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SOS1, a Genetic Locus Essential for Salt Tolerance and Potassium Acquisition

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To begin to determine which genes are essential for salt tolerance in higher plants, we identified four salt-hypersensitive mutants of Arabidopsis by using a root-bending assay on NaCl-containing agar plates. These mutants (*sos1-1*, *sos1-2*, *sos1-3*, and *sos1-4*) are allelic to each other and were caused by single recessive nuclear mutations. The *SOS1* gene was mapped to chromosome 2 at 29.5 ± 6.1 centimorgans. The mutants showed no phenotypic changes except that their growth was >20 times more sensitive to inhibition by NaCl. Salt hypersensitivity is a basic cellular trait exhibited by the mutants at all developmental stages. The *sos1* mutants are specifically hypersensitive to Na^+ and Li^+. The mutants were unable to grow on media containing low levels (below ~1 mM) of potassium. Uptake experiments using ^{86}Rb showed that *sos1* mutants are defective in high-affinity potassium uptake. *sos1* plants became deficient in potassium when treated with NaCl. The results demonstrate that potassium acquisition is a critical process for salt tolerance in glycophytic plants.

INTRODUCTION

Plants experience constant fluctuations in the availability and quality of soil water. The quality of soil water is influenced mostly by the concentrations of essential plant mineral nutrients as well as nonessential ions. Nonessential ions, such as Na^+, when present in high concentrations in the soil, adversely affect plant growth (Greenway and Munns, 1980). Excessive Na^+ in the soil, often referred to as salinity, is a major problem for a substantial portion of agricultural land in the world (Epstein et al., 1980). Recently, much effort has been directed toward understanding the molecular and cellular mechanisms by which plants tolerate salinity stress, with the eventual goal of improving salt tolerance of crop plants (Binzel and Reuveni, 1994). One important objective is to determine which genes are important for plant salt tolerance. A widely used approach has been to identify genes whose expression is regulated by salt stress. Many salt-regulated genes have been identified in the last decade. Identification of these genes has permitted a better understanding of the complexity of salt tolerance in higher plants (Cushman et al., 1990; Bray, 1993; Serrano and Gaxiola, 1994). However, the function of most of the gene products in salt tolerance has been difficult to establish (Bray, 1993; Serrano and Gaxiola, 1994). This approach is also unsuitable for identifying low-abundance mRNAs and proteins that might have important regulatory roles in salt responses. Clearly, a genetic approach is needed to determine which genes are necessary for salt tolerance.

Potassium is a major monovalent cationic essential nutrient. Potassium uptake plays a vital role in plant growth, development, stomatal movements, enzyme activation, and osmoregulation (Epstein, 1972; Kochian and Lucas, 1988). It is generally accepted that potassium uptake into plant cells is mediated by two mechanisms operating on the plasma membrane (Epstein, 1966). One is a low-affinity system that functions only when extracellular potassium concentration is high (millimolar range). The second is a high-affinity system that functions at low extracellular potassium concentrations (micromolar range). Physiological studies indicate that high levels of Na^+ inhibit K^+ uptake through the low-affinity system (Rains and Epstein, 1967). Recently, several K^+ channel homologs have been identified from Arabidopsis by complementation of K^+ uptake–deficient yeast mutants (Ko and Gaber, 1991) and patch clamp analysis with Xenopus (Anderson et al., 1992; Schachtman et al., 1992; Sentanec et al., 1992; Schroeder et al., 1994; Hadjeb and Berkowitz, 1995). Some of the inward-rectifying K^+ channels probably serve as the low-affinity K^+ uptake system (Schroeder et al., 1994). Using a similar approach of yeast complementation, Schachtman and Schroeder (1994) have cloned a high-affinity K^+ transporter from wheat that could be part of the high-affinity K^+ uptake system in higher plants. The functions of the cloned K^+ channels and transporters in K^+ acquisition and salt tolerance in plants, however, remain to be determined.

We have used a molecular genetic approach with Arabidopsis as a model system to understand salt tolerance mechanisms in glycophytic plants. We reasoned that genes essential for salt tolerance could be identified by selecting and characteriz-

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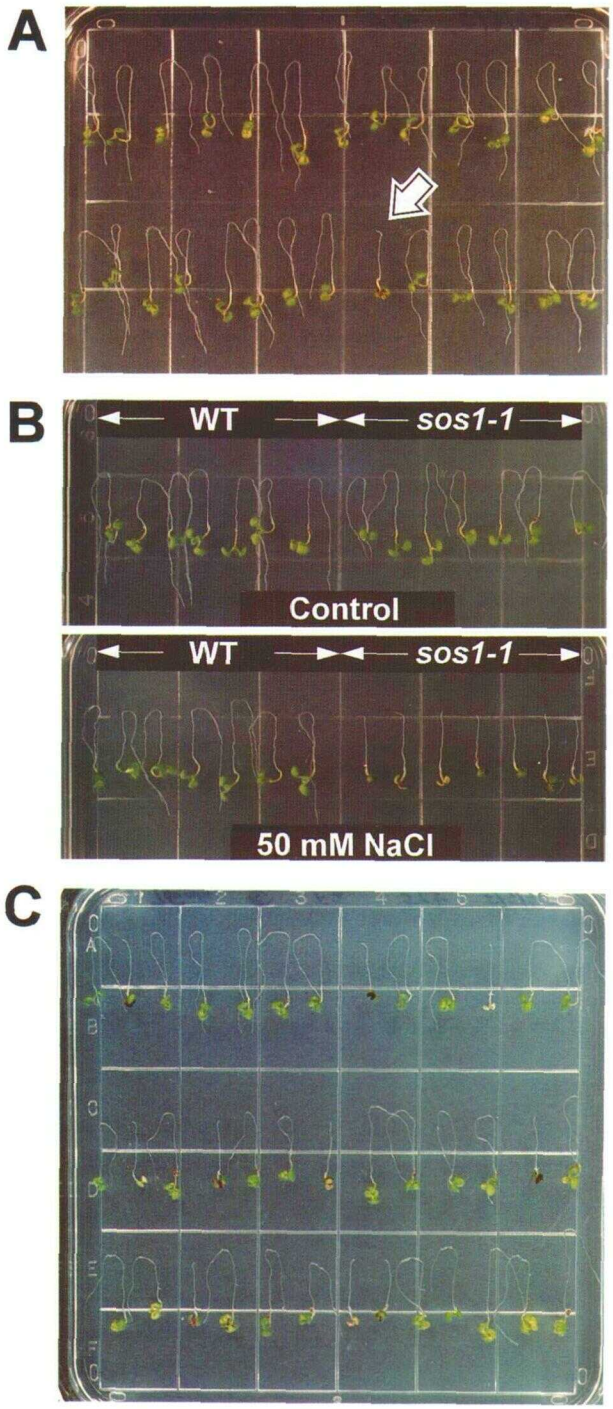

Figure 1. Use of the Root-Bending Assay for Selection and Characterization of Salt-Hypersensitive Mutants.

Four-day-old Arabidopsis seedlings with 1- to 1.5-cm-long roots, on vertical agar plates, were transferred to plates supplemented with NaCl and allowed to grow upside down.

(A) Seedlings from EMS-mutagenized M_2 seeds were screened on 50 mM NaCl plates for *sos* mutants. The arrow points to a putative *sos* mutant.

(B) Rescreening of putative *sos* mutants, using the root-bending assay. *sos1-1* seedlings shown here exhibited normal root bending on medium without NaCl (Control) but no root bending on medium supplemented with 50 mM NaCl (50 mM NaCl), thus demonstrating that it is a true salt-hypersensitive mutant. WT, wild type.

(C) F_2 segregation analysis, using the root-bending assay. Uniformly grown F_2 seedlings from a cross between the wild type and *sos1-1* were transferred onto medium supplemented with 75 mM NaCl. The wild type (with root bending)–to–*sos1-1* (without root bending) ratio is ~3:1, indicating that *sos1-1* is caused by a single recessive nuclear mutation.

ing salt-hypersensitive mutants. In this study, we isolated and characterized four Arabidopsis mutants whose growth is hypersensitive to NaCl inhibition. These mutants define one genetic locus that is essential for salt tolerance. We show that this locus is also required for high-affinity K^+ uptake.

RESULTS

Isolation of Salt-Hypersensitive Mutants

NaCl stress inhibits plant root and shoot growth. Arabidopsis is a glycophytic species that is sensitive to low to moderate levels of NaCl stress. The growth inhibition by NaCl of Arabidopsis seedlings can be conveniently observed as a reduction in root elongation as well as cotyledon and leaf expansion. We characterized the inhibition of seedling root elongation by using a root-bending assay modified from Howden and Cobbett (1992). In this assay, young seedlings growing uniformly in the absence of salt stress are transferred and placed with roots upside down onto vertical agar plates containing appropriate levels of NaCl as the stress agent. Continued growth on the salt plates results in bending of the roots due to gravitropism; thus, lack of root bending is a visual sign of growth inhibition by NaCl (Figures 1A to 1C). Agravitropic mutants can be easily distinguished from true salt-hypersensitive mutants because the former can exhibit continued upward root growth and uninhibited shoot growth. Murphy and Taiz (1995) have developed a vertical-mesh-transfer technique for rapid screening of heavy metal tolerance mutants that is related to this screening method. This new technique may also be useful for faster screening of NaCl-hypersensitive mutants in the future.

We screened ~50,000 ethylmethane sulfonate (EMS)–mutagenized M_2 seeds for NaCl-hypersensitive mutants, using the root-bending assay on 50 mM NaCl plates (Figure 1A). This amount (50 mM) of NaCl has no obvious effect on the root bending of wild-type (Columbia ecotype) seedlings (Figure 1) that could exhibit uniform root bending on plates containing up to 150 mM NaCl (data not shown). Of 21 putative mutants obtained, four remained hypersensitive to NaCl stress in the M_3 generation and were named salt overly sen-

Table 1. Genetic Analysis of Arabidopsis *sos* Mutants

Strains or Crosses (♀ × ♂)	Generation	Total Seedlings Tested	Resistant[a]	Sensitive[a]	χ^2
Wild-type Columbia		4982	4982	0	
sos1-1/sos1-1		1473	0	1473	
sos1-2/sos1-2		435	0	435	
sos1-3/sos1-3		423	0	423	
sos1-4/sos1-4		379	0	379	
Wild type × *sos1-1/sos1-1*	F_1	54	54	0	
	F_2	231	175	56	0.071[b]
sos1-2/sos1-2 × wild type	F_1	59	59	0	
sos1-3/sos1-3 × wild type	F_1	34	37	0	
sos1-4/sos1-4 × wild type	F_1	37	37	0	
sos1-2/sos1-2 × *sos1-1/sos1-1*	F_1	30	0	30	
sos1-1/sos1-1 × *sos1-3/sos1-3*	F_1	35	0	35	
sos1-1/sos1-1 × *sos1-4/sos1-4*	F_1	46	0	46	
sos1-2/sos1-2 × *sos1-3/sos1-3*	F_1	16	0	16	

[a] Resistant or sensitive was determined in the root-bending assay, using 75 mM NaCl.

[b] The calculated value was based on the expected ratio of three wild-type (i.e., resistant) seedlings to one mutant (i.e., sensitive) seedling; $P > 0.05$.

sitive (*sos*) mutants. Figure 1B shows the test of M_3 seedlings of *sos1-1*. With or without salt stress, the phenotypes of the other three independent mutants are identical to *sos1-1*.

Genetic Characterization of *sos1*

Genetic analysis showed that all four mutations are recessive and are allelic to each other (Table 1). The analysis of the selfed F_2 seedlings from a cross between *sos1-1* and the wild type revealed a 3:1 segregation ratio of wild type/*sos1-1* (Table 1). Therefore, the *sos1* mutants are caused by single recessive nuclear mutations. An example of the segregation analysis from the root-bending assay is shown in Figure 1C. Unless specified otherwise, all subsequent characterizations were performed with *sos1-1*.

Salt Hypersensitivity of *sos1* Mutants Is a Basic Cellular Trait and Is Expressed at All Developmental Stages

The salt sensitivity of the *sos1* mutant was quantified by measuring root elongation of seedlings placed on agar plates containing different levels of NaCl. Root elongation is a convenient and accurate indicator of plant growth because we found that root and shoot growth of the Arabidopsis lines presented in this study are always simultaneously inhibited by NaCl. The concentration of NaCl that decreased the root elongation rate by 50% relative to medium without salt (I_{50}) was estimated. The I_{50} concentrations for *sos1* and the wild type are ~4 and 96 mM, respectively (Figure 2). Thus, *sos1* seedlings are >20 times more sensitive to NaCl than are the wild-type seedlings.

Salt sensitivity of plants often depends on developmental stages. We determined whether *sos1* is also hypersensitive to NaCl at developmental stages other than the seedling. Germination of *sos1* seed was found to be hypersensitive to NaCl

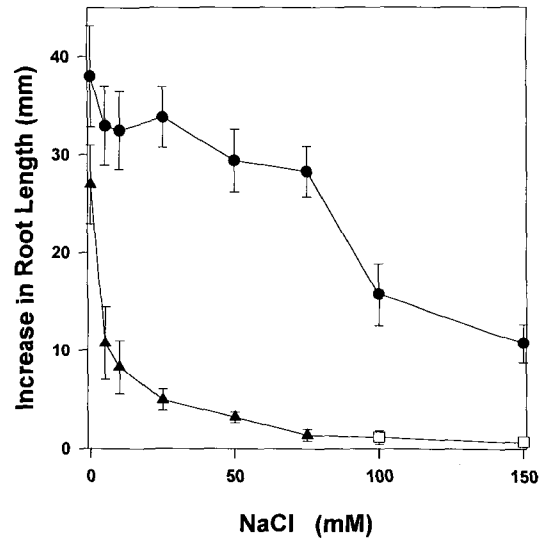

Figure 2. *sos1* Seedlings Are >20 Times More Sensitive to NaCl Than Are Wild-Type Seedlings.

Root elongation of *sos1* and wild-type seedlings was measured to quantify their sensitivities toward NaCl inhibition. Estimated I_{50} concentrations (NaCl concentration at 50% inhibition) are 4 and 96 mM for *sos1* and the wild type, respectively. Shown are root elongation data 7 days after transfer to NaCl plates. Root elongation data were also obtained on days 1 to 6, and they yielded similar I_{50} concentrations. ●, wild type; ▲, *sos1*; □, dead *sos1*.

inhibition (data not shown). At 100 mM NaCl, the germination rate of the wild type was not affected, whereas *sos1* was inhibited to ~35%. The wild-type seeds that could not germinate on 200 mM NaCl all were able to germinate after being transferred to media without NaCl. However, none of the *sos1* seeds that did not germinate on NaCl-containing media was able to germinate on media without NaCl, indicating that these mutant seeds had been permanently damaged by NaCl.

Rosette leaves of *sos1* and wild-type plants also were examined for NaCl responses. The growth of *sos1* plants was completely inhibited by watering with a solution containing 25 mM NaCl, whereas the wild type could grow in the presence of 100 mM NaCl (Figure 3).

To determine whether the NaCl hypersensitivity of *sos1* is a basic cellular trait or associated with only specific cell and tissue types of intact plants, calli were generated from mutant and wild-type seeds. In the absence of NaCl, growth of *sos1* calli was not different from that of the wild type. *sos1* calli could not grow on media containing 100 mM NaCl and were killed by 150 mM NaCl (Figure 4). In contrast, the wild-type calli could grow on 100 mM NaCl (Figure 4) and survive on 150 mM NaCl (data not shown). The results show that the NaCl hypersensitivity of *sos1* is a basic cellular trait and that it is expressed at all developmental stages and in cultured cells of the mutant.

sos1 Is Specifically Hypersensitive to Na^+ and Li^+

Because the salt that we used is NaCl, we wanted to determine whether *sos1* is hypersensitive to Na^+ or Cl^- or both. As shown in Figure 5A, *sos1* is hypersensitive to NaCl but not to KCl. Furthermore, *sos1* is hypersensitive to Na_2SO_4 but not to K_2SO_4 (Figure 5B). Therefore, *sos1* is hypersensitive to Na^+ but not to Cl^-, SO_4^{2-}, or K^+. In addition to Na^+, *sos1* is also hypersensitive to Li^+ (Figure 6A). Li^+ is generally considered a more toxic analog for Na^+ (Mendoza et al., 1994). Ten millimolars LiCl did not inhibit the wild type; however, it exerted >80% inhibition on *sos1* plants. Interestingly, *sos1* is not hypersensitive to Cs^+ (Figure 6B), another very toxic monovalent cation (Sheahan et al., 1993).

sos1 Cannot Grow on Low-Potassium Media

As part of our effort to characterize the responses of *sos1* to various salts, we tested *sos1* seedlings on media containing various levels of KCl. Regular Murashige and Skoog (MS) medium (Murashige and Skoog, 1962) contains ~20 mM K^+. *sos1* grew well with this level of K^+ (Figure 1). Adding additional KCl to MS media reduced the growth of both *sos1* and

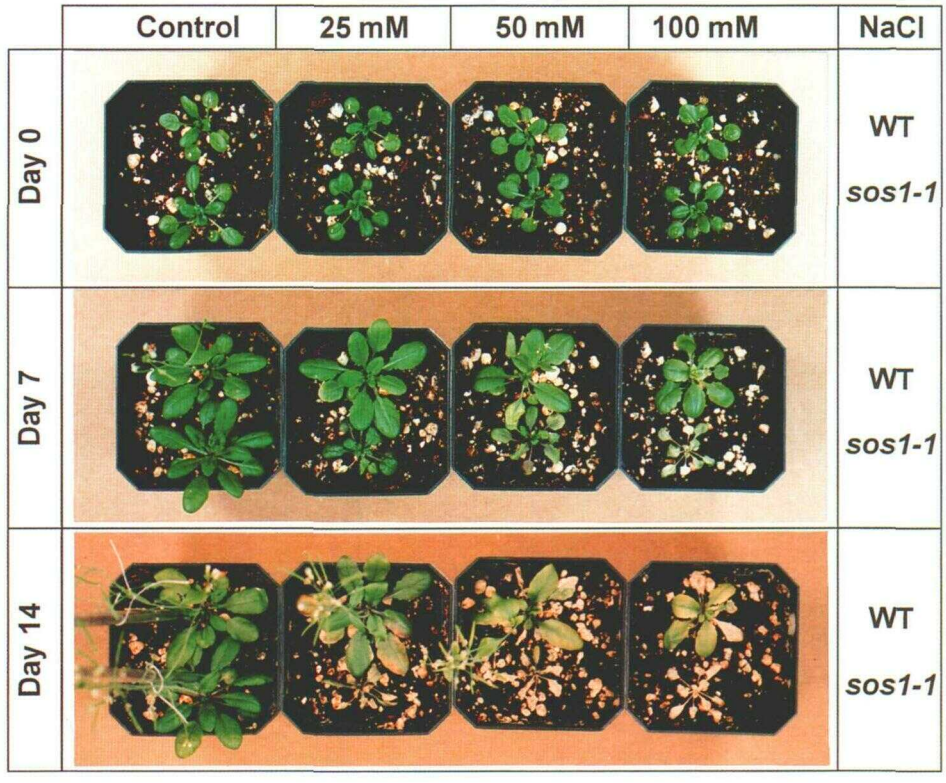

Figure 3. Soil-Grown *sos1* Plants Are Hypersensitive to NaCl.

Plants with eight to 10 rosette leaves were flooded every 3 days with either distilled water (Control) or 25, 50, or 100 mM NaCl solution. WT, wild type.

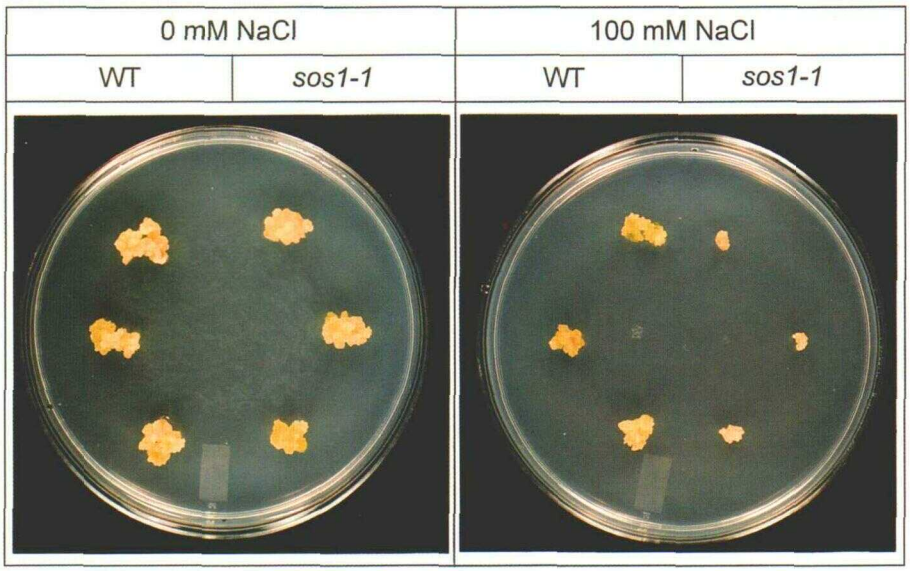

Figure 4. Callus Tissue Derived from *sos1* Is Hypersensitive to NaCl.

The calli were photographed 10 days after treatment. *sos1* calli did not grow at all on 100 mM NaCl medium and were killed within 1 week after being transferred to the NaCl medium. WT, wild type.

the wild type, presumably due to osmotic stress (data not shown). However, when the K^+ levels in the MS medium were reduced to 200 μM, *sos1* could not grow, whereas the wild type grew relatively well (Figure 7). Replacing NH_4NO_3 in the low-potassium medium with Tris–nitrate did not improve the growth of *sos1* or the wild type, indicating that the lack of growth of *sos1* on the 200 μM K^+ medium was not due to inhibition by NH_4^+ (data not shown). Quantitative measurement of root elongation indicated that *sos1* could not grow on media containing <1 mM K^+, whereas the growth of the wild type was not affected by K^+ levels (Figure 8). It should be noted that the agar medium could sustain the growth of wild-type seedlings even without any added K^+, presumably because of a low level of K^+ contamination in the agar (Sheahan et al., 1993).

sos1 Is Deficient in High-Affinity K^+ Uptake

The observation that *sos1* needs high levels of K^+ to grow indicates that the mutant may be deficient in high-affinity K^+ uptake. The capacity for K^+ absorption was determined by measuring $^{86}Rb^+$ uptake into *sos1* and the wild-type seedlings. The uptake rate for K^+ was at least twofold lower in *sos1* than in the wild type when the external K^+ concentration was 200 μM. However, there was no difference in K^+ uptake rate between *sos1* and the wild type when the external K^+ was 20 mM (Figure 9). These uptake results are very consistent with the growth data in Figure 8 and demonstrate that *sos1* is indeed defective in the high-affinity K^+ uptake system and that it contains a normal low-affinity K^+ uptake system.

K^+ uptake rates as a function of K^+ concentration in the external media were determined for both *sos1* and the wild type at the high-affinity range. The uptake in *sos1* was consistently lower than in the wild type (Figure 10A). Assuming that *sos1* is defective in a high-affinity K^+ transport system, then the difference between the uptake rates in the wild type and *sos1* may correspond to the uptake mediated through this high-affinity system. A plot of the difference in uptake rates, as a function of potassium concentration, approximates a saturable system (Figure 10B). The K_m and V_{max} concentrations for this system were estimated to be 256 μM and 796 nmol/hr/g fresh weight, respectively, based on the Hanes plot (Figure 10B). The estimated K_m value is lower than 420 μM as determined by Polley and Hopkins (1979) but higher than 30 μM obtained by Maathuis and Sanders (1994). The discrepancies could be caused by different methodologies as well as the status of the plants used.

Potassium Deficiency in NaCl-Stressed *sos1*

K^+ content in NaCl-treated *sos1* seedlings was measured to determine whether the mutant was deficient in K^+. After 24 hr of exposure to various levels of NaCl, the K^+ content was decreased in both *sos1* and the wild-type plants (Figure 11). More importantly, this decrease was greater in *sos1* at all NaCl concentrations (Figure 11). The K^+ content in the wild type did not decrease to <3% of the dry weight, whereas in *sos1* it decreased to ~1%. The results demonstrate that K^+ deficiency occurs in NaCl-treated *sos1* plants.

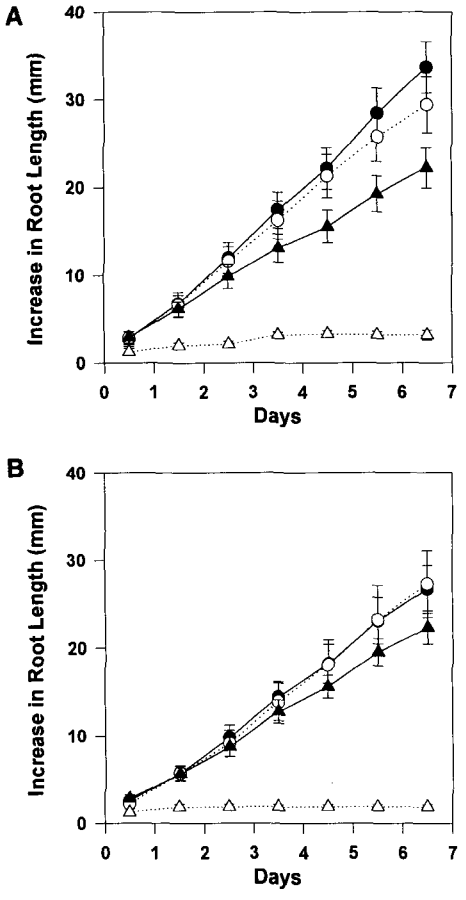

Figure 5. Growth Response of *sos1* and Wild-Type Seedlings toward NaCl, KCl, Na_2SO_4, and K_2SO_4.

sos1 is hypersensitive to Na^+ but not to Cl^-, SO_4^{2-}, or K^+.
(A) Growth response toward NaCl and KCl. ●, wild type on 50 mM KCl; ○, wild type on 50 mM NaCl; ▲, *sos1* on 50 mM KCl; △, *sos1* on 50 mM NaCl.
(B) Growth response toward Na_2SO_4 and K_2SO_4. ●, wild type on 25 mM K_2SO_4; ○, wild type on 25 mM Na_2SO_4; ▲, *sos1* on 25 mM K_2SO_4; △, *sos1* on 25 mM Na_2SO_4.

Map Position of *sos1*

As a first step toward cloning *SOS1*, the chromosomal map position of *sos1* was determined by using a combination of the visible *glabrous* (*gl1*) marker (Koornneef et al., 1982), microsatellite markers (Bell and Ecker, 1994), and cleaved amplified polymorphic sequence (CAPS) markers (Konieczny and Ausubel, 1993). Homozygous *sos1-1* plants in the Columbia *gl1* background were crossed to plants of the Landsberg *erecta* background. The resulting F_2 population segregated for the *sos1* and *gl1* phenotypes and for Landsberg *erecta* and Columbia microsatellite and CAPS markers. In 43 of the *sos1* mutants recovered, 36 were found to be *GL1*, indicating that *sos1* is not linked to this *gl1* locus on chromosome 3. DNA was isolated from *sos1* homozygotes from the F_2 population, and segregation of microsatellite markers was determined. *sos1* showed linkage to *nga168* on chromosome 2 (Table 2) and no linkage to *nga63* and *nga111* on chromosome 1, *nga8* on chromosome 4, or *nga225* and *nga76* on chromosome 5. Segregation of the CAPS marker *GPA1* on chromosome 2 was then determined. As shown in Table 2, *sos1* is more closely linked to *GPA1*. By using the Kosambi function (Koorneef and Stam, 1992), the map position of *sos1* was calculated to be 29.5 ± 6.1 centimorgans on the upper arm of chromosome 2.

DISCUSSION

In this study, we report the isolation and characterization of salt-hypersensitive mutants of Arabidopsis. The fact that the

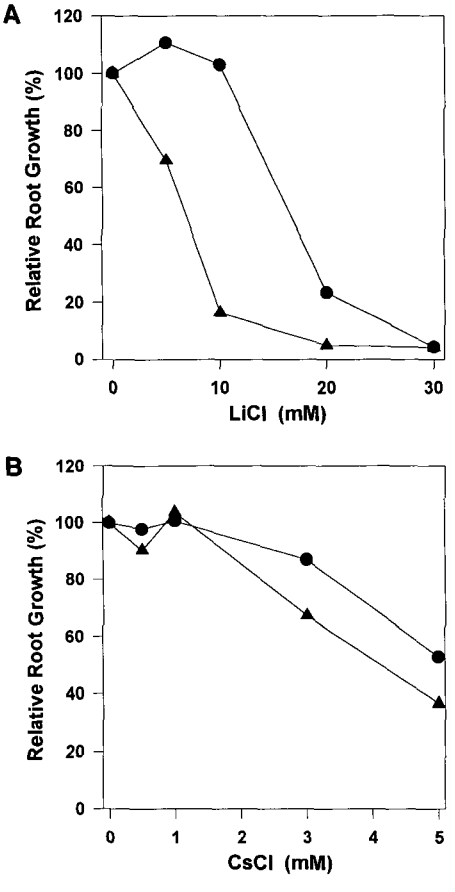

Figure 6. *sos1* Seedlings Are Hypersensitive to Li^+ but Not Cs^+.

Relative root growth was calculated as the percentage of growth in the presence of salt (i.e., Li^+ or Cs^+) over growth in the absence of salt.
(A) Relative growth on LiCl.
(B) Relative growth on CsCl.
●, wild type; ▲, *sos1*.

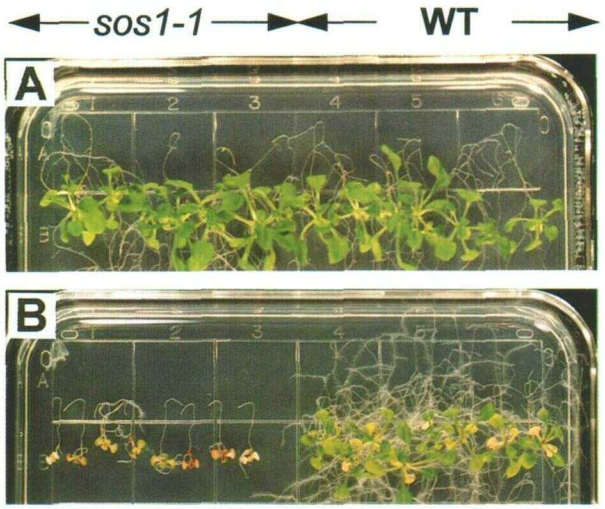

Figure 7. *sos1* Plants Cannot Grow with Low Levels of Potassium.

Four-day-old *sos1* and wild-type seedlings grown on MS medium were transferred to media containing various levels of K^+.
(A) Plants on 20 mM K^+.
(B) Plants on 200 μM K^+.
The plants were photographed 2 weeks after the transfer. WT, wild type.

mutants appear normal on regular growth media suggests that the affected locus is not essential for normal plant growth and development but is required for salt tolerance. Furthermore, we found that the underlying reason for the salt hypersensitivity is a defective high-affinity potassium uptake system. These mutants will serve as an invaluable tool for understanding salt tolerance mechanisms as well as the role of potassium and its high-affinity uptake in various physiological processes in plants.

Molecular Genetic Approach to Studying Salt Tolerance

Arabidopsis has proven to be a very powerful model system for developmental and hormonal research on higher plants (Meyerowitz and Sormerville, 1994). It also has been used successfully to analyze plant interactions with biotic as well as certain abiotic factors. However, its use in studies on plant salt responses has been very limited. Werner and Finkelstein (1995) as well as Saleki et al. (1993) have selected several Arabidopsis mutants that can germinate in the presence of high salt. However, these mutants were not more salt tolerant beyond the germination stage. An alternative approach to understanding salt tolerance is to select for mutants that have lost certain salt tolerance mechanisms and are thus more sensitive to salt stress than is the wild type. In practice, the application of this straightforward strategy has been hampered in part by the fact that wild-type Arabidopsis is very sensitive to NaCl. Nevertheless, we demonstrated in this study that it is possible to isolate mutants that are hypersensitive to NaCl and that the mutants are extremely useful for the identification of genes essential for salt tolerance. The mutants presented here define a gene (i.e., *SOS1*) involved in potassium acquisition. We have since isolated more NaCl-hypersensitive mutants (L. Ding and J.-K. Zhu, unpublished data). These mutants may define genes involved in other plant processes that are important in salt tolerance, such as Na^+ extrusion and compartmentation, the synthesis of compatible osmolytes, ionic and osmotic sensing and signal transduction, or even in some as yet unknown processes required for salt tolerance. Salt-sensitive yeast mutants defective in one of many of these processes have been identified recently (Brewster et al., 1993; Haro et al., 1993; Mendoza et al., 1994). The salt-sensitive yeast mutants have been indispensable, for example, in the dissection of osmotic signaling pathways (Brewster et al., 1993; Maeda et al., 1994, 1995).

Potassium Acquisition—A Critical Process for Salt Tolerance

We have shown that *sos1* mutants are hypersensitive to Na^+ stress and defective in high-affinity K^+ uptake. These two phenotypes always cosegregated with each other (data not shown). In addition to *sos1-1*, the other three alleles also appear deficient in high-affinity K^+ uptake because they were not able to grow on low K^+ media (data not shown). Therefore, we conclude that the high-affinity K^+ uptake system is required

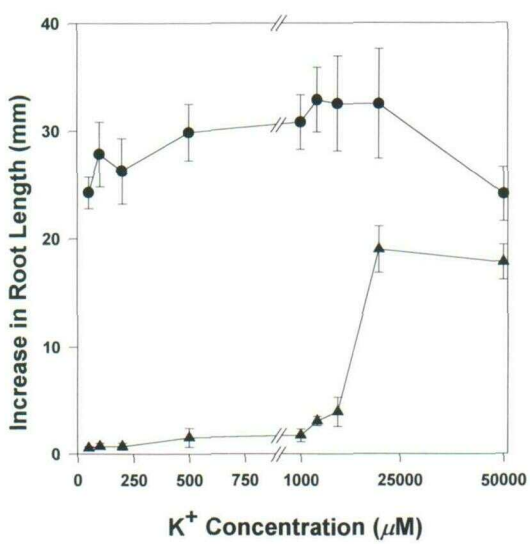

Figure 8. Growth of *sos1* and Wild-Type Seedlings as a Function of Medium Potassium Concentration.

Root elongation was measured 1 week after transferring the seedlings from MS medium to media containing various levels of K^+. Only at K^+ concentrations >1 mM was substantial growth of *sos1* plants observed. ●, wild type, ▲, *sos1*.

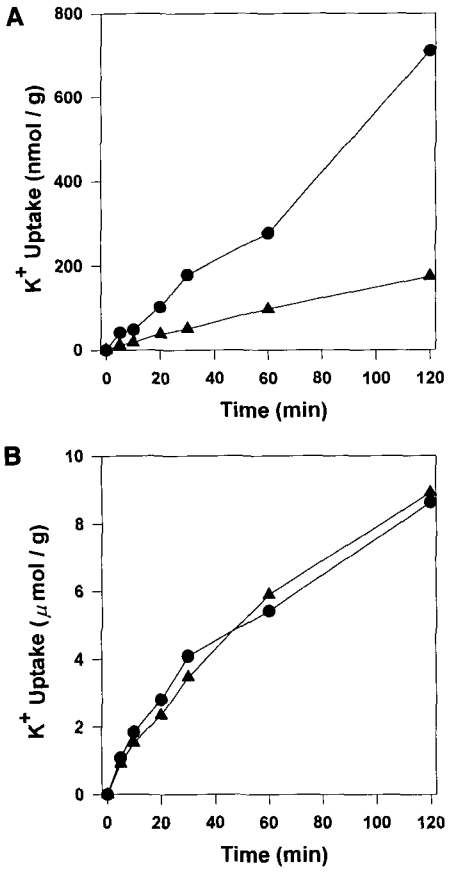

Figure 9. Potassium (^{86}Rb) Uptake in *sos1* and Wild-Type Seedlings as a Function of Time.

(A) Uptake at 200 μM external K^+.
(B) Uptake at 20 mM external K^+.
As shown in **(A)**, *sos1* is defective in high-affinity K^+ uptake. As shown in **(B)**, low-affinity K^+ uptake is normal in *sos1*. ●, wild type; ▲, *sos1*.

for salt tolerance. A direct consequence of a defective high-affinity K^+ uptake system is K^+ deficiency, which was evident in NaCl-stressed *sos1* plants (Figure 11). The difference in K^+ uptake under NaCl stress is probably even greater, considering that the wild type was actively growing and thus diluting the cellular pool of K^+. Potassium deficiency may in turn lead to increased cellular susceptibility to and damage by NaCl. This is suggested by the observation that NaCl-treated *sos1* seeds would not germinate even after being rescued to media without NaCl. Furthermore, K^+ deficiency alone could not prevent the germination of *sos1* or the wild-type seeds (data not shown). Our conclusion that high-affinity K^+ uptake is essential for salt tolerance also is supported by the observation that the yeast high-affinity K^+ transport mutant *trk1*$^-$ is NaCl sensitive (Haro et al., 1993).

The hypothesis of dual mechanisms of potassium uptake was formulated more than three decades ago (Epstein et al., 1963). Despite some reports of inconsistencies of experimental data with the hypothesis, it is supported by the majority of evidence in the literature. Recent studies indicate that the low-affinity uptake system is a K^+ channel(s), whereas the high-affinity system consists of a K^+–Na^+ symporter(s) (Schroeder et al., 1994; Rubio et al., 1995). Thermodynamic principles dictate that the low-affinity system can only function in the transport of K^+ down its electrochemical potential gradient. The high-affinity system, with the tight coupling of proton motive force, can function in transporting K^+ against its electrochemical potential gradient. Compared with the low-affinity system, the high-affinity system is slower and may be more costly in terms of cellular energy. However, this costly system is essential for plant survival when the external K^+

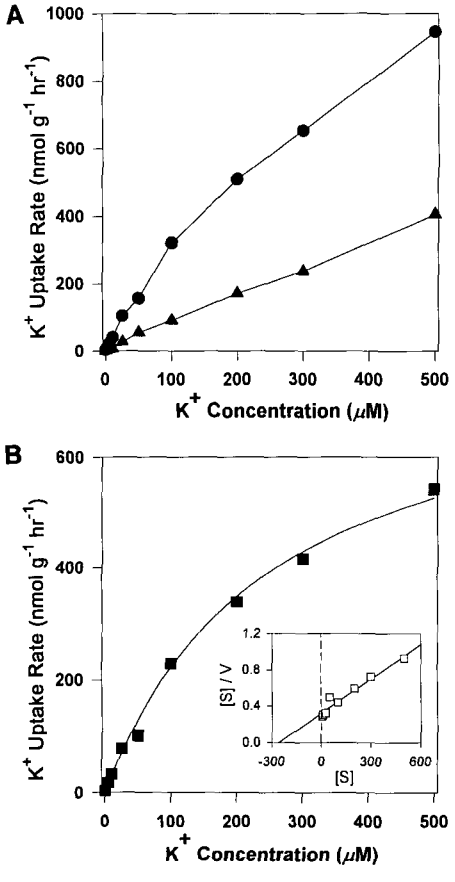

Figure 10. High-Affinity Potassium (^{86}Rb) Uptake in *sos1* and Wild-Type Seedlings.

(A) Uptake as a function of external K^+ concentration. ●, wild type; ▲, *sos1*.
(B) *SOS1*-dependent high-affinity potassium uptake as a function of external K^+ concentration. The *SOS1*-dependent K^+ uptake was obtained by subtracting the uptake in *sos1* plants from that in the wild-type seedlings used for results in **(A)**. The insert shows the Hanes plot of *SOS1*-dependent K^+ uptake. [S], K^+ concentration (micromolar); V, K^+ uptake rate (nanomoles per gram per hour).

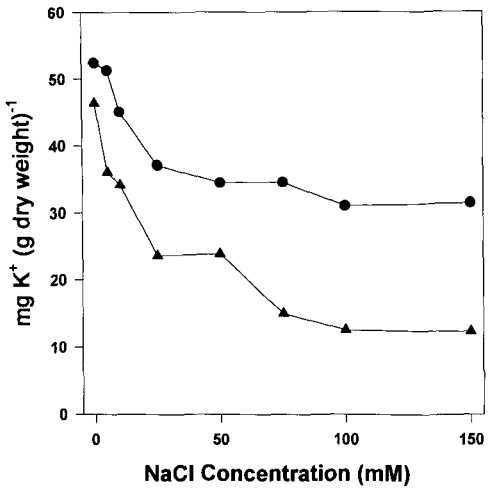

Figure 11. Potassium Content in *sos1* and Wild-Type Seedlings as a Function of NaCl Concentration in the External Media.

The media contain ~10 mM K^+ (see Methods for details). NaCl-treated *sos1* plants show potassium deficiency. ●, wild type; ▲, *sos1*.

concentration is low (Figures 7 and 8). It also is essential when plants are faced with high external Na^+ (Figure 1) even in the presence of 20 mM K^+ in the media. The addition of more K^+ (up to 100 mM) to the NaCl-containing media did not improve the growth of either *sos1* or the wild type (L. Ding and J.-K. Zhu, unpublished data). The low-affinity system must not be functioning under such a condition of high external Na^+. It is not known whether this is because it is thermodynamically unfavorable for the low-affinity system or because the plants actively turn off this system in response to Na^+ stress. An accurate thermodynamic calculation would require simultaneous measurement of membrane potential and cytoplasmic K^+ concentration. A switch from low-affinity to high-affinity K^+ uptake has been suggested to occur in yeast cells in response to Na^+ stress (Haro et al., 1993). In any case, high Na^+ has been shown to inhibit low-affinity K^+ uptake, whereas high-affinity uptake is relatively unaffected (Epstein et al., 1963).

K^+ uptake is not only an integral part of salt tolerance mechanisms in glycophytes, but it may hold a key to improving salt tolerance. It appears that increased salt tolerance in salt-adapted tobacco cells is accompanied by an enhanced K^+ uptake capacity (Watad et al., 1991). Increased K^+/Na^+ selectivity of the high-affinity transporter *HKT1* from wheat has been shown to improve salt tolerance of transfected *trk*$^-$ yeast cells (Rubio et al., 1995). The *Kna1* locus, which controls K^+/Na^+ selectivity in wheat, appears to be important in plant productivity under an excess of soil Na^+ (Dvorak and Gorham, 1992). In addition, the yeast *HAL1* gene, when overexpressed, confers osmotic and salt tolerance and enhances accumulation of K^+ in the presence of NaCl (Gaxiola et al., 1992).

It is tempting to speculate on the molecular nature of *SOS1*. One possibility is that *SOS1* encodes a high-affinity K^+ transporter similar to the cloned wheat gene *HKT1*. Alternatively, *SOS1* could encode a regulator of the high-affinity transporter. Although *HKT1* alone was sufficient to provide the injected Xenopus oocytes with K^+ flux (Schachtman and Schroeder, 1994), there may be another component(s) required for its proper function in plants.

METHODS

Plant Materials and Growth Conditions

Arabidopsis thaliana (ecotype Columbia) carrying the homozygous recessive *glabrous* (*gl1*) mutation (Koornneef et al., 1982) was the parental strain of ethylmethane sulfonate (EMS)–mutagenized seed. The wild-type Arabidopsis Landsberg *erecta* ecotype used for genetic mapping was from the Arabidopsis stock center (Columbus, OH).

Seeds were surface sterilized by soaking in a solution of Clorox plus 0.01% Triton X-100 for 10 min and rinsing five times with sterile water. The seeds were resuspended in sterile 0.3% (w/v) low-melting-point agarose before being sown in rows onto agar plates for germination. The agar medium contained Murashige and Skoog (MS) salts (Murashige and Skoog, 1962) with 3% (w/v) sucrose and 1.2% (w/v) agar, pH 5.7. The plates were then stored at 4°C for 48 hr to improve germination uniformity before being placed in a vertical position in a growth room for germination. When appropriate, seedlings (10 to 20 days old) were transferred to soil (Pro-Mix XB; Premier Brands Inc., Red Hill, PA) and grown to maturity. Plotted plants were watered twice a week, once with a nutrient solution (Peter's 15-16-17; Grace-Sierra Horticultural Products, Milpitas, CA). Temperature in the growth room was 23 ± 2°C. Light provided by cool-white fluorescent bulbs was 50 to 70 $\mu E\ m^{-2}\ sec^{-1}$ (constant) for seedlings in agar plates and ~100 $\mu E\ m^{-2}\ sec^{-1}$ (16-hr light/8-hr dark) for potted plants.

Isolation of Mutants and Genetic Analysis

EMS-mutagenized M_2 seed were obtained from Lehle Seeds (Round Rock, TX). Approximately 50,000 M_2 seeds representing ~6500 M_1 lines were screened for NaCl-hypersensitive mutants by using a root-bending assay. Four-day-old seedlings with 1- to 1.5-cm-long roots were transferred from the vertical agar plates, one by one, onto a second agar medium that was supplemented with 50 mM NaCl. The seedlings were arranged in rows, and the plates were oriented vertically

Table 2. Genetic Mapping of *sos1*

Markers	Chromosome	n^a	Recombination Frequency[b] (% ± SE)
nga63	I	28	44.6 ± 6.6
nga168	II	43	37.2 ± 5.2
GPA1	II	43	24.4 ± 4.6
nga8	IV	33	45.5 ± 6.1
nga76	V	28	50.0 ± 6.7

[a] Number of samples analyzed.
[b] Calculated by the Kosambi function (Koorneef and Stam, 1992).

with the roots pointing upward. Root growth and subsequent downward curving were apparent several hours after the transfer. Roots that did not show curving and apparent growth were noted, and the putative mutant seedlings were picked up 3 to 7 days later and placed on a 0.6% agar medium (without NaCl) to grow to six to seven rosette leaves. Twenty-one putative mutants were then transferred to soil to grow to maturity.

Aliquots of seeds from the putative mutants were screened again, using the above root-bending assay on agar plates with 50 mM NaCl or no NaCl (Figure 1B). Of the 21 putative mutants, four exhibited normal root bending on agar plates without NaCl, but no root bending was exhibited on plates with 50 mM NaCl; thus, the mutants were considered true salt-hypersensitive.

Mutants were crossed with the wild type (Columbia, *gl1*) by rubbing stamens from the mutants onto the stigma of emasculated wild-type flowers. The mutants also were crossed with each other for allelic tests. The NaCl sensitivity of F_1 and F_2 seedlings arising from the crosses was determined by the root-bending assay on 75 mM NaCl plates.

Growth Measurement

For stress treatments and growth measurement, 4-day-old seedlings from vertical plates were transferred and placed with roots pointing downward onto vertical agar plates supplemented with various salts. Each plate contained five mutant and five wild-type seedlings. Three replicate plates were used for each treatment. Increases in root length were measured with a ruler every day for 7 days.

Potassium-free medium was prepared by replacing MS salts with the following: 1650 mg/L NH_4NO_3, 440 mg/L $CaCl_2 \cdot 2H_2O$, 370 mg/L $MgSO_4 \cdot 7H_2O$, 165 mg/L $(NH_4)_2HPO_4$, 27.8 mg/L $FeSO_4 \cdot 7H_2O$, 37.3 mg/L disodium EDTA, 0.7495 mg/L NaI, 6.3 mg/L H_3BO_3, 16.9 mg/L $MnSO_4 \cdot H_2O$, 8.6 mg/L $ZnSO_4 \cdot 7H_2O$, 0.25 mg/L $Na_2MO_4 \cdot 2H_2O$, 0.016 mg/L $CuSO_4 \cdot 5H_2O$, and 0.0267 mg/L $CoSO_4 \cdot 6H_2O$. Where stated, NH_4NO_3 was replaced with an equal molar concentration of Tris–nitrate (Sigma) to determine any toxic effect of NH_4^+ on Arabidopsis root growth (Cao et al., 1993). Varying levels of potassium in the media were achieved by adding appropriate amounts of KCl to the K^+-free medium.

Determination of K^+ Content

Approximately 150 seeds were sterilized and incubated in a 250-mL flask containing 75 mL of medium (half-strength MS salts and 2% sucrose, pH 5.5). The flasks were shaken at 120 rpm in a chamber with continuous cool fluorescent light illumination and 22°C temperature. After 8 days, the appropriate amount of 5 M 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 collected, rinsed five times briefly with distilled water (total of 100 mL of water), weighed, and dried at 65°C for 24 hr. After dry weight measurement, K^+ content was determined by atomic absorption spectrophometry.

Measurement of Potassium (^{86}Rb) Uptake

For measurement of potassium uptake, using ^{86}Rb as a tracer, 4-day-old seedlings from vertical MS agar plates were transferred to vertical agar plates containing 5 μM K^+. After 2.5 days, 13 seedlings were collected, rinsed briefly in K^+-free medium, and then added to a 10-mL uptake solution containing K^+-free medium supplemented with appropriate amounts of KCl and 0.5 μCi/mL of ^{86}Rb (Amersham). The uptake was performed at 23°C under white fluorescent light. Uptake at different K^+ concentrations was performed for 30 min. Uptake at 200 μM or 20 mM K^+ was performed for 5, 10, 20, 30, 60, and 120 min. At the completion of uptake, the seedlings were rinsed twice (15 sec each) in 30 mL of K^+-free media and incubated for 15 min in 35 mL of ice-cold K^+-free media. In the case of uptake at 20 mM K^+, rinsing solutions were supplemented with 40 mM mannitol for osmotic balance. The seedlings were blotted dry on filter paper and weighed, and the radioactivity was measured in a scintillation counter.

Callus Initiation and Maintenance

Calli were generated from the wild type and mutants by sowing seeds directly on a medium containing the following: MS salts, 2% sucrose, 10 mg/L *myo*-inositol, 100 μg/L nicotinic acid, 1 mg/L thiamine-HCl, 100 μg/L pyridoxin-HCl, 400 μg/L glycine, 0.23 μM kinetin, 4.5 μM 2,4-D, and 1% (w/v) agar, pH 5.7. After 2 weeks, the calli were transferred to a callus maintenance medium with the above-mentioned components, except that the kinetin and 2,4-D concentrations were changed to 0.46 and 2.25 μM, respectively.

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