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Tomato TERF1 modulates ethylene response and enhances osmotic stress tolerance by activating expression of downstream genes

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Abstract The interaction between ethylene and osmotic stress pathways modulates the expression of the genes relating to stress adaptation; however, the mechanism is not well understood. In this paper, we report a novel ethylene responsive factor, tomato ethylene responsive factor 1 (TERF1), that integrates ethylene and osmotic stress pathways. Biochemical analysis indicated that TERF1 binds to the GCC box (an element responsive to ethylene) and to the dehydration responsive element, which is responsive to the osmoticum. Expression of *TERF1* was induced by ethylene and NaCl treatment. Under normal growth conditions, overexpression of *TERF1* in tobacco activated the expression of GCC box-containing pathogen related genes and also caused the typical ethylene triple response. Further investigation indicated that transgenic TERF1 tobacco exhibited salt tolerance, suggesting that TERF1 might function as a linker between the ethylene and osmotic stress pathways.

Keywords: Ethylene response; Expression of *PR* gene; GCC box; Tomato ethylene responsive factor 1; Salt tolerance

1. Introduction

Plants have unique systems for signal transduction and gene expression during development and responses against various environmental stresses [1]. A major target of signal transduction is the nucleus where the terminal signals lead to the transcriptional activation of numerous genes, and consequently to the de novo synthesis of a variety of proteins that have putative roles in stress adaptation. Plant hormones, such as ethylene, salicylic acid (SA), jasmonic acid (JA), and abscisic acid (ABA), have been intensively investigated as putative signals [2]. Communication between these signals might modulate the expression of the genes relating to stress adaptation but the interactions between these signal pathways are not well understood, nor are the mechanisms governing their cross-regulation.

Ethylene is a regulator of a variety of developmental and stress responses in plants, including seed germination, cell elongation, cell fate, sex determination, fruit ripening, flower senescence, and responses to biotic and abiotic stresses [3]. Ethylene accumulation during pathogen infection upregulates the expression of many genes in plants, such as basic *chitinase*, β-*1,3-glucanases*, *defensins*, and other *pathogenesis-related* (*PR*) genes [4]. Analysis of *PR* gene promoters revealed a common *cis*-acting ethylene response element called the GCC box, which contains a consensus sequence of AGCCGCC [4]. More recent evidence also demonstrated that the GCC box in *PDF1.2* of *Arabidopsis* is also responsive to JA [5]. Further, both JA and ethylene signaling appear to function synergistically to induce *PDF1.2*, *HEL* and *CHIB* in *Arabidopsis* [6,7], and *osmotin* and *PR1b* in tobacco [8]. Although sequences involved in response to JA/ethylene have been identified in a number of promoters [5,9], the upstream regulatory proteins that interact with the *cis*-acting element are not well understood.

Since the ethylene-responsive factor (ERF) proteins, ERF1–ERF4, were first isolated in tobacco that specifically binds to the GCC box [4], it has been documented that ERF proteins contain a highly conserved ERF DNA binding domain consisting of 58 or 59 amino acid residues [3,10]. Sequence analysis from the *Arabidopsis* genome indicates that numerous genes encode ERF proteins, which represent the second largest transcription factor family in plants [11], but only a few members of this family have been characterized. More importantly, some of these proteins have been shown to integrate signals from different plant hormone pathways and play roles in stress responses [12,13]. For example, *Arabidopsis* ERF1 is a downstream component of both ethylene and jasmonate pathways and plays a key role in the integration of both signals to regulate the expression of defense response genes [13]. Tomato Pti4 can mediate cross-talk between SA and ethylene/JA pathways and regulate defense-related gene expression via GCC box and non-GCC box genes [12,14]. Overexpression of tobacco *Tsi1* results in improved tolerance to salt and resistance to pathogen, suggesting that Tsi1 might be involved in two separate signal transduction pathways under abiotic and biotic stress [15]. In this paper, we report a novel member of ERF proteins from tomato that we designated tomato ethylene responsive factor 1 (TERF1), since it was found to bind to the ethylene responsive element GCC box. Its activity is induced by ethylene and NaCl treatment. Furthermore, in tobacco, the constitutive expression of *TERF1* induces the ethylene triple response and enhances osmotic stress tolerance by activating the expression of downstream genes.

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2. Materials and methods

2.1. Plant material and growth conditions

All plants were grown in growth chambers at 25 °C with a 16-h-light/dark cycle (except where mentioned in the text). 4-week-old tomato (*Lycopersicon esculentum* cv Lichun) and 6-week-old tobacco (*Nicotiana tabacum* cv NC89) were used for Northern blots. Ethylene treatments were performed with 2 ml of 40% ethephon and 1 g $NaHCO_3$ dissolving in 200 ml H_2O (in such conditions, ethephon will liberate ethylene gas). The control and treated tomato plants were placed in a sealed plexiglass chamber before whole plant tissues were harvested. For salt treatment, tomato plants were sprayed with 300 mM NaCl and the whole plants were used for extraction of total RNA. For detecting the expressions of *TERF1* and downstream genes in transgenic tobacco plants, leaves from normal growth 4–5-week-old plants were used.

2.2. Screening with yeast one-hybrid

The construction of reporter plasmids and tomato cDNA library, and the screening procedure with yeast one-hybrid are described in [16].

2.3. Binding assay in vitro

The *TERF1* full encoding area was cloned in frame into *Nde*I *Hin*dIII sites of the pET28a vector (Novagen). The resulting construct was transformed into *Escherichia coli* strain BL21. Expression and purification of recombinant TERF1 were conducted with a commercial kit following the manufacturer's instructions (Invitrogen). The oligonucleotides of the wild-type GCC box sequence AGTGCCAAAAGCCGCCACACCCCT and mutant GCC box sequence AGTGC- CAAAATCCACTACACCCCT (mutated nucleotides are underlined), or wild-type dehydration responsive element (DRE) box (ATTTCATGGCCGACCTGCTTTT) and mutant DRE box (ATTTCATAATCAACCTGCTTTT) (mutated nucleotides are underlined) were end labeled with ^{32}P-dATP. The assay mixtures contained 1.2 μg recombinant TERF1, 2 ng binding probe (8×10^4 cpm), 2 μg of salmon DNA, 20 mM HEPES–KOH, pH 7.5, 50 mM KCl, 0.1 mM EDTA, 10% glycerol, and 0.5 mM DTT in a 10 μl reaction volume. The mixtures were incubated at room temperature for 15 min and separated on a 4% polyacrylamide gel in 0.5× TBE buffer. Subsequently, the gel was exposed to Imaging Screening-K and visualized with the Molecular imager® FX (Bio-Rad).

2.4. Subcellular localization of TERF1

The coding region of *TERF1* and *DNLS*, which deleted the sequence containing the predicted nuclear localization signal (NLS), was amplified by polymerase chain reaction (PCR) to introduce *Xba*I at the 5′ end and *Sma*I at the 3′ end. The resulting fragments were digested with *Xba*I and *Sma*I and cloned into the expression vector pROK2 (derived from pBI121, Clontech), yielding the plasmids pTERF1 and pDNLS, respectively. Then, the coding region of green fluorescent protein (GFP) was amplified from pBIN 35S mGFP4 by PCR to introduce a *Sma*I site at 5′ end and a *Sac*I site at 3′ end. The PCR products were digested with *Sma*I and *Sac*I, and fused in frame into pTERF1 or pDNLS, resulting in TERF1-GFP or DNLS-GFP. Then, the above constructs were introduced into Agrobacterium LBA4404 for further analysis. Agrobacterium culture for transient expression in onion epidermal cells was prepared as described by Yang et al. [17]. The onion epidermal cells were dipped in the prepared Agrobacterium solution for 40 min, transferred to Murashige and Skoog (MS) plates, and incubated at 25 °C under light for 24–48 h. The localization of the fusion protein was observed by confocal microscopy (Bio-Rad).

2.5. Transactivation assay

Coding region of *TERF1* and deletion of the predicted activating domain fragment were fused in frame to the DNA binding domain vector pLexA, resulting in pLex-TERF1 and Plex-DAD, respectively. The fusion plasmids were transformed into EGY48 with p8op-lacZ as described by the manufacturer (Clontech). The transformants were selected by growth on selective medium plates at 30 °C for 3 days. The colony lift filter assay using *o*-nitrophenyl β-D-galactopyranoside as a substrate was performed subsequently to determine the ability of each translation product to activate transcription.

2.6. RNA transcript expression analysis

Total RNA was extracted from leaves. Twenty micrograms of total RNA was separated on a 1.5% formaldehyde-agarose gel and transferred onto nylon membranes. Using ^{32}P-labeling of *TERF1* 3′ flanking cDNA as a probe, the membrane was hybridized using the standard procedures of Northern blotting. The hybridized membrane was exposed to Imaging Screening-K and visualized with the Molecular imager® FX (Bio-Rad).

The probes for downstream genes' expression were cloned by RT-PCR using specific primers. These primers were used to clone tobacco *PR* genes: 5′-CTATTCCTTGTTTCTCACC-3′ and 5′-CCTTAACACATGACAAAGC-3 for *prb-1b* (*PR1*); 5′-GGGAATGAAATCAGCCCTG-3′ and 5′-GTCCCAAACTCCACCAGAG-3′ for *GLA* (*PR2*); 5′-CTCTCCTACTCCTCTCTGC-3′ and 5′-CACCAGGACTAACACCAAG-3′ for *CHN50* (*PR3*); and 5′-CTTCCTCCTTGCC TTTGTG-3′ and 5′-GCCACTTCATCACTTCCAG-3′ for *osmotin* (*PR5*).

2.7. Generation of transgenic tobacco

To analyze the roles of *TERF1*, the overexpressing vector was constructed by cloning the whole coding sequence of *TERF1* cDNA into pROK2 in the sense orientation under the control of the *cauliflower mosaic virus* 35S promoter, resulting in pROK-TERF1. The vector was introduced into tobacco (NC89) by Agrobacterium transformation. Transgenic plants were identified using Northern blots. The wild type of tobacco was named WT, while overexpressing *TERF1* in tobacco was named OE and the number following OE indicates the different transgenic line.

2.8. Assay of salt treatment

6 week tobacco plants were used in this experiment. The procedure was described in [18] with modifications. 25 WT tobacco and 25 of each transgenic lines were used for salt treatment. 100 ml of salt or water was watered every other day over the treatment. The concentrations of NaCl were increased stepwise by 50 mM every 4 days, starting from 50 mM. After 12 days, all plants were watered with 200 mM NaCl until the salt tolerance phenotype was observed.

2.9. Assays of seed germination

Seed germination was assayed as described by Beaudoin et al. [19]. Surface-sterilized seeds were plated on MS media containing various concentrations of NaCl and kept at 23 °C with a 16-h-light photoperiod. Germinated seeds were scored every day. For the triple-response assay, surface sterilized seeds were plated onto minimal media plates containing 1-aminocyclopropane-carboxylic acid (ACC) or $AgNO_3$, and incubated at 23 °C in darkness for 6 days.

3. Results

3.1. Isolation and characterization of TERF1

In order to clone the regulatory proteins that interact with the *cis*-acting element GCC box, 1.2×10^6 yeast transformants were screened by the yeast one hybrid method from a tomato expression cDNA library with a four times repeated GCC box present in the promoter (−74 to −51 bp) of tomato *NP24* [20] as bait. 14 positive clones were isolated. One positive clone with a full length cDNA was named as *tomato ethylene responsive factor 1* (*TERF1*), encoding a putative protein containing 224 amino acids. Database searches indicated that the TERF1 protein contains a 59 amino acid region that constitutes a DNA-binding domain, the ERF domain, which is highly conserved in members of the ERF family of plant transcription factors (Fig. 1). The predicted TERF1 protein contains a basic region in its N-terminal region that might function as a NLS. Also, TERF1 has an acidic C-terminal region that might act as an activation domain (AD) for transcription (Fig. 1). Sequence comparison analysis at the amino acid level showed that TERF1 shares very weak similarity outside this region with other reported members of the ERF

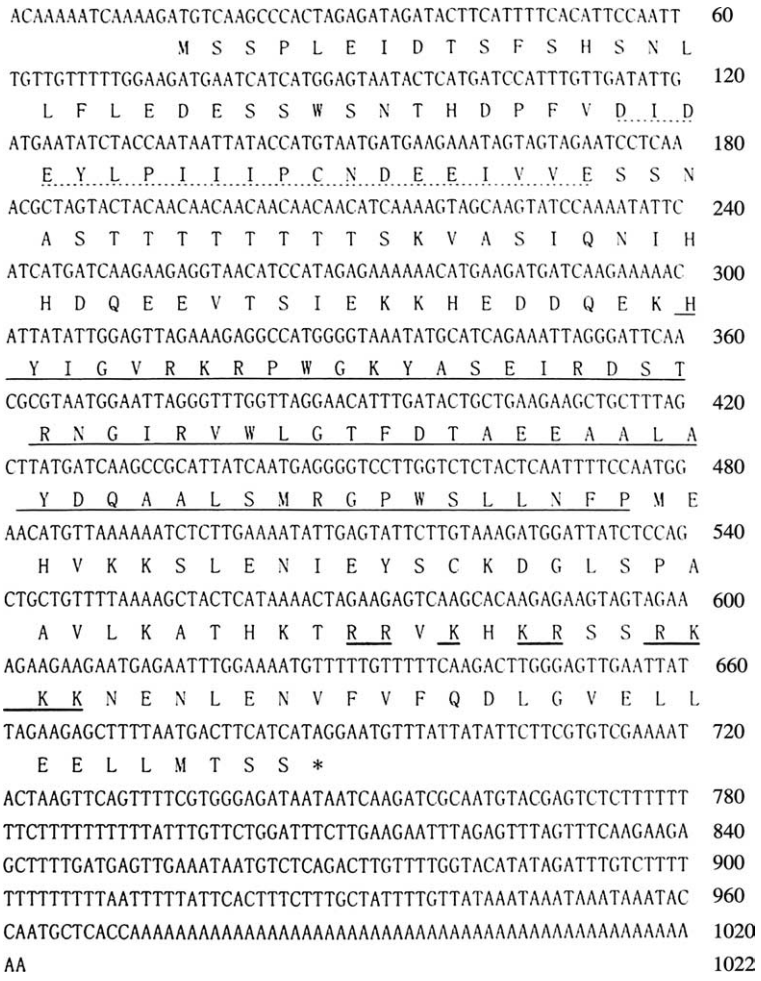

Fig. 1. Nucleotide and deduced amino acid sequences of TERF1. The ERF domain is underlined. A basic region that acted as a NLS is shown with dot and an acidic C-terminal region that acted as a transcriptional activator is shown with a short dash.

family, but a higher similarity in ERF domain (Fig. 2), suggesting that TERF1 is a novel member of the ERF family of plant transcription factors.

To test whether TERF1 can interact with the ethylene GCC box, we tested the binding affinity of TERF1 to the GCC box in vitro. Firstly, TERF1 fusion protein was expressed in pET28a and purified with a His-affinity column. The binding ability of recombinant TERF1 to the synthesized GCC box and the mutant GCC box was tested by using an electrophoresis mobility shift assay. As shown in Fig. 3A, the recombinant TERF1 protein could bind to the GCC box, but not to the mGCC box, suggesting specific interaction of TERF1 with the GCC box in vitro.

There is evidence that ERF proteins, such as CBF1, Tsi1 and DREB proteins, can bind to DRE [15,21,22]. In order to test whether TERF1 interacts with DRE, we tested the binding affinity of TERF1 with DRE using an electrophoresis mobility shift assay. Our results indicated that the recombinant TERF1 protein interacted with DRE, but not with the mutant DRE (Fig. 3A), further demonstrating that the TERF1 protein not only takes part in GCC box-mediated signaling pathway, but also DRE-mediated signaling.

To investigate the cellular distribution of TERF1, we performed an in vivo targeting experiment using GFP as a fluorescent marker. Using fluorescence microscopy, the fusion protein TERF1-GFP was localized to the nucleus of onion

Fig. 2. Sequence comparison derived from sequence alignment of the ERF domain region from tobacco, tomato and *Arabidopsis*. Boxes in black represent 100% similarity, gray for 75% conserved amino acids. Protein names are indicated at the left.

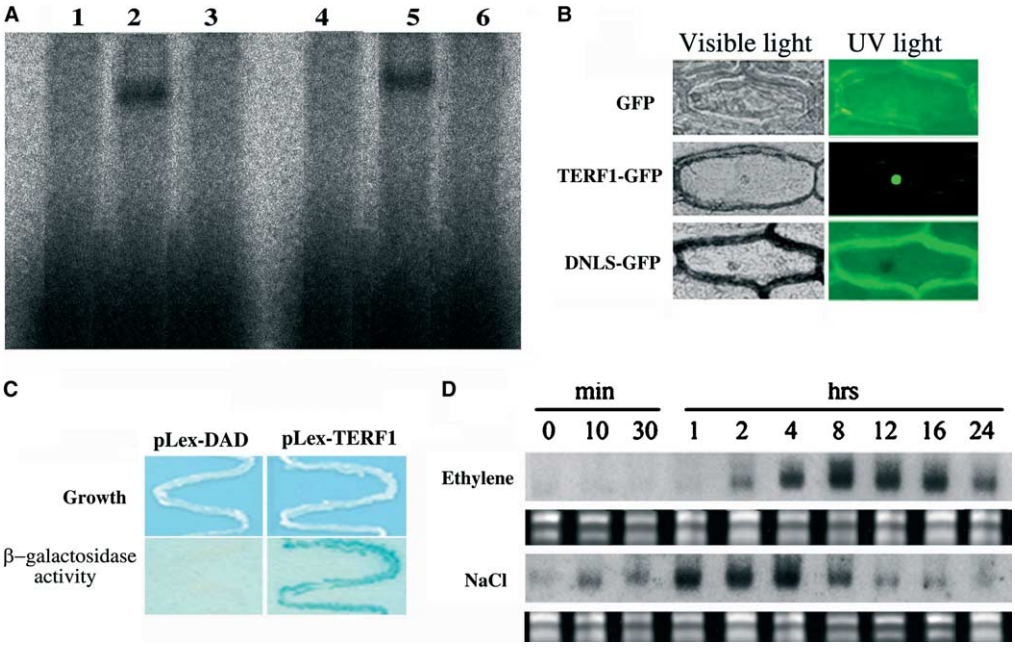

Fig. 3. Characterizations of TERF1 as transcription factor. (A) Binding activity of TERF1 to *cis*-acting elements of GCC box and DRE. Lanes 1 and 4: only the free probes of GCC box and DRE, respectively; lanes 2 and 5: 1.2 μg of recombinant TERF1 plus labeled GCC box and DRE, respectively; lanes 3 and 6: 1.2 μg of recombinant TERF1 plus labeled mGCC box and mDRE, respectively. (B) Subcellular localization of the TERF1 protein. The TERF1-GFP, DNLS-GFP and GFP were transiently expressed in onion epidermal cell by infiltration of Agrobacterium. (C) Transcriptional activation of TERF1 in yeast. plex-TERF1 or pLex-DAD was transformed into yeast strain EGY48 containing reporter plasmid. (D) Expression of *TERF1* in tomato. Each lane was loaded with 20 μg of total RNA. The RNA gel blots were hybridized with the *TERF1* cDNA 3′ flanking fragment. Equal loading was verified by ethidium bromide staining as loading control.

epidermal cells. By contrast, the fluorescence of the fusion protein DNLS-GFP, which deleted the NLS of TERF1, was distributed throughout the cell and was excluded from the nucleus. The control GFP was uniformly seen throughout the cell (Fig. 3B).

TERF1 has an acidic C-terminal half that might act as a transcriptional activator domain. To investigate the transcriptional activation activity of TERF1, we fused the coding regions for TERF1 to the LexA DNA binding domain expression vector and examined its behavior as a potential transcriptional activator in yeast (Fig. 3C). In the absence of the LexA activation domain, the wild-type TERF1 protein fused to the LexA DNA binding domain activated transcription of the lacZ reporter gene, indicating that the TERF1 functions as a transcriptional activator in yeast.

The expression of the *TERF1* in tomato leaves after ethylene and NaCl treatment was analyzed using RNA gel blot analysis. As shown in Fig. 3D, *TERF1* expression was induced within 2 h after exposure to ethylene gas and continued to increase over the next 4–8 h before gradually decreasing. However, *TERF1* gene expression was induced as early as 10 min after NaCl treatment and continued to increase over the next 1–4 h before gradually decreasing, suggesting that TERF1 might be involved in ethylene and osmotic stress signaling.

3.2. Overexpression of TERF1 in tobacco causes phenotypic changes associated with the ethylene response

To analyze the function of TERF1 in plants, tobacco transgenic plants constitutively expressing *TERF1* under the 35S promoter were generated. 19 individual transgenic plants determined by Southern and Northern blot analysis were obtained (data not shown). Six lines with a single insertion were confirmed by antibiotic selection in T1 seed germination. 3 of the 6 single copy insertion lines displayed consistent phenotypic changes, but the control transformed with an empty vector did not. This phenotype appeared at the seedling stage, and the phenotypic difference between WT and OE plants became more obvious as plants matured. The leaves of OE plants were slightly smaller and darker green than those of

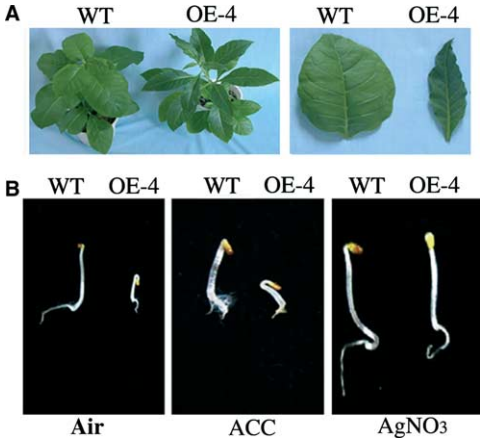

Fig. 4. Phenotype changes and triple response in transgenic *TERF1* tobacco. (A) Phenotype of transgenic *TERF1* tobacco (OE) and wild type (WT) 5 months after planted in soil. Left panel: mature plants; right panel: leaves. (B) Ethylene response phenotype in OE tobacco. 6-day OE seedlings were germinated on agar plates in the dark with or without 5 μM 1-aminocyclopropane-carboxylic acid (ACC) or 15 μM $AgNO_3$.

WT plants (Fig. 4A), which is very similar to the effect of Pti4 in transgenic *Arabidopsis* [12]. As discussed above, *TERF1* expression is inducible responsive to ethylene. In order to establish whether TERF1 is involved in ethylene signaling, we applied the triple-response assay [23]. Our results indicated that in the absence of ethylene, etiolated OE seedlings displayed inhibition of root and hypocotyl elongation, and more curvature of the apical hook than WT – a typical phenotype caused by ethylene treatment (Fig. 4B). Addition of the ethylene precursor, ACC, enhanced this ethylene typical response while it could be inhibited by, $AgNO_3$, a potent inhibitor of ethylene action (Fig. 4B), suggesting that expression of *TERF1* in tobacco might involve biosynthesis of ethylene.

Based on the above results, it was important to determine whether the ethylene morphology displayed by these plants was the consequence of ethylene overproduction or due to constitutive activation of the signaling pathway. Firstly, we checked the level of endogenous ethylene in wild type and transgenic *TERF1* plants. We found that the level of ethylene increased 3-fold in transgenic *TERF1* plants compared to wild type plants (data not shown), suggesting that the observed morphology evoked by the expression of *TERF1* was a consequence of ethylene production.

In view of the observation that the phenotypic changes in 35S::ERF1 were due to activation of ethylene responses [23], we therefore examined the expression of some ethylene-responsive genes in OE. As expected, no accumulation of the four ethylene-inducible genes, *prb-1b* [24,25], *CHN50* [26], *GLA* [27], and *osmotin* (GCC boxes locate at −83 to −77 and −179 to −172 bp), which are known to contain GCC box in their promoters, was detected in WT plants in the absence of ethylene. In contrast, high-level constitutive expressions of the four transcripts were observed in OE plants (Fig. 5), providing evidence that TERF1 is involved in the activation of ethylene responses.

3.3. Overexpression of TERF1 enhances osmotic stress tolerance

The fact that the expression of *TERF1* was induced after NaCl treatment (Fig. 3D), and some downstream genes, such as *osmotin*, were regulated by TERF1 in transgenic tobacco (Fig. 5), suggests that TERF1 may be involved in abiotic stress response. It has been reported that high concentration of salts

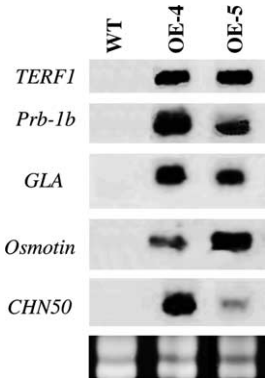

Fig. 5. Constitutive expression of *TERF1* in OE tobacco activates constitutive expression of GCC box-containing genes. Total RNA was isolated from leaves of 4-week-old tobacco plants. RNA gel blots were hybridized with the probes indicated beside the panel. Equal loading was verified by RNA stained with ethidium bromide.

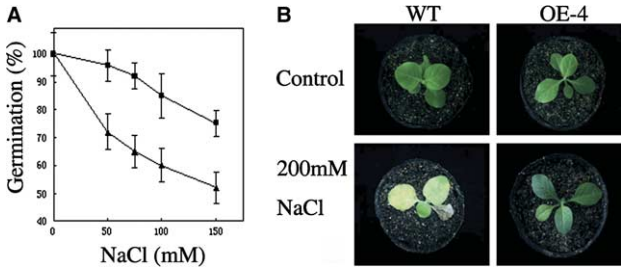

Fig. 6. Effects of NaCl on seed germination and plant tolerance to salt. (A) Germination response to indicated NaCl concentrations 5 days after plating seeds on minimal medium. About 100 seeds were used for each treatment. Results are the average of three replicates ± S.D. WT: black triangle curve; OE-4: black square curve. (B) Phenotypic differences after salt treatment between WT and OE. Salt treatment was conducted as described in [18]. Significant phenotypic differences were observed 33 days after treatment with 200 mM NaCl.

inhibits the germination of *Arabidopsis* [28,29]. We therefore used the seed germination assay to test whether OE changes the response of plants to salt. Under our experimental conditions, the germination rate of OE is significantly higher than WT from 50 to150 mM NaCl (Fig. 6A), suggesting that the OE enhances salt tolerance in seed germination. It has also been reported that salt tolerance in seed germination is not extended to vegetative stages [28,29]. To determine whether this salt tolerance in seed germination is consistent with the situation in mature seedlings, the 6-week old plants were watered with NaCl. After 33 days treatment with 200 mM NaCl, OE grew faster than WT but slower than plants irrigated with water. The leaves of WT turned yellow and bleached, but OE plants turned darker green than those of plants irrigated with water (Fig. 6B), suggesting that the overexpression of *TERF1* enhanced the salt tolerance in the vegetative stage.

4. Discussion

Accumulating evidence indicates that ERF proteins in plants belong to one of the largest transcription factor family [11,14], suggesting that ERF proteins might have crucial roles in regulating development and the responses to environmental stresses. Though genomic information for tomato is not available, the cloning and identification of tomato ERF proteins has recently made major advances [16,24,30]. In the present paper, *TERF1* was isolated and shown to encode an ERF protein, which contains an activation domain in its C-terminus and a NLS in its N-terminus. This analysis, based on the predicted protein sequence, was further confirmed by the activation of TERF1 in the expression of downstream genes, including *LacZ* in yeast, *PR* genes in transgenic *TERF1* tobacco, and the expression of *TERF1* in the nucleus of onion epidermal cells. Biochemical analysis revealed that TERF1 interacted with the GCC box and DRE in vitro, which is very similar to the characterization of tobacco *Tsi1* [15]; however, their protein sequences share less than 34% similarity, indicating that TERF1 is a novel member of ERF proteins as a transcriptional activator that might have a distinct role in response to stresses.

The interaction of TERF1 with the GCC box and DRE is consistent with the characterization of Pti4, Tsi1 and LeERF

proteins [15,30,31], suggesting that TERF1 might function in ethylene and osmotic stress pathways. For example, Tsi1 can bind to both the GCC box and the DRE/C-repeat, and may be involved in two separate signal transduction pathways under abiotic and biotic stresses [15]. In our present study, we provide evidence that the expression of *TERF1* is regulated by ethylene and osmotic stresses, suggesting that TERF1 has a distinctive regulation in ethylene and osmotic stress responses. Gain-of function mutations obtained by insertional mutagenesis of T-DNA or transposon elements carrying a CaMV 35S promoter have proved to be a powerful tool for assessing the function of a gene. *ERF1* overexpression causes a partial ethylene response and activates the expression of GCC box-containing genes, such as *PDF1.2* and β-*chintinase*. However, it does not activate *HOOKLESS1*, which also contains a GCC element in its promoter [23], suggesting that ERF1 is responsible for the activation of a subset of the ethylene-responsive GCC-box-containing target genes. In this subset of the ethylene pathway, it is possible that other ERF family members, or interactions with bZIP transcription factors, may be responsible for activating these target genes. In our investigation, the expression of *TERF1* in transgenic tobacco causes ethylene responses in seedlings and mature plants, and this ethylene response is the consequence of ethylene overproduction and the activation of constitutive expression of GCC box-containing *PR* genes. It has been found that one of the tomato ACC oxidase genes that contain the GCC box in its promoter region, which activates ethylene biosynthesis, was regulated by ERF protein Pti4/5/6 through the interaction with tomato Pto kinase [24]. Now, we are extensively investigating whether the expression of *TERF1* in tobacco activates the expression of ACC oxidase gene(s) through the interaction with the GCC box.

It has also been shown that the expression of some *PR* genes is a typical part of the plant's defense response to wound and pathogen attack [13,15,32] and can also be induced by water stress [1]. Our preliminary investigation also found that overexpressing *TERF1* in pepper greatly increased resistance to *Xanthomas campestris pv vesicatoria* and *Phytophthora capsoci* (Huang et al. unpublished data), further supporting the role of ERF protein in disease resistance. More recent research indicates that ERF proteins are also involved in abiotic stress, like the role of Tsi1 in salt tolerance [15]. These findings suggest that the GCC box may act as a cis-regulatory element for biotic and abiotic stress signal transduction [33]. In this paper, we demonstrated that overexpression of *TERF1* in tobacco plants also enhances tolerance to osmotic stresses like salt; this supports the hypothesis that there is interaction between the ethylene signaling pathway and the abiotic signaling pathway.

Interactions among transcription factors are thought to play an important part in mediating responses to various signaling events. It will be important to determine whether TERF1 and/or related ERF proteins can interact with other transcription factors that are involved in distinct signal transduction pathways. The adaptive role of ethylene in plant defense has been documented extensively. The tomato transcription factor Pti4 appears to regulate gene expression directly by binding to the GCC box and possibly a non-GCC-box element; it could also work indirectly by either activating the expression of transcription factor genes or interacting physically with other transcription factors [14]. It also has been reported that ethylene plays a role in mediating responses to abiotic stresses. Therefore, our results suggest that TERF1 might be a linker in ethylene and osmotic stress signaling pathways, providing new insight into the role of ERF proteins in the ethylene signaling and osmotic stress pathways.

5. Accession numbers

The gene bank accession numbers for the sequences mentioned in this article are AY044236 (*TERF1*), X66942 (*prb-1b*), M60402 (*GLA*), X51599 (*CHN50*), X95308 (*osmotin*), AF093743 (*NP24*), AF076277 (*ERF1*), AB008104 (*AtERF2*), AB008105 (*AtERF3*), D38123 (*EREBP1*), D38126 (*EREBP2*), U89255 (*Pti4*), U89256 (*Pti5*), and AF058827 (*Tsi1*).

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