Arabidopsis sos1 mutant in a salt-tolerant accession revealed an importance of salt acclimation ability in plant salt tolerance

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Keywords: salt tolerance, Arabidopsis, natural variation, salt acclimation

An analysis of the salinity tolerance of 354 Arabidopsis thaliana accessions showed that some accessions were more tolerant to salt shock than the reference accession, Col-0, when transferred from 0 to 225 mM NaCl. In addition, several accessions, including Zu-0, showed marked acquired salt tolerance after exposure to moderate salt stress. It is likely therefore that Arabidopsis plants have at least two types of tolerance, salt shock tolerance and acquired salt tolerance. To evaluate a role of well-known salt shock tolerant gene SOS1 in acquired salt tolerance, we isolated a sos1 mutant from ion-beam-mutagenized Zu-0 seedlings. The mutant showed severe growth inhibition under salt shock stress owing to a single base deletion in the SOS1 gene and was even more salt sensitive than Col-0. Nevertheless, it was able to survive after acclimation on 100 mM NaCl for 7 d following by 750 mM sorbitol for 20 d, whereas Col-0 became chlorotic under the same conditions. We propose that genes for salt acclimation ability are different from genes for salt shock tolerance and play an important role in the acquisition of salt or osmotic tolerance.

Introduction

Excess salt accumulation in soils causes both osmotic and ionic stresses, which greatly harm crop growth. In response to salt stress, plants express various genes encoding enzymes for osmolyte synthesis, ion channels, receptors and components of calcium or other regulatory signaling to confer salt tolerance. Genetic studies have revealed several essential genes conferring salt tolerance in a glycophyte accession of Arabidopsis thaliana, Col-0. These include genes for a plasma membrane Na+/H+ antiporter (SOS1), a vacuolar Na+/H+ antiporter (NHX1) and a plasma membrane Na⁺ transporter (HKT1). However, what confers salt tolerance in halophytes is poorly understood.

Arabidopsis thaliana is distributed widely across the world. This broad geographic distribution encompasses substantial variation in growth environments, so the phenotypic variation among accessions should reflect the genetic variation that allows adaptation to specific conditions. Considerable variation in potentially adaptive traits has been found in Arabidopsis, including resistance to biotic stresses and tolerance of abiotic stresses. Such accessions may be useful as sources of genetic material. We studied the salt tolerance of 354 Arabidopsis accessions during vegetative growth and found a wide range of variation in salt tolerance among them. Several accessions, including Bu-5, Bur-0, Ll-1, WI-0 and Zu-0, were much more tolerant than the salt-sensitive accession Col-0. These accessions showed not only greater salt-shock tolerance than Col-0, but also acclimation to salt stress after brief exposure to moderate salt stress, which Col-0 lacked. Genetic analyses indicated that the salt-shock tolerance is a quantitative trait under polygenic control. Although there has been a great deal of study on plant salt-stress responses, almost all reported genes are assumed to function in salt-shock tolerance. On the other hand, the salt acclimation ability is regulated by a single locus on chromosome 5 that is common among the markedly salt-tolerant accesses. To evaluate a role of salt-shock gene in acquired salt tolerance, we screened ion-beam-mutagenized Zu-0 seedlings for a salt-shock mutant.

Results and Discussion

Isolation of a salt-sensitive mutant of salt-tolerant Zu-0. The salt-tolerant accession Zu-0 shows not only salt-shock tolerance, but also salt acclimation ability. To dissect the contribution of the salt acclimation ability, we generated ion-beam-mutagenized seed pools from Zu-0 and screened mutants defective in salt shock tolerance with Zu-0 background from M2 seeds: 7-d-old seedlings grown on nylon mesh on MS agar were transferred to MS agar supplemented with 225 mM NaCl and grown on

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Submitted: 04/10/13; Revised: 04/22/13; Accepted: 04/23/13
Citation: Ariga H, Katori T, Yoshihara R, Hase Y, Nozawa S, Narumi I et al. Arabidopsis sos1 mutant in a salt-tolerant accession revealed an importance of salt acclimation ability in plant salt tolerance. Plant Signal Behav 2013; 8: e24779; http://dx.doi.org/10.4161/psb.24779
mutant. Seedlings of Zu-0 showed greater salt tolerance than those of Col-0, as reported, but the mutant seedlings showed hypersensitivity (Fig. 1).

**Na⁺ accumulation in shoots of Zu-0 mutant.** The restriction of the transport of Na⁺ from roots to shoots is important to salt tolerance. Using ion chromatography, we compared the Na⁺ content in shoots between the mutant and wild-type plants under salt stress. The Na⁺ content increased less in Zu-0 than in Col-0 (Fig. 2). A system for suppressing Na⁺ influx into shoots may account in part for the high salt-shock tolerance of Zu-0. On the other hand, the mutant accumulated more Na⁺ than Zu-0 (Fig. 2). The excess uptake of Na⁺ may account for the hypersensitive phenotype of the mutant.

**Genetic analysis of the salt-sensitive mutant.** We crossed the mutant with the salt-tolerant Bu-5 and used 46 F₂ progeny to map the mutation. The locus responsible for the salt sensitivity of the mutant was located near the distal end of the short arm of chromosome 2, near the simple-sequence-length polymorphic marker nga1145 (Fig. 3A). *SOS1*, a gene encoding a Na⁺/H⁺ antiporter, lies within 270 kb of nga1145. Because *SOS1* plays an essential role in salt tolerance in Col-0 and the Col-0 sos1 mutant shows severe defects in salt tolerance and excessive Na⁺ uptake, as does this mutant, we hypothesized that *SOS1* is the relevant gene. To test this hypothesis, we cloned a DNA fragment including *SOS1* from both the mutant and the wild type (Zu-0) and determined the sequences. We found a single base deletion in *SOS1* of the mutant, which caused a frame shift followed by a stop codon terminating the SOS1 protein in the 4th transmembrane domain (Fig. 3B and C).

To confirm whether the mutated *SOS1* is the causal gene, we crossed the mutant with the *sos1-1* mutant (Col-0 background). The F₁ seedlings showed the same hypersensitivity to salt stress as *sos1-1* and the mutant of Zu-0 (Fig. 4). This allelism test demonstrated that the *SOS1* mutation is the cause of the salt-sensitive phenotype of the mutant and that SOS1 plays an essential role in the salt-shock tolerance of Zu-0.

**Acquired salt tolerance of the mutant.** To test the salt acclimation ability of the mutant, we transferred 7-d-old seedlings onto MS agar supplemented with 100 mM NaCl (which does not affect the growth of Col-0 or Zu-0) to acclimate for 7 d and then onto MS agar supplemented with 750 mM sorbitol for a further 20 d. The mutant seedlings showed growth inhibition during the acclimation period. However, they were able to survive the subsequent severe osmotic stress, as were the Zu-0 wild-type seedlings, whereas Col-0 seedlings showed complete chlorosis (Fig. 5). The *sos1 × Zu-0* was therefore defective in salt-shock tolerance, but not in salt acclimation ability. It was more tolerant than the Col-0 plants, which cannot acclimate but have normal salt-shock tolerance mechanisms. Despite its clear effect of acclimation on acquired osmotic tolerance to 750 mM sorbitol (Fig. 5), *sos1 × (Zu-0)* did not show remarkable tolerance toward 300 mM NaCl after acclimation. This could be due to severe Na⁺ toxicity in the mutant, as Na⁺ accumulation in shoots is even higher than Col-0 (Fig. 2.) It is thought that the *sos1* mutation induces severe Na⁺ toxicity in the Zu-0 mutant. These data indicated that acclimation is not a process only to enhance tolerance to

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**Figure 1.** The hypersensitive phenotype of the mutant in the salt-shock assay. Seven-day-old seedlings grown on nylon mesh (990 μm) on MS agar (upper panel) were transferred to MS agar supplemented with 225 mM NaCl and grown on for 7 d (lower panel).

**Figure 2.** Na⁺ contents in shoots under salt stress. Seven-day-old seedlings of the mutant, Col-0 and Zu-0 were cultured on MS plates supplemented with 100 mM NaCl for 3 d. Values are means ± SD for five individual experiments (n = 5). Differences between mutant and Zu-0 (*), Zu-0 and Col-0 (+), or mutant and Col-0 (‡) were analyzed by Student’s t-test. *, ‡, ‡p < 0.05; ‡‡p < 0.001.
acquired thermotolerance was compromised in both hsa32 and hsfA2 knockout mutants. Here we propose that the salt acclimation ability is a novel salt tolerance, which is independent of the well known salt-shock tolerance including SOS1 and plays an important role in the acquisition of greater salt- or osmotic-stress tolerance.

**Material and Methods**

**Plant materials and culture.** Seeds of Arabidopsis thaliana Zu-0 (JA264) were provided by the RIKEN Bio Resource Center, Japan. They were sown on Murashige and Skoog (MS) agar plates containing salt shock, but is independent from SOS1 function in response to salt shock in Zu-0. We propose that salt acclimation ability is an important mechanism for higher salt stress tolerance in Arabidopsis, especially in osmotic stress tolerance. Among acquired stress tolerance systems, acclimation to temperatures is well known. Most temperate plants, including Arabidopsis, develop freezing tolerance after exposure to low, non-freezing temperatures (< 10°C), a process called cold acclimation. The expression of heat shock proteins induced by non-lethal heat treatment also confers tolerance to otherwise lethal high temperatures. Arabidopsis proteins Hsa32 and HsfA2 have been reported to play important roles in plant heat acclimation and acquired thermotolerance was compromised in both hsa32 and hsfA2 knockout mutants. Here we propose that the salt acclimation ability is a novel salt tolerance, which is independent of the well known salt-shock tolerance including SOS1 and plays an important role in the acquisition of greater salt- or osmotic-stress tolerance.
The plants, watered with 1:1,000 Hyponex nutrient solution, were selfed to set M2 seeds as a screening pool. Using a salt-shock-tolerance assay described in the experimental section 3.3, we screened ~35,000 M2 seeds for salt-sensitive mutants.

**Measurement of Na⁺.** Seven-day-old Col-0 and Zu-0 seedlings grown on nylon mesh on MS agar plates were transferred to MS agar supplemented with 100 mM NaCl and grown on for 3 d. The shoots were harvested at 0, 1 and 3 d after transfer and soaked in 5 mL of sterile distilled water for 5 sec. The solution with the plants was then boiled for 15 min, passed through a 0.2-μm filter (Toyo Roshi Kaisha, Ltd.), diluted 1:20 with distilled water and analyzed for Na⁺ using a Shim-pack IC-C3/C3 (S) column on a PIA-1000 Personal Ion Analyzer.

**Genetic mapping.** The single isolated mutant was crossed with salt-tolerant Bu-5 and the resulting F1 progeny were selfed to generate F2 populations. Genomic DNA was prepared from individual F2 seedlings with the salt-sensitive phenotype for use as PCR templates. For mapping we used the following simple-sequence-length polymorphism markers: Chr. 1, NT1G11, nga248 and NF5114; Chr. 2, nga1145, PLS8 and nga168; Chr. 3, nga162, CIW11 and nga6; Chr. 4, JV30/31, DET1.2 and nga1139; Chr. 5, nga151, SO262 and JV75/76. PCR conditions used an initial 94°C for 2 min; 40 cycles of 94°C for 20 sec, 54 or 57°C for 20 sec and 72°C for 5 sec; and a final 72°C for 2 min. The microsatellites were fractionated in 6% agarose gel and recombinant values were calculated from the band patterns.

**Conclusion**

*Arabidopsis thaliana* Zu-0 is a highly salt-tolerant accession with salt acclimation ability. We screened hypersensitive plants from Zu-0 M2 seeds and identified a mutant. The mutant was defective in salt-shock tolerance on account of a 1-bp deletion in *SOS1*, but not in salt acclimation ability. The Zu-0 mutant plants showed greater tolerance than the wild-type Col-0 plants, which defect in acquired salt tolerance. We suggest the importance of salt acclimation ability to severe salt- or osmotic-tolerance.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

**Acknowledgments**

We thank Akane Horiguchi (Tokyo University of Agriculture) for generating the ion-beam-mutagenized F2 seed pool. The *Arabidopsis* accessions used in this study were provided by the RIKEN Bio-Resource Center through the National Bio-Resource Project of MEXT. The *sos1-1* mutant was provided by Jian-Kang Zhu (University of California, Riverside). This work was supported by a Grant-in-Aid for Scientific Research on Innovative Areas (No. 23119518 to T.T.) and for Scientific Research (C) (No. 24580483 to T.T.) from the Ministry of Education, Culture, Sports, Science and Technology of Japan.
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