International Journal of Biological, Life and Agricultural Sciences
ISSN: 2415-6612
Vol:8, No:3, 2014

A Novel Gene Encoding Ankyrin-Repeat Protein, *SHG1*, is Indispensable for Seed Germination under Moderate Salt Stress

H. Sakamoto, J. Tochimoto, S. Kurosawa, M. Suzuki, S. Oguri

***Abstract*—**Salt stress adversely affects plant growth at various stages of development including seed germination, seedling establishment, vegetative growth and finally reproduction. Because of their immobile nature, plants have evolved mechanisms to sense and respond to salt stress. Seed dormancy is an adaptive trait that enables seed germination to coincide with favorable environmental conditions. We identified a novel locus of *Arabidopsis*, designated *SHG1* (*salt hypersensitive germination 1*), whose disruption leads to reduced germination rate under moderate salt stress conditions. *SHG1* encodes a transmembrane protein with an ankyrin-repeat motif that has been implicated in diverse cellular processes such as signal transduction. The *shg1*-disrupted *Arabidopsis* mutant died at the cotyledon stage when sown on salt-containing medium, although wild-type plants could form true leaves under the same conditions. On the other hand, this mutant showed similar phenotypes to wild-type plants when sown on medium without salt and transferred to salt-containing medium at the vegetative stage. These results suggested that *SHG1* played indispensable role in the seed germination and seedling establishment under moderate salt stress conditions. *SHG1* may be involved in the release of seed dormancy.

***Keywords*—**Germination, ankyrin repeat, *Arabidopsis*, salt tolerance.

I. Introduction

SOIL salinity is a critical environmental factor that adversely affects crop productivity and quality. Because of their immobile nature, plants have evolved mechanisms to sense and respond to salt stress.

Salt stress adversely affects plant growth at various stages of development including germination, seedling establishment, vegetative growth and finally reproduction. There have been numerous reports demonstrating that seed germination is inhibited by salt stress [1], [2]. Seed dormancy is an adaptive trait that enables seed to withstand the salt stress conditions and to germinate only when the conditions are suitable for seed germination and growth. Therefore, sensing environmental changes and retaining/releasing dormancy are important processes for adaptation to salt stress conditions.

Seed germination is a complicated process and is sensitive to many hormonal and environmental cues [3]. Abscisic acid (ABA) inhibits seed germination, while ethylene and gibberellin acid antagonize the ABA-induced inhibitory effect on seed germination. Although significant advances have been made in understanding salt tolerance mechanisms acting during vegetative growth, the underlying molecular mechanisms involved in the adaptation of seed to salt stress are unclear as yet.

In plants, proteins with Ankyrin-repeat domains, which often mediate protein–protein interactions, are involved in plant responses to biotic and abiotic stresses. Because of the importance of ankyrin-repeat proteins in plants, genome-wide localization, phylogenetic relationships and expression profiles have been analyzed in *Arabidopsis* [4] and rice [5]. In the *Arabidopsis* genome, 105 genes encoding ankyrin-repeat proteins have been identified. Becerra et al. [4] classified these genes in 16 groups based on their structural similarity. The most abundant group contains 37 genes encoding proteins with ankyrin repeats and transmembrane domains (named the AtANKTM family), and four of these genes, *ACD6*, *BDA1*, *ITN1* and *DRA1* have been functionally characterized as mediators of stress responses so far [6]-[11].

We previously demonstrated that ITN1 protein was a plasma membrane localized AtANKTM involved in ABA signal transduction and that the lack of this protein led to increased tolerance to salt stress in an *Arabidopsis* mutant at vegetative stage. ITN1 functions as a plasma membrane anchor of a nuclear protein RTV1 and partially inhibits the nuclear transport of RTV1, although possible effects of ITN1-RTV1 interaction on salt tolerance remain unclear [9], [10]. These findings raise a possibility that each member of the AtANKTM family may function as signaling components in responses to various environmental factors through interaction with (or release of) their respective partners.

In this study, a novel member of AtANKTM was identified as a positive regulator of seed germination under moderate salt stress conditions (named *SHG1*).

II. Materials and Methods

A. Plant Materials and Growth Conditions

All lines of Arabidopsis thaliana described here were derived from the Columbia wild type. The *SALK_049955* line is available from the Arabidopsis Biological Resource Center at Ohio State University. Unless otherwise stated, plants were routinely grown at 22°C under continuous white light on solid MS medium [12] containing 1% w/v sucrose and 0.5% w/v gellan gum.

H. Sakamoto is with Faculty of Bioindustry, Tokyo University of Agriculture, Japan (corresponding author; phone: +81-152-48-3834; fax: +81-152-48-3834; e-mail: h3sakamo@bioindustry.nodai.ac.jp).

J. Tochimoto, S. Kurosawa and M. Suzuki are with Faculty of Bioindustry, Tokyo University of Agriculture, Japan.

S. Oguri is with Faculty of Bioindustry, Tokyo University of Agriculture, Japan (phone: +81-152-48-3886; e-mail: s-oguri@bioindustry.nodai.ac.jp).

B. Measurement of Germination Rate

Seeds (>40 seeds in one experiment) were sown on solid MS medium supplemented with 100 or 125mM NaCl. Germination rate was measured at 2 weeks after sowing.

C. Salt Tolerance Assay during Vegetative Growth

Two-week-old plants sown on solid MS medium were transferred to medium supplemented with 150mM NaCl. At 13 days after transfer, electrolyte leakage from leaves was measured as described previously [13].

III. Results and Discussion

A. Salt Hypersensitive Phenotype of an Arabidopsis Mutant Deficient in At4g03490

In the *Arabidopsis* genome, 37 genes encoding proteins with ankyrin repeats and transmembrane domains (named the AtANKTM family) have been identified [4]. Each member of the family may function as signaling components in responses to various environmental factors, such as *ACD6*, *BDA1*, *ITN1* and *DRA1* [6]-[11]. We previously reported that the *ITN1* negatively affected plant tolerance to salt at vegetative stage [9].

In this study, to understand the possible involvement of AtANKTMs in tolerance to salt stress, we screened for mutants showing unusual germination rate on medium supplemented with NaCl among *Arabidopsis* T-DNA insertional mutants of the *AtANKTMs* gene.

Germination of a mutant line, *SALK_049955*, was strongly inhibited by 100 or 125mM NaCl, compared with wild-type plants (Fig. 1). After germination, this mutant died at the cotyledon stage under 125mM NaCl conditions, although wild-type plants could form true leaves and display green phenotype under the same conditions (Fig. 2). In the absence of exogenous NaCl, this mutant showed a similar germination rate (Fig. 1) and similar phenotypes to wild-type plants (data not shown).

In the mutant, T-DNA was inserted in an intron of *At4g03490* gene. The insertion of T-DNA was thought to disrupt this gene. We have designated this mutant as *shg1-1* (*salt hypersensitive germination 1-1*). These results suggested that wild-type *SHG1* played indispensable role in the seed germination and seedling establishment under moderate salt stress conditions.

It is well known that seed germination and seedling establishment is inhibited under non-optimal conditions such as excess salt. Seed dormancy is an adaptive trait that enables seed to withstand the salt stress conditions and to germinate only when the conditions are suitable for seed germination and growth. *SHG1* may be involved in sensing the levels of salt stress and triggering seed germination under no-excess salt conditions.

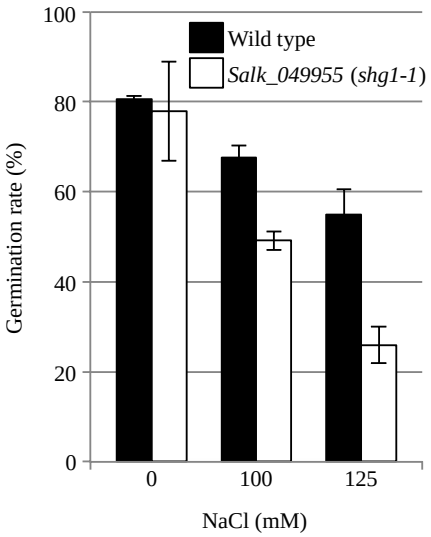

Fig. 1 Germination rate of *Salk_049955* (*shg1-1*) (white bar) and wild-type (black bar) plants on medium supplemented with NaCl. Data are means ± SE (n = 3)

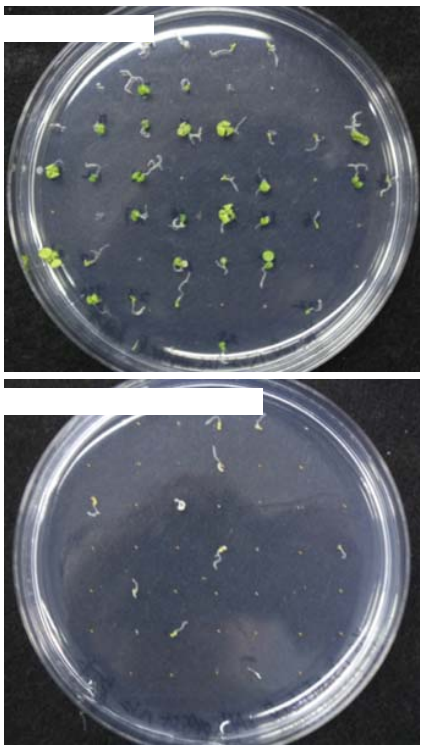

Fig. 2 Phenotypes of wild-type (a) and *Salk_049955* (*shg1-1*) (b) plants sown on medium supplemented with 125mM NaCl

B. Structure of SHG1

According to TAIR (http://www.arabidopsis.org/), the *SHG1* gene consists of 3440bp between the start and stop codons (Fig. 3). The deduced SHG1 protein consists of 690 amino acids. The N-terminal region contains nine ankyrin repeats, based on the SMART protein domain prediction program

(http://smart.embl-heidelberg.de). The middle part contains four predicted transmembrane helices. The C-terminal region (172 amino acids) does not match any peptides with known functions. In *Arabidopsis*, four of *AtANKTM* genes, *ACD6*, *BDA1*, *ITN1* and *DRA1* have been functionally characterized. Their deduced amino acid sequences show 23.5%, 17.0%, 18.0% and 24.9% identity with the SHG1 protein, respectively.

Ankyrin repeats often mediate protein-protein interaction. Like ITN1 protein, the SHG1 protein may function as a membrane anchor of its interacting partner. It is possible that the SHG1 protein regulates retaining/releasing dormancy through interaction with (or release of) its partners.

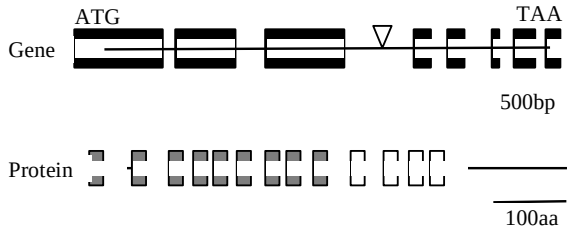

Fig. 3 The structures of the *SHG1* gene (upper) and the predicted SHG1 protein (lower). In the gene structure, black boxes indicate exons and lines indicate introns. The triangle indicates the T-DNA insertion site in *Salk_049955* (*shg1-1*). In the protein structure, gray boxes indicate ankyrin repeats and white boxes indicate transmembrane helices. The "aa" is an abbreviation for "amino acids."

C. Normal Responses to Salt Stress during Vegetative Growth in the shg1-1 Mutant

To investigate the sensitivity of the *shg1-1* mutant to salt stress at the vegetative stages, the mutant was shown on medium without NaCl and was transferred to medium supplemented with 150mM NaCl at 2 weeks after germination. There were no obvious differences in phenotypes between the mutant and wild-type plants (Fig. 4). A similar result was also obtained when tissue damage was monitored in leaves by electrolyte leakage, an indicator of plasma membrane damage (Fig. 5). These suggest that the sensitivity of this mutant to salt stress is similar to wild-type plants during vegetative growth.

Generally, salt stress is known to cause water deficit in plant cells and to activate ABA signaling. ABA signaling plays critical roles in tolerance to water deficit, including inhibition of seed germination, stomatal closure and osmolyte accumulation [14]. Enhanced inhibition of germination under salt stress conditions is frequently observed in ABA-hypersensitive plants, such as *ABF3*- or *ABF4*-overexpressing *Arabidopsis* [15]. The *ABF3* and *ABF4* encode transcription factors involved in ABA signaling, respectively. These transgenic lines also showed enhanced drought tolerance, probably resulting from the constitutive operation of a part of the ABA signaling. On the other hand, the *shg1-1* mutant did not show drought-tolerant phenotypes (data not shown) and salt-tolerant phenotype during vegetative growth (Figs. 4 and 5), suggesting the possibility that ABA signaling is not aberrantly activated in the mutant at least during vegetative growth. *SHG1* may be specifically involved in the release of seed dormancy and the seedling establishment.

A detailed study is required on the modes of action of *SHG1* in plant salt tolerance. A dissection of the biochemistry of the SHG1 protein, possibly including a study of its protein–protein interactions, will provide valuable information regarding the precise roles of AtANKTM family proteins in the fundamentally important adaptations of plants to abiotic stresses.

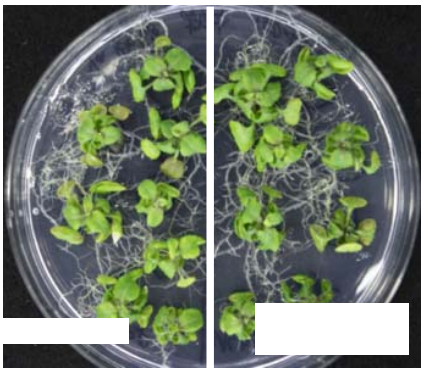

Fig. 4 Phenotypes of wild-type (left) and *Salk_049955* (*shg1-1*) (right) plants sown on medium withoutNaCl, transferred at 2 weeks onto a medium supplemented with 150 mMNaCl and grown for an additional 13 days

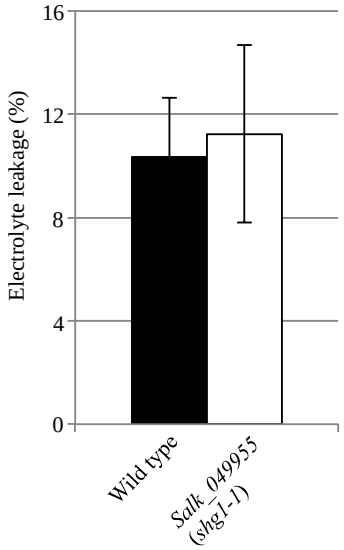

Fig. 5 Electrolyte leakage from leaves of *Salk_049955* (*shg1-1*) (white bar) and wild-type (black bar) plants used in Fig.4. Data are means ± SD (n = 6)

ACKNOWLEDGMENT

We thank the Arabidopsis Biological Resource Center for providing the *Salk_049955* line.

REFERENCES

[1] C. Chang, B. Wang, L. Shi, Y. Li, L. Duo, and W. Zhang, "Alleviation of Salt Stress-Induced Inhibition of Seed Germination in Cucumber (*Cucumis sativus* L.) by Ethylene and Glutamate," *J. Plant Phys.*, vol. 167, pp. 1152–1156, 2010.

[2] A. J. Vallejo, M. J. Yanovsky, and J. F. Botto, "Germination Variation in *Arabidopsis thaliana* Accessions under Moderate Osmotic and Salt Stresses," *Ann. Botany*, vol. 106, pp.833-842, 2010.
[3] W. E. Finch-Savage, and G. Leubner-Metzger, "Seed Dormancy and the Control of Germination," *New Phytol.*, vol. 171, pp. 501-523, 2006.
[4] C. Becerra, T. Jahrmann, P. Puigdomènech, and C.M. Vicient, "Ankyrin Repeat- Containing Proteins in Arabidopsis: Characterization of a Novel and Abundant Group of Genes Coding ankyrin-transmembrane proteins," *Gene*, vol. 340, pp. 111–121, 2004.
[5] J. Huang, X. Zhao, H. Yu, Y. Ouyang, L. Wang, and Q. Zhang, "The Ankyrin Repeat Gene Family in Rice. Genome-Wide Identification, Classification and Expression Profiling," *Plant Mol. Biol.*, vol. 71, pp. 207–226, 2009.
[6] H. Lu, D.N. Rate, J.T. Song, and J.T. Greenberg, "ACD6, a Novel Ankyrin Protein, is a Regulator and an Effector of Salicylic Acid Signaling in the *Arabidopsis* Defense Response," *Plant Cell*, vol. 15, pp. 2408–2420, 2003.
[7] H. Lu, Y. Liu, and J.T. Greenberg, "Structure–Function Analysis of the Plasma Membrane-Localized *Arabidopsis* Defense Component ACD6," *Plant J.*, vol. 44, pp. 798–809, 2005.
[8] Y. Yang, Y. Zhang, P. Ding, K. Johnson, X. Li, and Y. Zhang, "The Ankyrin-Repeat Transmembrane Protein BDA1 Functions Downstream of the Receptor-Like Protein SNC2 to Regulate Plant Immunity," *Plant Physiol.*, vol. 159, pp. 1857–1865, 2012.
[9] H. Sakamoto, O. Matsuda, and K. Iba, "ITN1, a Novel Gene Encoding an Ankyrin-Repeat Protein that Affects the ABA-Mediated Production of Reactive Oxygen Species and Is Involved in Salt-Stress Tolerance in *Arabidopsis thaliana*," *Plant J.*, vol. 56, pp. 411–422, 2008.
[10] H. Sakamoto, K. Sakata, K. Kusumi, M. Kojima, H. Sakakibara, and K. Iba, "Interaction between a Plasma Membrane-Localized Ankyrin-Repeat Protein ITN1 and a Nuclear Protein RTV1," *Biochem. Biophys. Res. Commun.*, vol. 423, pp. 392–397, 2012.
[11] H. Sakamoto, Y. Nakagawara, and S. Oguri, "The Expression of a Novel Gene Encoding an Ankyrin-Repeat Protein, DRA1, is Regulated by Drought-Responsive Alternative Splicing," *Int. J. Biol. Life Sci. Eng.*,vol. 7, pp. 81-84, 2013.
[12] T. Murashige, and F. Skoog, "A Revised Medium for Rapid Growth and Bioassays with Tobacco Tissue Culture," *Physiol. Plant.*, vol. 15, pp. 473–497, 1962.
[13] T. Nanjo, M. Kobayashi, Y. Yoshiba, Y. Kakubari, K. Yamaguchi-Shinozaki, and K. Shinozaki, "Antisense Suppression of Proline Degradation Improves Tolerance to Freezing and Salinity in *Arabidopsis thaliana*," *FEBS Lett.*, vol. 461, pp. 205–210, 1999.
[14] S. Mahajan, and N. Tuteja, "Cold, Salinity and Drought Stresses: An Overview," *Arch. Biochem. Biophys.*, vol. 444, pp. 139–158, 2005.
[15] J. Y. Kang, H. I. Choi, M. Y. Im, and S. Y. Kim, "Arabidopsis Basic Leucine Zipper Proteins That Mediate Stress-Responsive Abscisic Acid Signaling," *Plant Cell*, vol. 14, pp. 343–357, 2002.