Environmental and Experimental Botany 86 (2013) 9–16

Contents lists available at SciVerse ScienceDirect

Environmental and Experimental Botany

journal homepage: www.elsevier.com/locate/envexpbot

A new protein kinase gene *SSG1* is essential for adaptation of *Arabidopsis* to salt stress

Min Fan[a], Dingming Kang[a], Hua Guan[a], Zhangliang Chen[a,b], Li-Jia Qu[b,*]

[a] College of Agronomy and Biotechnology, China Agricultural University, Beijing 100094, People's Republic of China
[b] National Laboratory of Protein and Plant Gene Research, College of Life Sciences, Center for Life Sciences, Peking University, Beijing 100871, People's Republic of China

ARTICLE INFO

Article history:
Received 13 October 2011
Received in revised form
14 November 2011
Accepted 17 November 2011

Keywords:
Arabidopsis
Protein kinase
Salt stress
Adaptation

ABSTRACT

Soil salinity significantly limits plant productivity on agricultural lands, and salt tolerance of plant requires a complex mechanism in which many genes are involved. From an activation-tagging mutant collection, we identified a loss-of-function *Arabidopsis* mutant which exhibits hypersensitivity to NaCl during seed germination and is designated *ssg1* (salt sensitive during seed germination 1). Knocking down the expression of *SSG1* by RNAi recapitulated the phenotype of *ssg1*, suggesting that functional *SSG1* is necessary for normal seed germination in the presence of NaCl. The seed germination of *ssg1* was not sensitive to K^+, but was hypersensitive to osmotic stress. *SSG1* encodes a protein kinase, possibly with alternatively spliced forms. *In vitro* kinase assay indicated that SSG1 possessed protein kinase activity which can both auto-phosphorylate and phosphorylate substrate. Quantitative real-time RT-PCR analysis showed that, in the mutants *ssg1-1* and *ssg1-2*, the expression level of some salt-responsive marker genes, i.e., *SOS1*, *SOS2*, *SOS3*, *SOS4*, *AtNHX* and *AtNKT*, was down-regulated, whereas *SSG1* expression level was not changed in *sos* mutants. These results suggest that *SSG1* is possibly a component in the signaling pathway in response of plants to salt stress.

1. Introduction

Plants need essential mineral nutrients to grow and develop, but soil salinity significantly limits plant productivity on agricultural lands. Current efforts to improve plant stress tolerance by genetic transformation have obtained some important achievements, but the complex mechanisms of salt tolerance make the task considerable difficult (Vinocur and Altman, 2005). It is noteworthy that *Arabidopsis* as a model plant has played an important role in the study of those stress-associated genes and the genetically complex mechanisms of salt stress (Flowers et al., 1977). Like other glycophytic plant specials, *Arabidopsis* is sensitive to salt stress. This adverse sensitivity is indicated in the inhibition of germination, reduction of growth and disturbance of development (Lazof and Bernstein, 1999). Especially, the most evident of salt sensitivity in *Arabidopsis* is during seed germination and seeding stages (Xiong and Zhu, 2002).

Seed germination is an important developmental process in the plant life cycle under salt stress. Through analysis of salt tolerant mutants researchers may illuminate the mechanisms of salt tolerance and enhance resistance to salt stress in sensitive species. Several having been reported *Arabidopsis* mutants, *RS* mutants (Saleki et al., 1993), *rss* mutants (Werner and Finkelstein, 1995) and *san* mutants (Quesada et al., 2000), can germinate on highly saline conditions, but the tolerance of these mutants is same to the wild type in seedlings and mature graduate. Whereas some *Arabidopsis* salt tolerant mutant such as *pst1* (Tsugane et al., 1999) is not salt tolerant during seed germination, but maintains salt tolerance run through their seeding and mature development. And a mass of mutants such as *mkp1* (Ulm et al., 2002) is salt tolerance throughout the *Arabidopsis* life cycle. These results suggest that there are different salt tolerance mechanisms during seed germination and subsequent plant development. This has also been supported by other evidences (Foolad, 1999; Quesada et al., 2002; Gao et al., 2006). Alternatively through analysis of salt sensitive mutants researchers may also elucidate the mechanisms of salt tolerance. Some *Arabidopsis* mutants were identified, such as salt oversensitive (*sos*) mutants (Wu et al., 1996) and several *los* and *hos* mutants (Zhu, 2000), which were sensitive to salt stress all the life cycle. There are different salt tolerance mechanisms during seed germination and subsequent plant development. The above results have a certain extent explored the salt tolerance mechanism of *Arabidopsis*, but the functional identification of salt tolerance genes and tolerance mechanism in this model plant still requires further elucidation because stress may occur at multiple stages of plant development and there are different salt tolerance mechanisms during seed germination and subsequent plant development.

* Corresponding author. Fax: +86 10 6275 3018.
E-mail address: qulj@pku.edu.cn (L.-J. Qu).

0098-8472/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.envexpbot.2011.11.012

Regulation of cell ion homoeostasis and reestablishing ion homeostasis is a most important way to plant development and growth to salt stress. Some plasma membranes Na^+/H^+ antiporters have been fought and studied in barley, tomato and wheat (Blumwald, 2000). Until recently the salt-overly-sensitive (SOS) pathway has began reveal the molecular mechanism involved in the plants response to ionic stress. Three salt overly sensitive genes (*SOS1, SOS2, SOS3*) have been found in molecular analysis of *sos* mutants to share a common pathway (Zhu, 2000; Zhu et al., 1998; Liu and Zhu, 1997; Wu et al., 1996). *SOS3* encoding a Ca^{2+} binding protein is a Ca^{2+} sensor essential for transducing the salt stress induced Ca^{2+} signal. The *sos2* and *sos3* mutant are hypersensitive to salt stress (Ishitani et al., 2000; Liu and Zhu, 1998). *SOS2* encoding a serine/threonine protein kinase (Liu et al., 2000), which would be activated by *SOS3* and interact with *SOS3* in the presence of Ca^{2+} (Halfter et al., 2000). A FISH-motif in the regulatory domain of *SOS2* is necessary for interaction with *SOS3* and activation the *SOS2* kinase domain (Guo et al., 2001). Like *SOS2* and *SOS3*, *SOS1* is also hypersensitive to salt and is a first target of the *SOS3–SOS2* complexity in the pathway by molecular genetic analysis of the *sos1* mutant of *Arabidopsis*. *SOS1* encodes a plasma membrane Na^+/H^+ antiporter with a very long predicted cytoplasmic tail (Shi et al., 2000). Except for *SOS1* additional targets of *SOS3–SOS2* complexity in the SOS regulatory pathway, *AtNHX* (vacuolar Na^+/H^+ exchanger) (Qiu et al., 2004; Yokoi et al., 2002) and *AtHKT* transporter (Rus et al., 2001, 2004), are emerging. In addition, *SOS4* (pyridoxal-5-phosphate) also regulates ion homoeostasis by modulating the activities of ion transporters (Shi et al., 2002; Shi and Zhu, 2002). Above evidence indicates that the SOS pathway is a important signaling pathway for ion homeostasis responses under salt stress (Zhu, 2002). However, whether it includes all salt tolerance mechanisms during plant different development stages; whether it is an only pathway under salt stress; whether the SOS pathway controls other plant processes and cross-talk with other pathways are far from clear.

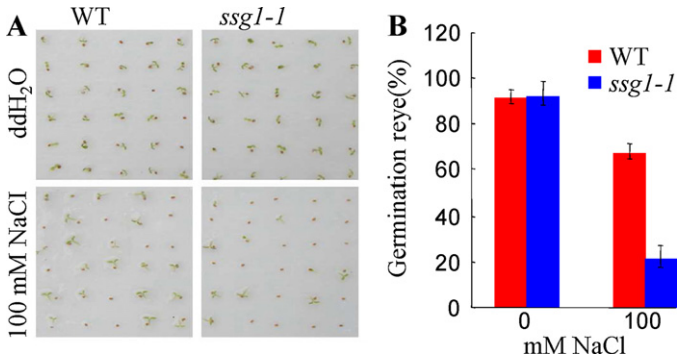

Fig. 1. Isolation of mutant *ssg1-1* by germination assay. The seeds were grown on filter papers saturated with ddH_2O and 100 mM NaCl and the germination rate of the seeds was satisfacted and the picture was taken in 8 days germination. (A) The top of picture is the germination of WT and *ssg1-1* under ddH_2O; the bottom of picture is the germination of WT and ssg*1-1* under 100 mM NaCl. (B) Germination rates of wild type and *ssg1-1* seeds under ddH_2O and 100 mM NaCl. Data are the means of three replicates of 100 seeds. Bar indicates the SE.

To address the mechanisms of salt tolerance during seed germination, we have performed a mutant screening from an *Arabidopsis* insertion-tagged mutant pool (Qin et al., 2003), and one mutant, designated *ssg1-1* (Salt Sensitive at Germination), has been isolated and showed high sensitivity to NaCl than the wild type under salt stress during seed germination. Furthermore, *SSG1* was identified as a functional kinase which can autophosphorylate and phosphorylate substrate. Salt stress marker genes were down regulated in mutant and *SSG1* transcript expression level in other mutants was similar to that in the wild type. These results indicate that SSG1

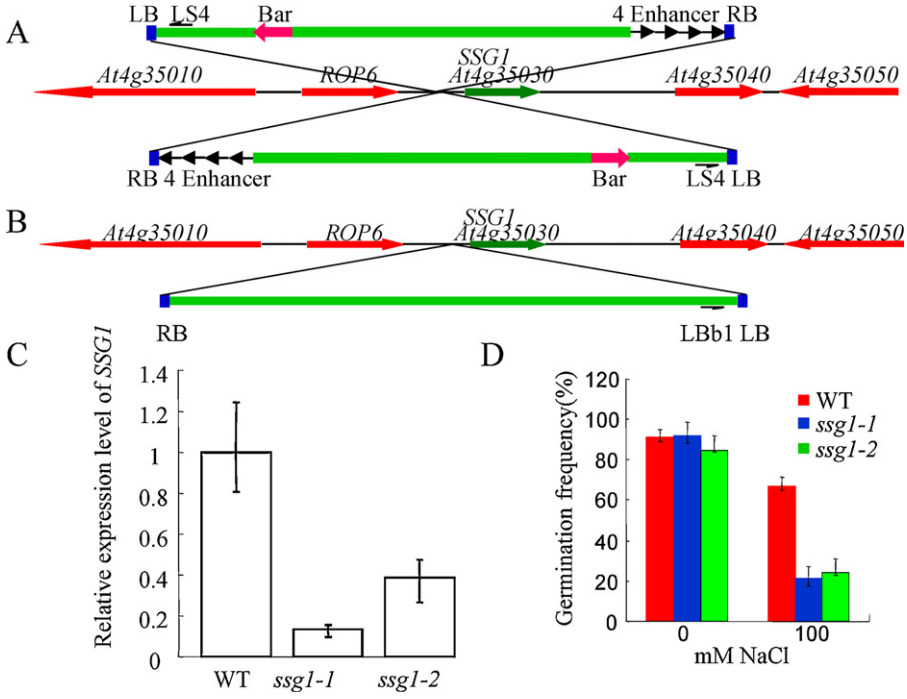

Fig. 2. Molecular cloning of the *SSG1*. (A) Scheme of the genomic region flanking the T-DNA insertion site in *ssg1-1*. Genes are represented by red and green rectangle, intergenic regions by lines. The arrow direction represents the transcriptional orientation of the genes. The four red arrowheads represent the four 35S enhancers from pSKI015. LB, T-DNA left border; bar, Basta resistance gene; 4Enhancer, CaMV 35S enhancer tetrad; RB, T-DNA right border. (B) Scheme of the genomic region flanking the T-DNA insertion site in *ssg1-2*. SALK_092926 line from ABRC was named *ssg1-2* because T-DNA of this line also insert in the promoter of At4g35030. (C) Expression of *SSG1* in the wild type and the two mutants by real time PCR with the primer pair SSGRT-1 and SSGRT-2. Tubling2 as an internal control. (D) Germination rates of wild type and *ssg1-1* and *ssg1-2* seeds under ddH_2O and 100 mM NaCl. Data are the means of three replicates of 100 seeds. Bar indicates the SE. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)