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An *Arabidopsis* mutant that requires increased calcium for potassium nutrition and salt tolerance

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ABSTRACT **Potassium (K^+) nutrition and salt tolerance are key factors controlling plant productivity. However, the mechanisms by which plants regulate K^+ nutrition and salt tolerance are poorly understood. We report here the identification of an *Arabidopsis thaliana* mutant, *sos3* (*s*alt-*o*verly-*s*ensitive *3*), which is hypersensitive to Na^+ and Li^+ stresses. The mutation is recessive and is in a nuclear gene that maps to chromosome V. The *sos3* mutation also renders the plant unable to grow on low K^+. Surprisingly, increased extracellular Ca^{2+} suppresses the growth defect of *sos3* plants on low K^+ or 50 mM NaCl. In contrast, high concentrations of external Ca^{2+} do not rescue the growth of the salt-hypersensitive *sos1* mutant on low K^+ or 50 mM NaCl. Under NaCl stress, *sos3* seedlings accumulated more Na^+ and less K^+ than the wild type. Increased external Ca^{2+} improved K^+/Na^+ selectivity of both *sos3* and wild-type plants. However, this Ca^{2+} effect in *sos3* is more than twice as much as that in the wild type. In addition to defining the first plant mutant with an altered calcium response, these results demonstrate that the *SOS3* locus is essential for K^+ nutrition, K^+/Na^+ selectivity, and salt tolerance in higher plants.**

Soil salinity presents an increasing threat to plant agriculture as more and more of the world's arable land becomes salinized (1). High salinity exerts its detrimental effects on plants because of ion toxicity as well as osmotic stress (2). In most saline soils, Na^+ is the major toxic cation. One harmful effect of Na^+ is that it disrupts K^+ nutrition (3, 4). K^+ is one of the three major components in fertilizers applied to soils and a key factor controlling crop productivity (5). Physiologic studies have established that K^+ uptake by plant roots is mediated by at least two mechanisms, i.e., the mechanism 1 (high-affinity) and 2 (low-affinity) transport systems (3, 6, 7). Because soil solutions often contain <1 mM K^+, the high-affinity system is thought to play the predominant role in plant potassium nutrition (5). High-affinity K^+ uptake is a tightly regulated process. When plant roots experience K^+ deficiency, the high-affinity system is induced and becomes essential for plant growth (8–10). Both high- (11, 12) and low-affinity (13–15) K^+ transporters have been cloned from plants. However, it remains to be shown which of these cloned transporters actually function in root K^+ uptake from the soil solutions. Furthermore, the mechanisms by which roots detect K^+ deficiency and the signaling pathway that up-regulates the high-affinity system are still unknown.

The importance of the mechanism 1 transport system in plant salt tolerance is underlined by recent genetic evidence suggesting that it is essential for plant survival and growth under salinity stress (10). The *Arabidopsis sos1* mutation which causes reduced mechanism 1 K^+ uptake also leads to increased sensitivity of plant growth to inhibition by NaCl (10). Kinetic analysis suggested that mechanism 1 uptake has a higher K^+/Na^+ selectivity than mechanism 2 (3). One factor known to be involved in the regulation of K^+/Na^+ selectivity of K^+ transport during NaCl stress is Ca^{2+} (16). Under NaCl stress, Ca^{2+} increases the selectivity of root K^+ transport systems (16). High external Ca^{2+} has been shown to improve plant salt tolerance (17). However, the cellular components that mediate these Ca^{2+} effects have not been identified genetically or biochemically.

We report here the identification of a genetic locus, *SOS3*, in *Arabidopsis thaliana* that is necessary for salt tolerance, K^+ nutrition, and Ca^{2+} response. A mutation in this locus (*sos3*) results in plant hypersensitivity toward NaCl inhibition and in an increased requirement for K^+. Surprisingly, high external Ca^{2+} can suppress the mutant phenotype. *sos3* represents the first higher plant mutant with an altered response to Ca^{2+}. The mutation reveals an important link between plant responses to Na^+ stress, K^+ deficiency, and Ca^{2+} regulation. Our results suggest that Ca^{2+} plays a fundamental role in regulating root potassium nutrition and plant salt tolerance.

MATERIALS AND METHODS

Isolation of the *sos3* Mutant. *A. thaliana* (ecotype Columbia) carrying the homozygous recessive *gl1* mutation was the parental strain of ethyl methanesulfonate- or fast neutron-mutagenized seeds. M2 populations from ethyl methanesulfonate- or fast neutron-mutagenized seeds or T4 pools from *Agrobacterium*-transformed lines (18), were screened using the root-bending assay of Wu *et al.* (10). Seeds were surface-sterilized and germinated on medium containing Murashige and Skoog (MS) salts (19), 3% (wt/vol) sucrose, and 1.2% (wt/vol) agar, pH 5.7. When appropriate, seedlings were transplanted to pot medium and grown to maturity. Growth conditions were as described (10).

Genetic Analysis. The *sos3* mutant was backcrossed with the wild-type Columbia *gl1* background, and the F1 seedlings were allowed to self-pollinate. F1 and F2 seedlings were scored for salt sensitivity using the root-bending assay (10). For mapping of the *SOS3* locus, homozygous *sos3* plants in the Columbia *gl1* background were crossed to plants of the Landsberg *erecta* background. From the segregating F2 generation, 426 homozygous *sos3* mutants were selected for mapping with molecular markers that are polymorphic between Columbia and Landsberg *erecta*.

Growth Measurement. Wild-type and *sos3* mutant seedlings (4 days old) grown on vertical MS agar plates were transferred to various agar media for stress treatment and growth measurements (10). To determine the K^+ requirement of mutant plants, seedlings were transferred to a modified MS medium A supplemented with various levels of KCl. Modified MS medium A contains potassium-free 1/20 strength MS major salts and 1× MS minor salts. For the determination of the Ca^{2+} requirement, seedlings were transferred to a modified MS medium B supplemented with various levels of $CaCl_2$. Modi-

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Abbreviations: MS, Murashige and Skoog; WT, wild type.
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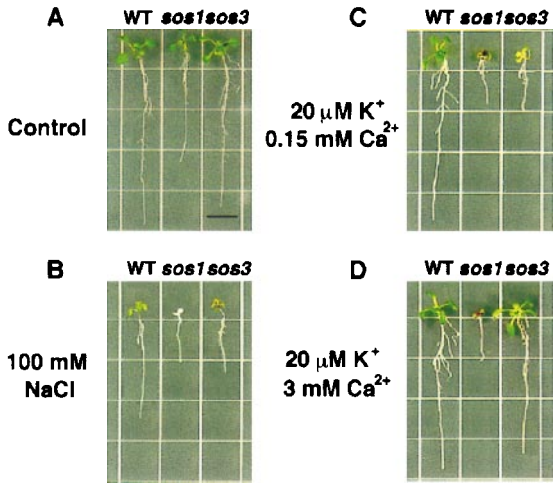

FIG. 1. Representative plants of wild-type (WT), *sos1*, and *sos3* mutants that were exposed to high-salt (100 mM NaCl) or low-K^+ (20 μM K^+) stresses. Seedlings were grown on vertical MS agar plates for 4 days (root length $\cong$ 1.5 cm) and then transferred to vertical agar plates containing control (*A,* MS nutrients), 100 mM NaCl (*B,* MS + 100 mM NaCl), or modified MS (see *Materials and Methods*) with 20 μM K^+ and 0.15 mM (*C*) or 3 mM Ca^{2+} (*D*) for 7 days. The growth of both *sos1* and *sos3* was inhibited by either NaCl (*B*) or low K^+ (*C*). However, the growth of *sos3* on low K^+ was restored by increased Ca^{2+} (*D*). (Bar = 1 cm.)

fied MS medium B contains potassium- and calcium-free 1/20 strength MS major salts, 1× MS minor salts, and 20 μM K^+ unless stated otherwise.

Determination of Ion Contents. Four-day-old seedlings grown on MS plates were transferred to 250-ml flasks containing a 50-ml solution of half-strength MS salts and 2% sucrose. The flasks were shaken at 120 rpm under constant fluorescent light for 5 days. The seedlings were transferred to 50-ml treatment solutions after being washed briefly twice with deionized water and once with appropriate treatment solutions. The treatment solutions were prepared by adjusting the K^+, Na^+, and Ca^{2+} concentrations of a calcium–potassium-free modified MS medium to the stated levels.

The calcium–potassium-free modified MS medium consists of the following: 1650 mg/liter NH_4NO_3, 370 mg/liter $MgSO_4{\cdot}7H_2O$, 165 mg/liter $(NH_4)_2HPO_4$, 27.8 mg/liter $FeSO_4{\cdot}7H_2O$, 37.3 mg/liter disodium EDTA, 0.7495 mg/liter NaI, 6.3 mg/liter H_3BO_3, 16.9 mg/liter $MnSO_4{\cdot}H_2O$, 8.6 mg/liter $ZnSO_4{\cdot}7H_2O$, 0.25 mg/liter $Na_2MO_4{\cdot}2H_2O$, 0.016 mg/liter $CuSO_4{\cdot}5H_2O$, and 0.0267 mg/liter $CoSO_4{\cdot}6H_2O$.

The seedlings were grown in the treatment solutions for 4 days, briefly rinsed three times with deionized water, and dried in a 80°C oven. The samples were digested in 3 ml of concentrated nitric acid overnight followed by boiling for ~1 h until the solution became completely clear. The solution was brought to 25 ml by adding 0.25% lanthanum in 2.5% nitric acid. The K^+, Na^+, and Ca^{2+} contents in the solution were determined by atomic absorption spectrophotometry.

RESULTS

Isolation of the *sos3* Mutant. The *sos3* mutant was identified from a large-scale screening of *sos* mutants. Altogether, ~260,000 seedlings from ethyl methanesulfonate- or fast neutron-mutagenized M2 seeds, or T4 seeds from T-DNA-mutagenized plants were screened on 50 mM or 75 mM NaCl by using the root-bending assay of Wu *et al.* (10). Of 41 *sos* mutants isolated (J.K.Z., unpublished data), one line defines a new locus, designated as *SOS3*. The *sos3* mutant was identified from fast neutron-mutagenized M2 seeds. Fig. 1 shows the phenotype of the *sos3* mutant under the conditions of NaCl stress or low K^+. On regular nutrient medium (MS salts), *sos3* seedlings were indistinguishable from the wild type. However, under high NaCl or low K^+ stresses, the growth of *sos3* plants was inhibited to a greater extent than that of the wild type (Fig. 1).

Table 1. Genetic analysis of the *sos3* mutant

Parental genotype	Seedlings tested, *n*	Resistant*	Sensitive*
SOS3/SOS3 × *sos3/sos3*	131	131	0
SOS3/sos3 × *SOS3/sos3*	1235	925	310
sos3/sos3 × *sos1-1/sos1-1*	34	34	0

*Resistance or sensitivity was determined in the root-bending assay, using 100 mM NaCl.

Genetic Analysis. The *sos3* mutant was backcrossed with the wild-type background. The resulting F1 plants all exhibited the wild-type phenotype in response to NaCl stress. F2 progeny from the cross segregated 925:310 (WT:*sos3*) (Table 1). This indicated that *sos3* is a monogenic recessive mutation in a nuclear gene. The *sos3* mutant was also crossed to *sos1-1*. The resulting F1 plants exhibited the wild-type phenotype under NaCl stress, indicating that the *sos3* mutant defines a locus that is different from *SOS1*.

Chromosome Mapping of *sos3*. To map the *sos3* mutation, the mutant in the Columbia background was crossed with wild-type Landsberg *erecta* plants. From the segregating F2 population, 426 salt hypersensitive plants were selected, and genomic DNA was extracted from each of the plants. Initial screening with simple sequence length polymorphism markers (20) from each of the five chromosomes of *Arabidopsis* indicated that the *sos3* mutation is linked to the marker *nga76* on chromosome V. Further analysis with additional markers on chromosome V showed that *sos3* is very tightly linked to *nga139*. Only 4 recombination events were detected among the 426 plants (equivalent of 852 chromosomes) examined. Therefore, the *SOS3* locus is ~0.5 cM away from *nga139*. Based on a recently published physical map of chromosome V (21), this genetic distance likely corresponds to <100 kb. As shown in Fig. 2, *sos3* is ~1.6 cM (14 recombination events detected among the 426 plants) from marker *CDPK9* which represents a locus encoding a calcium-dependent protein kinase (22).

sos3 Is Specifically Hypersensitive to Na^+ and Li^+. Root elongation is a convenient and accurate indicator of *Arabidopsis* seedling growth (10). Measurement of root elongation showed quantitatively that the *sos3* mutant is hypersensitive to NaCl stress (Fig. 3). The concentration of NaCl that decreased the root elongation rate by 50% relative to medium without salt (I_{50}) was estimated. The I_{50} for *sos3* and the wild-type seedlings are ~37 and 100 mM, respectively (Fig. 3). To determine whether the *sos3* mutant is hypersensitive to osmotic stress or to specific ions, the seedlings were also treated on media supplemented with KCl, LiCl, CsCl, or mannitol. The results show that *sos3* is also hypersensitive to LiCl, but not to KCl, CsCl, or mannitol (Fig. 3). Therefore, the *sos3* mutation does not result in hypersensitivity to general osmotic stress. Rather, the hypersensitivity is restricted to Na^+ and Li^+.

Potassium Requirement of *sos3*. Because the salt-hypersensitive phenotype of *sos1* mutants has been found to cosegregate with the inability to grow on low K^+ culture medium (10), we tested whether *sos3* is similarly affected. Although the *sos3* mutant seedlings grew normally on medium containing 20 mM K^+, they failed to grow on a medium containing 20 μM K^+ (Fig. 1). In contrast, wild type grew well on both 20 μM and 20 mM K^+ media (Fig. 1). Forty-five *sos3* seedlings were selected from a segregating F2 population based on their NaCl sensitivity. These seedlings all failed to grow on 20 μM K^+, indicating that the inability of *sos3* seedlings to grow on low K^+ cosegregates with their salt-hypersensitive phenotype. The results suggest an essential role of the *SOS3* gene in potassium nutrition under limiting K^+ availability.

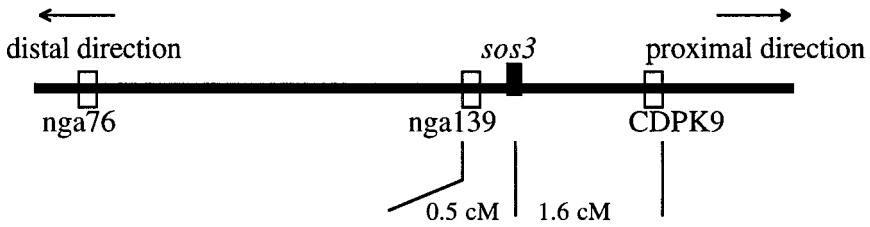

FIG. 2. Genetic map showing the position of the *SOS3* locus relative to selected physical markers on chromosome V.

To determine whether *sos3* seedlings require more or less K^+ than *sos1-1*, their root growth was measured over a wide range of K^+ levels. As shown in Fig. 4A, while *sos1-1* required 20 mM K^+ for significant root growth, ~1 mM was sufficient for *sos3*. For wild-type seedlings, only 0.1 mM K^+ was necessary. Both *sos3* and the wild type had maximal root growth at 10 mM K^+. K^+ levels above 10 mM inhibited *sos3* and wild-type root growth, whereas *sos1* root growth was improved by these high levels of K^+.

Calcium Requirement of *sos3*. Surprisingly, we found that the growth defect of *sos3* seedlings on low potassium growth medium could be corrected by raising the external Ca^{2+} concentration to >2 mM (Figs. 1*D* and 4*B*). Calcium also stimulated root growth of wild-type plants. However, the wild type required only 125 μM Ca^{2+} for maximal growth, whereas 1.25 mM Ca^{2+} was not enough for *sos3* mutant plants (Fig. 4*B*). In comparison, up to 10 mM Ca^{2+} did not significantly increase the growth of *sos1* plants on low potassium growth medium (Fig. 4*B*).

We determined whether increased Ca^{2+} could similarly correct the growth defect of *sos3* on medium containing high levels of NaCl. Indeed, Ca^{2+} substantially increased the salt tolerance of *sos3* plants (Fig. 5). On medium containing 50 mM NaCl, root growth of *sos3* was completely inhibited when no Ca^{2+} was supplemented. However, 1.5 mM or higher Ca^{2+} resulted in significant growth of *sos3* plants (Fig. 5). Although elevated Ca^{2+} also increased the salt tolerance of the wild type, the effect was not as great as for *sos3* mutant plants (Fig. 5). The wild type showed significant growth even without any added Ca^{2+}. Although <1.5 mM Ca^{2+} reduced the survival rate of *sos1* plants, high Ca^{2+} was not able to increase the growth of *sos1* on 50 mM NaCl (Fig. 5). Increased Ca^{2+} did not substantially increase the growth of *sos3*, *sos1*, or the wild-type plants on 100 mM NaCl (data not shown). Perhaps at 100 mM NaCl, the ameliorating effect of Ca^{2+} was simply swamped out.

Ca^{2+} Improves K^+/Na^+ Selectivity of *sos3* Seedlings. It has been suggested that high external Ca^{2+} mitigates NaCl effect by improving the K^+:Na^+ ratio of plant cells (16). To determine whether high Ca^{2+} rescues *sos3* growth under NaCl stress through such a mechanism, K^+ and Na^+ contents were measured in 50 mM NaCl-stressed *sos3* seedlings under high or low external Ca^{2+}. Similar to *sos1-1*, *sos3* seedlings exhibited K^+ deficiency under NaCl stress (Table 2). However, *sos3* seedlings accumulated more Na^+ than the wild type. In comparison, NaCl-stressed *sos1-1* accumulated less Na^+ than the wild type (23). There was virtually no difference between the K^+ or Na^+ content in *sos3* and wild-type seedlings that were grown on MS medium alone without NaCl (data not shown).

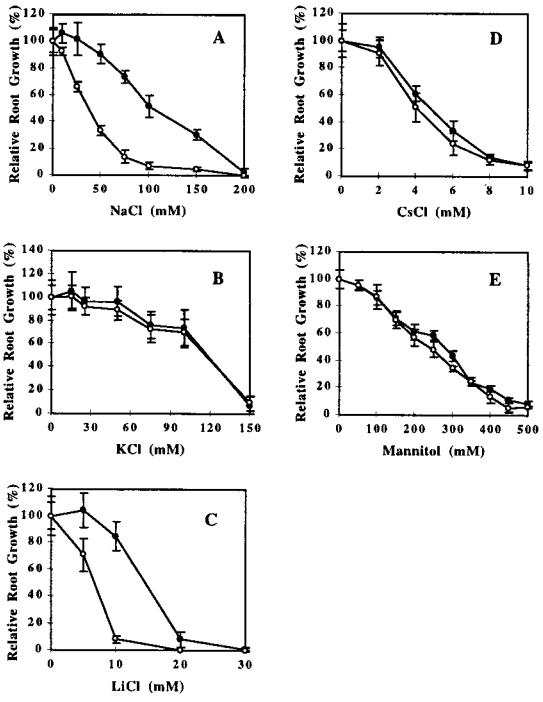

FIG. 3. The *sos3* mutant is hypersensitive to Na^+ and Li^+ but not to K^+, Cs^+, or general osmotic stress induced by mannitol. Wild-type (●) and *sos3* (○) seedlings were grown for 4 days on vertical agar plates containing MS nutrients and then transferred to vertical agar plates containing MS supplemented with NaCl, KCl, LiCl, CsCl, or mannitol. Root growth was measured 6 days after the transfer. Growth on MS nutrients was considered as 100%. Values are the averages of 15 seedlings.

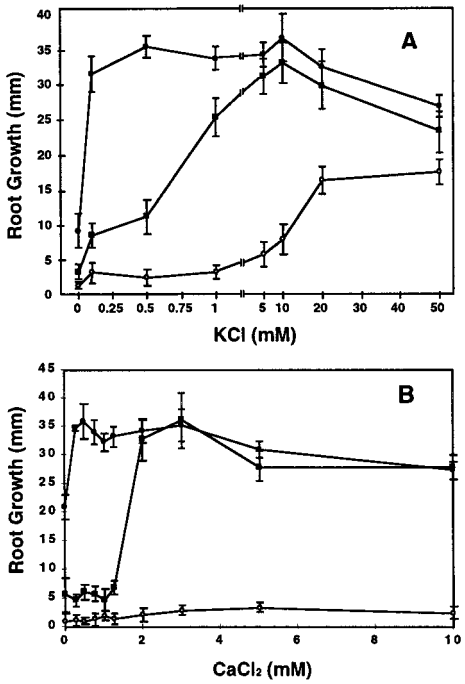

FIG. 4. High Ca^{2+} restores the growth of *sos3* on low-K^+ growth medium. (*A*) Optimal growth of wild type (●), *sos1* (○), and *sos3* (■) seedlings requires different external K^+ concentrations in the media. (*B*) Ca^{2+} dose-response curve of wild type (●), *sos1* (○), and *sos3* (■) seedlings. The medium contained 20 μM K^+. Media were modified from MS (see *Materials and Methods*). Growth measurement was similar to that in Fig. 3 except that the measurement was taken 3 days after the seedling transfer to minimize the effect of residual K^+ and Ca^{2+} carried by the plants from MS medium. [Bars = SD (n = 15).]

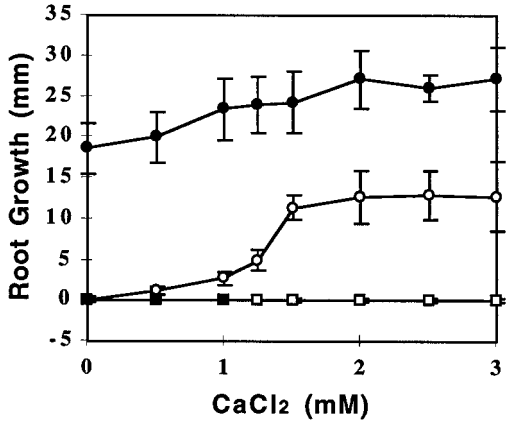

FIG. 5. Effect of Ca^{2+} on salt tolerance of wild-type (●), *sos1* (■, □), and *sos3* (○) seedlings. ■, *sos1* seedlings that were killed by the treatments. Four-day-old seedlings grown on MS plates were transferred to grow on nutrient media supplemented with 50 mM NaCl and various levels of Ca^{2+}. The nutrient media were the same as for MS except that Ca^{2+} was removed. Growth measurement was similar to that in Fig. 3. [Bars = SD (n = 15).]

High external Ca^{2+} increased cellular K^+ content in both *sos3* and wild- type plants (Table 2). This Ca^{2+} effect was more than twice as large in *sos3* (30%) than in the wild type (14%). High Ca^{2+} also reduced Na^+ content in both *sos3* and the wild type. Again, this effect was more pronounced in *sos3* plants. In summary, high Ca^{2+} increased the K^+:Na^+ ratio by 57% and 26% in *sos3* and the wild type, respectively. The data suggest that the Ca^{2+} effect observed on *sos3* may be mediated at least partially through the improvement of potassium nutrition and K^+/Na^+ selectivity.

***sos1* Is Epistatic to *sos3*.** The *sos3* and *sos1* mutants exhibit similar phenotypes in that they are hypersensitive to Na^+ and Li^+ and not capable of growing on low K^+ media. Thus, the two *SOS* genes may function in the same pathway that regulates K^+ nutrition and salt tolerance. To determine the epistatic relationship between *sos3* and *sos1*, we constructed *sos1sos3* double mutant. Thirty *sos* mutants were selected from the selfed F2 progeny of a cross between *sos3sos3* and *sos1-1sos1-1*. To identify a double mutant, these *sos* mutants were each test crossed to *sos3sos3* and *sos1-1sos1-1*. One *sos1sos3* double mutant line was identified because the F1 progenies from both of the test crosses showed a NaCl hypersensitive phenotype.

The response of *sos1sos3* to NaCl is similar to that of *sos1* (Fig. 6*A*). Without NaCl, the growth rate of *sos1sos3* seedlings was similar to that of *sos1*. With 10 or 25 mM NaCl, growth of *sos1sos3* seedlings was reduced to similar levels as *sos1*. The response of *sos1sos3* seedlings to Ca^{2+} is also the same as that of *sos1*. As shown in Fig. 6*B*, the growth of *sos3* on low K^+ culture medium was restored by high Ca^{2+}, whereas high Ca^{2+} had little effect on the growth of *sos1* or *sos1sos3*. Therefore, increased Ca^{2+} suppressed *sos3* but not *sos1* or *sos1sos3* mutations. Taken together, these results suggest that *sos1* is epistatic to *sos3*.

DISCUSSION

The *sos3* mutation defines a genetic locus that is essential for salt tolerance as well as growth on low-K^+ culture media. It is

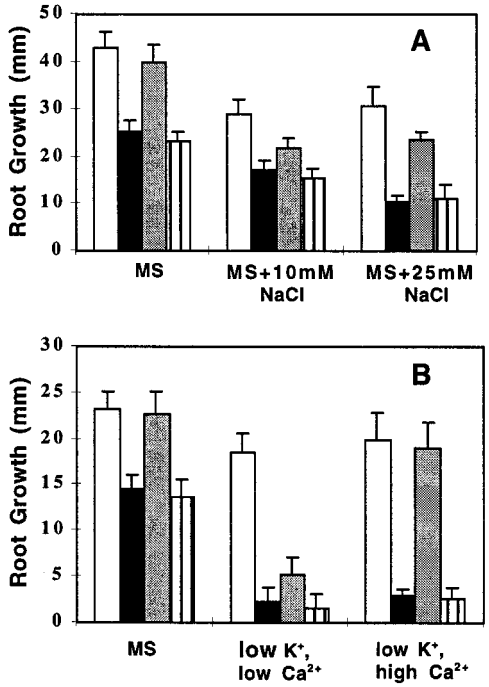

FIG. 6. *sos1sos3* double mutant resembles *sos1* both in salt sensitivity and in response to Ca^{2+}. (*A*) Growth of wild-type (□), *sos1* (■), *sos3* (▨), and *sos1sos3* (▩) seedlings on MS medium, MS medium supplemented with extra 10 or 25 mM NaCl. (*B*) Growth of wild-type (□), *sos1* (■), *sos3* (▥), and *sos1sos3* (▦) seedlings on MS (20 mM K^+, 3 mM Ca^{2+}), low K^+–low Ca^{2+} (20 μM K^+, 0.15 mM Ca^{2+}), or low K^+–high Ca^{2+} (20 μM K^+, 3 mM Ca^{2+}) media. Growth measurement was conducted on the seventh day after the transfer. For Ca^{2+}-responsive treatment (*B*), the measurement was taken 3 days after seedling transfer to minimize the effect of residual K^+ and Ca^{2+} carried by the plants from MS medium. [Bars = SD (n = 15).]

surprising that increased external Ca^{2+} could completely suppress the growth defect on low-K^+ culture medium and partially suppress the salt-hypersensitive phenotype of the *sos3* mutant. The *sos3* mutation thus uncovers a fundamental role of calcium in regulating potassium nutrition and salt tolerance in plant roots. A beneficial effect of external Ca^{2+} on plant salt tolerance was reported decades ago (16, 17). Despite of intensive efforts, the underlying mechanisms of this effect remain unclear. The identification of the *sos3* mutation provides an excellent opportunity to resolve the molecular nature of this important calcium response.

The calcium effect is specific to *sos3* and was not observed for *sos1* (Figs. 4*B* and 5). Because *sos1* and *sos3* exhibit similar phenotypes, the two genes probably function in a common pathway that regulates potassium nutrition and salt tolerance. Genetic evidence suggests that *sos1* is epistatic to *sos3*. Thus, *SOS1* may encode a component upstream of *SOS3*.

The failure of *sos3* seedlings to grow on low-K^+ culture medium suggests that the mutant may be defective in high-affinity K^+ uptake. We measured K^+ uptake (10) in *sos3* seedlings or excised seedling roots over a wide range of

Table 2. Effects of external Ca^{2+} levels on K^+/Na^+ selectivity of *sos3* and wild-type seedlings treated with 50 mM NaCl

	K^+ content, mg/g dry wt % increase		Na^+ content, mg/g dry wt % decrease		K^+/Na^+ ratio % increase	
Ca^{2+} level	Low*	High†	Low*	High†	Low*	High†
WT	53.4 ± 2.0	60.9 ± 0.4 (+14%)	29.8 ± 2.2	27.0 ± 1.3 (−9%)	1.8	2.26 (+26%)
sos3	36.5 ± 0.7	47.6 ± 2.0 (+30%)	51.8 ± 1.8	43.4 ± 3.0 (−16%)	0.7	1.10 (+57%)

Data represent mean ± SE (n = 3).
*Low Ca^{2+} medium contains 0.3 mM $CaCl_2$.
†High Ca^{2+} medium contains 2 mM $CaCl_2$.

external K^+ concentrations with low or high Ca^{2+}. No substantial difference in K^+ uptake between *sos3* and the wild type was found (data not shown). This could be a result of technical limitations of the uptake assay if the *SOS3* gene controls a K^+ uptake system with an extremely high affinity (e.g., submicromolar range) or if the altered uptake is restricted to particular cells or tissues responsible for the growth defect. Alternatively, the *sos3* mutation may affect K^+ utilization and does not affect K^+ uptake. We also found that there is no substantial difference in K^+ efflux between *sos3* and wild-type seedlings (data not shown).

The *sos3* mutation clearly causes a decrease in K^+ content and K^+/Na^+ selectivity in NaCl-stressed seedlings. High external Ca^{2+} greatly improves tissue K^+ content and K^+/Na^+ selectivity of *sos3* plants. This may explain the observed partial Ca^{2+} suppression of NaCl hypersensitivity of *sos3*. The *SOS3* gene could encode a calcium-sensing protein such as calmodulin, calcium-dependent–calmodulin-independent protein kinase or calcium/calmodulin-dependent protein phosphatase (i.e., calcineurin) (24, 25). Calmodulin is known to mediate calcium regulation of potassium channels in *Paramecium* (26). Of particular relevance is that, in higher plants, it has been shown that the activation of a vacuolar K^+ channel requires 10 times more Ca^{2+} without calmodulin than in the presence of calmodulin (27). Calcineurin is an essential component for salt tolerance in yeast by controlling sodium efflux and potassium uptake (28). Data suggest that in NaCl-stressed yeast cells, calcineurin functions to switch off low-affinity potassium uptake and turn on the high-affinity potassium uptake system thereby increase K^+/Na^+ selectivity (28). Pharmacologic evidence indicates that a calcineurin homolog is also present in plant cells and mediates the calcium inhibition of inward-rectifying K^+ channels in guard cells (29). However, the molecular nature of calcineurin remains elusive in plants.

Alternatively, the observed Ca^{2+} effect could be mediated through an extracellular target. Aside from abundant Ca^{2+} ligands in the pectic component of the cell wall and lipids of the plasma membrane, no Ca^{2+}-binding protein is known in the wall or the outer surface of the plasma membrane. A general beneficial effect of external Ca^{2+} on plant salt tolerance is often attributed to the notion that Ca^{2+} is necessary for maintaining plasma membrane integrity and improving K^+/Na^+ selectivity of potassium uptake systems (16). The general beneficial effect of Ca^{2+} was also observed here for wild type and *sos1* plants (Fig. 5). However, the effect on *sos3* is much more dramatic and specific. If the Ca^{2+} effect reported here is extracellular, then there could be a specific cell wall or plasma membrane calcium sensor controlling potassium acquisition. Extracellular Ca^{2+}-sensing receptors that respond to millimolar concentrations of Ca^{2+} have been found in mouse keratinocyte as well as bovine parathyroid (30, 31). Whatever Ca^{2+} sensor *SOS3* may encode, the *sos3* mutation appears to increase the threshold of Ca^{2+} activation of the sensor or its downstream target.

Yet another possibility is that *sos3* is defective in general cellular Ca^{2+} homeostasis as a result of impaired uptake or insufficient intracellular pools of stored Ca^{2+}. This appears unlikely because defective cellular Ca^{2+} homeostasis would be expected to cause pleiotropic phenotypes, since calcium is thought to be required for numerous signaling reactions critical for normal plant growth and development (24, 32). *sos3* plants are normal except when challenged with low potassium or salt stress (Fig. 1). We have also measured Ca^{2+} uptake in *sos3* and wild-type seedlings using $^{45}Ca^{2+}$. No difference was detected between *sos3* and the wild type (data not shown). There was also no significant difference in Ca^{2+} content between NaCl-stressed *sos3* and wild-type seedlings (data not shown). Cloning of the *SOS3* gene is expected to provide insights into the structure and function of the gene product. We have taken a big step toward the positional cloning of *SOS3* by placing the mutation within a distance of probably <100 kb from the physical marker *nga139* (Fig. 2).

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