mitogen-activated protein (MAP) kinase pathway used for adapting to hyperosmotic stress (O'Rourke et al., 2002). The other is the calcineurin pathway, which is a phosphatase enzymatic (calcineurin) cascade used for adjusting to ionic stress (Fig. 2). With regard to the HOG1 pathway, in yeast this is initiated by the activation of one of two osmosensors, either the low-osmolarity sensor SHO1 or the high-osmolarity sensor SLN1 (Maeda et al., 1994, 1995). These osmosensors activate a MAP kinase cascade that ultimately leads to the transcriptional activation of glycerol-biosynthetic genes and an increase in glycerol concentrations that adjusts the osmotic balance of the cell. The MAP kinases in this cascade are inactivated by dephosphorylation mediated by various tyrosine phosphatase and serine/threonine phosphatases (Saito and Tatebayashi, 2004).

With regard to the calcineurin pathway, it is triggered by Na^+ ionic stress, which elevates levels of intracellular calcium. Ca^{2+}/calmodulin then activates calcineurin, a serine/threonine protein phosphatase, that in turn regulates the transporters involved in Na^+ influx and efflux (Fig. 2). Consequently, calcineurin-deficient yeast mutants are unable to convert their K^+ transport pump Trk1p to the high-affinity

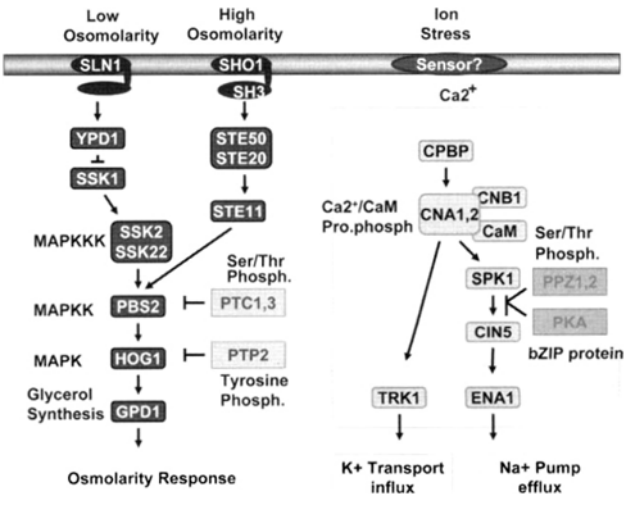

Figure 2. Osmotic and Na^+ ion stress-mediated signaling pathways in yeast *S. cerevisiae*. Two signaling cascades mediate osmotic adjustment and appropriate ion homeostasis under NaCl stress: mitogen-activated protein (MAP) kinase cascade and one regulated by calcineurin, a Ca^{2+}/Calmodulin-dependent protein phosphatase. At high osmolarity, two distinct cell-surface osmosensors activate MAP kinase cascade that eventually up-regulates glycerol production and accumulation. Na^+ stress also activates calcineurine-dependent pathway involved in regulation of Na^+ influx (TRK1) activity and efflux transporters (ENA1). Calcineurin-deficient mutants fail to convert K^+ transport system to high-affinity state that facilitates better discrimination for K^+ over Na^+ (Fig. 1). Mutants show reduced expression of ENA1 gene encoding a plasma membrane sited P-type ATPase essential for Na^+ efflux. Net result is substantially greater Na^+ accumulation and, consequently, an extremely salt-sensitive phenotype.

state needed to cope with greater extracellular NaCl concentrations, where the pump has increased affinity for K^+ but an unchanged Michaelis constant (K_m) for Na^+ or Li^+. In addition, Na^+ efflux in the mutant cell is reduced by low expression of ENA1, a P-type ATPase essential for NaCl tolerance. As a result, calcineurin-deficient cells are salt-sensitive because their uptake of Na^+ increases in the presence of high extracellular NaCl. Two other phosphatases, PPZ1 and PPZ2, negatively regulate the calcineurin pathway (Mendoza et al., 1994).

Cytosolic Ca^{2+} homeostasis is possibly achieved by a sophisticated feedback mechanism (Cunningham and Fink, 1996) that involves three Ca^{2+} transporters: PMC1, PMR1, and PMR2A. Expression of all three is promoted by calcineurin, which also inhibits the low-affinity vacuolar H^+/Ca^{2+} exchanger VCX1. Thus, these transporters not only control Ca^{2+} signal transduction, but are also partially responsible for the physiological consequences of Na^+ ionic stress-induced changes in cytosolic Ca^{2+} concentrations.

SALT-STRESS SIGNALING IN PLANTS

When plants perceive stress (either abiotic or biotic), they activate signal-transduction pathways that allow them to adapt to even minor environmental changes. As shown by many physiological and biochemical analyses, these adaptive responses involve numerous alterations that are generated in a highly organized manner. Thus, salt stress activates mechanisms that induce osmotic homeostasis and osmotic adjustment as well as those that control stress damage and induce repair or detoxification, and/or control growth (Anas and Vivekanandan, 2000; Kashem et al., 2000; Parida et al., 2002; Zhu, 2002; Parida and Das, 2005). Research programs now often involve direct analyses of the higher plant *Arabidopsis* (Somerville and Koornneef, 2002). This is because, apart from gene replacement by homologous recombination, almost everything that can be done with yeast can now be performed with *Arabidopsis*, including mutagenesis, mutant screening, positional cloning, and gene-tagging.

Arabidopsis is a glycophyte that is damaged by saline stress and shows inhibited growth in the presence of high salt levels. These plants can be harmed at all developmental stages. For instance, embryos treated with 150 mM NaCl show callose accumulation, abnormal ovule and embryo formation, and even cell death. Their seed germination and seedling stages are the most sensitive, with germination being strongly inhibited at about 75 mM NaCl. However, even at 50 mM NaCl, germination and subsequent plant growth are markedly impaired, and cell death is common.

The enormous advances made in developmental and disease resistance research with this genus have led to many studies of *Arabidopsis*-based salt tolerance (Saleki et al., 1993; Quesada et al., 2000). One approach has involved the isolation of mutant plants with reduced or increased sensitivity to salt (Tsugane et al., 1999; Zhu, 2000). Another approach, depicted in Figure 3, assesses *Arabidopsis* transformed with a chimeric gene promoter–LUC construct, where the expression of a firefly luciferase reporter gene is driven by a salt stress-inducible promoter, such as *RD29A*. The resulting transgenic plants emit bioluminescence in response to salt. Their seeds are then mutated by treatment with a mutagen such as ethyl methanesulfonate (EMS), after which the seedlings are screened for altered bioluminescence patterns in the presence of salt (Chinnusamy et al., 2002). This promoter-reporter approach has been very useful for identifying genes involved in seed germination and osmotic signal-transduction (Ishitani et al., 1997), and has also greatly improved our understanding of salt-stress signaling in higher plants.

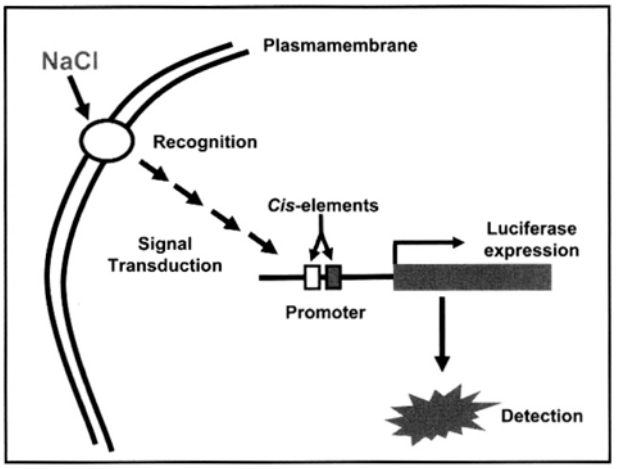

Figure 3. Identification of NaCl stress-signaling component using RD29::Luciferase system. One approach for identifying genes that function in salinity stress is to introduce into *Arabidopsis* plants a chimeric gene construct consisting of firefly luciferase coding sequence (LUC) under control of stress-responsive RD29A promoter. LUC activity in transgenic plants, as assessed via *in vivo* luminescence imaging, faithfully reports expression of endogenous RD29A gene. The *cos* (for *constitutive expression of osmotically responsive* genes), *los* (for *low expression of osmotically responsive* genes), and *hos* (for *high expression of osmotically responsive* genes) mutants were identified by Zhu's group using high-throughput luminescence imaging system. Because this approach utilizes screen for genes that function in stress-response signaling, this system makes it possible to identify genes that substantially impact adaptation to abiotic stress.

SOS pathway

Use of the *Arabidopsis* model system has revealed the existence of a pathway that regulates ionic homeostasis under salt stress. This pathway (Fig. 4) was discovered by the Zhu group after they cloned the salt overly sensitive (SOS) genes from a pool of *Arabidopsis* mutants. One of these mutants, the recessive *sos3*, is particularly interesting. While hypersensitive to Na^+, this trait is partially ameliorated by elevated Ca^{2+} levels in the culture medium (Liu and Zhu, 1998). SOS3 shares high sequence identity with the B-subunit of calcineurin in yeast, and belongs to a novel subfamily of EF-hand-type calcium-binding proteins (Guo et al., 2001). SOS1 and SOS2 genes also have been cloned from mutant pools of *Arabidopsis*. Molecular analyses have revealed that they are components of the cellular ion homeostasis pathway that transduces the salt stress-induced Ca^{2+} signal (Fig. 4; Shabala et al., 2005), as follows. The Ca^{2+}-dependent

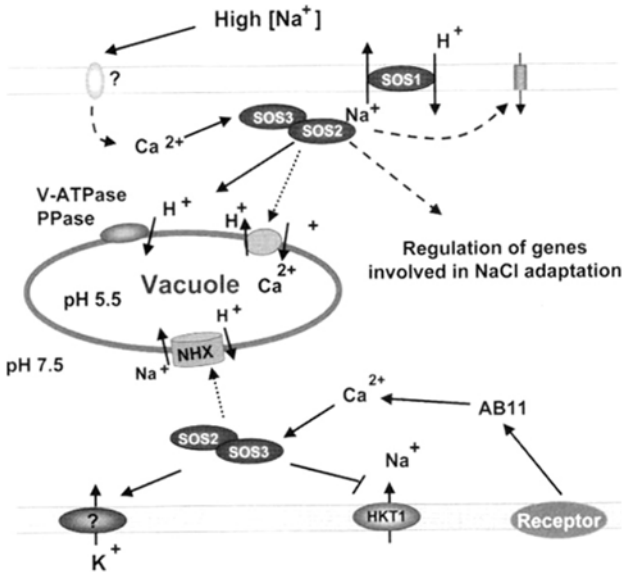

Figure 4. SOS pathway (modified from Zhu, 2003). Ca^{2+}-activated Salt Overly Sensitive (SOS) signal-transduction pathway facilitates Na^+ homeostasis *in planta* (Zhu, 2003). Hypersaline stress induces transient increase in $[Ca^{2+}]$ that is decoded by components of SOS pathway to facilitate Na^+ homeostasis. Recognition of Ca^{2+} signals by SOS3 is facilitated by interactions between divalent cation and EF structures in SOS3, which then activates serine/threonine kinase SOS2, followed by phosphorylation of plasma membrane-localized SOS1 to induce SOS1 Na^+/H^+ antiporter activity. SOS1 has been suggested as Na^+ sensor but determinant(s) responsible for salt-induced transient increase in $[Ca^{2+}]$ has yet to be identified (see text for details).

SOS signal-transduction pathway involves the recruitment and activation by SOS3 of SOS2, an effector kinase (Shi et al., 2000; Zhu, 2003). The SOS3-SOS2 protein kinase complex then phosphorylates and activates SOS1, an Na^+/H^+ exchanger in the plasma membrane that contributes to cellular sodium homeostasis by transporting sodium ions out of the cell. Activation of SOS3 elevates its Na^+/H^+ antiporter activity. The SOS3-SOS2 protein kinase complex also up-regulates the steady-state levels of *SOS1* transcript and stabilizes SOS1 expression (Shi et al., 2000; Zhu, 2003)

The *sos1*, *sos2*, and *sos3* mutants are hypersensitive not only to NaCl, but also to K^+ deficiency. This indicates that the SOS-mediated signal-transduction cascade also positively regulates the acquisition of K^+. Although *sos1* mutant plants display defective K^+ uptake at low external concentrations, the activated SOS1 protein does not show K^+ transport activity *in vivo*, which suggests that SOS1 regulates K^+ uptake only indirectly (Quintero et al., 2002). Notably, pretreatment with 50 mM NaCl inhibits the K^+ permeability of *sos1* root cells and *sos1* seedling growth under K^+-limiting conditions (Qi and Spalding, 2004). Consequently, it has been hypothesized that the elevated content of cytoplasmic Na^+ results from that loss of SOS1 function, impairing the permeability of K^+, and that AKT1 is a target of salt stress in *sos1* plants (Qui et al., 2002; Quintero et al., 2002; Qi and Spalding, 2004). Moreover, the Na^+ influx transporter AtHKT1 suppresses the *sos3-1* phenotype of Na^+ accumulation and hypersensitivity (Rus et al., 2001, 2004). In addition, *hkt1* mutations suppress the hypersensitivity of *sos1-1* and *sos2-2* to K^+-deficient conditions by blocking the accumulation of Na^+ ions in the cytosol (Rus et al., 2001, 2004). In contrast, plants that over-express AtHKT1 readily accumulate Na^+ ions and are hypersensitive to K^+ deficiency (Rus et al., 2001). Thus, it appears that AtHKT1 controls Na^+ homeostasis and, in doing so, also regulates K^+ nutrient status (Rus et al., 2004). Therefore, the SOS signal-transduction pathway is involved in K^+ acquisition either directly or indirectly.

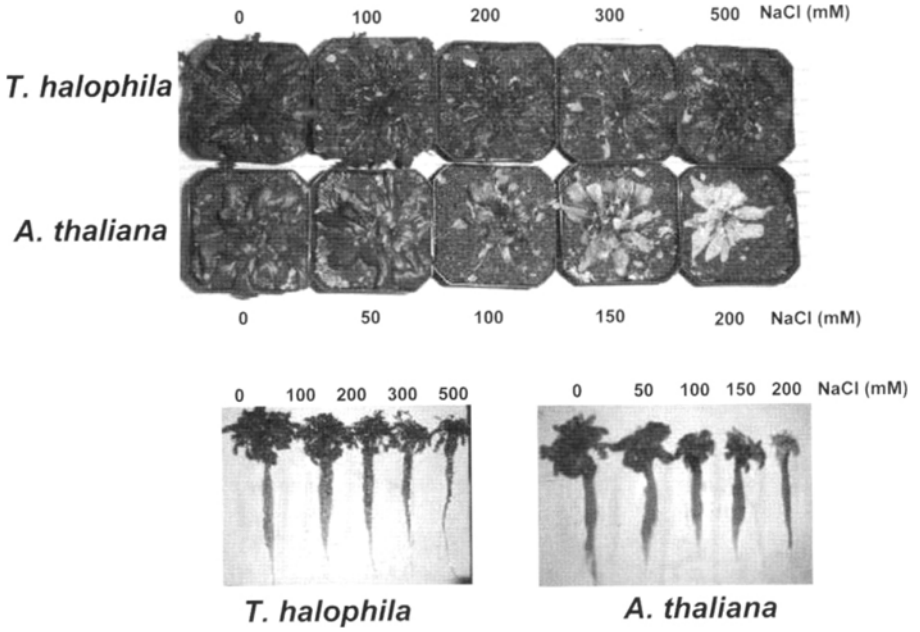

Figure 5. Comparison of NaCl tolerances between salt cress (*Thellungiella haloplia*) and *Arabidopsis* plants. Survival capacities were evaluated in Turface hydroponic growth media after NaCl concentration was increased from 0 to 500 mM, in 100-mM increments every 5 d. Photographs were provided by Dr. Ray A. Bressan (Center for Plant Environmental Stress Physiology, Purdue University).

Role of ABA in salt-stress responses

As shown by analyses of ABA-insensitive *Arabidopsis* mutants, such as *abi1* and *abi2*, plants that are challenged by external osmotic stress initiate their adaptive response by recruiting ABA (Himmelbach et al., 2003). Other phytohormones, e.g., salicylic acid, jasmonic acid, and ethylene (which are known to regulate the protective response of plants against diverse stresses), do not play a major role in osmotic-stress responses. After its recruitment, ABA modulates the expression of stress-responsive genes. Notably, not all NaCl stress-inducible genes are affected by ABA, which indicates that NaCl stress-inducible gene expression involves two different pathways, namely, an ABA-dependent pathway and an ABA-independent pathway (Zhu, 2002).

NaCl stress not only induces ABA-mediated gene expression, it also promotes ABA biosynthesis by transcriptionally regulating ABA-biosynthetic genes (Xiong and Zhu, 2003), leading to increased levels of abscisic acid in plant vegetative tissues. In particular, NaCl stress as well as dehydration up-regulate the transcript levels of genes for the ABA biosynthesis pathway that encode zeaxanthin epoxidase (ZEP), 9-cis-epoxycarotenoid dioxygenase (NCED), ABA aldehyde oxidase (AAO), and molybdenum cofactor sulfurylase (MCSU) (Audran et al., 1998; Xiong et al., 2001; Himmelbach et al., 2003; Xiong and Zhu, 2003). ZEP is involved in the first biosynthetic step more specific to this pathway, where zeaxanthin is epoxidated to violaxanthin by ZEP (Audran et al., 1998). NECD is involved in the rate-limiting step of ABA biosynthesis, namely, the oxidative cleavage of the major epoxycarotenoid 9-cis-neoxanthin that generates xanthoxin. Notably, ABA can also positively regulate its own biosynthesis by activating ABA-biosynthetic genes. This may be amplified in ABA-mediated stress responses (Xiong and Zhu, 2003).

Generation of salt stress-resistant plants

As indicated above, the adaptation of plants to salt stress involves a complex network of different mechanisms with disparate effects. These mechanisms include those of ion homeostasis, which control Na^+ influx and efflux through the plasma membrane and compartmentalize toxic Na^+ in the vacuole. Other mechanisms induce the production and accumulation of osmoprotectants, the scavenging of toxic radicals, and the transport of water (see previous section). Mechanisms for coordinated long-distance responses are also involved. Their elucidations have revealed which components of salt-stress signal-transduction and ion homeostasis transporters may be used in generating salt stress-resistant crops (Cassells and Doyle, 2003).

CONCLUDING REMARKS

This review surveys our current understanding of the mechanisms involved in salt-stress adaptation and resistance, as revealed by research with the yeast model *Saccharomyces cerevisiae* and the glycophyte model plant *Arabidopsis*. These studies have led to the identification of many mechanisms and components associated with these phenomena. Future experiments will continue the identification of salt stress-related genes and determine how plants sense such stress and how these known components are regulated.

It is generally believed that, while analysis of *Arabidopsis*, a typical glycophyte, is likely to point to many salt-resistance mechanisms also implemented by halophytes, some mechanisms used by particularly salt-tolerant species can be determined only by actively studying those individual species (Bressan et al., 2001; Zhu, 2001). This is because halophytes are likely to be resistant to salt stress not only because they utilize known salt-adaptation components more effectively than do glycophytes, but also because they have evolved additional salt-tolerance mechanisms not present in glycophytes (Bressan et al., 2001; Zhu, 2001). Therefore, further research using halophyte model plants may also be valuable (Zhu, 2000; Bressan et al., 2001). The recently discovered halophytic plant species *Thellungiella halophila* is likely to be a good model for such analyses because it self-fertilizes, has a small genome, and can be transformed and mutated quite easily (Fig. 5; Inan et al., 2004). Moreover, it shares over 90% cDNA sequence identity with *Arabidopsis* and has a similar morphology and life history, namely, a small size, a short life cycle, self pollination, and prolific seed production. Systemic genetics comparisons of *T. halophila* and *Arabidopsis* may thus reveal the novel salt-tolerance determinants and pathways that operate in halophytes (Vera-Estrella et al., 2005). These efforts might involve identifying the differences in signal-transduction pathways or determining the functions of genes that are more strongly expressed in the halophyte upon NaCl stress.

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