



# Salt Stress Tolerance of Plants

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## Abstract

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Salinity stress negatively impacts agricultural yield throughout the world affecting production whether it is for subsistence or economic gain. The plant response to salinity consists of numerous processes that must function in coordination to alleviate both cellular hyperosmolarity and ion disequilibrium. In addition, crop plants must be capable of satisfactory biomass production in a saline environment (yield stability). Tolerance and yield stability are complex genetic traits that are difficult to establish in crops since salt stress may occur as a catastrophic episode, be imposed continuously or intermittently, or become gradually more severe, and at any stage during development. However, cell biology and molecular genetics research is providing new insight into the plant response to salinity and is identifying genetic determinants that effect salt tolerance. Recent confirmation that many salt tolerance determinants are ubiquitous in plants has led to the use of genetic models, like *Arabidopsis thaliana*, to further dissect the plant salt stress response. Since many of the most fundamental salt tolerance determinants are those that mediate cellular ion homeostasis, this review will focus primarily on the functional essentiality of ion homeostasis mechanisms in plant salt tolerance. The transport systems that facilitate cellular capacity to utilize Na<sup>+</sup> for osmotic adjustment and growth and the role of the Salt-Overly-Sensitive (SOS) signal transduction pathway in the regulation of ion homeostasis and salt tolerance will be particularly emphasized. A perspective will be presented that integrates cellular based stress signaling and ion homeostasis mechanisms into a functional paradigm for whole plants and defines biotechnology strategies for enhancing salt tolerance of crops.

*Keywords: Salt adaptation, ion homeostasis, transport determinants, stress signaling*

## Introduction

Soil salinity is a major constraint to food production because it limits crop yield and restricts use of land previously uncultivated. The United Nations Environment Program estimates that approximately 20% of agricultural land and 50% of cropland in the world is salt-stressed (Flowers and Yeo, 1995). Natural boundaries imposed by soil salinity also limit the caloric and the nutritional potential of agricultural production. These constraints are most acute in areas of the world where food distribution is problematic because of insufficient infrastructure or political instability. Water and soil management practices have facilitated agricultural production on soils marginalized by salinity but additional gain by these approaches seems problematic. On the horizon are crop improvement strategies that are based on the use of molecular marker techniques and biotechnology, and can be used in

conjunction with traditional breeding efforts (Ribaut and Hoisington, 1998). DNA markers should enhance the recovery rate of the isogenic recurrent genome after hybridisation and facilitate the introgression of quantitative trait loci necessary to increase stress tolerance. Molecular marker techniques were used successfully to transfer alleles of interest from wild relatives into commercial cultivars (Tanksley and McCouch, 1997).

The basic resources for biotechnology are genetic determinants of salt tolerance and yield stability. Implementation of biotechnology strategies to achieve this goal requires that substantial research effort be focused to on identify salt tolerance effectors and the regulatory components that control these during the stress episode (Hasegawa et al., 2000b). Further knowledge obtained about these stress tolerance determinants will be additional resource information for the dissection of the plant response to salinity, which

will reveal how plants sense salt stress, transduce signals to mediate a defensive response and define the signal pathway outputs or effectors that accomplish the processes required for stress survival and alleviation, and steady-state growth in the saline environment. Molecular genetic and plant transformation advances have made it feasible to assess biotechnological strategies based on activated signal cascades, engineered biosynthetic pathways, targeted gene or protein expression or alteration of the natural stress responsiveness of genes for development of salt tolerant crops (Hasegawa et al., 2000b; Zhu, 2001).

The molecular identities of key ion transport systems that are fundamental to plant salt tolerance are now known (Hasegawa et al., 2000b). More recently, the SOS salt stress signalling pathway was determined to have a pivotal regulatory function in salt tolerance, fundamental of which is the control of ion homeostasis (Hasegawa et al., 2000b; Sanders, 2000; Zhu, 2000). This review will summarize research on plant ion homeostasis in saline environments and present a model that integrates current understanding of salt stress sensing, which leads to the activation of the SOS pathway and the regulation of ion transport systems that facilitate ion homeostasis.

### **Genetic Diversity for Salt Tolerance in Plants**

The extensive genetic diversity for salt tolerance that exists in plant taxa is distributed over numerous genera (Flowers et al., 1986; Greenway and Munns, 1980). Most crops are salt sensitive or hypersensitive plants (glycophytes) in contrast to halophytes, which are native flora of saline environments. Some halophytes have the capacity to accommodate extreme salinity because of very special anatomical and morphological adaptations or avoidance mechanisms (Flowers et al., 1986). However, these are rather unique characteristics for which the genes are not likely to be introgressed easily into crop plants.

Research of recent decades has established that most halophytes and glycophytes tolerate salinity by rather similar strategies often using analogous tactical processes (Hasegawa et al., 2000b). The cytotoxic ions in saline environments, typically  $\text{Na}^+$  and  $\text{Cl}^-$ , are compartmentalized into the vacuole and used as osmotic solutes (Blumwald et al., 2000; Niu et al., 1995). It follows then that many of the molecular entities that mediate ion homeostasis and salt stress signaling are similar in all plants (Hasegawa et al., 2000b). In the fact, the paradigm for ion homeostasis that facilitates plant salt tolerance resembles that described for yeast (Bressan et al., 1998; Serrano et al., 1999). The fact that cellular ion homeostasis is controlled and effected by common molecular entities made it feasible to use of

model genetic organismal systems for the dissection of the plant salt stress response (Bressan et al., 1998; Serrano et al., 1999; Hasegawa et al., 2000a; Sanders, 2000; Zhu, 2000; 2001). Research on the plant genetic model *Arabidopsis* has increased greatly our understanding of how cellular salt tolerance mechanisms are integrated and coordinated in an organismal context, and are linked to essential phenological adaptations. Since *Arabidopsis* is a glycophyte, a salt tolerant genetic model will be required to delineate if salt tolerance is affected most by form or function of genes or more by differences in the expression of common genes due either to transcriptional or post-transcriptional control (Zhu, 2001).

### **Cellular Mechanisms of Salt Stress Survival, Recovery and Growth**

High salinity causes hyperosmotic stress and ion disequilibrium that produce secondary effects or pathologies (Hasegawa et al., 2000b; Zhu, 2001). Fundamentally, plants cope by either avoiding or tolerating salt stress. That is plants are either dormant during the salt episode or there must be cellular adjust to tolerate the saline environment. Tolerance mechanisms can be categorized as those that function to minimize osmotic stress or ion disequilibrium or alleviate the consequent secondary effects caused by these stresses. The chemical potential of the saline solution initially establishes a water potential imbalance between the apoplast and symplast that leads to turgor decrease, which if severe enough can cause growth reduction (Bohnert et al., 1995). Growth cessation occurs when turgor is reduced below the yield threshold of the cell wall. Cellular dehydration begins when the water potential difference is greater than can be compensated for by turgor loss (Taiz and Zeiger, 1998).

The cellular response to turgor reduction is osmotic adjustment. The cytosolic and organellar machinery of glycophytes and halophytes is equivalently  $\text{Na}^+$  and  $\text{Cl}^-$  sensitive; so osmotic adjustment is achieved in these compartments by accumulation of compatible osmolytes and osmoprotectants (Bohnert, 1995; Bohnert and Jensen, 1996). However,  $\text{Na}^+$  and  $\text{Cl}^-$  are energetically efficient osmolytes for osmotic adjustment and are compartmentalized into the vacuole to minimize cytotoxicity (Blumwald et al., 2000; Niu et al., 1995). Since plant cell growth occurs primarily because of directional expansion mediated by an increase in vacuolar volume, compartmentalization of  $\text{Na}^+$  and  $\text{Cl}^-$  facilitates osmotic adjustment that is essential for cellular development.

Movement of ions into the vacuole might occur directly from the apoplast into the vacuole through

membrane vesiculation or a cytological process that juxtaposes the plasma membrane to the tonoplast (Hasegawa et al. 2000b). Then compartmentalization could be achieved with minimal or no exposure of the cytosol to toxic ions. However, it is not clear presently the extent to which processes like these contribute to vacuolar ion compartmentalization. The bulk of  $\text{Na}^+$  and  $\text{Cl}^-$  movement from the apoplast to the vacuole likely is mediated through ion transport systems located in the plasma membrane and tonoplast. Presumably, tight coordinate regulation of these ion transport systems is required in order to control net influx across the plasma membrane and vacuolar compartmentalization. The SOS signal pathway is a pivotal regulator of, at least some, key transport systems required for ion homeostasis (Hasegawa et al., 2000a; Sanders, 2000; Zhu, 2000).

### Osmolytes and Osmoprotectants

As indicated previously, salt tolerance requires that compatible solutes accumulate in the cytosol and organelles where these function in osmotic adjustment and osmoprotection (Rhodes and Hanson, 1993). Some compatible osmolytes are essential elemental ions, such as  $\text{K}^+$ , but the majority are organic solutes. Compatible solute accumulation as a response to osmotic stress is an ubiquitous process in organisms as diverse as bacteria to plants and animals. However, the solutes that accumulate vary with the organism and even between plant species. A major category of organic osmotic solutes consists of simple sugars (mainly fructose and glucose), sugar alcohols (glycerol and methylated inositols) and complex sugars (trehalose, raffinose and fructans) (Bohnert and Jensen, 1996). Others include quaternary amino acid derivatives (proline, glycine betaine,  $\beta$ -alanine betaine, proline betaine), tertiary amines (1,4,5,6-tetrahydro-2-methyl-4-carboxyl pyrimidine), and sulfonium compounds (choline *o*-sulfate, dimethyl sulfonium propionate) (Nuccio et al., 1999). Many organic osmolytes are presumed to be osmoprotectants, as their levels of accumulation are insufficient to facilitate osmotic adjustment. Glycine betaine preserves thylakoid and plasma membrane integrity after exposure to saline solutions or to freezing or high temperatures (Rhodes and Hanson, 1993). Furthermore, many of the osmoprotectants enhance stress tolerance of plants when expressed as transgene products (Bohnert and Jensen, 1996; Zhu, 2001). An adaptive biochemical function of osmoprotectants is the scavenging of reactive oxygen species that are by-products of hyperosmotic and ionic stresses and cause membrane dysfunction and cell death (Bohnert and Jensen, 1996).

A common feature of compatible solutes is that these

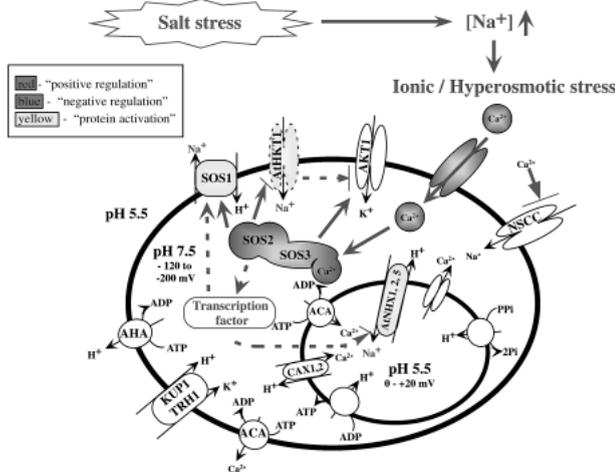
compounds can accumulate to high levels without disturbing intracellular biochemistry (Bohnert and Jensen, 1996). Compatible solutes have the capacity to persevere the activity of enzymes that are in saline solutions. These compounds have minimal effect on pH or charge balance of the cytosol or luminal compartments of organelles. The synthesis of compatible osmolytes is often achieved by diversion of basic intermediary metabolites into unique biochemical reactions. Often, stress triggers this metabolic diversion. For example, higher plants synthesize glycine betaine from choline by two reactions that are catalyzed in sequence by choline monoxygenase (CMO) and betaine aldehyde dehydrogenase (BADH) (Rhodes and Hanson, 1993). Pinitol is synthesized from myo-inositol by the sequential catalysis of inositol-*o*-methyltransferase and ononitol epimerase (Bohnert and Jensen, 1996).

### Ion Homeostasis - Transport Determinants and Their Regulation

Since NaCl is the principal soil salinity stress, a research focus has been the transport systems that are involved in utilization of  $\text{Na}^+$  as an osmotic solute (Blumwald et al., 2000; Hasegawa et al., 2000b; Niu et al., 1995). Research of more than 30 years previously, established that intracellular  $\text{Na}^+$  homeostasis and salt tolerance are modulated by  $\text{Ca}^{2+}$  and high  $[\text{Na}^+]_{\text{ext}}$  negatively affects  $\text{K}^+$  acquisition (Rains and Epstein, 1967).  $\text{Na}^+$  competes with  $\text{K}^+$  for uptake through common transport systems and does this effectively since the  $[\text{Na}^+]_{\text{ext}}$  in saline environments is usually considerably greater than  $[\text{K}^+]_{\text{ext}}$ .  $\text{Ca}^{2+}$  enhances  $\text{K}^+/\text{Na}^+$  selective intracellular accumulation (Maathuis et al., 1996; Rains and Epstein, 1967).

Research of the last decade has defined many of the molecular entities that mediate  $\text{Na}^+$  and  $\text{K}^+$  homeostasis and given insight into the function of  $\text{Ca}^{2+}$  in the regulation of these transport systems. Recently, the SOS stress-signaling pathway was identified to be a pivotal regulator of plant ion homeostasis and salt tolerance (Hasegawa et al., 2000b; Sanders, 2000). This signaling pathway functionally resembles the yeast calcineurin cascade that controls  $\text{Na}^+$  influx and efflux across the plasma membrane (Bressan et al., 1998). Expression of an activated form of calcineurin in yeast or plants enhances salt tolerance further implicating the functional similarity between the calcineurin and the SOS pathways (Mendoza et al., 1996; Pardo et al., 1998). A diagram of the relevant transporters and  $\text{Ca}^{2+}$ -dependent stress signaling pathway involved in  $\text{Na}^+$  homeostasis is shown in Figure 1. Little is known about the mechanistic entities that are responsible for  $\text{Cl}^-$  transport or the regulation of  $\text{Cl}^-$  homeostasis (Hedrich, 1994).

**Salt Stress Signaling that Mediates Na<sup>+</sup> Homeostasis**



**Fig. 1. Salt stress-induced, Ca<sup>2+</sup>-dependent signalling that mediates Na<sup>+</sup> homeostasis and salt tolerance.**

SOS1, plasma membrane Na<sup>+</sup>/H<sup>+</sup> antiporter; SOS2, serine/threonine kinase; SOS3, Ca<sup>2+</sup> binding protein; AHA, plasma membrane H<sup>+</sup>-ATPase; ACA, plasma/tonoplast membrane Ca<sup>2+</sup>-ATPase; KUP1/TRH1, high-affinity K<sup>+</sup>-H<sup>+</sup> co-transporter; AKT1, K<sub>in</sub> channel; NSCC, non selective cation channel; CAX1 or 2, Ca<sup>2+</sup>/H<sup>+</sup> antiporter; NHX1, 2 or 5, endomembrane Na<sup>+</sup>/H<sup>+</sup> antiporter; red - positive regulation, blue - negative regulation, yellow - protein activation

**Ion Transport Systems that Mediate Na<sup>+</sup> Homeostasis**

**H<sup>+</sup>-Pumps.** H<sup>+</sup> pumps in the plasma membrane and tonoplast energize solute transport necessary to compartmentalize cytotoxic ions away from the cytoplasm and to facilitate the function of ions as signal determinants (Maeshima, 2000; Maeshima, 2001; Morsomme and Boutry, 2000; Ratajczak, 2000). That is these pumps provide the driving force (H<sup>+</sup> electrochemical potential) for secondary active transport and function to establish membrane potential gradients that facilitate electrophoretic ion flux (Figure 1). The plasma membrane localized H<sup>+</sup> pump is a P-type ATPase and is primarily responsible for the large (pH and membrane potential gradient across this membrane (Morsomme and Boutry, 2000). A vacuolar type H<sup>+</sup>-ATPase and a vacuolar pyrophosphatase generate the ΔpH and membrane potential across the tonoplast (Drozdowicz and Rea, 2001; Maeshima, 2001). The activity of these H<sup>+</sup> pumps is increased by salt treatment and induced gene expression may account for some of the upregulation (Hasegawa et al., 2000b; Maeshima, 2001).

Recently, the plasma membrane H<sup>+</sup>-ATPase was confirmed as a salt tolerance determinant based on analyses of phenotypes caused by the semi-dominant *aha4-1* mutation (Vitart et al., 2001). The mutation to *AHA4*, which is expressed predominantly in the roots,

causes a reduction in root and shoot growth (relative to wild type) of plants that are grown on medium supplemented with 75 mM NaCl. Decreased root length of salt treated *aha4-1* plants is due to reduce cell length. In NaCl supplemented medium, leaves of *aha4-1* plants accumulate substantially more Na<sup>+</sup> and less K<sup>+</sup> than those of wild type. It is postulated that *AHA4* functions in the control of Na<sup>+</sup> flux across the endodermis (Vitart et al., 2001).

**Na<sup>+</sup> Influx and Efflux Across the Plasma Membrane.**

Recently, much insight has been gained about Na<sup>+</sup> transport systems that are involved in net flux of the cation across the plasma membrane (Amtmann and Sanders, 1999; Blumwald et al., 2000; Hasegawa et al., 2000b). Transport systems with greater selectivity for K<sup>+</sup> are presumed to facilitate Na<sup>+</sup> "leakage" into cells. Specifically, Na<sup>+</sup> is a competitor for uptake through plasma membrane K<sup>+</sup> inward rectifying channels, such as those that are in the Shaker type family, e.g. AKT1 (Schachtman, 2000). K<sup>+</sup> outward rectifying channels also may facilitate Na<sup>+</sup> influx (Schachtman, 2000). The high affinity K transporter (HKT) from wheat and low affinity cation transporter (LCT) also may be responsible for Na<sup>+</sup> influx across the plasma membrane (Schachtman, 2000; Amtmann and Sanders, 1999; Blumwald et al., 2000). Both HKT and LCT transport Na<sup>+</sup> when expressed in heterologous systems, providing evidence of their function in Na<sup>+</sup> uptake. Wheat HKT1 is a Na<sup>+</sup>-H<sup>+</sup>-dependent K<sup>+</sup> transporter. Modifications to HKT1 that enhance K<sup>+</sup> transport also reduce Na<sup>+</sup> influx and enhance salt tolerance further establishing that Na<sup>+</sup> conductance occurs through this protein (Rubio et al., 1999). Physiological data also implicate nonselective cation (NSC) channels in Na<sup>+</sup> influx (Amtmann and Sanders, 1999).

Recently, the properties of HKT proteins from *Arabidopsis* (Kato et al., 2001; Uozumi et al., 2000), rice (Horie et al., 2001) and eucalyptus (Fairbairn et al., 2000) have been characterized. AtHKT1 is the only member of the *Arabidopsis* gene family while both rice and eucalyptus have at least two genes. AtHKT1 expression increases NaCl sensitivity of a yeast strain deleted for the plasma membrane Na<sup>+</sup> efflux system (*ena1-4Δ*) but does not suppress the K<sup>+</sup> deficiency of *trk1 trk2* mutant cells that have attenuated uptake of this essential cation. However, AtHKT1 expression does suppress the K<sup>+</sup> deficient phenotype of an *E. coli* mutant for which acquisition of the cation is disrupted. Electrophysiological data indicate that AtHKT1 expressed in *Xenopus* oocytes specifically transports Na<sup>+</sup> and conductance is K<sup>+</sup>, H<sup>+</sup> and voltage independent (Uozumi et al., 2000).

The *in planta* function of AtHKT1 as an effector of Na<sup>+</sup> influx has been confirmed recently (Rus et al., 2001). T-DNA insertional and deletion mutations of AtHKT1 were identified in a screen for suppressors of

NaCl sensitivity of the *sos3-1* mutant (Liu and Zhu, 1997; Rus et al., 2001). Suppression of *sos3-1* NaCl sensitivity is correlated with reduced cellular accumulation of Na<sup>+</sup> and capacity to maintain [K<sup>+</sup>]<sub>int</sub>. Together, these results establish that AtHKT1 controls Na<sup>+</sup> influx into plants. It is likely that AtHKT1 is a Na<sup>+</sup> influx system but its function as a regulator of Na<sup>+</sup> and K<sup>+</sup> influx systems cannot be precluded. Since the transcript is expressed predominantly in the roots, AtHKT1 most probably functions in the control of Na<sup>+</sup> into the xylem for export to the shoot (Rus et al., 2001; Uozumi et al., 2000).

Rice (*Oryza sativa* L. Indica) OsHKT1 and OsHKT2 were identified based on sequence similarity with wheat HKT (Horie et al., 2001). *OsHKT1* and 2 transcripts accumulate in response to low K<sup>+</sup> but their steady-state abundance is reduced by treatment with 30 mM NaCl. Yeast complementation and *Xenopus* oocyte expression data indicate that OsHKT1 functions as a Na<sup>+</sup> influx system like AtHKT1 but OsHKT2 is a Na<sup>+</sup>/K<sup>+</sup> symporter. The two Eucalyptus camaldulensis HKT1 (EcHKT1 and 2) orthologs have similar transport characteristics as the wheat protein (Fairbairn et al. 2000). Interestingly, activation of these proteins occurs in response to hypotonic treatment, implicating an osmosensing capacity.

The recent identification of *sos3-1 hkt1* double mutations in *Arabidopsis* has confirmed the existence of a Na<sup>+</sup> entry system(s) different than HKT1 that functions *in planta*. Reduction in [Ca]<sub>ext</sub> to μM concentrations abrogates the capacity for an *hkt1* knockout mutation to suppress Na<sup>+</sup> sensitivity of *sos3-1*. These results indicate the presence of a Ca<sup>2+</sup>-inhibited Na<sup>+</sup> influx system. For the last several years, physiological research has indicated the presence of Ca<sup>2+</sup> insensitive and sensitive Na<sup>+</sup> conductance in analysis of plant cell patches (Amtmann and Sanders, 1999). The Ca<sup>2+</sup> sensitive component of Na<sup>+</sup> uptake recently has been attributed to NSC channels (Davenport and Tester, 2000). The combined electrophysiological and *in planta* mutant dissection data indicate that there are least two Na<sup>+</sup> influx systems, one whose activity is directly inhibited by Ca<sup>2+</sup> and the other HKT, for which genetic evidence indicates may be regulated negatively by the SOS signal pathway (Figure 1).

Energy-dependent Na<sup>+</sup> transport across the plasma membrane of plant cells is mediated by the secondary active Na<sup>+</sup>/H<sup>+</sup> antiporter SOS1. Phylogenetically, SOS1 is similar to SOD2 of *Saccharomyces pombe*, NHA1 of *S. cerevisiae* and NhaA and NhaP of *Pseudomonas aeruginosa* (Shi et al., 2000). The SOS1 protein contains a large C-terminal domain (1162 amino acid) that distinguishes it from other members of the phylogenetic family. The SOS1 antiporter is distantly related to the plant endomembrane NHX type of antiporters. *SOS1* gene expression is upregulated by

NaCl stress and this is dependent on other components of the SOS signal pathway (see below).

**Na<sup>+</sup> Vacuolar Compartmentalization.** A Na<sup>+</sup>/H<sup>+</sup> antiporter that is energized by the ΔpH across the tonoplast facilitates vacuolar compartmentalization of the cation. The *Arabidopsis AtNHX1* was isolated by functional genetic complementation of a yeast mutant defected for the endosomal Na<sup>+</sup>/H<sup>+</sup> antiporter yeast (ScNHX1) and has sequence similarity to mammalian NHE transporters (Apse et al., 1999; Gaxiola et al., 1999; Quintero et al., 2000). Transgenic *Arabidopsis* and tomato plants that over express AtNHX1 accumulate abundant quantities of the transporter in the tonoplast and exhibit substantially enhanced salt tolerance (Apse et al., 1999; Quintero et al., 2000; Zhang and Blumwald, 2001). These results implicate the pivotal function of the AtNHX family in vacuolar compartmentalization of Na<sup>+</sup>.

Predicted amino acid sequence and topological similarities to AtNHX1 led to the categorization of six loci in *Arabidopsis* as *AtNHX* gene family members (Yokoi et al., submitted). Phylogenetically, the proteins can be categorized into two subgroups, one containing four (AtNHX1-4) and the other two (AtNHX5 and 6) members. AtNHX2 and AtNHX5 expression suppresses the Na<sup>+</sup>/Li<sup>+</sup> sensitive phenotype of a salt sensitive yeast mutant (*enal-4 nha1 nhx1Δ*) indicating that both AtNHX2 and AtNHX5 are orthologous to yeast ScNHX1 and AtNHX1. AtNHX2 suppresses the Na<sup>+</sup>/Li<sup>+</sup> sensitive phenotype of the yeast mutant to a greater extent than *AtNHX1*. *AtNHX1* and *AtNHX2* are expressed constitutively in shoot and roots. Transcript abundance of *AtNHX1* and *AtNHX2* is induced by hyperosmotic stress (NaCl, sorbitol) and this osmotic response is dependent on the hormone abscisic acid (ABA). NaCl but not ABA induces *AtNHX5* transcript abundance (Yokoi et al., submitted). Steady-state transcript abundance of *AtNHX1*, 2 and 5 is greater in *sos* mutants than wild type Col-0 *gll* indicating that the SOS pathway negatively regulates transcriptional expression of these Na<sup>+</sup>/H<sup>+</sup> antiporters genes. Yeast complementation and expression profiling data indicate that AtNHX2 and 5, like AtNHX1, are functional salt tolerance determinants. A common hyperosmotic stress signal pathways regulates the expression of *AtNHX1* and 2 but a different cascade controls *AtNHX5* expression. Post-transcriptional mechanisms that control AtNHX antiporter activation are still not known.

### Ca<sup>2+</sup> Signaling and the Activation of the Salt Overly Sensitive (SOS) Signal Transduction Pathway

Jian-Kang Zhu and co-workers identified three genetically linked *Arabidopsis* loci (*SOS1*, *SOS2* and *SOS3*), which are components of a stress-signaling

pathway that controls ion homeostasis and salt tolerance (Hasegawa et al. 2000a; Sanders, 2000; Zhu, 2000; 2001). Genetic analysis of  $\text{Na}^+/\text{Li}^+$  sensitivity established that *sos1* is epistatic to *sos2* and *sos3* (Zhu, 2000). These *sos* mutants also exhibit a  $\text{K}^+$  deficient phenotype in medium supplemented with  $\mu\text{M}$   $[\text{K}^+]_{\text{ext}}$  and  $[\text{Ca}^{2+}]_{\text{ext}}$ .  $\text{Na}^+$  and  $\text{K}^+$  deficiency of *sos2* and *sos3* is suppressed with  $\text{mM}$   $[\text{Ca}^{2+}]_{\text{ext}}$  (Zhu et al., 1998). *sos1* exhibits hyperosmotic sensitivity unlike *sos3* and *sos2*. Together, these results indicate that the SOS signaling pathway regulates  $\text{Na}^+$  and  $\text{K}^+$  homeostasis and is  $\text{Ca}^{2+}$  activated.

SOS3 encodes a  $\text{Ca}^{2+}$  binding protein with sequence similarity to the regulatory B subunit of calcineurin (protein phosphatase 2B) and neuronal  $\text{Ca}^{2+}$  sensors (Ishitani et al., 2000; Liu and Zhu, 1998). Interaction of SOS3 with the SOS2 kinase (Liu et al., 2000) and SOS2 activation is  $\text{Ca}^{2+}$  dependent (Halfter et al., 2000). The *in planta* function of SOS3 as a salt tolerance determinant is dependent on  $\text{Ca}^{2+}$  binding and N-myristoylation (Ishitani et al., 2000).

The SOS2 serine/threonine kinase (446 amino acids) has a 267 amino acid N-terminal catalytic domain that is similar in sequence to yeast SNF1 (sucrose nonfermenting) kinase and the mammalian AMPK (AMP-activated protein kinase) (Liu et al., 2000; Zhu, 2000). The kinase activity of SOS2 is essential for its salt tolerance determinant function (Zhu, 2000). The SOS2 C-terminal regulatory domain interacts with the kinase domain to cause autoinhibition. A 21 amino acid motif in the regulatory domain of SOS2 is the site where SOS3 interacts with the kinase and is the autoinhibitory domain of the kinase (Guo et al., 2001). Binding of SOS3 to this motif blocks autoinhibition of SOS2 kinase activity. Deletion of the autoinhibitory domain results in constitutive SOS2 activation, independent of SOS3. Also, a Thr<sup>168</sup> to Asp mutation in the activation loop of the kinase domain constitutively activates SOS2.

Genetic and biochemical evidence indicates that components of the SOS signal pathway function in the hierarchical sequence outlined in Figure 1 (Hasegawa et al., 2000b; Sanders, 2000; Zhu, 2001).  $\text{Ca}^{2+}$  binds to SOS3, which leads to interaction with SOS2 and activation of the kinase. Among the SOS signal pathway outputs are transport systems that facilitate ion homeostasis. The plasma membrane sited  $\text{Na}^+/\text{H}^+$  antiporter SOS1 is controlled by the SOS pathway at the transcriptional and post-transcriptional level (Guo et al., 2001; Zhu, 2001). Recently, functional disruption of *AtHKT1* was shown to suppress the salt sensitive phenotype of *sos3-1*, indicating that the SOS pathway negatively controls this  $\text{Na}^+$  influx system (Rus et al., 2001). Also, the SOS pathway negatively controls expression of *AtNHX* family members that are implicated as determinants in the salt stress response (Yokoi et al., submitted).

$[\text{Ca}^{2+}]_{\text{ext}}$  enhances salt tolerance and salinity stress elicits a transient  $[\text{Ca}^{2+}]_{\text{cyt}}$  increase, from either an internal or external source, that has been implicated in adaptation (Knight et al., 1997, Lauchli, 1990). Data from recent experiments with yeast has provided insight into  $\text{Ca}^{2+}$  activation of salt stress signaling that controls ion homeostasis and tolerance (Matsumoto et al., 2001). The hyperosmotic component of high salinity induces a short duration (1 min) rise in  $[\text{Ca}^{2+}]_{\text{cyt}}$  that is due substantially to influx across the plasma membrane through the Cch1p and Mid1p  $\text{Ca}^{2+}$  transport system. The transient increase in  $[\text{Ca}^{2+}]_{\text{cyt}}$  activates the PP2B phosphatase calcineurin (a key intermediate in salt stress signaling controlling ion homeostasis) leading to the transcription of *ENA1*, which encodes the P-type ATPase that is primarily responsible for  $\text{Na}^+$  efflux across the plasma membrane (Nakamura et al., 1993; Mendoza et al., 1994; Matsumoto et al., submitted). The model proposes that the hyperosmotically-induced localized  $[\text{Ca}^{2+}]_{\text{cyt}}$  transient activates calmodulin that is tethered to Cch1p-Midp (Elhers et al., 1999; Sanders et al., 1999; Matsumoto et al., submitted). Calmodulin in turn activates signaling through the calcineurin pathway, which mediates ion homeostasis and salt tolerance (Matsumoto et al., submitted). From these results, a paradigm for salt-induced  $\text{Ca}^{2+}$  signaling and the activation of the SOS pathway can be suggested (Figure 1). Components of the SOS pathway, either SOS3 or upstream elements, might be associated with an osmotically responsive channel through which  $\text{Ca}^{2+}$  influx could initiate signaling through the pathway.

It is notable that a new elevated  $[\text{Ca}^{2+}]_{\text{cyt}}$  steady state is established in yeast cells, that are maintained in medium supplemented with NaCl, after the hyperosmotic induction of the short duration  $[\text{Ca}^{2+}]_{\text{cyt}}$  transient (Matsumoto et al., submitted). It is likely that the newly established  $[\text{Ca}^{2+}]_{\text{cyt}}$  contributes to cellular capacity for growth in salinity. The vacuolar membrane  $\text{H}^+/\text{Ca}^{2+}$  antiporter Vcx1p and endomembrane localized  $\text{Ca}^{2+}$ -ATPases are pivotal effectors that regulate the amplitude and duration of the  $[\text{Ca}^{2+}]_{\text{cyt}}$  transient (Miseta et al., 1999). The  $[\text{Ca}^{2+}]_{\text{cyt}}$  steady state established in salt containing medium presumably also involves coordination of channel activation that facilitates influx from external and internal sources and energy dependent transport systems that compartmentalize the divalent cation. It is reasonable to assume that the salt induced  $[\text{Ca}^{2+}]_{\text{cyt}}$  transient detected in plant cells (Knight, 1996) and, perhaps, a new  $[\text{Ca}^{2+}]_{\text{cyt}}$  steady-state are controlled by the ECA and ACA  $\text{Ca}^{2+}$ -ATPases and CAX1 and 2 transporters which are orthologs of Vcx1p (Sze et al., 2000). Nevertheless,  $\text{Ca}^{2+}$  has at least two roles in salt tolerance, a pivotal signaling function in the salt stress response leading to adaptation and a direct inhibitory effect on a  $\text{Na}^+$  entry system.

## Perspectives

Database analysis indicates that there are at least seven additional SOS2 isoforms (PKS:Protein Kinase S) and six SOS3 isoforms (SCaBPs:SOS3-like Calcium Binding Proteins) of SOS3. Whether these isoforms also are salt tolerance determinants has yet to be elucidated. One can speculate that these proteins have similar signalling intermediate functions as the prototype proteins but in different cell types or at unique stages of development. Perhaps these isoforms are constituents of signal pathways that respond to different inducers but are still components of the plant response to salt stress. Notwithstanding, it is likely that these proteins have both unique and overlapping functions. It is plausible also that some of these isoforms act as negative regulators of SOS signal transduction by physical interaction with the positive effector or competition for substrates required for signalling. Such positive and negative regulation of signal modulation may constitute a "fine tuning" necessary to achieve the appropriate plant response for stress adaptation and yield stability. Further insight regarding these suggestions may establish how the plant salt stress response is coordinated through gene families. The control system probably is even more complicated since other SOS signal pathway intermediates and outputs and other signalling cascades necessary for salt tolerance may exist.

Recent progress in the elucidation of salt stress signalling and effector output determinants that mediate ion homeostasis has uncovered some potential biotechnology tactics that may be used to obtain salt tolerant crop plants, i.e. enhance the yield stability under salinity. Two basic strategies are feasible; regulate the salt stress signal pathway that controls tolerance effectors or modulate effector activity or efficacy. The recent demonstration that a constitutively activated SOS2 kinase can be achieved by deletion of the auto inhibitory domain or by site-specific modifications to the catalytic domain of the protein kinase (Guo et al., 2001) offers an approach to regulate stress signaling that controls ion homeostasis. Constitutive activation of yeast calcineurin in the host or in plants increases salt tolerance by predisposing the plants to survive the stress episode (Mendoza et al., 1996; Pardo et al., 1998). Furthermore, overexpression of AtNHX1 enhances plant salt tolerance, presumably by increasing vacuolar Na<sup>+</sup> compartmentalization that minimizes the toxic accumulation of the ion in the cytosol and facilitates growth in the saline environment (Apse et al., 1999; Zhang and Blumwald, 2001). Perhaps regulating net Na<sup>+</sup> influx across the plasma membrane would enhance salt tolerance efficacy achieved by overexpressing the vacuolar antiporter. Control of net Na<sup>+</sup> flux across the plasma membrane should be achieved by modulating the expression or

activation of SOS1 (Na<sup>+</sup> efflux) and/or HKT1 (Na<sup>+</sup> influx). Or, by expressing more efficacious forms of the Na<sup>+</sup> transport proteins. For example, mutant variant forms of HKT1 transport more K<sup>+</sup> at the expense of Na<sup>+</sup> and render greater salt tolerance (Rubio et al., 1999). Promoters that direct tissue and inducer specific regulation of the target genes can condition the expression of the signal intermediates and the effectors. Thus regulation of the numerous salt tolerance determinants can be coordinated for an effective plant response but many of the costs associated with salt tolerance in nature might be minimized because some essential evolutionary necessities can be compensated for by agricultural practices.

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