Plant Physiol. (1997) 114: 591–596

Proline Accumulation and Salt-Stress-Induced Gene Expression in a Salt-Hypersensitive Mutant of Arabidopsis[1]

Jiping Liu and Jian-Kang Zhu*

Department of Plant Sciences, University of Arizona, Tucson, Arizona 85721

The *sos1* mutant of *Arabidopsis thaliana* is more than 20 times more sensitive to NaCl stress than wild-type Arabidopsis. Because proline (Pro) is generally thought to have an important role in plant salt tolerance, the *sos1* mutant and the wild type were compared with respect to their capacity to accumulate Pro under NaCl stress, and *sos1* mutant plants accumulated more Pro than the wild type. The *P5CS* gene, which catalyzes the rate-limiting step in Pro biosynthesis, is induced by salt stress to a higher level in *sos1* than in the wild type. Although a defective high-affinity K uptake system in *sos1* causes K deficiency and inhibits growth in NaCl-treated plants, this decrease is not a sufficient signal for Pro accumulation and *P5CS* gene expression. Not all salt-stress-induced genes have a higher level of expression in *sos1*. The expression levels of *AtPLC* and *RD29A*, which encode a phospholipase C homolog and a putative protective protein, respectively, are the same in *sos1* as in the wild type. However, the expression of *AtMYB*, which encodes a putative transcriptional factor, is induced to a much higher level by salt stress in *sos1*. Thus, the *SOS1* gene product serves as a negative regulator for the expression of *P5CS* and *AtMYB*, but has no effect on *AtPLC* and *RD29A* expression.

Osmotic stress severely limits plant growth and agricultural productivity (Boyer, 1982). In response to drought and salinity stress, many plant species accumulate high levels of Pro, which is thought to function in stress adaptation (Adams and Frank, 1980; Greenway and Munns, 1980; Hanson and Hitz, 1982; Rhodes, 1987; Delauney and Verma, 1993). It has been suggested that Pro protects plant tissues against osmotic stress because it is an osmosolute, a source of nitrogen compounds, a protectant for enzymes and cellular structures (Stewart and Lee, 1974; Le-Rudulier et al., 1984; McCue and Hanson, 1990; Serrano and Gaxiola, 1994), and a scavenger for hydroxyl radicals (Smironff and Cumbes, 1989). Recently, it was reported that constitutive production of Pro could confer osmotolerance in transgenic tobacco plants (Kishor et al., 1995). However, the lack of correlation between Pro level and salt tolerance in certain plant species has also led to the conclusion that Pro accumulation is merely a consequence of stress and does not lead to salt tolerance (Bar-Nun and Poljakoff-Mayer, 1977; Hanson et al., 1979; Richards and Thurling, 1979; Moftah and Michel, 1987).

In higher plants either the glutamate or the Orn pathway is used for Pro biosynthesis (Adams and Frank, 1980; Delauney and Verma, 1993). Under osmotic stress, Pro accumulation is mainly a result of de novo synthesis from glutamate (Delauney and Verma, 1993). Genes encoding Pro biosynthesis enzymes have recently been cloned from higher plants (Delauney and Verma, 1990; Hu et al., 1992). The first two steps beginning from glutamate are catalyzed by P5CS (Adams and Frank, 1980), which is the rate-limiting enzyme (Hu et al., 1992; Zhang et al., 1995). Expression of *P5CS* has been found to be induced by salt stress, dehydration, and ABA (Hu et al., 1992; Yoshiba et al., 1995).

The mechanism by which osmotic stress induces *P5CS* gene expression and Pro accumulation is not understood. The gram-negative bacteria *Escherichia coli* and *Salmonella typhimurium* also accumulate osmolytes such as Pro and betaine in response to osmotic stress (Csonka and Hanson, 1991). The *proU* gene, which encodes a high-affinity betaine transport system, is induced by osmotic stress at the transcriptional level (Sutherland et al., 1986). This induction has been found to depend not on changes in turgor elicited by osmotic stress, but on the intracellular concentration of K^+ ions (Sutherland et al., 1986). Therefore, when bacterial cells are osmotically stressed by NaCl, the consequent changes in cell turgor lead to an increase in intracellular K^+ concentrations, which then induces *proU* expression. In salt-stressed plant cells, while the concentration of osmolytes such as Pro increases, the intracellular K^+ concentration decreases (Greenway and Munns, 1980). It is possible that intracellular K^+ depletion serves as an intermediate signal that mediates salt-stress-induced Pro accumulation in higher plants.

In addition to *P5CS*, many other genes are induced by salt stress in Arabidopsis. Some of the salt-stress-induced genes such as *RD29A* encode putative protective proteins (Yamaguchi-Shinozaki and Shinozaki, 1993), whereas others, such as *AtMYB* and *AtPLC*, encode proteins that share sequence identities to transcription factors and signaling components (Urao et al., 1993; Hirayama et al., 1995). The expression patterns of these genes have been extensively analyzed. However, no cellular component that positively or negatively regulates their expression has been identified.

Our laboratory has recently isolated an Arabidopsis mutant, *sos1*, which exhibits growth that is hypersensitive to

[1] This work was supported by a grant from the U.S. Department of Agriculture National Research Initiative Competitive Grants Program (no. 95-37100-2628).

* Corresponding author; e-mail jkzhu@ag.arizona.edu; fax 1–520–621–7186.

Abbreviations: MS, Murashige and Skoog; P5CS, Δ^1-pyrroline-5-carboxylate synthetase.

NaCl inhibition (Wu et al., 1996). Availability of this single-gene mutant provides an excellent opportunity to address whether Pro accumulation is obligatory for salt tolerance. We report here that *sos1* mutant plants accumulate more Pro than wild-type plants in response to salt stress. The *P5CS* gene is found to be overexpressed in salt-stressed *sos1* plants. Our results demonstrate that more Pro production does not necessarily lead to increased salt tolerance. Furthermore, we found that salt stress induced overaccumulation of Pro and the *P5CS* message is not mediated by an intracellular depletion of K^+. Several other salt-stress-induced genes were also examined in the *sos1* mutant, and the results suggest that the *SOS1* gene plays a negative role in the expression of some of these genes.

MATERIALS AND METHODS

Arabidopsis thaliana (ecotype Columbia) carrying the homozygous recessive *glabrous* (*gl1*) marker (Koornneef et al., 1982) was used as the wild-type control. The *sos1* mutant used for this work was the *sos1–1* allele (Wu et al., 1996). Seeds were surface-sterilized, suspended in sterile, 0.3% (w/v), low-melting-point agarose, and germinated in vertical agar plates containing MS salt (Murashige and Skoog, 1962), 3% (w/v) Suc, and 1.2% (w/v) agar, pH 5.7. Plants were grown at 22 to 24°C with continuous cool-fluorescent illumination.

Salt Stress and Low-K Treatments

Four-day-old seedlings from vertical plates were transferred onto vertical agar plates containing either the MS medium supplemented with different levels of NaCl or low-K (200 μM) medium. Low-K medium was prepared as described by Wu et al. (1996). After 5 to 7 d, the transferred plants were collected for Pro analysis. For RNA extraction, approximately 100 4-d-old seedlings from vertical plates were transferred to 250-mL flasks with 75 mL of medium containing one-half-strength MS salts and 2% (w/v) Suc, pH 5.5. The flasks were shaken at 120 rpm under constant light. After 2 d, the solution in the flasks was replaced with the same solution supplemented with different concentrations of NaCl or with the low-K solution. The plants were treated for 12 h before being harvested for RNA extraction, or were treated for 2 d in the case of Pro analysis.

RNA-Blot Analysis

Seedlings were harvested from the flasks, blotted dry with paper towels, and immediately frozen in liquid nitrogen. The samples were ground in liquid nitrogen and extracted on ice with 4.5 mL of extraction buffer containing 50 mM Tris-HCl, pH 8.0, 300 mM NaCl, 5 mM EDTA, 2% SDS, 2 mM aurintricarboxylic acid, and 10 mM β-mercaptoethanol. After the addition of 0.7 mL of cold 3 M KCl, the mixture was incubated on ice for 15 min and then centrifuged at 9000*g* for 20 min. RNA was precipitated from the supernatant by the addition of 2 mL of 8 M LiCl and incubation at 4°C overnight. The precipitate was pelleted by centrifugation at 9000*g* for 20 min and then resuspended in 2 mL of water. The suspension was extracted with phenol and chloroform and RNA was precipitated from the aqueous phase with ethanol. The pellet was washed with 80% ethanol, dried, and resuspended in 200 μL of water. RNA was separated on formaldehyde-agarose gels and blotted onto nylon membrane. The membrane was first stained with methylene blue to verify equal loading and transfer. Blots were then hybridized with fragments of *RD29A, P5CS, AtMYB,* or *AtPLC* that were labeled with ^{32}P by random primer labeling. The *P5CS* probe was a 1.6-kb cDNA fragment showing sequence identity to the *P5CS* gene reported in Yoshiba et al. (1995). *RD29A* (Yamaguchi-Shinozaki and Shinozaki, 1993), *AtMYB* (Urao et al., 1993), and *AtPLC* (Hirayama et al., 1995) probes were cloned from genomic DNA of wild-type Columbia plants by PCR. The PCR primer pairs used for the amplification of *RD29A, AtMYB,* and *AtPLC* were 5′ CCC GGA TCC TTT TCT GAT ATG GTT GCC 3′ and 5′ GCC CTC GAG CCG AAC AAT TTA TTA ACC 3′, 5′ GCG CAA TAT CTA CCG GGA AG 3′ and 5′ ATG TCG TAT CGG GGC AGA AC 3′, and 5′ GGC TTG AGA CTC TGG CAA AAA C 3′ and 5′ AAT ACC TGG CCT TAC CTC CGA C 3′, respectively. The *RD29A* fragment was a gene-specific probe of 0.5 kb from the 3′ noncoding region. Hybridizations were carried out at 55°C. Blots were washed at 55°C in 3× saline sodium citrate (Ausubel et al., 1987) plus 0.1% SDS.

mRNA bands on radiographic films were scanned with a densitometer (model 300A, Molecular Dynamics, Sunnyvale, CA). rRNA bands probed with labeled 18S rRNA were also scanned as controls. mRNA intensities were normalized against the corresponding rRNA levels.

Pro Analysis

Seedlings were harvested, frozen in liquid nitrogen, and then dried by lyophilization. Approximately 50 mg of dry seedling tissue was ground in 3% sulfosalicylic acid to extract free Pro. Pro concentration was determined as described by Bates et al. (1973).

RESULTS

sos1 Plants Overaccumulate Pro under Salt Stress

In terms of growth inhibition, the *sos1* mutant is more than 20 times more sensitive to salt stress than the wild type (Wu et al., 1996). Because Pro accumulation has been suggested to be important for salt tolerance, we were interested in determining the level of Pro in *sos1* plants. Free Pro in wild-type and *sos1* seedlings exposed to different levels of NaCl were extracted and measured. Figure 1A shows that Pro levels increased in both wild-type and *sos1* plants in response to 50 mM NaCl treatment for 2 d in solution culture. However, the Pro level in *sos1* was nearly twice as much as that in the wild type after 50 mM NaCl treatment (Fig. 1A). Without NaCl treatment, *sos1* contained slightly more Pro than the wild type (Fig. 1A). Because most of the growth measurements and estimates of NaCl sensitivity *sos1* were carried out on plants that were treated on NaCl-containing agar plates (Wu et al., 1996), the Pro contents of wild-type and *sos1* seedlings treated for 5 d on plates containing 0 or 50 mM NaCl were determined (Fig. 1B). Similarly, Pro levels increased in both wild-type

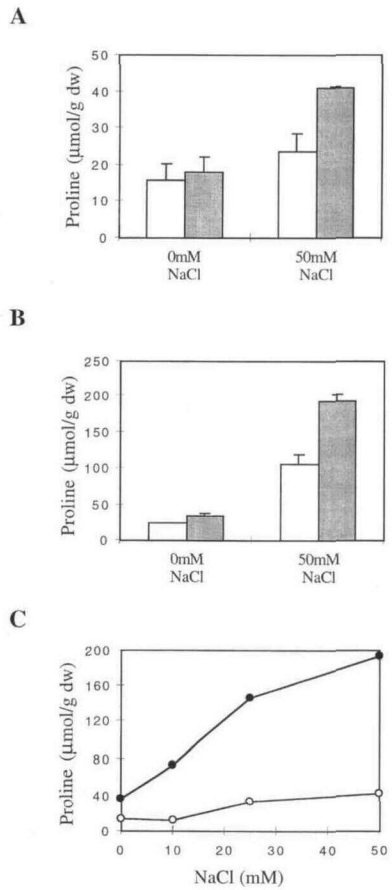

Figure 1. *sos1* plants produce higher levels of Pro than the wild type in response to salt stress. A, Pro levels in seedlings treated in solution culture for 2 d ($n = 3$); B, Pro levels in seedlings treated in agar plates for 5 d ($n = 3$); C, Pro content as a function of external NaCl concentration. The plants were treated in agar plates for 7 d. Open bar, Wild type; shaded bar, *sos1*; ○, wild type; ●, *sos1*; dw, dry weight.

and *sos1* plants upon NaCl treatment. The levels were approximately three to four times higher in plants treated in the agar plates compared with plants from the solution culture (Fig. 1). Again, the Pro level in *sos1* was about twice as much as that in wild-type plants when treated with 50 mM NaCl. Pro content in plants treated with different concentrations of NaCl was also measured. Although the absolute values varied 2- to 3-fold among different experimental runs, the trend was always that *sos1* contained at least twice as much Pro as the wild type when they were both treated with NaCl. Figure 1C presents results from a typical experiment in which the mutant and the wild-type seedlings were treated for 7 d on agar plates containing different levels of NaCl.

P5CS, a Gene Encoding a Key Enzyme in Pro Biosynthesis, Is Expressed at a Higher Level in *sos1* Plants under Salt Stress

The rate-limiting step in Pro biosynthesis from glutamate is catalyzed by P5CS (Hu et al., 1992; Zhang et al., 1995). In Arabidopsis, the *P5CS* gene is induced by salt stress and other forms of osmotic stress (Yoshiba et al., 1995). To investigate if the overaccumulation of Pro in *sos1* plants under salt stress could be due to changes in *P5CS* gene expression, northern analysis was performed on RNA extracted from seedlings treated with different concentrations of NaCl for 12 h. Figure 2 shows that the steady-state levels of*P5CS* mRNA increased in both wild-type and *sos1* plants as a function of external NaCl concentration. However, the increase was higher in *sos1* plants (Fig. 2). A quantitative analysis of the results presented in Figure 3 showed that the *P5CS* message abundance is approximately 1 to 2 times higher in *sos1* plants than in the wild-type plants. For example, both *sos1* and the wild type had maximal levels of *P5CS* expression at 150 mM NaCl. However, the abundance in *sos1* is about 2.5 times higher than in wild-type plants (Fig. 3). The results suggest that Pro overaccumulation in *sos1* is due at least partially to increased *P5CS* expression.

Low K Inhibits the Growth of *sos1* Plants, But Has No Effect on Pro Production and *P5CS* Gene Expression

The *sos1* mutant has a defective high-affinity K uptake system, which results in intracellular K deficiency under salt stress (Wu et al., 1996). Since increased K content during salt stress serves as a signal for Pro and betaine accumulation in bacteria (Sutherland et al., 1986), we hypothesized that a decreased level of K in salt-stressed plants could be a signal for Pro accumulation in plants as well. Thus, the increased Pro content and *P5CS* expression in *sos1* could be due to a more pronounced decrease in K^+ concentration (Wu et al., 1996) when the mutant is treated with NaCl. Phenotypically, low K^+ and high NaCl both inhibit the growth of *sos1* plants (Wu et al., 1996). *sos1* and wild-type plants grown on MS media were transferred to medium containing either 20 mM K^+ in MS medium, 100 mM NaCl in MS medium, or 200 μM K^+ for 5 d. As shown in Figure 4A, 100 mM NaCl induced Pro accumulation. However, neither wild-type nor *sos1* plants treated with 200 μMK^+ had significantly increased Pro levels (Fig. 4A), even though 200 μM K^+ and 100 mM NaCl caused similar growth inhibition in *sos1* plants (Fig. 4B). RNA-blot analysis showed that *P5CS* expression was not induced by low-K treatment in either wild-type or *sos1* plants (Fig. 4C). These data do not support our original hypothesis, and instead suggest that Pro accumulation and *P5CS* expression are

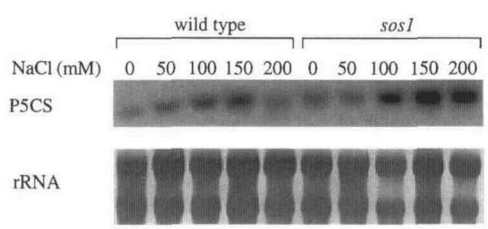

Figure 2. Expression of the *P5CS* gene is induced to higher levels by salt stress in *sos1* than in the wild type. Wild-type and *sos1* seedlings were treated with various levels of NaCl for 12 h in solution culture. The lower panel shows rRNA stained with methylene blue as a control for loading and transfer.

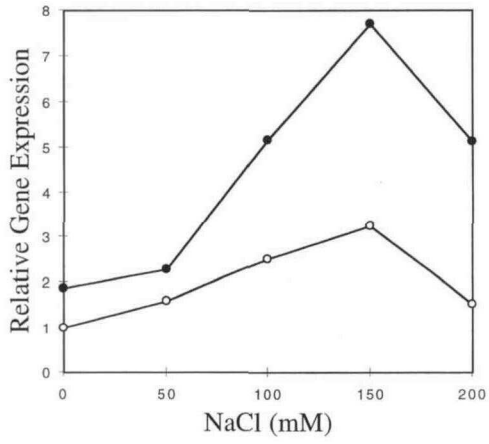

Figure 3. Quantitative representation of the data in Figure 2. *P5CS* band intensities were scanned and normalized against the corresponding rRNA level. ○, Wild type; ●, *sos1*.

unlikely to be caused by decreased cellular K^+ content in salt-stressed cells.

The *sos1* Mutation Differentially Affects Salt-Induced Gene Expression

A large number of plant genes are induced under salt-stress conditions (Bray, 1993; Serrano and Gaxiola, 1994). Because the *sos1* mutation increases the salt-stress-induced expression of *P5CS*, we examined several other salt-induced genes to determine the effect of the mutation on their expression. All of the three genes (*RD29A*, *AtMYB*, and *AtPLC*) examined here are known to be induced by salt-stress conditions in Arabidopsis (Yamaguchi-Shinozaki and Shinozaki, 1993; Urao et al., 1993; Hirayama et al., 1995). *RD29A* encodes a putative protective protein (Yamaguchi-Shinozaki and Shinozaki, 1993). *AtMYB* encodes a putative transcriptional factor related to MYB protein (Urao et al., 1993). *AtPLC* is a gene coding for phosphatidylinositol-specific phospholipase C, which functions in signal transduction (Hirayama et al., 1995). Neither the mechanisms of salt-stress regulation nor the exact functions of these genes in salt tolerance are known.

As shown in Figure 5, all of the three genes are induced by NaCl treatments. Expression levels of *AtPLC* and *RD29A* are not significantly different between *sos1* and wild-type plants. The level of *AtMYB* expression is substantially higher in *sos1* compared with the wild type. At 100 mM NaCl, *AtMYB* mRNA abundance in wild-type plants did not increase significantly compared with the control treatment (0 mM NaCl). However, 100 mM NaCl strongly increased *AtMYB* expression in *sos1*. Figure 5B also shows that low-K treatment did not induce the expression of *RD29A*.

DISCUSSION

In this paper we examined Pro accumulation and salt-stress-induced gene expression in the salt-hypersensitive Arabidopsis mutant, *sos1*. The fact that a mutant more sensitive to salt stress contains more Pro suggests that Pro content is not a factor limiting salt tolerance in this plant. The data strongly support the notion that Pro accumulation is a symptom of stress injury rather than an indicator of stress tolerance.

The overaccumulation of Pro in this single-gene mutant also provides an opportunity to study how Pro increases under salt stress. We hypothesized that overaccumulation of Pro could be due to a much lower cellular K content in salt-stressed *sos1*. The hypothesis was based on the observation that salt stress leads to more K in bacterial cells, which in turn serves as a signal for betaine accumulation (Sutherland et al., 1986). Salt stress results in decreased K content in plant cells. Despite a good correlation between Pro accumulation and K deficiency in salt-stressed *sos1* plants, our results indicate that decreased K content is not an intermediate signal for salt regulation of Pro accumulation or of *P5CS* gene expression. What, then, is the signal for Pro accumulation and *P5CS* gene expression? Apparently, Pro production is not simply a consequence of growth inhibition. This is because, although low-K treat-

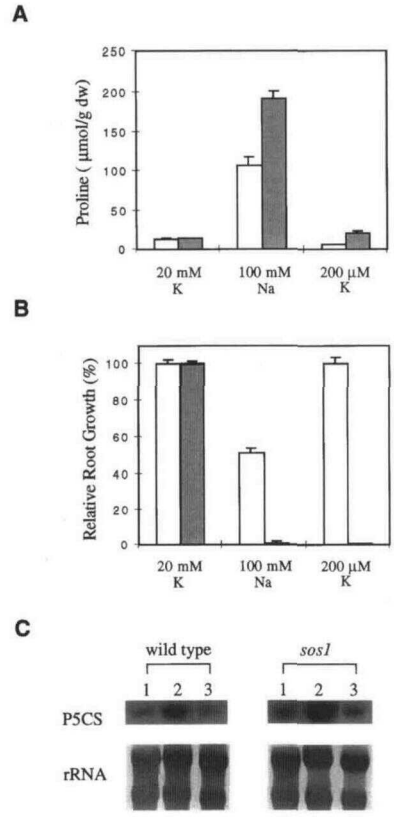

Figure 4. Low-K treatment does not induce *P5CS* gene expression or Pro accumulation in wild-type and *sos1* plants. A, Pro content of *sos1* and wild-type plants treated with control (20 mM K), salt (100 mM NaCl), and low-K (200 μM K) media (n = 3). Notice that low-K treatment does not induce Pro accumulation. B, Relative root growth of *sos1* and wild-type plants treated with control, salt, and low-K media (n = 18). Note that both low-K and salt treatments inhibit *sos1* growth. Open bars, Wild type; shaded bar, *sos1*; dw, dry weight. C, *P5CS* gene expression. Note that low-K treatment does not induce *P5CS* expression. Lanes 1, Control; lanes 2, salt treatment; lanes 3, low-K treatment.

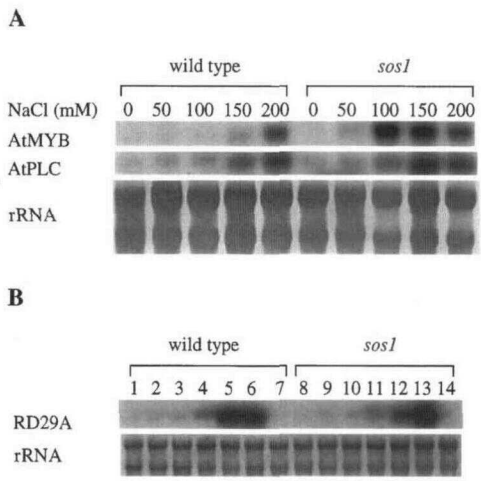

Figure 5. Expression of *AtMYB*, *AtPLC*, and *RD29A* as a function of external NaCl concentration in wild-type and *sos1* plants. Plants were treated with NaCl for 12 h in solution culture. A, Patterns of expression of *AtMYB* and *AtPLC*. B, Expression of *RD29A*. Lanes 1 and 8, Control (0 mM NaCl, 20 mM K^+); lanes 2 and 9, 25 mM NaCl; lanes 3 and 10, 50 mM NaCl; lanes 4 and 11, 100 mM NaCl; lanes 5 and 12, 150 mM NaCl; lanes 6 and 13, 200 mM NaCl; lanes 7 and 14, 200 μM K^+.

ment caused near complete inhibition of *sos1* growth (Fig. 4), it did not significantly induce Pro accumulation. One possibility is that the signal could be an increased Na^+ content in salt-treated plants. However, we found that *sos1* has a significantly lower Na^+ content than the wild type when treated with NaCl (Ding and Zhu, 1996). We suggest that turgor reduction is an intermediate signal for Pro accumulation in salt-stressed plant cells. When treated with NaCl stress, *sos1* plants contain less K^+ and less Na^+ compared with wild-type plants. Because Pro and other organic osmolytes only increase slightly (Ishitani et al., 1996), K^+ and Na^+ are the predominant osmolytes in salt-stressed Arabidopsis. Therefore, less cellular K^+ and Na^+ in *sos1* plants probably leads to higher cellular osmotic potential and, consequently, more pronounced turgor reduction. The greater turgor reduction could then cause higher *P5CS* gene expression and Pro production. In this regard, increased Pro production in *sos1* may partially compensate its deficiency in inorganic osmolytes (i.e. K^+ and Na^+). Future investigations into the water relations in the *sos1* mutant might provide clues regarding this speculation.

It is intriguing that the level of *AtMYB* expression is higher in *sos1*. Because *AtMYB* encodes a putative transcriptional factor, its increased expression may result in higher levels of expression of a group of its target genes. None of the genes regulated by *AtMYB* has been identified. It is possible that *P5CS* is one such gene, because it also has a higher level of expression in *sos1*.

One would expect that the level of *RD29A* expression would be higher in *sos1* because the *RD29A* gene product is generally considered a stress protein (although its function is unknown). It is surprising that this stress gene is not expressed at a higher level in salt-treated *sos1*, even though this mutant is much more severely stressed or inhibited by NaCl (Fig. 5B), which might indicate that *RD29A* expression is caused by a stress factor that is not preferentially activated in *sos1*. In any case, our results suggest that the *SOS1* gene is a negative regulator for the salt-stress-regulated expression of *P5CS* and *AtMYB*, but not *AtPLC* or *RD29A*. The results also imply that the signal transduction pathways for the salt induction of *P5CS* and *AtMYB* are different from those used by *AtPLC* and *RD29A*.

ACKNOWLEDGMENTS

The authors would like to acknowledge the excellent technical assistance of Lei Ding and Becky Stevenson. We are also grateful to Albino Maggio and Dr. Ray A. Bressan for the Arabidopsis *P5CS* probe.

Received November 25, 1996; accepted March 13, 1997.

LITERATURE CITED

Adams E, Frank L (1980) Metabolism of proline and the hydroxyprolines. Annu Rev Biochem **49:** 1005–1061

Ausubel FM, Brent R, Kingston RE, Moore DD, Seidman JG, Smith JA, Struhl K, eds (1987) Current Protocols in Molecular Biology. John Wiley and Sons, New York

Bar-Nun N, Poljakoff-Mayer A (1977) Salinity stress and the contents of proline in roots of *Pisum sativum* and *Tamarix tetragyna*. Ann Bot **41:** 173–179

Bates LS, Waldren RP, Teare ID (1973) Rapid determination of free proline for water stress studies. Plant Soil **39:** 205–207

Boyer JS (1982) Plant productivity and environment. Science **218:** 443–448

Bray EA (1993) Molecular responses to water deficit. Plant Physiol **103:** 1035–1040

Csonka LN, Hanson AD (1991) Prokaryotic osmoregulation: genetics and physiology. Annu Rev Microbiol **45:** 569–606

Delauney AJ, Verma DPS (1990) A soybean gene encoding Δ^1-pyrroline-5-carboxylate reductase was isolated by functional complementation in *Escherichia coli* and is found to be osmoregulated. Mol Gen Genet **221:** 299–305

Delauney AJ, Verma DPS (1993) Proline biosynthesis and osmoregulation in plants. Plant J **4:** 215–223

Ding L, Zhu J-K (1996) Reduced Na^+ uptake in the NaCl-hypersensitive *sos1* mutant of *Arabidopsis thaliana*. Plant Physiol **113:** 795–799

Greenway H, Munns R (1980) Mechanism of salt tolerance in nonhalophytes. Annu Rev Plant Physiol **31:** 149–190

Hanson AD, Hitz WD (1982) Metabolic responses of mesophytes to plant water deficits. Annu Rev Plant Physiol **33:** 163–203

Hanson AD, Nelson CE, Pedersen AR, Everson EH (1979) Capacity for proline accumulation during water stress in barley and its implications for breeding for drought resistance. Crop Sci **19:** 489–493

Hirayama T, Ohto C, Mizoguchi T, Shinozaki K (1995) A gene encoding a phosphatidylinositol-specific phospholipase C is induced by dehydration and salt stress in *Arabidopsis thaliana*. Proc Natl Acad Sci USA **92:** 3903–3907

Hu C-AA, Delauney AJ, Verma DPS (1992) A bifunctional enzyme (Δ^1-pyrroline-5-carboxylate synthetase) catalyzes the first two steps in proline biosynthesis in plants. Proc Natl Acad Sci USA **89:** 9354–9358

Ishitani M, Majumder AL, Bornhouser A, Michalowski CB, Jensen RG, Bohnert HJ (1996) Coordinate transcriptional induction of *myo*-inositol metabolism during environmental stress. Plant J **9:** 537–548

Kishor PBK, Hong Z, Miao G-H, Hu C-A, Verma DPS (1995) Overexpression of Δ^1-pyrroline-5-carboxylate synthetase increases proline products and confers osmotolerance in transgenic plants. Plant Physiol **108:** 1387–1394

Koornneef M, Dellaert LWM, van der Veen JH (1982) EMS- and radiation-induced matation frequencies at individual loci in *Arabidopsis thaliana* (L.) Heynh Mutat Res **93:** 109–123

Le-Rudulier D, Strom AR, Dandekar AM, Smith LT, Valentine RC (1984) Molecular biology of osmoregulation. Science **224:** 1064–1068

McCue KF, Hanson AD (1990) Drought and salt tolerance: towards understanding and application. Trends Biotech **8:** 358–362

Moftah AE, Michel BE (1987) The effect of sodium chloride on solute potential and proline accumulation in soybean leaves. Plant Physiol **83:** 238–240

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol Plant **15:** 473–479

Rhodes D (1987) Metabolic responses to stress. *In* PK Stumpf, EE Conn, DD Davies, eds, The Biochemistry of Plants: A Comprehensive Treatise, Vol 12. Physiology of Metabolism. Academic Press, New York, pp 201–241

Richards RA, Thurling N (1979) Genetic analysis of drought stress response in rapeseed (*Brassica campestris* and *B.napus*). III. Physiological characters. Euphytica **28:** 755–759

Serrano R, Gaxiola R (1994) Microbial models and salt stress tolerance in plants. Crit Rev Plant Sci **13:** 121–138

Smironff N, Cumbes QJ (1989) Hydroxyl radical scavenging activity of compatible solutes. Phytochemistry **28:** 1057–1060

Stewart GR, Lee JA (1974) The role of proline accumulation in halophytes. Planta **120:** 279–289

Sutherland L, Cairney J, Elmore MJ, Booth IR, Higgins CF (1986) Osmotic regulation of transcription: induction of the *proU* betaine transport gene is dependent on the intracellular potassium. J Bacteriol **168:** 805–814

Urao T, Yamaguchi-Shinozaki K, Urao S, Shinozaki K (1993) An *Arabidopsis* myb homolog is induced by dehydration stress and its gene product binds to the conserved MYB recognition sequence. Plant Cell **5:** 1529–1539

Wu S-J, Ding L, Zhu J-K (1996) *SOS1*, a genetic locus essential for salt tolerance and potassium acquisition. Plant Cell **8:** 617–627

Yamaguchi-Shinozaki K, Shinozaki, K (1993) Characterization of the expression of a desiccation-responsive *rd29* gene of *Arabidopsis thaliana* and analysis of its promoter in transgenic plants. Mol Gen Genet **236:** 331–340

Yoshiba Y, Kiyosue T, Katagiri T, Ueda H, Mizoguchi T, Yamaguchi-Shinozaki K, Wada K, Harada Y, Shinozaki K (1995) Correlation between the induction of a gene for Δ^1-pyrroline-5-carboxylate synthetase and the accumulation of proline in *Arabidopsis thaliana* under osmotic stress. Plant J **7:** 751–760

Zhang C-S, Lu Q, Verma DPS (1995) Removal of feedback inhibition of Δ^1-pyrroline-5-carboxylate synthetase, a bifunctional enzyme catalyzing the first two steps of proline biosynthesis in plants. J Biol Chem **270:** 20491–20496