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Effect of *LeERF1* and *LeERF2* overexpression in the response to salinity of young tomato (*Solanumlycopersicum* cv. Micro-Tom) seedlings

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Abstract Ethylene responsive factors (ERFs) are important transcriptional regulators involved in plant responses to abiotic stress. *LeERF1* and *LeERF2*, two members of the ERF family in tomato (*Solanum lycopersicum*), have previously been cloned. In this study, we investigated the salt-stress tolerance of transgenic tomato overexpressing *LeERF1* and *LeERF2*. The transgenic lines had longer roots than wild-type (WT) plants under salt stress conditions. Furthermore, we examined physiological and biochemical indexes in the plants and found that overexpression of *LeERF1* and *LeERF2* enhanced the release of chlorophyll and free proline, but decreased the malondialdehyde contents of the plants. Transgenic tomato displayed higher superoxide dismutase and guaiacol peroxidase activity than WT tomato under high salinity conditions. Moreover, quantitative RT-PCR analysis revealed that the expression levels of salt stress-related genes, including *TAS14*, *HVA22*, *LHA1*, *PR5*, and *RBOHC*, which were upregulated in the transgenic plants. Therefore, overexpression of *LeERF1* and *LeERF*2 positively modulates the ethylene-mediated response to salt stress in tomato.

Keywords *LeERF1* · *LeERF2* · Salt tolerance · Stress-regulated genes · Transgenic tomato

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Introduction

Abiotic stress restricts the growth and development of plants. Although the approaches and mechanisms used by plants to deal with adverse environmental conditions differ, the strategies employed by plants under these conditions are basically the same (Potters et al. 2009). Among the various sources of abiotic stress, soil salinity presents an increasing threat to plants and agriculture (Stevens et al. 2006). Therefore, improving salt tolerance in plants is a significant task of stress-resistance research.

In recent years, the genetic engineering of stress tolerant plants has become an increasing focus of study; ethylene has surfaced as one of the most popular research issues (Cramer et al. 2011). Ethylene responsive factors (ERFs) function as transacting elements in plant ethylene responses. ERFs, which regulate downstream genes by binding to their *cis*-acting elements, play important roles during plant development and increase a plant's ability to fight against environmental stress (Mizoi et al. 2012). ERFs are members of the AP2 (APETALA2)/ERF family, a unique transcription factor family in plants. ERF subfamily members (comprising 65 members in *Arabidopsis thaliana*) contain a well-conserved DNA-binding domain (Allen et al. 1998). Previous 3D structural analysis of the ERF/AP2 domain in *AtERF1* showed that this domain consists of a three-stranded antiparallel β-sheet that can bind to the GCC box complex in the *cis*-acting elements of

downstream genes, as well asan α-helix located approximately parallel to the β-sheet (Allen et al. 1998). ERF genes are not only induced by ethylene, but they can also respond to NaCl (Zhang et al. 2004), wounding (Tournier et al. 2003) and other abiotic stressors. For example, in rice, the overexpression of *OsBIERF1* to *OsBIERF4* (*Oryza sativa* benzothiadiazole-induced ERF) increases plant resistance to various abiotic stress conditions (Cao et al. 2006). In addition, overexpression of *JcERF*, a transcription factor isolated from *Jatrophacurcas*, increases salt and freezing tolerance in transgenic *Arabidopsis* (Tang et al. 2007). Moreover, genome-wide expression analyses of ERF subfamily members have been performed in poplar (*Populus trichocarpa*), soybean (Zhuang et al. 2009), tomato (Sharma et al. 2010) and rice (Sharoni et al. 2011), which revealed that these genes are induced by low or high temperature, dehydration or high salinity. Some EREBP/AP2-type transcription factors protect plants from pathogen attack as well as osmotic stress, such as *Tsi1* in tobacco (Grichko and Glick 2001). These results indicate that ERFs play an important role in abiotic stress responses.

In a previous study, we isolated *LeERF1* and *LeERF2* from tomato fruits and found that their products were able to specifically bind to the tomato osmotin promoter GCC box (Tournier et al. 2003). Sequence analysis clearly indicated that these factors were capable of specifically binding to GCC box-containing *cis*-elements and that they belong to the large ERF family of transcription factors, which are unique to plants (Tournier et al. 2003). Subsequently, transgenic tomatoes overexpressing *LeERF1* and *LeERF2* were obtained. Although *LeERF1* and *LeERF2* were previously isolated and characterized, the function of these genes in salt resistance is still unclear.

In this study, we used Micro-Tom tomato (*Solanum lycopersicum* cv. Micro-Tom) as model plant (Marti et al. 2006). To further explore the roles of *LeERF1* and *LeERF2* in tomato under salt stress, we employed transgenic tomato overexpressing *LeERF1* and *LeERF2*. We measured physiological and biochemical indexes such as chlorophyll, malondialdehyde (MDA) and proline content, as well as superoxide dismutase (SOD) and guaiacol peroxidase (POD) activity, in the transgenic plants under high salinity conditions. Moreover, we also analyzed the expression of salt stress-related genes in transgenic tomato by qRT-PCR, including *TAS14*, *HVA22*, *LHA1*, *PR5* and *ROBHC*. Specifically, *TAS14*, encoding a tomato dehydrin, is induced by NaCl treatment in leaves (Godoy et al. 1994). *HVA22* encodes a protein synthesis inhibitor that shares little homology with any of the reported ABA-inducible genes or cycloheximide (Shen et al. 2001). *LHA1*, which encodes plasma membrane (PM) H^+-ATPase, increases PM H^+-ATPase activity under salt stress conditions (Tomasi et al. 2009). *PR5* is a pathogenesis-related genes that is associated with systemic acquired resistance (Ward et al. 1991). Finally, *ROBHC*, a member of the respiratory burst oxidase homologs (RBOH) family, is induced by abiotic stresses and protects the plants from damage (Cramer and Jones 1996). The results of this study help elucidate the molecular signaling pathway that functions during stress responses and reveal possible candidate genes that can be used to breed stress-resistant plants.

Materials and methods

Plant materials and treatments

Tomato seeds (*Solanum lycopersicum* cv. Micro-Tom; obtained from INP-ENSA Toulouse, France) were employed, including seeds from wild type (WT) and transgenic plants that were homozygous and overexpressed *LeERF1* or *LeERF2*. The seeds were pretreated using several steps. First, the seeds were surface-sterilized in 75 % ethanol for 30 s and washed 3–5 times in sterile water. The seeds were then immersed in 5 % NaClO for 15 min and washed 3–5 times with sterile water. The entire process was performed under a laminar flow hood. Finally, the seeds were placed on filter paper and germinated in a growth chamber at 25 ± 2 °C for 3–4 days. The seeds were used for further experiments after germination; the lengths of the roots were approximately 0.5 cm.

The seeds were transferred to MS medium and the roots were pressed gently after sterilizing. The lengths of the roots were measured after 5 days. Approximately 100 seeds were used per line. MS medium supplemented with NaCl (0, 100 and 150 mM) was applied to both the WT and transgenic plants. All of the plants were grown in a plant growth chamber under a 14 h photoperiod at a daytime temperature of 25 ± 2 °C with a light intensity of 250 mmol m^{-2} s^{-1} and a nighttime temperature of 20 ± 2 °C; the relative humidity was 80 %.

A 4-week-old tomato plants were used for salt-stress treatment under greenhouse conditions at 25 ± 2 °C with a day/night cycle of 16/8 h, 80 % relative humidity and 250 μmol m^{-2} s^{-1} light intensity. Culture solution (Yamasaki nutrient solution) (Prescott et al. 1992) with NaCl (0, 100 and 150 mM) was applied to the plants. Control plants were irrigated with nutrient solution only. The fourth true leaves of select lines were harvested and frozen immediately in liquid nitrogen after 0, 5 and 15 days for further experiments. There were three replicates for each salt concentration and each period; six plants were used per replicate.

cDNA preparation and expression analysis by qRT-PCR

RNA was extracted using Trizol reagent according to the manufacturer's instructions (Invitrogen, USA), and DNase treatment was performed using a value of 2 μg DNA/sample. Total RNA (~10 g per lane) was separated in 1.2 % agarose-formaldehyde gels prior to purification, and the gels were stained with methylene blue to assess the quality and quantity of the RNA. Then, cDNA was reverse transcribed from 2 μg RNA per sample using a RevertAid^{TM} First-strand cDNA Synthesis Kit (Fermentas, UK).

Using the *actin* gene as the internal standard and the cDNA as the template, qRT-PCR was performed in an ABI PRISM 7000 Sequence Detection System (Applied Biosystems) using Bio-Rad Chromo 4 (USA). The PCR amplification conditions were as follows: 95 °C for 10 min, 95 °C for 15 s and 60 °C for 60 s, with 40 cycles per step. Three biological and three technical replicates were performed for each reaction. The gene-specific primers are described in Table 1. The data were analyzed using the comparative CT method (Schmittgen et al. 2008).

Measurement of chlorophyll and carotenoid contents

Leaf chlorophyll and carotenoid contents were determined after salt treatment for 0 and 15 days according to the methods of Tang (Tang et al. 2007). Briefly, 0.3 g of fresh leaves was weighed and extracted with 25 ml of 95 % alcohol for 36 h in the dark. The extracts were examined using a V/VIS 752 Spectrophotometer; the absorbance was measured at 663, 645 and 470 nm.

Assay of MDA and proline contents

The MDA content was estimated according to the methods of Hara (Hara et al. 2003). A 1 g of tissue was ground in liquid nitrogen and combined with 10 % TCA and 6 % TBA. The absorbance was determined at 440, 532 and 600 nm using a V/VIS 752 Spectrophotometer.

Proline levels were determined according to Bates; the absorbance was determined at 520 nm (Bates et al. 1973).

Determination of antioxidant enzyme activities

POD and SOD in leaves were extracted according to the methods of Beyer and Fridovich (Beyer Jr and Fridovich 1987) with minor modifications; 0.5 g of leaves were ground in ice-cold 5 ml PBS (0.05 M, pH 7.8). The supernatant was collected after centrifugation (10,000*g*, 20 min) for further analysis.

POD activity was assayed at 470 nm in 1 ml of reaction mixture containing 0.3 % H_2O_2. The reaction was started by adding 0.05 ml of enzyme extract to the reaction mixture.

SOD activity was assayed using the NBT method according to Beyer (Beyer et al. 1994).

Results

Root length in transgenic tomato under salt stress

The transgenic tomato lines with overexpression of *LeERF1* (L1, L2 and L3) and *LeERF2* (L4, L5 and L6)

Table 1 Gene-specific primers were used in the qRT-PCR

Genes	Accession no.	Primers	Sequence (5′–3′)
LeERF1	AY077626.1	*Le-ERF1F*	CGGTATCATCAGCTTCGGAAA
		Le-ERF1R	TCTCAACTTCTAATTCGGCTTGCT
LeERF2	AY496704.1	*Le-ERF2F*	GTTCCTCTCAACCCCAAACG
		Le-ERF2R	TTCATCTGCTCACCACCTGTAGA
TAS14	NM001247109.1	*TAS14F*	CTCTAGCTCGTCGGAGGATGAT
		TAS14R	CTTCATGTTGTCCAGGCATCTTC
HVA22	XM004250118.1	*HVA22F*	GATATTTGTGGCATGGCTAGTT
		HVA22R	TTGGATTTGGCTTTAGGAGAC
LHA1	NM001247846.1	*LHA1F*	CTGAGGAAGCGAAGAGGAGA
		LHA1R	CGAGACCCTTCAACTTCACA
PR5	XM004238172.1	*PR5F*	ATTGTTGCACTCAAGGTCCA
		PR5R	CTTGTTGGATCGTCTTGAGG
RBOHC	NM001247342.1	*RBOHCF*	GACATTGTTTCTGGCACGAG
		RBOHCR	TCCAACTTTAGCCTCTGGGT
Actin	AB695290.1	*SlactinF*	TGTCCCTATTTACGAGGGTTATGC
		SlactinR	CAGTTAAATCACGACCAGCAAGAT

were employed for germination experiments. The mRNA levels of the *LeERF1* and *LeERF2* genes in the transgenic tomato lines were shown in Fig. 1. The lines (L2 and L4) which had high expression level were subjected to RNA analysis and physiological studies.

Seeds of WT and transgenic tomato were germinated on MS medium supplemented with NaCl (100 and 150 mM). As is known to us, the root is the most sensitive organ in the plant for perceiving salt stress (Jaleel et al. 2009). After 5 days, the lengths of the roots were measured. As shown in Fig. 2, there was no obvious difference in root length between the WT and transgenic plants in the absence of NaCl. As the concentration of NaCl increased, the root growth was significantly inhibited in both the WT and transgenic plants. However, under 100 mM of NaCl conditions, the inhibition of roots in WT was greater than in transgenic plants. As shown in Fig. 2b, compared to the length of roots in WT, the ratio was 2.269 and 2.608 of *LeERF1* and *LeERF2*, respectively. Indicating that salt stress-induced inhibition of root elongation was attenuated in the transgenic lines. These results suggest that transgenic tomato overexpressing *LeERF1* and *LeERF2* had greater salinity tolerance than WT.

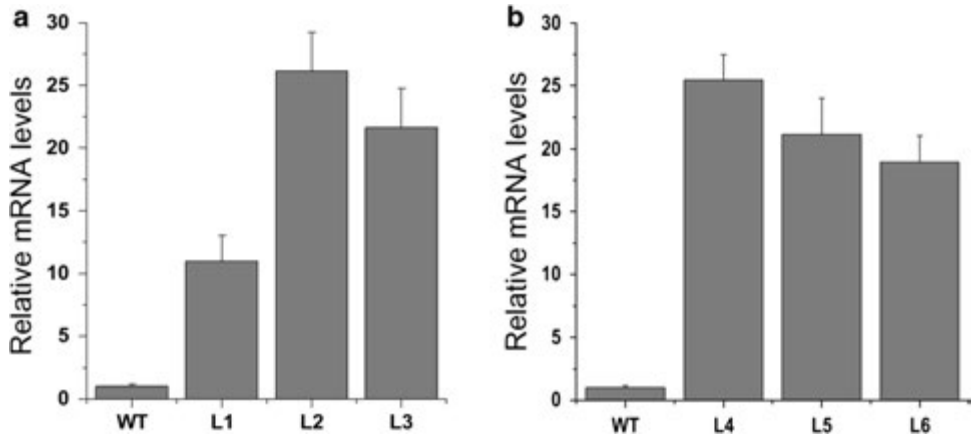

Fig. 1 qRT-PCR analysis of mRNA expression levels of the *LeERF1* and *LeERF2* genes in WT and transgenic tomato. The results are the mean ± SD of three individual measurements. Standard errors are indicated by *vertical bars*. **a** The expression levels of the *LeERF1* gene. L1, L2 and L3 are *LeERF1*-overexpressing transgenic tomato lines. **b** The expression levels of the *LeERF2* gene. L4, L5, L6 and L7 are *LeERF2*-overexpressing transgenic tomato lines

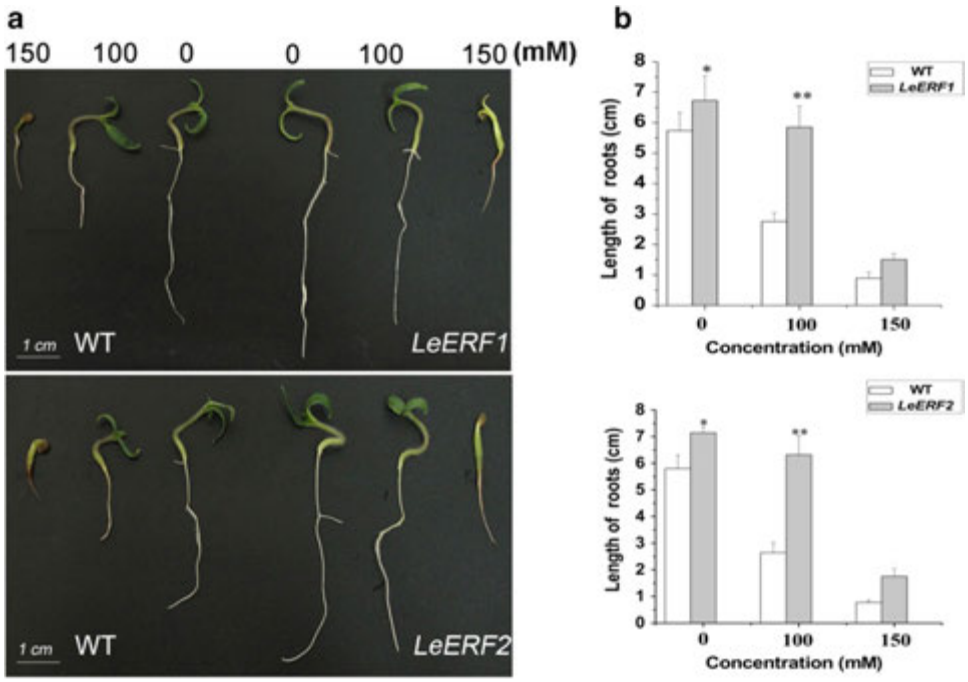

Fig. 2 Root elongation of WT and transgenic tomato grown on MS medium supplemented with 100 and 150 mM NaCl for 5 days. The lengths of roots in **a** WT and *LeERF1*-overexpressing transgenic tomato and **b** WT and *LeERF2*-overexpressing lines under salt stress. Standard errors are indicated by *vertical bars*. *Asterisks* indicate a significant difference at $*P < 0.05$ or $**P < 0.01$ levels as determined by *t* test

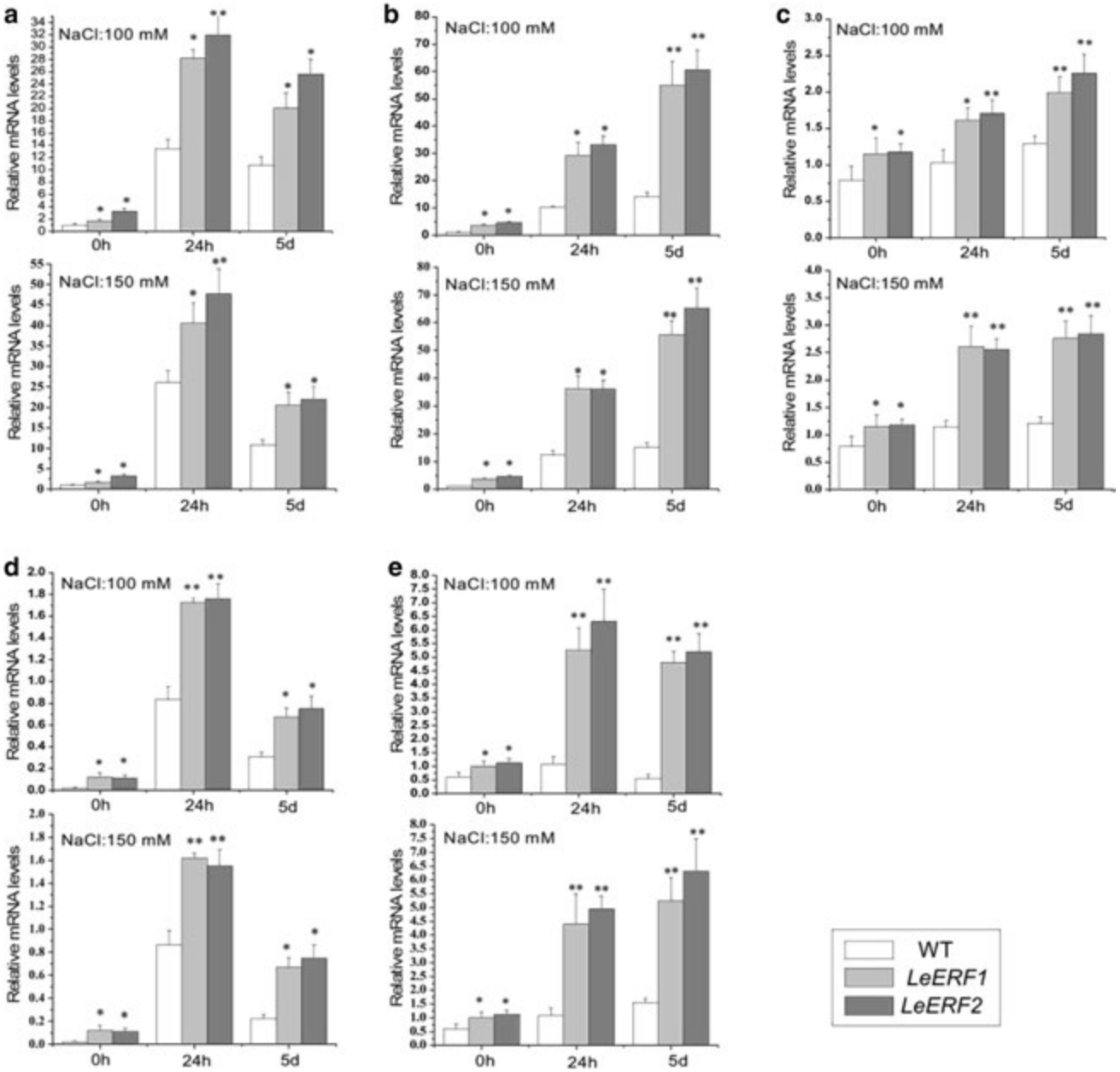

Fig. 3 Expression profiles of stress-related genes in WT and transgenic tomato under salt-stress conditions. **a** *TAS14*; **b** *HVA22*; **c** *LHA1*; **d** *PR5*; **e** *RBOHC*; the *actin* gene was used as an internal control. The results are the mean ± SD of three individual measurements. Standard errors are indicated by *vertical bars*. *Asterisks* indicate statistical difference at the $*P < 0.05$ or $**P < 0.01$ level as determined by *t* test

The expression levels of salt-related genes in tomato under salt stress

To further identify the roles of *LeERF1* and *LeERF2* in tomato under salt stress, we analyzed the expression levels of several stress-related genes, including *TAS14*, *HVA22, LHA1, PR5* and *RBOHC*, in WT and transgenic tomato using qRT-PCR. We examined the expression levels at 0, 24 h and 5 days, as which had done in a previous study (Sharma et al. 2010). As shown in Fig. 3, in plants not subjected to salt treatment at 0 h, the overexpression of *LeERF1* and *LeER*F2 strongly increased the mRNA expression levels of *TAS14, HVA22, LHA1, PR5* and *RBOHC* as compared to WT. After 24 h, the expression levels of these five genes were significantly upregulated under NaCl treatment (at both 100 and 150 mM). In addition, after 5 days of treatment, the tomato leaves had higher or similar levels of *HVA22, LHA1* and *RBOHC*

expression as compared to those after 24 h of treatment. However, the transcript levels of *TAS14* (Fig. 3a) and *PR5* (Fig. 3d) decreased at day 5 rather than staying at a relatively high level. As a whole, the expression levels of the five genes were notably higher in the transgenic tomato plants under salt stress than in the WT. These results indicate that these genes are mutually regulated and suggest that *LeERF1* and *LeERF2* play important roles in the plant response to abiotic stress.

Changes in tomato pigments under salt stress

Salt stress has severe effects on the efficiency of photosynthesis. In addition, salt stress can also reduce the effects of photosynthetic assimilation. One of the main reasons for this is that salt stress can accelerate the degradation rates of photosynthesis-related components (Moradi and Ismail 2007). Hence, chlorophyll and carotenoid contents are important indexes for evaluating salt tolerance in plants (Larré et al. 2013). Therefore, we investigated the effects of salt stress (using various concentrations of NaCl) on the contents of pigments including chlorophylls and carotenoids in WT and transgenic tomato, as shown in Fig. 4.

There were no marked differences in pigment contents between WT and transgenic plants grown in the absence of supplemental NaCl. However, the contents of chlorophyll a, total chlorophyll and carotenoids were significantly higher in transgenic plants overexpressing *LeERF1* and *LeERF2* than in WT plants under 100 and 150 mM NaCl conditions (Fig. 4a, c, d), whereas NaCl treatment did not have a noticeable effect on the chlorophyll b contents in WT or transgenic plants (Fig. 4b).

Effects of salt stress on MDA and proline contents in tomato

The concentration of MDA in a plant can reflect the effect of salt stress on membrane-lipid peroxidation and indicate the degree of damage in the cell membrane (Sairam et al. 2005). Figure 5a shows the contents of MDA in WT and transgenic tomato subjected to various concentrations of NaCl for 15 days. We observed an upward trend for MDA content in both WT and transgenic plants with increasing NaCl concentration. However, the variation in MDA content was larger in WT than in the transgenic plants. Moreover, the MDA contents in transgenic plants overexpressing *LeERF1* and *LeERF2* were markedly lower than those in WT plants under 100 and 150 mM NaCl treatment (Fig. 5a), which indicated that the transgenic plants had lower levels of lipid peroxidation than

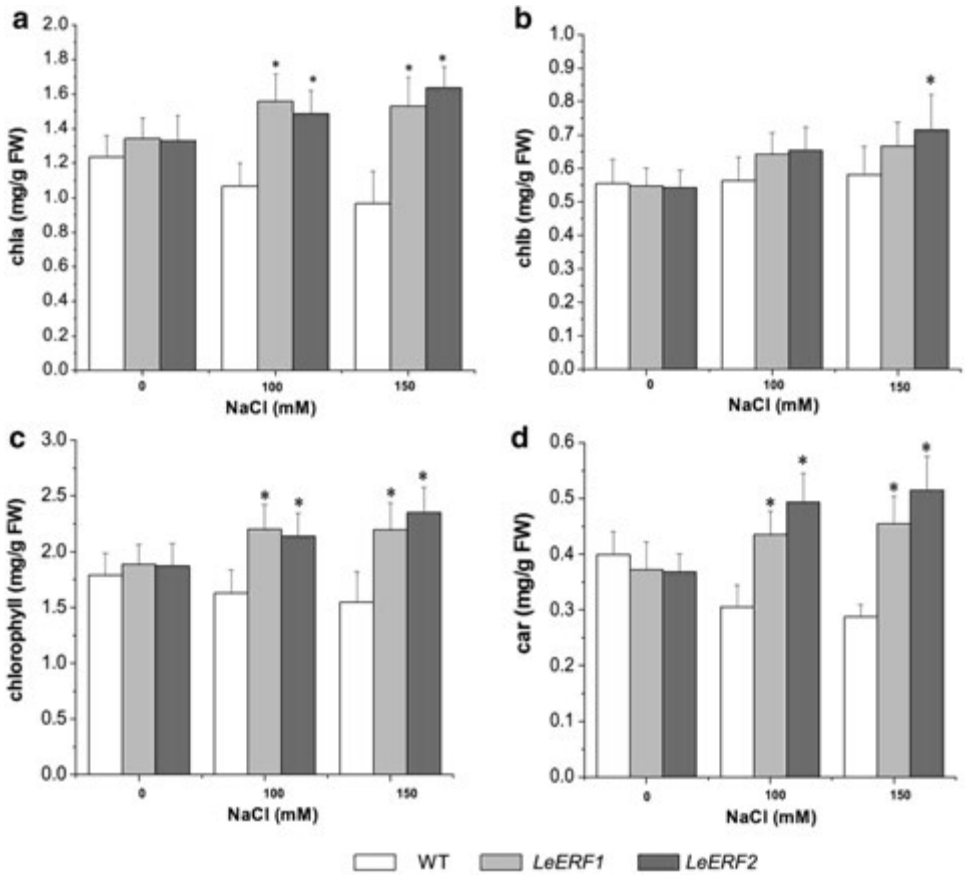

Fig. 4 Contents of chlorophylls and carotenoids in WT and transgenic tomato under various levels of NaCl treatment for 15 days. **a** Contents of chlorophyll a, **b** chlorophyll b and **c** total chlorophyll in leaves of WT, *LeERF1* and *LeERF2* transgenic plants under salt stress. **d** Contents of carotenoids in leaves of WT, *LeERF1* and *LeERF2* transgenic plants under salt stress. Independent experiments were performed in triplicate. The results are the mean ± SD of three individual measurements. Standard errors are indicated by *vertical bars*. *Asterisks* indicate statistical difference at the $*P < 0.05$ or $**P < 0.01$ levels as determined by *t* test

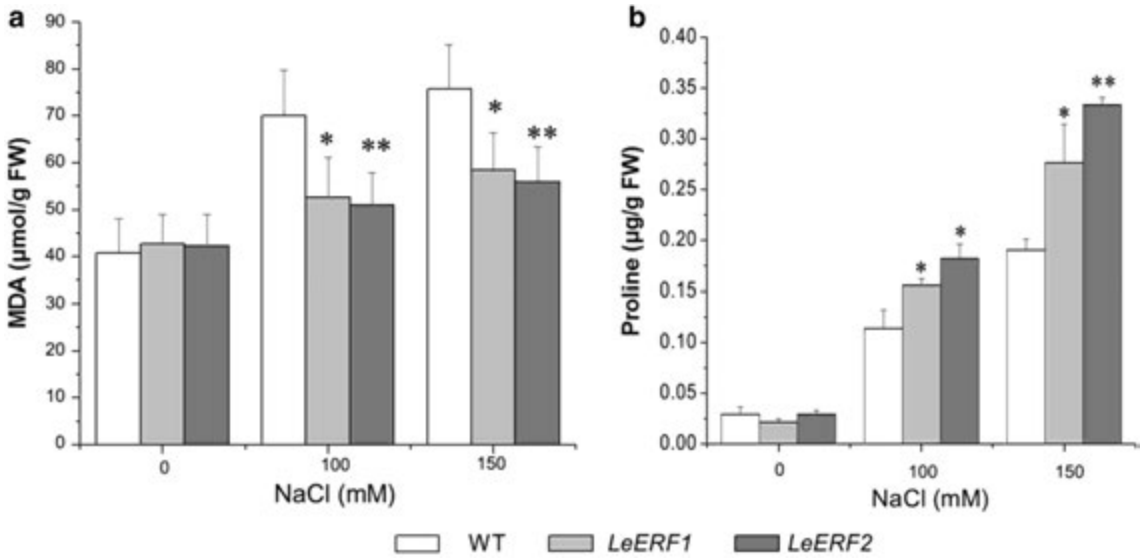

Fig. 5 Contents of MDA and proline in WT and transgenic tomato plants under various levels of NaCl treatment for 15 days. **a** Contents of MDA and **b** contents of proline in WT and transgenic tomato plants under various concentrations of NaCl treatment for 15 days; independent experiments were performed in triplicate. The results are the mean ± SD of three individual measurements. Standard errors are indicated by *vertical bars*. *Asterisks* indicate statistical difference at the $*P < 0.05$ or $**P < 0.01$ levels as determined by *t* test

WT, which was helpful to maintain the normal functioning of membranes.

Proline, a biochemical indicator of plant stress tolerance, can help maintain the osmotic pressure in the cell and maintain the integrity of the cell membrane (Liu and Zhu 1997). Figure 5b shows the contents of proline in WT and transgenic tomato grown under various concentrations of NaCl for 15 days. It was difficult to detect proline in both WT and transgenic plants under normal conditions due to the low proline content in tomato. However, the free proline contents rose sharply with increasing NaCl supply. Furthermore, the concentrations of proline in the transgenic lines were significantly higher than that in WT plants under salt stress conditions. As mentioned above, higher proline contents can increase salinity tolerance. Therefore, we can conclude that transgenic plants that overexpress *LeERF1* and *LeERF2* have better salt tolerance than WT, which the latter has stronger effect.

Effects of salt stress on antioxidant enzymes in tomato

SOD and POD are the main antioxidant enzymes that protect membrane-lipid peroxidation in organisms. As shown in Fig. 6a, the SOD activity was similar in WT and transgenic plants in the absence of salt treatment. However, as the supply of salt increased, the SOD activity increased rapidly in the transgenic plants while it remained nearly unchanged in WT. In addition, very little POD activity was detected in WT or transgenic plants in the absence of salt treatment, whereas POD activity was obviously higher in the transgenic lines than in WT plants under salt-stress conditions.

Discussion

In this study, the results revealed that overexpression of *LeERF1* and *LeERF2* alleviated the inhibitory effects of salt on root growth at a NaCl concentration of 100 mM. This result is consistent with a previous study indicating that overexpression of *ERF* enhanced tolerance to salt stress in tomato. However, there was no significant difference in root length at a NaCl concentration of 150 mM. This result suggests that there may be different responses to salt stress among different tomato genotypes.

Overexpression of ERF family members can increase the mRNA levels of salt-related genes under various salt-stress conditions (Véry and Davies 2000; Tomasi et al. 2009; Kalifa et al. 2004; Basu et al. 2002). As shown in Fig. 3, we selected five genes representing all phylogenetic groups for expression analysis under various salt-stress conditions, including *RBOHC, TAS14*, *HVA22*, *PR5* and *LHA1*. Previous studies have indicated that these genes exhibit differential accumulation patterns in response to salt treatment. We found that the expression levels of these genes were higher in the transgenic plants than in WT plants under the same salt-stress conditions. These results may be due to the fact that the overexpression of *LeERF1* and *LeERF2* can increase the expression levels of several genes that help increase salt tolerance and positively

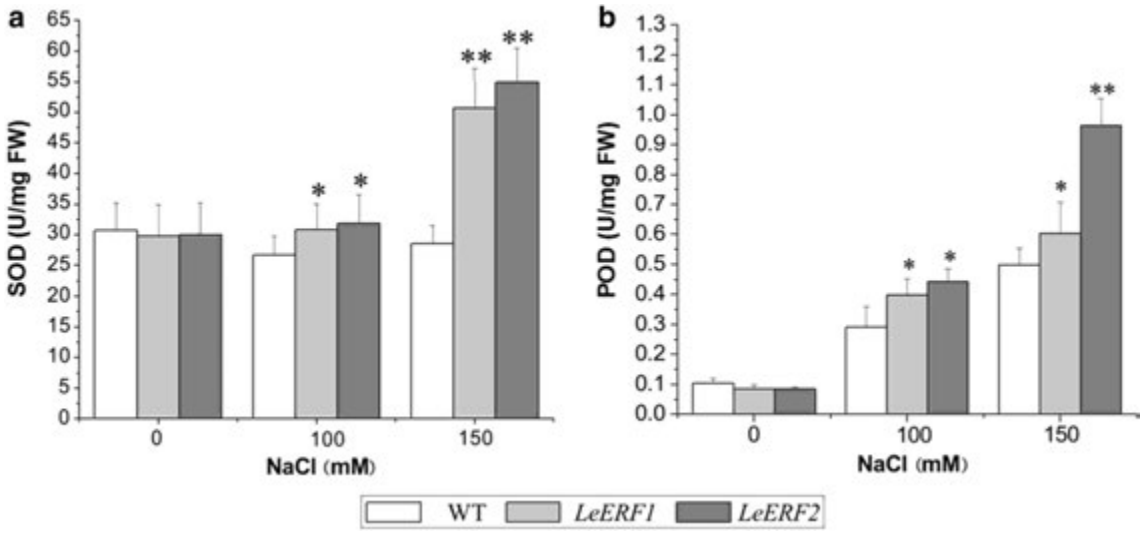

Fig. 6 SOD and POD activity in WT and transgenic tomato plants under various levels of NaCl treatment for 15 days. **a** SOD activity in WT and transgenic tomato under salt stress; **b** POD activity in WT and transgenic tomato under salt stress; independent experiments were performed in triplicate. The results are the mean ± SD of three individual measurements. Standard errors are indicated by *vertical bars*. *Asterisks* indicate statistical difference at the $*P < 0.05$ or $**P < 0.01$ levels as determined by *t* test

mediate the activation of salt-stress signaling in tomato plants. The results also indicate that *LeERF1* and *LeERF2* can activate the stress response in plants.

Under a variety of abiotic stress, reactive oxygen species (ROS) are one of the most important and earliest signals of the response. Increasing evidence (Kunkel and Brooks 2002; Sakuma et al. 2002; Singh 2002) has demonstrated the function of ROS in abiotic stress signaling pathways. With the increase in electron transport in plants under salt stress, much more ROS are produced by chloroplasts and mitochondria, which leads to oxidative damage and causes chlorophyll degradation, membrane structure disfiguration and protein and nucleotide denaturation, ultimately resulting in cell death (Fukao and Bailey-Serres 2004). In our work according to the method, the forth leaf of tomato plants was used for index detection. The fresh weight of these leaves was about 150 mg. The results indicated that transgenic plants overexpression *LeERF1* and *LeERF2* had significantly higher contents of chlorophyll and carotenoids than in WT plants with salt (100 and 150 mM) (Fig. 4a, c, d). Because maintaining higher levels of chlorophyll and carotenoid means the lower level of ROS in the transgenic plants, meanwhile, salt stress inhibits the rate of photosynthesis by decreasing the cellular water potential, and the rate of water evaporation decreases as the salinity level increases in the leaves, which results in a decline in chlorophyll content (Moradi and Ismail 2007). These indicated that transgenic plants overexpression *LeERF1* and *LeERF2* were more tolerant to salt stress than WT plants.

Oxidative stress is one of the main factors that influence plant growth (Lin and Kao 2000). Figure 5 shows that the overexpression of *LeERF1* and *LeERF2* elevated the free proline levels, but decreased the MDA content in tomato. On a weight basis, transgenic tomato displayed higher SOD and POD activities than WT tomato under high salinity conditions (Fig. 6). Under these circumstances, ROS can be eliminated by POD and SOD in the cell, which would increase the plant's resistance to salt stress (Sairam et al. 2005). MDA content is one of the most important indexes that indicate oxidative stress in injured cells. Proline is regarded as a free radical scavenger and it also represents a source of carbon and nitrogen in the cell membrane (Ehsanpour and Fatahian 2003; Lutts et al. 1996). Again, our results suggest that the physiological indexes were much better in the transgenic plants than in WT tomato, which indicates that *LeERF1*- and *LeERF2*-overexpressing transgenic plants have stronger resistance to salt that WT.

Indeed, the overexpression of *LeERF1* and *LeERF2* can lead to many changes in plant physiological indexes, as well as the increased expression of several stress-related genes. The results of this study indicate that the overexpression of *LeERF1* and *LeERF2* can enhance salt resistance in tomato. However, it is important to clarify the mechanisms of these regulatory steps and to understand how *LeERF1* and *LeERF2* affect the expression of downstream genes under salt stress. Furthermore, the interaction between *LeERF1* and *LeERF2* will be an important subject of future studies.

Author contribution NH Jointly conceived the study with ZL, performed and designed experiments, analyzed data and wrote the manuscript. ZL helped with further

experimentation and revisions to the manuscript. NT contributed to data analysis and manuscript writing. FY wrote the paper. All authors read and approved the final version of the manuscript.

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