Biochemical and Biophysical Research Communications 450 (2014) 794–801

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Function of wheat *Ta-UnP* gene in enhancing salt tolerance in transgenic *Arabidopsis* and rice

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ARTICLE INFO

Article history:
Received 7 June 2014
Available online 19 June 2014

Keywords:
Wheat
Salt tolerance
qPCR
RNAi

ABSTRACT

Based on microarray analysis results of the salt tolerant wheat mutant, we identified and cloned an unknown salt-induced gene *Ta-UnP* (*Triticum aestivum* unknown protein). Quantitative PCR results revealed that *Ta-UnP* expression was induced not only by salt but also by polyethylene glycol, abscisic acid, and other environmental stress factors. Under salt stress, transgenic *Arabidopsis* plants that overexpressed *Ta-UnP* showed superior physiological properties (content of proline, soluble sugar, MDA, and chlorophyll) compared with the control. Subcellular localization demonstrated that Ta-UnP was mainly localized on the cell membrane. The expressions of nine salt tolerance-related genes of *Arabidopsis* in *Ta-UnP*-overexpressed *Arabidopsis* were analyzed via qPCR, and the results revealed that the expressions of *SOS2*, *SOS3*, *RD29B*, and *P5CS* were significantly up-regulated, whereas the other five genes only slightly changed. The results of the salt tolerance analysis indicated that *Ta-UnP* can enhance the salt tolerance of transgenic rice plants, and RNAi transgenic rice plants became highly susceptible to salt stress. The results from this study indicate that this novel *Ta-UnP* may be useful in improving of plant tolerance to salt stress.

1. Introduction

Salt stress is a major constraint in crop growth and yields in the surrounding environment [1]. More than 20% of cultivated land area in the world has excess salt content that may cause salt stress [2]. Exposure to high-salinity conditions greatly inhibits plant growth and development [3]. To address these problems, the functions of salt-tolerance genes in plants should be explored, and transgenic salt-resistant varieties should be cultivated [4].

Wheat is an important staple crop. However, a high-salt environment can significantly affect the yield and quality of wheat. As a result, studies on the salt tolerance mechanism and salt stress resistance genes of wheat have great significance in addressing issues related to increased food demand. Therefore, the cloning and functional validation of stress tolerance genes deepen our understanding of signaling network in response to stress in wheat and may eventually lead to the development of more stress-tolerant crops.

For this study, we employed microarray analysis [5] and studied the global gene expression of the salt-tolerant wheat mutant RH8709-49 [6], under salt stress. The results showed that the expression of 61 215 genes was regulated under salt stress [7]. For this report, one of those up-regulated genes was cloned, and its function, as it relates to salt-stress responses, was further characterized.

2. Materials and Methods

The salt-tolerant wheat mutant RH8706-49 and salt-susceptible mutant RH8706-34 used in this study are near-isogenic lines with highly similar genetic backgrounds but significantly different salt tolerances. *Arabidopsis thaliana* ecotype "Columbia", *Oryza sativa* group Japonica and the tobacco plants were from our laboratory stock.

2.1. Cloning of Ta-UnP cDNA sequence

Based on a partial SOM clustering analysis chart of the gene chip of wheat salt-tolerance mutant RH8706-49 under salt stress, this research selected the expressed sequence tag (EST) probe with increasing expression (gb:CA599246) after salt stress. A BLAST search in National Center for Biotechnology Information (NCBI) EST libraries yielded several highly homologous wheat EST clones related to salt stress. The full-length cDNA sequence was built by

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http://dx.doi.org/10.1016/j.bbrc.2014.06.055
0006-291X/

assembling these clone sequences. Total RNA was extracted from RH8706-49 plants at the two-leaf stage and reverse-transcribed into cDNA [8]. *Ta-UnP* was cloned by taking cDNA as the template, and the sequence was deposited in GenBank (accession No. ADR63289).

2.2. Expression pattern of Ta-UnP

Wheat plants at the two-leaf stage were treated with 0.8% NaCl, 50 μmol/L ABA, and 15% PEG for 0, 1, 6, 12, and 72 h. Total RNA extracts from each sample were reverse-transcribed. The cDNA was used in the qPCR analysis [9]. The primers used for the quantitative reverse transcription-PCR are shown in Table 1.

2.3. Subcellular localization of Ta-UnP in transgenic tobacco plants

The cloned *Ta-UnP* sequence was subcloned into the expression vector pCAMBIA2300-35S-GFP-OCS (abbrev. p2300-GFP). An empty pCAMBIA2300 vector was used as the control sample. The leaves of six-week-old tobacco plants growing at normal conditions were infiltrated with *Agrobacterium* cultures that harbor one of the two transformation vectors. After 45–48 h, the infected leaves were placed on slides and visualized using a laser confocal microscope at an excitation wavelength of 488 nm. The primers used are shown in Table 1.

2.4. Salt tolerance of Ta-UnP overexpressing Arabidopsis plants

The cloned *Ta-UnP* cDNA was subcloned into the expression vector pCAMBIA1300. The freeze–thaw method was used to transfer the vector into *Agrobacterium tumefaciens* strain GV3101. Positive clones were used to transform *Arabidopsis* [10]. The wild-type plant was used as the control. Positive transgenic *Arabidopsis* plants were selected on an MS plate containing hygromycin (25 mg/L) [11], and their transgenic nature was confirmed via RT-PCR. The primers used are shown in Table 1.

Transgenic homozygous and control seeds were surface-sterilized and sown on 0 and 180 mM NaCl MS plates. These seeds were vernalized for 3 d at 4 °C and then cultured at normal conditions for 3–5 d to determine the germination rate.

Transgenic homozygous and control seeds were grown vertically on an MS plate in a 22 °C incubator for 3 d, after which they were moved to an MS plate containing 150 mM NaCl and then placed vertically to allow the roots of the seedlings to elongate along the surface of the agar. The root length was recorded after 3 d of culture.

After 10 d, the seedlings of the transgenic and control seeds were transferred to pots filled with vermiculite and were grown under normal light at 22 °C. After 2 weeks, the plants were watered with plain water and water containing 180 mM NaCl. Phenotypic changes were recorded for both treated and control plants.

Table 2
Primers used in QRT-PCR.

Genes	Forward primers(5′ → 3′)	Reverse primers(5′ → 3′)
Actin	TCGCTGACCGTATGAGCAAAG	TGTGAACGATTCCTGGACCTG
RD29B	GTGAAGATGACTATCTCGGTGGTC	TACCAAGAGACTCAGCAATCTCTG
KIN2	GTCAGAGACCAACAAGAATGCC	TGACTCGAATCGCTACTTGTTC
P5CS1	TTCTCAGATGGTTTCCAGGTTG	TGGGAATGTCCTGATGGGTG
COR15a	ACTCAGTTCGTCGTCGTTTCTC	TCTCACCATCTGCTAATGCCTC
ADH	CTCTTGGTGCTGTTGGTTTAGG	AATTGGCTTGTCATGGTCTTTC
FRY1	CGCAGTAGCACTAGGATTG	TTGACACCGAGTTTATTGG
SAD1	GCGAACAATCCTTCACAG	CTTCGGGAGACCCACCT
SOS2	ATTGAGGCTGTAGCGAAC	GGTATTCCTTCTGTTGCC
SOS3	GGAGGAATCTCTTCGCTG	CACGAAAGCCTTATCCACC

2.5. Salt tolerance of Ta-UnP-overexpressing and RNAi knockdown transgenic rice plants

The amplified *Ta-UnP* was cloned into the pTCK303 overexpression vector for transformation into rice lines. An RNA interference (RNAi) knockdown construct was prepared using the *Ta-UnP* homologous sequence *Os03g0407100* in rice and then cloned into the expression vector pTCK303 as a hairpin construct. Rice plants were transformed with these two constructs, and the control plants were transformed with the empty vector pTCK303. Seeds harvested from the transformed and control plants were soaked in a solution containing 100 mmol/L of hygromycin. After 7 d, total RNA was isolated from germinated and rooted plants and assayed via RT-PCR to validate gene overexpression. Positive transgenic plants were grown in either water or water supplemented with 170 mM NaCl at 28 °C in an illuminated incubator in a 16/8 h (light/dark) photoperiod. Phenotypic characteristics were recorded after 10 d of treatment. The primers used in RT-PCR are shown in Table 1.

2.6. Content of proline, soluble sugar, MDA, and chlorophyll in transgenic plants

Seeds from transgenic lines and control plants were plated on MS plates and cultured in an illuminated incubator at 22 °C. After 10 d, the seedlings were transferred to pots filled with vermiculite and grown at 22 °C with normal light conditions. After another 14 d, the plants were subjected to salt treatment with 180 mM NaCl solution. The treatments were terminated after 10 d, at which time the above-ground tissues were harvested to analyze the levels of proline [12], soluble sugars [13], malondialdehyde (MDA) [14], and chlorophyll [15].

2.7. Content of sodium and chloride in Ta-UnP-overexpressing and RNAi knockdown transgenic rice plants

Seeds harvested from transformed plants and control plants were soaked in a solution containing 1 lg/mL hygromycin. After 7 d, the positive plants were grown in water for another 7 d at 28 °C under a 16 h photoperiod. The plants were then grown in either water or water containing 170 mM NaCl for an additional 5 days. The whole plants were harvested to analyze the levels of sodium [16] and chloride [17].

Table 1
Primers for reverse-transcription PCR.

	Forward primers(5′ → 3′)	Reverse primers(5′ → 3′)
Arabidopsis overexpression	TCTAGAGCGAGCATGGAGGTGGA	GGATCCATCCTTCGCCTCCGGCT
β-Actin	TGCTATCCTTCGTTTGGACCTT	AGCGGTTGTTGTGAGGGAGT
Ta-UnP	GAAGAAGGCGTCCGAGGA	CAGGCGTCGTCAGCGTC
Subcellular localization	GGTACCATGGAGGTGGAGGCCG	TCTAGAGGCGTCGTCAGCGTCC
Rice overexpression	GGTACCGCGAGCATGGAGGTGGA	ACTAGTATCCTTCGCCTCCGGCT
Rice RNAi	GAGCTCGGATCCCCGCCGTGCCTCCG	ACTAGTGGTACCCGAAGCAGCTTCCCGTG

2.8. Expression of stress-related genes in transgenic Arabidopsis

Nine genes that are closely associated with salt tolerance in *Arabidopsis*—*SOS2*, *SOS3*, *FRY1*, *SAD1*, *COR15a*, *RD29B*, *KIN2*, *ADH*, and *P5CS*—were selected for expression analysis.

Total RNA was extracted from transgenic and control plants grown in an MS plate for 10 d. The cDNA transcribed from the RNA was used in the qPCR analysis of stress-inducible genes in transgenic *Arabidopsis* plants. The primers for these qPCR reactions are shown in Table 2.

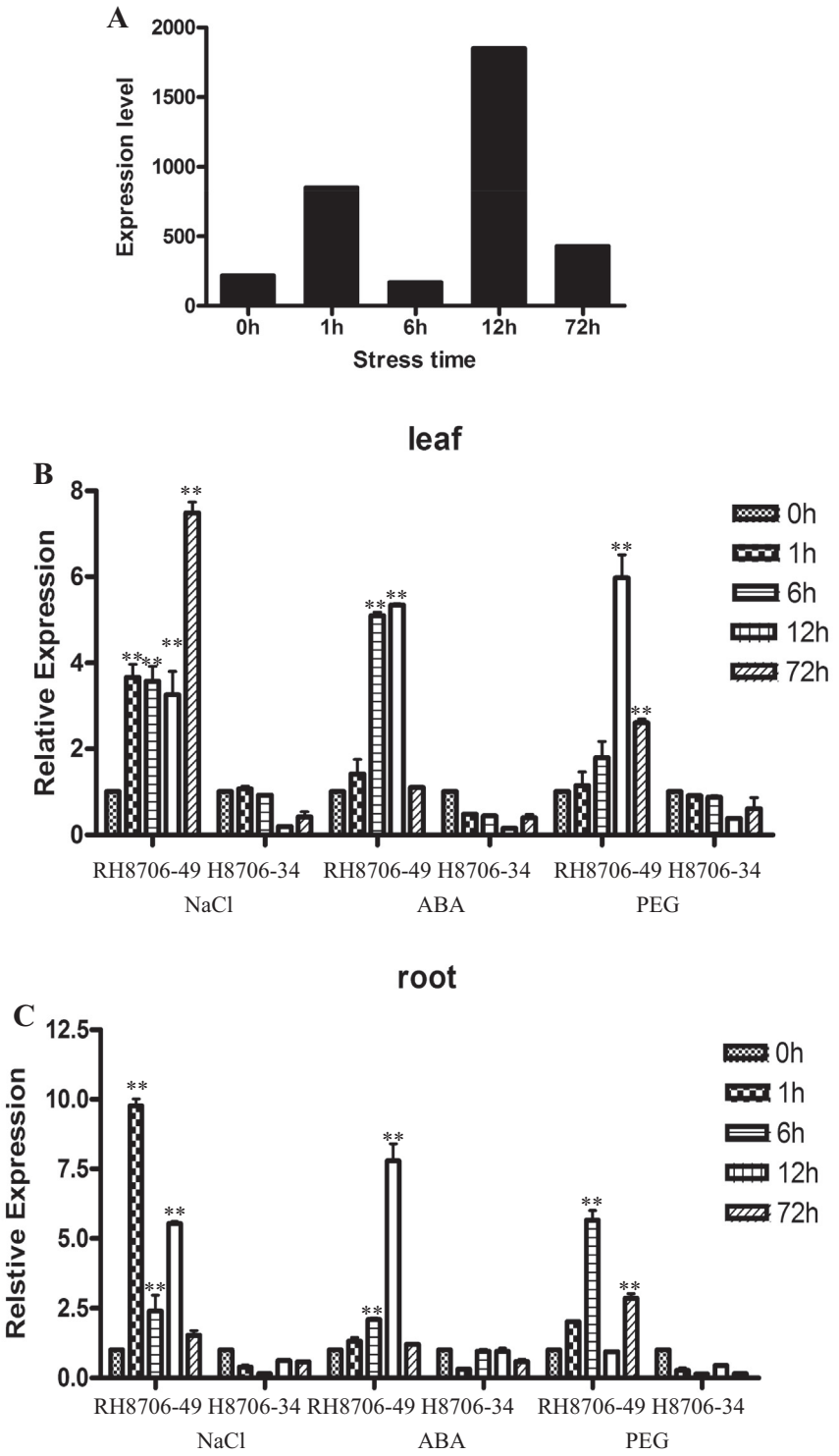

Fig. 1. Expression of newly identified *Triticum aestivum* unknown protein (*Ta-UnP*) gene in salt-tolerant and salt-susceptible wheat line. (A) Microarray analysis of the salt-tolerant wheat cultivar RH8706-49 subjected to salt treatment identified the salt-inducible probe CA599246. (B) Expression of *Ta-UnP* in leaves of salt-tolerant RH8706-49 and salt-susceptible wheat cultivar RH8706-34 at 0, 1, 6, 12, and 72 h following treatment with 0.8% NaCl, 50 μmol/L abscisic acid (ABA), and 15% polyethylene glycol (PEG). (C) Expression of *Ta-UnP* in roots of salt-tolerant RH8706-49 and salt-susceptible RH8706-34 following treatment with NaCl, PEG, or ABA at 0, 1, 6, 12, and 72 h. $^{*}P < 0.05$ and $^{**}P < 0.01$. Values are mean ± standard deviation (SD) ($n = 3$ independent experiments).

3. Results

3.1. Cloning of Ta-UnP cDNA sequence

Based on the microarray analysis of the salt-tolerant RH8706-49 wheat line following salt treatment (our laboratory, unpublished data; Fig. 1A) and the subsequent self-organizing mapping results identified a salt-inducible probe (gb:CA599246). The cDNA sequence of wheat with a complete coding region was cloned using the probe with increasing expression after salt stress. The sequence contained a 375 bp open reading frame. The protein was 125 amino acids in length and contained no known putative conserved domain. It was a novel gene of unknown function, and Ta-UnP is 70% homologous to rice Os03g0407100 protein. It was named *Ta-UnP* (*Triticum aestivum* unknown protein) (GenBank accession No. ADR63289).

3.2. Analysis of Ta-UnP expression

Based on the qPCR results, *Ta-UnP* was induced in leaves of salt-tolerant wheat cultivar with the highest *Ta-UnP* transcript level (7.49-fold that of the untreated control) detected after 72 h of treatment. ABA and PEG treatment also induced gene expression at most of the time points following treatment, and the highest level (5.34-fold and 5.97-fold that of the untreated control plant) was observed 12 h after treatment. In the leaves of the non-salt-tolerant RH8706-34 plants, salt, PEG, or ABA treatment only slightly changed the *Ta-UnP* transcript levels (Fig. 1B).

The expression of *Ta-UnP* in the roots of RH8706-49 did not follow the same trend as that in the leaves. Salt treatment induced the expression of *Ta-UnP* in roots at most of the time points of treatment, and the highest expression level (9.77-fold that of the control sample) was observed 1 h after salt treatment. Under ABA stress, the expression of *Ta-UnP* increased at most of the time points, and the highest level (7.79-fold that of the control) was reached after 12 h of treatment. For PEG stress, the expression of *Ta-UnP* fluctuated, and its highest level (5.56-fold that of the control) was reached after 6 h of treatment. Similar to that in the leaves, the expression of *Ta-UnP* in the roots of RH8706–34 under salt treatment did not significantly change (Fig. 1C).

3.3. Subcellular localization of Ta-UnP in transgenic tobacco plants

In tobacco leaves infiltrated with *Agrobacterium* harboring a *Ta-UnP*-GFP fusion construct, microscopic results revealed that fluorescence was mainly localized in the cell membrane (Fig. 2A–C).

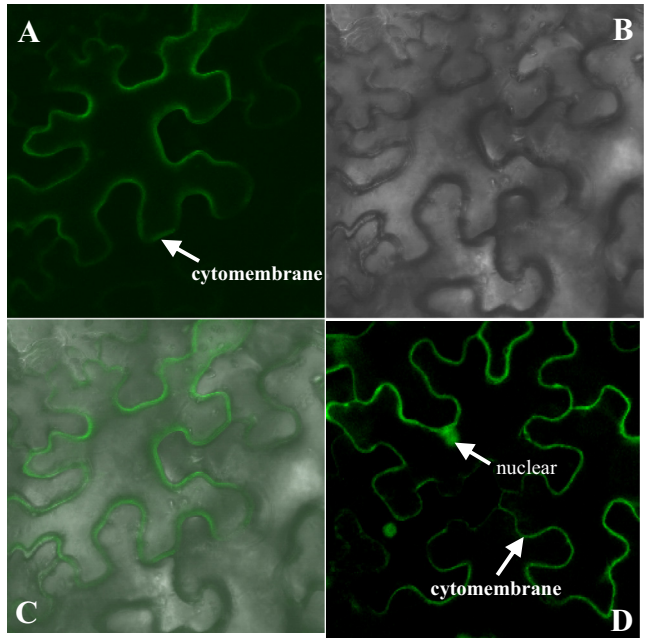

Fig. 2. Subcellular localization of Ta-UnP in tobacco plants via immunofluorescence assay. (A). Fluorescence image of Ta-UnP-GFP (B). Bright-field microscopic image of the Ta-UnP-GFP-expressing cell (C). Merged image of fluorography and bright-field microscopic images of Ta-UnP-GFP (D). Fluorescence image of GFP in control plants. GFP green fluorescent protein. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

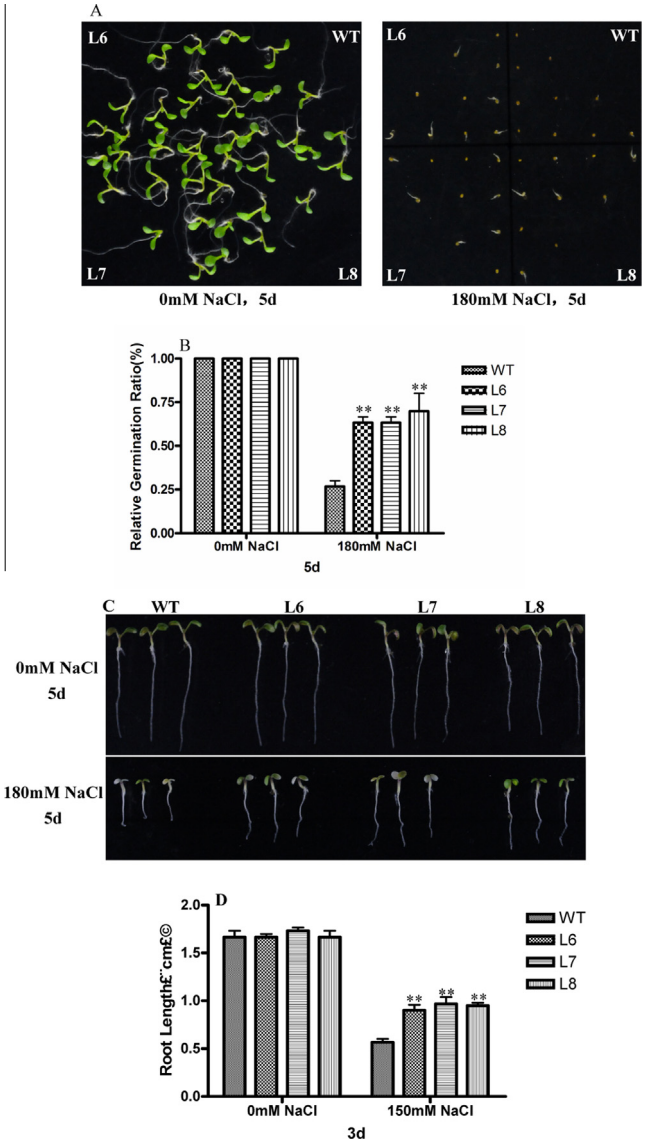

Fig. 3. Salt tolerance of *Ta-UnP* transgenic plants. (A) and (B) Seed germination rates before and after salt treatment in transgenic and control *Arabidopsis* plants. WT, wild type (control plant); L6, L7, L8: three transgenic *Ta-UnP* lines. $^{*}P < 0.05$ and $^{**}P < 0.01$, according to the *t* test. (C) and (D) Root growth before and after salt treatment in transgenic and control *Arabidopsis* plants. WT, wild type (control plant); L6, L7, L8: three transgenic *Ta-UnP* lines. $^{*}P < 0.05$ and $^{**}P < 0.01$, according to the *t* test. (E) Phenotypes of WT and L6, L7, and L8 *Ta-UnP* transgenic *Arabidopsis* plants either in the absence of salt stress (0 mM) or following salt stress caused by watering the plants with 180 mM NaCl for 10 d. Values are mean ± SD ($n = 3$ independent experiments) (F) Phenotype of *Ta-UnP*-overexpressed (L2, L3, L6) and RNAi (I2, I8, I11) plants: at normal growth conditions or following salt stress caused by watering the plants with 170 mM NaCl for 10 d. Control plants (CK) were transformed with the empty vector pTCK303. Values are mean ± SD ($n = 3$ independent experiments).

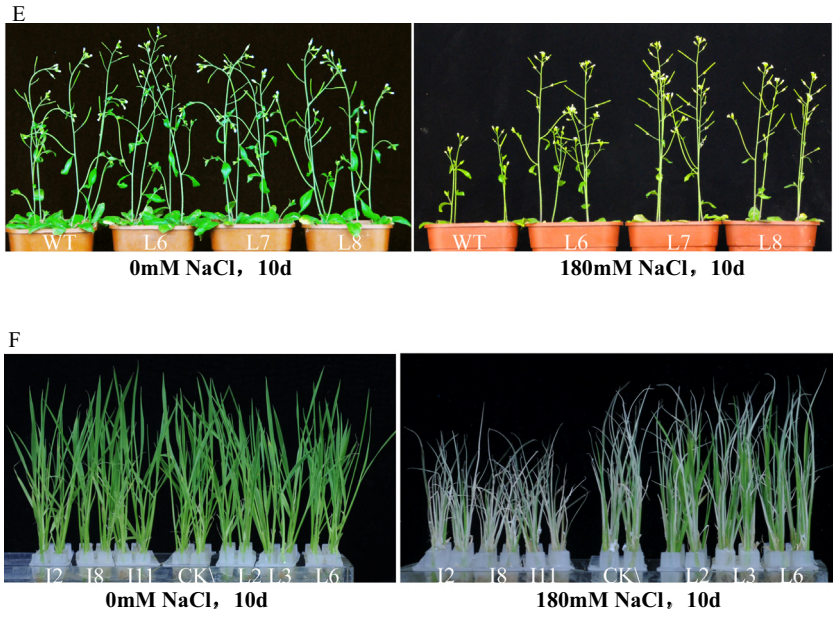

Fig. 3 (*continued*)

In cells inoculated with the empty pCAMBIA2300 vector plasmid, fluorescence was localized in the nucleus and cell membrane (Fig. 2D). These results indicate that Ta-UnP was mainly localized in the cell membrane.

3.4. Salt tolerance of Ta-UnP overexpressing Arabidopsis plants

Detection of seed germination: The transgenic plants and WT control plants presented similar seed germination on the 0 mM NaCl MS plate, whereas the germination of *Ta-UnP* homozygous seeds was much better than that of the WT control plants on the 180 mM NaCl plate (Fig. 3A and B).

Detection of root length: Root growth was similar between the transgenic lines and WT plants on the 0 mM NaCl MS plate. After 3 d of treatment with 150 mM NaCl, the *Ta-UnP* transgenic plants produced roots that were 1.66-fold longer than the WT plants (Fig. 3C and D).

Detection of salt tolerance in transgenic *Arabidopsis* plants: At normal culture conditions, the transgenic and WT *Arabidopsis* plants grew in a similar pattern. However, after treatment with 180 mM NaCl for 10 d, the transgenic plants maintained a relatively strong growth; however, the non-transgenic WT plants wilted, and the bolts were stunted. The overexpression of *Ta-UnP* significantly enhanced the salt tolerance of transgenic *Arabidopsis* (Fig. 3E).

3.5. Salt tolerance of overexpressing and RNAi knockdown transgenic rice plants

At normal culture conditions, both the overexpressing and RNAi knockdown transgenic lines grew well and had green leaves without obvious physiological differences from transgenic plants. After 10 d of treatment with 170 mM NaCl *Ta-UnP* overexpressing rice, L2, L3, and L6 grew well, whereas the control plants wilted. More severe wilting occurred in the RNAi lines (I2, I8, I11) than that in the control plants, resulting in the death of several RNAi plants (Fig. 3F).

3.6. Content of proline, soluble sugar, MDA, and chlorophyll in transgenic plants

The detected physiological indices were present at significantly different levels in the WT and *Ta-UnP* transgenic plants. Following treatment with 180 mM NaCl, the levels of proline and soluble sugars were higher in the *Ta-UnP* transgenic plants than those in the WT plants. In the salt-stressed WT plants, the content of proline, soluble sugars, and chlorophyll increased by 1.46-, 1.03-, and 0.61-fold, respectively, compared with those in the untreated WT plants. In the salt-treated *Ta-UnP* transgenic plants, the levels of these three compounds increased by 1.56-, 1.26-, and 0.91-fold, which were all significantly higher compared with those in the WT plants. The MDA content increased by 2.41- and 2.12-fold in the treated WT and transgenic plants, respectively, but the change was significant only in the WT plants (Fig. 4A–D).

3.7. Content of sodium and chloride in Ta-UnP-overexpressing and RNAi knockdown transgenic rice plants

Under normal culture conditions, no differences in the contents of Na^+ or Cl^- was detected between transformed rice plants and control. Although bothlines showed some increase in Na^+ and Cl^- content after salt treatment, the content of Na^+ and Cl^- in the *Ta-UnP* overexpressing rice plants was significantly lower than that in the CK control plants, the content of Na^+ and Cl^- in the RNAi lines was significantly higher than that in the CK plants. Following NaCl treatment, the Na^+ levels increased by 1.77-fold in the CK plants, by 1.34-fold in the *Ta-UnP* overexpressing rice and by 2.99-fold in RNAi lines compared with the levels before salt treatment (Fig. 4E).

The Cl^- levels increased by 3.61-fold in the CK plants, by 3.39-fold in the *Ta-UnP* overexpressing rice and by 4.60-fold in RNAi lines compared with the levels before salt treatment (Fig. 3F).

3.8. Expression of stress-related genes in Ta-UnP overexpressing Arabidopsis plants

The expression of nine genes that are closely associated with salt tolerance in *Arabidopsis* was selected for expression analysis.

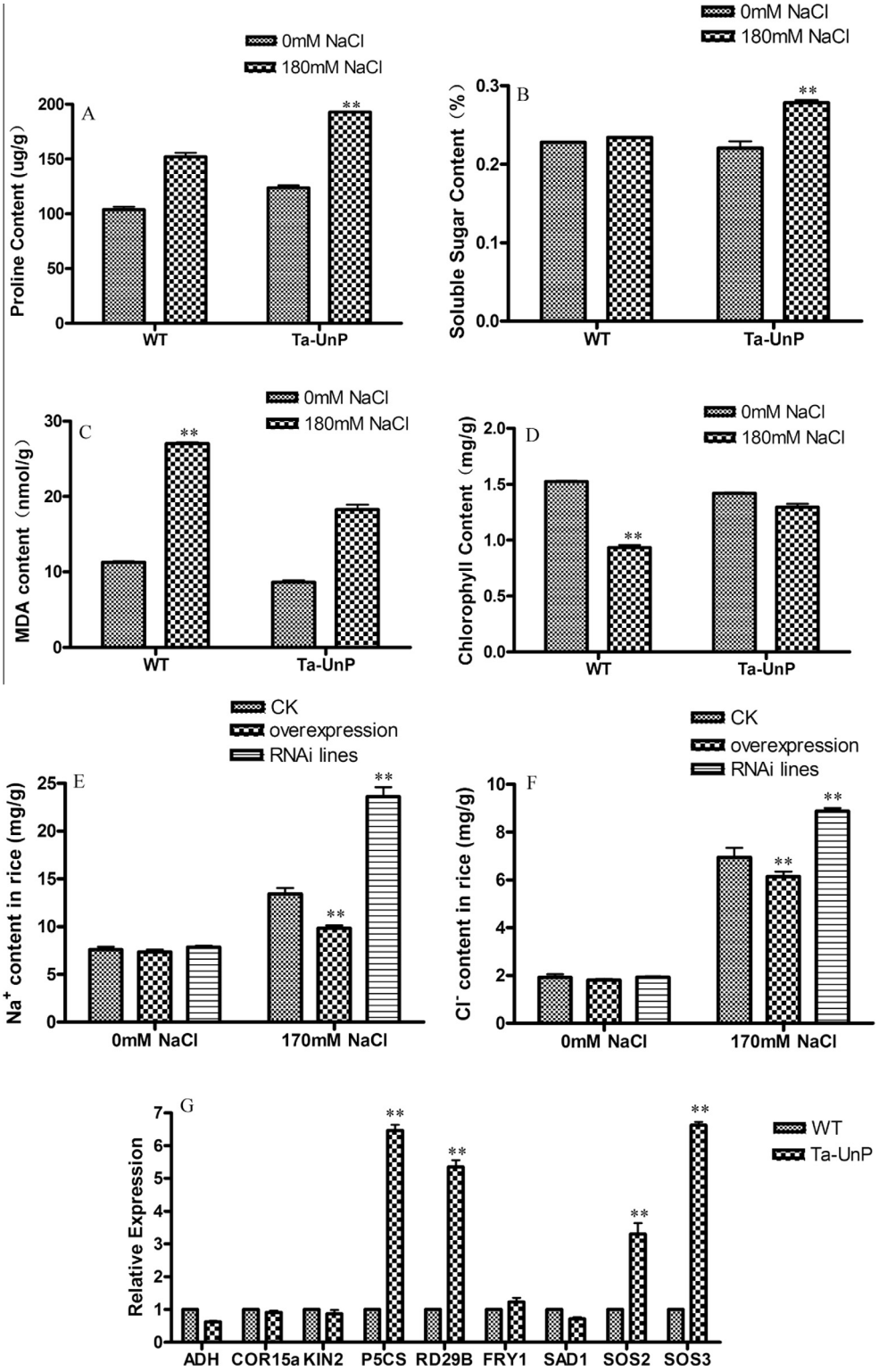

Fig. 4. Contents of ions, proline, soluble sugars, MDA, chlorophyll and relative expression of nine stress-related genes in *Ta-UnP* transgenic *Arabidopsis* plants. Physiological analyses in *Ta-UnP* transgenic and WT *Arabidopsis* plants before and after salt treatment. (A) proline content, (B) soluble sugar content, (C) malondialdehyde content, (D) chlorophyll content. Content of sodium and chloride in *Ta-UnP*-overexpressing and RNAi knockdown transgenic rice plants. (E) Na^+ content, (F) Cl^- content. (G) Relative expression of nine stress-related genes—*SOS2*, *SOS3*, *FRY1*, *SAD1*, *COR15a*, *RD29B*, *KIN2*, *ADH*, and *P5CS*—in *Ta-UnP* transgenic *Arabidopsis* plants at salt stress conditions. Comparable WT plants were used as control plants, and the transcript level in WT plants was arbitrarily assigned a value of 1. $^{**}P < 0.01$. Values are mean ± SD ($n = 3$ independent experiments).

The qPCR results indicate that following salt stress, *SOS2*, *SOS3*, *RD29B*, and *P5CS* transcripts were more abundant in transgenic *Arabidopsis* than in WT plants, whereas the *FRY1*, *SAD1*, *COR15a*, *KIN2*, and *ADH* transcripts did not significantly change (Fig. 4G).

4. Discussion

EST, that was significantly induced after a 12 h salt stress was selected from the gene expression profile chip of RH8706-49. The

EST was used as a probe, and abundant homologous ESTs were acquired from the Blast search in NCBI. A wheat full-length cDNA sequence (*Ta-UnP*) with a complete coding region was cloned via gene splicing.

The expression of *Ta-UnP* was induced by salt in salt-tolerant mutant RH8706-49, which suggests that *Ta-UnP* may participate in pathways responding to salt stress and thus confer salt tolerance to the isogenic line. Under adverse stresses (e.g., high salt content, drought, and low temperature), plants accumulate much ABA, which can increase the free Ca^{2+} content in cells, deactivate inwardly rectifying K^+ channels, and close the stomata [18]. The expression of *Ta-UnP* in RH8706-49 was highly induced after 50 μM ABA treatment, which suggests that *Ta-UnP* may participate in the salt stress response mechanism through the ABA signal transduction pathway. The expression of *Ta-UnP* was also significantly induced after PEG stress, thereby indicating that *Ta-UnP* may close the stomata through the ABA signal transduction pathway to reduce water loss and thus improve the drought tolerance of transgenic plants.

The following analysis of salt tolerance in *Ta-UnP* overexpressing *Arabidopsis* plants demonstrated that transgenic *Arabidopsis* exhibited better performance in terms of germination rate, root length, plant height, leaf color, and fructification than the control plants. This finding confirms the effectiveness of *Ta-UnP* in enhancing the salt tolerance of transgenic plants.

Rice was selected to further explore the salt tolerance mechanism of *Ta-UnP* because of the closer genetic relationship of rice with wheat than with *Arabidopsis*. In this research, *Ta-UnP* functions are further discussed based on the interference homologous gene (Os03g0407100) of *Ta-UnP* and overexpressed *Ta-UnP* in rice plants. In this experiment, *Ta-UnP* overexpression can enhance the salt tolerance of transgenic rice lines. RNAi knockdown transgenic lines become susceptible to salt stress because RNAi technology successfully inhibits the expression of *Ta-UnP* homologous genes in rice lines, which confirms that *Ta-UnP* can enhance the salt tolerance of transgenic plants.

To explore the salt tolerance mechanism of *Ta-UnP*, we measured the physiological indices of transgenic plants. When the external osmotic pressure is changed, an osmoprotectant can protect the cell structure and keep the osmotic equilibrium (maintaining continuous water inflow or reducing outflow) [19]. These coexisting organic osmotic substances mainly include proline and soluble sugar [20]. After salt stress, transgenic plants accumulated abundant proline and soluble sugar, thereby indicating that *Ta-UnP* can facilitate the accumulation of osmotic substances, such as proline and soluble sugar, in plants under salt stress to decrease intracellular osmotic pressure and protect plants from damage caused by salt stress. Membrane lipid peroxidation caused by free radicals is a reflection of stress-induced cell damage [21]. Therefore, MDA content generated during membrane lipid peroxidation is often used as a measurement index of oxidative damage [22]. After salt treatment, the MDA content in *Ta-UnP*-overexpressed *Arabidopsis* was significantly lower than that in the control plants, which indicates that the transgenic plants experienced less severe membrane damage from lipid peroxidation than the WT plants. As such, the salt tolerance of plants is improved. Crop yields decline because excessive salt stress reduces photosynthetic ability. Although many factors can reduce photosynthesis under salt stress, such an inhibition mechanism is still unknown [23]. Salt stress decreased chlorophyll content in both transgenic and control plants. However, the chlorophyll content of the transgenic plants was still higher than that of the control. The detection result confirmed that *Ta-UnP* can reduce the damage caused by salt stress to the plant photosynthetic system, thereby improving its salt tolerance.

Maintaining a relatively low level of Na^+ in cells is very important to the salt tolerance of plants [24]. Cl^- inhibits growth and development of plants mainly by destroying cell ultrastructure [25]. Salt tolerance of plants can be improved by decreasing Cl^- accumulation in photosynthetic cells [26]. Detection of Na^+ and Cl^- contents in *Ta-UnP* overexpressing rice plants and RNAi lines shows that Na^+ and Cl^- contents significantly higher in RNAi lines than that in CK control, but keep low in *Ta-UnP*-overexpressing rice plants, under salt stress. This detection result further confirmed that *Ta-UnP* can reduce damages to plants caused by salt stress and enhance salt tolerance of transgenic plants.

The qPCR results showed that *SOS2*, SOS3, *RD29B*, and *P5CS* were induced to a greater extent in the transgenic plants than that in the control plants, which indicates that *Ta-UnP* may affect their expression in the upstream region. The expression of *RD29B* is mainly controlled by ABA and as a response to drought stress. Accordingly, the expression levels of *Ta-UnP* in the roots and leaves of RH8706-49 increased after ABA and PEG treatment. *P5CS* is the key to ABA biosynthesis in plants [26]. The activity of P5CS represents a rate-limiting step in proline biosynthesis, which is controlled at the level of *P5CS* transcription and through the feedback inhibition of P5CS by proline [27]. This result is in accordance with the significant increase in proline content in transgenic plants after salt treatment. Under salt stress, cellular ion balance is regulated through the salt overly sensitive (SOS) signal transduction pathway, which includes enzymes SOS2 and SOS3 (which directly control the K^+/Na^+ ion balance). The protein kinase complex including SOS3 and SOS2 can be activated by Ca^{2+} produced under salt stress. The protein kinase complex then phosphorylates and activates the Na^+/H^+ antiporter protein SOS1 on the plasma membrane. Based on these results, we postulate that *Ta-UnP* may enhance salt tolerance via the SOS and ABA signal transduction pathways.

Acknowledgments

This work was supported by the National Natural Science Fund (30971766) and Hebei Provincial Natural Science Fund (No. C2009000278).

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