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Mechanisms of salt tolerance in plants

Surekha Katiyar-Agarwal, Paul Verslues and Jian-Kang Zhu*

Institute for Integrative Genome Biology and Department of Botany and Plant Sciences, University of California, Riverside, California 92521

**For correspondence jian-kang.zhu@ucr.edu*

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Abstract

The increasing prevalence of soil salinity is one of the most significant obstacles to improving crop productivity. Recent advances in genetic and molecular analysis of *Arabidopsis thaliana* mutants, ion transporters and stress signaling proteins have improved our understanding of the mechanisms of cellular ion homeostasis and its regulation in plants. Since Na toxicity is the principal stress component in saline soils, much research has focused on the identification of ion transporters and regulatory mechanisms that mediate Na+ homeostasis and maintenance of a high cytoplasmic K^+/Na^+ ratio. The SALT OVERLY SENSITIVE (SOS) signaling pathway, composed of the SOS1, 2 and 3 proteins, has emerged as a key factor in the detection of and tolerance to salt stress. Recent evidence suggests that the SOS pathway may regulate several ion transport mechanisms critical for salt tolerance.

Salt stress is a serious environmental factor limiting crop productivity and affects about 20% of irrigated agricultural land (Flowers and Yeo, 1995). Progress in breeding for salt-tolerant crops has been limited because of inadequate understanding of the molecular basis of salt tolerance mechanisms in plants. The adverse effects of saline soil include ① nutrient constraints caused by reduced uptake of potassium, phosphorous, nitrate and calcium; ② ion cytotoxicity due to accumulation of sodium, chloride and sulfate; ③ osmotic stress that results from relatively high solute concentrations in the soil; and ④ oxidative stress caused by the accumulation of reactive oxygen species that damage membrane lipids, proteins and nucleic acids.

Ion homeostasis is a fundamental cellular mechanism by which all living cells maintain an intracellular ionic composition. One of the most detrimental effects of salt stress is to disrupt the ion homeostasis mechanisms of the plant. In particular, the similar radii of Na^+ and K^+ make it difficult for transport proteins to distinguish between these two ions. Thus, under conditions of high external Na^+, there is substantial uptake of Na^+ through K^+ transporters or channels (Blumwald *et al.* 2000). This Na^+ entry disrupts the normally high cytosolic K^+/Na^+ ratio and causes numerous detrimental effects. Therefore, maintenance of a high cytosolic K^+/Na^+ concentration is a key requirement for plant salt tolerance (Glenn *et al.* 1999). There are several possible strategies that plants could employ to avoid a damaging decrease in the K^+/Na^+ ratio: reduce entry of Na^+ into the cell, remove Na^+ from the cell, or compartmentalize Na^+ into the vacuole where it cannot disrupt cellular function. In this article, we discuss recent advances in understanding the molecular mechanisms of ion homeostasis in plants and, in particular, the role of *Arabidopsis thaliana* SALT OVERLY SENSITIVE (SOS) pathway in the maintenance of ion homeostasis under saline conditions.

Sodium uptake

Both low affinity and high affinity transport systems are involved in determining the cellular K^+/Na^+ ratio during salt stress. Both AKT1, an inward-rectifying K^+ channel of the Shaker type family, as well as LCT1, a low-affinity cation transport channel in wheat, have been shown to mediate low affinity Na^+ influx during salt stress (Schachtman, 2000). In contrast, the high-affinity carrier HKT1, a Na^+-K^+ symporter, is required for continued K^+ uptake during salt stress (Uozumi *et al.* 2000). High external Na^+ concentrations inhibited HKT1-mediated K^+ influx (Rubio *et al.* 1995) and transgenic wheat with reduced *HKT1* expression exhibited significantly less Na^+ uptake and enhanced tolerance to salinity (Laurie *et al.* 2002). This suggests that HKT1 is an important determinant of salt tolerance.

Sodium extrusion and the SOS pathway

The *Arabidopsis sos1*, *2* and *3* mutants, were isolated in a genetic screen for plants hypersensitive to NaCl. Subsequent work has shown that these three SOS proteins are components of a stress-signaling pathway controlling ion homeostasis (Zhu, 2003). SOS1 was the first protein to be identified as a plasma membrane Na^+/H^+ antiporter involved in Na^+ efflux (Shi *et al.* 2000, Qiu *et al.* 2002). SOS1 plays a crucial role in sodium efflux from root cells and long-distance Na^+ transport from roots to shoots (Shi *et al.* 2002). Transgenic plants overexpressing SOS1 exhibited enhanced tolerance to NaCl because they accumulated less Na^+ in the transpirational stream and in shoots as compared with wild-type plants (Shi *et al.* 2002). An additional role for SOS1 as a Na^+ sensor, possibly involving the cytoplasmic, hydrophilic C-terminal domain of the protein, has been suggested (Zhu, 2003).

SOS2 is a ser/thr protein kinase capable of autophosphorylation. It has an N-terminal catalytic domain similar to that of yeast sucrose nonfermenting 1 (SNF1) and mammalian AMP-activated protein kinase (AMPK) and a

C-terminal regulatory domain (Liu *et al*. 2000). SOS3 is a myristoylated calcium-binding protein that is thought to respond to salt-induced Ca^{2+} transients in the cytosol (Liu and Zhu, 1998). SOS3 shares significant sequence similarity with yeast calcineurin B subunit and animal neuronal calcium sensors (Liu and Zhu, 1998). SOS3 physically interacts with the regulatory region of SOS2, activates its protein kinase activity in a Ca^{2+}-dependent manner and recruits it to the plasma membrane (Halfter *et al.* 2000). The SOS3/SOS2 complex then phosphorylates and activates SOS1, which results in the efflux of Na^+ ions, thereby restoring cellular ionic balance (Quintero *et al.* 2002). Co-expression of SOS1, 2 and 3 in a salt-sensitive yeast mutant led to enhanced tolerance to Na^+ (Qiu *et al.* 2002), further demonstrating the importance of the SOS signaling pathway in salt tolerance.

In addition to activating Na^+ efflux by regulating the activity of the SOS1 antiporter, the SOS pathway may also turn off Na+ influx. The salt-sensitive phenotype of *sos3* was suppressed in an *athkt1* mutant (Rus *et al.* 2001). This suggests that the SOS3/SOS2 complex may also negatively regulate HKT1 function. It is possible that additional salt tolerance factors regulated by the SOS signaling pathway remain to be identified.

Vacuolar compartmentalization

Vacuolar compartmentalization of Na^+ ions is an efficient mechanism to maintain a lower concentration of Na^+ in the cytosol. Transport of Na^+ into the vacuole is mediated by a Na^+/H^+ antiporter. The proton-motive force for this transport is provided by both a proton pumping ATPase and a H^+-pyrophosphatase. Overexpression of a vacuolar H^+-pyrophosphatase (AVP1) in Arabidopsis enhanced sequestration of Na^+ into vacuoles and led to increased salt tolerance (Gaxiola *et al.* 2001). Likewise, transgenic plants overexpression the tonoplast Na^+/H^+ antiporter, AtNHX1 also exhibited increased salt tolerance (Apse *et al*. 1999). Here again, the SOS regulatory pathway may have a role. A study by Qiu *et al.* (2003) showed that the addition of a constitutively active SOS2 allele into a *sos2* mutant background increased tonoplast Na^+/H^+ antiporter activity. However, the antiporter activity was not affected by the *sos3* mutant. Regulation of tonoplast Na^+ transport may instead occur through SOS3-like calcium binding proteins (SCaBPs that have been shown to interact with SOS2).

Conclusions

The identification and characterization of sodium transporters in plants has helped in understanding the mechanism of ion homeostasis. Systematic genetic, molecular, and biochemical studies carried out with the *Arabidopsis* mutants has demonstrated the existence of the SOS signaling pathway that senses salt stress, either through the Ca^{2+} sensing activity of SOS3 or direct sensing of Na^+ by SOS1, and regulates Na^+ efflux and other ion transport and salt tolerance mechanisms. It is hoped that these studies, along with further work on the molecular mechanisms of ion homeostasis and stress sensing and signaling, will uncover the most promising approaches to improve the performance of crop plants under saline conditions.

References

Apse MP, Aharon GS, Snedden WS and Blumwald E 1999. Salt tolerance conferred by overexpression of a vacuolar Na^+/H^+ antiport in *Arabidopsis*. Science, 285: 1256-1258

Blumwald E, Aharon GS and Apse P 2000. Sodium transport in plant cells. Biochim. Biophys. Acta, 1465: 140-151

Flowers TJ, and AR Yeo 1995. Breeding for salinity resistance in crop plants. Where next? Aust. J. Plant Physiol, 22: 875-884

Gaxiola RA, Li J, Undurraga S, Dang V, Allen GJ, Alper SL and Fink GR 2001. Proc. Natl. Acad. Sci. USA, 98: 11444-11449

Glenn E, Brown JJ and Blumwald E 1999. Salt tolerance and crop potential of halophytes. Crit. Rev. Plant Sci, 18: 227–255

Halfter U, Ishitani M and Zhu J-K 2000. Proc. Natl. Acad. Sci. USA, 97: 3735-3740

Laurie S, Feeney KA, Maathuis FJM, Heard PJ, Brown SJ and Leigh RA 2002. A role for HKT1 in sodium uptake by wheat roots. Plant J, 32: 139-149

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 CS 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

Qiu, QS, Guo Y, Dietrich MA, Schumaker KS and Zhu J-K 2002. Regulation of SOS1, a plasma membrane Na+/H+ exchanger in *Arabidopsis thaliana*, by SOS2 and SOS3. Proc. Natl. Acad. Sci. USA, 99: 8436-8441

Quintero FJ, Ohta M, Shi H, Zhu, J-K and Pardo JM 2002. Reconstitution in yeast of the *Arabidopsis* SOS signaling pathway for Na^+ homeostasis. Proc. Natl. Acad. Sci. USA, 99: 9061-9066

Rubio F, Gassmann W and Schroeder JI 1995. Sodium driven potassium uptake by the plant potassium transporter HKT1 and mutations conferring salt tolerance. Science, 270: 1660-1663

Rus A, Yokoi S, Sharkhuu A, Reddy M, Lee B-H, Matsumoto TK, Koiwa H, Zhu J-K, Bressan RA and Hasegawa PM 2001. Proc. Natl. Acad. Sci. USA, 98: 14150-14155

Schachtman DP 2000. Molecular insights into the structure and function of plant K^+ transport mechanisms. Biochim. Biophys. Acta, 1465: 127-139

Shi H, Ishitani M, Kim C-S and Zhu J-K 2000. Proc. Natl. Acad. Sci. USA, 97: 6896-6901

Shi H, Quintero FJ, Pardo JM and Zhu J-K 2002. Plant Cell, 14: 465-477

Uozumi N, Kim EJ, Rubio F, Yamaguchi T, Muto S, Tsuboi A, Bakker EP, Nakamura T and Schroeder JI 2000. Plant Physiol, 122: 1249-1259

Zhu, J-K 2003. Regulation of ion homeostasis under salt stress. Curr. Opin. Plant Biol, 6: 441-445