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An overexpression of the *AP2/ERF* transcription factor from *Iris typhifolia* in *Arabidopsis thaliana* confers tolerance to salt stress

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Abstract

The roles of ethylene responsive factors (ERFs) and their positive and negative regulations of abiotic stress tolerance have been widely reported. This study reports the characterization of *ItERF* from *Iris typhifolia* Kitag with respect to molecular and functional properties. The 867 bp cDNA fragment of *ItERF* was cloned by reverse transcription PCR from *I. typhifolia*. Real-time quantitative PCR revealed that *ItERF* expression was induced in the roots, stems, and leaves of *I. typhifolia* after NaCl treatment, and that *ItERF* expressions were significantly higher in the leaves and roots than in the stems. A green fluorescent protein marker revealed that *ItERF* was located to the nucleus. Plant survival and root growth of *ItERF* transgenic *Arabidopsis thaliana* L. seedlings were much better than those of the wild type under NaCl stress. Malondialdehyde content in the transgenic lines was significantly lower than that in the wild type. Growth of yeast transformants showed an enhanced tolerance to salt stress than non-transformed yeast cells. All of the results verified that the expression of *ItERF* had effects on plant growth under salt stress.

Additional key words: ethylene responsive factor, NaCl, transgenic plants.

Introduction

Transcription factors (TFs) are important for maintaining the expression of genes that encode functional proteins in the genome. Ethylene responsive factors (ERFs) are members of a novel family of transcription factors, which are specific to plants. The ERFs themselves are a large family of plant-specific stress-responsive TFs, which are characterized by a conserved 58-59 amino acid DNA-binding domain (designated as the AP2/ERF domain). They can specifically bind to GCC *cis*-elements (AGCCGCC) and other related elements in the promoters of target genes to modulate their expression (Fujimoto *et al*. 2000, Nakano *et al*. 2006, Sharma *et al.* 2010).

To date, ERFs have been shown to play crucial roles in regulating a wide range of plant defense- and stress-related genes, which are associated with a variety of biological processes in plants including metabolism, growth, development, flowering, and responses to environmental stimuli (Mizoia *et al*. 2012, Núñez-Pastrana *et al*. 2013). In particular, they can modulate multiple responses to abiotic stresses (Jack *et al*. 1997, Ohta *et al*. 2001, Song *et al*. 2005, Yang *et al*. 2005). For example, Sub1A, an ERF-like protein, enhances recovery from drought through the reduction of leaf water loss and lipid peroxidation in transgenic rice plants at the vegetative stage (Fukao *et al*. 2011). Soybean GmERF7 is induced by drought, salt, methyl jasmonate, ethylene, and abscisic acid treatments, and GmERF7 enhances salt tolerance in transgenic plants (Zhai *et al*. 2013). Overexpression of *PsAP2* from *Papaver somniferum* in transgenic tobacco plants exhibits increased tolerance to both abiotic and biotic stresses (Mishra *et al*. 2015). Gao *et al.* (2008) studied the tomato *TERF1* gene and found that transgenic rice plants have an increased salt and drought tolerance due to an enhanced proline accumulation and a reduced water loss. Furthermore, plants overexpressing *AtERF71* have an increased tolerance to NaCl, mannitol, flooding, and methyl viologen, and have a reduced reactive oxygen species accumulation under high salt stress (Park *et al*. 2011). Overexpression of *GmERF7* significantly improves salt tolerance in transgenic tobacco with an increased chlorophyll and sugar content in transgenic plants (Zhai *et al*. 2013). The roles of ERF transcription factors in the regulation of salt stress responses appear to be complex, and the biological functions of these factors and the ERF-mediated signal transduction pathway are unclear. Therefore, further studies are warranted.

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Abbreviations: ERF - ethylene responsive factor; GFP - green fluorescent protein; MDA - malodialdehyde; TF - transcription factor; WT - wild type.

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Iris typhifolia grows in warm areas and has tolerance to salt and alkaline stress. These features, together with its bright color and high ornamental value, make it popular for group planting and flower borders. However, there has been no research to date to clarify the role of the *ERF* signal transduction pathway in salt and alkaline tolerance in *I. typhifolia*. In this study, a novel *ERF* gene, *ItERF*, was cloned from *I. typhifolia* and it was transferred into *Arabidopsis.* The adaptability of the gene to salt and alkaline was studied by detecting the changes of plant survival rate, root length and malondialdehyde (MDA). Our study provides clear insights into the regulation of *ItERF* activity in abiotic salt stress tolerance.

Materials and methods

Plants and treatments: *Iris typhifolia* Kitag. seeds were maintained at the Northeast Agricultural University. Seeds were placed in small pots containing soil and *Vermiculite* (3:1) to germinate under an ambient temperature of 25 °C, an air humidity of 45 %, a 16-h photoperiod, and an irradiance of 47 μmol m^{-2} s^{-1} using sodium lamps. Leaves from two-month-old seedlings were harvested and frozen in liquid nitrogen and then stored at -80 °C until subsequent extraction of total RNA and gene cloning.

To examine their responses to salt stress, two-month-old *I. typhifolia* seedlings were treated with 150 mM NaCl and leaves, stems, and roots were harvested at 0, 6, 12, 24, and 48 h. Samples were immediately frozen in liquid nitrogen and stored at -80 °C.

Strains, vectors, and reagents: *Escherichia coli* strain JM109 (*Transgen Biotech*, Beijing, China), *Agrobacterium tumefaciens* strain EHA105, and *Saccharomyces cerevisie* strain INVScI were steadily maintained in our laboratory. The pMD18-T vector was procured from *TaKaRa* (Dalian, China) and the pBI121 and pBI121-MCS-GFP expression vectors were prepared previously in our laboratory.

The restriction enzymes and gel recovery reagents were obtained from *TaKaRa* and T_4-DNA ligase from *Fermentas* (Burlington, Canada). The *Ex-Taq* DNA polymerase and reverse transcription kits were purchased from *Invitrogen* (Carlsbad, USA). Real-time quantitative fluorescent dyes and *SYBR Green QPCR Master Mix* were purchased from *Toyobo* (Osaka, Japan). The northern blot kits were purchased from *Roche* (Shanghai, China). Antibiotics were purchased from *Sigma-Aldrich* (St. Louis, MO, USA). All other analytical reagents were purchased from suppliers in China.

Cloning *ERF* gene of *I. typhifolia* and vector construction: The *ItERF* cDNA fragment containing an open-reading frame was amplified by reverse transcription PCR using primers *ItERF*-1F and *ItERF*-1R (Table 1 Suppl.). A high-fidelity thermostable DNA *Ex-Taq* polymerase was used to amplify *ItERF*, which was then ligated into the pMD18-T vector (*TaKaRa*) and transformed into *E. coli* strain JM109 cells. Recombinant pMD18-T-*ItERF* plasmids were extracted from the transformants and verified by *Xba* I and *Spe* I digestion and sequencing.

Using the recombinant pMD18-T-*ItERF* plasmid as a template, primers containing restriction sites (Table 1 Suppl.) were used for PCR amplification. The target fragments were ligated with pMD18-T and sequenced. The pBI121-MCS-GFP plasmid and the recombinant plasmid pMD18-T-*ItERF* were digested with *Xba* I and *Spe* I, and the recombinant plasmid pBI121-*ItERF*-GFP was constructed after gel extraction and purification. The recombinant plasmid was transformed into JM109 cells and sequenced and was then used to investigate the subcellular localization of proteins.

The target *ItERF* fragment was PCR-amplified from pMD18-T-*ItERF* using gene-specific primers and the *Xba* I and *Sac* I restriction sites were added (Table 1 Suppl.). The pBI121 plasmid was digested with *Xba* I and *Sac* I, and subsequently ligated with the target *ItERF* fragment. The recombinant plasmid pBI121-*ItERF* was transformed into JM109 cells, extracted from transformants, and sequenced. It was then transformed into *A. tumefaciens* strain EHA105 and genetically transformed into *Arabidopsis thaliana* L. (the ecotype Columbia).

Bioinformatics: The open reading frame and amino acid sequences of *ItERF* were obtained from the National Center for Biotechnology Information (*NCBI*; https://www.ncbi.nlm.nih.gov). The sequences of *ERF* members from *Arabidopsis thaliana*, *Oryza sativa*, *Nicotiana tabacum*, *Lycopersicon esculentum*, and *Solanum tuberosum* were used to create a phylogenetic tree with the *MEGA6* software. The subcellular localization of the ItERF protein was predicted using *PSORT* (http://psort.hgc.jp/form2.html).

Real-time quantitative PCR: Total RNA were extracted from the leaves, stems, and roots of *I. typhifolia* and reverse transcribed to produce cDNA. The cDNA was diluted 10 times and used as a template for real-time quantitative PCR. The assays were performed using *SYBR Green QPCR Master Mix* (*Toyobo*, Osaka, Japan). *Iris typhifolia Actin2* (NCBI accession number EX953716) was used as a housekeeping gene (Gu *et al*. 2014). Primers (*ItERF*-Q-F, *ItERF*-Q-R) were designed using *Primer 5.0* (Table 1 Suppl.). The relative expressions of *ItERF* were calculated using the ΔΔCt method (Pfaffl 2001).

Subcellular localization: The plasmid pBI121-*ItERF*-GFP was introduced to *A. thaliana* (the ecotype Columbia) using the vacuum infiltration method of *Agrobacterium*-mediated transformation (Bechtold and Pelletier 1998). Seeds were collected and screened on agar plates containing a half-strength Murashige and Skoog medium supplemented with 50 mg·dm^{-3} kanamycin. Protoplasts were isolated from fresh *Arabidopsis* leaves and GFP fluorescence was examined by fluorescence microscopy (*DM2500, Leica*, Bensheim, Germany) with amplification 200 and taken photographs with a camera (*Leica DFC420 C*, Wetzlar, Germany) (Wu *et al*. 2009).

Analysis of sensitivity to NaCl in yeast: For yeast transformation, the coding region of the *ItERF* gene amplified by PCR with primers pYES2-*ItERF*-F and pYES2-*ItERF*-R was fused to the pYES2 vector (under the control of a separate *recombinant galectin 1* promoter) to construct the plasmid pYES2-*ERF*. It was expressed in yeast by using a galactose-inducible protein. The plasmid pYES2-*ERF* and pYES2 empty vector were transformed into yeast strain INVScI by using the chemical transformation method (Gietz *et al*. 1988). To examine the effects of NaCl stress, we incubated the yeast cells containing pYES2-*ERF* and the pYES2 vector in yeast extract peptone dextrose medium plus overnight at 30 °C. Then, the cultures were adjusted to absorbance A_{600} = 0.3 and diluted to 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5} concentrations with yeast peptone glucose medium. Approximately 5 mm^3 of each serial dilution was spotted on yeast peptone glucose medium plates supplemented with different concentrations of NaCl as indicated; an yeast extract peptone dextrose medium plus agar plate without NaCl

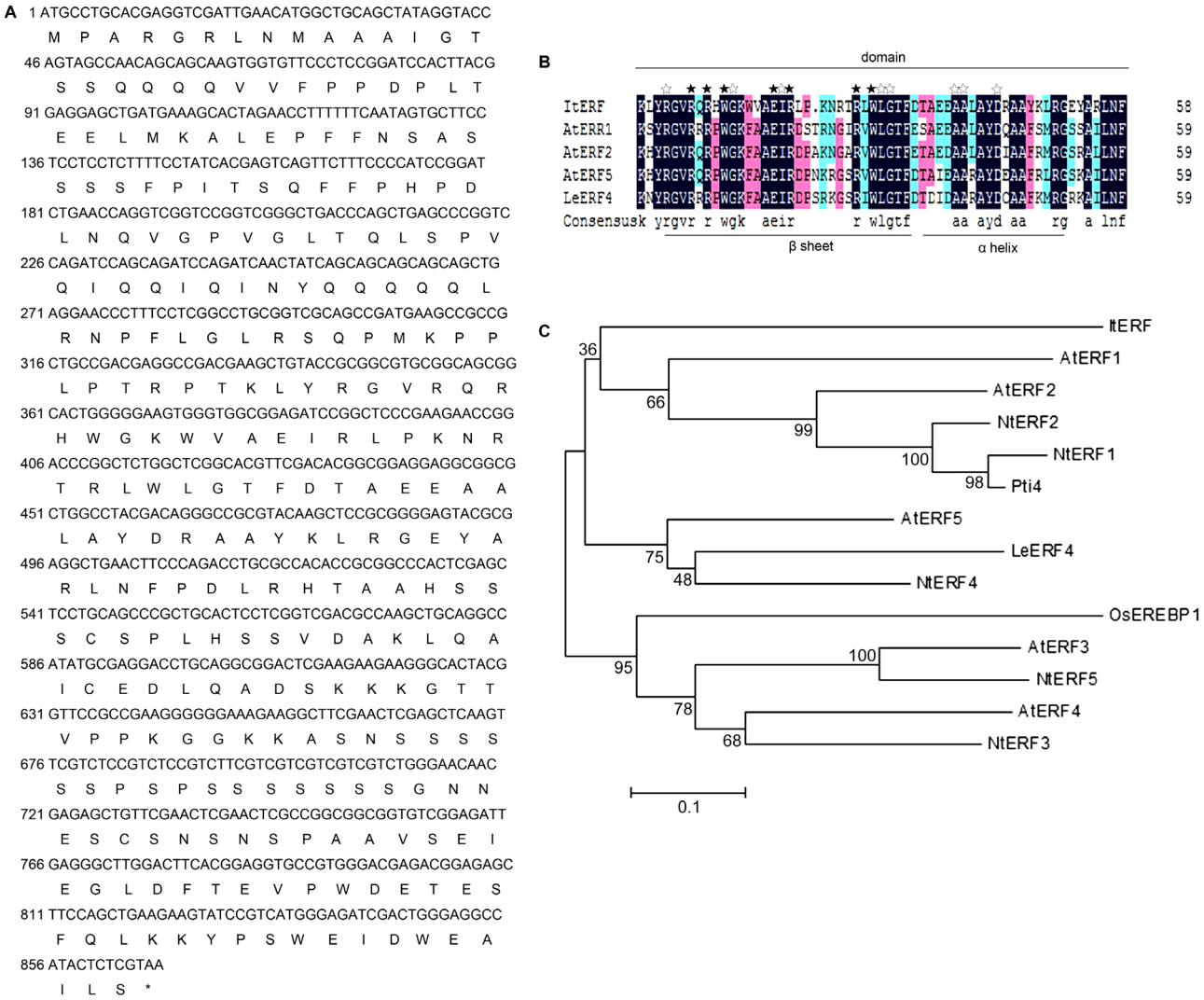

Fig. 1. Multiple sequence alignment and phylogenetic relationships of *Iris typhifolia ethylene responsive factor* (*ItERF*) homologs: Analysis of the open reading frame (*A*) and the coding amino acid sequence of the *ItERF* (*B*). The *asterisks* represent amino acid residues that were shown to bind to the GCC box. The n*umbers at right* indicate the amino acid position of the ERF domain in each protein. The structure of the ERF domain is shown *below* the sequence; Amino acid residues identified by nuclear magnetic resonance analysis (Allen *et al*. 1998) to interact with nucleotides within the GCC box are *emphasized* in the ERF domain consensus sequence. The *bar* and *black arrows* indicate the β sheet and the β strands within the β sheet, respectively. The *cross-hatched box* indicates the α helix. *C* - A neighbor-joining phylogenetic tree of the *ERF* gene family as created by *DNAMAN*. The bootstrap value (above 50 %) supports from 1 000 replicates are indicated at each branch, the *scale* is 0.2. All amino acid sequences were retrieved from *GenBank*: *I. typhifolia ItERF* (MF508603); *Arabidopsis thaliana* AtERF1/2/3/4/5 (BAA32418, BAA32419, NP_175479, BAA32421, BAA32422, and NP_188666); *Oryza sativa* OsEREBP1 (AAF23899); *Nicotiana tabacum* NtERF2/3/4/5 (NP_001311965, BAJ72663, XP_016467940, and AAU81956); *Lycopersicon esculentum* LeERF4 (NP_001234313); *Solanum tuberosum* Pti4 (ACF57857).

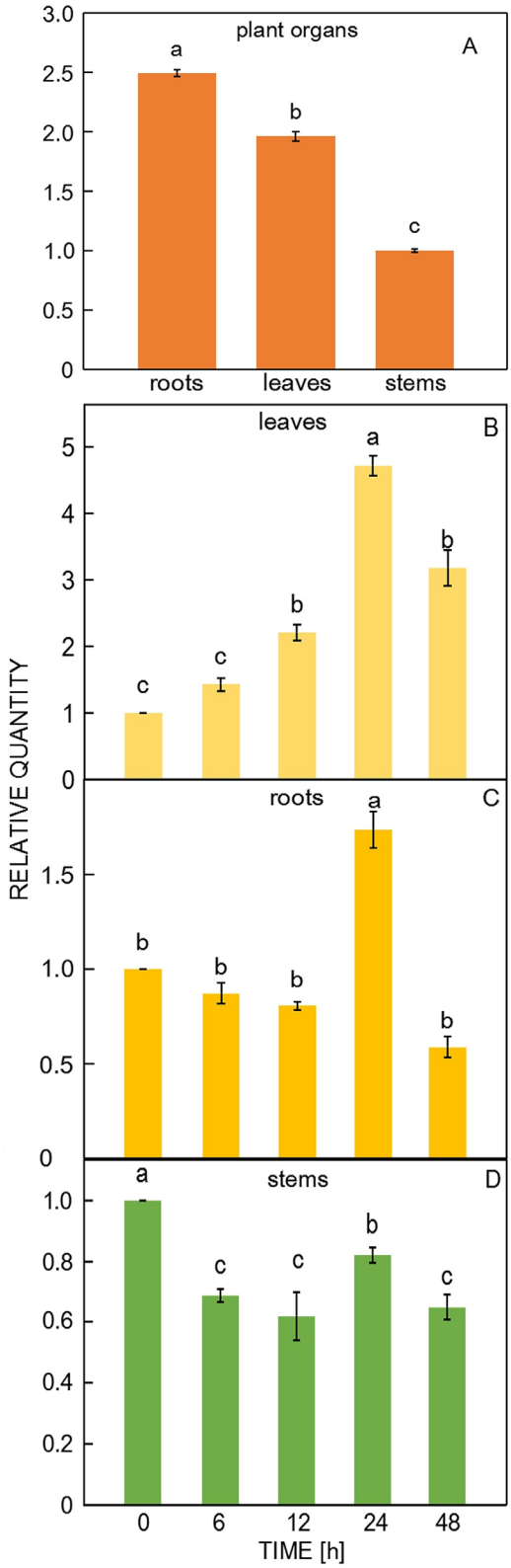

Fig. 2. Quantitative real-time PCR analyses of *Iris typhifolia ethylene responsive factor* (*ItERF*) transcription (relative to the housekeeping gene *Actin2*) in leaves, roots, and stems of control plants (*A*) and under salt stress (150 mM NaCl) in leaves (*B*), roots (*C*), and stems (*D*). Means ± SEs, $n = 3$, different letters indicate significant differences according to *ANOVA* ($P < 0.05$).

was used as a control. Growth was monitored after 3 d at 30 °C.

Northern blot analysis: To confirm *ItERF* gene expression, total RNA was extracted from plant tissues using *TRIzol*. Denaturing gel electrophoresis was conducted followed by an RNA gel blot analysis using a digoxigenin probe (Sambrook *et al*. 1989).

Stress tolerance analysis of transgenic *Arabidopsis*: The full-length *ItERF* cDNA was cloned into pBI121 under the *cauliflower mosaic virus 35S* promoter and introduced into *A. thaliana* (the ecotype Columbia) using the floral dip method (Bechtold *et al*. 1993). Transformants were selected by screening successive generations on kanamycin and by PCR confirmation. The resulting T_3 homozygous transgenic lines were selected for further analyses. Firstly, seeds from three transgenic T3 lines (#1, #2, and #3) and the WT were surface-sterilized and plated onto a half-strength Murashige and Skoog agar to germinate and grow for 7 d, after which they were transferred into 10-cm-diameter pots containing compost soil. After another 7 d of growth at 22 °C, an irradiance of 47 μmol m^{-2} s^{-1}, and a 16-h photoperiod, the seedlings were watered with 100 cm^3 of 0, 50, 100, 150, 200, or 250 mM NaCl solution every 2 d for 20 d, and then the survival rate was determined. In addition, 7-d-old seedlings were grown on a half-strength Murashige and Skoog medium supplemented with 0, 50, 100, 150, or 200 mM NaCl under the same environmental conditions, and the root lengths and the MDA content of samples were measured following the protocol as described by Wang et al. (2010) at 20 d. Malondialdehyde was extracted with trichloroacetic acid and detemined using thiobarbituric acid. Each sample contained at least 12 plantlets, and 3 biological repetitions were performed.

Statistical analysis: All experiments were repeated three times. Values represent means ± standard errors (SEs) of three independent experiments. Differences were analyzed by *ANOVA* using the *SPSS18.0* program. Significance of differences was evaluated at the probability level $\alpha = 0.05$.

Results

The 867 bp cDNA fragment of *ItERF* (GenBank number: MF508603) was cloned by real-time PCR from *I. typhifolia.* The cDNA fragment containing an open reading frame encoded a 288 amino-acid residue protein with a 31.82-kDa molecular mass (Fig. 1*A*). The ItERF protein contained a conserved 58-amino acid ERF DNA-binding domain and a conserved 7-amino acid sequence motif referred to as the WV (A) AEIRD box. The ERF domain comprised three β-sheets and an α-helix (Fig. 1*B*). We speculate that the ItERF protein belongs to the EREBP-like branch of the *RAP2* gene family (Okamuro *et al*. 1997). Sequence alignment analysis shows that ItERF shared 58 - 78 % identity with the AP2/ERF domain from different plants (Fig. 1*A*). Multiple sequence alignments and phylogenetic analysis revealed that ItERF had the

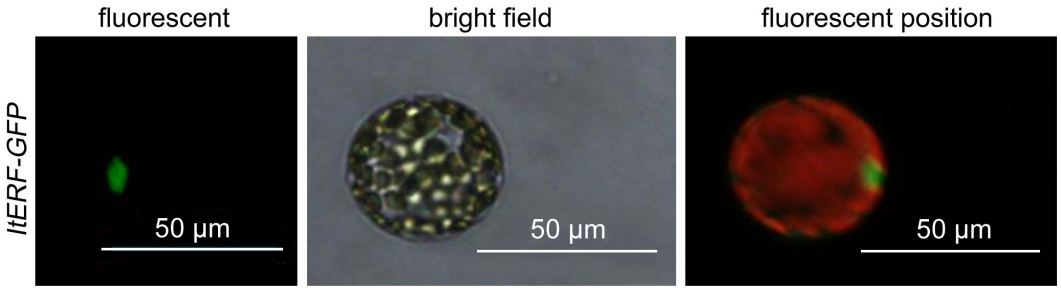

Fig. 3. Subcellular localization of *Iris typhifolia* ethylene responsive factor (ItERF) using green fluorescent protein (GFP). The magnification is 200.

greatest amino acid sequence homology with *Lycopersicon esculentum* LeERF4 and *Arabidopsis* AtERF5 (Fig. 1*C*).

An *ItERF* relative expression was examined by quantitative PCR after 48 h of treatment with 150 mM NaCl (Fig. 2). Under normal growth conditions, the lowest *ItERF* expression in the stems (the highest ΔCt value) was used as a calibrator (designated as 1) to determine relative expressions in the other tissues (Fig. 2*A*). With 150 mM NaCl stress, leaf *ItERF* mRNA increased gradually over time and reached its highest value at 24 h, and this value was 4.65-times higher than that in the control without NaCl. However, the *ItERF* expression decreased at 48 h (Fig. 2*B*). In contrast, *ItERF* expression in roots decreased initially and then increased and peaked after 24 h of treatment (Fig. 2*C*). The expression in stems was not significantly changed (Fig. 2D). These results show that the *ItERF* gene participated in salt stress responses in roots and leaves. Moreover, *ItERF* expression strongly correlated with salt stress in roots and leaves.

Analysis using *PSORT* predicted that *ItERF* localized to the nucleus (47.8 %), extracellularly including the cell wall (21.7 %), and in the mitochondria (8.7 %). To confirm the subcellular localization, the *ItERF*-GFP fusion gene driven by the *cauliflower mosaic virus 35S* promoter was transformed into *A. thaliana* by *Agrobacterium*-mediated

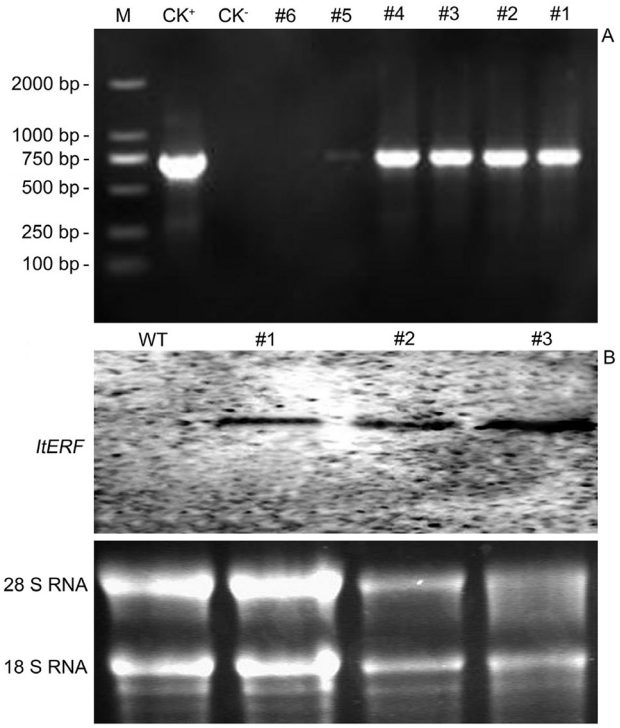

Fig. 4. Identification of *Iris typhifolia ethylene responsive factor* (*ItERF*) in transgenic *Arabidopsis* plants: *A* - PCR amplification of *ItERF* and *B* - Northern blot analysis of *ItERF* expression in leaves of wild-type (WT) and transgenic lines. After isolating the total RNA, 20 μg of it was loaded in each lane. Photographs of the ethidium bromide-stained gel that was used as loading controls. M - marker (5 kb), CK^+ - positive plasmid, CK^- - wild-type.

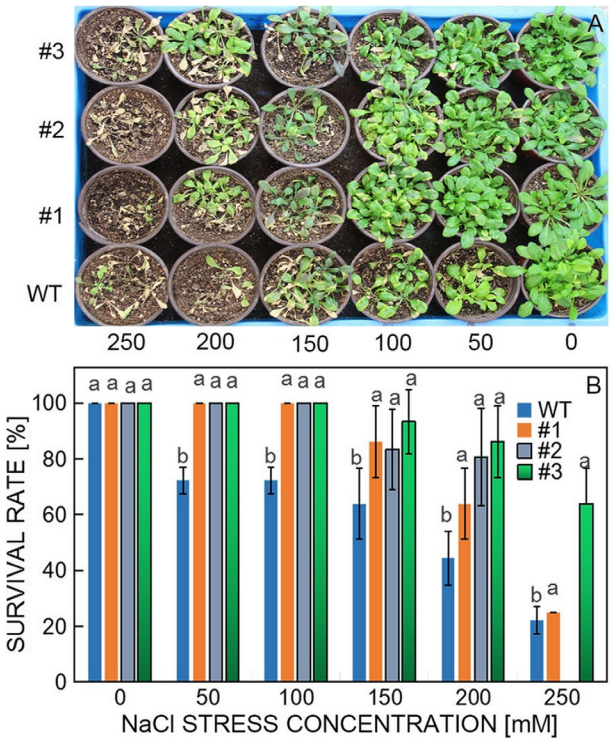

Fig. 5. Transgenic plants overexpressing *Iris typhifolia ethylene responsive factor* (*ItERF*) exhibiting tolerance to NaCl stress: *A* - growth of wild-type (WT) and transgenic *Arabidopsis* lines (#1, 2, and 3); *B* - survival rates of WT and transgenic *Arabidopsis* lines (#1, #2, and #3) after salt stress. Means ± SEs, $n = 3$, different letters indicate significant differences according to *ANOVA* ($P < 0.05$).

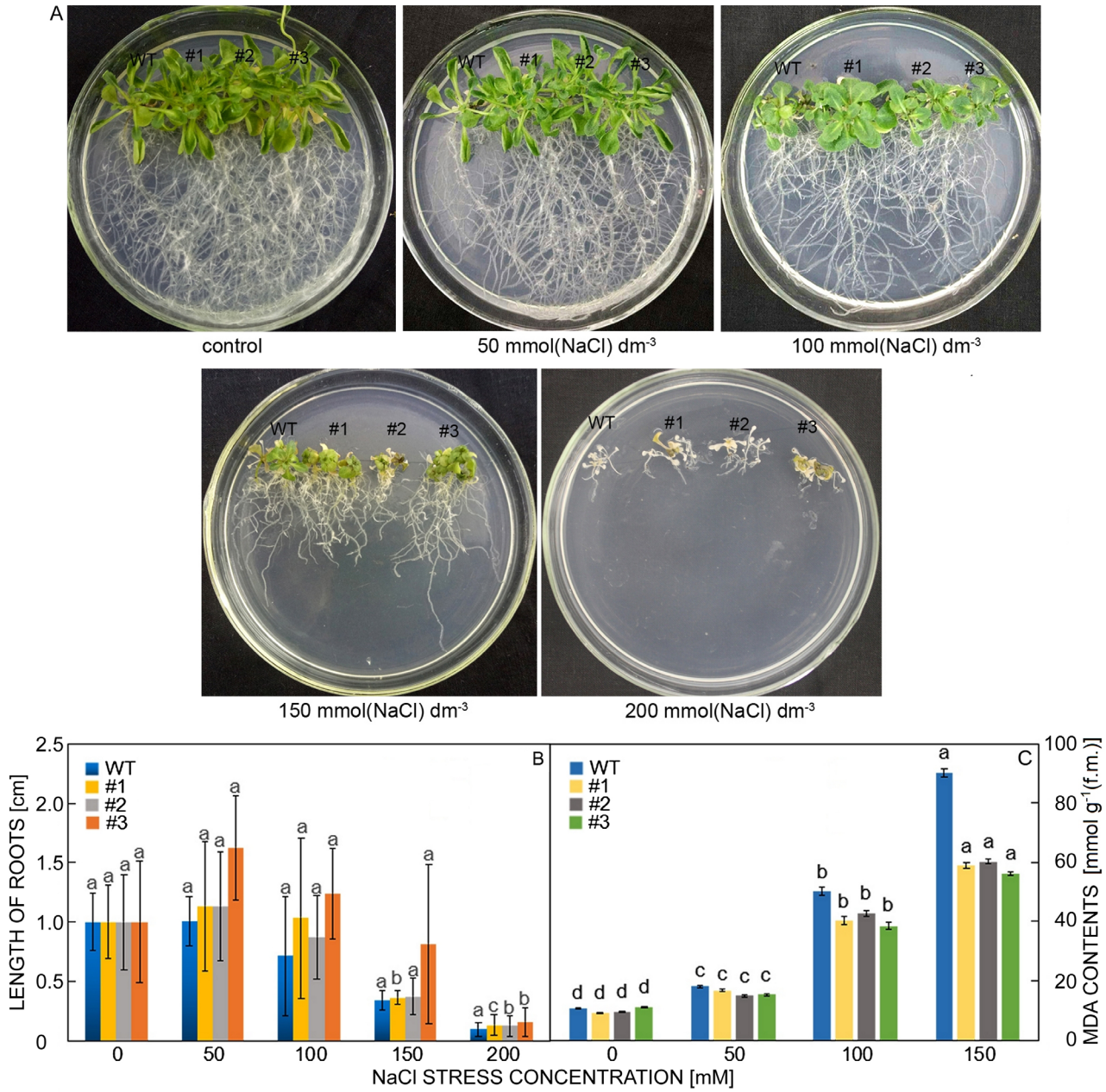

Fig. 6. Resistance of transgenic *Arabidopsis* lines and wild-type (WT) to 0, 50, 100, 150, and 200 NaCl applied to *in vitro* culture after germination for 20 d: *A* - plantlet growth, *B* - lengths of roots, and *C* - malondialdehyde (MDA) content. Means ± SEs, $n = 3$, different letters indicate significant differences according to *ANOVA* ($P < 0.05$).

transformation. As shown in Fig. 3, the *ItERF*-GFP fusion protein was exclusively localized to the nucleus (Chen *et al.* 2009).

To further determine the functions of *ItERF* in responses to salt stress, we generated T_3 homozygous transgenic *Arabidopsis* plants overexpressing *ItERF* and confirmed its expression using PCR of genomic DNA (Fig. 4*A*). Northern blot analysis detected high amounts of *ItERF* mRNA transcripts in the transgenic lines but none in the WT (Fig. 4*B*). Three representative transgenic lines (lines #1, #2, and #3) with medium and high *ItERF* expression levels were selected for the subsequent salt tolerance experiment.

Arabidopsis plants (20-d-old) with apparently normal growth characteristics were used for analysis of tolerance to salt stress (Fig. 5*A*). Under normal growth conditions, a plant survival rate was almost 100 %. During exposure to low salt stress (50 and 100 mM NaCl), almost all of the plants from the three transgenic lines kept growing vigorously whereas the WT plants became withered, chlorotic, and bleached. As the salt concentration increased to 150 and 200 mM NaCl, the WT survival rate decreased significantly whereas more than 60 % of the transgenic plants survived and continued to grow (Fig. 5*A*,*B*). These

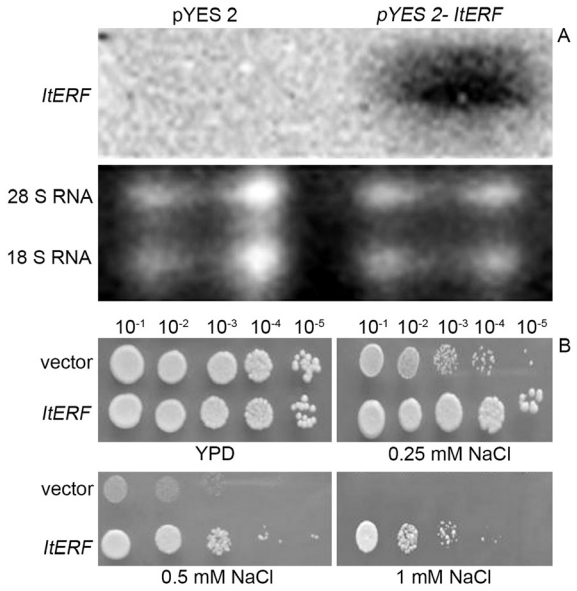

Fig. 7. Overexpressionof *Iris typhifolia ethylene responsive factor* (*ItERF*) in yeast cells and their resistance to NaCl stress. *A* - The overexpression of *ItERF* in yeast cells was confirmed by Northern blot analysis. *B* - Yeast cells containing the plasmid pYES2-*ItERF* or only the vector pYES2 were incubated in yeast peptone glucose medium (YPG) with different concentrations of NaCl at 30 ° and growth was monitored after 3 d.

results indicate that the *ItERF* gene increased tolerance to salt stress in transgenic *Arabidopsis*.

To examine the salt tolerance of transgenic plants overexpressing *ItERF*, we compared the root lengths of the transgenic and WT plants grown on Murashige and Skoog plates with or without NaCl. The root lengths of the transgenic and WT plants were similar under normal growth conditions indicating that overexpression of *ItERF* did not obviously affect plant growth or the phenotype (Fig. 6*A*). Salt treatment significantly inhibited plant growth; however, the transgenic lines showed higher root lengths in comparison with the WT (Fig. 6*A*,*B*). Content of MDA serves as an indicator of plant cell oxidative damage; NaCl stress often increases MDA content in plants. Content of MDA was low and not significantly different between the WT and overexpressing lines not treated with NaCl. However, MDA content positively correlated with NaCl concentration and was significantly higher in the WT than in the transgenic lines. Overexpression of *ItERF* alleviated salt stress-initiated oxidative stress in plant cells.

Two transformed yeast lines were constructed. One was transformed with an empty vector pYES2 as a control and the other was transformed with a recombined plasmid *ItERF* (Fig. 7*A*) as confirmed by Northern blot analysis. Under each growing condition, the cells were plated at five serial dilutions (corresponding to the five columns in each panel in Fig. 7*B*). Under control conditions, the growth of the yeast cells transformed with pYES2-*ItERF* was similar to that with the pYES2 empty vector. At 1 mM NaCl, the pYES2 vector transformant could hardly grow. By contrast, the *ItERF* transformant grew well. At 0.5 mM NaCl, the pYES2 vector transformant did not grow well compared with the *ItERF* transformant. These results indicate that yeast cells expressing *ItERF* were more resistant to NaCl stress.

Discussion

To date, most of the *ERF* family members have been identified in certain plant species including *Arabidopsis* (Oñate-Sánchez and Singh 2002), tomato (Tournier *et al*. 2003), and tobacco (Fischer and Dröge-Laser 2004); but only a few members have been cloned and characterized. In this study, a novel *ERF* gene, *ItERF*, was identified from *I. typhifolia*. Like ERF proteins from other plant species, ItERF has a conserved ERF DNA-binding domain, which consists of three antiparallel β-sheets and an α-helix (Fig. 1*B*). Phylogenetic analysis indicated that ItERF has the highest amino acid sequence homology to *Lycopersicon esculentum* LeERF4 and *Arabidopsis* AtERF1/2/5 (Fig. 1*C*). Cellular compartmentalization is an important regulatory mechanism for some transcriptional factors (Igarashi *et al*. 2001). Most of the ERFs identified so far are localized to the nucleus (Park *et al*. 2001, Zhang *et al*. 2009, 2010). In this study, the *PSORT* software was used to predict the subcellular localization of ItERF. The probability of the ItERF amino acid sequence containing a nuclear sorting signal was predicted at 47.8 %. We constructed the plant expression vector pBI121-*ItERF*-GFP that contained a fusion of *ItERF* with GFP. Following transformation of the plasmid DNA into *Arabidopsis*, we found that the fusion protein was expressed only in the nucleus (Fig. 3).

Increasing evidence suggests that ERFs can act as activators of transcription. Tobacco NtERF2/4, *Arabidopsis* AtERF1/2/5, and potato StEREBP1 have been found to act as transcriptional activators (Zhou *et al*. 1997, Fujimoto *et al*. 2000, Lee *et al*. 2007). Expression of *ItERF* was induced in the roots, stems, and leaves of *I. typhifolia* after salt treatment (Fig. 2). The leaves and roots had significantly higher *ItERF* expressions than the stems indicating that *ItERF* was involved in salt stress tolerance. Therefore, plants may respond to salt stress by increasing *ItERF* expression in exposed roots and leaves.

In previous studies, overexpressions of several *ERF* genes, such as *GmERF7*, *HvEAF*, and *ERF96*, improved the tolerance of transgenic plants to NaCl. These studies provided the rationale for testing the salt tolerance of *ItERF* transgenic plants (Jung *et al*. 2007, Zhang *et al*. 2009, Zhai *et al*. 2013, Wang *et al*. 2017). In this study, transgenic *Arabidopsis* overexpressing *ItERF* had an increased tolerance to salt stress. The *ItERF* transgenic lines treated with NaCl showed increased survival rates (Fig. 5) and root growth in comparison with the WT plants. Content of MDA in the transgenic lines was significantly lower than in the WT (Fig. 6). In order to better prove that transgenic plants have a higher salt tolerance, we did yeast related experiments (Ardie *et al*. 2009). Expression of *ItERF* in the transgenic yeast has revealed that the recombinant yeast exhibited a higher stress tolerance to salt than the

control yeast cells (Fig. 7).

In conclusion, our results suggest that ItERF acts as a transcription activator in the nucleus. It is involved in multiple signal transduction pathways by activating the expression of stress-related genes through binding to the GCC-box. The *ItERF* can improve the salt tolerance of plants.

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