Reduced Na\textsuperscript{+} Uptake in the NaCl-Hypersensitive sos1 Mutant of Arabidopsis thaliana\textsuperscript{1}

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sos1 is an Arabidopsis thaliana mutant with \ (>20 times higher sensitivity toward Na\textsuperscript{+} inhibition due to a defective high-affinity potassium-uptake system. We report here that sos1 accumulates less Na\textsuperscript{+} than the wild type in response to NaCl stress. The Na\textsuperscript{+} contents in sos1 seedlings exposed to 25 mM NaCl for 2 or more d are about 43\% lower than those in the wild type. When assayed at 20 mM external NaCl, sos1 seedlings pretreated with low potassium have 32\% lower Na\textsuperscript{+} uptake than the wild type. However, little difference in Na\textsuperscript{+} uptake could be measured when the seedlings were not pretreated with low potassium. Low-potassium treatment was shown to induce high-affinity potassium-uptake activity in Arabidopsis seedlings. No substantial difference in Na\textsuperscript{+} efflux between sos1 and the wild type was detected. The results show that the reduced Na\textsuperscript{+} accumulation in sos1 is due to a lower Na\textsuperscript{+} influx rate. Therefore, the sos1 mutation appears to disrupt low-affinity Na\textsuperscript{+} uptake in addition to its impairment of high-affinity K\textsuperscript{+} uptake.

Salinization of irrigated lands is an increasing threat to agriculture (Epstein et al., 1980; Tanji, 1990). In saline soils Na\textsuperscript{+} is the principal toxic ion, the concentrations of which often exceed 25 mM (Greenway and Munns, 1980). Many cellular activities are sensitive to Na\textsuperscript{+} inhibition (Greenway and Munns, 1980). Thus, maintaining a low cytosolic Na\textsuperscript{+} concentration is important for many plants growing in NaCl-affected environments. Plant cells maintain a low cytosolic Na\textsuperscript{+} concentration through Na\textsuperscript{+} exclusion, extrusion, or compartmentation (Niu et al., 1995). Compartmentation of Na\textsuperscript{+} into the vacuole is likely through the action of Na\textsuperscript{+}-H\textsuperscript{+} antiporters in the tonoplast (Barkla and Blumwald, 1991). Na\textsuperscript{+} extrusion via Na\textsuperscript{+}-ATPase contributes the major portion of NaCl tolerance in the yeast Saccharomyces cerevisiae (Haro et al., 1991). In plants no Na\textsuperscript{+} pump activity has been detected, and Na\textsuperscript{+} extrusion is thus likely achieved through Na\textsuperscript{+}-H\textsuperscript{+} antiporters on the plasma membrane (DuPont, 1992).

Na\textsuperscript{+} exclusion requires restricting Na\textsuperscript{+} uptake at the plasma membrane. The molecular mechanisms of Na\textsuperscript{+} uptake are poorly understood (Niu et al., 1995). Physiological studies suggest that Na\textsuperscript{+} influx occurs through the mechanism 2 (low-affinity) potassium-uptake system (Rains and Epstein, 1965, 1967). Cloned potassium channels, which are presumed to function in low-affinity potassium uptake, however, are all very selective against Na\textsuperscript{+} (Schroeder et al., 1994). It is possible that other potassium channels exist that are less selective between potassium and Na\textsuperscript{+}. Schachtman et al. (1991) have suggested that Na\textsuperscript{+} entry into plant cells may be via outward-rectifying cation channels. Under saline conditions Na\textsuperscript{+} depolarizes the plasma membrane, which increases the open probability of outward-rectifying cation channels, thereby allowing Na\textsuperscript{+} influx to occur down its steep electrochemical gradient. More recently, Rubio et al. (1995) reported that the high-affinity potassium transporter HKT1 (Schachtman and Schroeder, 1994) from wheat contributes to Na\textsuperscript{+} influx because it functions as a K\textsuperscript{+}-Na\textsuperscript{+} symporter. It was demonstrated that at physiologically toxic Na\textsuperscript{+} concentrations, high-affinity potassium uptake through HKT1 was blocked and low-affinity Na\textsuperscript{+} uptake occurred. Therefore, mechanism 1 (high-affinity) potassium-uptake systems may also mediate Na\textsuperscript{+} influx.

The NaCl-hypersensitive mutant of Arabidopsis thaliana, sos1, is defective in high-affinity potassium uptake (Wu et al., 1996). NaCl-stressed sos1 seedlings contain less potassium than the wild type. We report here that sos1 also contains less Na\textsuperscript{+} when treated with NaCl. The results suggest that the reduced Na\textsuperscript{+} accumulation is due to a decreased Na\textsuperscript{+} influx into sos1 seedlings. Thus, the SOS1 gene is probably also involved in Na\textsuperscript{+} uptake into plant cells.

MATERIALS AND METHODS

The sos1 mutant of Arabidopsis thaliana and its wild-type background (ecotype Columbia) used in this work are described by Wu et al. (1996). The sos1-1 allele was used in all experiments reported here. Seeds were surface-sterilized and planted on agar plates for germination. The germination medium contained MS salts, 3\% Suc, and 1.2\% agar, pH 5.7. Plants were grown in a growth room at 22 to 24°C with continuous, cool-fluorescent illumination.

Potassium-Uptake Assays (\textsuperscript{86}Rb\textsuperscript{+})

For measurement of potassium uptake using \textsuperscript{86}Rb\textsuperscript{+} as a tracer, 4-d-old seedlings from vertical MS agar plates were transferred to vertical agar plates containing 200 \textmu M potassium. After 2 to 3 d, approximately 30 seedlings were collected, rinsed briefly in solution A, and then added to 10

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Abbreviation: MS, Murashige-Skoog.
mL of solution A supplemented with 200 μM KCl and 0.5 μCi/mL 86Rb⁺ (Amersham). The uptake was performed for 30 min at 25 ± 1°C under a white fluorescent light. At the completion of uptake, the seedlings were rinsed twice (15 s each) in 30 mL of solution A and then incubated for 15 min in 35 mL of ice-cold solution A plus 3 mM CaCl₂. The seedlings were then blotted dry on filter paper and weighed, and the radioactivity was measured in a scintillation counter.

Solution A is constituted of potassium-free one-twentieth-strength MS major salts with regular amounts of minor nutrients. It was prepared as follows: 82.5 mg/L NH₄NO₃, 22 mg/L CaCl₂·2H₂O, 18.5 mg/L MgSO₄·7H₂O, 7.2 mg/L NH₄H₂PO₄, 1.39 mg/L FeSO₄·7H₂O, 1.865 mg/L disodium EDTA, 0.7495 mg/L NaI, 6.3 mg/L H₂BO₃, 16.9 mg/L MnSO₄·H₂O, 8.6 mg/L ZnSO₄·7H₂O, 0.25 mg/L Na₂MoO₄·2H₂O, 0.016 mg/L CuSO₄·5H₂O, and 0.0267 mg/L CoSO₄·6H₂O, pH 5.7.

Measurement of 22Na⁺ Uptake

22Na⁺ was used as a tracer for Na⁺ uptake. The seedlings for 22Na⁺ uptake were pretreated the same way as those for 86Rb⁺ uptake. After approximately 30 seedlings were rinsed in solution B, they were put into an uptake solution composed of solution B plus 20 mM NaCl and 0.5 μCi/mL 22Na⁺ (Amersham). The uptake was performed for 30 min at 25 ± 1°C under a white fluorescent light. At the completion of uptake, the seedlings were rinsed twice (15 s each) in 30 mL of solution B plus 40 mM mannitol and then incubated for 15 min in 35 mL of ice-cold solution B plus 40 mM mannitol and 5 mM NaCl. The seedlings were then blotted dry on filter paper and weighed, and the radioactivity was measured in a scintillation counter.

Solution B is a potassium-free MS solution, which was prepared as follows: 1650 mg/L NH₄NO₃, 440 mg/L CaCl₂·2H₂O, 370 mg/L MgSO₄·7H₂O, 144 mg/L NH₄H₂PO₄, 27.8 mg/L FeSO₄·7H₂O, 37.3 mg/L disodium EDTA, 0.7495 mg/L NaI, 6.3 mg/L H₂BO₃, 16.9 mg/L MnSO₄·H₂O, 8.6 mg/L ZnSO₄·7H₂O, 0.25 mg/L Na₂MoO₄·2H₂O, 0.016 mg/L CuSO₄·5H₂O, and 0.0267 mg/L CoSO₄·6H₂O, pH 5.7.

Measurement of 22Na⁺ Efflux

22Na⁺ was used as a tracer for Na⁺ efflux. The seedlings for Na⁺ efflux were also pretreated the same way as those for 86Rb⁺ uptake. After being rinsed in solution B, approximately 150 seedlings were put into 20 mL of a loading solution composed of solution B plus 0.5 mM NaCl and 2 μCi/mL 22Na⁺ (Amersham). The loading lasted for 17 h at 25 ± 1°C on a shaker (75 rpm) under a white fluorescent light.

The 22Na⁺-loaded seedlings were washed briefly to remove surface radioactivity and placed into 15 mL of an efflux solution containing solution B plus 20 mM NaCl. At predetermined times, the seedlings were taken out, adhering solution was blotted, and the seedlings were placed into the fresh efflux solution. Aliquots of each efflux solution were analyzed for radioactivity. At the end of the efflux experiment, the seedlings were rinsed three times in solution B, blotted dry on filter paper, and weighed, and radioactivity remaining in the seedlings was determined.

Determination of Na⁺ Content

Seeds (100–150) were sterilized and incubated in a 250-mL flask containing 75 mL of medium (one-half-strength MS salts and 2% Suc, pH 5.5). The flasks were shaken at 120 rpm in a chamber with continuous, cool-fluorescent illumination at 22°C. After 8 d, 5 mM NaCl was added to give a desired NaCl concentration, and the seedlings were allowed to continue to grow for specified time periods. The seedlings were then collected, briefly rinsed five times with distilled water (total 500 mL), weighed, and dried at 65°C for 24 h. After the dry weight measurement, Na⁺ content was determined by atomic absorption spectrophotometry.

RESULTS

Reduced Na⁺ Accumulation in NaCl-Stressed sosl

When sosl and wild-type seedlings were exposed to NaCl stress for 24 h, their Na⁺ contents increased (Fig. 1). The increases in Na⁺ content were higher with increasing external NaCl concentrations. Below 10 mM NaCl, the increase in Na⁺ content was almost proportional to the external NaCl concentration. Surprisingly, the increases in sosl were substantially smaller than in the wild type. This is also true when Na⁺ content was expressed as a percentage of fresh weight (data not shown). At 50 mM external NaCl, the Na⁺ content in sosl is approximately 70% of that in the wild type.

Wild-type and sosl seedlings were also treated at 25 mM NaCl for different time periods to determine whether the difference in Na⁺ content is transient. The Na⁺ contents in

![Figure 1](https://www.plantphysiol.org)
both sos1 and the wild type increased with longer exposure to NaCl (Fig. 2). The Na⁺ contents reached near peak levels after 2 to 3 d of NaCl treatments. sos1 plants contained less Na⁺ at all times after the treatment. The peak level of Na⁺ content in sos1 is about 43% lower than that of wild-type plants.

**sos1 Has a Lower Na⁺-Uptake Rate**

The reduced accumulation of Na⁺ in sos1 plants suggests that the mutant has a lower influx rate and/or a higher efflux rate for Na⁺. Na⁺-uptake assays were carried out at 20 mM external Na⁺ using 22Na⁺ as a tracer. When plants grown on nutrient agar plates with 20 mM potassium were used for the assay, no difference was detected between sos1 and wild-type seedlings (Fig. 3). Both had a Na⁺-uptake rate of approximately 3 μmol g⁻¹ fresh weight h⁻¹ (Fig. 3). However, when the seedlings were pretreated for 2 d on a medium containing 200 μM potassium, the wild type had a Na⁺-uptake rate approximately 1.5 times as much as that of sos1 (Fig. 4). The Na⁺-uptake rate of the wild type was increased by about 60% after the low-potassium treatment. In contrast, low-potassium treatment increased the Na⁺-uptake rate of sos1 by only 4%.

We found that the difference in Na⁺ uptake between sos1 and the wild type was correlated with the expression of the high-affinity potassium-uptake system. Without low-potassium treatment, very low (<0.35 μmol g⁻¹ h⁻¹) potassium-uptake rates were detected in wild-type and sos1 seedlings at 200 μM external potassium (Fig. 5). After low-potassium treatment, potassium-uptake rates were increased in both the wild type and sos1. But the rate in sos1 was only about 40% of that in the wild type (Fig. 5). This is in agreement with a previous measurement that indicated that sos1 is defective in high-affinity potassium uptake (Wu et al., 1996). The results indicate that high-affinity potassium uptake is induced by low-potassium treatment in Arabidopsis. A lower Na⁺ influx rate in the mutant can be observed only when the high-affinity potassium-uptake system is expressed under low-potassium treatment.

Na⁺ efflux rates were also determined for wild-type and sos1 seedlings. As shown in Figure 6, no substantial difference was found between the efflux kinetics of the wild type and sos1. Especially in the time frame (10-90 min) that indicates a cytoplasmic efflux, the rates in sos1 and the wild type are almost identical. The results show that there is no difference in the Na⁺ efflux rate between sos1 and the wild type. Therefore, the reduced Na⁺ accumulation in the mutant is due to a lower Na⁺ influx rate.

**DISCUSSION**

In this study we showed that the sos1 mutant accumulates less Na⁺ than the wild type when they are exposed to NaCl stress. The lower Na⁺ content in sos1 appears to be a
result of reduced Na⁺-uptake rate in the mutant. For example, after treatment with 20 mM NaCl for 1 d, the difference in Na⁺ content between sos1 and the wild type is estimated to be approximately 0.56% of dry weight (Fig. 1), which is 0.28 mg g⁻¹ fresh weight d⁻¹ (the water content in sos1 and the wild type is approximately 95%). At 20 mM NaCl, the difference in Na⁺-uptake rate between sos1 and the wild type is 1.6 μmol g⁻¹ fresh weight h⁻¹, which equals 0.88 mg g⁻¹ fresh weight d⁻¹. Thus, the difference in Na⁺ uptake is sufficient to account for the lower Na⁺ content in sos1 plants.

sos1 is defective in high-affinity potassium uptake (Wu et al., 1996). But the defect cannot be seen on plants grown on nutrient medium containing 20 mM potassium (Fig. 5), probably because only the low-affinity potassium-uptake system is expressed at high external potassium concentrations. Induction of high-affinity potassium uptake by potassium deprivation has also been observed in other plant species (Drew et al., 1984; Fernando et al., 1992). When high-affinity potassium uptake is induced by a low-potassium treatment, the defect in the potassium uptake of sos1 is revealed (Fig. 5). Similarly, the lower Na⁺-uptake rate of sos1 can be seen only after a low-potassium treatment. The results suggest that whatever is causing the defective high-affinity potassium uptake in sos1 is also responsible for the reduced Na⁺ uptake in the mutant.

One possibility is that the high-affinity potassium-uptake system controlled by the SOS1 gene also mediates Na⁺ uptake. This is supported by the recent discovery that the high-affinity potassium transporter HKT1 also mediates Na⁺ influx when the gene is expressed in yeast and Xenopus oocytes (Rubio et al., 1995). Although no K⁺-Na⁺ symport activity has yet been clearly demonstrated in higher plants, the molecular evidence with HKT1 strongly suggests that Na⁺-driven potassium symport is possible. Our results indicate that the high-affinity potassium-uptake system could contribute up to approximately 35% of the Na⁺ influx in wild-type Arabidopsis. This is because (a) the sos1 mutation resulted in approximately 32% less Na⁺ uptake compared with the wild type, and (b) approximately 63% of the Na⁺ influx in low-potassium-treated wild-type plants can be detected even when the high-affinity potassium-uptake system is not expressed. The fact that the low-potassium treatment did not significantly increase the Na⁺ influx rate of sos1 indicates that the mutation nearly completely eliminated Na⁺ uptake contributed by the high-affinity potassium-uptake system. The other 65% of Na⁺ uptake in wild-type Arabidopsis is likely contributed by the low-affinity potassium-uptake system. Because the low-affinity potassium-uptake system is normal in sos1 (Wu et al., 1996), the mutant retains about 65% of Na⁺ uptake.

Our data are very consistent with the notion of K⁺-Na⁺ symport. However, it is important to point out that the results do not necessarily imply such a K⁺-Na⁺ symport mechanism. It could be that the high-affinity potassium-uptake system in Arabidopsis consists of K⁺-H⁺ symport (Maathuis and Sanders, 1994) and that it is not very selective against Na⁺. Another possibility is that the SOS1 gene controls, in addition to high-affinity potassium uptake, another transport system(s) that contributes to Na⁺ influx. The results suggest that the SOS1 gene is a player in Na⁺ uptake into plant cells.

Our finding that sos1 accumulates less rather than more Na⁺ in response to NaCl stress revives the question of why the mutant is hypersensitive to NaCl stress. We believe that the main reason is potassium deficiency. Cellular potassium content above a certain threshold level could be vital for plant growth. Because of the defective high-affinity potassium-uptake system, potassium content in sos1 decreases much more than in the wild type when they are exposed to NaCl stress (Wu et al., 1996). Thus, NaCl-stressed sos1 may not be able to absorb potassium to a level above the threshold that is required for active growth. First, a low-potassium treatment (e.g., 200 μM potassium) in the absence of high Na⁺ could completely inhibit sos1 growth (Wu et al., 1996). Wild-type plants are not inhibited by a low-potassium treatment because they can absorb
enough potassium for active growth, owing to a functional high-affinity potassium-uptake system. Second, a lower potassium content may render sos1 much more sensitive to Na+, even though its Na+ level is not as high as that of the wild type. Murguia et al. (1995) have shown that the activity of 3'(2'),5'-bisphosphate nucleotidase is much more inhibited by Na+ when there is less potassium in the assay solution. Third, the lower potassium and Na+ contents in sos1 together may result in a less negative osmotic potential and thus may make it more difficult for the mutant to restore turgor after NaCl shock.

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**LITERATURE CITED**


