J. Plant Biol. (2013) 56:375-382
DOI 10.1007/s12374-013-0154-y

ORIGINAL ARTICLE

Overexpression of the *AtSTK* Gene Increases Salt, PEG and ABA Tolerance in *Arabidopsis*

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Received: April 20, 2013 / Accepted: July 25, 2013

Abstract *AtSTK* (At5g02800), which is a serine-threonine protein kinase gene of *Arabidopsis thaliana*, was cloned, and its function was studied. The study found that the overexpression of *AtSTK* could significantly improve the ability of *A. thaliana* to tolerate salt, PEG, and ABA stresses. RT-PCR analysis revealed that the expression of the *AtSTK* gene could be obviously induced by salt, PEG, and ABA. The examination of the physiological characteristics showed that the overexpression of *AtSTK* in *Arabidopsis* significantly reduced the plasma membrane permeability, significantly increased the proline content, and decreased the MDA content. These changes may reflect the physiological mechanisms through which *AtSTK* overexpression improves stress resistance in *Arabidopsis*. In addition, the overexpression of the *AtSTK* gene significantly antagonised the inhibitory effect of high concentrations of exogenous ABA on *Arabidopsis* seed germination. The subcellular localisation results showed that AtSTK is located in both the cytosol and the nucleus. The examination of its tissue-specific expression showed that *AtSTK* is expressed in various *Arabidopsis* tissues and is particularly strongly expressed in the vessels. The signalling pathway analysis indicated that AtSTK might transfer the salt stress signal in *Arabidopsis* through the MAPK pathway.

Keywords: Abiotic resistance, *AtSTK*, Overexpression, Signalling pathway

Introduction

Soil salinity is one of the major constraints on food production because it limits crop yields and restricts the use of previously uncultivated land. It has been estimated that approximately 20% of agricultural land and 50% of cropland worldwide is exposed to salt stress (Flowers and Yeo 1995). Recent advances in plant molecular biology techniques offer a new opportunity to understand the genetics of stress resistance genes and their contribution to plant performance under stress. These biotechnological advances will provide new tools for breeding in stressful environments. Recently, an increasing number of plant disease-resistance genes and stress-induced genes have been isolated from different species (Munns 1993; Bohnert et al. 1995; Zhu 2001). Increasing evidence shows that serine-threonine kinase genes play crucial roles in plant responses to abiotic stress (Liu and Zhu 1997; Liu and Zhu 1998; Diedhiou et al. 2008; Mao et al. 2010).

TaSTK, which is a wheat serine-threonine protein kinase gene, was discovered and cloned from mutant wheat lines using cDNA-AFLP (cDNA-amplified restriction fragment length polymorphism), RACE (rapid amplification of cDNA ends), and other amplification methods (Ge et al. 2007). This protein was found to be upregulated in response to salt stress and appeared to contribute to salt resistance in the examined plants. However, the wheat genome is exceptionally large, and it is difficult to further study the mechanism of *TaSTK* regulation in wheat. Therefore, the *AtSTK* (At5g02800) gene, which is the homolog of *TaSTK* in *A. thaliana* (76% amino acid homology within the coding region), was cloned. In this study, the function, subcellular localisation, and tissue-specific expression of *AtSTK* and its associated signalling pathway were examined.

Results

ATSTK Overproduction Increases Salt Resistance in *Arabidopsis*

The total cellular RNA was isolated from vector control

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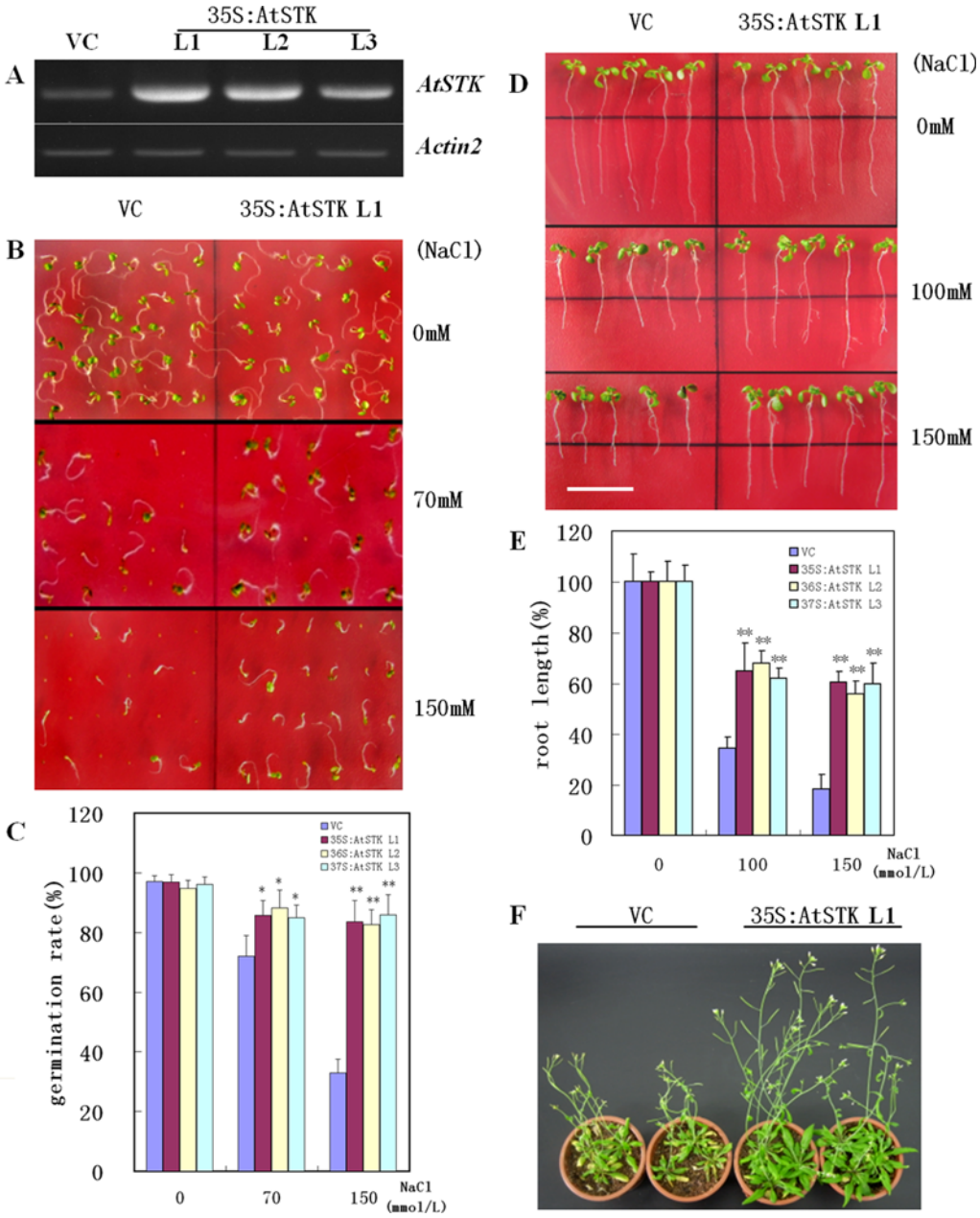

Fig. 1. Salt resistance in transgenic *Arabidopsis* plants overexpressing *AtSTK*. (A) The total RNA was extracted from transgenic plants carrying the p1300:35S:AtSTK or the p1300:35S vector (vector control: VC) and wild-type plants (Col); the level of *AtSTK* expression was detected through RT-PCR. (B, C) *AtSTK* gene-modified or VC *A. thaliana* were incubated in MS medium, MS medium + 70 mM NaCl, or MS medium + 150 mM NaCl and cultured for 5 d. The germination rate was then calculated. The values presented are the means ± SD (n = 60). The asterisks indicate the statistically significant differences in the comparisons between the VC and the 35S:AtSTK lines, which were determined through t-tests (*$P < 0.05$, **$P < 0.001$). (D, E) The 35S:AtSTK and VC plants were grown in MS for 4 d. The young seedlings were then transferred to MS medium, MS medium + 100 mM NaCl, or MS medium + 150 mM NaCl and incubated for 7 d. The relative root lengths were measured and are shown as a percentage of the root length in the absence of salt. Bars, 1 cm. (F) VC and transgenic *Arabidopsis* plants were grown in MS medium for 10 d and then transferred to pots. Two weeks later, all of the plants were irrigated with a 100 mM NaCl nutrient solution, and the changes in the plant phenotypes were then analysed.

(VC) and *AtSTK* gene-modified *A. thaliana*, transcribed into cDNA, and detected through semi-quantitative RT-PCR. Using the same quantity of the template, the expression of *AtSTK* in the transgenic *A. thaliana* was found to be significantly higher than that observed in the VC plants, which indicates that the *AtSTK* gene was successfully overexpressed in the transgenic *A. thaliana* (Fig. 1A).

Seeds of *AtSTK* gene-modified or p1300 vector control-

modified (VC) *A. thaliana* were sown in MS medium, MS medium supplemented with 70 mmol/L NaCl, or MS medium supplemented with 150 mmol/L NaCl and cultured for 5 d. The results showed that the germination of *A. thaliana* was greatly inhibited under increasing salt concentrations. In the MS medium supplemented with 70 mmol/L NaCl, the germination rates of the two types of plants exhibited statistically significant differences. In the MS medium supplemented with 150 mmol/L NaCl, both the seed germination and the plant growth exhibited significant differences. The germination rate of the VC plants was lower, and their cotyledons were almost completely white; in contrast, most of the transgenic plants exhibited green cotyledons and survived. The statistical analysis showed that the germination rate in the MS medium for both the VC and the transgenic *Arabidopsis* plants was approximately 100%, whereas, in the MS medium supplemented with 70 mmol/L NaCl, the germination rates of the VC and the transgenic plants were 72% and approximately 85%, respectively. In the MS medium supplemented with 150 mmol/L NaCl, the seed germination rate of VC plants was approximately 33%, whereas that of the transgenic plants was approximately 81.4%. Thus, the overexpression of the *AtSTK* gene significantly increased the salt resistance of *A. thaliana* (Figs. 1B and 1C).

The transgenic plants, including the VC plants, were grown on MS-agar plates for 4 d under normal conditions and then transferred to MS medium supplemented with 100 mmol/L NaCl or MS medium supplemented with 150 mmol/L NaCl. The growth marks were then made, and the plants were vertically cultivated for 7 d. The results showed that, under increasing salt concentrations, the root growth in the *AtSTK* transgenic and the VC plants exhibited significant differences: the root growth in the transgenic plants was significantly greater, and these plants clearly produced a greater number of lateral roots compared to the VC plants (Figs. 1D and 1E).

After being irrigated with a 100 mmol/L NaCl nutrient solution, the adult vector control plants become yellow, grew slowly, and died after 16 d. In contrast, although some of the leaves turned yellow, the *35S:AtSTK* transgenic plants were still able to grow, flower, and ultimately seed. This finding further illustrates that the overexpression of the *AtSTK* gene significantly improved salt resistance in *A. thaliana* adult plants (Fig. 1F).

Overexpression of the *AtSTK* Gene Improves the Tolerance of *Arabidopsis* to Osmotic Stress and ABA

Seeds of p1300:35S:AtSTK or p1300-transformed *Arabidopsis* were sown in MS medium, MS medium supplemented with 5% PEG6000, MS medium supplemented with 10% PEG6000, MS medium supplemented with 0.5 μmol/L ABA, or MS medium supplemented with 1.0 μmol/L ABA and cultured in an illuminated incubator at 22°C for 5 d. Neither the germination nor the growth of the two types of plants showed any significant differences in the MS medium. In

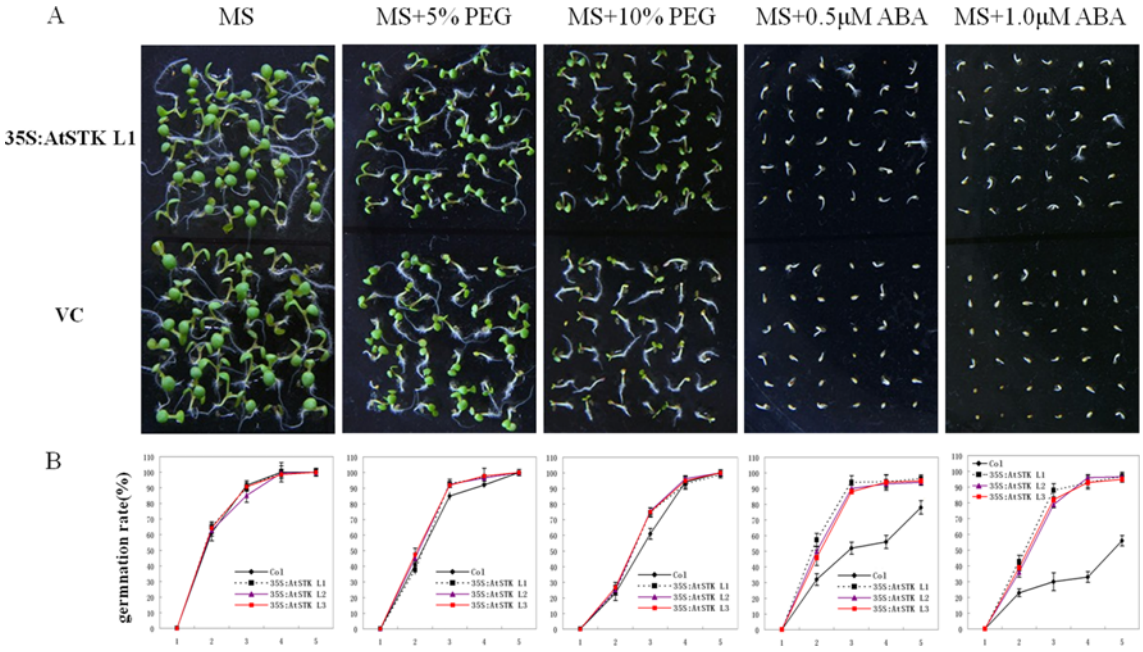

Fig. 2. Overexpression of *AtSTK* improved the tolerance of *Arabidopsis* to PEG6000 and ABA, (A) Seeds of p1300:35S:AtSTK and control transgenic plants were sown in MS medium, MS medium + 5% PEG6000, MS medium + 10% PEG6000, MS medium + 0.5 μmol/L ABA, or MS medium + 1.0 μmol/L ABA and cultured in an illuminated incubator at 22°C for 5 d, (B) The daily germination rates of the two types of *Arabidopsis* seeds under different conditions were plotted as linear graphs.

contrast, the germination rates of the two types of plants in the MS medium containing PEG6000 exhibited differences within the first 3 d. The average germination rates of the *AtSTK*-overexpressing plant seeds in the media containing 5% PEG and 10% PEG were 93% and 74.67%, respectively, and the corresponding germination rates of the control *Arabidopsis* seeds were 85% and 61%, respectively. The cotyledons of the control *Arabidopsis* plants remained white for a long period of time, and sprout growth was retarded in these plants (Fig. 2). In contrast, the cotyledons of the *AtSTK*-overexpressing plants rapidly became green and retained normal growth.

In the media containing 0.5 μmol/L and 1.0 μmol/L ABA, the germination rates of the two types of plant seeds exhibited significant differences. The average germination rates of the p1300:35S:AtSTK transgenic plants in these media were 94.1% and 88%, respectively. Correspondingly, the germination rates of the control *Arabidopsis* were 52% and 30%, respectively (Fig. 2). Therefore, the overexpression of the *AtSTK* gene significantly reduced the inhibitory effect of ABA on *Arabidopsis* seed germination.

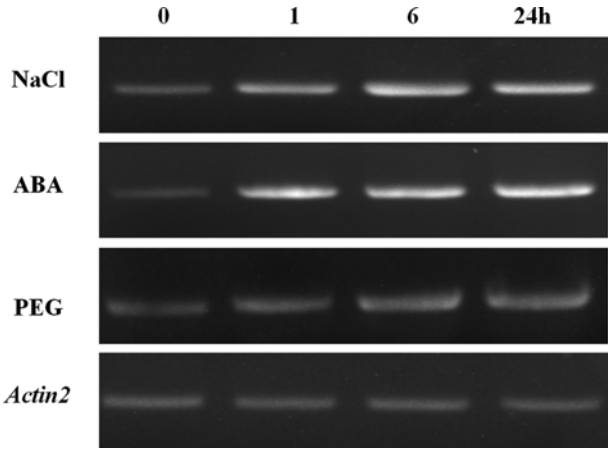

Fig. 3. Expression patterns of *AtSTK* in the seeding leaves under NaCl, PEG and ABA. The 4-week-old seedings were treated by H_2O, 250 mM NaCl, 0.1 mM ABA and 15% PEG6000 for 0, 1, 6, 24 h. Total RNA of the seeding leaves was extracted by Trizol after each treatment. Ethidium bromide staining of PCR products using *AtSTK* specific primers, and the RT-PCR products with *Actin2* specific primers (bottom). Each experiment point was repeated at least three times.

The Expression Patterns of AtSTK Gene under High Salt, PEG and ABA Stress

To investigate the expression patterns of the *AtSTK* gene under stress such as high salinity, PEG, and ABA, the analysis with RT-PCR was performed, respectively. The concentration of the *AtSTK* mRNA increased rapidly and peaked within 6 h by NaCl treatment. By ABA treatment, the expression level of the *AtSTK* gene increased rapidly and reached the maximum after 1 h and maintained the stable high level for 24 h. The expression of the *AtSTK* gene increased and peaked within 6 h in response to PEG treatment (Fig. 3). RT-PCR analysis revealed that the expression of the *AtSTK* gene could be obviously induced by salt, PEG, and ABA.

Membrane Permeability and Proline and MDA Contents of *AtSTK*-overexpressing *Arabidopsis*

The p1300:35S:AtSTK and control *Arabidopsis* plants were

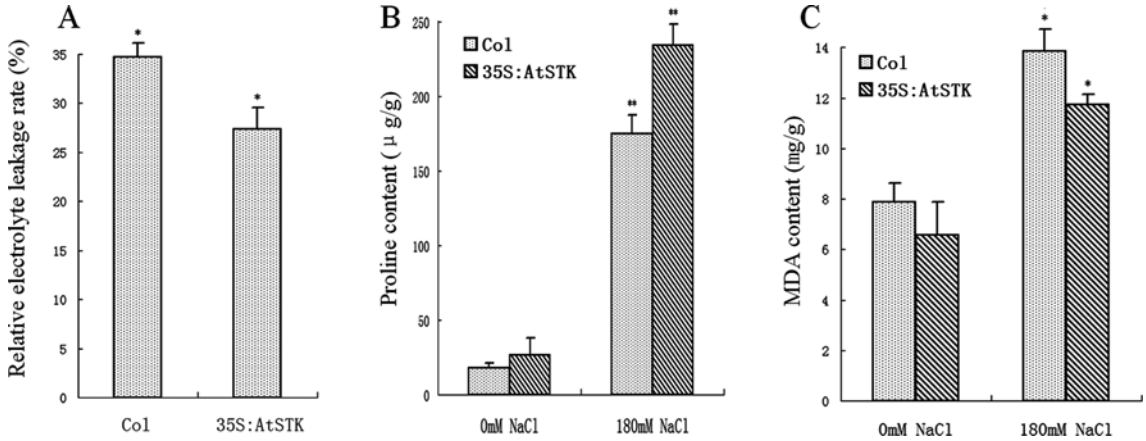

Fig. 4. Physiological characteristics of *AtSTK*-overexpressing plants. (A) Leaves of 3-week-old p1300:35S:AtSTK and control *Arabidopsis* seedlings were cleaned and rinsed with deionised water. Then, 0.2 g of samples from the leaves were transferred to 10 mL of deionised water, vacuum pumped for 30 min, and allowed to stand for 1 h at room temperature. The membrane permeability of the samples was then determined. *AtSTK*-overexpressing and control seedlings were irrigated with 0 mM or 180 mM NaCl solutions for 10 d, and the proline content (B) and the MDA content (C) in the aerial plant parts were then measured. The data were analysed using t-tests (*$P < 0.05$, **$P < 0.001$).

cultured for 3 weeks, and the membrane permeability was then examined. The results showed that the membrane permeability was significantly lower in *AtSTK*-overexpressing *Arabidopsis* compared with the control *Arabidopsis* plants (Fig. 4A). This finding suggests that fewer Na^+ ions enter the *AtSTK*-overexpressing plant cells under salt stress, and this hypothesis may be one of the reasons that the overexpression of *AtSTK* increases salt tolerance in *Arabidopsis* plants.

The proline and MDA contents of the p1300:35S:AtSTK and control *Arabidopsis* leaves were determined before and after the plants were subjected to salt stress. It was found that the proline content of *AtSTK*-overexpressing leaves was significantly higher than that of control plant leaves (Fig. 4B). The increasing content of proline, which is involved in osmotic stress resistance, might also improve the salt tolerance ability of *Arabidopsis* plants. The results of the MDA content analysis showed that the levels of MDA in the *AtSTK*-overexpressing plants were lower than those in the control plants (Fig. 4C). Therefore, the overexpression of *AtSTK* was able to reduce peroxide damage in plant cells under salt stress.

Subcellular Localisation of AtSTK in Transgenic *Arabidopsis*

We experimentally determined the subcellular localisation of AtSTK in transgenic *Arabidopsis* plants expressing the AtSTK-GFP fusion protein. The p1300-AtSTK-GFP and p1300-GFP transgenic plants were cultured for 6 d, and their root cells were observed under a confocal microscope. According to the green fluorescence observed in the cells, it was found that the GFP-AtSTK fusion protein exhibited a similar localisation in the cytosol and nucleus (Fig. 5).

Detection of the Tissue-specific Expression of the *AtSTK* Gene

To further investigate the physiological functions of the transgenic plants, the expression pattern of the *AtSTK* gene was detected in promoter:GUS transgenic plants. The full-length promoter sequence (~900 bp) of the *AtSTK* gene was used to drive the expression of GUS. The Pro:AtSTK:GUS transgenes were found to be expressed in various parts of the adult transgenic plants, including the roots, stems, leaf blades, floral organs, and mature pods. In the roots, GUS

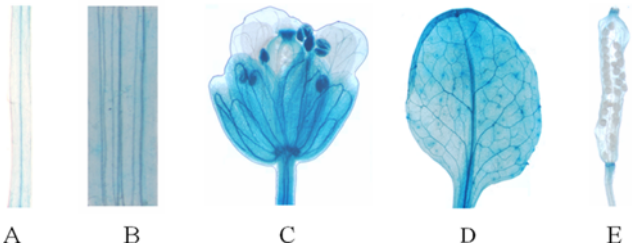

Fig. 6. Tissue-specific expression of *AtSTK* in *Arabidopsis* plants. To study the tissue-specific expression of the *AtSTK* gene, different tissues from 21-day-old promoter:GUS transgenic *Arabidopsis* plants were examined: (A) root, (B) petiole, (C) flower, (D) leaves, and (E) mature pod.

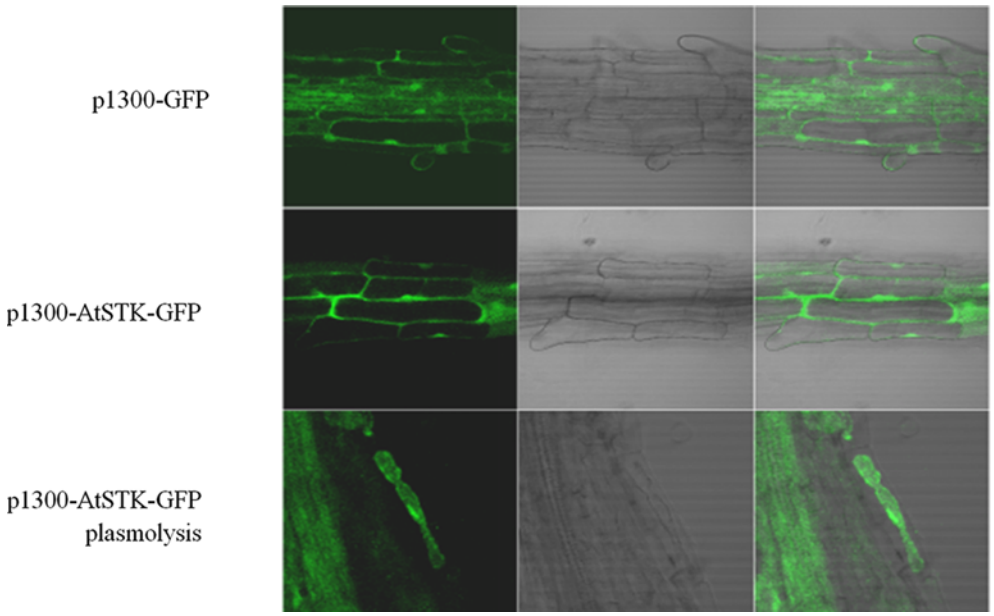

Fig. 5. Localisation of the AtSTK-GFP fusion protein in *Arabidopsis* root cells. Roots of 6-day-old p1300-AtSTK-GFP and p1300-GFP transgenic seedlings were grown in MS medium, and the root tip cells were then observed under a confocal microscope and plasmolysed using 2 mol/L sugar.

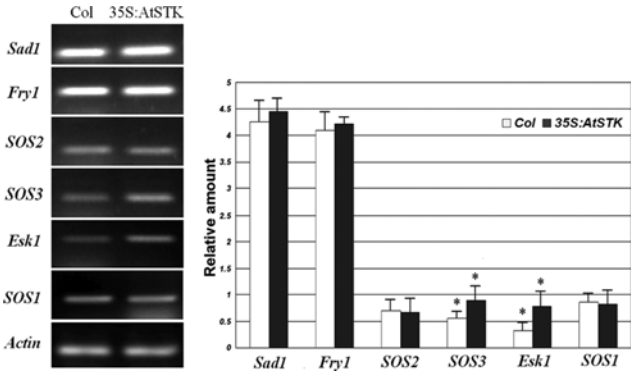

Fig. 7. Semi-quantitative RT-PCR analysis of salt resistance-related genes in *Arabidopsis*. Representative semi-quantitative RT-PCR results for genes belonging to three major signalling pathways. The amount of mRNA from the wild-type (Col) and the 35S: AtSTK *Arabidopsis* plants, which was adjusted based on the level of β-actin and is expressed as the ratio of the densitometric measurement obtained for the sample to the corresponding internal standard (β-actin), is shown in the right panels. The data were analysed using t-tests (*P < 0.05, **P < 0.001).

staining was mainly detected in the lateral roots. These results collectively suggest that *AtSTK* is expressed in various *Arabidopsis* tissues and is particularly strongly expressed in the vessels (Fig. 6).

Analysis of the *AtSTK* Gene Signalling Pathway

The expression patterns of several abiotic stress response genes in wild-type (Col) and *AtSTK* gene-overexpressing plants were detected through semi-quantitative RT-PCR. The results showed that the expression of the *Sad1*, *Fry1*, *SOS1*, and *SOS2* genes exhibited no significant differences under consistent template levels. However, the *Esk1* and *SOS3* genes showed upregulated expression in the *AtSTK* gene-overexpressing *Arabidopsis* plants (Fig. 7).

Because the *Esk1* gene acts downstream of the MAPK pathway, the overexpression of the *AtSTK* gene may affect the expression of a series of genes in the MAPK pathway, which would ultimately lead to increases in the expression of the *Esk1* gene.

Discussion

Serine-threonine protein kinases play key roles in stress signal transduction in plants (Ferreira 1991; Stone and Walker 1995; Chenk and Snaar-Jagalska 1999; Rudrabhatla and Rajasekharan 2002; Chinchilla et al. 2003; Rudrabhatla and Rajasekharan 2003). To date, a total of 57 serine-threonine protein kinases have been identified in *A. thaliana*, and 23 of these have been demonstrated to participate in numerous stress responses, such as the responses to salt, high osmolarity, abscisic acid, salicylic acid, cold, and heat stresses (Rudrabhatla et al. 2006). The *TaSTK* gene was identified as a salt resistance serine-threonine protein kinase gene in a previous study conducted by our group (Ge et al. 2007). The *A. thaliana AtSTK* gene is the homolog of *TaSTK*.

Functional analyses of stress resistance showed that the overexpression of the *AtSTK* gene significantly improved the tolerance of *Arabidopsis* plants to salt, PEG, and ABA stresses. It was found that the expression of this gene could be obviously induced by salt, PEG and ABA. Additionally, physiological analyses indicated that the overexpression of *AtSTK* in *Arabidopsis* resulted in a significant reduction in the plasma membrane permeability, a significant increase in the proline content, and a relative decrease in the MDA content.

A decrease in membrane permeability could reduce the levels of harmful molecules found in *Arabidopsis* cells when plants are under abiotic stresses, such as salt, PEG, and ABA stresses, which would therefore reduce the stress and improve the stress resistance. Osmotic stress is an important type of injury caused by salt stress in plants. Previous studies have shown that the main function of proline is to balance high concentrations of salt in the vacuole to avoid dehydration of the cytoplasm (Santa-Cruz et al. 1999). Therefore, the accumulation of proline in the *AtSTK*-overexpressing *Arabidopsis* cells can be considered a mechanism that the plant uses to protect itself against salt stress.

ABA has the obvious effect of inhibiting seed germination. It has been shown that protein phosphatase 2C (PP2C), which is an important negative regulator of the ABA signalling pathway, is a serine-threonine protein phosphatase (Cohen et al. 1999). AtSTK also belongs to the family of serine-threonine protein kinases. The present study found that the overexpression of the *AtSTK* gene has the same negative regulatory function as exogenous ABA and significantly improved the germination of *Arabidopsis* seeds in media with a high concentration of ABA.

The MAPK, CDPK, and SOS signal transduction pathways are the three main signal transduction pathways involved in the responses to cold, drought, and salt stress in *Arabidopsis*. SOS signalling appears to be closely related to plant ion salt stress because it is mainly involved in the control of the ionic balance under salt stress. The *SOS3*, *SOS2*, and *SOS1* genes all belong to this pathway. Mutations in these genes have been shown to result in increased sensitivity to salt stress (Zhu et al. 1998). Our research found that the overexpression of *AtSTK* increased the expression of the *SOS3* gene. However, the expression of the *SOS1* and *SOS2* genes did not exhibit significant differences. Studies have shown that SOS3 and SOS2 must be combined into a complex to activate the expression of the downstream gene *SOS1* (Hwang et al. 2002). Therefore, the fact that SOS2 did not

show an increase could be the main reason that the stress signal did not pass through SOS1.

The MAPK pathway is involved not only in many different processes that occur during growth and development but also in the signal transduction pathways that respond to high salt, drought, low temperature, ABA, and pathogenic and oxidation reactions in plants (Bogre et al. 1997; Kovtun et al. 1998). The downstream genes *Eskimo1* (*Esk1*) and *Pst1* in the MAPK pathway have been found to be closely related to plant cold tolerance and salt tolerance (Xin and Browse 1998; Xiong et al. 2002). The RT-PCR results showed that the overexpression of *AtSTK* led to a significant upregulation of the expression of the *Esk1* gene. Therefore, AtSTK may act mainly through the MAPK signalling pathway to regulate stress resistance in *Arabidopsis*.

Materials and Methods

Construction of Transgenic Plants

The full-length cDNA for *AtSTK* (At5g02800) was cloned into the binary expression vector pCAMBIA1300, which contains the modified cauliflower mosaic virus (CaMV) 35S promoter. The expression plasmid p1300:35S:AtSTK was then incorporated into *Agrobacterium tumefaciens* GV3101 to perform the transformation of *Arabidopsis*. The transformed *Arabidopsis* plants were selected and detected through PCR using the primers sp1 (5'-TGGGTTGGATCCCGTGTTCT-3') and sp2 (5'-CTCTCCAAGAAAGACAGCAC-3'). We subsequently obtained 30 transgenic T2 lines overexpressing the *AtSTK* gene.

The expression of *AtSTK* in the transgenic *A. thaliana* was detected through RT-PCR. The leaves of 4-week-old seedlings were collected and subjected to RNA extraction using TRIzol, and 1 μg of total RNA was reverse transcribed into cDNA using the MMLV reverse transcriptase and an Oligo dT(18) primer. The primers sp1 and sp2 were also employed in the RT-PCR analysis, and the β-actin gene was used as an internal control. The lines showing the highest exogenous *AtSTK* expression were then selected for further experiments.

Salt Resistance Analysis of *AtSTK* Gene-transformed *A. thaliana*

The seeds of p1300:35S:AtSTK or p1300-transformed *A. thaliana* were surface-sterilised, sown on MS medium, MS medium supplemented with 70 mmol L^{-1} NaCl, or MS medium supplemented with 150 mmol L^{-1} NaCl, and cultured in an illuminated incubator at 22°C. The germination rate was then calculated. A subset of the sterilised seeds was vertically cultured in MS medium for 4 d at 22°C. The plants were then transferred to MS medium containing 0, 100, or 150 mmol L^{-1} NaCl. The root length was measured after 7 d of continuous vertical culture.

Tolerance to Osmotic Stress and ABA in *AtSTK*-overexpressing *Arabidopsis*

Seeds of P1300:35S:AtSTK and P1300 vector-transformed *Arabidopsis* were surface sterilised, sown on MS medium, MS medium supplemented with 5% PEG6000, MS medium supplemented with 10% PEG6000, MS medium supplemented with 0.5 μmol L^{-1} ABA, or MS medium supplemented with 1.0 μmol L^{-1} ABA medium, and cultured in an illuminated incubator at 22°C for 5 d. The germination rates were calculated every day and plotted as a line chart.

The expression Patterns Analysis of *AtSTK* Gene Under High Salt, PEG and ABA Stress

Total RNA was isolated from the seeding leaves at 0, 1, 6, 24 h after 250 mM NaCl, 0.1 mM ABA and 15% PEG6000 treatment. The first strand cDNA was synthesized with 1 μg total RNA and 1 μL Superscript II enzyme (Invitrogen, USA) according to the manufacturer's protocol. As a control, *Actin2* gene was amplified. The primers used for detecting *AtSTK* gene expression were sp1 and sp2. All RT-PCR experiments were repeated at least three times.

Determination of the Physiological Characteristics of *AtSTK*-overexpressing Plants

AtSTK-overexpressing and vector control *Arabidopsis* seedlings were cultured for 3 weeks at 22°C. Their leaves were then cleaned and rinsed with deionised water for 10 min. We then drilled discs with a hole puncher and placed 0.2 g of the leaf blades into 10 mL of deionised water. This mixture was then vacuum pumped for 30 min and allowed to stand for 1 h at room temperature. The membrane permeability was then measured using a DDSJ-318 conductivity meter.

Additionally, the two types of seedlings were irrigated with 0 mmol/L and 180 mmol/L NaCl solutions for 10 d, and the proline and MDA contents in the aerial parts of the plants were detected (Troll and Lindsley 1955).

Subcellular Localisation of AtSTK in *Arabidopsis*

The *AtSTK* sequence was amplified using the primers G1 (5'-CG*TCTAGA*TGGGTTGGATCCCGTGTTCT-3') and G2 (5'-GG*G-GTACC*CGACCCTCTTTGATCTAGGTGG-3'; italics indicate the restriction sites), and the vector p1300-AtSTK-GFP was constructed. The vector was then transfected into *Arabidopsis* using *Agrobacterium* GV3101. Selected roots of 6-day-old p1300-GFP and p1300-AtSTK-GFP transgenic *Arabidopsis* plants were analysed through laser scanning confocal microscopy.

Detection of the Tissue-specific Expression of the *AtSTK* Gene

The full-length *AtSTK* gene promoter was obtained through PCR amplification using the primers LP1 (5'-G*CTGCAG*CGGAAAATC-AACAAAACCC-3') and RP (5'-C*GGATCC*TAAGCTTCTCACG-ATCTTCTTC-3'; italics indicate the restriction sites). A P1300: promoter:GUS vector was then constructed and incorporated into *Agrobacterium tumefaciens* GV3101 for the transformation of *A. thaliana*. The mature pods, roots, leaves, petioles, and flowers of the transgenic *Arabidopsis* plants were cleaned with distilled water in a small EP tube. Dyeing liquid (50 μL of X-Gluc mother liquor and 950 μL of base fluid) was then added to the tube, and the tube was incubated at 37°C overnight. The experimental materials were then treated two to three times with 70% or 95% ethanol to remove the chlorophyll.

Expression Analysis of the Stress-tolerant Signalling Pathway Genes in the 35S:AtSTK *Arabidopsis*

Total RNA was extracted from the 4-week-old seedlings of wild-type (Col) and 35S: AtSTK *Arabidopsis*, and was reverse transcribed into cDNA using the MMLV reverse transcriptase. According to a genetic analysis of three stress-tolerant signalling pathways, six downstream stress-tolerant genes were selected. The expression of these genes in wild-type (Col) and *AtSTK*-overexpressing *Arabidopsis* plants was detected through semi-quantitative RT-PCR. The primer pairs used to amplify these genes in the semi-quantitative PCR analysis are listed in Table 1. The *β-actin* gene was also amplified for calibration purposes,

Table 1. Primers designed to amplify the downstream genes of three salt stress-induced signalling pathways

Gene	Upstream primer (5'-3')	Downstream primer (5'-3')
Actin	TCGCTGACCGTGAGCAAAG	TGTGAACGATTCCTGGACCTG
Sad1	GCGAACAATCCTTCACAG	CTTCGGGAGACCCACCT
Fry1	CGCAGTAGCACTAGGATTG	TTGACACCGAGTTTATTGG
SOS1	GGCAGCATGGTTAATGTGTAC	CTCGGAGAATCGATTCTCACA
SOS2	ATTGAGGCTGTAGCGAAC	GGTATTCCTTCTGTTGCC
SOS3	GGAGGAATCTCTTCGCTG	CACGAAAGCCTTATCCACC
Esk1	CGTCTTCCGAGTCGAGGATTAT	GACAGTGGTACGTAGAGGATCAAT

and three determinations were performed for each sample. Ten microlliters of PCR products were loaded into 1% agarose gel and visualized after staining with ethidium bromide. The ratio between gel bands is calculated by the GeneTools software (Syngene).

Acknowledgements

This work was supported by the National Natural Science Fund (No. 30900104) and the Hebei Provincial Natural Science Fund (No. C2009000271).

Author's Contributions

LB constructed transgenic plants, detected the salt resistance of *TaSTK* overexpressing plants, detected the subcellular localisation and the tissue-specific expression of *TaSTK*, detected the expression level of the stress-tolerant signalling pathway genes in 35S: *TaSTK* plants; CCF detected the tolerance to osmotic stress and ABA; JLL detected the expression patterns of *TaSTK* under high salt, PEG and ABA stress; XXL detected the physiological characteristics of *AtSTK* overexpressing plants; BCZ, YZS and ZJH revised the manuscript; RCG designed the experimental plan and revised the manuscript. All the authors agreed on the contents of the paper and post no conflicting interest.

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