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Evaluation of *Thellungiella halophila ST7* for improving salt tolerance in cotton

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Abstract

Background: *Gossypium hirsutum* (upland cotton) is one of the principal fiber crops in the world. Cotton yield is highly affected by abiotic stresses, among which salt stress is considered as a major problem around the globe. Transgenic approach is efficient to improve cotton salt tolerance but depending on the availability of salt tolerance genes.

Results: In this study we evaluated salt tolerance candidate gene *ST7* from *Thellungiella halophila*, encoding a homolog of *Arabidopsis* aluminum-induced protein, in cotton. Our results showed that *ThST7* overexpression in cotton improved germination under NaCl stress as well as seedling growth. Our field trials also showed that *ThST7* transgenic cotton lines produced higher yield under salt stress conditions. The improved salt tolerance of the transgenic cotton lines was partially contributed by enhanced antioxidation as shown by diaminobenzidine (DAB) and nitrotetrazolium blue chloride (NBT) staining. Moreover, transcriptomic analysis of *ThST7* overexpression lines showed a significant upregulation of the genes involved in ion homeostasis and antioxidation, consistent with the salt tolerance phenotype of the transgenic cotton.

Conclusions: Our results demonstrate that *ThST7* has the ability to improve salt tolerance in cotton. The *ThST7* transgenic cotton may be used in cotton breeding for salt tolerance cultivars.

Keywords: *Gossypium hirsutum*, Aluminum-induced protein, Salinity, *Thellungiella halophila*, *ST7*, Salt tolerance

Background

Salinity stress is one of the most acute and critical abiotic stress that limits the growth and yield of crops (Tester and Davenport 2003; Parida et al. 2004). There is an immediate requirement to develop new varieties of salt tolerant crops that can cope with high salinity environments (Ma et al. 2020). To withstand the salt stress, plants have developed intricate mechanisms (Blumwald 2000). The higher concentration of sodium chloride in the environment disturbs the overall cellular homeostasis in plants (Zhu 2002). Plants have developed strategies to avoid the injuries from high NaCl levels (Flowers and Colmer 2008), for instance, sequestration of NaCl into large vacuole (Martinoia et al. 2007). Alteration of the ion transport mechanisms may also help plants in response against salt stress (Darko et al. 2020). The mechanisms of salt tolerance also include the acclimatization to the osmotic stress, cytoplasmic Na^+ exclusion, Na^+ and Cl^- accumulation tolerance and compartmentalization, occurring in a disciplined manner (Munns and Tester 2008).

Cotton is considered as one of the principal fibers and oil seed crop. It is also used in medicinal products, household stuff, and as major raw material in textile industry. It fulfils 35% of world's fiber need (Martinez et al. 2018).

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Abiotic stresses cause about 50% reduction of the global cotton yield. Salinity stress is among the most important restriction for cotton crop productivity and growth (Wang et al. 2003).

A number of studies have been done in order to develop salt tolerant cotton varieties via transgenic approach. For instance, *TsVP*, a H^+-PPase gene from *Thellungiella halophilla,* was demonstrated to improve the growth of root and shoot and photosynthetic activity under high salinity in cotton (Lv et al. 2008). The transgenic plants showed decreased membrane ion leakage and malondialdehyde level as compared with the wild type because the transgene assisted the sequestration of Na^+ and Cl^- in the vacuoles (Lv et al. 2008). The expression of *TsVP* also enhanced the emergence and survival rate of cotton, improved fiber quality under high saline environment (Zhang et al. 2016a, b). Another study demonstrated that the expression of *AVP1* encoding vacuolar pyrophosphatase from *Arabidopsis thaliana* improved growth of the transgenic cotton and the fiber yield under salt stress (Pasapula et al. 2011). Cheng et al. (2018) showed the co-expression of *AtNHX1* gene from *Arabidopsis thaliana* and *TsVP* gene from *Thellungiella halophilla* in cotton enhanced emergence rate and yield of the transgenic cotton under high saline environment (Cheng et al. 2018).

Using a functional gene mining method, Du et al. (2008) identified *SALT TOLERANCE* (*ST*) genes from salt cress (*ThST7*). *ThST7,* one of the identified genes, encodes an auxin/aluminum-responsive protein (Du et al. 2008). The Arabidopsis homologue of *ThST7*gene is *At5G19140* with 94% similarity to the ThST7 amino acid sequence (Du et al. 2008). A study conducted by Lin et al. showed that the transcript level of *At5G19140* gene increased in hypoxia stress (Lin et al. 2017). This aluminum-induced protein was also found responsive to PEG stress in wheat (Pál et al. 2018). Putrescine treatment was found to upregulate the aluminum induced protein genes (Agarwal et al. 2009).

In this study, we aimed to evaluate the salt tolerance conferred by *ThST7* gene we previously isolated from *Thellungiella halophilla* in cotton. Our results not only showed improved germination and growth performance of transgenic cotton lines under salt stress in controlled environment but also showed better performance in field trials.

Results

ThST7 enhanced cotton seed germination under salt stress

To evaluate *ThST7*-conferred salt tolerance in cotton, we made the overexpression construct and obtained transgenic lines by *Agrobacterium*-mediated transformation (Additional file 1: Fig. S1A). The expression of the transgene *ThST7* was verified in the transgenic cotton lines by reverse transcription polymerase chain reaction(RT-PCR) analysis (Additional file 1: Fig. S1B). T_3 transgenic cotton seeds were assessed for germination either with 250 mmol·L^{-1} or without NaCl stress. Both the control (CK) and transgenic (*ST7*-OE1 and *ST7*-OE2) cotton seeds were germinated at almost equal rate in absence of salt stress. Similarly, both CK and transgenic seeds attained stable germination rate at day 5–8 with initiation at the same time. While at 250 mmol·L^{-1} NaCl stress, germination rate of *ST7*-OE1 and *ST7*-OE2 transgenic cotton seeds was significantly higher (~37% and 33%, respectively) than CK seeds (~20%) on the 8th day of seed sowing. Transgenic seeds germinated 1 day earlier than the CK (Fig. 1A, B).

Under normal growth condition, both CK and the transgenic lines revealed no obvious difference after growing for 2 weeks in soil, while in soil containing 250 mmol·L^{-1} NaCl, survival rate of *ST7*-OE1 (~32%) and *ST7*-OE2 (~38%) seedlings was significantly higher than that of the CK seedlings (~20%) (Fig. 1C, D). These results indicated that *ThST7* overexpression in cotton improved seed germination under salt stress.

ThST7 enhanced seedling growth under salt stress

To demonstrate the *ThST7*-conferred salt tolerance in seedlings, we conducted seedling growth and survival in hydroponic culture with 0 or 140 mmol·L^{-1} NaCl as described in "Methods" section. After 140 mmol·L^{-1} NaCl treatment for 3 days, the survival rate of transgenic seedlings (~70%~78%) was significantly higher than that of CK (~20%), while in absence of NaCl stress, transgenic and CK seedlings survived almost equally (100%) (Fig. 2A, B). We also examined the salt tolerance of transgenic seedlings grown in soil. The 20-days-old seedlings were treated without or with 300 mmol·L^{-1} NaCl for 10 days (Fig. 2C). Under no salt control condition, no apparent difference was observed between the CK and the transgenic lines, while under salt stress condition the CK was more sensitive to the salt stress compared with the transgenic plants. The transgenic limes exhibited significantly higher survival rates (~76%~78%) than the CK (~40%) (Fig. 2C, D).

ThST7 enhanced the tolerance against oxidative stress

To detect the level of reactive oxygen species (ROS) under salt treatment in transgenic lines, we performed diaminobenzidine (DAB) and nitrotetrazolium blue chloride (NBT) staining of cotton leaf. DAB staining visualized the accumulation of H_2O_2 while the NBT staining depicted the presence of superoxide radicals. Under the normal condition, the DAB and NBT staining were similar between the CK and transgenic lines. However, under the salt treatment (0.6% NaCl), less DAB staining

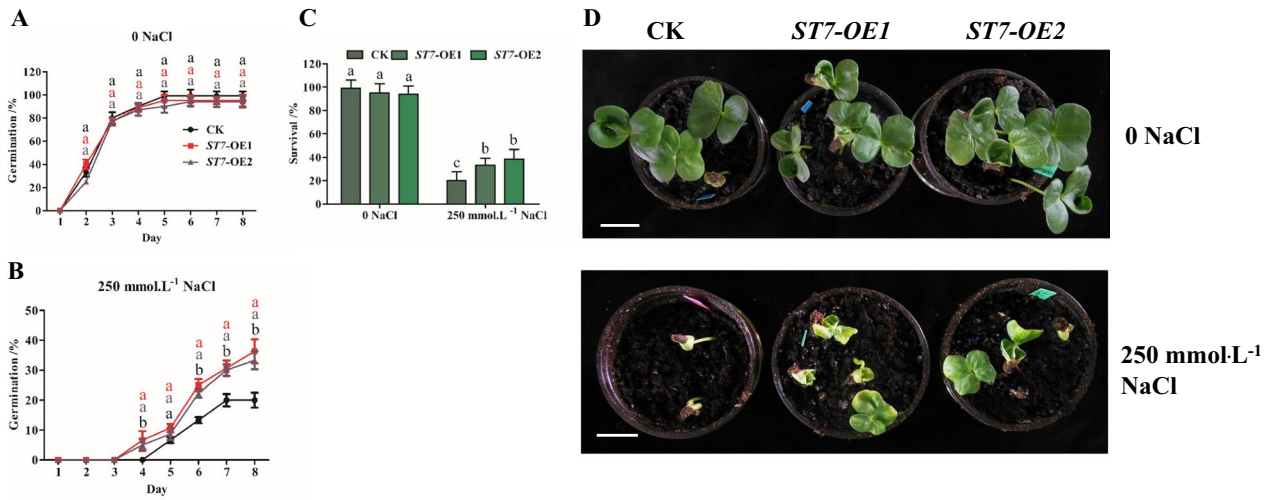

Fig. 1 Expression of *ThST7* gene in cotton confers salt tolerance phenotype in seed germination. **A** Seed germination curve. Seeds of the wild type (CK) and *ST7*-OE lines were germinated in soil watered with 0 or 250 mmol·L^{-1} NaCl for 8 days and germination percentage was recorded every day. Values are mean ± SD (10 seeds per replicate, five replicates per treatment). Different letters denote significant differences ($P < 0.05$) from Duncan's multiple range tests. **B** Seedling survival ratio. The above germinated seeds were grown for 2 weeks before seedling survival ratio was recorded. Values are mean ± SD (10 plants per replicate, five replicates per treatment). Different letters denote significant differences ($P < 0.05$) from Duncan's multiple range tests. **C** Growth of germinated seeds. Bar = 4 cm

signals were observed in the transgenic leaves compared with the control (Fig. 3A). Similarly, the blue NBT staining signals were significantly more and darker in the CK leaf compared with that in *ST7*-OE1 and *ST7*-OE2 lines (Fig. 3B). These results demonstrated that the transgenic cotton plants had a better ability to scavenge H_2O_2 and superoxide radicals compared with the CK plants.

ThST7 improved cotton yield in field

To detect the effect of *ThST7* on cotton yield, we performed a field trial in saline soil as described in "Methods" section. Under normal condition, there was no significant difference in cotton yield per plot between the CK and transgenic lines, while under 0.4% NaCl stress, cotton yield of CK was lower than that of *ST7*-OE1 and *ST7*-OE2 lines (Fig. 4A). The average boll weight of CK and transgenic lines was almost similar in normal conditions, but under 0.4% NaCl stress, boll weight of transgenic lines (*ST7*-OE1 and *ST7*-OE2) was heavier than that of CK (Fig. 4B). These results indicated that the overexpression of *ThST7* increased cotton yield under salt stress in the field conditions.

The genes involved in ion homeostasis and antioxidation were enriched and upregulated in the transgenic line

To investigate the molecular mechanism by which *ThST7* confers salt tolerance in cotton, we compared the transcriptomes between *ThST7*-OE and CK under both normal and salt stress. Based on the RNA-seq read counts, a total of 9 182 differentially expressed genes (DEGs) were observed under the salt-stress treatment versus 1 918 DEGs under normal condition. A total of 790 genes were upregulated and 1 128 were downregulated under normal conditions, while 4 608 were upregulated and 4 574 were downregulated under salt stress (Fig. 5A).

Transcriptomic differences between CK and *ThST7*-OE were determined by performing pairwise comparisons (CK-N vs. *ThST7*-OE1-N, CK-N vs. CK-S, and CK-S vs. *ThST7*-OE1-S). Based on these pairwise comparisons we have categorized into three groups: total number of genes found in the CK-N versus *ThST7*-OE1 are 73 from which 53 overlapped with CK-N versus CK-S. The total number of genes found in CK-N versus CK-S were 7 573, out of which 2 707 overlapped with CK-S versus *ThST7*-OE1-S. The total number of genes found in CK-S versus *ThST7*-OE1-S were 1 346, and out of which 10 overlapped with CK-N versus *ThST7*-OE1. Only 5 genes were found overlapped in all of these groups (Fig. 5B).

The DEGs were grouped into several biological processes under normal (Gig. 6A) and salt treatment (Fig. 6B). Abiotic stress-related genes were significantly upregulated in the transgenic plants as compared with the CK under normal and salt stress conditions (Additional file 1: Tables S1 and S2). *ThST7*-OE plants exhibited a drastic upregulation of genes involved in salt-tolerance such as *KEA2*, *KEA3*, *NHX2*, *NHD1*, and *AKT2* under salt stress conditions as shown by the heat map (Fig. 7). Moreover, the genes encoding antioxidant enzymes such

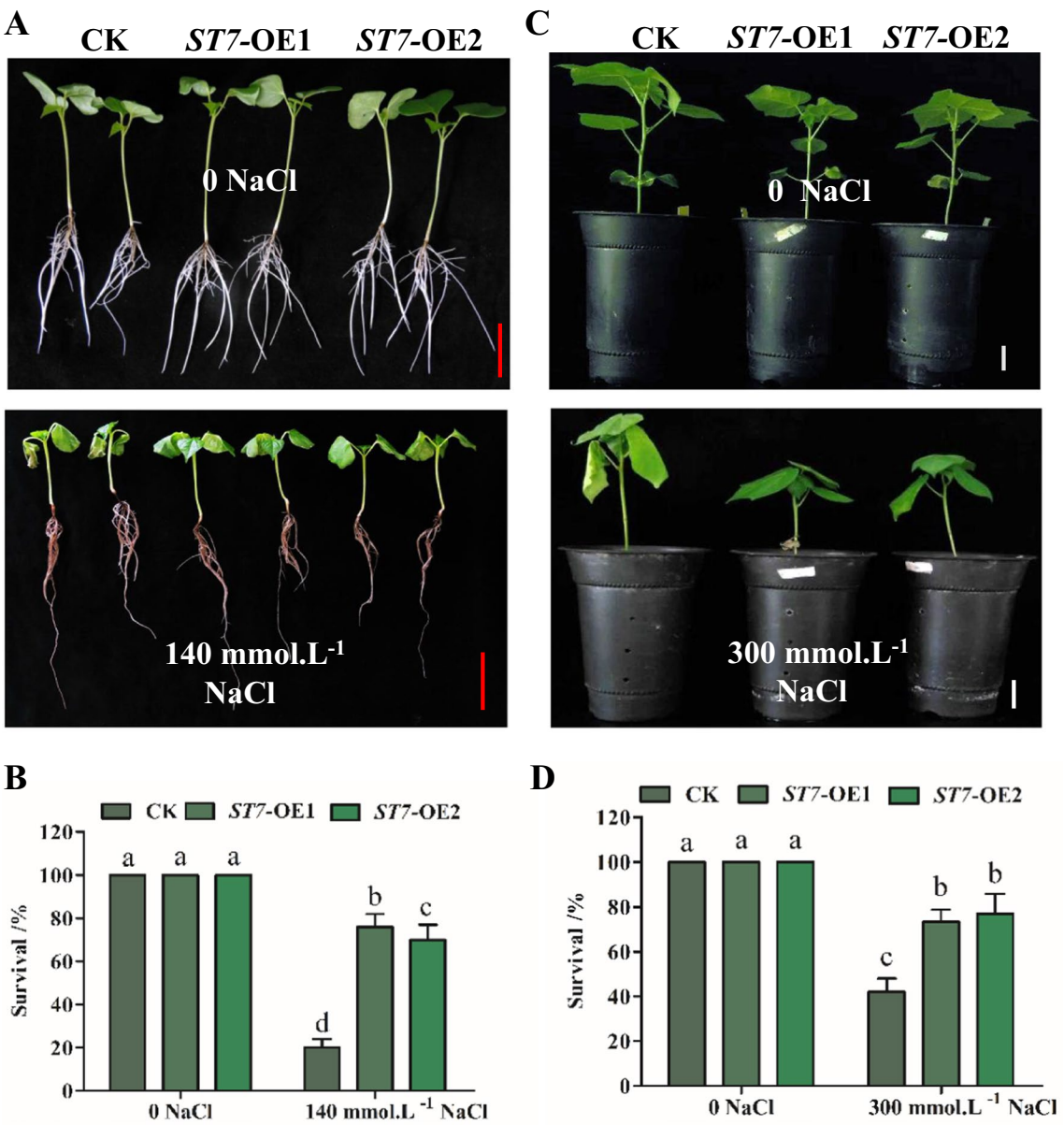

Fig. 2 *ThST7* overexpression improved salt tolerance of 2-weeks-old cotton seedlings grown in hydroponic culture or in soil under different salt concentrations. **A**, **B** Seeds of the CK and *ST7*-OE lines germinated in soil for 4 days, transferred to hydroponic culture for 1 week and then treated with 140 mmol·L^{-1} NaCl for 3 days. Bar = 4 cm. Values are mean ± SD (7 plants per replicate, four replicates per treatment). Different letters denote significant differences ($P < 0.05$) from Duncan's multiple range tests. **C** The 20-days-old seedlings grown in soil were treated with either 0 or 300 mmol·L^{-1} NaCl for 10 days before the images were recorded. Bar = 4 cm. **D** Survival rate of the seedlings as in **C**. Values are mean ± SD (10 plants per replicate, five replicates per treatment). Different letters denote significant differences ($P < 0.05$) from Duncan's multiple range tests

as ascorbate peroxidase, catalase, peroxidase, and glutathione S-transferase were prominently upregulated in *ThST7*-OE (Fig. 7, Additional file 1: Table S2). These results demonstrated that *ThST7* affected the transcription of an array of salt-responsive genes in cotton.

To find out whether there are homologues of *ThST7* in cotton genome, we performed a Blast search using ThST7 protein sequence against cotton genome. The BLAST results showed that there are 23 genes homologous to *ThST7* in the *Gossypium hirsutum* L. genome. Among the 23 genes, 21 genes belong to Stem-specific protein TSJT1 family while the remaining 2 genes are

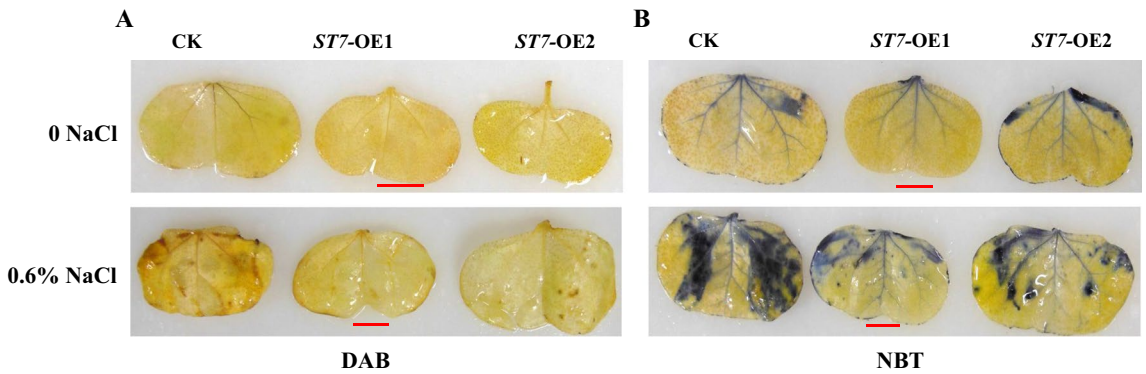

Fig. 3 DAB and NBT staining. The leaves of control, *ST7*-OE1, and *ST7*-OE2 plants treated with either 0 or 140 mmol·L^{-1} NaCl for one day before DAB (**A**) or NBT staining (**B**). Bar = 1 cm

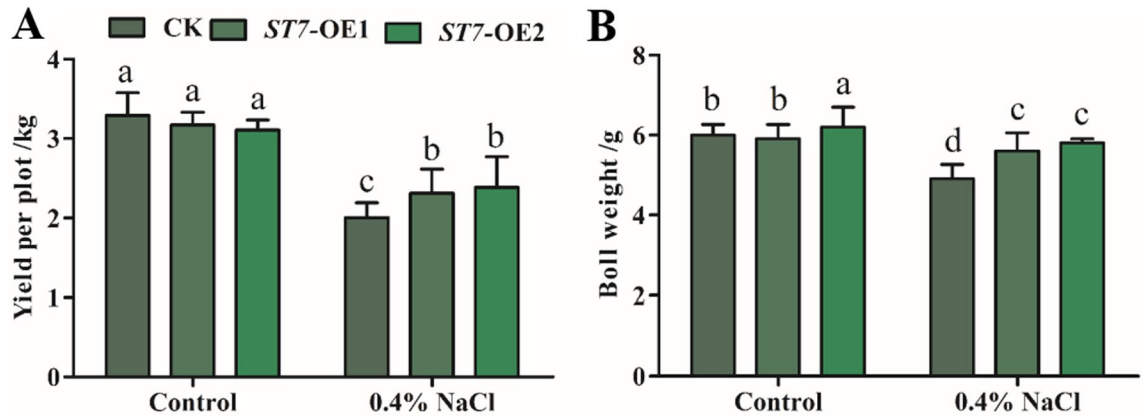

Fig. 4 *ThST7* overexpression improved cotton yield in saline field. **A** Yield per plot of wild type, *ST7*-OE1 and *ST7*-OE2 lines in normal soil conditions (0.1% NaCl) and saline soil with 0.4% NaCl. **B** Average boll weight of the CK and *ST7*-OE lines as in **A**

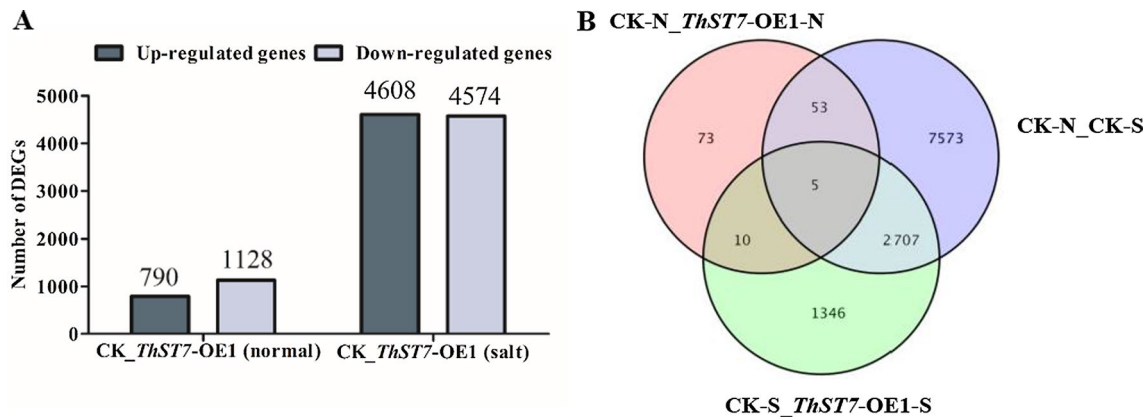

Fig. 5 RNA-seq of the CK and *ST7*-OE seedlings under normal and salt stress conditions. **A** Number of upregulated and downregulated genes in CK and *ST7*-OE under normal and salt stress condition as revealed by RNA sequencing. **B** Venn diagrams of the common and specific genes unique and shared between the different pairwise comparisons

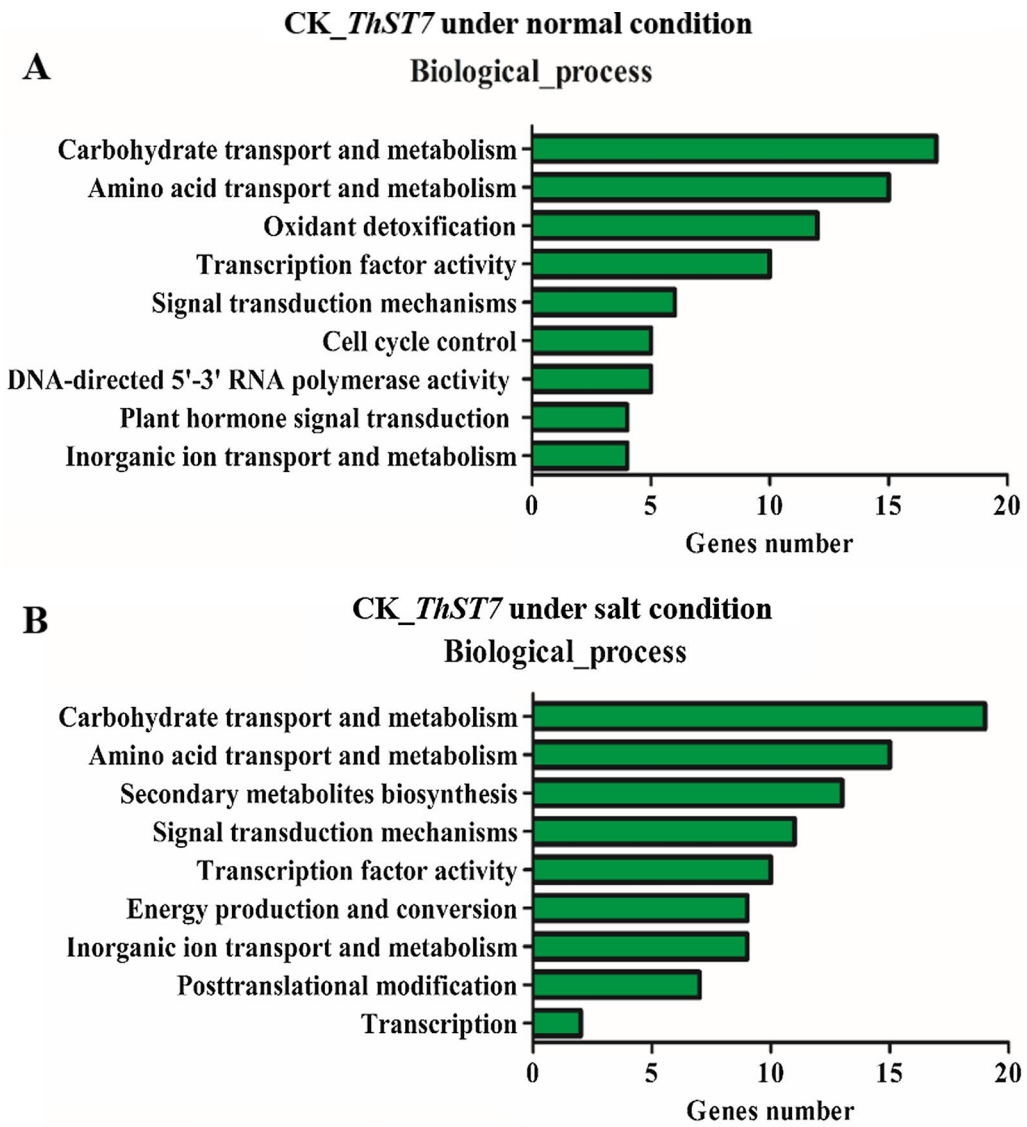

Fig. 6 The Gene ontology(GO) grouped up-regulated genes in different biological processes. **A** The number of genes involved in the biological processes under normal conditions. **B** The number of genes involved in the biological processes under salt conditions

annotated as hypothetical protein ERO13 (Additional file 1: Table S3), suggesting that ThST7 proteins might be functionally conserved in cotton.

Discussion

In this study we evaluated the salt tolerance conferred by *ThST7* from *Thellungiella halophila* and demonstrated the feasibility of this gene improving salt tolerance in cotton.

Cotton is most sensitive to saline stress at germination stage (Peng et al. 2018). A significant reduction in cotton germination was seen in cotton upon exposure to salt stress (Khorsandi and Anagholi 2009; Ma et al. 2011), even a complete inhibition under high salinity stress (Sattar et al. 2010). In our study we observed that the *ThST7* transgenic lines displayed early and increased germination under salt stress compared with CK (Fig. 1A, B, C). Thus, *ThST7* confers a desirable trait—salt tolerance germination in cotton.

Seedling stage is a significant phase in plant life cycle and vulnerable to environmental stresses including salt stress, which significantly decreases seedling survival (Sattar et al. 2010). Our results showed that upon exposure to salt stress *ThST7*-OE lines displayed significantly enhanced seedling growth and survival rate as compared with CK both in soil and hydroponic culture

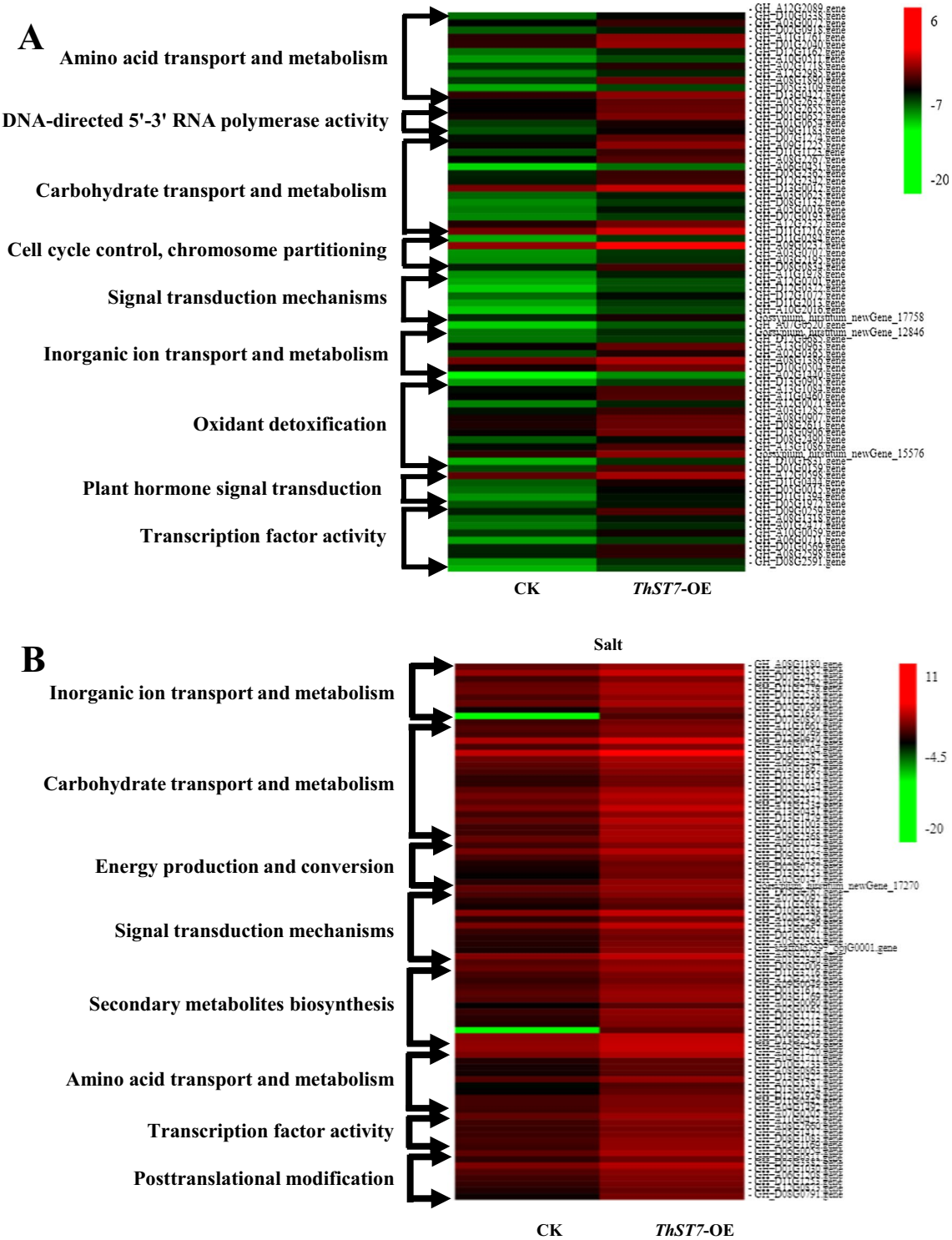

Fig. 7 Heatmap of genes upregulated by in *ST7*-OE plants under normal conditions. Genes involved in different biological processes in CK and *ST7*-OE plants under normal conditions (**A**) and salt stressed conditions (**B**) were shown. The results were presented as log(2) ratios. Only results with a *P* value < 0.05 and that have been confirmed in three independent experiments were included. A color code was used to visualize the data

(Fig. 2). This demonstrates that *ThST7* is capable of enhancing salt tolerance of cotton seedlings, which would greatly benefit cotton seedling establishment in saline soil.

ROS accumulation is induced in plants under salt stress (Choudhury et al. 2017; Luo et al. 2021). An augmented production of ROS (including OH^-, H_2O_2, O_2, and O_2^-) generates cellular oxidative stress that harms membranes and macromolecules in plants (Lin et al. 2020). In our study we observed that *ThST7*-OE cotton exhibited higher ability of ROS scavenging as evidenced by DAB and NBT staining (Fig. 3). These results signify that *ThST7* enhances antioxidant capabilities and protects cotton plants from oxidative damage.

One major goal of crop genetic engineering is to increase crop yield (Bao et al. 2016). High salt content in soil significantly decreases reproductive and vegetative growth of cotton that cause poor fiber quality and low yield (Dong 2012). The yield of cotton decreases with the reduction in boll number and weight (Longenecker 1974). Under saline conditions, decrease in mature bolls takes place due to reduction of fruit bearing position, augmented flower and boll shedding along with delayed flowering (Bernstein and Hayward 1958). Increase in the salt content of soil leads to decrease in the movement of sucrose towards developing bolls thus causing reduction in boll weight (Peng et al. 2016). In our study we demonstrated that in field trails the *ThST7*-OE lines produced higher yield and average boll weight than CK (Fig. 4) under salinity.

We also carried out RNA transcriptomic analysis of *ThST7*-OE lines under salt stress. It was revealed that transcript levels of various genes involved in tolerance against salinity stress were upregulated including *NHX2, KEA2, KEA3, NHD1,* and *AKT2* (Fig. 7, Additional file 1: Table S2). Under salinity stress, plants adapt many strategies to main ion homeostasis via reduction in concentration of Na^+ and increase in concentration of K^+ (Cui et al. 2020). The accumulation of Na^+ in vacuole by sodium/hydrogen exchangers (*NHXs*) aids in the maintenance of cellular Na^+ homeostasis (Zhang et al. 2016a, b). The Na^+ compartmentalization is controlled by Na^+/H^+ antiporter (*NHX*) (Apse and Blumwald 2007; Guo et al. 2020). It has been demonstrated that under salinity stress, *NHX* participates in the Na^+ partition into vacuoles, which aids the cells to uptake water and sustain the osmotic balance, decreases the noxious effect of salt ions and regulates the cytoplasmic Na^+ concentration and pH (Apse et al. 1999; Yamaguchi et al. 2001; Guo et al. 2020). In our study we observed that the transcript level of *NHX2* is upregulated in transgenic OE lines of cotton compared with that in CK. The function of *NHX2* is linked to accumulation of Na^+ in vacuole and thus leading to salt tolerance in saline environment (Yarra and Kirti 2019).

Along with this, we also observed the transcript levels of *KEA2* and *KEA3* were upregulated under salt stress in transgenic cotton lines as compared with CK. KEAs are potassium efflux antiporters. *KEA2* works as a mediator for monovalent cation/proton exchange and is localized in chloroplast inner envelop membrane (Aranda-Sicilia et al. 2012). However, potassium transporter *KEA3* is highly expressed to transport Na^+ and Cl^- into the vacuole for ion absorption and balance adjustments under salt stress (Zhang et al. 2020). It was shown that under osmotic stress *KEA2* and *KEA3* were involved in hyperosmotic-induced Ca^{2+} responses. It was demonstrated that *KEA2* and *KEA3* mutants displayed reduced Ca^{2+} levels during the hyperosmotic-induced Ca^{2+} response, thus depicting that *KEA2* and *KEA3* act as sensors of osmotic stress that are able to regulate the enhancement of Ca^{2+} (Stephan et al. 2016).

We also observed that the transcript level of *AKT2* was also upregulated under salt stress in transgenic cotton lines. *AKT2* (*Arabidopsis* K^+ transporter) is a K^+ channel that is permeable to K^+ but not Na^+ and is located in leaf phloem tissue (Tian et al. 2021). The maintenance of Na^+/K^+ homeostasis is important for the survival of plants under salt stress, which is dependent upon the function of Na^+ and K^+ transporters (Assaha et al. 2017). The *AKT2* knockout rice plants were more sensitive to saline environment compared with the wild types (Tian et al. 2021). In another study it was demonstrated that under salt stress the upregulation of *AKT2* led to augmented K^+ in vascular bundles along with its redistribution between roots and shoots of Arabidopsis (Pilot et al. 2003).

Conclusions

In conclusion, *ThST7* improves cotton seed germination and growth under salt stress. The transgenic cotton lines exhibits increased yield in saline soil in field trial. The salt tolerance phenotype of *ThST7*-OE plants is supported by the transcriptomic analyses results. Therefore, *ThST7* is a candidate gene for salt tolerance improvement in cotton.

Methods

ThST7 gene cloning and plant transformation

Full length complimentary *ThST7* gene was successfully cloned into a plant binary expression vector (pCB2004) under the control of constitutive promoter *CaMV* (cauliflower mosaic virus) 35S. *ThST7* gene was followed by Nos terminator (Additional file 1: Fig. S1A). *NPTII* (neomycin phosphotransferase) gene of the vector was used as a selection marker both in the initial screening of transformant bacterial colonies and cotton plants. Transgenic

cotton lines were obtained by *Agrobacterium*–mediated transformation (Li et al. 2002) of vector construct into callus segment of explant. The regenerated pallets after transformation were transferred to soil to get T_0 seeds. T_1 seed were harvested by grafting the T_0 shots in wild type plants. T_3 generation of transformed cotton along with wildtype (CK) was used to carry out this study.

RNA isolation and RT-PCR

Fresh leaves were taken from plants of both genotypes and ground in mortar and pestle with the use of liquid nitrogen. Total RNA was isolated using Eastep Super Total RNA Extraction Kit (Promega Biotech Co. Ltd., Beijing). Isolated RNA (1 µg) from each sample was subjected for reverse transcription reaction. The cDNA was used for RT-PCR analysis to check the expression level of transgenic lines with primers *ST7*-F (ATGTTGGGAATT TTCAGCGGAG) and *ST7*-R (TCAATCTGCAAGAAC TGCTGCT). *GhHis*3 (AF024716) was used as internal control (Forward: TGGGAAGGCTCCAAGGAAGCA, Reverse: CGAGCCAACTGGATGTCCTTG) (Additional file 1: Fig. S1B).

Plant materials

Cotton (*Gossypium hirsutum* L.) cultivar R15 was used in this study. Experiments were conducted in the green house facility of School of Life Sciences, University of Science and Technology of China, Hefei, China.

Seed germination

Transgenic cotton lines along with CK seeds were germinated in long day/night (16/8 h) conditions with temperature 25~26 °C. For germination under salt tolerance, seeds were germinated under irrigation with 0 or 250 mmol·L^{-1} of NaCl for 8 days. Germination rate was observed for each day after sowing.

Survival rates

Transgenic cotton lines were grown for 4 days in normal growth conditions in soil (16/8 h of light and 28°C). On the fifth day of germination, seedlings were transferred to hydroponic solution (Hoagland and Arnon 1950) for 1 week and treated with 140 mmol·L^{-1} NaCl for another 3 days in the green house under controlled conditions and survival rate was counted. Likewise, transgenic and CK lines were grown in soil for 20 days and treated with 0 or 300 mmol·L^{-1} NaCl for another 10 days before survival rate was counted.

DAB and NBT protocol

To analyze the ROS scavenging capacity of the transgenic lines, DAB and NBT staining were conducted as described (Alvarez et al. 1998). Briefly, the leaves from the *ST7*-OE1 *and ST7*-OE2 transgenic lines and CK plants were treated with 0 or 0.6% NaCl, then stained in the 0.1% (w/v) DAB solution in the dark for 18 h. Afterwards, leaves were submerged in 96% (v/v) ethanol to remove chlorophyll. For NBT staining, the leaves were stained in NBT staining solution for 12 h, then treated with 96% (v/v) ethanol to remove chlorophyll.

Field trial of cotton

To check the performance of *ThST7* transgenic cotton, field trials were performed in Experiment Station of Shanxi Agricultural Academy, Yuncheng, Shanxi Province, China from April 2020 to September 2020. Experimental design contained 3 replicate plots per genotype per treatment with plot size of 2.5 X 6 m^2 with random arrangement. During the whole growth period, the control group was grown in the soil with salt (NaCl) content < 0.1%, while the salt stress treatment group was grown in the soil with 0.4% NaCl. Cotton yield (fiber plus seeds) was recorded at the end of the field trial.

RNA sequencing

The plants were grown hydroponically with the conditions described above as CK and salt treated lines (140 mmol·L^{-1} NaCl). The 16-days-old seedlings were sampled for RNA sequencing. A total of 20 seedlings of the salt treated, and controls were collected. RNA library construction and sequence analysis were conducted as described (Hu et al. 2016).

Accession numbers

Sequence data from this article can be found in the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov/) or *Arabidopsis* TAIR database (https://www.arabidopsis.org/) under the following accession numbers: *AtNHX7: NM_126259, ThST7-5–2: EU714069, EU714068, ST225*: *EU714080, GhHIS3: LOC107951735, AT5G19140: AK318630, AtNHX1: AT5G27150*.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s42397-021-00108-1.

Additional file 1: Fig. S1. Overexpression construct of *ThST7* and verification of the transgenic lines. **Table S1.** Abiotic stress-related genes that are significantly upregulated in the transgenic line compared to wild type control under normal condition. **Table S2.** Abiotic stress-related genes that are significantly upregulated in the transgenic line compared to wild type control under salt stress condition. **Table S3.** *ThST7* homologues in cotton genome.

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Authors' contributions
Alfatih A, Xiang CB and Wu SJ designed the experiments. Ali M, Nazish T, Javaid A, Wu J performed most of the experiments and data analyses. Zhu YH, Li J, Zhang HY, and Wu SJ conducted field trials and data analyses. Ali M and Alfatih A wrote the manuscript. Alfatih A, Wu SJ and Xiang CB revised the manuscript and supervised the project. All authors read and approved the final manuscript.

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Availability of data and materials
All data generated or analyzed during this study are included in this published article [and its supplementary information files].

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
The manuscript has not been published or submitted for publication elsewhere.

Competing interests
The authors declare no competing interests.

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