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Involvement of SlSOS2 in tomato salt tolerance

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The Ca^{2+}-dependent SOS pathway has emerged as a key mechanism in the homeostasis of Na^+ and K^+ under saline conditions. We recently identified and functionally characterized by complementation studies in yeast and Arabidopsis the gene encoding the calcineurin-interacting protein kinase of the SOS pathway in tomato, *SlSOS2*.[1] We also show evidences on the biotechnological potential of *SlSOS2* conferring salt tolerance to transgenic tomato. The increased salinity tolerance of *SlSOS2* overexpressing plants is associated with higher sodium content in stems and leaves. SlSOS2 overexpression upregulates the Na^+/H^+ exchange at the plasma membrane (SlSOS1) and K^+, Na^+/H^+ antiport at the endosomal and vacuolar compartments (LeNHX2 and LeNHX4). Therefore, SlSOS2 seems to be involved in tomato salinity tolerance through regulation of Na^+ extrusion from the root, active loading of Na^+ into the xylem and Na^+ and K^+ compartmentalization.

Keywords: Na^+ and K^+ homeostasis, salt tolerance, SlSOS2 overexpression, *Solanum lycopersicum*, transgenic tomato

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Soil salinity has become one of the major worldwide agricultural problems limiting crop productivity. Thus, understanding plant tolerance to salinity is of significant importance and represents one of the major research topics in Plant Biology today. Plants can recognize salt stress and trigger appropriate responses involving changes in metabolism, growth and development because they have evolved signal transduction pathways. In Arabidopsis, the SOS signal transduction pathway is a key for Na^+ homeostasis and salinity tolerance.[2] The SOS pathway is constituted by the Na^+/H^+ exchanger SOS1 and the regulatory proteins SOS2 and SOS3. SOS3 is a calcium-binding protein sensing cytosolic calcium changes elicited by salt stress,[3] whereas SOS2 is a serine/threonine protein kinase which can interact with SOS3 upon its activation by Ca^{2+}.[4] This interaction results in the formation of the SOS3/SOS2 complex and the activation of its kinase activity, which eventually leads to phosphorylation and subsequent activation of the plasma membrane Na^+/H^+ exchanger SOS1, involved in Na^+ extrusion from the cell and long distance Na^+ transport.[5-8] In Arabidopsis, it was demonstrated that SOS2 also regulates the activity of vacuolar transporters such as the V-ATPase,[9] cation/proton antiporters of the NHX family[10] or the Ca^{2+}/H^+ exchanger CAX1.[11] Thus, SOS2 seems to be a key regulatory component of salt tolerance and therefore can be considered a candidate gene to enhance salinity tolerance in crops such us tomato. The objective of the work by Huertas et al. was to explore the biotechnological potential of SlSOS2 to improve salt stress tolerance of tomato and, at the same time, to search for the targets of SlSOS2 in this plant species.

SOS2 (CIPK24) belongs to the SnRK3/calcineurin-interacting protein kinase-(CIPK) subfamily with 25 and 30 members in Arabidopsis and rice, respectively[12] and a likely similar number in tomato.[1] Reconstitution of the tomato SOS system in appropriate yeast mutant strains together with functional complementation of the salt-hypersensitive phenotype of Arabidopsis *sos2* mutant by SlSOS2 allowed us to demonstrate that the isolated tomato SOS2-like nucleotide sequence is the true ortholog of *AtSOS2* and therefore the encoded protein should operate in a tomato SOS salt signal

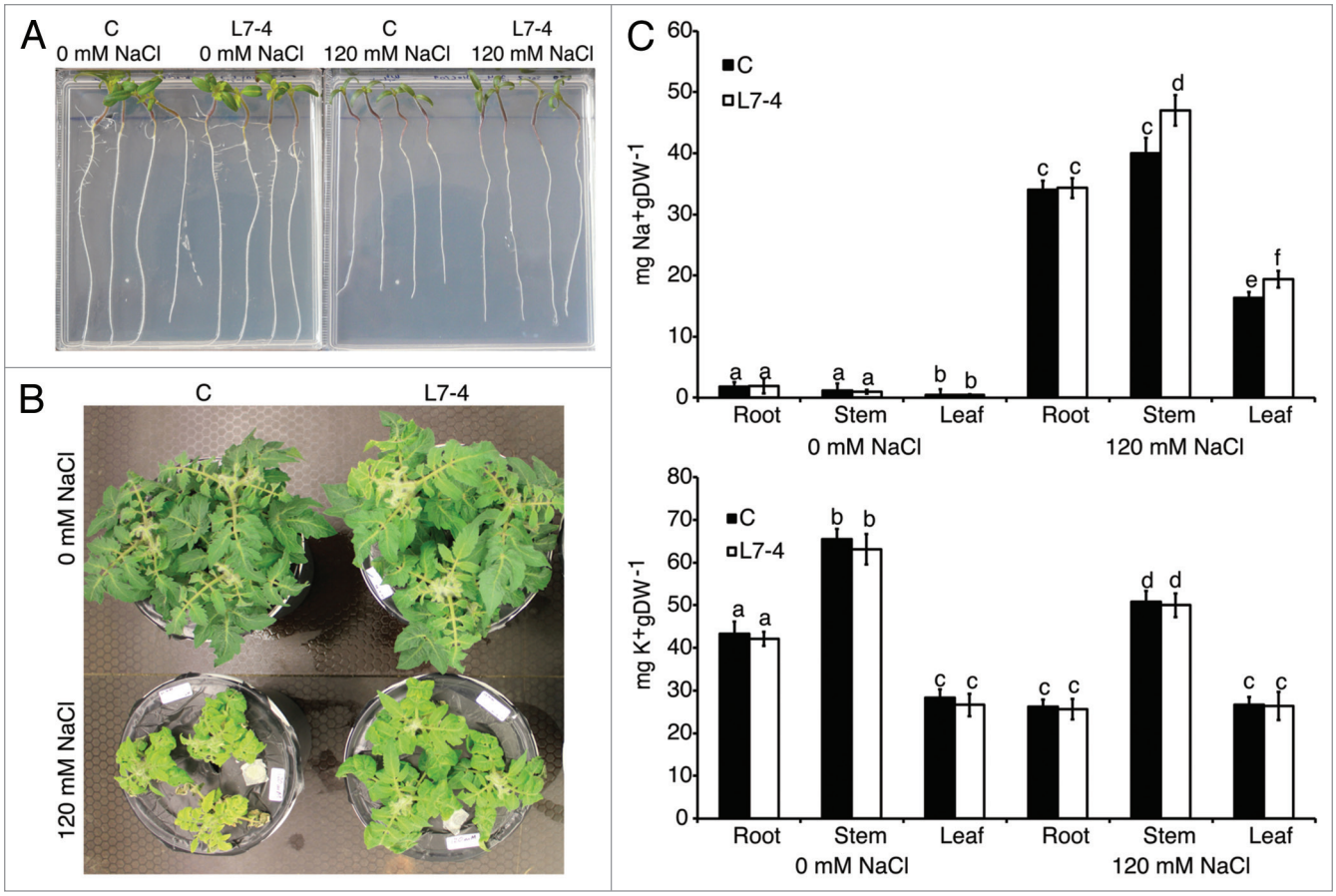

Figure 1. Effect of *SlSOS2* overexpression on growth (A and B) and Na^+ and K^+ content (C) of tomato plants cultivated with or without NaCl. Untransformed (line C) and T2 seedlings overexpressing SlSOS2 (line L7-4) were grown on agar plates in MS medium for four days and further cultivated in the same medium supplemented with 0 and 120, mM NaCl for 5 additional days (A). Twenty five day-old untransformed (line C) or transgenic plants were grown in hydroponics in ¼ Hoagland nutrient solution to which 0 or 120 mM NaCl was added for the last 10 d of cultivation (B). Na^+ and K^+ contents were determined in plants grown in as in (B) and after 5 d treatment with 0 and 120 mM NaCl (C) and data were expressed as means ± SD of three independent experiments, using five plants per line and treatment.

transduction pathway. We undertook a preliminary approach to studying the role of SlSOS2 in salinity tolerance by measuring its transcript levels in two tomato species differing in salt tolerance and grown in the presence and absence of NaCl. The expression of endogenous *SlSOS2* was found to be induced by NaCl in salt-sensitive but not in salt-tolerant tomato species, which showed a high *SlSOS2* expression even in the absence of stress. Therefore, a high constitutive expression of *SlSOS2* seems to be part of a plant strategy to overcome an eventual increase of NaCl in the external medium, suggesting the potential impact of this protein to confer salt tolerance when overexpressed in tomato. The better salt tolerance of transgenic tomato overexpressing *SlSOS2* over untransformed plants (**Fig. 1A and B**) supports the above hypothesis. The increased salt tolerance of transgenic plants was linked to a higher Na^+ content without changes in K^+ content in leaves and stems (**Fig. 1C**), suggesting that regulation of ion transporters responsible for Na^+ partitioning at whole plant level and Na^+ compartmentalization at cell level might be the basis of the salt tolerant phenotype. In this regard, it was previously shown that salt-tolerant tomato species accumulate Na^+ mainly in the stems and older leaves, while salt-sensitive species/varieties accumulate Na^+ preferentially in roots.[13,14]

Overall, the results of Huertas et al. point to the regulatory role of SlSOS2 on the activity of transporters responsible for Na^+ and K^+ homeostasis (**Fig. 2**). Indeed, expression of the plasma membrane Na^+/H^+ antiporter SlSOS1,[8] and the endosomal and vacuolar K^+, Na^+/H^+ antiporters LeNHX2 and LeNHX4 [14-16] were found to be higher in roots and shoots of the transgenic plants than in those of untransformed controls, particularly under saline conditions. Accordingly, a higher Na^+/H^+ exchange activity was found in the plasma membrane, tonoplast and endosomal membrane vesicles isolated from plants overexpressing *SlSOS2* as compared with untransformed controls. The study of Na^+ transport in different cell types and plant organs can be critical to assess the role of the SOS pathway in xylem loading/unloading, Na^+ export by roots, retention in stems and the differential distribution/accumulation in leaves.[17] In this respect, tomato represents a good model to study the involvement of the SOS pathway in long-distance Na^+ transport, since its anatomical structure with a well developed stem allows a precise dissection of the relative content of Na^+ in stem vs. leaf.

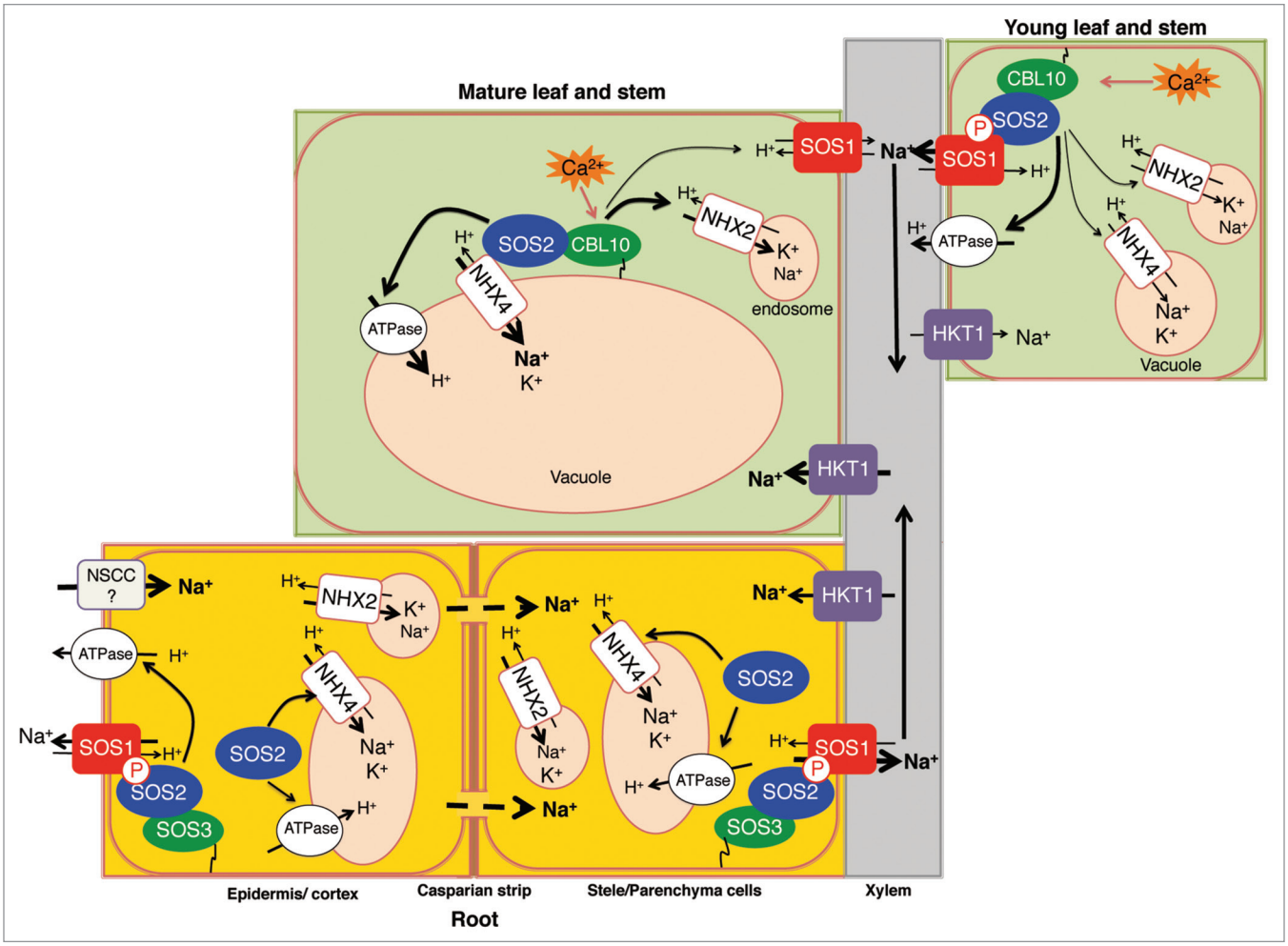

Figure 2. Schematic diagram showing the regulation of some plasma membrane Na^+/H^+ and intracellular membrane K^+, Na^+/H^+ antiporters in tomato plants overexpressing *SlSOS2*. Activation of SlSOS1 by SlSOS2 will promote Na^+ extrusion out of the root, Na^+ loading into the xylem and Na^+ efflux from leaves. Activation of LeNHX2 and LeNHX4 by SlSOS2 will cause Na^+ (and K^+) accumulation in the vacuolar and endosomal compartments of roots and leaves. SlSOS2 also activates the plasma membrane and tonoplast H^+-ATPase responsible for energizing the antiport activity of SlSOS1, LeNHX2 and LeNHX4. It is hypothesized that in aerial parts CBL10/SlSOS2 kinase complex would promote cell Na^+ extrusion by SOS1 in young/developing leaf and stem tissues and intracellular Na^+ compartmentalization by NHXs transporters in mature/old leaf and stem tissues. Compartmentalization of Na^+ in old tissues would be favored by a more active Na^+ retrieval systems from xylem through HKT1-like Na^+ transporters (see more explanations in the text). Arrow thickness indicates the hypothetical dominance of the ion flux.

In this sense, we have previously shown that besides its main action in extruding Na^+ out the root, SlSOS1 is critical for the partitioning of Na^+ in plant organs and the ability of tomato plants to retain Na^+ in the stems, thus preventing Na^+ from reaching the photosynthetic tissues.[8] In accordance with these findings, our work indicates that SlSOS2-induced expression and activity of SlSOS1 in roots and shoots might promote the efflux of Na^+ out of the root epidermal cells as well as the active loading of Na^+ into the xylem and/or its retention in the stem and distribution to leaves (**Fig. 2**). It has been hypothesized that the transport function of SOS1 in xylem loading should be coordinated to that of HKT1-like Na^+ transporters in xylem unloading for long-distance transport of Na^+ and its subsequent distribution in photosynthetic tissues.[17-19] Thus, SlSOS1 could mediate the transfer of Na^+ from the xylem parenchyma cells to xylem, preferentially in roots, while tomato HKT1-like transporters could mediate the reverse flow in more differentiated root tissues and parts of the mature/developed leaf and stem. In this respect, we have recently isolated two isoforms of HKT1-like transporters in tomato, SlHKT1;1 and SlHKT1;2, which could operate together with SlSOS1 to promote Na^+ transport to the green tissues involved in cellular compartmentation of Na^+ (Belver et al., unpublished results).

To prevent Na^+ toxicity at the cellular level it is important not only to extrude Na^+ out of the cell but to efficiently sequester this ion in subcellular compartments like the vacuoles. As mentioned before, AtSOS2 could interact with proteins at the tonoplast membrane of Arabidopsis roots, like NHX1, CAX1 and the V-ATPase.[9-11] Work in our laboratory has identified four NHX isoforms in tomato responsible for Na^+ and K^+ compartmentation, LeNHX1 to LeNHX4. The more active isoforms are the endosomal K^+, Na^+/H^+ antiporter

LeNHX2 and the vacuolar Na^+, K^+/H^+ antiporter LeNHX4 that were also found to be NaCl induced.[14-16] As expected, we found an induced expression of LeNHX2 and LeNHX4 in both roots and shoots as well as an increased Na^+/H^+ and K^+/H^+ antiport activity at the endosomes and vacuolar membranes of *SlSOS2* overexpressing tomato roots, eventually leading to the accumulation of Na^+ (and also K^+) in cell compartments of the roots and shoots. It is worth noting that vacuolar sequestration of Na^+ (and K^+) not only lowers its concentration in the cytoplasm, but can also contribute to osmotic adjustment in order to maintain water absorption from saline solutions thus allowing plant cells to keep turgor and expansion under salt stress. In this respect, the capacity to sequester excessive Na^+, and other ions such as K^+, into the vacuole could be one of the key features of mature plants overexpressing *SlSOS2* in order to maintain growth, flowering and fruit production under salt stress (**Fig. 1B**). However, Na^+ extrusion to the external medium by SlSOS1 might be the main salt tolerance mechanism in the transgenic young seedlings, in which the tissues responsible for long-distance Na^+ transport and intracellular Na^+ accumulation are not well developed (**Fig. 1A**).

Since excess Na^+ is especially harmful for young leaves, Na^+ accumulation in shoots of tomato plants follows a different pattern in young and mature/old leaves.[13] Based on our results we can speculate that regulation of Na^+ and K^+ homeostasis by SlSOS2 is also different in young and mature/old leaves (**Fig. 2**). While in young leaves SlSOS1 would have a major role in Na^+ detoxification, in old leaves SlSOS1 would likely have a minor role, since a Na^+ efflux system at the plasma membrane is not needed to prevent Na^+ toxicity in cells having an efficient ion compartmentation mechanism. In the mature/old leaves the main mechanism to prevent Na^+ accumulation in the cytosol could probably be linked to the combined action of HKT1-like transporters mediating Na^+ unloading from the xylem[20] and NHX-type transporters such as LeNHX4 and LeNHX2 promoting Na^+ and K^+ accumulation in vacuoles and endosomes (**Fig. 2**).

In Arabidopsis, SOS2 (CIPK24) has been identified as a multi-functional protein kinase that regulates different aspects of salt tolerance by interacting with distinct CBL calcium sensor proteins.[21] While SOS2 is expressed throughout the plant, the CBL proteins that activate its kinase activity are localized in different parts of the plant. In roots SOS3 (CBL4) activates SOS2 to positively regulate SOS1 to extrude Na^+ out of the cell.[5,6] Our results demonstrate that SlSOS2 is activating NHX-type transporters in roots either independently or by interacting with a yet unknown CBL protein. In Arabidopsis, it is CBL10 (SCABP8) that functions in the shoot activating SOS2.[22-24] It seems likely that in tomato there is a functional counterpart to AtCBL10 activating SlSOS2 in aerial part. Nevertheless, it is not clear whether the CBL10-SOS2 kinase complex interacts with and activates NHX-type transporters in vacuolar compartments responsible for storage and detoxification of Na^+[22] or recruits SOS2 to the plasma membrane to activate SOS1.[23,24] As stated by Luan et al. it cannot be ruled out that in addition to regulating the activity of ion transporters, CBL10 could also regulate the location of the signaling event to initiate specific cell responses to salt stress in tomato. Our data on *SlSOS2* overexpressing plants indicate that both mechanisms could coexist and suggest that the prevalence of one mechanism over the other would depend on the ontogeny of the tissue and the external salt conditions, as discussed above.

In summary, we provide evidences that overexpression of *SlSOS2* would reinforce the salt signal transduction pathway that eventually leads to efflux and/or compartmentation of toxic Na^+. In fact, our results indicate that SlSOS2 can regulate some of the mechanisms of ion (Na^+ and/or K^+) uptake/exclusion, translocation and compartmentation in tomato. Moreover, this work shows that study of the intricate signaling pathways involved in the plant response to environmental stress is a promising area of research, which may ultimately lead to improvements in plant stress resistance through genetic manipulation.

Acknowledgments

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