



## Salt Tolerance

Authors: Xiong, Liming, and Zhu, Jian-Kang

Source: The Arabidopsis Book, 2002(1)

Published By: The American Society of Plant Biologists

URL: <https://doi.org/10.1199/tab.0048>

---

BioOne Complete ([complete.BioOne.org](https://complete.BioOne.org)) is a full-text database of 200 subscribed and open-access titles in the biological, ecological, and environmental sciences published by nonprofit societies, associations, museums, institutions, and presses.

Your use of this PDF, the BioOne Complete website, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at [www.bioone.org/terms-of-use](https://www.bioone.org/terms-of-use).

Usage of BioOne Complete content is strictly limited to personal, educational, and non - commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

---

BioOne sees sustainable scholarly publishing as an inherently collaborative enterprise connecting authors, nonprofit publishers, academic institutions, research libraries, and research funders in the common goal of maximizing access to critical research.

First published on September 30, 2002: e0048. doi: 10.1199/tab.0048

# Salt Tolerance

Liming Xiong and Jian-Kang Zhu<sup>a</sup>

Department of Plant Sciences, University of Arizona, Tucson, AZ 85721, Tel. (520) 626-2229, Fax (520) 621-7186, E-mail: jkzhu@ag.arizona.edu

<sup>a</sup> Corresponding author

## Abstract

Studying salt stress is an important means to the understanding of plant ion homeostasis and osmo-balance. Salt stress research also benefits agriculture because soil salinity significantly limits plant productivity on agricultural lands. Decades of physiological and molecular studies have generated a large body of literature regarding potential salt tolerance determinants. Recent advances in applying molecular genetic analysis and genomics tools in the model plant *Arabidopsis thaliana* are shedding light on the molecular nature of salt tolerance effectors and regulatory pathways.

## Introduction

Plants need essential mineral nutrients to grow and develop. However, excessive soluble salts in the soil are harmful to most plants. In fact, no toxic substance restricts plant growth more than does salt on a world scale. It is estimated that salinity affects at least 20% of world's arable land and more than 40% of irrigated land to various degrees (Rhoades and Loveday, 1990). In extreme cases, productive agricultural land could no longer sustain agricultural production and had to be abandoned. This may have contributed to the decline of some human civilizations in history. In coastal areas periodic invasions of seawater directly add salts to the soil. Soils in other semi-arid or arid regions, especially those with ineffective drainage, accumulate salts as irrigation water evaporates, leaving deposits of soluble salts.

Based on their capacity to grow on high salt medium, plants are traditionally classified as glycophytes or halophytes (Flowers et al., 1977). Halophytes are tolerant to high concentrations of NaCl; some can withstand salts that are more than twice the concentration of seawater. Most plants, including the majority of crop species, are glycophytes and cannot tolerate high salinity. For glycophytes, salinity imposes ionic stress, osmotic stress, and secondary stresses such as nutritional disorders and oxidative stress (Zhu, 2001a). Sodium toxicity represents

the major ionic stress associated with high salinity. Additionally, some plant species are also sensitive to chloride, the major anion found in saline soils. In certain saline soils, ion toxicity is worsened by alkaline pH. The low osmotic potential of saline solutions hampers plant water uptake, resulting in "physiological drought." For halophytic plants that are tolerant of sodium toxicity, osmotic stress may be the main cause of growth inhibition.

Understanding the mechanisms of plant salt tolerance will lead to effective means to breed or genetically engineer salt tolerant crops. Salt tolerance research also represents an important part of basic plant biology, contributing to our understanding of subjects ranging from gene regulation and signal transduction to ion transport, osmoregulation and mineral nutrition. Additionally, some aspects of salt stress responses are intimately related to drought and cold stress responses (Zhu, 2001b). Plant salt tolerance studies thus contribute to understanding mechanisms of cross-tolerance of abiotic stresses.

Salt stress studies generally fall into the following four categories: 1) Physiology of salt toxicity and salt tolerance. This includes cellular and metabolic responses to salt (Bohnert and Sheveleva, 1998; Hasagawa et al., 2000), as well as whole plant responses (Flowers et al., 1997; Greenway and Munns, 1980; Yeo, 1998). 2) Mechanisms

of salt transport across cellular membranes and over long distances in plants. This includes physiological and molecular characterization of various ion transporters involved in salt uptake, extrusion, compartmentalization, and in the control of long distance transport (Blumwald et al, 2000; Schachtman and Liu, 1999). 3) Survey of genes whose expression is regulated by salt stress (Zhu et al., 1997; Xiong and Zhu, 2001; Shinozaki and Yamaguchi-Shinozaki, 1997; Ingram and Bartels, 1996; Bray, 1997; Bohnert et al., 1995). More recently, research in this aspect is being accelerated by using gene chips and cDNA microarrays (Seki et al., 2001; Kawasaki et al., 2001; Bohnert et al., 2001). 4) Mutational analysis of salt tolerance determinants and salt stress signaling (Zhu, 2000; 2001a, b; Xiong and Zhu, 2001). This overview will emphasize recent progress in the genetic analysis of salt tolerance in Arabidopsis.

### Salt Stress and Arabidopsis

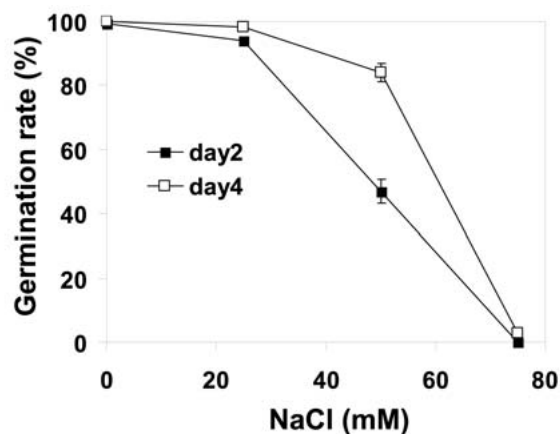
Arabidopsis is a glycophytic plant that is sensitive to growth inhibition and damage by salt stress. Although salt sensitivity among Arabidopsis ecotypes/accessions may exist, a systematic comparison among different ecotypes has not been reported. Given that the Arabidopsis genome has been fully sequenced, this comparison between ecotypes may localize salt tolerance QTLs (quantitative trait loci) and potentially lead to the molecular identification of some major loci, if relatively large differences in salt tolerance exist between ecotypes. Since Arabidopsis is a relatively new model for salt tolerance studies, and is not an ideal subject for physiological analysis, information on salt tolerance in Arabidopsis is rather limited. Research with other glycophytic plant species has shown that upon exposure to high salinity, plants may exhibit a reduced growth rate, accelerated development and senescence or death if the stress is severe or prolonged (reviewed by Lazof and Bernstein, 1999). Like many other glycophytes, the sensitivity of Arabidopsis to salt stress is exhibited at all stages of development. For example, a brief (8 hr) treatment with 150 mM NaCl during seed development stage resulted in callose deposition and abnormal changes in ovule and embryo structures indicative of cell death (Sun and Hauser, 2001). However, in Arabidopsis salt sensitivity is most evident at the seed germination and seedling stages. Even after cold stratification of imbibed seeds to break dormancy, Arabidopsis seed germination was greatly impaired at salt concentrations at or above 75 mM (Figure 1). After germination, seedling growth is also very sensitive to NaCl. Although lower concentrations of NaCl

(< 50 mM NaCl) may have a slightly stimulating effect on fresh weight gain in culture media, higher than 50 mM of NaCl clearly inhibits plant growth (Figure 2) and eventually kills the plants.

### Arabidopsis As A Model Plant To Study Salt Tolerance

With their high levels of salt tolerance, halophytes would appear to be the plants of choice to search for tolerance genes and to study salt tolerance mechanisms. A number of research groups have been studying various halophytes in an effort to clone the genes responsible for salt tolerance (Bohnert et al., 1995). However, because of the complexity of salt tolerance mechanisms and the lack of a well-defined genetic system, the potential of halophytes in revealing salt tolerance mechanisms largely remains to be realized.

Because Arabidopsis is a glycophyte and is very sensitive to salt, one might assume that this plant is not suitable for studying the mechanisms of salt tolerance. However, previous studies with cultured glycophytic plant cells indicated that these cells could be adapted to tolerate high concentrations of salt that would kill un-adapted cells. For instance, normal tobacco cells cannot grow in the pres-



**Figure 1.** Germination of Arabidopsis seeds is sensitive to NaCl. Seeds of the ecotype C24 (one of the salt sensitive ecotypes) were planted on filter paper saturated with indicated concentrations of NaCl and incubated at 4°C for 2 days before being moved to room temperature (23°C) under white light for germination. Germination (radical emergence) was scored 2 (closed squares) or 4 (open squares) days after the plates being incubated at room temperature.

ence of 100 mM NaCl. After salt adaptation, some tobacco cells are able to tolerate five times that much salt (Hasegawa et al., 1994). Similarly, alfalfa cells as well as intact plants have been adapted to tolerate very high levels of NaCl (Winicov, 1991). In fact, like cold acclimation, whole plants can also become salt tolerant by being exposed to lower non-lethal concentrations of salt (e.g., Amzallag et al., 1990). Although salt adaptation process did not receive as much attention as cold acclimation did, presumably because of technical difficulties in quantitatively implementing the 'salt acclimation' treatments. Nevertheless, the fact that adaptation can increase plant salt tolerance suggests that glycophytes do have salt tolerance machinery that may not be operating effectively in un-adapted conditions. Therefore, the difference in salt tolerance between glycophytes and halophytes appears to be quantitative rather than qualitative, and basic salt tolerance mechanisms are probably conserved in all plant species. The difference in salt sensitivity/tolerance may have resulted from differences in regulatory circuits or from gene alleles coding for key salt tolerance effectors. For example, the vacuolar  $\text{Na}^+/\text{H}^+$  antiporter gene *AtNHX1* is not as highly inducible in *Arabidopsis* as its homologous gene is in halophytes, and high level *AtNHX1* expression driven by the strong CaMV 35S promoter could significantly improve *Arabidopsis* salt tolerance (Apse et al., 1999; Hamada et al., 2001; Shi and Zhu, 2002).

The success of *Arabidopsis* as a genetic model plant for developmental and disease resistance studies has

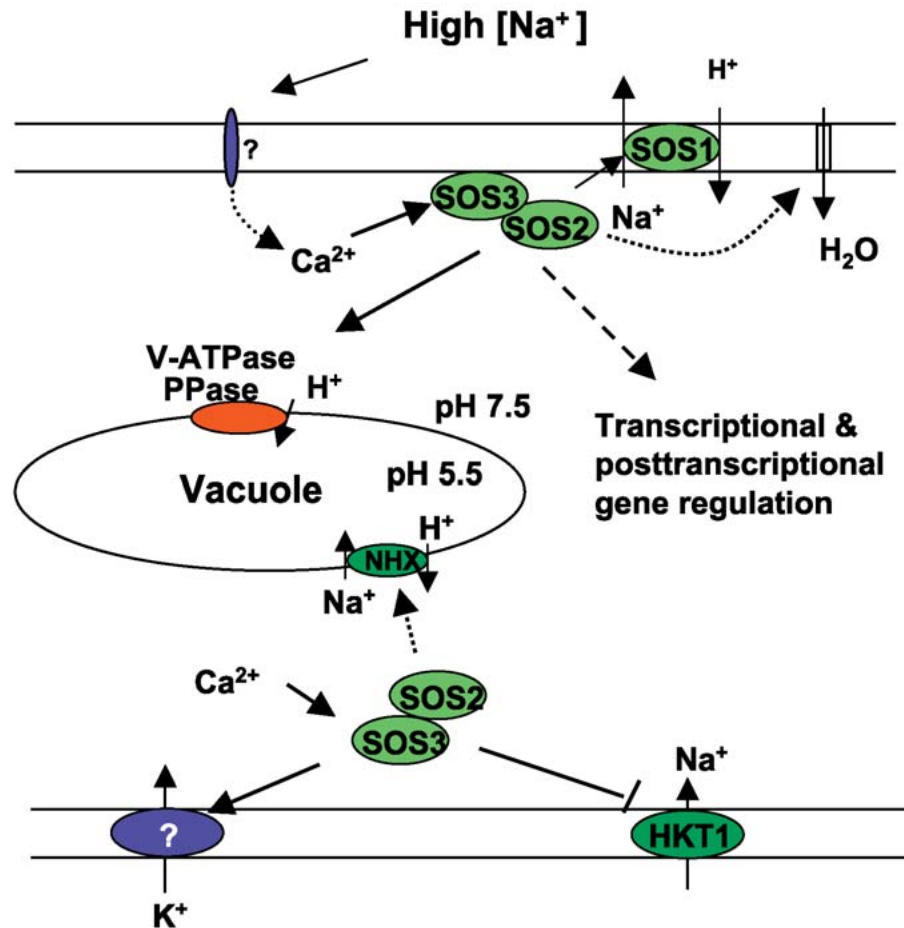
attracted investigators to test its feasibility as a model to study plant salt tolerance. Screening for mutants that are tolerant to high concentrations of salt during germination represented the first such attempt. The germination salt tolerant mutants were named as *rs* (Saleki et al., 1993), *rss* (Werner and Finkelstein, 1995), or *san* (Quesada et al., 2000). Other mutant screens used the inhibitory effect of salts on root growth (*sos* mutants) (Zhu, 2000) or general seedling growth (e.g., Tsugane et al., 1999). The identification of *Salt Overly Sensitive* (SOS) loci has uncovered an important regulatory pathway that controls ion homeostasis and salt tolerance (Zhu, 2000). More sophisticated genetic screens such as the one using a firefly luciferase reporter driven by a salt stress-responsive promoter have been successful in identifying salt tolerance determinants that are difficult to isolate from growth inhibition-based screens (Ishitani et al., 1997). It is expected that the use of *Arabidopsis* as a model will continue to unravel the salt tolerance mechanisms in higher plants (Sander, 2000; Zhu, 2000).

### Mechanisms of Salt Entry into Root Cells

Sodium ions are not required for the growth of most land plants. Land plants do not seem to have transport systems specifically for  $\text{Na}^+$  uptake. However,  $\text{Na}^+$  can still enter plant cells via several routes. Since the concentration of  $\text{Na}^+$  in the soil solution is usually much higher than that in the cytosol of root cells,  $\text{Na}^+$  movement into root cells is passive. Current evidence suggests that  $\text{Na}^+$  enters root cells mainly through various cation channels. These channels could be voltage-dependent cation channels or voltage-independent cation channels (VIC). Among them, VIC channels are considered the major route for  $\text{Na}^+$  entry into plant cells (Amtmann and Sanders, 1999; Schachtman and Liu, 1999; Tyerman and Skerrett, 1999; White, 1999). Whereas the molecular identities of the VIC type non-selective cation channels are not known, those of voltage-dependent ion channels that facilitate  $\text{Na}^+$  entry have been understood to some extent. Due to the similarity between  $\text{Na}^+$  and  $\text{K}^+$ , voltage-dependent  $\text{K}^+$  inward rectifiers or outward rectifiers appear to be one path for  $\text{Na}^+$  entry into root cells (reviewed in Blumwald et al., 2000). For example, in wheat roots, the  $\text{K}^+$  transporter HKT1 may function as a low affinity  $\text{Na}^+$  transporter at high external  $\text{Na}^+$  (Rubio et al., 1995). The homolog in *Arabidopsis*, AtHKT1, mediated  $\text{Na}^+$  uptake when expressed in *Saccharomyces cerevisiae* or *Xenopus* oocytes (Uozumi et al., 2000). However, the function of AtHKT1 *in planta* is unclear. In a screen for second site suppressor mutations



**Figure 2.** Sensitivity of *Arabidopsis* plants to salt during the vegetative stage. Two-week-old seedlings (ecotype Columbia) growing in the soil were irrigated with 0, 50, 75, and 100 mM NaCl, respectively. Pictures were taken three weeks (upper panel) and four weeks (low panel) after the treatment. Note anthocyanin accumulation in leaves, the bleaching of leaves, and retarded growth and delayed development of seedlings treated with higher concentrations of the salt.



**Figure 3.** The SOS pathway functions in ion homeostasis under salt stress. High extracellular concentrations of salt elicit a rise in cytosolic  $\text{Ca}^{2+}$  concentrations. The  $\text{Ca}^{2+}$  sensor SOS3 upon the perception of this  $\text{Ca}^{2+}$  signal interacts with and activates the protein kinase SOS2. Activated SOS2 then regulates the activities of ion transporters or transcriptional activators to regulate ion homeostasis or gene expression. The SOS2 targets include the SOS1  $\text{Na}^+/\text{H}^+$  antiporter, the vacuolar  $\text{Na}^+/\text{H}^+$  exchangers NHX, and the  $\text{Na}^+$  transporter HKT1. Other potential targets include tonoplast ATPase and pyrophosphatases, water channels, and potassium transporters.

of salt sensitive phenotypes of *sos3* (see later sections), one of the suppressors was identified as a lesion in AtHKT1 (Rus et al., 2001). The *hkt1* suppressor mutant has a lower  $\text{Na}^+$  content, implying that AtHKT1 mediates  $\text{Na}^+$  uptake into plants. This study also suggests that wild type SOS3, likely by acting together with SOS2, may repress the activity of AtHKT1 in allowing  $\text{Na}^+$  entry into root cells (Figure 3). It is thus expected that *hkt1* mutations would also suppress the salt sensitive phenotype of *sos2*.

Besides being regulated by the SOS module (see later sections),  $\text{Na}^+$  uptake or exclusion and hence plant salt tol-

erance are also directly or indirectly modulated by many other factors. These factors include membrane potential, acidity of the external solution, variety and concentration of salts, and plant hormones (see below) (reviewed in Xiong and Zhu, 2002a). Here we briefly discuss the effect of one of these factors, membrane potential, on plant salt tolerance.

Under normal conditions, the plasma membrane potential (MP) of root cells is maintained at  $\sim -130$  mV. Apparently, a more negative potential would facilitate entry of the positively charged  $\text{Na}^+$  into cells. MP in plant cells



is generated and maintained by P-type ATPases, which pump  $H^+$  out of the cell and create an electrochemical potential facilitating the uptake of solutes. Some transporters affect salt sensitivity indirectly by altering MP as a result of regulation of ion flux. For example, in yeast *trk1/trk2* mutants that are defective in  $K^+$  uptake, the plasma membrane becomes hyperpolarized and this greatly enhances the uptake of cations and rendered the mutants more sensitive to  $Na^+$ ,  $Li^+$ , and low pH (Serrano et al., 1999). An interesting observation was reported also in yeast where a deletion in the *PMP3* gene resulted in hyperpolarization of the membrane as measured by methylammonium uptake. The yeast mutant was more sensitive to salt stress due to increased uptake of  $Na^+$  (Navarre and Goffeau, 2000). As expected, this effect on uptake was not specific to  $Na^+$ . The *pmp3* mutation also suppressed the increased  $K^+$  requirement in the *trk1* and *trk2* mutants, presumably because membrane hyperpolarization enabled  $K^+$  uptake through other transporters. Interestingly,  $Ca^{2+}$  can reverse the salt sensitivity in the *pmp3* mutant. PMP3 is a small hydrophilic protein predicted to be localized on the plasma membrane. It is not known how this protein can regulate membrane potential. PMP3 is homologous to the plant proteins RCI2A and RCI2B in Arabidopsis (Medina et al., 2001; Nylander et al., 2001) and BLT101 in barley (Goddard et al., 1993). In fact, the Arabidopsis RCI2A can complement the yeast *pmp3/sna1* salt sensitive phenotype (Navarre and Goffeau, 2000; Nylander et al., 2001). Interestingly, the expression of *RCI2A* and *RCI2B* genes in Arabidopsis was transiently induced by low temperature or salt stress (Medina et al., 2001; Nylander et al., 2001). However, Nylander et al. (2001) reported that a mutation in the yeast homologous gene *SNA1*, which is the same gene as *PMP3*, makes the yeast sensitive specifically to NaCl, since the mutant was not sensitive to either 1 M KCl or 0.6 M LiCl (Nylander et al., 2001). It would be interesting to see whether mutations in Arabidopsis *RCI2A* and *RCI2B* also yield similar salt sensitive phenotypes. Future studies on the mechanism of RCI2 function might shed light on salt stress regulation by membrane potentials.

### Dealing with Ion Toxicity

Restoring ion homeostasis in plants disturbed by salt stress represents an acute response, both in terms of time and the importance in dealing with overall salt stress effects. Plants employ several ways to combat ionic stress imposed by high salinity. These include restricting salt uptake, increased extrusion and compartmentalization, and controlled long distance transport to aerial parts.

Additionally, to avoid cellular damage and nutrient deficiency, plant cells need to maintain adequate  $K^+$  nutrition and a favourable  $K^+/Na^+$  ratio in the cytosol (Niu et al., 1995; Serrano et al., 1999).

Excessive sodium ions at the root surface may disrupt plant potassium nutrition that is vital for the maintenance of cell turgor, membrane potential, and the activities of many enzymes (reviewed by Lazof and Bernstein, 1999). Because of the similar physic-chemical properties of sodium and potassium ions, sodium at a high concentration has a strong inhibitory effect on potassium uptake by the root. For example,  $K^+$  uptake via Arabidopsis KUP1 (Kim et al., 1998; Fu and Luan, 1998), which mediates both high and low affinity  $K^+$  transport, was inhibited by 5 mM or higher concentrations of NaCl (Fu and Luan, 1998). Plants use both low and high affinity systems for potassium uptake. Sodium ions may have a more damaging effect on the low affinity system that has low potassium/sodium selectivity. Under sodium stress, it is necessary for plants to operate the more selective high affinity potassium uptake system in order to maintain adequate potassium nutrition. It is a general phenomenon that salt treatment of plants causes a decrease in cellular  $K^+$  content (Figure 4), which may be partly responsible for reduced growth and vigour under salt stress.

Sodium, once enters into the cytoplasm, has a strong inhibitory effect on the activity of many enzymes. This inhibition is also dependent on potassium level in the cytoplasm: a high sodium/potassium ratio is most damaging. Even for halophytes that accumulate large quantities of sodium inside the cell, their cytosolic enzymes are just as sensitive to sodium as enzymes of glycophytes. This implies that halophytes have to compartmentalize sodium into the vacuole, away from cytosolic enzymes, which is precisely what has been found (Flowers et al., 1977).

An important determinant for plant salt tolerance that is particularly relevant to sodium and potassium homeostasis is calcium. It has long been observed both in solution culture and in soils that increased calcium supply has a protective effect on plants under sodium stress. Yet these experiments did not distinguish whether  $Ca^{2+}$  acted extracellularly or intracellularly. Recently, experiments with altered cellular  $Ca^{2+}$  homeostasis imply that internal/cytosolic  $Ca^{2+}$  is important to salt sensitivity regulation. For example, when the Arabidopsis *ACA4* gene that codes for a vacuolar  $Ca^{2+}$ -ATPase was expressed in yeast, it increased the salt tolerance of the yeast cells (Geisler et al., 2001). Arabidopsis vacuolar  $Ca^{2+}/H^+$  antiporter gene *CAX1*, when overexpressed in tobacco, led to increased sensitivity to ionic stress in the transgenics. These transgenic plants appeared to be  $Ca^{2+}$ -deficient despite a higher total  $Ca^{2+}$  content (Hirschi, 1999). Similarly, overexpression of the Arabidopsis ionotropic glu-

tamate receptor GluR2 reduced the utilization efficiency of  $\text{Ca}^{2+}$  and resulted in  $\text{Ca}^{2+}$ -deficient and salt sensitive phenotypes (Kim et al., 2001). The resulting physiological deficiency of  $\text{Ca}^{2+}$  may have been due to an alteration in the threshold requirement for this cation. Since different organelles within the cell may have different tolerance to  $\text{Na}^+$ , it is of interest to know whether the tolerance has to do with  $\text{Ca}^{2+}$  concentration in organelles.

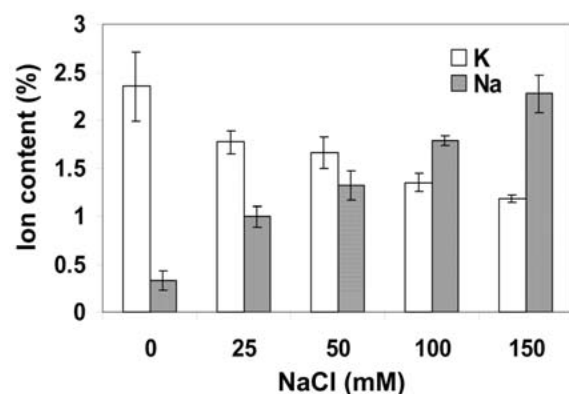
Calcium also sustains potassium transport and potassium/sodium selectivity in sodium-challenged plants. The underlying mechanism for maintaining at least a threshold level of cytosolic potassium in plant cells under sodium stress seems to be similar to that operating in the unicellular yeast *Saccharomyces cerevisiae*. Sodium stress is sensed by an as yet unknown receptor(s). An early detectable response to sodium stress is a rise in the cytosolic free calcium concentration (Knight, 2000). This calcium signal serves as a second messenger that is believed to turn on machineries that control, for example, potassium uptake and potassium/sodium discrimination. In Arabidopsis, genetic studies suggested that the sensor protein for this salt-induced calcium signature is the  $\text{Ca}^{2+}$ -binding protein SOS3. A loss-of-function mutation in this protein renders the plant hypersensitive to salt stress. SOS3 is functionally similar to the B subunit of the protein phosphatase calcineurin (Liu and Zhu, 1998), although SOS3 does not function through a phosphatase (Halfter et al., 2000). In yeast cells, calcineurin upon activation by

calcium modifies potassium transporters (Mendoza et al., 1994). As a result, low affinity potassium transport that cannot sufficiently discriminate potassium from sodium is turned off, while high affinity potassium transport is turned on. High affinity potassium transporters have the ability to take up potassium in the presence of higher concentrations of  $\text{Na}^+$ . Although it has not been demonstrated experimentally, similar processes are believed to occur in plant cells following calcium activation of SOS3. Besides the regulation of potassium transport, the SOS3 pathway in plants or calcineurin pathway in yeast is also important in activating transporters that remove sodium from cells. Such transporters in Arabidopsis include for example, sodium/proton antiporters such as SOS1. Consistent with this, the  $\text{Na}^+$  efflux rate in *sos3* was much slower than that in the wild type (Elphick et al., 2001).

Extrusion of sodium out of the cell is a straightforward way to avoid  $\text{Na}^+$  accumulation in the cytosol. It is expected that sodium extrusion may be more important in certain cells, for example, root epidermal cells. This is because most other cells in the plants are surrounded by neighbouring cells and sodium extruded by one cell would become a problem for its neighbouring cells. Indeed, SOS1 promoter-GUS analysis showed that the plasma membrane  $\text{Na}^+/\text{H}^+$  antiporter gene is preferentially expressed in epidermal cells surrounding the root tip, and in parenchyma cells bordering the xylem throughout the plant (Shi et al., 2002a).

Sodium extrusion is achieved by sodium/proton antiporters on the plasma membrane. In Arabidopsis, this is accomplished by the plasma membrane-localized  $\text{Na}^+/\text{H}^+$  antiporter SOS1 (Shi et al., 2000; 2002a). Mutations in *SOS1* rendered the mutant plants very sensitive to  $\text{Na}^+$  (Wu et al., 1996). Recent studies (Qiu et al., 2002) indicate that plasma membrane vesicles from Arabidopsis plants have a  $\text{Na}^+/\text{H}^+$  antiporter activity. This activity is enhanced by pre-treatment with salt stress. More importantly, the *sos1* mutation reduces the antiporter activity drastically, demonstrating that SOS1 does function as a plasma membrane  $\text{Na}^+/\text{H}^+$  antiporter.

To further explore the role of SOS1 in combating salt stress, the gene was overexpressed in wild type Arabidopsis. It was found that plants overexpressing SOS1 have markedly improved tolerance to salt stress (H. Shi and J.-K. Zhu, unpublished). Analysis of the transgenic plants revealed that these lines had a lower  $\text{Na}^+$  content in the shoot upon treatment with  $\text{Na}^+$  (H. Shi and J.-K. Zhu, unpublished). These results provide evidence that SOS1 as a  $\text{Na}^+$  efflux carrier is vital for plant salt tolerance.



**Figure 4.** Salt stress impairs K nutrition in Arabidopsis. With increased concentration of NaCl in the culture medium,  $\text{Na}^+$  content in plants increases whereas K content decreases. Arabidopsis seedlings (ecotype Columbia) growing in ? strength of the MS solution (pH 5.3) for two weeks were treated with NaCl by adding NaCl into the culture solution to the indicated final salt concentrations. Seedlings were allowed to grow for 3 days before harvesting and analyzing ion contents (dry weight basis). Data are means  $\pm$  SE (n=3).

### Salt Compartmentalization and Long Distance Transport

Another important mechanism to reduce accumulation of cytosolic  $\text{Na}^+$  is to store it in the vacuole where the  $\text{Na}^+$  no longer contacts cytosolic enzymes. Compartmentalization of sodium into the vacuole not only separates  $\text{Na}^+$  from cytosolic enzymes, it also helps balance against the low extracellular osmotic potential created by salt stress. The ability to compartmentalize  $\text{Na}^+$  in the vacuole is likely the principle determinant of salt tolerance capacity in most plant species. Vacuolar compartmentalization of  $\text{Na}^+$  is achieved by the action of  $\text{Na}^+/\text{H}^+$  antiporters on the tonoplast - the vacuolar membrane. The proton gradient that drives the antiporter is generated by tonoplast  $\text{H}^+$ -ATPases and pyrophosphatases (Figure 3). Transgenic Arabidopsis plants overexpressing the vacuolar  $\text{H}^+$ -pyrophosphatase AVP1 showed increased tolerance to salt stress presumably by increasing the sequestration of salt in vacuoles (Gaxiola et al., 2001). Similarly, overexpression of one of the vacuolar  $\text{Na}^+/\text{H}^+$  antiporters, AtNHX1, increases the salt tolerance of the transgenic Arabidopsis plants (Apse et al., 1999).

The ability to restrict  $\text{Na}^+$  transport from the root to the aerial parts is another important determinant of salt tolerance. It was thought that growth inhibition is mainly restricted to the shoot under moderate salt stress (Lazof and Bernstein, 1999). One related process in the aerial parts is to retain  $\text{Na}^+$  in older leaves, which reduces transport to young organs. Amongst several steps that control the long distance transport of  $\text{Na}^+$  are the radial transport of  $\text{Na}^+$  across the root cortical cells and the loading of  $\text{Na}^+$  into the xylem vessel. One component of radial transport of  $\text{Na}^+$  in Arabidopsis root is *SAS1*. Mutations in *SAS1* resulted in several times increased  $\text{Na}^+$  content in aerial parts while the  $\text{Na}^+$  content in roots remained unchanged (Nublat et al., 2001). Cloning of the *SAS1* locus has not been reported. Another component of  $\text{Na}^+$  xylem loading is *SOS1*. In *sos1* mutants,  $\text{Na}^+$  concentration in the xylem sap is higher than that in the wild type, suggesting that *SOS1* controls  $\text{Na}^+$  loading into and/or retrieval from the xylem, in addition to its role in extruding  $\text{Na}^+$  in root epidermal cells (Shi et al., 2002a). *SOS1* is expressed around vascular tissues, consistent with its function in  $\text{Na}^+$  loading/retrieval (Shi et al., 2002a).

### Dealing with Osmotic Stress and Salt Stress Injuries

When plants are challenged with hyperosmolarity, accumulation of ions such as  $\text{Na}^+$  in the vacuoles can serve as a means to lower osmotic potential of the cells, and this process is perhaps cost-effective with regard to the amount of energy and resources spent (Yeo, 1983). A related strategy used to lower the osmotic potential of the cell cytosol is to accumulate compatible osmolytes. For glycophytes, the capacities for  $\text{Na}^+$  compartmentalization and accumulation of osmolytes are both limited. Various compatible osmolytes such as proline, glycine betaine, and polyols can greatly reduce stress damage to plant cells. An increased production of osmolytes is a general phenomenon found in all plants in response to salt stress. This is clearly an adaptive strategy, and transgenic plants with increased osmolyte production or reduced degradation (e.g., Nanjo et al., 1999) show improved salt tolerance. Besides reducing the osmotic potential of the cytosol to facilitate water uptake, many compatible osmolytes have additional functions such as protecting proteins from misfolding and alleviating the toxic effect of reactive oxygen species generated by salt stress (Smirnoff and Cumber, 1989; for reviews see Nuccio et al., 1998; Hong et al., 2000). In fact, the direct protective effect of osmolytes on cellular structures against oxidative stress or protein misfolding may outweigh their role in osmotic adjustment, since their concentration in the transgenic plants overexpressing osmolyte biosynthetic genes is generally very low and thus not very significant for osmotic adjustment (Zhu, 2000).

The beneficial role of compatible osmolytes was suggested by early studies showing that the activities of certain enzymes were less inhibited by excessive salts in the presence of compatible osmolytes (Greenway and Munns, 1980). The major compatible osmolyte in Arabidopsis is proline. One interesting Arabidopsis mutant with altered proline accumulation is *eskimo1* that exhibits increased freezing tolerance (Xin and Browse, 1998). The *eskimo1* mutant has a high content of proline, resulting from both enhanced production and reduced degradation (Xin and Browse, 1998). It was not reported in that study whether this mutant also has enhanced tolerance to salt stress. Nonetheless, the role of osmolytes in plant salt tolerance was supported with experimental evidence that salt tolerance was increased to various extents in transgenic plants engineered to produce new osmolytes absent in the parental lines or in plants that overexpressed the genes whose products limit the production of these osmolytes (Nuccio et al., 1999; Bohnert and Sheveleva, 1998).

As osmolytes have general protective effects, the benefit seen in transgenic plants goes beyond salt stress toler-



ance. For example, expression of a bacterial choline oxidase gene *CodA* in *Arabidopsis* conferred accumulation of glycinebetaine and increased tolerance to cold and salt stress (Hayashi et al., 1997). In another example, Holmstrom et al. (2000) expressed bacterial glycine betaine biosynthesis genes in tobacco, a species that does not accumulate glycine betaine. The transgenic plants accumulated glycine betaine, and were more tolerant to salt stress, to photoinhibition by high light, as well as to low temperature stress. Nevertheless, it should be pointed out that an enhanced production of osmolytes is not the whole story of salt tolerance. As discussed in earlier sections, there are many other determinants that are also important. In fact, it is likely that osmolyte accumulation may initially result from cellular damage (Delauney and Verma, 1993). For example, it was found that there is an increased accumulation of proline in the *sos1* mutant under salt stress, which probably reflects increased cellular damage by salt stress in the mutant (Liu and Zhu, 1997).

Whereas the types of osmolytes that plants produce are plant species-dependent, the magnitude of the accumulation is influenced by other factors including environmental conditions such as salt stress. Although the signal transduction pathways leading to the production of osmolytes are not understood at the moment (Hare et al., 1999), it is possible that these pathways are similar to those operating in yeast, i.e., the HOG1 pathway. Upregulation by salt or drought stress of genes in proline biosynthesis such as P5CS was widely reported (Savoure et al. 1997; Strizhov et al., 1997; Yoshida et al., 1999; Xiong et al., 2001c). In yeast cells, genes that encode glycerol transporters are also down regulated by osmotic stress with an outcome of reduced glycerol loss. In *Arabidopsis*, genes that encode enzymes in proline catabolism are also down regulated by osmotic stress. For example, expression of the proline dehydrogenase gene is up-regulated by proline and down-regulated by dehydration (Kiyosue et al., 1996; Raymond et al., 2000). Additionally, the production of osmolytes is modulated by feedback regulation. For example, the biosynthesis of proline in plants involves a feedback inhibitory regulation. Under salt stress, the P5CS protein, which is responsible for the limiting step in proline biosynthesis, may lose this negative regulation due to conformational changes in the protein. Tobacco plants expressing a mutated form of P5CS protein that disabled this negative regulation accumulated more proline under both stress and unstressed conditions and showed increased salt tolerance (Hong et al., 2000).

## Secondary Stresses

The ionic and osmotic stresses imposed by high salinity on plants may create secondary stresses. These secondary or derived stresses include for example the accumulation of toxic or unwanted compounds, perturbation in cellular metabolism, and nutritional disorders. Among these, oxidative stress is an important constraint for salt tolerance.

Studies implicated that salt stress could generate reactive oxygen species (ROS) in plants (e.g., Burdon et al., 1996; Shen et al., 1997; Tsugane et al., 1999; Hong et al., 2000), which include, for example, hydrogen peroxide, hydroxyl radicals, and superoxide anions. The mechanism responsible for the generation of ROS upon abiotic stress in plants is not clear. In phagocytic cells,  $H_2O_2$  generation is via NADPH oxidase that is triggered by growth factors (Rhee et al., 2000). In plants, NADPH oxidases are likely also responsible for the generation of  $H_2O_2$  in hypersensitive responses (Kawasaki et al., 1999). ROS can have damaging effects on cellular structures and macromolecules such as lipids, enzymes and DNA. Detoxification of these compounds therefore contributes to salt and other stress tolerance. As mentioned above, one major function of compatible osmolytes is to alleviate oxidative stress damage, as suggested by transgenic studies (Reviewed in Bohnert et al., 1998; Nuccio et al., 1999). Thus, transgenic plants overexpressing ROS scavenging enzymes such as superoxide dismutase, catalase, and GST showed somewhat increased tolerance to salt stress and other stresses (reviewed in Bohnert et al., 1998). The role of ROS scavengers in plant salt tolerance is also supported by genetic evidence. For example, a genetic screen for salt tolerant growth in *Arabidopsis* resulted in the isolation of several salt tolerant *pst* mutants. The enhanced salt tolerance in *pst1* mutants is associated with increased ROS detoxification as a result of activation of superoxide dismutase and ascorbate peroxidase (Tsugane et al., 1999).

Salt stress, like some other stresses, may also cause genotoxicity, i.e. DNA damage. In murine kidney cells, it was reported that hyperosmolarity from NaCl causes DNA double strand breaks within 15 min, which is not attributable to apoptosis (Kultz and Chakravarty, 2001). In plants, although direct observation on DNA damage caused by salt stress has not been reported, a connection between DNA damage and salt stress is demonstrated by the isolation of the *Arabidopsis* *uvs66* mutant. This mutant is hypersensitive to UV-C and to the DNA damaging agents methyl methane sulfonate and mitomycin (Albinsky et al., 1999). Interestingly, root growth of the mutant seedlings is also sensitive to NaCl and ABA. The salt sensitivity is specific to NaCl and not to general osmotic stress. This suggests that the genotoxic signal

pathway and salt stress signal pathways may use some common components, for example, the MAPK (mitogen activated protein kinase) module.

Studies in yeast and animal cells have implicated connections between oxidative stress and osmotic stress, both of which use common components of the MAPK module (for review, see Kyriakis and Avruch, 1996; Xiong and Zhu, 2002b). Similar connections were recently also revealed in Arabidopsis. A number of studies showed that several MAPK components are activated or their gene expression induced by salt and other stresses (reviewed in Meskiene and Hirt, 2000; Xiong and Zhu, 2001). The role of MAPK modules in plant tolerance to abiotic stresses was further illustrated by the study where a tobacco MAPK kinase (MAPKK) ANP orthologue, NPK1, was expressed in an active form in Arabidopsis. NPK1 mediates  $H_2O_2$ -regulated gene expression in plants (Kovtun et al., 2000). The engineered Arabidopsis plants showed increased tolerance to freezing, heat shock and salt stress (Kovtun et al., 2000), yet the expression of the stress-responsive gene *RD29A* was not affected. This suggests that in addition to the connections among MAPK modules used by osmotic stress and ROS signaling, there is specificity in the individual pathways (Knight and Knight, 2001). For example, an Arabidopsis mutant defective in a MAPK kinase phosphatase exhibited hypersensitivity to UV-C, but not to salt stress (Ulm et al., 2001).

### Salt Stress Regulation of Gene Expression

The expression of numerous plant genes is regulated by salt stress. Investigations of salt induced transcript changes in fact opened the era of molecular biology of plant salt stress. By studying which genes are regulated and how they are regulated, investigators are most interested in the functions of the gene products in plant stress tolerance. A general assumption has been that stress up-regulated genes may be important for stress tolerance. Early works employed several model plants such as the common ice plant, the resurrection plant, several crop plants, as well as Arabidopsis. These studies have been comprehensively reviewed (Skriver and Mundy, 1990; Ingram and Bartel, 1996; Bray, 1997; Zhu et al., 1997) and readers are referred to these reviews for details. Here we briefly outline the main findings in Arabidopsis and also discuss some recent progress.

Genes that are upregulated by salt stress mainly belong to several groups, based on their possible functionality. These genes encode the LEA proteins, enzymes (involved in the biosynthesis of osmolytes, hormones, detoxifica-

tion, and general metabolism), transporters (ion transporters, ABC transporters, and aquaporins), and regulatory molecules such as transcription factors, protein kinase and phosphatases. The most common and widely reported genes that are stress-regulated are perhaps the *LEAs* or *LEA*-like genes. *LEA* genes encode late embryogenesis abundant proteins (Baker et al., 1988; Dure et al., 1989). These genes are highly expressed in seeds during the desiccation stage following maturation, and in vegetative tissues in response to water deficit. They have been described in many plant species. *LEA*-like genes are also found in bacteria, yeast and animals (Close and Lambers, 1993; Garay-Arroyo et al., 2000; Shen et al., 2001), suggesting that they may represent some common stress-responses since the *LEA*-like genes in these non-plant systems are also induced by osmotic stress (Garay-Arroyo et al., 2000). Despite their wide occurrence, the functions of this group of polypeptides are ill defined except in a few cases where overexpression of individual *LEA* genes resulted in some degree of stress protection (e.g. Xu et al., 1996).

One group of genes in Arabidopsis that are termed *RD* (responsive to dehydration), *COR* (cold-regulated) or *LTI* (low temperature-induced), or *KIN* (cold induced), are strongly induced by salt, drought, low temperature, and ABA. The products of many of these genes may not be structurally related to but share some features with *LEA* proteins and are therefore can be loosely classified as *LEA*-like proteins. These proteins are highly soluble and remain soluble even after being boiled. Because these genes are not expressed under normal (non-stress) growth conditions, they may not be required for normal cellular functions. Since they are strongly expressed following stress treatments such as salt, drought, or low temperature, their products are thought to have some function in protecting cellular structures under stress. One major hypothesis is that these proteins could prevent the denaturation of key proteins by acting as chaperones. The finding that enhanced expression of the transcription factors that regulate the expression of these *LEA*-like genes can increase the tolerance of transgenic plants to cold, drought, or salt stress demonstrates that these proteins do have a protective effect against abiotic stresses (Jaglo-Ottesen et al, 1998; Liu et al., 1998; Kasuga et al., 1999).

Traditional expression profiling approach to search for salt-regulated genes is being replaced with cDNA microarrays or oligo chips. Microarray analysis of gene expression under salt stress was reported for the yeast *S. cerevisiae* (Yale and Bohnert, 2001; Rep et al., 2000; Posas et al., 2000). Microarray has the potential to monitor gene expression profiles on a genome scale, yet current application of this technology in Arabidopsis still falls short of this expectation, partly because only a limited number of cDNAs are available. Seki et al. (2001) examined

Arabidopsis 1,300 genes under drought or cold stresses. Kawasaki et al. (2001) and Bohnert et al. (2001) examined the expression of more genes in rice and Arabidopsis and several other organisms, respectively. The significance of the results generated can only be appreciated with a careful consideration of the experimental conditions and after confirmatory experiments.

With the availability of the microarray technology, the large number of genes that are regulated may at first please the investigators. However, caution should be exercised in interpreting the results. For example, salt stress treatment results in a rapid decline in photosynthesis within minutes (e.g., Kawasaki et al., 2001). Therefore, genes that are regulated by photosynthetic activities may be affected but they are not regulated by salt stress *per se*. In an example, Reymond et al. (2000) studied the expression of 150 genes in response to wounding, pathogen inoculation and drought treatment. It was found that many of the wounding-induced genes are also up-regulated by drought treatment. Therefore, some of these genes may be primarily induced by drought stress instead of the mechanical stimulus intended. The dosage of stress and the time course of gene induction are also important factors to be considered. The primary stresses and secondary stresses may induce genes in different time frames.

### ABA and Other Plant Hormones in Salt Stress Responses

The inhibitory effect of salt stress on plant growth is exhibited at several levels and involves an array of cellular processes. Salt stress affects the expression of cell cycle progression genes (e.g., Burssens et al., 2000) and thus affects cell division. A perturbed turgor may also affect cell expansion and inhibits growth. All these cellular processes may be regulated by an altered hormone homeostasis under salt stress. One well-documented observation is that abscisic acid (ABA) level increases upon salt stress. ABA may have to do with cell cycle regulation (e.g., Wang et al., 1998). However, a major physiological effect of ABA is the stimulation of stomatal closure. As a result, photosynthesis declines (e.g., Kawasaki et al., 2001) and photoinhibition and oxidative stress occur. Another factor that contributes to decreased photosynthesis is the inhibitory effect of salt stress on the efficiency of translocation and assimilation of photosynthetic products; both processes are regulated by plant hormones. Therefore, a perturbed growth, evidenced particularly by inhibition of cell expansion under salt stress, may be controlled to a large extent by hormone home-

ostasis. Accordingly, quite a number of reports have indicated that applications on crops of growth regulators, such as gibberellic acid and cytokinin, had some benefits in alleviating the adverse effects of salt stress.

ABA is involved in many aspects of plant responses to salt stress. Besides inducing the expression of many salt-responsive genes (Rock, 2000; Bray, 1997; Skriver and Mundy, 1990; Ingram and Bartel, 1996; Bray, 1997; Zhu et al., 1997; Chandler and Robertson, 1994), exogenous ABA application was shown in some experiments to increase salt tolerance of the treated plants or plant tissues. ABA may regulate plant salt responses at several levels. ABA synthesized in the root under drought or salt stress may be an endogenous signal that ascends with transpirational flow to regulate shoot growth, stomatal aperture and hence stress tolerance (reviewed in Davies and Zhang, 1991). At the whole plant level, ABA can relieve the growth inhibition caused by decreased water potential ( $\Psi_w$ ) under salt or drought stress. Under these conditions, root activity is very important for coordinating the whole plant to combat stress. Increased endogenous ABA levels were shown to be important to maintain maize primary root elongation at low  $\Psi_w$  (Saab et al., 1990). This effect may have to do with ABA inhibition of ethylene production (Sharp, 2002). Spollen et al. (2000) reported that at a  $\Psi_w$  of  $-1.6$  MPa, inhibition of maize primary root elongation by the ABA synthesis inhibitor fluridone was prevented by applying inhibitors of ethylene biosynthesis or of ethylene signaling. As in ABA deficient mutants, the production of ethylene in fluridone-treated seedlings was increased. Re-application of ABA can reduce ethylene production back to the non-fluridone-treated control levels. Similarly, ABA also stimulates leaf expansion and this is not related to the control of water potentials in the leaf, but instead to the control of ethylene production in the leaf, as was demonstrated with the tomato ABA deficient mutants *flacca* (Sharp et al., 2000). These results convincingly demonstrated that one role of ABA in promoting root and shoot growth at low  $\Psi_w$  is to restrict the production of ethylene, and thereby reduce its inhibitory effect on root elongation and leaf expansion. In Arabidopsis, one interesting observation regarding the relation of ethylene production and root elongation was made with the *sos4* mutants. While root and shoot growth of *sos4* seedlings were more inhibited by salt stress than found in wild type, root growth of *sos4* was more tolerant to mannitol (Shi et al., 2002b). Because SOS4 catalyzes the production of pyridoxal phosphate (Shi et al., 2002b), a co-factor required by ACC (aminocyclopropane-1-carboxylic acid) synthase in ethylene synthesis, *sos4* mutant plants may have a reduced ethylene production relative to the wild type under the osmotic stress imposed by high concentrations of mannitol.

The role of ethylene in inhibiting primary root elongation

may not be the whole story of ethylene in drought or salt stress responses. Root elongation obviously helps plants explore further soil regions which may have untapped water, and is therefore important against severe water shortages. However root hairs may be more relevant to combating less severe or temporary water shortages. Although ethylene inhibits primary root elongation, it also aids root hair differentiation and promotes root hair elongation. Interestingly, the *sos4* mutation diminishes root hair elongation, possibly through diminishing ethylene production. In support of this concept, supplying the ethylene precursor ACC can overcome the short root hair phenotype in this mutant (J.-K. Zhu, unpublished). Further studies on the relationship between root hair elongation, primary root elongation and salt or drought tolerance by using *sos4* may help understand the fine balance between root development and plant drought and salt tolerance.

The interaction between ABA and ethylene in vegetative growth as well as in seed germination was further demonstrated by genetic analysis of ABA signal transduction in Arabidopsis. Characterization of mutants with altered responses to ABA during germination and analysis of ethylene response mutants indicated that ethylene counteracts ABA signaling during seed germination, but it positively regulates ABA action in root inhibition (Beaudoin et al., 2000; Ghassemian et al., 2000; Gazzarrini and McCourt, 2001).

At the molecular level, ABA induces the expression of numerous plant genes (reviewed by Rock, 2000). Some of these genes encode various signal transduction components such as putative receptors, protein kinases/phosphatases and transcription factors that may participate in salt stress signaling; others encode effectors for stress tolerance. These effectors include, for example, H<sup>+</sup>-ATPases, P5CS, and aquaporins (e.g., Kaldenhof et al., 1993; Tyerman et al., 1999). ABA-regulated and salt stress-regulated pathways that lead to the activation of these genes are at least partially separate because the level of gene expression for combined treatment with ABA and salt could be additive (e.g. Chak et al., 2000). Additionally, these two pathways appear to interact at certain points since in some experiments the signaling output from combined treatment with ABA and salt is synergistic relative to individual treatments (Bostock and Quatrano, 1992; Xiong et al., 1999b; Xiong et al., 2001c).

Plant mutants defective in ABA and other hormone production or signal transduction are excellent tools for analyzing the function of the plant hormones in salt stress tolerance. In a genetic study of abiotic stress signal transduction, we recovered a group of mutants that exhibit reduced expression of the luciferase reporter gene under control of the ABA- and salt-stress responsive promoter *RD29A* (Ishitani et al., 1997; Xiong et al., 2001c). Expression of the endogenous *RD29A* and many other

stress-responsive genes are also changed in these mutants. This is particularly evident in the *los5* mutant. In *los5*, the expression of several genes such as *RD29A*, *COR15*, *COR47*, *RD22*, and *P5CS* under salt stress was severely reduced or even completely blocked (Xiong et al., 2001c). Surprisingly, several of these mutants are found to be ABA deficient (Xiong et al., 2001a; Xiong et al., 2002). When ABA was re-introduced, the expression of *RD29A-LUC* was restored to the wild type level, demonstrating that ABA-deficiency is responsible for blocking the expression of these stress responsive genes (Xiong et al., 2001c). Positional cloning of two of mutant loci, *LOS6* and *LOS5*, identified that they are allelic to *ABA DEFICIENT 1 (ABA1)* and *ABA3* respectively (Xiong et al., 2001c; Xiong et al., 2002). These findings suggest that salt induction of these stress-responsive genes is almost completely dependent on ABA, which contradicts with the previous model that osmotic stress signaling uses both ABA-dependent and ABA-independent pathways.

The studies on *los5* and *los6* mutants demonstrated a critical role of ABA in regulating salt-regulated gene expression. However, there has been ambiguity in the relationship between ABA and salt tolerance. Like other ABA-deficient or ABA-insensitive mutants, both *los5* and *los6* are more tolerant to NaCl during germination. This is because salt induces ABA biosynthesis, which contributes to the salt sensitivity of seed germination. In *aba* or *abi* mutants, the biosynthesis or the responses to ABA is defective, and hence salt stress has less inhibition on germination. Genetic screens that attempted to isolate salt tolerant mutants using the seed germination phenotype also support this notion, since some of the salt tolerant mutants are alleles of *abi* mutants (Quesada et al., 2000). The tolerance to salt found in mutants isolated by seed germination screening often does not extend to seedling or adult stages (Saleki et al., 1993; Quesada et al., 2000). Thus salt tolerance must involve some different processes at different stages of plant development.

In the *aba* mutants, salt stress responses are also complicated. Using *los5/aba3* and *los6/aba1* mutants, we tested their responses to salt in vegetative growth. We found little difference in root elongation between *los6/aba1* and the wild type at various concentrations of NaCl. However, root elongation in *los5/aba3* plants was more sensitive to NaCl. With prolonged exposure to high concentrations of salt (e.g. 100 mM NaCl for 3 weeks), *los5/aba3* plants showed a higher sensitivity to NaCl, as indicated by leaf yellowing or eventual death after longer exposure (Xiong et al., 2001c), whereas *los6/aba1* did not differ much from the wild type in appearance. The reason for the difference in salt sensitivity between *los6/aba1* and *los5/aba3* is not very clear, although both mutants have similar ABA contents as measured in bulk leaf samples. One possibility is that the ABA levels thus measured may not be accurate in



reflecting cell or tissue-specific ABA contents, which may be important for salt tolerance. Another possibility is that *los5/aba3* might have other roles unrelated to ABA biosynthesis (Xiong et al., 2001c). These additional roles could be related to the fact that the sulfurylated MoCo, the LOS5/ABA3 catalyzed product, is required both by aldehyde oxidases and xanthine dehydrogenase. It is known that aldehyde oxidases also participate in the synthesis of auxin and that xanthine dehydrogenases function in nitrogen metabolism. Currently, it is not clear whether these enzymatic functions affect salt stress signaling/ tolerance or not. Additionally, it is likely that MoCo synthases may be required for the anchoring of certain receptors to membrane patches.

Like *aba* mutants, Arabidopsis *abi* mutants also showed an increased tolerance to NaCl during seed germination, yet their sensitivity or tolerance to salt stress at vegetative stages was not reported. Our study indicates that the salt responses in root and shoot growth in *abi1* or *abi2* mutants are rather complicated: both sensitivity and tolerance to salt relative to the wild type seedlings were observed, depending on the severity and duration of salt stress and seedling ages (J.-K. Zhu, unpublished).

### Aspects of Salt Stress Signal Transduction in Arabidopsis

We refer to the processes involved in the perception of salt stress and subsequent initiation of physiological responses as salt stress signal transduction. For abiotic stresses such as low temperature, drought, and salinity, the nature of the primary signals that are recognized by plants is unclear. Salt stress may have either an ionic, osmotic, or a mechanical impact on plant cells. One possibility is that all these different signals have their own specific receptors (Xiong and Zhu, 2001). These receptors can either operate independently or cooperatively to initiate downstream signaling events. This multiplicity of signals originated from one stress may account for part of the complexity of the signaling pathway that is ascribed as 'crosstalk' or connectedness (Xiong and Zhu, 2001). For ionic signals, candidate receptors could be various ion transporters that harbor ion-binding sites on either the extracellular and/or intracellular side. Along with the primary ionic and osmotic signals, secondary signals such as stress hormones (ABA and ethylene) and ROS are generated. These secondary signals may act as ligands and bind to cognate receptors. These receptors include the receptor-like protein kinases, two-component sensor kinases, and G-protein coupled receptors (Xiong and Zhu, 2001). Despite this

complexity, three types of signaling pathways are apparent. These are the ionic stress signaling pathway, the osmolyte accumulation pathways, and the stress-responsive LEA-like gene regulation pathways. Here we mainly discuss the ionic stress signal transduction pathway under salt stress.

It has been well documented that salt stress, like many other abiotic stresses, can elicit a transient increase in cytosolic  $\text{Ca}^{2+}$  (for review, see Knight, 2000; Sanders et al., 1999). Higher plants, like yeast, also use  $\text{Ca}^{2+}$  to signal and combat salt stress (Bressan et al., 1998). The source of this  $\text{Ca}^{2+}$  could be extracellular or intracellular, both of which have been demonstrated experimentally (Bush, 1995; Muir and Sander, 1997). For ligand-mediated intracellular  $\text{Ca}^{2+}$  release, the three ligands known in animal cells, i.e., inositol 1,4,5-trisphosphate ( $\text{IP}_3$ ), cyclic ADP-ribose (cADPR), and nicotinic acid adenine dinucleotide phosphate (NAADP) have now been implicated to release  $\text{Ca}^{2+}$  from intracellular stores in plant cells as they do in animal cells (NaVazio et al., 2000; Wu et al., 1997; Allen et al., 1995; Cote and Grain, 1994). Evidence suggests that intracellular  $\text{Ca}^{2+}$  transient upon hyperosmotic stress of plant cells involves inositol 1,4,5- trisphosphate ( $\text{IP}_3$ ) gated  $\text{Ca}^{2+}$  release from internal stores such as endoplasmic reticulum (ER) and vacuoles. It has been shown previously that exogenous  $\text{IP}_3$  was able to release  $\text{Ca}^{2+}$  from vacuolar vesicles or isolated vacuoles (e.g., Schumaker and Sze, 1987) and to mediate transient increases in cytosolic  $\text{Ca}^{2+}$  (e.g., Allen et al., 1995). Both  $\text{IP}_3$  and  $\text{Ca}^{2+}$  were implicated in ABA and environmental stress responses (for review, see Munnik et al., 1998; Sanders et al., 1999). In addition, the transcript levels of several enzymes involved in the generation of  $\text{IP}_3$  are induced by ABA, osmotic stress or cold (Hirayama et al., 1995).  $\text{PIP}_2$  levels as well as  $\text{IP}_3$  levels increased during salt stress of Arabidopsis plants and the time frame for these changes correlates with the transient increase in cytosolic  $\text{Ca}^{2+}$  (DeWald et al., 2001). Microinjection as well as pharmacological experiments showed that increased cytoplasmic  $\text{Ca}^{2+}$  can activate the expression of stress responsive genes such as *RD29A*, *KIN1*, and *cas15* (Monroy and Dhindsa, 1995; Sheen, 1996; Wu et al., 1997). In animal cells, the d forms of phosphatide-specific phospholipase C (PLC), which are structurally similar to the plant PLCs, is activated by G-protein  $\alpha$  subunit ( $\text{G}\alpha$ ), resulting in the cleavage of phosphatidylinositol 4,5-bisphosphate ( $\text{PIP}_2$ ) to produce  $\text{IP}_3$ . Whereas plant G-protein associated receptors are poorly characterized (Xiong and Zhu, 2001), recent identification of Arabidopsis *gpa1* mutant with lesions in the prototypic  $\text{G}\alpha$  gene showing impaired ABA-regulated stomatal aperture (Wang et al., 2001) may provide a clue to the identification of G protein-associated receptors in plants. It has not been reported whether this sole prototypic  $\text{G}\alpha$  in Arabidopsis also mediates salt and other stress signal



transduction.

Although molecular and pharmacological studies have implicated phosphoinositides in many cellular responses in plants, genetic evidence on the involvement of phosphoinositide signaling in abiotic stress signal transduction has been lacking until recently. In a genetic screen using the stress-responsive *RD29A* promoter driven firefly luciferase as a reporter (Ishitani et al., 1997; Xiong et al., 1999a), we isolated a mutant, *fiery1* (*fry1*) that exhibits much enhanced reporter gene expression in response to cold, salt, drought, and ABA (Xiong et al., 2001b). The *FRY1* gene encodes an enzyme with both 3'(2'),5'-bisphosphate nucleotidase and inositol polyphosphate 1-phosphatase activities. This gene was identical to the previously characterized *SAL1* gene that was isolated by its ability to confer increased salt tolerance when expressed in yeast cells (Quintero et al., 1996). Because the *fry1* mutant plants did not show sulfur deficiency symptoms, the bisphosphate nucleotidase activity of *FRY1* in sulfur assimilation appears dispensable. Therefore, it is hypothesized that lack of the inositol polyphosphate 1-phosphatase activity is responsible for the enhanced gene expression in response to stress and ABA treatments. In animal cells, in vitro studies suggest that  $IP_3$  is degraded through either a 5-phosphatase pathway or a 3-kinase pathway (Figure 5) (Majerus, 1992; Berridge and Irvine, 1989). These pathways in plant cells are not yet demonstrated. In a recent study, overexpression of an inositol polyphosphate 5-phosphatase resulted in reduced ABA- and stress-responsive gene expression in transgenic plants (Burnette et al., 2001). Two independent studies reported that feeding plants with [ $^3H$ ]-labeled  $IP_3$  yielded inositol 4,5-bisphosphate as the primary and immediate catabolite (Brearly et al., 1997; Drabak et al., 1991), demonstrating that in these plant cells  $IP_3$  was likely first hydrolyzed through a 1-phosphatase pathway. However, the inositol polyphosphate 1-phosphatase responsible for this early termination of  $IP_3$  signal has not been isolated or characterized. Added to this complexity is the fact that the inositol polyphosphate 1-phosphatase characterized in animal cells is not active against  $IP_3$  (Inhorn et al., 1987; Majerus, 1992). Whereas the *FRY1*/*SAL1* activity against  $Ins(1,4)P_2$  and  $Ins(1,3,4)P_3$  was demonstrated previously (Quintero et al., 1996), its activity on  $IP_3$  was not known. We measured the activity against  $IP_3$  of the *FRY1* recombinant protein and found that it had a measurable although limited activity against  $IP_3$  (~13% of that against  $Ins(1,4)P_2$  or  $Ins(1,3,4)P_3$ ) (Xiong et al., 2001b). The in vivo activity of *FRY1* on  $IP_3$  and its significance in overall metabolism of  $IP_3$  are still unknown. Nevertheless, loss of *FRY1* activities would inevitably slow down  $IP_3$  degradation (Figure 5) and lead to enhanced stress gene expression. To test the impact of *fry1* mutations on  $IP_3$  metabolism, we measured  $IP_3$  levels in *fry1* and wild type plants treated with ABA, and found that

whereas the ABA-induced  $IP_3$  rise was transient in wild type plants, it was higher and more sustained in *fry1* mutant plants (Xiong et al., 2001b).

Whatever the mechanisms involved, transient increases in cytosolic  $Ca^{2+}$  must be coupled with downstream signaling events to mediate stress adaptation. In Arabidopsis salt stress signaling, the  $Ca^{2+}$  signal is perceived by the calcineurin B-like  $Ca^{2+}$  sensor SOS3 (Liu et al., 2000). However, different from the calcineurin B in yeast that acts through activation of a protein phosphatase, SOS3 interacts with and activates the protein kinase SOS2 (Halfter et al., 2000). Thus, SOS3 in some aspects resembles an adaptor or scaffold protein that mediates the interaction of SOS2 with other proteins such as ion transporters. This property of SOS3 is suggested by the requirement of myristoylation for full function in salt tolerance (Ishitani et al., 2000). It appears that SOS3, by anchoring SOS2 to particular plasma membrane patches, may facilitate SOS2 phosphorylation of ion transporters or regulatory partners of transporters. This is further supported by the study that introducing SOS1, SOS2 and SOS3 all together into a yeast mutant deleted of endogenous  $Na^+$  transporters greatly improved salt tolerance of the yeast transformants, whereas expressing SOS1 alone only slightly improved salt tolerance of the yeast cells (Quintero et al., 2002).

To understand the interaction between SOS3 and SOS2, the functional domains of both proteins were analyzed. The regulatory and catalytic domains of SOS2 interact, resulting in autoinhibition of the kinase (Guo et al., 2001) (Figure 6). A 21 amino acid motif (FISL motif, refers to the conserved amino acid residues) in the regulatory domain mediates SOS2 interaction with SOS3. It seems that the interaction between SOS3 and SOS2 results in the release of autoinhibition and provides substrate access to the SOS2 kinase domain (Figure 6) (Guo et al., 2001). Presumably through phosphorylation, the activated SOS2 can modulate the activity of ion transporters such as SOS1 and HKT1 (Figure 3), as discussed before.

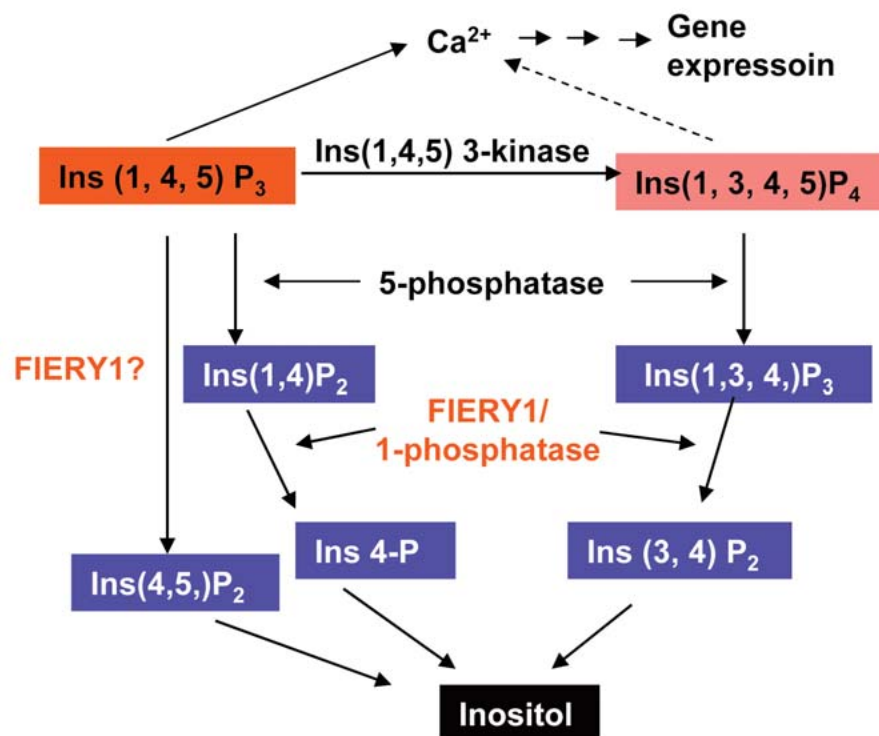
### Strategies for Finding Salt Tolerance Determinants in Arabidopsis

Several approaches are commonly used to identify genes important for plant salt tolerance (Xiong and Zhu, 2001). They can be classified as either biochemical, expression profiling or genetic. The biochemical approach relies on previous knowledge that a particular enzyme or biochemical pathway is important for salt tolerance. These studies are typically supported by transgenic studies where the genes of interest are overexpressed and/or underex-

pressed and the resultant transgenic plant phenotypes analyzed. The gene expression profiling approach has been the most popular one for the last decade and a half. In this approach, gene expression under stress conditions is compared to that in the absence of stress. Genes that show increased expression under stress are cloned by using a variety of differential or subtractive screening techniques. More recently, microarray or gene chip analysis is employed to probe gene expression on larger scales as described in earlier sections. A major limit of the expression profiling approach is that many genes critical for plant salt tolerance are not induced by salt stress. In addition, many of the induced genes may not be important for salt tolerance, e.g., their increased expression under salt stress may be a consequence of stress injury. Eventually, functional analysis through transgenic over-expression or under-expression, or through reverse

genetics to identify knockout mutants is needed to establish whether an induced gene is functionally important for plant salt tolerance.

The genetic approach utilizes natural or induced variations in stress tolerance or stress gene regulation. Gene mutations in Arabidopsis plants can be easily created by using one of several mutagens such as ethylmethane sulfonate, fast neutrons, transposons or T-DNA. Mutations in salt tolerance genes are expected to cause decreased salt tolerance in plants, unless they are functionally redundant. Therefore, one can search for plant mutants that are hypersensitive to salt stress, and these mutants will lead to the identification of salt tolerance genes. The *sos* mutants were isolated in such a screen. Of course, screening for the opposite phenotype (i.e. increased salt tolerance) would identify negative regulators of stress tolerance or gain-of-function mutations. To overcome the shortage of



**Figure 5.** Pathways for the degradation of inositol 1,4,5-trisphosphate (IP<sub>3</sub>). In animal cells, IP<sub>3</sub> can be catabolized by either Ins (1,4,5) 3-kinase or inositol polyphosphate 5-phosphatase (5-phosphatase). The products Ins (1,3,4,5)P<sub>4</sub> and Ins(1,4)P<sub>2</sub> are further dephosphorylated by FIERY1-like inositol polyphosphate 1-phosphatase (1-phosphatase) and finally to inositol. In plants, it is purposed that FIERY1 dephosphorylates both Ins (1,4)P<sub>2</sub> and Ins (1,3,4)P<sub>3</sub> and thus mediates the degradation of IP<sub>3</sub>. Additionally, there may be a 1-phosphatase(s) that can directly dephosphorylate the IP<sub>3</sub> at the 1-position. The in vivo ability of FIERY1 in this direct catabolism of IP<sub>3</sub> is yet to be determined. A higher and sustained level of IP<sub>3</sub> may contribute to a higher level of gene expression.

salt stress-specific visible phenotypes, a stress-responsive promoter driven reporter can be used to facilitate genetic screening based on molecular phenotypes. Our studies have found this promoter-reporter approach to be efficient, and especially powerful in identifying gene mutations that have only subtle visible or tolerance phenotypes (Ishitani et al, 1997; Xiong et al., 1999a). In Table 1, we summarize some of the cloned genetic loci that affect plant sensitivity to salt stress.

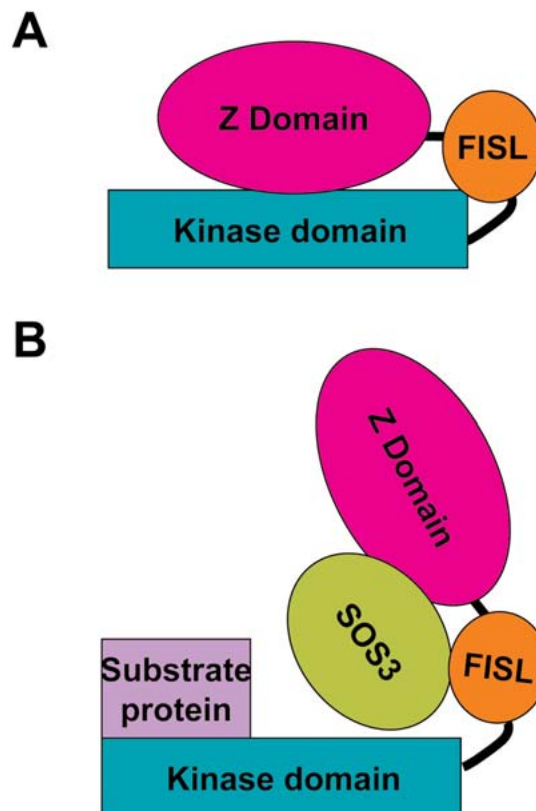
Several other methods such as transgenic plant analysis and yeast complementation can be used to complement mutational studies. RNAi knockout of candidate genes is a new and much more efficient way to suppress the expression of genes of interest, compared to the traditional antisense approach (Chuang and Meyerowitz, 2000; Smith et al., 2000).

Transgenic plants expressing candidate genes under the control of a constitutive promoter (such as CaMV 35S) have been widely used to examine gene function in salt and other stress tolerance. The use of inducible promoters or tissue-specific promoters may avoid the adverse side effects of constitutive or ubiquitous expression. Kasuga et al (1999) found that plants expressing *CBF/DREB* under the stress-responsive *RD29A* promoter were more stress-tolerant but were not compromised in growth, unlike CaMV 35S promoter-driven expression. These studies are exciting in providing leads for the genetic engineering of stress tolerance in crop plants. However, caution should be taken when the overexpression results are used to infer the native function of the endogenous plant genes. For example, overexpression of a transcription factor in a tissue or condition where it is normally not expressed may activate other target genes and thus lead to phenotypes unrelated to its normal function.

It is also worth noting that high expression of stress-responsive genes in some plant mutants does not necessarily mean that the mutants are more stress-tolerant. In our screen of signal transduction mutants using the *RD29A-LUC* reporter, not only mutants with reduced stress gene induction were more sensitive to respective stresses (e.g., *los5*, Xiong et al., 2001c), but also some mutants with a high level of stress gene induction are stress-sensitive. The latter mutants include *hos1* (Ishitani et al., 1998; Lee et al., 2001), *hos2* (Lee et al., 1999), *hos5* (Xiong et al., 1999b), *sad1* (Xiong et al., 2001a), and *fry1* (Xiong et al., 2001b). One explanation is that the stress gene products may in fact provide some protection against stress damage in the wild type background, but in the mutant, their high level of expression may be a compensatory response to reduced stress tolerance as a result of the primary mutation, and thus the enhanced stress gene expression response may be triggered by stress injury. In the mutant backgrounds, the protective effect of the stress gene products simply cannot overcome stress sensitivity

caused by the primary lesions.

Yeast complementation often has been the method of choice in identifying plant genes with similar functions. This approach was very successful in the isolation of plant ion transporters, some of which are related to salt tolerance. Examples of putative plant salt tolerance determinants isolated by yeast complementation are presented in Table 2. Readers are referred to the references for details.



**Figure 6.** Activation of SOS2 protein kinase by SOS3  $\text{Ca}^{2+}$ -binding protein. (A) In the absence of activated SOS3, the kinase activity of SOS2 is inhibited by interaction between the C-terminus and the kinase domain through the FISL motif. (B) Upon binding to  $\text{Ca}^{2+}$ , SOS3 becomes active and then interacts with the FISL motif and releases its inhibition of SOS2 kinase activity. This also provides substrate accessibility to SOS2 kinase domain. Through protein phosphorylation, SOS2 regulates the activity of ion transporters and restore ion homeostasis under salt stress. By myristoylation, SOS3 may also help to recruit SOS2 to specific membrane localization (not shown). The Z domain of SOS2 does not have a defined function yet. Figure is a courtesy of Dr. Yan Guo.

**Table 1.** Selected Arabidopsis genetic loci that affect plant responses to salt stress. Please refer to the text for details.

Locus symbol	Encoded product	GeneBank accession	TAIR accession
<i>HKT1</i>	Na <sup>+</sup> transporter	<a href="#">AF237672</a>	At4g10310
<i>SOS1</i>	Na <sup>+</sup> /H <sup>+</sup> antiporter	<a href="#">AF256224</a>	At2g01980
<i>SOS2</i>	Ser/thr protein kinase	<a href="#">AF237670</a>	At5g35410
<i>SOS3</i>	Ca <sup>2+</sup> sensor	<a href="#">AF060553</a>	At5g24270
<i>SOS4</i>	pyridoxal 5-kinase	<a href="#">AF400125</a>	At5g37850
<i>ABA1/LOS6</i>	zeaxanthin epoxidase	<a href="#">AY093145</a>	At5g67030
<i>ABA3/LOS5</i>	molybdenum cofactor sulfurase	<a href="#">AY034895</a>	At1g16540
<i>ABII</i>	Type 2C protein phosphatase	<a href="#">X77116</a>	At4g26080
<i>ABI2</i>	Type 2C protein phosphatase	<a href="#">Y08965</a>	At5g57050
<i>FRY1</i>	Inositol polyphosphate 1-phosphatase/3'(2')5'-bisphosphate nucleotidase	<a href="#">AY034894</a>	At5g63980
<i>HOS1</i>	novel protein with a RING finger domain	<a href="#">AAB87130</a>	At2g39810
<i>SAD1</i>	Lsm5-like snRNP	<a href="#">AY034896</a>	At5g48870

**Table 2.** Some Arabidopsis genes that compliment salt sensitive phenotypes of yeast mutants or increase yeast salt tolerance when expressed in respective yeast strains.

Genes	Products	References
<i>ACA4</i>	Vacuolar membrane Ca <sup>2+</sup> -ATPase	Geisler et al., 2001
<i>NHX1</i>	Vacuolar Na <sup>+</sup> /H <sup>+</sup> antiporter	Gaxiola et al., 1999
<i>AVP1</i>	Vacuolar H <sup>+</sup> -pyrophosphate	Gaxiola et al., 1999
<i>SAL1</i>	3'5'-bisphosphate nucleotidase and inositol polyphosphate phosphatase	Quintero et al., 1996
<i>STO, STZ</i>	Zinc finger-like transcription factors	Lippuner et al., 1996
<i>SLT1</i>	Unknown protein	Matsumoto et al., 2001
<i>GSK1</i>	GSK2/Shaggy-like kinase	Piao et al., 1999.
<i>CBL1</i>	Calcineurin B-like	Kudla et al., 1999.
<i>HK1</i>	Histidine kinase	Urao et al., 1999.
<i>MEKK1</i>	MAP kinase kinase kinase	Covic and Lew, 1996; Mizoguchi et al., 1996; Covic et al., 1999.
<i>RCI2A</i>	Unknown membrane protein	Nylander et al., 2000.
<i>HAL3</i>	FMN-binding protein	Espinosa-Ruiz et al., 1999



## Salt Tolerance beyond Arabidopsis

What this review on salt tolerance is all about is Arabidopsis, a plant after all very sensitive to salt. With growing knowledge on salt tolerance in Arabidopsis, it is high time to tackle the salt tolerance mechanisms in halophytes. An ideal halophytic model organism has been sought after by many researchers. Commonly used salt tolerant organisms such as the common ice plant *Mesembryanthemum crystallinum*, and the unicellular alga *Dunaliella salina* are not amenable to genetic analysis. One genetically tractable halophyte is *Thellungiella halophila* that is native to the coastal regions of eastern China. This plant can complete its life cycle in the presence of 300 mM NaCl. More importantly, its genome size is small, and its cDNAs share over 90% identity to their counterparts from Arabidopsis (Zhu, 2001). Genetic analysis of *Thellungiella halophila* in comparison with that of Arabidopsis should shed light on how the ionic and osmotic homeostasis and detoxification mechanisms are fine tuned to achieve extreme salt tolerance in halophytic plants.

## Acknowledgements

We would like to thank Dr. Andre Jagendorf for critical reading of the manuscript.

## References

- Albinsky, D., Masson, J.E., Bogucki, A., Afsar, K., Vass, I., Nagy, F., and Paszkowski, J. (1999). Plant responses to genotoxic stress are linked to an ABA/salinity signaling pathway. *Plant J.* **17**, 73-82.
- Allen, G.J., Muir, S.R., and Sanders, D. (1995). Release of  $\text{Ca}^{2+}$  from individual plant vacuoles by both  $\text{InsP}_3$  and cyclic ADP-ribose. *Science* **268**, 735-737.
- Amtmann, A., and Sanders, D. (1999). Mechanisms of  $\text{Na}^+$  uptake by plant cells. *Adv. Bot. Res.* **29**, 76-112.
- Amzallag, G.N., Lemer, H.R., and Poljakoff-Mayber, A. (1990). Induction of increased salt tolerance in *Sorghum bicolor* by NaCl treatment. *J. Exp. Bot.* **41**, 29-34.
- Apse, M.P., Aharon, G.S., Snedden, W.A., and Blumwald, E. (1999). Salt tolerance conferred by overexpression of a vacuolar  $\text{Na}^+/\text{H}^+$  antiporter in Arabidopsis. *Science* **285**, 1256-1258.
- Baker, J., Steele, C., and Dure, L. III. (1988). Sequence and characterization of 6 Lea proteins and their genes from cotton. *Plant Mol. Biol.* **11**, 277-291.
- Beaudoin, N., Serizet, C., Gosti, F., and Giraudat, J. (2000). Interactions between abscisic acid and ethylene signaling cascades. *Plant Cell* **2**, 1103-1015.
- Berridge, M.J., Irvine, R.F. (1989). Inositol phosphates and cell signalling. *Nature* **341**: 197-205.
- Blumwald, E., Aharon, G., and Apse, M.P. (2000). Sodium transport in plant cells. *Biochim. Biophys. Acta* **1465**, 140-151.
- Bostock, R.M., and Quatrano, R.S. (1992). Regulation of *Em* gene expression in rice, interaction between osmotic stress and abscisic acid. *Plant Physiol.* **98**, 1356-1363.
- Bohnert, H.J., Ayoubi, P., Borchert, C., Bressan, R.A., Burnap, R.L., Cushman, J.C., Cushman, M.A., Deyholos, M., Fischer, R., Galbraith, D.W., Hasegawa, P.M., Jenks, M., Kawasaki, S., Koiwa, H., Kore-eda, S., Lee, B.H., Michalowski, C.B., Misawa, E., Nomura, M., Ozturk, N., Postier, B., Prade, R., Song, C.P., Tanaka, Y., Wang, H., and Zhu, J.K. (2001). A genomics approach towards salt stress tolerance. *Plant Physiol. Biochem.* **39**, 295-311.
- Bohnert, H.J., Nelson, D.E., and Jensen, R.G. (1995). Adaptations to environmental stresses. *Plant Cell* **7**, 1099-1111.
- Bohnert, H.J., and Sheveleva, E. (1998). Plant stress adaptations, making metabolism move. *Curr. Opin. Plant Biol.* **1**, 267-274.
- Bohnert, H.J., Su, H., and Shen, B. (1999). Molecular mechanisms of salinity tolerance. In *Molecular responses to cold, drought, heat, and salt stress in higher plants*, Shinozaki, K., and Yamaguchi-Shinozaki, K. (ed.). R.G. Landes Company, Austin, p. 29-62.
- Bray E.A. (1997). Plant responses to water deficit. *Trends in Plant Sci.* **2**, 48-54.
- Brearey, C.A., Parmar, P.N., and Hanke, D.E. (1997). Metabolic evidence for  $\text{PtdIns}(4,5)\text{P}_2$ -directed phospholipase C in permeabilized plant protoplasts. *Biochem J.* **324**, 123-131.
- Bressan, R.A., Hasegawa, P.M., and Pardo, J.M. (1998). Plants use calcium to resolve salt stress. *Trends Plant Sci.* **3**, 411-412.
- Burdon, R.H., O'Kane, D., Fadzillah, N., Gill, V., Boyd, P.A., and Finch, R.R. (1996). Oxidative stress and responses in *Arabidopsis thaliana* and *Oryza sativa* subjected to chilling and salinity stress. *Biochem. Soc. Trans.* **24**, 469-472.
- Burnette, R., Guneseckara, B., Ecertin, M., Berdy, S., and Gillasp, G. (2001). A signal terminating gene from Arabidopsis can alter ABA signaling. The 12<sup>th</sup> International Meeting on Arabidopsis Research. June 23-27. Madison, WI. USA. Abstract No. 373.
- Bursens, S., Himanen, K., van de Cotte, B., Beeckman, T., van Montagu, M., Inze D., and Verbruggen, N. (2000). Expression of cell cycle regulatory genes and morphological alterations in response to salt stress in Arabidopsis. *Planta* **211**, 632-640.
- Bush, D.S. (1995). Calcium regulation in plant cells and its role in signaling. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **46**, 95-122.



- Chak, R.K.F., Thomas, T.L., Quatrano, R.S., and Rock, C.D.** (2000). The genes *ABI2* and *ABI2* are involved in abscisic acid- and drought-inducible expression of the *Daucus carota* L. *Dc3* promoter in guard cells of transgenic *Arabidopsis thaliana* (L.) Heynh. *Planta* **210**, 875-883.
- Chandler, P.M., and Robertson, M.** (1994). Gene expression regulation by abscisic acid and its relation to stress tolerance. *Ann. Rev. Plant Physiol. Plant Mol. Biol.* **45**, 113-141.
- Chuang C.F., and Meyerowitz E.M.** (2000). Specific and heritable genetic interference by double-stranded RNA in *Arabidopsis thaliana*. *Proc. Natl. Acad. Sci. USA* **97**, 4985-4990.
- Close T.J., and Lammers P.J.** (1993). An osmotic protein of cyanobacteria is immunologically related to plant dehydrins. *Plant Physiol.* **101**, 773-779.
- Cote, G.G., and Crain, R.** (1994). Why do plants have phosphoinositides? *BioEssays* **16**, 39-46.
- Covic, L., and Lew, R.R.** (1996). *Arabidopsis thaliana* cDNA isolated by functional complementation shows homology to serine/threonine protein kinases. *Biochim. Biophys. Acta* **1305**, 125-129.
- Covic, L., Silva, N.F., and Lew, R.R.** (1999). Functional characterization of ARAKIN (ATMEKK1): a possible mediator in an osmotic stress response pathway in higher plants. *Biochim. Biophys. Acta* **1451**, 242-252.
- Davies, W.J., and Zhang, J.** (1991). Root signals and the regulation of growth and development of plants in drying soil. *Ann. Rev. Plant Physiol.* **42**, 55-73.
- Delauney, A., and Verma, D.P.S.** (1993). Proline biosynthesis and osmoregulation in plants. *Plant J.* **4**, 215-223.
- DeWald, D.B., Torabinejad, J., Jones, C.A., Shope, J.C., Cangelosi, A.R., Thompson, J.E., Prestwich, G.D., and Hama, H.** (2001). Rapid accumulation of phosphatidylinositol 4,5-bisphosphate and inositol 1,4,5-trisphosphate correlates with calcium mobilization in salt-stressed *Arabidopsis*. *Plant Physiol.* **126**, 759-769.
- Dröbak, B.K., Watkins, P.A.C., Chattaway, J.A., Roberts, K., and Dawson, A.P.** (1991). Metabolism of inositol (1,4,5) trisphosphate by a soluble enzyme fraction from pea (*Pisum sativum*) roots. *Plant Physiol.* **95**, 412-419.
- Dure, L., III, Crouch, M., Harada, J., Ho., T.-H. D., Mundy, J., Quatrano, R., Thomas, T., and Sung, Z. R.** (1989). Common amino acid sequence domains among the LEA proteins of higher plants. *Plant Mol. Biol.* **12**, 475-486.
- Elphick, C. H., Sanders, D., and Maathuis, F. J. M.** (2001). Critical role of divalent cations and Na<sup>+</sup> efflux in *Arabidopsis thaliana* salt tolerance. *Plant Cell Environ.* **24**, 733-740.
- Espinosa-Ruiz, A., Belles, J.M., Serrano, R., and Gulianez-Macia, F.A.** (1999). *Arabidopsis thaliana* AtHAL3: a flavoprotein related to salt and osmotic tolerance and plant growth. *Plant J.* **20**, 529-539.
- Flowers, T.J., Troke, P.F., and Yeo, A.R.** (1977). The mechanism of salt tolerance in halophytes. *Annu. Rev. Plant Physiol.* **28**, 89-121.
- Fu, H.H., and Luan, S.** (1998). AtKUP1: a dual-affinity K<sup>+</sup> transporter from *Arabidopsis*. *Plant Cell* **10**, 63-74.
- Garay-Arroyo A., Colmenero-Flores J.M., Garcarrubio A., and Covarrubias A.A.** (2000). Highly hydrophilic proteins in prokaryotes and eukaryotes are common during conditions of water deficit. *J. Biol. Chem.* **275**, 5668-5674.
- Gaxiola, R.A., Rao, R., Sherman, A., Grisafi, P., Alper, S.L., and Fink, G.R.** (1999). The *Arabidopsis thaliana* proton transporters, AtNhx1 and Avp1, can function in cation detoxification in yeast. *Proc. Natl. Acad. Sci. USA* **96**, 1480-1485.
- Gaxiola, R.A., Li, J., Undurraga, S., Dang, L.M., Allen, G.J., Alper, S.L., and Fink, G.R.** (2001). Drought- and salt-tolerant plants result from overexpression of the AVP1 H<sup>+</sup>-pump. *Proc. Natl. Acad. Sci. USA* **98**, 11444-11449.
- Gazzarrini, S. and McCourt, P.** (2001). Genetic interaction between ABA, ethylene and sugar signaling pathways. *Curr. Opin. Plant Biol.* **4**, 387-391.
- Geisler, M., Frange, N., Gomes, E., Martinoia, E., and Palmgren, M.G.** (2000). The *ACA4* gene of *Arabidopsis* encodes a vacuolar membrane calcium pump that improves salt tolerance in yeast. *Plant Physiol.* **124**, 1814-1827.
- Ghassemian, M., Nambara, E., Cutler, S., Kawaide, H., Kamiya, Y., and McCourt, P.** (2000). Regulation of abscisic acid signaling by the ethylene response pathway in *Arabidopsis*. *Plant Cell* **12**, 1117-1126.
- Goddard, N.J., Dunn, M.A., Zhang, L., White, A.J., Jack, P.L., and Hughes, M.A.** (1993). Molecular analysis and spatial expression pattern of a low-temperature-specific barley gene, *blt101*. *Plant Mol Biol.* **23**, 871-879.
- Greenway, H., and Munns, R.** (1980). Mechanisms of salt tolerance in non-halophytes. *Ann. Rev. Plant Physiol.* **31**, 149-180.
- Guo, Y., Halfer, U., Ishitani, M., and Zhu, J.-K.** (2001). Molecular characterization of functional domains in the protein kinase SOS2 that is required for plant salt tolerance. *Plant Cell* **13**, 1383-1399.
- Halfer, U., Ishitani, M., and Zhu, J.K.** (2000). The *Arabidopsis* SOS2 protein kinase physically interacts with and is activated by the calcium-binding protein SOS3. *Proc. Natl. Acad. Sci. USA* **97**, 3735-3740.
- Hamada, A., Shono, M., Xia, T., Ohta, M., Hayashi, Y., Tanaka, A., and Hayakawa, T.** (2001). Isolation and characterization of a Na<sup>+</sup>/H<sup>+</sup> antiporter gene from the halophyte *Atriplex gmelini*. *Plant Mol. Biol.* **46**, 35-42.
- Hare, P.D., Cress, W.A., and van Staden, J.** (1999). Proline synthesis and degradation: a model system for elucidating stress-related signal transduction. *J. Exp. Bot.* **50**, 413-434.
- Hasegawa, P.M., Bressan, R.A., Nelson, D.E., Samaras, Y., and Rhodes, D.** (1994). Tissue culture in the improvement of salt tolerance in plants. In: *Soil Mineral Stresses. Approaches to crop improvement, Monographs on Theoretical and Applied Genetics* (edited by Yeo, A.R.; Flowers, T.J.), Vol. 21, Springer-Verlag, Berlin, pp. 83-125.
- Hasegawa, P.M., Bressan, R.A., Zhu, J.K. and Bohnert, H.J.** (2000). Plant cellular and molecular responses to high salinity. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **51**, 463-499.
- Hayashi, H., Alia, Mustardy, L., Deshnum, P., Ida, M., and Murata, N.** (1997). Transformation of *Arabidopsis thaliana* with the *CodA* gene for choline oxidase: accumulation of glycine-betaine and enhanced tolerance to salt and cold stress. *Plant J.* **12**, 133-132.

- Hirayama, T., Ohto, C., Mizoguchi, T., and Shinozaki, K.** (1995). A gene encoding a phosphatidylinositol-specific phospholipase C is induced by dehydration and salt stress in *Arabidopsis thaliana*. *Proc. Natl. Acad. Sci. USA* **92**, 3903-3907.
- Hirschi, K.D.** (1999). Expression of *Arabidopsis* CAX1 in tobacco: altered calcium homeostasis and increased stress sensitivity. *Plant Cell* **11**, 2113-2122.
- Holmstrom, K.O., Somersalo, S., Mandal, A., Palva, T.E., and Welin, B.** (2000). Improved tolerance to salinity and low temperature in transgenic tobacco producing glycine betaine. *J. Exp. Bot.* **51**, 177-185.
- Hong, Z., Lakkineni, K., Zhang, Z., and Verma, D.P.S.** (2000). Removal of feedback inhibition of D1-pyrroline-5-carboxylate synthetase results in increased proline accumulation and protection of plants from osmotic stress. *Plant Physiol.* **122**, 1129-1136.
- Inhorn, R.C., Bansal, V.S., and Majerus, P.** (1987). Pathway for inositol 1,3,4-trisphosphate and 1,4-bisphosphate metabolism. *Proc. Natl. Acad. Sci. USA* **84**, 2170-2174.
- Ingram, J., and Bartels, D.** (1996). The molecular basis of dehydration tolerance in plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **47**, 377-403.
- Ishitani, M., Liu, J., Halfter, U., Kim, C.S., Shi, W., and Zhu J.K.** (2000). SOS3 function in plant salt tolerance requires N-myristoylation and calcium binding. *Plant Cell* **12**, 1667-1678.
- Ishitani, M., Xiong, L., Stevenson, B., and Zhu, J.-K.** (1997). Genetic analysis of osmotic and cold stress signal transduction in *Arabidopsis*: Interactions and convergence of abscisic acid-dependent and abscisic acid-independent pathways. *Plant Cell* **9**, 1935-1949.
- Ishitani, M., Xiong, L., Lee, H., Stevenson, B., and Zhu, J.-K.** (1998). HOS1, a genetic locus involved in cold-responsive gene expression in *Arabidopsis*. *Plant Cell* **10**, 1151-1161.
- Jaglo-Ottosen, K.R., Gilmour, S.J., Zarka, D.G., Schabenberger, O., and Thomashow, M.F.** (1998). *Arabidopsis* CBF1 overexpression induces COR genes and enhances freezing tolerance. *Science* **280**, 104-106.
- Kaldenhoff, R., Kolling, A., and Ritchter, G.** (1993). A novel blue light-inducible and abscisic acid-inducible gene of *Arabidopsis thaliana* encoding an intrinsic membrane protein. *Plant Mol. Biol.* **23**, 1187-1198.
- Kasuga, M., Liu, Q., Miura, S., Yamaguchi-Shinozaki, K., and Shinozaki, K.** (1999). Improving plant drought, salt, and freezing tolerance by gene transfer of a single stress-inducible transcriptional factor. *Nature Biotechnol.* **17**, 287-291.
- Kawasaki, S., Borchert, C., Deyholos, M., Wang, H., Brazille, S., Kawai, K., Galbraith, D., and Bohnert, H.** (2001). Gene expression profiles during the initial phase of salt stress in rice. *Plant Cell* **13**, 889-905.
- Kawasaki, T., Henmi, K., Ono, E., Hatakeyama, S., Iwano, M., Satoh, H., and Shimamoto, K.** (1999). The small GTP-binding protein Rac is a regulator of cell death in plants. *Proc. Natl. Acad. Sci. USA* **96**, 10922-10926.
- Kim, E.J., Kwak, J.M., Uozumi, N., and Schroeder, J.I.** (1998). *AtKUP1*, an *Arabidopsis* gene encoding high-affinity potassium transporter activity. *Plant Cell* **10**, 51-62.
- Kim, S.A., Kwak, J.M., Jae, S.K., Wang, M.H., and Nam, H.G.** (2001). Overexpression of the *AtGluR2* gene encoding an *Arabidopsis* homolog of mammalian glutamate receptors impairs calcium utilization and sensitivity to ionic stress in transgenic plants. *Plant Cell Physiol.* **42**, 74-84.
- Kiyosue, T., Yoshida, Y., Yamaguchi-Shinozaki, K., and Shinozaki, K.** (1996). A nuclear gene encoding mitochondrial proline dehydrogenase, an enzyme involved in proline metabolism, is upregulated by proline but downregulated by dehydration in *Arabidopsis*. *Plant Cell* **8**, 1323-1335.
- Knight, H.** (2000). Calcium signaling during abiotic stress in plants. *International Rev. Cytol.* **195**, 269-325.
- Knight, H., and Knight, M.R.** (2001). Abiotic stress signaling pathways: specificity and cross-talk. *Trends Plant Sci.* **6**, 262-267.
- Kovtun, Y., Chiu, W.L., Tena, G., and Sheen, J.** (2000). Functional analysis of oxidative stress-activated mitogen-activated protein kinase cascade in plants. *Proc. Natl. Acad. Sci. USA* **97**, 2940-2945.
- Kudla, J., Xu, Q., Harter, K., Griessem, W., and Luan, S.** (1999). Genes for calcineurin B-like proteins in *Arabidopsis* are differentially regulated by stress signals. *Proc. Natl. Acad. Sci. USA* **9**, 4718-4723.
- Kultz, D., and Chakravarty, D.** (2001). Hyperosmolarity in the form of elevated NaCl but not urea causes DNA damage in murine kidney cells. *Proc. Natl. Acad. Sci.* **98**, 1999-2004.
- Kyriakis, J. M., and Avruch, J.** (1996). Sounding the alarm: protein kinase cascades activated by stress and inflammation. *J. Biol. Chem.* **271**, 24313-24316.
- Lazof, D.B., and Bernstein, N.** (1999). The NaCl induced inhibition of shoot growth: the case for distributed nutrition with special consideration of calcium. *Adv. Bot. Res.* **29**, 113-189.
- Lee, H., Xiong, L., Ishitani, M., Stevenson, B., and Zhu, J.K.** (1999). Cold-regulated gene expression and freezing tolerance in an *Arabidopsis thaliana* mutant. *Plant J.* **17**, 301-308.
- Lee, H., Xiong, L., Gong, Z., Ishitani, M., Stevenson, B., and Zhu, J.-K.** (2001). The *Arabidopsis* HOS1 gene negatively regulates cold signal transduction and encodes a RING finger protein that displays cold-regulated nucleo-cytoplasmic partitioning. *Genes Dev.* **15**, 912-924.
- Lippuner, V., Cyert, M.S., and Gasser, C.S.** (1996). Two classes of plant cDNA clones differentially complement yeast calcineurin mutants and increase salt tolerance of wild type yeast. *J. Biol. Chem.* **271**, 12859-12866.
- Liu, J., and Zhu, J.-K.** (1997). Proline accumulation and salt-stress-induced gene expression in a salt-hypersensitive mutant of *Arabidopsis*. *Plant Physiol.* **114**, 591-596.
- Liu, J. and Zhu, J.-K.** (1998). A calcium sensor homolog required for plant salt tolerance. *Science* **280**, 1943-1945.
- Liu, J., Ishitani, M., Halfter, U., Kim, C.S., and Zhu J.K.** (2000). The *Arabidopsis thaliana* SOS2 gene encodes a protein kinase that is required for salt tolerance. *Proc. Natl. Acad. Sci. USA* **97**, 3730-3734.

- Liu, Q., Kasuga, M., Sakuma, Y., Abe, H., Miura, S., Yamaguchi-Shinozaki, K., and Shinozaki, K.** (1998). Two transcription factors, DREB1 and DREB2, with an EREBP/AP2 DNA binding domain separate two cellular signal transduction pathways in drought- and low-temperature-responsive gene expression, respectively, in *Arabidopsis*. *Plant Cell* **10**, 1391-1406.
- Liu, W., Schachtman, D.P., and Zhang, W.** (2000). Partial deletion of a loop region in the high affinity K<sup>+</sup> transporter HKT1 changes ionic permeability leading to increased salt tolerance. *J. Biol. Chem.* **275**, 27924-27932.
- Majerus, P.W.** (1992). Inositol phosphate biochemistry. *Annu. Rev. Biochem.* **61**, 225-250.
- Matsumoto, T.K., Pardo, J.M., Takeda, S., Bressan, R.A., and Hasegawa, P.M.** (2001). Tobacco and *Arabidopsis* SLT1 mediate salt tolerance of yeast. *Plant Mol Biol.* **45**, 489-500.
- Medina, J., Catala, R., and Salinas, J.** (2001). Developmental and stress regulation of *RCI2A* and *RCI2B*, two cold-inducible genes of *Arabidopsis* encoding highly conserved hydrophobic proteins. *Plant Physiol.* **125**, 1655-1666.
- Mendoza, I., Rubio, F., Rodríguez-Navarro, A., and Pardo, J.M.** (1994). The protein phosphatase calcineurin is essential for NaCl tolerance of *Saccharomyces cerevisiae*. *J. Biol. Chem.* **269**, 8792-8796.
- Meskiene, I., and Hirt, H.** (2000). MAP kinase pathways: molecular plug-and-play chips for the cell. *Plant Mol Biol.* **42**, 791-806.
- Mizoguchi, T., Irie, K., Hirayama, T., Hayashida, N., Yamaguchi-Shinozaki, K., Matsumoto, K., and Shinozaki, K.** (1996). A gene encoding a MAP kinase kinase kinase is induced simultaneously with genes for a MAP kinase and an S6 kinase by touch, cold and water stress in *Arabidopsis thaliana*. *Proc. Natl. Acad. Sci. USA* **93**, 765-769.
- Monroy, A.F., and Dhindsa, R.S.** (1995). Low-temperature signal transduction: induction of cold acclimation-specific genes of alfalfa by calcium at 25 degrees C. *Plant Cell* **7**, 321-331.
- Muir, S.R., and Sanders, D.** (1997). Inositol 1, 4, 5-trisphosphate-sensitive Ca<sup>2+</sup>-releasing across nonvacuolar membrane in cauliflower. *Plant Physiol.* **114**, 1511-1521.
- Munnik, T., Irvine, R.F., and Musgrave, A.** (1998). Phospholipid signaling in plants. *Biochim. Biophys. Acta* **1389**, 222-272.
- Nanjo, T., Kobayashi, M., Yoshida, Y., Kakubari, Y., Yamaguchi-Shinozaki, K., and Shinozaki, K.** (1999). Antisense suppression of proline degradation improves tolerance to freezing and salinity in *Arabidopsis thaliana*. *FEBS Lett* **461**, 205-210.
- Navarre, C., and Goffeau, A.** (2000). Membrane hyperpolarization and salt sensitivity induced by deletion of PMP3, a highly conserved small protein of yeast plasma membrane. *EMBO J.* **19**, 2515-2524.
- NaVazio, L., Bewell, M.A., Siddique, A., Dickinson, G.D., Galione, A., and Sanders, D.** (2000). Calcium release from the endoplasmic reticulum of higher plants elicited by the NADP metabolite nicotinic acid adenine dinucleotide phosphate. *Proc. Natl. Acad. Sci.* **97**, 8693-8698.
- Niu, X., Bressan, R.A., Hasegawa, P.M., and Pardo, J.M.** (1995). Ion homeostasis in NaCl stress environment. *Plant Physiol.* **109**, 735-742.
- Nublat, A., Desplans, J., Casse, F., and Berthomieu, P.** (2001). *sas1*, an *Arabidopsis* mutant overaccumulating sodium in the shoot, shows deficiency in the control of the root radial transport of sodium. *Plant Cell* **13**, 125-137.
- Nuccio, M.L., Rhodes, D., McNeil, S.D., and Hanson, A.D.** (1999). Metabolic engineering of plants for osmotic stress resistance. *Curr. Opin. Plant Biol.* **2**, 128-134.
- Nylander, M., Heino, P., Helenius, E., Palva, E.T., Ronne, H., and Welin, B.V.** (2001). The low-temperature- and salt-induced *RCI2A* gene of *Arabidopsis* complements the sodium sensitivity caused by a deletion of the homologous yeast gene *SNA1*. *Plant Mol. Biol.* **45**, 341-352.
- Piao, H.L., Pih, K.T., Lim, J.H., Kang, S.G., Jin, J.B., Kim, S.H., and Hwang, I.** (1999). An *Arabidopsis* GSK3/shaggy-like gene that complements yeast salt stress-sensitive mutants in induced by NaCl and abscisic acid. *Plant Physiol.* **119**, 1527-1534.
- Posas, F., Chambers, J.R., Heyman, J.A., Hoeffler, J.P., de Nadal, E., and Arino, J.** (2000). The transcriptional response of yeast to saline stress. *J. Biol. Chem.* **275**, 17249-17255.
- Qiu, Q.S., Guo, Y., Dietrich, M.A., Schumaker, K.S., and Zhu, J.K.** (2002). Regulation of SOS1, a plasma membrane Na<sup>+</sup>/H<sup>+</sup> exchanger in *Arabidopsis thaliana*, by SOS2 and SOS3. *Proc. Natl. Acad. Sci. USA* **99**, 8436-8841.
- Quesada, V., Ponce, M.R., and Micol, J.L.** (2000). Genetic analysis of salt-tolerant mutants in *Arabidopsis thaliana*. *Genetics* **154**, 421-436.
- Quintero, F.J., Garcíadeblás, B., and Rodríguez-Navarro, A.** (1996). The *SAL1* gene of *Arabidopsis*, encoding an enzyme with 3'(2'), 5'-bisphosphate nucleotide and inositol polyphosphate 1-phosphatase activities, increases salt tolerance in yeast. *Plant Cell* **8**, 529-537.
- Quintero, F.J., Ohta, M., Shi, H., Zhu, J.K., Pardo, J.M.** (2002). Reconstitution in yeast of the *Arabidopsis* SOS signaling pathway for Na<sup>+</sup> homeostasis. *Proc. Natl. Acad. Sci. USA* **99**, 9061-9066.
- Rep, M., Krantz, M., Thevelein, J.M., Hohmann, S.** (2000). The transcriptional response of *Saccharomyces cerevisiae* to osmotic shock. Hot1p and Msn2p/Msn4p are required for the induction of subsets of high osmolarity glycerol pathway-dependent genes. *J. Biol. Chem.* **275**, 8290-8300.
- Reymond, P., Weber, H., Damond, M., and Farmer, E.E.** (2000). Differential gene expression in response to mechanical wounding and insect feeding in *Arabidopsis*. *Plant Cell* **12**, 707-719.
- Rhee, S.G., Bae, Y.S., Lee, S.R., and Kwon, J.** (2000). Hydrogen peroxide: a key messenger that modulates protein phosphorylation through cysteine oxidation. *Science's STKE*, [www.stke.org/cgi/content/full/OC\\_sigtrans;2000/53/pe1](http://www.stke.org/cgi/content/full/OC_sigtrans;2000/53/pe1)
- Rhoades, J.D. and Loveday, J.** (1990). Salinity in irrigated agriculture. in *American Society of Civil Engineers, Irrigation of Agricultural Crops* (Steward, B.A. and Nielsen, D.R., eds), Am. Soc. Agronomists, Monograph 30, 1089-1142.
- Rhodes, D., and Hanson, A.D.** (1993). Quaternary ammonium and tertiary sulfonium compounds in higher plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **44**, 357-384.
- Rock, C.D.** (2000). Pathways to abscisic acid-regulated gene expression. *New Phytol.* **148**, 357-396.

- Rubio, F., Gassmann, W., and Schroeder, J.I. (1995). Sodium-driven potassium uptake by the plant potassium transporter HKT1 and mutations conferring salt tolerance. *Science* **270**, 1660-1663.
- Rubio, F., Schwarz, M., Gassmann, W., and Schroeder, J.I. (1999). Genetic selection of mutations in the high affinity K<sup>+</sup> transporter HKT1 that define functions of a loop site for reduced Na<sup>+</sup> permeability and increased Na<sup>+</sup> tolerance. *J. Biol. Chem.* **274**, 6839.
- Rus, A., Yokoi, S., Sharkhuu, A., Reddy, M., Lee, B.-h., Matsumoto, T.K., Koiwa, H., Zhu, J.-K., Bressan, R.A., and Hasegawa, P.M. (2001). AtHKT1 is a salt tolerance determinant that controls Na<sup>+</sup> entry into plant roots. *Proc. Natl. Acad. Sci. USA* **98**, 14150-14155.
- Saab, I.N., Sharp, R.E., Pritchard, J., and Voetberg, G.S. (1990). Increased endogenous abscisic acid maintains primary root growth and inhibits shoot growth of maize seedlings at low water potential. *Plant Physiol.* **93**, 1329-1336.
- Saleki, R., Young, P., and Lefebvre, D.D. (1993). Mutants of *Arabidopsis thaliana* capable of germination under saline conditions. *Plant Physiol.* **101**, 839-845.
- Sanders, D., Brownlee, C., and Harper, J.F. (1999). Communicating with calcium. *Plant Cell* **11**, 691-706.
- Sanders, D. (2000). The salty tale of *Arabidopsis*. *Curr. Biol.* **10**, R486-488.
- Savoure, A., Hua, X.-J., Bertauche, N., Van-Montagu, M. and Verbruggen, N. (1997). Absciscic acid-independent and abscisic acid dependent regulation of proline biosynthesis following cold and osmotic stresses in *Arabidopsis thaliana*. *Mol. Gen. Genet.* **254**, 104-109.
- Schachtman, D., and Liu, W. (1999). Molecular pieces to the puzzle of the interaction between potassium and sodium uptake in plants. *Trends Plant Sci.* **4**, 281-287.
- Schena, M., Shalon, D., Davis, R.W., and Brown, P.O. (1995). Quantitative monitoring of gene expression patterns with a complementary DNA microarray. *Science* **270**, 467-470.
- Schumaker, K.S., and Sze, H. (1987). Inositol 1,4,5-trisphosphate releases Ca<sup>2+</sup> from vacuolar membrane vesicles of oat roots. *J. Biol. Chem.* **262**, 3944-3946.
- Seki, M., Narusaka, M., Abe, H., Kasuga, M., Yamaguchi-Shinozaki, K., Carninic, P., Hayashizaki, Y., and Shinozaki, K. (2001). Monitoring the expression pattern of 1300 *Arabidopsis* genes under drought and cold stresses by using a full-length cDNA microarray. *Plant Cell* **13**, 61-72.
- Serrano, R., Mulet, J.M., Rios, G., Marguez, J.A., de Larrinoa, I.F., Leube, M.P., Mendizabal, I.M., Pascual-Ahuir, A., Proft, M., Ros, R., and Montesinos, C. (1999). A glimpse of the mechanisms of ion homeostasis during salt stress. *J. Exp. Bot.* **50**, 1023-1036.
- Sharp, R.E., LeNoble, M.E., Else, M.A., Thorne, E.T., and Gherardi, F. (2000). Endogenous ABA maintains shoot growth in tomato independently of effects on plant water balance: evidence for an interaction with ethylene. *J. Exp. Bot.* **51**, 1575-1584.
- Sharp, R.E. (2002). Interaction with ethylene: changing views on the role of abscisic acid in root and shoot growth responses to water stress. *Plant, Cell Environ.* **25**, 213-224.
- Sheen, J. (1996). Ca<sup>2+</sup> dependent protein kinases and stress signal transduction in plants. *Science* **274**, 1900-1902.
- Shen, Q., Chen, C.-N., Brands, A., Pan, S.-M., Ho, and T.-H. D. (2001). The stress- and abscisic acid- induced barley gene *HVA22*: developmental regulation and homologues in diverse organisms. *Plant Mol. Biol.* **45**, 327-340.
- Shen, B., Jensen, R.G., and Bohnert, H. (1997). Increased resistance to oxidative stress in transgenic plants by targeting mannitol biosynthesis to chloroplasts. *Plant Physiol.* **113**, 1177-1183.
- Shi, H., Ishitani, M., Kim, C., and Zhu, J.K. (2000). The *Arabidopsis thaliana* salt tolerance gene SOS1 encodes a putative Na<sup>+</sup>/H<sup>+</sup> antiporter. *Proc. Natl. Acad. Sci. USA*, **97**, 6896-6901.
- Shi, H., Quintero, F.J., Pardo, J.M., and Zhu, J.-K. (2002a). The putative plasma membrane Na<sup>+</sup>/H<sup>+</sup> antiporter SOS1 controls long distance Na<sup>+</sup> transport in plants. *Plant Cell* **14**, 465-477.
- Shi, H., Xiong, L., Stevenson, B., Lu, T. and Zhu, J.-K. (2002b). The *Arabidopsis salt overly sensitive 4* mutants uncover a critical role for vitamin B6 in plant salt tolerance. *Plant Cell* **14**, 575-588.
- Shi, H., and Zhu, J.-K. (2002). Regulation of the vacuolar Na<sup>+</sup>/H<sup>+</sup> antiporter gene *AtNHX1* expression by salt stress and abscisic acid. *Plant Mol. Biol.* (in press).
- Shinozaki, K., and Yamaguchi-Shinozaki, K. (1997). Gene expression and signal transduction in water-stress response. *Plant Physiol.* **115**, 327-334.
- Skriver, K., and Mundy, J. (1990). Gene expression in response to abscisic acid and osmotic stress. *Plant Cell* **2**, 503-512.
- Smirnoff, N., and Cumber, Q.J. (1989). Hydroxyl radical scavenging activity of compatible solutes. *Phytochemistry* **28**, 1057-1060.
- Smith, N.A., Singh, S.P., Wang, M.B., Stoutjesdijk, P.A., Green, A.G., and Waterhouse, P.M. (2000). Gene expression: Total silencing by intron-spliced hairpin RNAs. *Nature* **407**, 319-320.
- Spollen, W.G., LeNoble, M.E., Samuel, T.D., Bernstein, N., and Sharp, R.E. (2000). Absciscic acid accumulation maintains maize primary root elongation at low water potentials by restricting ethylene production. *Plant Physiol.* **122**, 967-976.
- Strizhov, N., Abraham, E., Okresz, L., Blickling, S., Zilberstein, A., Schell, J., Koncz, C., and Szabados, L. (1997). Differential expression of two *P5CS* genes controlling proline accumulation during salt-stress requires ABA and is regulated by ABA1, ABI1 and AXR2 in *Arabidopsis*. *Plant J.* **12**, 557-569.
- Sun, K., and Hauser, B. (2001). Salt stress induces anatomical changes in ovules and embryos, ultimately resulting in seed abortion. The 12<sup>th</sup> International Meeting on *Arabidopsis* Research. June 23-27. Madison, WI. USA. Abstract No. 420.
- Tarczynski, M.C., Jensen, R.G., and Bohnert, H. (1993). Stress protection of transgenic tobacco by production of the osmolyte mannitol. *Science* **259**, 508-510.
- Tsugane, K., Kobayashi, K., Niwa, Y., Ohba, Y., Wada, K., and Kobayashi, H. (1999). A recessive *Arabidopsis* mutant that grows photoautotrophically under salt stress shows enhanced active oxygen detoxification. *Plant Cell* **11**, 1195-1206.
- Tyerman, S.D., Bohnert, H.J., Maurel, C., Steudle, E., and Smith, J.A.C. (1999). Plant aquaporins: their molecular biology, biophysics and significance for plant water relations. *J. Exp. Bot.* **50**, 1055-1071.
- Tyerman, S.D., and Skerrett, I.M. (1999). Root ion channels and salinity. *Sci. Hort.* **78**, 175-235.



- Ulm, R., Revenkova, E., di Sansebastiano, G.-P., Bechtold, N., and Paszkowski, J.** (2001). Mitogen-activated protein kinase phosphatase is required for genotoxic stress relief in Arabidopsis. *Genes Dev.* **15**, 699-709.
- Uozumi, N., Kim, E.J., Rubio, F., Yamaguchi, T., Muto, S., Tsuboi, A., Bakker, E.P., Nakamura, T., and Schroeder, J.I.** (2000). The Arabidopsis *HKT1* gene homolog mediates inward Na<sup>+</sup> currents in *Xenopus laevis* oocytes and Na<sup>+</sup> uptake in *Saccharomyces cerevisiae*. *Plant Physiol.* **122**, 1249-1259.
- Urao, T., Yakubov, B., Satoh, R., Yamaguchi-Shinozaki, K., Seki, B., Hirayama, T., and Shinozaki, K.** (1999). A transmembrane hybrid-type histidine kinase in Arabidopsis functions as an osmosensor. *Plant Cell* **11**, 1743-1754.
- Wang, H., Qi Q., Schorr, P., Cutler, A.J., Crosby, W.L., and Fowke, L.C.** (1998). ICK1, a cyclin-dependent protein kinase inhibition from *Arabidopsis thaliana* interacts with both Cdc2a and CycD3, and its expression is induced by abscisic acid. *Plant J.* **15**, 501-510.
- Wang, X.Q., Ullah, H., Jones, A.M., and Assmann, S.M.** (2001). G protein regulation of ion channels and abscisic acid signaling in Arabidopsis guard cells. *Science* **292**, 2070-2072.
- Werner, J., and Finkelstein, R.R.** (1995). Arabidopsis mutants with reduced response to NaCl and osmotic stress. *Physiol. Plant.* **93**, 659-666.
- White, P.J.** (1999). The molecular mechanisms of sodium influx to root cells. *Trends Plant Sci.* **7**, 245-246.
- Winicov, I.** (1991). Characterization of salt tolerant alfalfa (*Medicago sativa* L) plants regenerated from salt tolerant cell lines. *Plant Cell Reports* **10**, 561-564.
- Wu, S.-J., Ding, L., and Zhu, J.-K.** (1996). *SOS1*, a genetic locus essential for salt tolerance and potassium acquisition. *Plant Cell* **8**, 671-627.
- Wu, Y., Kuzma, J., Marechal, E., Graeff, R., Lee, H.C., Foster, R., and Chua, N.H** (1997). Abscisic acid signaling through cyclic ADP-ribose in plants. *Science* **278**, 2126-2130
- Xin, Z., and Browse, J.** (1998). *eskimo1* mutants of Arabidopsis are constitutively freezing-tolerant. *Proc. Natl. Acad. Sci. USA* **95**, 7799-7804.
- Xiong, L., David, L., Stevenson, B., and Zhu, J.-K.** (1999a). High throughput screening of signal transduction mutants with luciferase imaging. *Plant Mol. Biol. Rep.* **17**, 59-170.
- Xiong, L., Ishitani, M., Lee, H., and Zhu, J.K.** (1999b). HOS5, a negative regulator of osmotic stress-induced gene expression in *Arabidopsis thaliana*. *Plant J* **19**, 569-578.
- Xiong, L., Ishitani, M., and Zhu, J.K.** (1999c). Interaction of osmotic stress, temperature, and abscisic acid in the regulation of gene expression in Arabidopsis. *Plant Physiol* **119**, 205-211.
- Xiong, L., Gong, Z., Rock, C.D., Subramanian, S., Guo, Y., Xu, W., Galbraith, D., and Zhu, J.K.** (2001a). Modulation of abscisic acid signal transduction and biosynthesis by an Sm-like protein in Arabidopsis. *Dev. Cell* **1**, 771-781.
- Xiong, L., Lee, B.H., Ishitani, M., Lee, H., Zhang, C., and Zhu, J.K.** (2001b). *FIERY1* encoding an inositol polyphosphate 1-phosphatase is a negative regulator of abscisic acid and stress signaling in Arabidopsis. *Genes Dev.* **15**, 1971-1984.
- Xiong, L., Ishitani, M., Lee, H., and Zhu, J.-K.** (2001c). The Arabidopsis *LOS5/ABA3* locus encodes a molybdenum cofactor sulfurase and modulates cold and osmotic stress responsive gene expression. *Plant Cell* **13**, 2063-2083.
- Xiong, L., and Zhu, J.-K.** (2001). Abiotic stress signal transduction in plants: molecular and genetic perspectives. *Physiol. Plant.* **112**, 152-166.
- Xiong, L., and Zhu, J.-K.** (2002a). Salt-stress signal transduction. In: Scheel D and Wernernack C (eds) *Plant Signal Transduction*. Frontiers in Molecular Biology Series. Oxford University Press. pp 165-197.
- Xiong, L., and Zhu, J.-K.** (2002b). Molecular and genetic aspects of osmotic stress responses in plants. *Plant, Cell Environ.* **25**, 131-140.
- Xiong, L., Lee, H., Ishitani, M., and Zhu, J.K.** (2002). Regulation of osmotic stress-responsive gene expression by the *LOS6/ABA1* locus in Arabidopsis. *J Biol. Chem.* **277**, 8588-8596.
- Xu, D., Duan, X., Wang, B., Hong, B., Ho, T.H.D., and Wu, R.** (1996). Expression of a late embryogenesis abundant (LEA) protein gene, *HvA1*, from barley confers tolerance to drought and salinity in transgenic rice. *Plant Physiol.* **110**, 249- 257.
- Yale, J. and Bohnert, H.J.** (2001). Transcript expression in *Saccharomyces cerevisiae* at high salinity. *J. Biol. Chem.* **276**, 15996-16007.
- Yoshida, Y., Nanjo, T., Miura, S., Yamaguchi-Shinozaki, K., and Shinozaki, K.** (1999). Stress-responsive and developmental regulation of D1-pyrroline-5-carboxylate synthetase 1 (*P5CS1*) gene expression in *Arabidopsis thaliana*. *Biochem. Biophys. Res. Comm.* **261**, 766-772.
- Yeo, A.R.** (1983). Salinity resistance: Physiologies and prices. *Physiol. Plant.* **58**, 214-222.
- Yeo, A.** (1998). Molecular biology of salt tolerance in the context of whole-plant physiology. *J. Exp. Bot.* **49**, 913-929.
- Zhu, J.-K., Liu, J., and Xiong, L.** (1998). Genetic analysis of salt tolerance in Arabidopsis: Evidence for a critical role of potassium nutrition. *Plant Cell* **10**, 1181-1191.
- Zhang, J., and Davies W.J.** (1991). Antitranspirant activity in xylem sap of maize plants. *J. Exp. Bot.* **42**, 317-321.
- Zhu, J.-K.** (2000). Genetic analysis of plant salt tolerance using *Arabidopsis*. *Plant Physiol.* **124**, 941-948.
- Zhu, J.-K.** (2001a). Plant salt tolerance. *Trends Plant Sci.* **6**, 66-71.
- Zhu J.-K.** (2001b). Cell signaling under salt, water and cold stresses. *Curr. Opin Plant Biol.* **4**, 401-406.
- Zhu J.-K., Hasegawa P.M., and Bressan, R.A.** (1997). Molecular aspects of osmotic stress in plants. *Crit. Rev. Plant Sci.* **16**, 253-277.