Isolation and characterization of a plasma membrane Na$^+$/H$^+$ antiporter gene *TaSOS1* from wheat

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ABSTRACT

We have cloned *TaSOS1* gene from *Triticum aestivum L.* which encodes a plasma membrane Na$^+$/H$^+$ antiporter. TaSOS1 encodes a polypeptide of 1,142 amino acid residues with a theoretical molecular mass of 126 kDa. TaSOS1 protein is a plasma membrane-bound protein which enhanced Na$^+$ and Li$^+$ tolerance when overexpressed in yeast salt sensitive mutants. TaSOS1 transcripts were detected in all investigated tissues, including roots, shoots, spikes and anthers and was up-regulated by both ABA and salt. Those results indicate TaSOS1 may play an important role in salt stress response in common wheat.

Keywords: *wheat, TaSOS1, Na$^+$/H$^+$ antiporter, plasma membrane, yeast, salt stress*

Soil salinity is a major abiotic stress worldwide. High levels of sodium ion (Na$^+$) are deleterious to cellular functions because of adverse effects on K$^+$ nutrition, cytosolic enzyme activities, photosynthesis, and other aspects of metabolism. In wheat, sensitivity to Na$^+$ has been shown to be correlated with Na$^+$ accumulation in the shoot (Gorham, 1990; Schachtman & Munns, 1992). Exclusion of shoot Na$^+$ can be achieved by increasing Na$^+$ efflux and restriction of Na$^+$ influx from the soil, as well as compartmentalization of Na$^+$ in the vacuole. Na$^+$/H$^+$ antiporters play an important role in these processes.

Na$^+$/H$^+$ antiporters catalyze the exchange of Na$^+$ and H$^+$ across membranes. In plants, Na$^+$/H$^+$ antiporters have been characterized in the vacuolar and plasma membranes where they function to remove Na$^+$ from the cytoplasm by transport into the vacuole or out of the cell thus preventing toxic cellular accumulations of Na$^+$ (Blumwald *et al*., 2000; Hasegawa *et al*., 2000). *AtSOS1* was cloned from *Arabidopsis* salt overly sensitive (SOS) mutants (Shi *et al*., 2000). The function of AtSOS1 was also indicated by enhancing salt tolerance in transgenic plants. Although AtSOS1, as the first identified plant plasma membrane Na$^+$/H$^+$ antiporter, has been studied in detail, plasma membrane Na$^+$/H$^+$ antiporters and their roles in other plants are still unclear. In this paper, we report on the isolation and characterization of a plasma membrane Na$^+$/H$^+$ antiporter gene *TaSOS1* in wheat and the expression patterns of *TaSOS1* and another putative vacuolar Na$^+$/H$^+$ antiporter, *TaNHX2* (GenBank accession No. AY040246) under various salt and ABA stress conditions.

MATERIALS AND METHODS

1 Plant growth conditions and NaCl stress treatments

Seeds of *Triticum aestivum L.* cv. Keyi26 were germinated in Petri dishes, then cultivated hydroponically in full strength Hoagland solution in a greenhouse. Two-week-old seedlings were transferred to fresh Hoagland medium supplemented with 150 mM , 250 mM NaCl or 20 μM abscisic acid (ABA). After exposure to the stress treatments for 3 h, 7 h and 24 h, shoots and roots were collected in liquid nitrogen and stored at −80 °C until required.

2 Putative partial cDNA cloning and RACE

Total RNA was extracted with Trizol reagent (GIBCO-BRL, USA) and 2 μg RNA was used for reverse transcription. Two μl of 5-fold diluted reverse-transcription products was used in the PCR (PCR system 9700, Applied Biosystems, USA) with the following cycling conditions: 94 °C for 5 minutes, then 35 cycles of 94 °C for 30 seconds; 54 °C for 30 seconds; 72 °C for 1 minute; after cycling a final step incubate the mixture at 72 °C for 10 minutes. Primers S1F (5'-TTT TCC TCG AGC AAC CCA GTC TTC-3') and S1R (5'-AGG GAC GAA TAA CTC AAT CTA CAG-3') were used to amplify the putative partial cDNA of *TaSOS1*. The PCR products were purified and cloned into pGEM-T vector (Promega) for sequencing. S1F was used to amplify the 3′-end of *TaSOS1* cDNA. The specific primers S1R1 (5'-GAC CGC AAA CCC TTC AAA TCA-3'), S1R2 (5'-GGA GGA AGA CGG CCA GAA GGA GAT-3') and S1R3 (5'-TGA CGG CTA AAA CAC CAG AGA CCT-3') were designed to amplify the 5′-end of *TaSOS1* cDNA. RACE PCR was performed by 5' RACE System and 3' RACE System Kit (GIBCO-BRL) according to the manufacturer's instructions. The isolated products were cloned into the plasmid pGEM-T vector for sequencing.

3 Transient expression of TaSOS1 gene

The coding region of *TaSOS1* was amplified by PCR using the oligonucleotide primers GS1F (5'-AGT TCT AGA ATG GAG ACG GGG GAG GCC G-3') and GS1R (5'-AGT GAT ATC CAG CTG CCT CGC GGT GGG C-3'), which included *Xba*I and *Eco*RV cut sites at their 5'-ends. After digestion with XbaI and EcoRV, the PCR products were directly integrated into pBIN 35S-mGFP4 vector to generate a TaSOS1-GFP fusion protein

1

transformation vector. 5 µg of the plasmid was used to bombard onion epidermis cells to investigate the subcellular localization of TaSOS1-GFP fusion protein in plant cells by transient expression, Microscopic detection of the TaSOS1-GFP fusion protein in transformed onion cells was performed using an Olympus CONFOCAL microscope.

4 Functional expression of TaSOS1 in yeast

S.cerevisiae strains ANT3 (*ena1::HIS3::ena4, nha1::LEU2*), GX1(*ena1::HIS3::ena4, nhx1::TRP1*) and AXT3 (*ena1::HIS3::ena4, nha1::LEU2, nhx1::TRP1*) are derivatives of W303. *TaSOS1*-coding sequence at the full length was cleaved using *Eco*RI from pTaSOS1 vector. For yeast complementation, *TaSOS1* was subcloned into the yeast expression vector pYES2.0 (Invitrogen, USA) to generate pYES2.0-*TaSOS1* expression plasmids. They were then transformed into a yeast strain AXT3 using the lithium acetate method according to the manufacturer's manual (Invitrogen, USA). For cation tolerance testing, 5 µL aliquots from saturated yeast cultures or 10-fold serial dilutions were spotted onto AP plates (8 mM phosphoric acid, 10 mM L-arginine, 2 mM MgSO4, 0.2 mM CaCl$_2$, 2% glucose, plus vitamins and trace elements) supplemented with 1 mM KCl with or without NaCl or LiCl as indicated. Resistance to hygromycin B was assayed in YPD medium (1% yeast extract, 2% peptone and 2% glucose)
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5 RT-PCR analysis

Total RNA was extracted with Trizol reagent (Invitrogen, USA). 2 µg of total RNA was used for reverse transcription as above. Two µl of 10-fold diluted reverse-transcription products were initially subjected to PCR reaction. A fragment of *α-Tubulin* gene (GenBank accession No. U76558) was used as the control for RT-PCR analysis. The PCR was performed at the profile: 94 °C for 5 min, then 25 cycles of 94 °C for 0.5 min; 57 °C for 0.5 min; 72 °C for 0.5 min; after cycling a final step of 72 °C for 7 min. Specific primers S1RTF (5'-GCT CGA GGA AAA GGA AAT AG-3') and S1RTR (5'-TCC ACT CTG ACG ATG ACT TC-3') were designed to amplify a 111 bp TaSOS1 fragment. The reaction is 94 °C for 5 min, then 31 cycles of 94 °C for 0.5 min; 57 °C for 0.5 min; 72 °C for 0.5 min; after cycling a final step of 72 °C for 5 min.

RESULTS

Isolation, alignment, and phylogenetic analysis of TaSOS1

The reconstituted cDNA of *TaSOS1* (GenBank accession No. AY326952) is 3,728 bp long, consisting of a 139 bp 5'-untranslated region (UTR) and 160 bp 3'-UTR. It encodes a polypeptide of 1,142 amino acid residues with a predicted molecular mass of 126 kD and pI value of 7.5. TaSOS1 is predicted to contain 12 putative transmembrane segments, although the actual number of transmembrane segments and the topology remain to be experimentally determined. TaSOS1 appears to be most closely related to the plasma membrane Na$^+$/H$^+$ antiporters of *Arabidopsis*, *Cymodocea nodosa*, and rice.

Localization analysis of TaSOS1 protein using green fluorescent protein (GFP)

Subcellular localization of TaSOS1 protein was determined by transient expression analysis. Onion cells transformed by tissue bombardment with a *TaSOS1-GFP* fusion clearly demonstrated targeting of the protein to the plasma membrane. Control transformations with GFP alone showed fluorescence throughout the cytoplasm and the nucleus.

Complementation of yeast with TaSOS1

The coding sequences TaSOS1 and TaNHX2 were cloned into the yeast expression vector pYES2.0 under the control of GAL1 promoter. The resulting plasmids were then transformed into salt sentitive AXT3 yeast cells which lack the Na$^+$ efflux proteins ENA1 to 4 and NHA1, as well as the vacuolar Na$^+$/H$^+$ antiporter NHX1. *TaSOS1* expression enhanced Na$^+$ tolerance equivalent to endogenous NHX1 and NHA1. Moreover, it also mediated greater tolerance to Li$^+$. But TaSOS1 failed to recover the sensitivity to hygromycin B and high external KCl. The putative vacuolar Na$^+$/H$^+$ antiporter TaNHX2 also conferred Na$^+$ tolerance, but the recovery was not as great as with TaSOS1. TaNHX2 didn't demonstrate an ability to transport Li$^+$ but TaNHX2 can partly restore the sensitivity to hygromycin B, suggesting that TaNHX2 is located in the vacuolar membrane.

Expression of *TaSOS1* in wheat seedlings under various stress conditions

To investigate the expression patterns of *TaSOS1* in wheat, total RNA was extracted from shoots and roots of two-week-old seedlings and from young spikes and anthers before undergoing RT-PCR analysis. It was found that *TaSOS1* was expressed at high levels in roots and shoots of young seedlings but at lower levels in young spikes and anthers.

The effects of salt and ABA on the expression of *TaSOS1* and *TaNHX2* in the roots and shoots of two-week-old wheat seedlings, after 3, 7 or 24 h treatment with NaCl and ABA, were determined. In contrast to *Arabidopsis AtSOS1* which was significantly up-regulated by salt stress but not by ABA, *TaSOS1* was only slightly up-regulated by both salt and ABA in wheat seedling roots and shoots. In contrast to *TaSOS1*, *TaNHX2* expression was significantly up-regulated by both salt and ABA.

DISCUSSION

Transport processes that regulate ion efflux from the cell, and vacuolar compartmentation, are critically

important to reducing Na⁺ accumulation in the plant cytosol. In higher plants, Na$^+$ efflux and compartmentalization are achieved by Na$^+$/H$^+$ antiporters located in both the plasma and vacuolar membranes. Recently, overexpression of either the *A. thaliana* vacuolar Na$^+$/H$^+$ antiporter *AtNHX1* or a plasma membrane Na$^+$/H$^+$ antiporter *AtSOS1* was reported to confer salt tolerance in transgenic plants (Apse *et al*.,1999; Shi *et al*., 2003). This suggests that Na$^+$/H$^+$ antiporters play a key role in salt durable in plants. The work here reports the cloning and functional characterization of a plasma membrane Na$^+$/H$^+$ antiporter *TaSOS1* from wheat which shows a high degree of similarity with the *Arabidopsis* AtSOS1.

Hydrophobicity plot analysis indicates that the N-terminal portion of TaSOS1 has 12 predicted transmembrane domains consisting approximately of 500 amino acid residues. TaSOS1 also has a long cytoplasmic C-terminal tail of 700 residues. Conserved domain analysis has shown the presence of a cyclic nucleotide-binding motif in the middle of the TaSOS1 protein, which was not evident in AtSOS1. Comparison of the TaSOS1 amino acid sequence with three other plant genes indicated that the four functional essential amino acids in *AtSOS1*, identified by sequence analysis of *sos1* mutant alleles are conserved in plant cytoplasm Na$^+$/H$^+$ antiporters. Two essential amino acids are located in the Na$^+$/H$^+$ exchange domain and the other two are located in the cyclic nucleotide-binding motif. The cNMP binding domain is also found in the mouse sperm-specific Na$^+$/H$^+$ exchanger spermNHE. The sequence alignment and phylogenetic tree for various Na$^+$/H$^+$ antiporter sequences show that the five putative plant plasma membrane Na$^+$/H$^+$ antiporters are closely related to spermNHE. The suggested cNMP binding domain may play an important role in the functions of plasma membrane Na$^+$/H$^+$ antiporters.

The function of TaSOS1 and another putative vacuolar Na$^+$/H$^+$ antiporter TaNHX2 were investigated using yeast mutants. Both TaSOS1 and TaNHX2 had the ability to suppress the Na$^+$ sensitivity of AXT3yeast mutants. This indicated that TaSOS1 and TaNHX2 function as Na$^+$ transporters, however, neither of these two genes suppressed the K$^+$ sensitivity of AXT3. TaSOS1 had Li$^+$ transporter activity suggesting a role in transporting smaller atoms while transient expression analysis of TaSOS1-GFP fusion proteins in onion indicted that TaSOS1 is primarily located on the plasma membrane.

TaSOS1 mRNA was detected in all investigated tissues of wheat (roots, shoots, young spikes and anthers), which is consistent with the *Arabidopsis* expression patterns identified by promoter–β-glucuronidase fusion analysis. The plasma membrane Na$^+$/H$^+$ antiporters may therefore be highly conserved in wheat and *Arabidopsis* and may function not only for excluding Na$^+$ from root but also for other biological functions such as for controlling long-distance Na$^+$ transport from root to shoot (Shi *et al*., 2002).

Unlike *AtSOS1* expression which was up-regulated by salt stress but not by ABA, *TaSOS1* was up-regulated by both salt stress and ABA. This suggests the regulation of transcription may be not the only thing important control in TaSOS1 activity as in *Arabidopsis*, Na$^+$/H$^+$ activity of *AtSOS1* was regulated at both the mRNA and protein levels. Although the expression was up-regulated by salt stress, post-transcriptional activation may be more important. As indicted in *sos* mutants, yeast and highly purified plasma membrane vesicles, the phosphorylation of AtSOS1 by the SOS3-SOS2 protein kinase complex is essential for fully Na$^+$/H$^+$ antiporter activity and salt tolerance (Qiu *et al*., 2002, 2003; Quintero *et al*., 2002). In wheat, by means of EST database searching, we have identified many genes that may be homologous to *SOS2* and *SOS3*. Although the function of these needs further investigation, it is possible that these have the same functions as SOS2 and SOS3 and will regulate TaSOS1 antiporter activity. There may also be a similar pathway involved in regulation of the Na$^+$/H$^+$ antiporter activity and activation of TaSOS1 in wheat.

ACKNOWLEDGEMENTS

REFERENCES

1. Apse MP, Aharon GS, Snedden WA, Blumwald E. 1999. *Science* 285: 1256-1258.

2. Blumwald E, Aharon GS, Apse MP. 2000. *Biochim Biophys Acta* 1465: 140-151.

3. Gorham J. 1990. *J Exp Bot* 41: 623-627.

4. Hasegawa PM, Bressan RA, Zhu JK, Bohnert HJ. 2000. *Annu Rev Plant Physiol Plant Mol Biol* 51: 463-499.

5. Qiu QS, Barkla BJ, Vera-Estrella R, Zhu JK, Schumaker KS. 2003. *Plant Physiol* 132: 1041-1052.

6. Qiu QS, Guo Y, Dietrich MA, Schumaker KS, Zhu JK. 2002. *Proc Natl Acad Sci USA* 99: 8436-8441.

7. Quintero FJ, Ohta M, Shi H, Zhu JK, Pardo JM. 2002. *Proc Natl Acad Sci USA* 99: 9061-9066.

8. Schachtman DP, Munns R. 1992. *Australian J of Plant Physiol* 19: 331-340.

9. Shi H, Ishitani M, Kim C, Zhu JK. 2000. *Proc Natl Acad Sci USA* 97: 6896-6901.

10. Shi H, Quintero FJ, Pardo JM, Zhu JK. 2002. *Plant Cell* 14: 465-477.

11. Shi H, Lee BH, Wu SJ, Zhu JK. 2003. *Nat Biotechnol* 21: 81–85.