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Cloning and expression analysis of *Arabidopsis TRR14* gene under salt and drought stress

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Abstract

TRR14 is a novel protein important in trehalose (α-D-glucosyl-[1,1]-α-D-glucopyranoside) signaling in *Arabidopsis*. In this research, we provided evidences to demonstrate that *TRR14* plays role in *Arabidopsis* responses to salt and drought stress. Transgenic *Arabidopsis* plants over-expressing *TRR14* under the control of CaMV 35S promoter were generated. The transformed lines showed higher transcript levels of *TRR14* than that of the Wild Type (WT) *Arabidopsis* plants. The RT-PCR results showed that *TRR14* transcript level increased markedly by salt and drought stress both in WT and transformed lines. Further experiments indicated that the *TRR14* transformed lines had unchanged seed germination, root length and chlorophyll content under stress conditions. In addition, the activity of oxidative enzymes like peroxidase and catalase were significantly induced in transformed lines under salt and drought treatments. Thus, the present data indicate that a novel protein, TRR14, is involved in plant salt and drought tolerance.

Keywords**:** *TRR14*, over-expression, *Arabidopsis,* salt, drought

Introduction

Abiotic stresses, especially salinity and drought, influence plant growth and limit agricultural crop production throughout the word. Plants have biochemical and physiological mechanisms to adapt and survive against these kinds of stresses (Zhu, 2001). One of these adaptive mechanisms is the synthesis or accumulation of osmolytes such as proline (Kishore et al., 2004), fructan (Pilson-Smits et al., 1995), glycinebetaine (Holmstrom et al., 2000), sorbitol (Gao et al., 2001), and or trehalose (Garg et al., 2002).

The TRR14 protein is an unknown protein that is encoded by At4g10300. It was first identified as a component of *Arabidopsis* responses to trehalose treatment (Aghdasi et al., 2012). Phylogenetic analyses revealed that TRR14 is a member of a small and divergent gene-family in *Arabidopsis* with a single Cupin domain. In plants, proteins containing a single Cupin domain and of similar size as TRR14, about 150 amino acids (aa), include diverse functions including: phosphomanose isomerase, polyketide synthase, dioxygenase, oxalate oxidase (germins), auxin binding protein and the somewhat larger 185 aa epimerase (Dunwell, 1998; Dunwell et al., 2001). TRR14 has six homologues in *Arbidopsis*. Its closest homologues are found in plants and cyanobacteria. TRR14 is located in chloroplastd and its expression is ubiquitous (Aghdasi et al., 2012).

Our previous work showed that TRR14 may involve in plant salt and drought tolerance. We isolated two *trr14* T-DNA insertion mutants from SALK collection. Characterization of *trr14* mutants revealed that *trr14* mutants have reduced seed germination, root length, survival rate and chlorophyll content under stress conditions. Furthermore the activity of oxidative enzyme, such as peroxidase, catalase and polyphenol oxidase, was decreased under salt and drought treatments.

In current research, we indicated that over-expression of *AtTRR14* in *Arabidopsis* leads to improvement of plant salt and drought tolerance. These results confirm the previous results that TRR14 involves in stress tolerance.

Materials and Methods

Plant materials and growth conditions

The *Arabidopsis thaliana* wild type (WT) plants accession Columbia-0 (COL-0) was used in this study. Seeds were surface sterilized for 5 min with 70% (v/v) ethanol followed by 10 min in 20% commercial bleach (4% (w/v) chlorine) and washed 5 times in sterile milli-Q water. Sterilized seeds were planted on half strength MS medium (Murashige and Skoog, 1962) supplemented with either 100 mM trehalose or sorbitol and solidified

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with 0.8% (w/v) agar. Seeds were stratified in darkness at 4oC for 2 days before the plates were transferred to a growth chamber at 25°C under a 16-h-light/8-h-dark photoperiod.

DNA extraction and PCR analysis

Three small leaves of seedlings were frozen in liquid nitrogen, pulverized with glass beads for 2 minutes at 2800 rpm in a dismembranator (Braun, Melsungen, Germany), and then genomic DNA was extracted using the Pure Gene DNA isolation kit (Amersham Pharmaciabiotec, England) following the manufacturer's instructions. PCR was performed with specific primers: 5'-acccaactcggtgttcgtag-3' (forward) and 5'-tgatagcagccattcactcg-3' (Reverse). After denaturation for 2 minutes at 94 ºC, DNA amplification was performed with 35 cycles (30 sec 94ºC, 30 sec 56ºC and 2 min 72ºC) followed by a final step at 72ºC for 5 minutes.

Plasmid construction of the gene, its plant transformation

For over-expression cDNA was first amplified by PCR, and ligated into pGEM-T easy vector (Promega). Briefly the CaMV35S expression cassette was isolated by digestion with *Eco*RV from pUC-18 vector: Amp resistant. The cassette was filled with Klenow and dNTPs and subsequently ligated into the pBin19 (*Hin*dIII/*Eco*RI) vector to yield pBin-35S. Purified fragments were cloned into the pBin-35S expression cassette using restriction enzymes, resulting in pBin35S/cDNA/NOS. The construct was introduced by electroporation into *Agrobacterium tumefaciens*, containing pGV2260 plasmid. The floral dip method (Clough and Bent, 1998) was used for transformation of the plant material, Col-0 WT bolting plants with *Agrobacterium tumefaciens.* Re-transformed seedlings were selected on ½ MS media containing 50 mg/L Kanamycin.

Sequence analysis

Sequences obtained from analysis with forward and reverse primers (T7: tatttaggtgacactatag and SP6: taatacgactcactataggg) were aligned and the PCR fragment structure was reconstructed by BLAST (Basic Local Alignement Search Tool) searches in TAIR (http://www.arabidopsis.org/Blast/).

RNA Isolation, RT-PCR and Real-Time PCR

Total RNA from Re-transformed lines and WT were isolated with RNeasy plant mini kit (QIAGEN USA, Valencia, CA). RNA concentration and purity were determined by measuring absorbtion at 260 nm. 10 ng RNA was treated with 2 U DNAse I (DNA- free, Ambion, Austin, USA) to remove genomic DNA. Absence of DNA was analyzed by performing a PCR reaction (40 cycles, similar to the real–time PCR program) on the DNaseI- treated RNA using Taq-DNA polymerase. RT-PCR experiments were performed using 1 ng of total RNA extracted and used for first-strand cDNA synthesis with 60 units M-MLV Reverse Transcriptase (promega, Madison, WI), 0.5 µg of odT16v (custom oligo from invitrogen, Carlsbad, CA) and 0.5 µg random hexamer (invitrogen). The gene specific primers used were: 5'-aacttgatcgggatatggagtg-3' (forward) and 5'-aacttgatcgggatatggagtg-3' (Reverse) for *TRR14* and 5'- gacccaaagacggagactctt-3' and 5'-gccaagtgattgtggagactc-3' for *AtACTIN2* as reference gene.

Stress treatments

The *Arabidopsis thaliana* accession Columbia-0 (COL-0) and re-transformed lines, seeds were sterilized 5 minutes with 70% ethanol followed by 10 minutes in 20 % commercial bleach (4% w/v chlorine) and washed 5 times in sterile Milli-Q water. The seeds were grown on MS medium solidified with 0.8% agar. Seeds were stratified in darkness at 4ºC for 2 days before the plates were transferred to a growth chamber at 22ºC under a 16-h-light /8-h-dark photoperiod. For determining the salt effect on germination, MS medium was supplemented with 0, 75, 100 or 150 mM NaCl. To determine the effect of dehydration on germination, medium was supplemented with 200 or 300 mM manitol. Germination assays were carried out with three replicates of 50 seeds. Germination rates was determined by measuring the time of radical emergence and germinating seeds were selected in the same period to measure root length.

For survival test, WT and re-transformed lines, seeds were fully germinated on MS medium and 3 days old seedlings were transferred to solid MS medium, supplemented with different levels of NaCl or manitol concentrations, and seedlings, growth was monitored for 14 days.

To measure root length under stress condition, the seedlings were grown on vertically positioned plates for 14-days. Then photographs were taken and root length measured with the Image J program (Wayne Rasband, NIH Maryland, USA).

Chlorophyll measurements

Chlorophyll levels were measured spectrophotometrically as described by Jeffery and Humphrey, (1975). In brief, 14 day old seedlings

were ground in liquid nitrogen and extracted with 80% (V/ V) acetone. Absorbance was determined at 647, 652 and 664 nm and the data were used to calculate chlorophyll content.

Oxidative enzymes activity assay

For preparation of crude enzyme extract, 0.05 g of fresh leaves were ground in 2 mM of 0.1 M cool phosphate buffer (pH 6.8) on ice bath as described by Kar and Mishra (1976). Homogenates were centrifuged at 15000 rpm for 15 min at 4ºC. The supernatants were used for catalase, peroxidase and polyphenoloxidase activity assays. The protein concentration of the supernatant was measured according to Lowery et al. (1951).

Catalase activity was determined by the consumption of H_2O_2 (Chensy and Mehler 1955). The reaction mixture (3 ml total volume) contained 50 mM phosphate buffer (pH 6.8), 100 µL enzyme extract and 15 mM H_2O_2. Absorbance decline in 240 nm was scanned automatically with spectrophotometer (Shimadzu UV-160) in kinetic mode. Peroxide reaction mixture (3 ml) contained 20 mM guaiacol, 25 mM phosphate buffer (pH: 6.8), 40 mM and 10 µL enzymes extract. Guaiacol peroxidase activity was scanned in 470 nm. Polyphenol oxidase assay mixture (3 mL) contained 10 mM pirogalol, 25 mM phosphate buffer (pH 6.8) and 200 µL enzymes extract. Enzyme activity was determined at 420 nm.

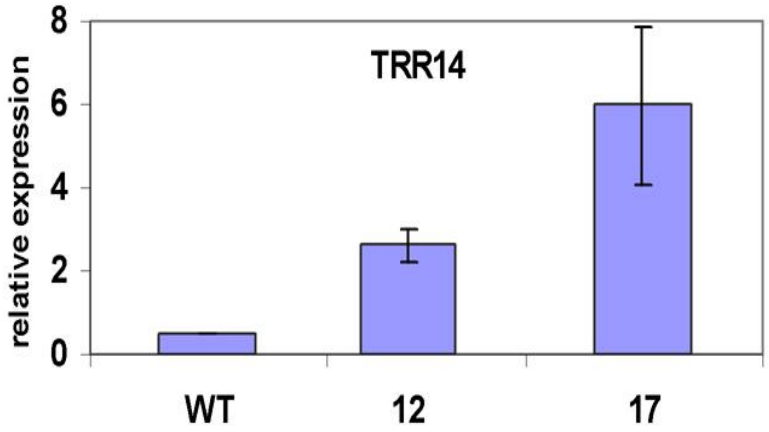


Figure 1. Q-PCR analysis of *TRR14* gene expression in WT and transformed lines (12 and 17).

Statistical analysis

The data presented in the figures and tables are the mean of three independent experiments with calculated standard deviations. A Duncan test has been done to identify statistical differences between pairs of means at a confidence level of 95% for each set of data.

Results

Transformation of cDNA constructs into WT

To analyze the function of *TRR14* in response to salt and drought stress, cDNA was constructed into binary vector pBin19 under the control of CaMV 35S promoter and were then transformed into the WT seedlings. Transformations with the full length cDNAs of *TRR14* yielded 20 independent lines per construct with resistance to the selection marker. The transformed lines did not show different phenotype compared to WT, on soil and under long-day conditions. The transformed lines remained unaltered with respect to flowering time and were fully fertile. Transformation yielded plants with higher expression levels of the *TRR14* gene. The mRNA level of *TRR14* was more than 2 times higher in the transformed lines compared to the WT (figure 1).

TRR14 expression under salt and drought stress

To investigate the effect of salt and drought stresses on the transcript level of *TRR14* gene, WT and transformed lines were subjected to the salt and dehydration stress. *TRR14* expression level was very low in unstressed control plants. After treatment with 100 mM NaCl, transcript level increased in control plants (figure 2A). Drought stress also sharply induced the expression of the *TRR14* gene in WT plants. The expression level of *TRR14* gene in manitol treatment was much higher than that in NaCl treatment (figure 2B). Similarly, *TRR14* expression was also induced by NaCl and manitol treatment in the transformed plants.

Salt response of Re-transformed lines

Physiological characteristics including seed

germination rate, root length, survival rate, chlorophyll content and oxidative enzyme activity were investigated in the transformed lines (lines 12 and 17) and WT plants under salt treatment.

To investigate the function of TRR14 under salt stress, the transformed lines and WT seeds were grown on MS medium supplemented with various levels of NaCl for 14 days. There was no difference in seed germination between the WT and transformed lines on MS medium. The germination rate of WT seeds was largely decreased under salt stress (figure 3, 4a). When the seeds were imbibed in 100 mM NaCl, about 23% of WT seeds were germinated, but the germination rate of the transformed lines was 75% (T12) and 80% (T17). While seed germination of the WT was retarded by 150 mM NaCl treatment, 10% and 8% of the transformed seeds were germinated (figure 4a).

To determine the effect of *TRR14* over-expression on plant growth under salt stress, the root length was measured in 14-day-old seedlings growing on MS medium containing various levels of NaCl. The root length of WT seedlings was significantly reduced in response to salt stress. There was no significant difference between root lengths of transformed lines growing on MS medium containing various NaCl concentrtion (figure 4b).

We also examined whether TRR14 affected *Arabidopsis* plant survival under salt stress. Seeds of WT and transformed lines were allowed to germinate on MS medium for 3 days, and then transferred to medium containing 75, 100 and 150 mM NaCl. The whole WT seedlings died after 100 mM NaCl treatment. In contrast about 25% of seedlings from the transformed lines survived at the same concentration of NaCl. All transformed lines seedlings died after 150 mM NaCl treatment (figure 4c).

There was no difference in chlorophyll a, chlorophyll b and the total chlorophyll contents of the transformed lines and WT seedlings on MS medium. When NaCl concentration increased to 100 mM, these three parameters decreased in WT seedlings. In contrast transformed seedlings were still green and the Chl a level significantly increased in response to 100 mM NaCl treatment (figure 3 and table 1). Chl a/b ratio was decreased in WT seedlings in response to 75 mM NaCl treatment. Whereas by adding 100 mM NaCl to the MS medium, this ratio was increased in WT seedlings (table 1).

Catalase, peroxidase and polyphenol oxidase activities were assayed as an indicator of oxidative stress. There was no significant difference in catalase, peroxidase and polyphenol oxidase activities between the WT and transformed lines on MS medium (table 1). When WT seedlings were grown in the presence of NaCl, catalase, peroxidaxe and polyphenol oxidase activities were significantly reduced compared with plants grown under normal condition. For transformed lines, the oxidative enzymes activity was induced by increasing NaCl concentration. The highest enzyme activity observed at 100 mM NaCl treatment (table 1).

Dehydration response of the Re-transformed lines

We further investigated the effect of TRR14 on seed germination under dehydration stress. The transformed lines (12 and 17) and WT seeds were grown on MS medium containing 200 or 300 mM mannitol for 14 days. Upon 200 mM manitol treatment, the seed germination of the transformed lines was bout 65%, whereas only 48% of the WT seeds were germinated by the 14th day. Seed germination was more decreased at 300 mM mannitol (figure 5a).

To test the possible role of *TRR14* overexpression on *Arabidopsis* growth under dehydration stress, root length was measured in 14-day-old seedlings growing on 200 or 300 mM mannitol. The root length of the WT seedlings significantly reduced in response of manitol stress. While no difference in root length was observed in the transformed lines when grown on different concentration of manitol (figure 5b)

In the next experiment, when 3-day-old WT and transformed seedlings were treated to 300 mM mannitol, most (87%) of the WT seedlings died. While about 49% of the transformed lines seedlings subjected to the same dehydration treatment survived (figure 5c)

Upon manitol stress, chlorophyll a and total chlorophyll contents were decreased and the Chl a/b ratio and Chl b content was increased in WT seedlings. While Chl a and total chlorophyll was increased in the transformed seedling, growing on 200 or 300 mM mannitol. Surprisingly the Chl a level was unchanged, but Chl a/b ratio was decreased in the transformed seedlings under manitol treatment (table 2).

While catalse and peroxidase activities were significantly decreased in WT seedlings, growing in 200 or 300 mM mannitol, they were significantly increased in the transformed lines. Significant difference was not observed in polyphenol oxidase activity in WT seedlings under different concentrations of mannitol. Polyphenol oxidase activity was significantly increased sharply in the transformed seedlings after 300 mM manitol treatment (table 2).

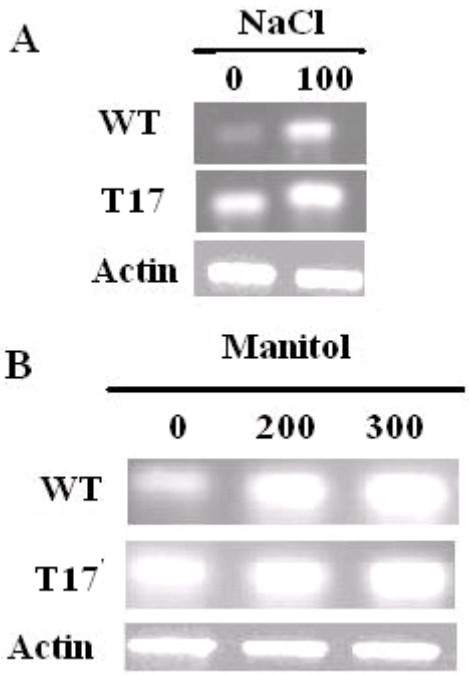


Figure 2. Expression analysis of *TRR14* in (**A**) salt stress condition (100 mM) and (**B**) drought stress condition (200 and 300 mM) from 14-days WT and Re-transformed line17 (T17) by RT-PCR. *AtACTIN2* expression levels are shown as control.

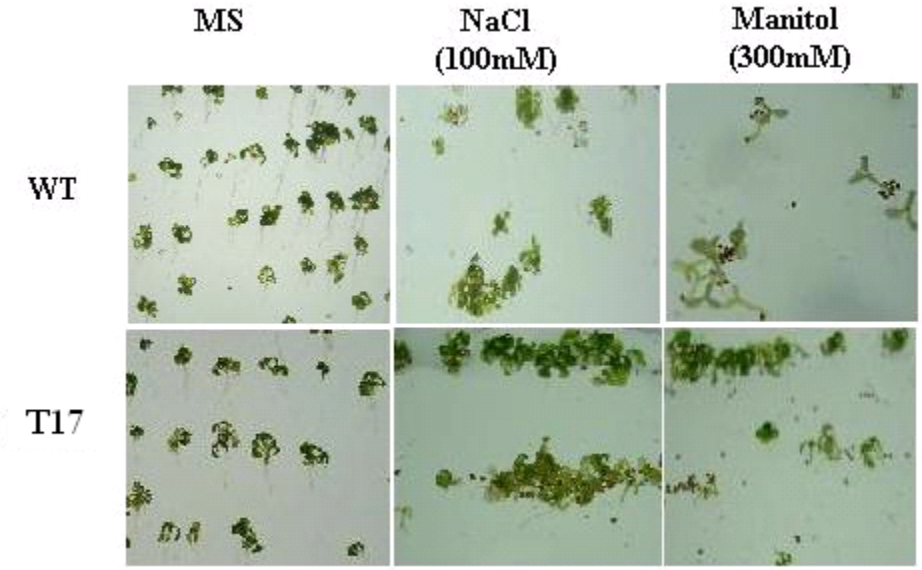
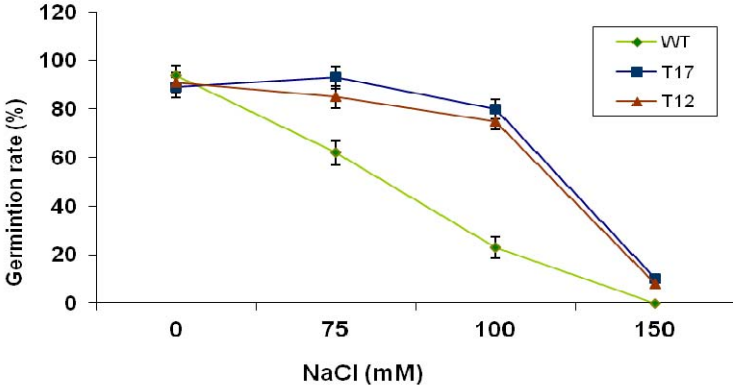
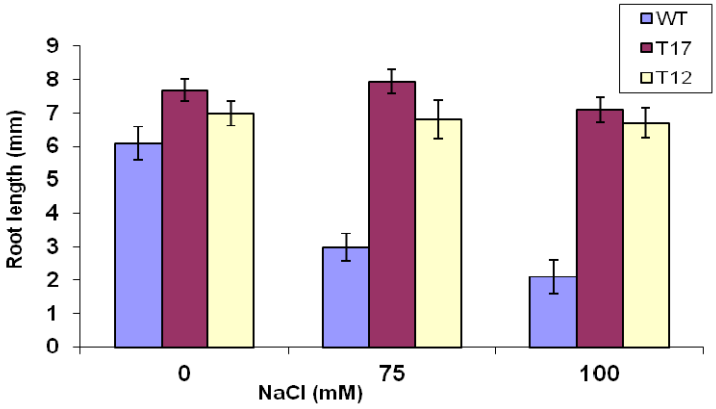


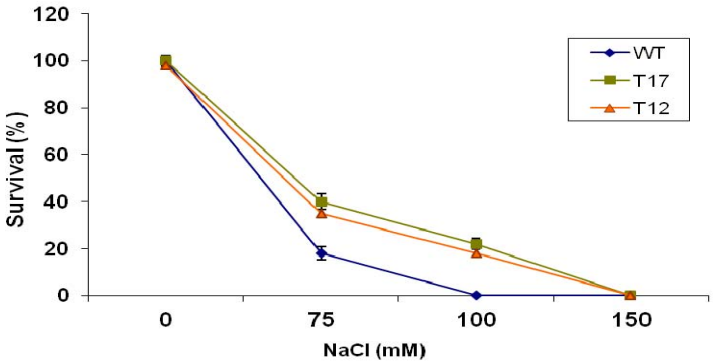
Figure 3. Effect of salt or drought stress on germination and seedling growth of WT and Re-transformed line 17.



a)

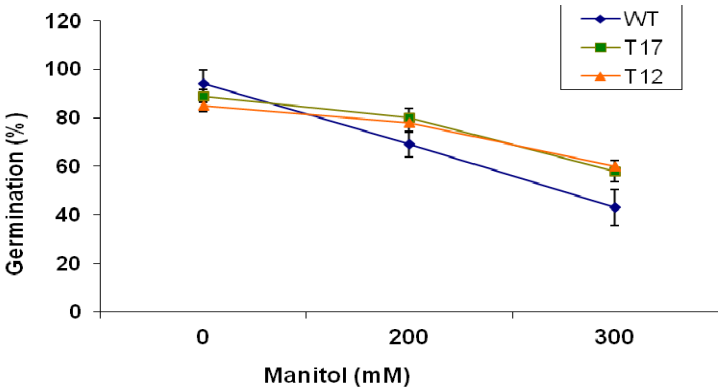


b)

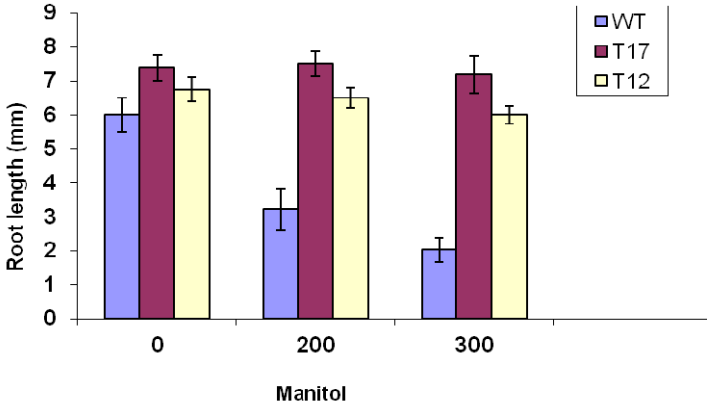


c)

Figure 4. Effect of salt stress on germination and seedling growth in WT and Re-transformed lines (T). Germination rate (a) and root length (b) of WT and transformed seeds grown on MS medium supplemented with 0, 75, 100 and 150 mM mannitol for 14 days. (c) The seeds of WT and Re-transformed lines were grown on MS medium, the 3-day-old seedlings were transferred to medium containing different concentrations of NaCl and the survival rate of the seedlings were measured after 14 days. The data were obtained from three independent experiments. Data are means ± SE. WT wild type, T12 and T17 Re-transformed lines.



a)



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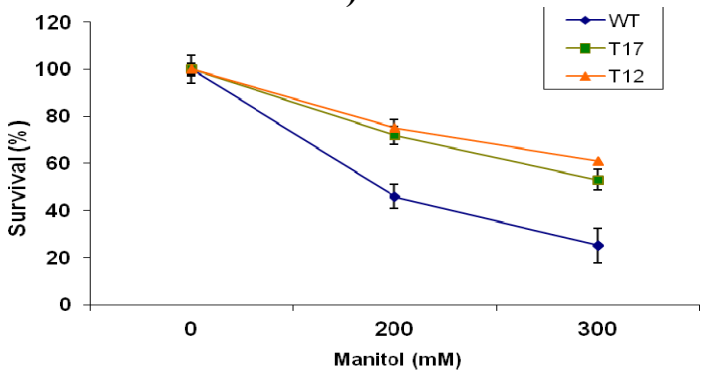

c)

Figure 5. Effect of drought stress on germination and seedling growth in WT and Re-transformed lines (T). Germination rate (**a)** and root length (**b**) of WT and transformed seeds grown on MS medium supplemented with 0, 200 and 300 mM mannitol for 14 days. **(c)** The seeds of WT and Re-transformed lines were grown on MS medium, the 3-day-old seedlings were transferred to medium containing different concentrations of mannitol and the survival rate of the seedlings were measured after 14 days. The data were obtained from three independent experiments. Data are means ± SE. WT wild type, T12 and T17 Re-transformed lines.

Table 1. Chlorophyll contents and enzyme activities in WT and Re-transformed lines (T) under salt stress

		Chlorophyll content (mg/g FW)				Enzyme activity (µmol min^{-1} mg $protein^{-1}$)		
	Salt (mM)	Chla	Chlb	Chla/b	Total Chl	Catalase	Peroxidse	Polyphenol oxidase
WT	0	0.58±0.04^{a}	0.25±0.02^{a}	2.3	0.75±0.05^{a}	0.33±0.03^{a}	37±4.6^{a}	3.6±0.36^{a}
	75	0.32±0.02^{b}	0.21±0.01^{b}	1.4	0.49±0.04^{b}	0.26±0.04^{a}	31.6±2.8^{b}	2.5±0.58^{b}
	100	0.24±0.05^{c}	0.11±0.02^{c}	2	0.24±0.03^{d}	0.15±0.04^{d}	24±3.7^{d}	1.9±0.44^{b}
T12	0	0.54±0.03^{a}	0.22±0.02^{a}	2.2	0.72±0.03^{a}	0.34±0.02^{a}	42.1±3.1^{a}	3.7±0.45^{a}
	75	0.59±0.03^{a}	0.29±0.02^{d}	2	0.77±0.02^{c}	0.44±0.02^{b}	48.5±2.4^{c}	4.1±0.21^{c}
	100	0.74±0.02^{d}	0.30±0.01^{d}	2.3	0.84±0.03^{e}	0.52±0.04^{bc}	60.1±2.4^{e}	4.8±0.34^{cd}
T17	0	0.56±0.04^{a}	0.26±0.02^{a}	2.1	0.74±0.04^{a}	0.36±0.03^{a}	41.17±5^{a}	3.9±0.32^{a}
	75	0.6±0.05^{a}	0.27±0.02^{d}	1.9	0.78±0.03^{c}	0.46±0.03^{b}	50±4.14^{c}	4±0.35^{c}
	100	0.72±0.03^{d}	0.31±0.02^{d}	2.1	0.86±0.03^{e}	0.54±0.04^{c}	59.5±2.8^{e}	5±0.36^{cd}

Seeds of WT and Re-transformed lines were grown on MS medium supplemented with 0, 75 and 100 mM salt for 14 days, then chlorophyll contents and enzymes activity were assayed. The data were obtained from three independent experiments. Data are means ± SE. Similar upper case letter in each column indicate no significant differences (Duncan test, P<0.05).

Table 2. Chlorophyll contents and enzyme activities in WT and Re-transformed lines (T) under drought stress.

		Chlorophyll content (mg/g FW)				Enzyme activity (µmol min^{-1} mg $protein^{-1}$)		
	Manitol (mM)	Chla	Chlb	Chla/b	Total Chl	Catalase	Peroxidse	Polyphenol oxidase
WT	0	0.58±0.02^{a}	0.25±0.02^{a}	2.3	0.75±0.05^{a}	0.33±0.03^{a}	37±4.6^{a}	3.6±0.36^{a}
	200	0.50±0.03^{b}	0.2±0.02^{b}	2.5	0.64±0.03^{b}	0.26±0.04^{c}	33±4.08^{a}	3.8±0.49^{ab}
	300	0.46± 0.05^{b}	0.14±0.03^{c}	3.1	0.58±0.05^{b}	0.1±0.03^{d}	30±2.9^{b}	3±0.41^{a}
T12	0	0.57±0.03^{a}	0.25±0.03^{a}	2	0.73±0.03^{a}	0.4±0.02^{b}	40.7±2.4^{a}	3.6±0.12^{a}
	200	0.6±0.02^{a}	0.32±0.02^{d}	1.9	0.78±0.02^{a}	0.48±0.02^{e}	51.7±2.8^{c}	4.4±0.28^{b}
	300	0.62±0.03^{a}	0.35±0.03^{d}	1.6	0.84±0.03^{c}	0.66±0.03^{f}	60.2±3.4^{d}	5.2±0.32^{bc}
T17	0	0.55±0.02^{a}	0.26±0.01^{a}	2.1	0.74±0.03^{a}	0.4±0.03^{b}	41.7±4.6^{a}	3.9±0.45^{a}
	200	0.6±0.01^{a}	0.3±0.02^{d}	1.9	0.8±0.03^{a}	0.47±0.04^{e}	54.3±3.2^{c}	4.5±0.5^{ab}
	300	0.63±0.05^{a}	0.34±0.02^{d}	1.5	0.89±0.05^{c}	0.65±0.04^{f}	62.5±3.7^{d}	5.7±0.4^{c}

Seeds of WT and Re-transformed lines were grown on MS medium supplemented with 0, 200 and 300 mM mannitol for 14 days, then chlorophyll contents and enzymes activity were assayed. The data were obtained from three independent experiments. Data are means ± SE. Similar upper case letter in each column indicate no significant differences (Duncan test, P<0.05).

Discussion

Drought and salinity are the major environmental limiting factors of agricultural production systems. To minimize yield losses, it is necessary to improve crop production under stressful conditions. One way is identification and over-expression of regulatory genes in signaling pathway that improve plant salt and drought tolerance. The obtained results indicated for the first time the involvement of *TRR14* in the plant response to the environmental stresses. In current study, we re-transformed *AtTRR14* into the *Arabidopsis* seedlings to study the physiological role of this novel gene in stress tolerance.

TRR14 expression level was very low in unstressed control plants. After treatment with 100 mM NaCl, transcript level increased in control plants (figure 2a). Drought stress also sharply induced expression of the *TRR14* gene in WT plants. The expression level of *TRR14* in manitol treatment was much higher than that in NaCl treatment (figure 2b). Similarly, *TRR14* expression was also induced by NaCl and Manitol treatment in the transformed plants.

Since salt and drought stress induced expression of TRR14, it was expected that *TRR14* could increase *Arabidopsis* seed germination under stressful conditions. Results obtained from seed germination on MS medium, supplemented with NaCl or mannitol, indicated that the transformed lines have higher germination rate than that of WT_s (figures 4a, 5a). The up-regulation of *TRR14* expression by salt or dehydration stress and the enhancement of germination rate of *TRR14* transformed lines under the salt or dehydration stress indicated that *TRR14* affects the germination of *Arabidopsis* in a positive way. But it is not known how *TRR14* affects germination and seedling growth under salt or dehydration stress conditions. These findings confirm our previous results that *TRR14* may affect seed germination in *Arabidopsis* (Aghdasi et al., 2012).

Abiotic stress affects different aspects of plant growth and development such as seed germination (Albuquerque and DeCarvalho, 2003), root length (Fan and Neumann, 2004; Fan et al., 2006; Jamil et al., 2006; Ma et al., 2006), photosynthetic activity, as well as chlorophyll synthesis (Agastian et al., 2000; Yang et al., 2006). Our data indicated that the transformed lines have unchanged root length, Chl a, Chl b and total chlorophyll level when grown on different concentrations of salt or manitol, compared with plants grown under normal condition. In addition, the observed unchanged chlorophyll contents of the transformed lines under stress may suggest that the transformed lines has unchanged photosynthetic capacities than WT. Reduction in chlorophyll and photosynthesis could be a reason for WT growth inhibition under stress.

One of the major effects of stress is induction of reactive oxygen species (ROS) production in plants (Smirnoff, 1996; Noctor and Foyer, 1998; Baier et al., 2005). Plants have enzymatic antioxidant defense system for scavenging of ROS (Blokhina et al., 2003). Our results showed that *TRR14* transformed lines have higher catalase and peroxidase activities than WT when plants grown under stress conditions (tables 1 and 2). It has been shown that tolerant cultivars have increased catalase activity under salt and drought stress (Hernandez and Almansa, 2002; Toorhan et al., 2006). Our results, which indicate an increase in peroxidase activity in transformed lines, are in accordance with those of Wang et al. (2009) and Toorhan et al. (2006), who found that salinity and drought stress lead to an increase in peroxidase activity in tolerant cultivars.

Trehalose is a non-reducing disaccharide which is one of the major osmoprotectants in nature (Crowe et al., 1998; Elbain, 2003). The role of trehalose in abiotic stress tolerance was demonstrated in many plants, but the detailed mechanism is not clear (Goddijn and Van Dun, 1999; Pramanic and Imai, 2005; Shima et al., 2007). Over-expression of trehalose-6-phosphate synthase (TPS) in tobacco and rice improved stress tolerance (Holmstrom et al., 1996; Garg et al., 2002; Jang et al., 2003). TRR14 is a novel protein that suppresses T6P-mediated growth arrest (our unpublished data). Characterization of TRR14 function will provide new insights into trehalose metabolism and stress pathway.

In conclusion, the present study has identified a novel gene, *TRR14,* that can be induced by salt and dehydration stress. The salt and dehydration tolerance of *TRR14* transformed *Arabidopsis* seedlings provide utility of this novel gene in genetic engineering to improve abiotic stress tolerance in plants. More investigation on the transformed plants of other *TRR14* family members will provide better understanding of the functional roles of *TRR14* in plants under abiotic stress conditions.

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