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ORIGINAL PAPER

Identification of ERF genes in peanuts and functional analysis of *AhERF008* and *AhERF019* in abiotic stress response

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Abstract Ethylene-responsive factor (ERF) play an important role in regulating gene expression in plant development and response to stresses. In peanuts (*Arachis hypogaea* L.), which produce flowers aerially and pods underground, only a few ERF genes have been identified so far. This study identifies 63 ERF unigenes from 247,313 peanut EST sequences available in the NCBI database. The phylogeny, gene structures, and putative conserved motifs in the peanut ERF proteins were analysed. Comparative analysis revealed the absence of two subgroups (A1 and A3) of the ERF family in peanuts; only 10 subgroups were identified in peanuts compared to 12 subgroups in *Arabidopsis* and soybeans. AP2/ERF domains were found to be conserved among peanuts, *Arabidopsis*, and soybeans. Outside the AP2/ERF domain, many soybean-specific conserved motifs were also detected in peanuts. The expression analysis of ERF family genes representing each clade revealed differential expression patterns in response to biotic and abiotic stresses. Overexpression of *AhERF008* influenced the root gravity of *Arabidopsis*, whereas overexpression of *AhERF019* enhanced tolerance to drought, heat, and salt stresses in *Arabidopsis*. The information generated in this study will be helpful to further investigate the function of ERFs in plant development and stress response.

Keywords ERF family · Gene function · Phylogeny · Peanut · Stress response · Plant development

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Introduction

Plant-specific TFs of the APETALA2/ethylene response factor (AP2/ERF) superfamily are defined by the presence of a conserved AP2/ERF domain consisting of approximately 60 amino acid residues (Ohme-Takagi and Shinshi 1995; Nakano et al. 2006). AP2/ERF transcription factors, which are characterised by the presence of the AP2/ERF DNA-binding domain, play significant roles in regulating plant biotic and abiotic stress-responsive gene expression (Sakuma et al. 2002). AP2/ERF genes comprise a large superfamily that has been divided into three groups (AP2, ERF, and RAV) based on their sequence similarities and number of AP2/ERF domains (Nakano et al. 2006). ERF family proteins contain a single AP2/ERF domain and can further be divided into two major subfamilies, namely, the CBF/DREB and the ERF subfamilies (Sakuma et al. 2002). Genes in the CBF/DREB subfamily have been observed to play an important role in enhancing abiotic stress tolerance by recognising the dehydration-responsive element (DRE) with a core motif of A/GCCGAC (Yamaguchi-Shinozaki and Shinozaki 1994; Thomashow 1999; Xu et al. 2011) and are involved in the response to biotic stresses, such as pathogenesis, through the recognition of the *cis*-acting element AGCCGCC, known as the GCC box (Hao et al. 1998; Xu et al. 2011). The proteins of the CBF/DREB and ERF subfamilies can be grouped into six subgroups: A-1 to A-6 and B-1 to B-6, respectively (Sakuma et al. 2002). The availability of genomic sequences has resulted in the identification of ERF family transcription factors in various plant species, including *Arabidopsis* (Liu et al. 1998; Nakano et al. 2006), rice (Cao et al. 2006), cotton (Huang et al. 2007; Jin and Liu 2008), soybeans (Zhang et al. 2008), tomatoes (Sharma et al. 2010), cucumbers (Hu and Liu 2011), and Chinese wild grapevine (Zhu et al. 2013). The roles of the ERF and CBF/DREB proteins in the response to biotic and abiotic stresses have also been extensively documented (Gutterson and Reuber 2004; Agarwal et al. 2006; Mizoi et al. 2012.). For example, Sub1A is an ERF-like protein that confers tolerance to submergence and drought in rice by affecting ethylene synthesis (Xu et al. 2006; Fukao et al. 2011). It has been observed that rice ERF proteins (namely, SNORKEL1 and SNORKEL2) can trigger remarkable internode elongation via gibberellins to avoid submergence (Hattori et al. 2009). These proteins in *Arabidopsis* (WIN1/SHN1), *Medicago truncatula* (WXP1/2), and rice (WR1) induce the production of epidermal waxes when overexpressed in plants (Aharoni et al. 2004; Broun et al. 2004; Zhang et al. 2005, 2007; Wang et al. 2012). Similarly, ERF activators such as CBF1/DREB2A, DREB1A, and OsDREB1F could enhance tolerance to salt, drought, and low temperatures in both rice and *Arabidopsis* (Kasuga et al. 1999; Wang et al. 2008). Despite our understanding of the potential role of DREBs/ERFs in improving crop stress tolerance, the exact functions of the majority of the DREBs/ERFs are still unknown. Therefore, identification and functional characterisation of new DREB/ERF genes will provide useful information on their potential role in peanuts, in which only a few members of the ERF family have been characterised.

Peanuts (*Arachis hypogaea* L.) grow in more than 100 countries throughout the world and serve as an important source of oil and protein. Aside from its importance in agriculture and food security, the peanut is also a plant species that produces flowers aerially but buries the recently fertilised ovules into the soil to produce pods underground. Peanuts are comparatively more tolerant to various stresses than most other related plant species. Ethylene is a key signalling molecule that regulates a variety of developmental processes and stress responses in plants (Philosoph-Hadas et al. 2005; Buer et al. 2006; Licausi et al. 2013). Studies focused on the ethylene transduction pathway in peanuts would be useful to better understand pod development and the peanut plant's superior stress tolerance. Although six ERF family transcription factor genes (*AhERF1–6*) were cloned and their expression levels during abiotic stress in peanuts were analysed (Chen et al. 2012), their functions remain unknown. Therefore, the present study focused on acquiring additional information regarding the AP2/ERF superfamily in peanuts using the peanut ESTs in the NCBI database, which resulted in the identification of 63 members in this superfamily (including 24 DREB subfamily and 39 ERF subfamily members). Furthermore, phylogenetic and protein motif structural analyses of the ERF and CBF/DREB subfamilies were also conducted along with the expression profiling of all of the peanut ERF genes. Additionally, the biological function of an ERF gene *AhERF019* was investigated in transgenic *Arabidopsis*.

Materials and methods

Sequence data and data processing

A total of 247,313 expressed sequence tags (ESTs), which were used as the primary sequence data set, were retrieved from the NCBI website (http://www.ncbi.nlm.nih.gov/nucest) on 20 September 2012. The Transeq program from the EMBOSS package was used to translate the DNA sequences into protein sequences. Based on the HMMER user's guide (http://hmmer.wustl.edu/; Version 2.3.2; Oct 2003), the Hmmpfam program was used to annotate various domains in the query sequence followed by the use of the Hmmfetch program to retrieve an HMM as a seed model from an HMM database, including the AP2/ERF domain. Finally, the Hmmalign program was used to align multiple EST sequences to the seed profile HMM to obtain EST sequences containing the AP2/ERF domain. We sequenced the target

complementary deoxyribonucleic acid (cDNA) clone in our cDNA library and performed a PCR using one primer complementary to the library vector and another primer complementary to the target gene using our yeast two-hybrid cDNA library as a template. We also executed a blast search with our transcriptome data to identify any missing sequences of the ERF genes that did not have a full-length CDS. To confirm the full-length genes, primers were designed as shown in ESM Table 1. To clone the full-length genes, total RNA was reverse-transcribed using the SuperScript™ II Reverse Transcriptase (Invitrogen, USA). The full-length open reading frame (ORF) was confirmed by sequencing more than three clones. The soybean gene data set was downloaded from the DFCI Soybean Gene Index (http://compbio.dfci.harvard.edu/tgi/cgi-bin/tgi/gimain.pl?gudb=soybean).

Alignment, phylogenetic analysis, and motif detection

All similarity searches were executed using the BlastN, BlastX, or BlastP tools at the NCBI database and MEGA 5.1. Conserved domain searches were performed against the conserved domain database at NCBI (http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi?). A phylogenetic tree was constructed with the aligned peanut AP2/ERF protein sequences using MEGA 5.1 and the neighbour-joining (NJ) method with the following parameters: Poisson correction, pairwise deletion, and bootstrapping (1,000 replicates; random seed). Motif detection was performed using MEME (http://meme.sdsc.edu/meme/meme.html) (Bailey et al. 2006).

Plant materials, stress treatments, and real-time PCR analysis

The peanut line '06-4104' (bearing the characteristics of high oil content and high resistance to bacterial wilt) was used in this study for expression analysis. *Ralstonia solanacearum* strain 2C1 was isolated from wilted peanut samples from the Hubei province (China). *R. solanacearum* was cultured at 200 rpm at 28 °C in PSA medium (200 g potato, 20 g sucrose, 3 g beef extract, 5 g tryptone, and 1 L of double-distilled H_2O) and homogenised in sterile 10 mM $MgCl_2$ for 36 h, with a calculated cell density of 108 cfu/mL [optical density at 600 nm (OD_{600}) = 0.8].

Plant treatments were performed at six fully expanded leaf stages. After seed germination, the seedlings were transplanted in sandy soil in growth chambers at 28 °C with a 14/10-h light/dark cycle. Flowering plants were used for tissue-specific expression analyses. Stress treatments were performed as follows: (1) the roots of the seedlings were immersed in water containing 200 mM NaCl for salt stress; (2) the seedlings were placed in a 4 °C growth chamber for cold treatment; (3) the root systems of intact plants were washed gently with water to remove soil and then placed on filter paper for rapid dehydration to induce drought stress; (4) seedlings were subjected to ABA, SA, Me-JA, and ET treatments by spraying with 100 μM ABA dissolved in 0.01 % ethanol, 100 μM SA in water, 100 μM JA in 0.01 % ethanol, and 100 μM ACC in water, respectively; and (5) the roots of the seedlings were immersed in an *R. solanacearum* suspension to promote *R. solanacearum* infection.

After exposure to one of these five stressors, peanut seedlings were harvested at various time points (0, 3, 6, 9, 12, 15, 18, 24 h), frozen in liquid nitrogen, and kept at –80 °C until further analysis. Total RNA was isolated from these samples at various time points with an RNA extraction kit (Tiangen Biotech, Beijing, China) according to the manufacturer's instructions. Poly(A) + RNA was used as the template for synthesis of the initial cDNA strands using reverse transcriptase (Invitrogen). Gene-specific primers (ESM Table 1) were designed to avoid any conserved regions. The specific primer pair (5′-TAAGAACAATGTTGCCATACAGA-3′ and 5′-GTTGCCTTGGATTATGAGC-3′) for peanut actin was used as an internal control, and real-time PCR were performed using the Bio-Rad IQ5 tHERMAL cycler.

Plant transformation and stress tolerance analysis of transgenic *Arabidopsis* plants

AhERF008 and *AhERF019* were introduced into the *Xba* I and *Bam*H I restriction endonuclease sites of the pCAMBIA1307 (a derivative of pCAMBIA1300 carrying the 2*CaMV 35S promoter and the OCS terminator) vector to overexpress these genes using the primers listed in ESM Table 1. The vectors were then transformed into *Arabidopsis* Col-0 via the *Agrobacterium tumefaciens*-mediated floral dip method (Clough and Bent 1998). Transgenic plants were grown in soil under a 14/10-h light/dark cycle at 22/20 °C and 60 % relative humidity. Transformed lines were selected by growing them on one-half Murashige and Skoog (MS) agar plates containing 40 μg/mL hygromycin under similar growth conditions. Homozygous T3 progenies, which were bred from a T2 population segregated into three hygromycin-resistant individuals to one hygromycin-sensitive individual, were used for phenotypic analysis. *Arabidopsis* Col-0 was used as the wild-type control for comparison. Seeds harvested from both homozygous transgenic plants carrying *AhERF008*/*AhERF019* (OE lines) and *Arabidopsis* Col-0 were surface-sterilised, placed on plates of MS medium containing 0.5 % phytagel, and kept at 4 °C for 3 days to break dormancy. These plants were then transferred to growth chambers under a 14/10-h light/dark cycle at 22 °C and 70 % humidity. Five-day-old seedlings from the MS medium plates were transferred to either regular MS plates or MS plates containing 200 mM NaCl and 325 mM mannitol and incubated at 22 °C with a 14/10-h light/dark cycle for certain day to record the phenotypic data. For heat stress, transgenic seedlings that were grown in

the 90-cm MS solid plates for 14 days were incubated at 42 °C for 3 h and recovered at 22 °C for 1 week. All of the aforementioned experiments were conducted in triplicate to obtain reliable results.

Results

Identification of unigenes possessing an AP2/ERF domain in peanut

After conducting comprehensive analysis of 247,313 ESTs, a total of 63 unigenes that contained only one AP2/ERF domain(s) were identified. Details regarding the identified unigenes in the present study are shown in ESM Tables 2 and 3. All 63 unigenes identified were further classified into two subgroups based on the similarity of the amino acid sequences of the AP2/ERF domains. A total of 24 unigenes encoding CBF/DREB-like proteins were assigned to the CBF/DREB subfamily, whereas 39 unigenes encoding ERF-like proteins were assigned to the ERF subfamily. All 63 unigenes of the ERF family containing a complete AP2/ERF domain were further analysed and are referred to as *AhERF001* to *AhERF063* (ESM Tables 2 and 3).

Phylogenetic comparison of ERF factors from peanuts and soybeans

Zhang et al. (2008) compared the ERF factors from *Arabidopsis*, soybeans, and rice (*Oryza sativa*) and found that the AP2/ERF domains were conserved among these three plants. Outside the AP2/ERF domain, the majority of the motifs were conserved among *Arabidopsis*, soybean, and rice. Zhang et al. (2008) also identified several soybean-specific conserved motifs. To analyse the phylogenetic relationships of the peanut ERF family, multiple alignment analyses of the amino acid sequences of the 63 peanut and 98 soybean ERF proteins were performed as described by Zhang et al. (2008) (ESM Fig. 1) because peanuts and soybeans belong to the Leguminosae family. Residues Arg27, Gly31, Ala39, Ala40, Asp44, and Gly52 were completely conserved among the 161 proteins in both species (ESM Fig. 2). Additionally, more than 90 % of the ERF family members contain Gly5, Arg7, Arg9, Trp11, Gly12, Glu17, Ile18, Arg19, Trp29, Leu30, Ala42, Ala46, Ala47, Ala55, Asn59 Phe60, and Pro61 residues. Based on an alignment, an NJ phylogenetic tree was generated with bootstrap analysis (1,000 replicates). As shown in ESM Fig. 1 and ESM Fig. 3, the phylogenetic tree divided the ERF family proteins from soybeans into 12 subgroups, designated as A-1 to A-6 and B-1 to B-6, in accordance with the classification described by Zhang et al. (2008). However, subgroups A-1 and A-3, which are present in *Arabidopsis* and soybeans, were absent in peanuts, with 24 DREB subfamily members and 39 ERF subfamily members in peanuts. The number of ERF subfamily members was almost twice that of DREB subfamily members.

Conserved motifs outside of the AP2/ERF domain in peanuts

The conserved motifs of ERF family proteins in both peanuts and soybeans were investigated using MEME, and the results are listed in ESM Table 4. The majority of the members within the same group shared one or more motifs outside the AP2/ERF domain (ESM Figs. 4 and 5). Some of the conserved motifs identified in the *Arabidopsis* and soybean ERF families were also examined in the AhERF unigenes. For example, the ERF-associated amphiphilic repression (EAR) motif was identified in members of subgroup A-5 as the CMA-5-2 motif and in members of subgroup B-1 as the CMB-1-3 motif in both *Arabidopsis* and soybeans (ESM Fig. 6 and ESM Table 4). The MCGGAII/L motif, which was normally designated as CMB-1-1 and was identified as a transcriptional activation domain (Liu et al. 1999), was also found in peanuts (ESM Fig. 5 and ESM Table 4). Zhang et al. (2008) reported that there were some soybean-specific motifs in the ERF family upon comparison of soybean ERF factors with those in *Arabidopsis*. For example, the CMA-6-10 motif in subgroup A-6, the CMB-1-4 motif in subgroup B-1, and the CMB-2-2 motif in subgroup B-2 were identified in peanuts as CMA-6-9, CMB-1-4, and CMB-2-4 (ESM Fig. 7), respectively. The BlastP analysis with the motif sequence QNFIGFEQ from AhERF022 in the NCBI database identified several ethylene-responsive transcription factors from *Medicago truncatula* (Accession No. XP_003615022, XP_003638785, ACJ83281) and *Cucumis sativus* (Accession No. XP_004142609, XP_004163671), indicating that these motifs might be specific to legumes.

Expression profiles of peanut ERF genes in different tissues

Many important genes are selectively expressed in specific tissues during various physiological and developmental processes. Of the 247,313 peanut ESTs (*A. hypogaea* 178,490, *Arachis duranensis* 35,291, *Arachis ipaensis* 32,787, *A. hypogaea* subsp. *fastigiata* 745) analysed, 20.78 % (51,403), 18.49 % (45,745), 8.34 % (20,628), and 2.3 % (5,724) of the peanut ESTs were isolated from root, seed, developing embryos, and gynophore, respectively. Thus, approximately 50 % of the studied EST set was isolated from underground tissues. Our analysis found that among the 544 peanut ESTs that were shown to encode ERF factors, 36.03 % (196), 23.52 % (128), 5.14 % (28), and 3.13 % (17) were from roots, seeds, developing embryos, and gynophores, respectively. In total, 67.82 % of the verified ERF factors were from underground tissues, indicating the important role of peanut ERF factors in the development of underground tissues. To investigate the detailed spatial transcript profiles of the peanut

ERF genes, real-time PCR was used to detect the expression patterns of all of the peanut ERF genes in the roots, stems, leaves, gynophores, calyces, flowers, and seeds. The expression profiles of the 63 peanut ERF genes showed different patterns of tissue-specific expression (Fig. 1). All 63 peanut ERF genes except *AhERF045* could be detected in different tissues. A few of the peanut ERF genes observed showed high expression levels in one or more tissues, but low expression in others. For example, *AhERF008* and *AhERF037* showed higher expression levels in the seed, but lower levels in the other tissues. Similarly, *AhERF004*, *AhERF012*, *AhERF030*, and *AhERF036* were significantly expressed in the flowers.

Screening the stress-responsive genes by the expression patterns of the 63 peanut ERF genes under different stresses

Peanuts are extensively grown in various environments where several abiotic and biotic stresses could greatly affect their productivity and seed quality. The primary abiotic factors affecting peanut production include drought stress, salinity, and extreme temperatures, whereas the major biotic constraints for peanuts include diseases caused by fungi, viruses, bacteria, and nematodes. The phytohormones SA (salicylic acid), JA (jasmonic acid), and ET (ethylene) play crucial roles in biotic stress signalling upon pathogenic infection. Previous reports indicated a significant predominant role of the A group (CBF/DREB subfamily) of transcription factors in regulating the abiotic stress response, whereas the B group (ERF subfamily) is involved in biotic and/or abiotic stress responses. The expression pattern of peanut ERF genes was investigated in the present study using real-time PCR under various stress conditions, including drought, salt, cold, high temperature, submergence, ethylene, MeJA, SA, and ABA. The results showed a response for all 63 genes to at least one of these treatments. Of the 63 genes, 41 genes were related to high salt, 39 genes to drought, 38 genes to cold, 54 genes to heat stress, 30 genes to submergence, 31 genes to exogenetic ABA, 32 genes to ACC, 30 genes to MeJA, 21 genes to SA, and 23 genes to *R. solanacearum* stress treatments (ESM Table 5). Peanuts are a summer crop and are normally subjected to heat stress throughout their growth period; interestingly, we observed that 51 of the 63 peanut ERF genes were induced by heat stress. Seven genes, namely, *AhERF005*, *AhERF007*, *AhERF008*, *AhERF014*, *AhERF018*, *AhERF050*, and *AhERF052*, showed increased expression by 100-fold when the plant was subjected to heat stress (Fig. 2).

Overexpression of *AhERF008* conferred root gravitropism to transgenic *Arabidopsis* plants

The function of the peanut ERF factors has not been studied to date; thus, *AhERF008* from the A-4 subgroup was overexpressed in *Arabidopsis* to verify its function. We transferred the 5-day-old *AhERF008* overexpressing and wild-type seedlings to 90-cm plates containing MS medium. After 2 weeks, we noticed that *AhERF008* overexpressing plants displayed reduced root gravitropism (Fig. 3), indicating the involvement of *AhERF008* in plant gravity. Because *AhERF008* was highly expressed in the peanut seed and embryo, its role in peanut gynophore and seed development requires further analyses.

Overexpression of *AhERF019* increased tolerance to various abiotic stresses in transgenic *Arabidopsis*

To understand the role of peanut ERF genes in plant abiotic stress responses, the stress-inducible gene *AhERF019* was overexpressed in *Arabidopsis* under the control of the CaMV 35S promoter. Overexpression of *AhERF019* did not affect the development of *Arabidopsis* (Fig. 4g). Upon analysis of the *AhERF019* transgenic plants for heat stress, the 3-week-old plants were subjected to a temperature of 42 °C for 180 min, and observations were recorded after 7 days. The results showed that almost all of the leaves in the wild-type plants were bleached, but there were only a few bleached leaves in the transgenic lines (Fig. 4a, b). To investigate whether *AhERF019* was involved in osmotic stress responses, *AhERF019*-transgenic and wild-type *Arabidopsis* were transferred to plates containing 325 mM mannitol. The growth of the *AhERF019* transgenic plant was more vigorous compared to the wild type, as the relative root weight of the *AhERF019* transgenic plants ranged from 63 to 95 %, whereas the wild-type root weight was approximately 22 % (Fig. 4c, d), suggesting that *AhERF019* transgenic plants were more tolerant to mannitol stress. For salt tolerance, significant phenotypic differences between wild-type and transgenic plants were observed after 10 day of stress. Leaves from the wild-type plants gradually turned dark and lost their greenness; however, leaves from the transgenic plants turned dark but maintained their green colour (Fig. 4e, f). The survival rate of the transgenic plants ranged from 58 to 100 %, whereas the wild-type plant survival was approximately 35 %, indicating that the transgenic plants exhibited tolerance against salt stress.

Discussion

Peanuts are an important oil and food legume crop grown in over 100 countries. Peanut plants cover 24 million hectares worldwide, with a total production of 38 million tons in 2010 (FAOSTAT 2010). Unfortunately, their sustained production is severely hampered by several biotic and abiotic stresses, such as fungi, bacteria, viruses, insect pests, drought, salt, and heat stress. It is estimated that 30 % of the yield loss of peanuts is due to various diseases and adverse physiological

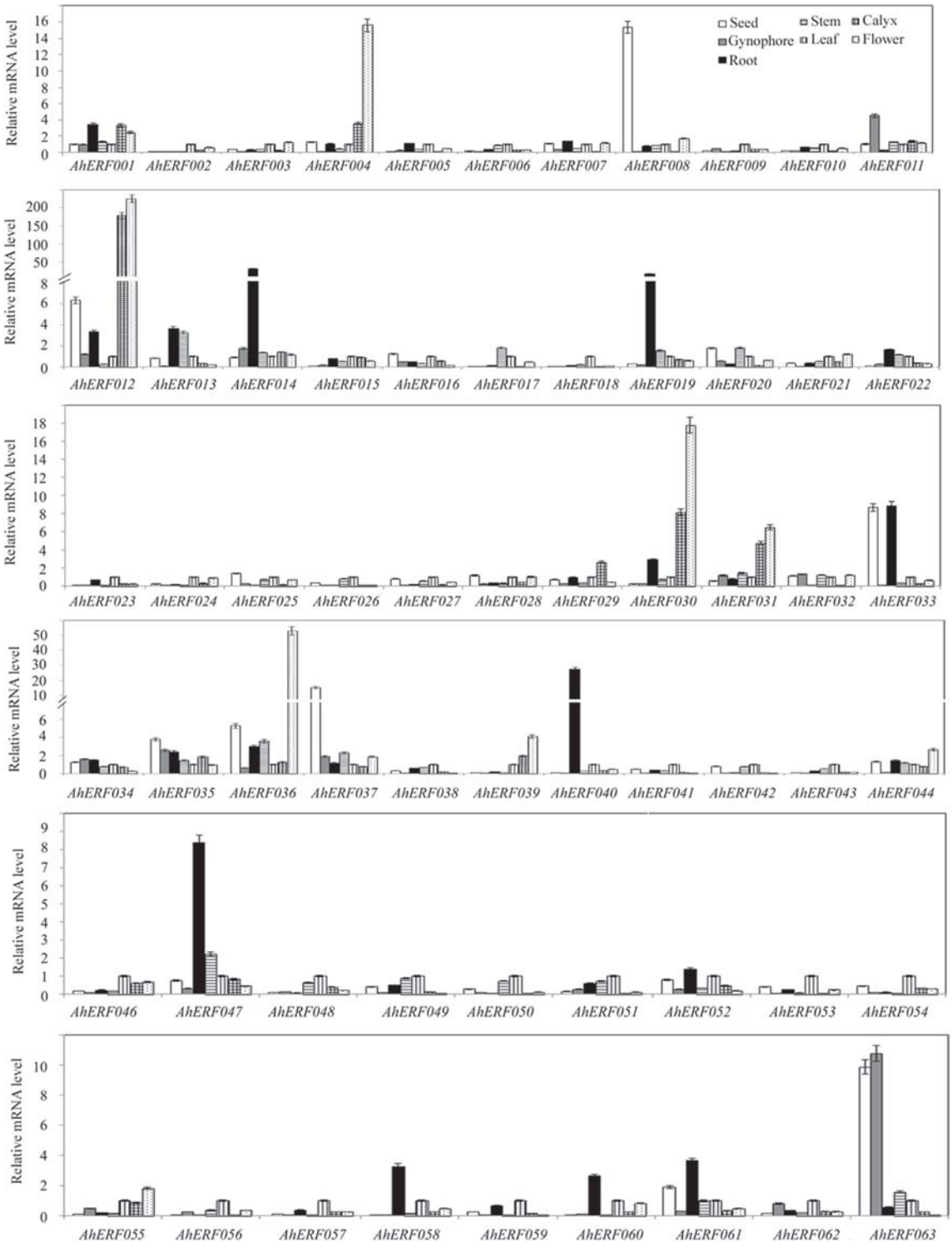

Fig. 1 Tissue expression patterns of peanut ERF genes. Total RNA was isolated from the peanut seed, gynophore, root, stem, leaf, calyx, and flower. Total RNA was reverse-transcribed into cDNA for use in real-time PCR analysis, with peanut actin as the control

conditions (Nelson et al. 1989). Thus, identification and characterisation of resistant genes in peanuts could provide insight into their functions, which would facilitate their judicious use in developing improved cultivars with higher resilience to these crippling stressors. Ethylene response factor (ERF) proteins play important roles in regulating the plant stress response and development.

Nakano et al. (2006) systematically surveyed the gene structure, phylogeny, and conserved motifs of the ERF gene family in *Arabidopsis* and rice, but relatively few peanut ERF

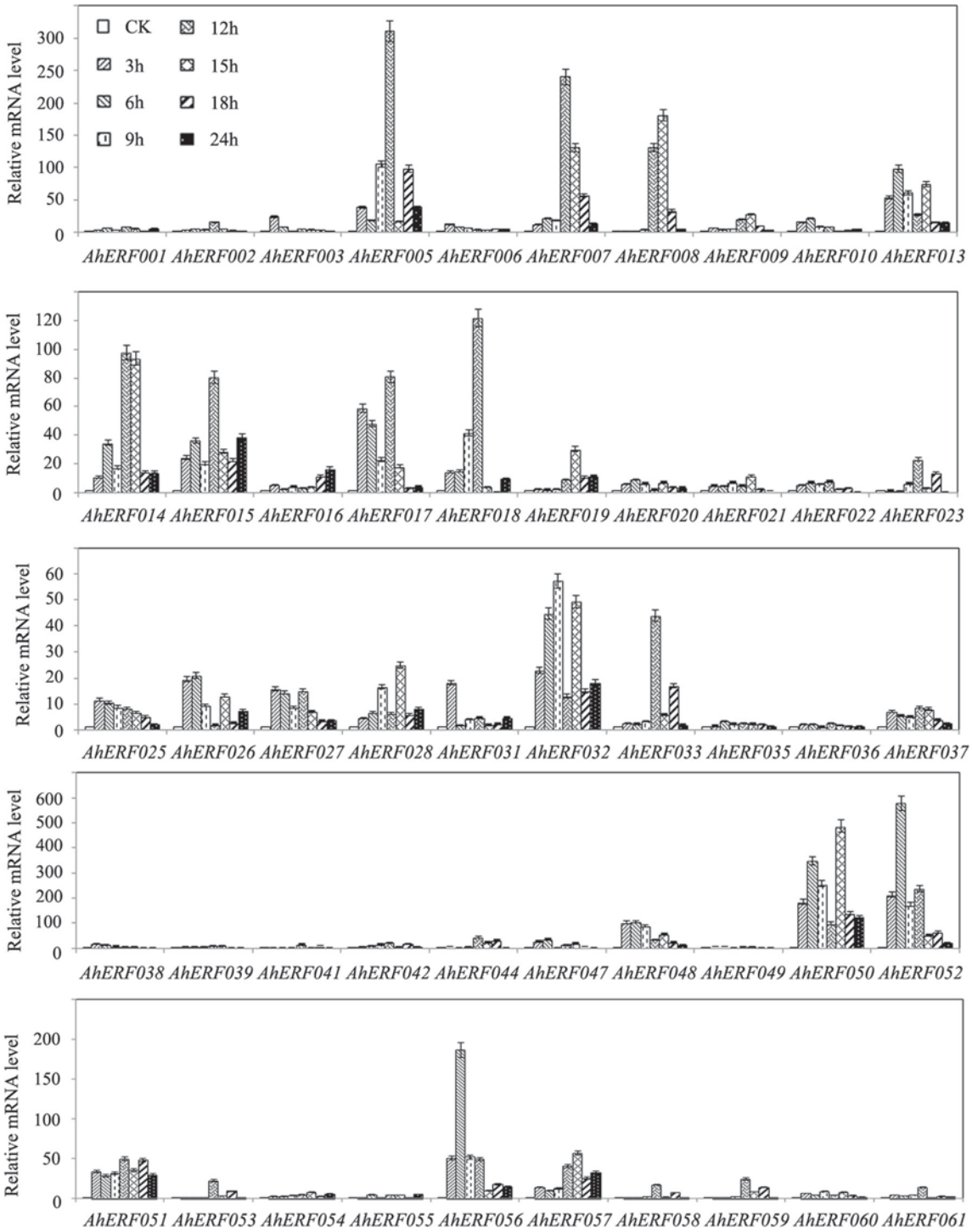

Fig. 2 Expression patterns of peanut ERF genes under heat stress. Total RNA was isolated from peanut seedlings exposed to 42 °C for the indicated amount of time. Total RNA was reverse-transcribed into cDNA for use in real-time PCR, with actin as the control

genes had been studied. To gain further knowledge regarding the ERF family in peanuts, ESTs of the AP2/ERF superfamily were identified from the NCBI EST database, including 63 members of the ERF family. Because we analysed the available peanut ESTs in the public domain, some ERF genes with low expression levels or tissue-specific expression patterns might have been missed in the present study, thus decreasing the likely number of ERF family members in peanuts. Some of the unigenes have high sequence similarities with registered proteins in the NCBI database (Supplementary Table 6). For example, *AhERF002*, *AhERF008*, *AhERF011*, *AhERF014*, *AhERF017*, *AhERF019*, *AhERF036*, and *AhERF044* encode

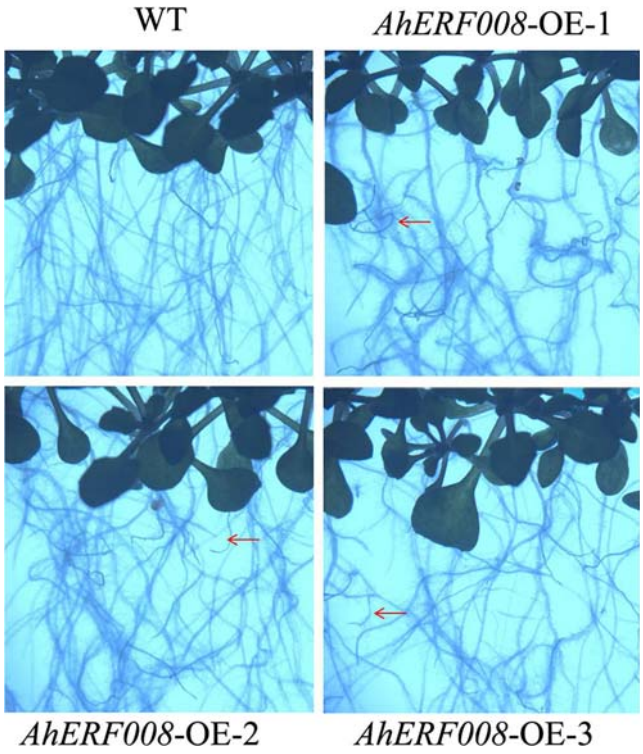

Fig. 3 AhERF008 conferred plant gravity. Five-day-old seedlings from MS medium plates were transferred to 90 cm MS plates and incubated at 22 °C on a 14/10-h light/dark cycle for 14 days to record any phenotypic changes

proteins that share 100 % amino acid sequence identities with registered peanut proteins (ESM Table 6). Therefore, the unigenes of the ERF family acquired in this study reflect the general status of ERF family members in peanuts and can be subjected to further analyses.

The conserved motif analysis of the ERF family demonstrated that most motifs were conserved in peanuts, soybeans, *Arabidopsis*, and rice. Proteins within a subgroup that share these conserved motifs are likely to have similar functions. For example, the EAR motif is essential for gene repression (Ohta et al. 2001; Yang et al. 2005). Zhang et al. reported that there were soybean-specific motifs in the ERF family when they compared soybean ERF factors with those in *Arabidopsis* such as the CMA-6-10 motif in subgroup A-6, the CMB-1-4 motif in subgroup B-1, and the CMB-2-2 motif in subgroup B-2. Interestingly, these motifs were all found in peanuts (ESM Fig. 7), suggesting that these motifs might be specific to leguminous plants. The actual function of these motifs requires additional study.

Plants have evolved many mechanisms to cope with a range of environmental stressors. There are multiple stress perception and signalling pathways, some of which are specific and others of which cross-talk at various stages. This signalling crosstalk not only occurs in biotic stress signalling but also in abiotic stress signalling (Kunkel and Brooks 2002; Chinnusamy et al. 2004; Fujita et al. 2006). Recent studies have revealed ERF subfamily transcription factors as

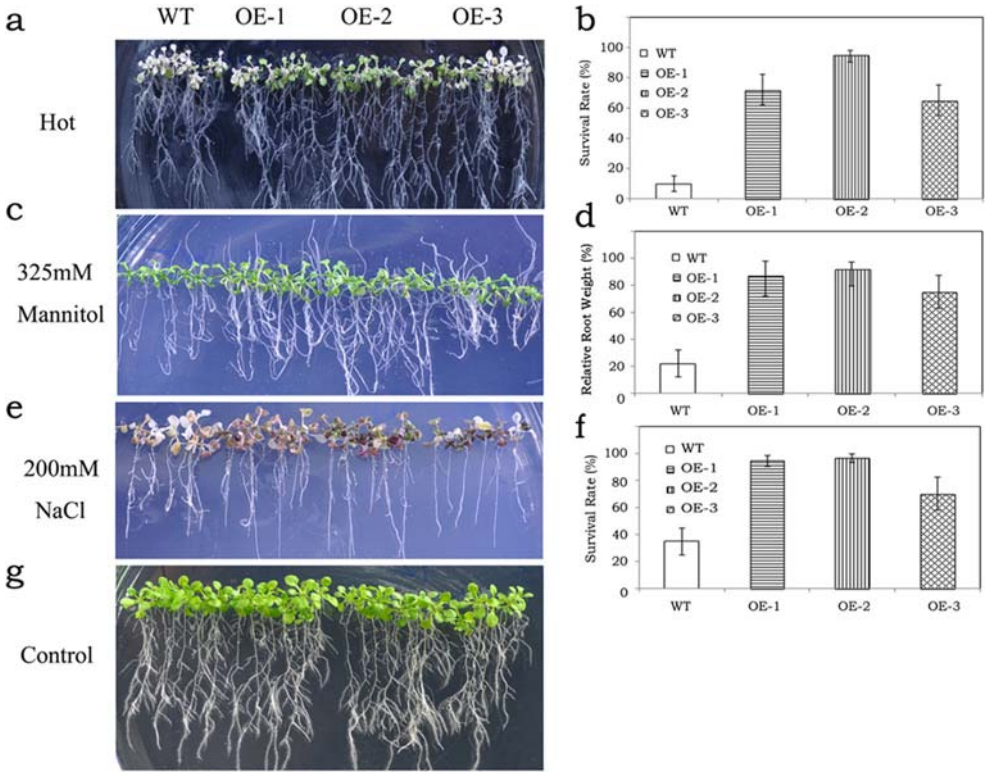

Fig. 4 AhERF019 increased abiotic stress tolerance in *Arabidopsis*. Five-day-old seedlings from the MS medium plates were transferred to MS plates containing 200 NaCl and 325 mM mannitol and incubated at 22 °C on a 14/10-h light/dark cycle for 7 days to record any phenotypic changes. For heat stress, 14-day-old seedlings were grown on horizontal MS solid plates, and transgenic seedlings were incubated at 42 °C for 3 h and recovered at 22 °C for 1 week

promising candidates for proteins involved in the crosstalk between stress signalling pathways. The phytohormones SA, JA, and ET play key roles in biotic stress signalling following pathogen infection. ERF genes integrate different pathogens and disease-related (i.e. ET, JA, and SA) signalling pathways (reviewed by Gutterson and Reuber 2004). The SA signal transduction pathway can act antagonistically with the ET/JA pathway (Leon-Reyes et al. 2010a; b; An and Mou 2011), whereas several ERFs are induced by SA, JA, or ET (Gu et al. 2000; Oñate-Sánchez and Singh 2002; Zhang et al. 2004; Seo et al. 2010; Zarei et al. 2011). These results indicate that ERFs can synergistically integrate the SA and the ET/JA pathways, but not antagonise them to finely modulate the defence response during a pathogen challenge. For example, the ethylene and jasmonate pathways converge in the transcriptional activation of ethylene response factor 1 (ERF1), which regulates the expression of a large number of genes that are responsive to both ethylene and jasmonate. ERF1 acts downstream of the intersection between the ethylene and jasmonate pathways, suggesting that this transcription factor is a key element in the integration of both signals for the regulation of defensive response genes (Solano et al. 1998; Lorenzo et al. 2003). Under biotic stress, the AP2/ERF transcription factor ORA59 acts as the integrator of the JA and ET signalling pathways and is the key regulator of JA- and ET-responsive PDF1.2 expression (Pre et al. 2008; Zarei et al. 2011). ABA is a phytohormone that is extensively involved in responses to abiotic stresses such as drought, low temperature, and osmotic stress. ABA also governs a variety of growth and developmental processes, including seed development, dormancy, germination, and stomatal movement. Some ERFs are activated by ABA (Qin et al. 2004; Wang et al. 2004; Zhang et al. 2004; Wan et al. 2011), indicating a cross-talk pathway between abiotic and biotic stress responses. Therefore, ERF genes encode multifunctional factors that respond to multiple stressors, integrate various signal transduction cascades, and potentially play dual roles in abiotic and biotic stresses in plants. In this study, the expression patterns of all the peanut ERF genes under various stress treatments were analysed. The abiotic stresses of drought, cold, heat, submergence, and high salinity induced the expression of 30, 34, 51, 26, and 41 peanut ERF genes, respectively. Exposure to *R. solanacearum* enhanced the expression of 21 peanut ERF genes. The expression of 9, 28, 24, and 21 peanut ERFs was induced by treatments with SA, ET, JA, and ABA, respectively. The ERF factors in peanuts may be related to elements involved in the crosstalk between the stress signalling pathways, and further studies on these ERF factors with regard to functional genomics would aid in better understanding the precise role of ERFs in plant development and stress response. Surprisingly, 30/63 (47.6 %), 34/63 (54 %), 51/63 (81 %), and 41/63 (65.1 %) of the peanut ERF genes are induced by drought, cold, heat, and high salinity, respectively. Heat and drought are severe stressors that peanuts always encounter in the summer during the flowering period; these stresses cause great yield loss. Approximately 80 and 50 % of the ERF genes were induced by heat and drought stress, respectively, indicating that many peanut ERF genes could be involved in the heat and drought stress responses and might contribute to peanut heat and drought stress adaption during the summer to produce a stable yield.

The availability of the entire genome sequence of several plant species has made it possible to confirm the relatively well-conserved organisation of the AP2/ERF superfamily with 147, 149, 202, 180, and 146 genes in *Arabidopsis thaliana*, *Vitis vinifera*, *Populus trichocarpa*, *Oryza sativa*, and *Solanum lycopersicon*, respectively, mostly represented by the ERF family (Nakano et al. 2006; Zhuang et al. 2008; Licausi et al. 2010; Zhuang et al. 2011; Pirrello et al. 2012). The ERF subfamily has also been characterised in tobacco (Park et al. 2001; Fischer and Droge-Laser 2004;), *Arabidopsis* (Broun et al. 2004; Yang et al. 2005; Mehrnia et al. 2013), peppers (Yi et al. 2004; Youm et al. 2008), tomatoes (Zhang et al. 2009; Upadhyay et al. 2013), corn (Chuck et al. 2002; Qin et al. 2004), and rice (Cao et al. 2006; Xu et al. 2011). Overexpression of some ERF genes enhanced the plants' resistance to biotic and abiotic stresses (Berrocal-Lobo et al. 2002; Fischer and Dröge-Laser 2004; Xu et al. 2011). Thus far, only a few ERF genes from this subfamily have been isolated from peanuts. Several studies have shown that different ERF genes in peanuts have different expression patterns and transcription abundances during abiotic stress. Our results also showed that peanut ERF genes respond to various abiotic and biotic stresses, indicating that these peanut ERF genes may play different roles in different stress conditions. As a stress-tolerant crop, peanuts develop by producing flowers aerially and burying the recently fertilised ovules in the soil to allow the fruit and seeds to mature underground. Gravity perception and the gravitropic response are essential for the completion of the reproductive cycle of the plant. Recent studies showed that ethylene and ERF factors play an important role in plant gravity and root development (Oliva and Dunand 2007). Ethylene and ERF factors are also involved in peanut gynophore and seed development (Chen et al. 2013). As an important oil and cash crop, the seed size and seed number are crucial in peanut yield. Our results showed that there are several root- and seed-specific ERF genes that are highly expressed, and these ERF factors might play an important role in root and seed development.

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References

Agarwal PK, Agarwal P, Reddy MK, Sopory SK (2006) Role of DREB transcription factors in abiotic and biotic stress tolerance in plants. Plant Cell Rep 25(12):1263–1274

Aharoni A, Dixit S, Jetter R, Thoenes E, van Arkel G, Pereira A (2004) The SHINE clade of AP2 domain transcription factors activates wax biosynthesis, alters cuticle properties, and confers drought tolerance when overexpressed in *Arabidopsis*. Plant Cell 16(9):2463–2480

An C, Mou Z (2011) Salicylic acid and its function in plant immunity. J Integr Plant Biol 53(6):412–428

Bailey TL, Williams N, Misleh C, Li WW (2006) MEME: discovering and analyzing DNA and protein sequence motifs. Nucleic Acids Res 1(34 (Web Server issue)):W369–W373

Berrocal-Lobo M, Molina A, Solano R (2002) Constitutive expression of ETHYLENE-RESPONSE-FACTOR1 in *Arabidopsis* confers resistance to several necrotrophic fungi. Plant J 29(1):23–32

Broun P, Poindexter P, Osborne E, Jiang CZ, Riechmann JL (2004) WIN1, a transcriptional activator of epidermal wax accumulation in *Arabidopsis*. Proc Natl Acad Sci U S A 101(13):4706–4711

Buer CS, Sukumar P, Muday GK (2006) Ethylene modulates flavonoid accumulation and gravitropic responses in roots of *Arabidopsis*. Plant Physiol 140(4):1384–1396

Cao Y, Song F, Goodman RM, Zheng Z (2006) Molecular characterization of four rice genes encoding ethylene-responsive transcriptional factors and their expressions in response to biotic and abiotic stress. J Plant Physiol 163:1167–1178

Chen N, Yang Q, Su M, Pan L, Chi X, Chen M, He Y, Yang Z, Wang T, Wang M, Yu S (2012) Cloning of six ERF family transcription factor genes from peanut and analysis of their expression during abiotic stress. Plant Mol Biol Report 30(6):1415–1425

Chen X, Zhu W, Azam S, Li H, Zhu F, Li H, Hong Y, Liu H, Zhang E, Wu H, Yu S, Zhou G, Li S, Zhong N, Wen S, Li X, Knapp SJ, Ozias-Akins P, Varshney RK, Liang X (2013) Deep sequencing analysis of the transcriptomes of peanut aerial and subterranean young pods identifies candidate genes related to early embryo abortion. Plant. Biotechnol J 11(1):115–127

Chinnusamy V, Schumaker K, Zhu JK (2004) Molecular genetic perspectives on cross-talk and specificity in abiotic stress signalling in plants. J Exp Bot 55(395):225–236

Chuck G, Muszynski M, Kellogg E, Hake S, Schmidt RJ (2002) The control of spikelet meristem identity by the branched silkless1 gene in maize. Science 298(5596):1238–1241

Clough SJ, Bent AF (1998) Floral dip: a simplified method for Agrobacterium-mediated transformation of *Arabidopsis thaliana*. Plant J 16(6):735–743

FAOSTAT (2010) FAO Statistical Database. From Food and Agricultural Organization, Rome, Italy, www.faostat.fao.org/site/339/default.aspx

Fischer U, Dröge-Laser W (2004) Overexpression of NtERF5, a new member of the tobacco ethylene response transcription factor family enhances resistance to tobacco mosaic virus. Mol Plant-Microbe Interact 17(10):1162–1171

Fujita M, Fujita Y, Noutoshi Y, Takahashi F, Narusaka Y, Yamaguchi-Shinozaki K, Shinozaki K (2006) Crosstalk between abiotic and biotic stress responses: a current view from the points of convergence in the stress signaling networks. Curr Opin Plant Biol 9(4):436–442

Fukao T, Yeung E, Bailey-Serres J (2011) The submergence tolerance regulator SUB1A mediates crosstalk between submergence and drought tolerance in rice. Plant Cell 23:412–427

Gu YQ, Yang C, Thara VK, Zhou J, Martin GB (2000) *Pti4* is induced by ethylene and salicylic acid, and its product is phosphorylated by the Pto kinase. Plant Cell 12(5):771–786

Gutterson N, Reuber TL (2004) Regulation of disease resistance pathways by AP2/ERF transcription factors. Curr Opin Plant Biol 7(4):465–471

Hao DY, Ohme-Takagi M, Sarai A (1998) Unique mode of GCC box recognition by the DNA-binding domain of ethyleneresponsive element-binding factor (ERF domain) in plants. J Biol Chem 273:26857–26861

Hattori Y, Nagai K, Furukawa S, Song XJ, Kawano R, Sakakibara H, Wu J, Matsumoto T, Yoshimura A, Kitano H, Matsuoka M, Mori H, Ashikari M (2009) The ethylene response factors SNORKEL1 and SNORKEL2 allow rice to adapt to deep water. Nature 460:1026–1030

Hu L, Liu S (2011) Genome-wide identification and phylogenetic analysis of the ERF gene family in cucumbers. Genet Mol Biol 34(4):624–633

Huang B, Jin L, Liu J (2007) Molecular cloning and functional characterization of a DREB1/CBF-like gene (*GhDREB1L*) from cotton. Sci China Ser C—Life Sci 50:7–14

Jin LG, Liu JY (2008) Molecular cloning, expression profile and promoter analysis of a novel ethylene responsive transcription factor gene *GhERF4* from cotton. Plant Physiol Biochem 46:46–53

Kasuga M, Liu Q, Miura S, Yamaguchi-Shinozaki K, Shinozaki K (1999) Improving plant drought, salt, and freezing tolerance by gene transfer of a single stress-inducible transcription factor. Nat Biotechnol 17:287–291

Kunkel BN, Brooks DM (2002) Cross talk between signaling pathways in pathogen defense. Curr Opin Plant Biol 5:325–331

Leon-Reyes A, Van der Does D, De Lange ES, Delker C, Wasternack C, Van Wees SC, Ritsema T, Pieterse CM (2010a) Salicylate-mediated suppression of jasmonate-responsive gene expression in *Arabidopsis* is targeted downstream of the jasmonate biosynthesis pathway. Planta 232(6):1423–1432

Leon-Reyes A, Du Y, Koornneef A, Proietti S, Körbes AP, Memelink J, Pieterse CM, Ritsema T (2010b) Ethylene signaling renders the jasmonate response of *Arabidopsis* insensitive to future suppression by salicylic acid. Mol Plant-Microbe Interact 23(2):187–197

Licausi F, Giorgi FM, Zenoni S, Osti F, Pezzotti M, Perata P (2010) Genomic and transcriptomic analysis of the AP2/ERF superfamily in *Vitis vinifera*. BMC Genomics 11:719

Licausi F, Ohme-Takagi M, Perata P (2013) APETALA2/Ethylene Responsive Factor (AP2/ERF) transcription factors: mediators of stress responses and developmental programs. New Phytol 199(3):639–649

Liu Q, Kasuga M, Sakuma Y, Abe H, Miura S, Yamaguchi Shinozaki K, Shinozaki K (1998) Two transcription factors, DREB1 and DREB2, with an EREBP/AP2 DNA binding domain, separate two cellular signal transduction pathways in drought and low temperature-responsive gene expression, respectively, in *Arabidopsis*. Plant Cell 10:1391–1406

Liu L, White MJ, MacRae TH (1999) Transcription factors and their genes in higher plants functional domains, evolution and regulation. Eur J Biochem 262:247–257

Lorenzo O, Piqueras R, Sanchez-Serrano JJ, Solano R (2003) ETHYLENE RESPONSE FACTOR1 integrates signals from ethylene and jasmonate pathways in plant defense. Plant Cell 15(1):165–178

Mehrnia M, Balazadeh S, Zanor MI, Mueller-Roeber B (2013) EBE, an AP2/ERF transcription factor highly expressed in proliferating cells, affects shoot architecture in *Arabidopsis*. Plant Physiol 162(2):842–857

Mizoi J, Shinozaki K, Yamaguchi-Shinozaki K (2012) AP2/ERF family transcription factors in plant abiotic stress responses. Biochim Biophys Acta 1819(2):86–96

Nakano T, Suzuki K, Fujimura T, Shinshi H (2006) Genomewide analysis of the ERF gene family in *Arabidopsis* and rice. Plant Physiol 140:411–432

Nelson SC, Simpson CE, Starr JL (1989) Resistance to *Meloidogyne arenaria* in *Arachis spp.*. Germplasm J Nematol 21(4S):654–660

Ohme-Takagi M, Shinshi H (1995) Ethylene-inducible DNA binding proteins that interact with an ethylene-responsive element. Plant Cell 7(2):173–182

Ohta M, Matsui K, Hiratsu K, Shinshi H, Ohme-Takagi M (2001) Repression domains of class II ERF transcriptional repressors share an essential motif for active repression. Plant Cell 13:1959–1968

Oliva M, Dunand C (2007) Waving and skewing: how gravity and the surface of growth media affect root development in *Arabidopsis*. New Phytol 176(1):37–43

Oñate-Sánchez L, Singh KB (2002) Identification of Arabidopsis ethylene-responsive element binding factors with distinct induction kinetics after pathogen infection. Plant Physiol 128(4):1313–1322

Park JM, Park CJ, Lee SB, Ham BK, Shin R, Paek KH (2001) Overexpression of the tobacco *Tsi1* gene encoding an EREBP/AP2-type transcription factor enhances resistance against pathogen attack and osmotic stress in tobacco. Plant Cell 13(5):1035–1046

Philosoph-Hadas S, Friedman H, Meir S (2005) Gravitropic bending and plant hormones. Vitam Horm 72:31–78

Pirrello J, Prasad BC, Zhang W, Chen K, Mila I, Zouine M, Latché A, Pech JC, Ohme-Takagi M, Regad F, Bouzayen M (2012) Functional analysis and binding affinity of tomato ethylene response factors provide insight on the molecular bases of plant differential responses to ethylene. BMC Plant Biol 12(1):190

Pre M, Atallah M, Champion A, De Vos M, Pieterse CM, Memelink J (2008) The AP2/ERF domain transcription factor ORA59 integrates jasmonic acid and ethylene signals in plant defense. Plant Physiol 147(3):1347–1357

Qin F, Sakuma Y, Li J, Liu Q, Li YQ, Shinozaki K, Yamaguchi-Shinozaki K (2004) Cloning and functional analysis of a novel DREB1/CBF transcription factor involved in cold-responsive gene expression in *Zea mays* L. Plant Cell Physiol 45(8):1042–1052

Sakuma Y, Liu Q, Dubouzet JG, Abe H, Shinozaki K, Yamaguchi-Shinozaki K (2002) DNA-binding specificity of the ERF/AP2 domain of *Arabidopsis* DREBs, transcription factors involved in dehydration- and cold-inducible gene expression. Biochem Biophys Res Commun 290:998–1009

Seo YJ, Park JB, Cho YJ, Jung C, Seo HS, Park SK, Nahm BH, Song JT (2010) Overexpression of the ethylene-responsive factor gene *BrERF4* from *Brassica rapa* increases tolerance to salt and drought in *Arabidopsis* plants. Mol Cells 30(3):271–277

Sharma MK, Kumar R, Solanke AU, Sharma R, Tyagi AK, Sharma AK (2010) Identification, phylogeny, and transcript profiling of ERF family genes during development and abiotic stress treatments in tomato. Mol Gen Genomics 284(6):455–475

Solano R, Stepanova A, Chao Q, Ecker JR (1998) Nuclear events in ethylene signaling: a transcriptional cascade mediated by ETHYLENE-INSENSITIVE3 and ETHYLENE-RESPONSE-FACTOR1. Genes Dev 12(23):3703–3714

Thomashow MF (1999) Plant cold acclimation: freezing tolerance genes and regulatory mechanism. Annu Rev Plant Physiol Plant Mol Biol 50:571–599

Upadhyay RK, Soni DK, Singh R, Dwivedi UN, Pathre UV, Nath P, Sane AP (2013) *SlERF36*, an EAR-motif-containing ERF gene from tomato, alters stomatal density and modulates photosynthesis and growth. J Exp Bot. doi:10.1093/jxb/ert162

Wan L, Zhang J, Zhang H, Zhang Z, Quan R, Zhou S, Huang R (2011) Transcriptional activation of OsDERF1 in *OsERF3* and *OsAP2-39* negatively modulates ethylene synthesis and drought tolerance in rice. PLoS One 6(9):e25216

Wang H, Huang Z, Chen Q, Zhang Z, Zhang H, Wu Y, Huang D, Huang R (2004) Ectopic overexpression of tomato *JERF3* in tobacco activates downstream gene expression and enhances salt tolerance. Plant Mol Biol 55(2):183–192

Wang Q, Guan Y, Wu Y, Chen H, Chen F, Chu C (2008) Overexpression of a rice *OsDREB1F* gene increases salt, drought, and low temperature tolerance in both *Arabidopsis* and rice. Plant Mol Biol 67:589–602

Wang Y, Wan L, Zhang L, Zhang Z, Zhang H, Quan R, Zhou S, Huang R (2012) An ethylene response factor OsWR1 responsive to drought stress transcriptionally activates wax synthesis related genes and increases wax production in rice. Plant Mol Biol 78(3):275–288

Xu K, Xu X, Fukao T, Canlas P, Maghirang-Rodriguez R, Heuer S, Ismail AM, Bailey-Serres J, Ronald PC, Mackill DJ (2006) *Sub1A* is an ethylene-response-factor-like gene that confers submergence tolerance to rice. Nature 442(7103):705–708

Xu ZS, Chen M, Li LC, Ma YZ (2011) Functions and application of the AP2/ERF transcription factor family in crop improvement. J Integr Plant Biol J53(7):570–585

Yamaguchi-Shinozaki K, Shinozaki K (1994) A novel cis-acting element in an *Arabidopsis* gene is involved in responsiveness to drought, low-temperature, or high-salt stress. Plant Cell 6(2):251–264

Yang Z, Tian L, Latoszek-Green M, Brown D, Wu K (2005) *Arabidopsis* ERF4 is a transcriptional repressor capable of modulating ethylene and abscisic acid responses. Plant Mol Biol 58:585–596

Yi SY, Kim JH, Joung YH, Lee S, Kim WT, Yu SH, Choi D (2004) The pepper transcription factor CaPF1 confers pathogen and freezing tolerance in *Arabidopsis*. Plant Physiol 136(1):2862–2874

Youm JW, Jeon JH, Choi D, Yi SY, Joung H, Kim HS (2008) Ectopic expression of pepper *CaPF1* in potato enhances multiple stresses tolerance and delays initiation of in vitro tuberization. Planta 228(4):701–708

Zarei A, Korbes AP, Younessi P, Montiel G, Champion A, Memelink J (2011) Two GCC boxes and AP2/ERF-domain transcription factor ORA59 in jasmonate/ethylene-mediated activation of the PDF1.2 promoter in *Arabidopsis*. Plant Mol Biol 75(4–5):321–331

Zhang H, Huang Z, Xie B, Chen Q, Tian X, Zhang X, Zhang H, Lu X, Huang D, Huang R (2004) The ethylene-, jasmonate-, abscisic acid- and NaCl-responsive tomato transcription factor JERF1 modulates expression of GCC box-containing genes and salt tolerance in tobacco. Planta 220(2):262–270

Zhang JY, Broeckling CD, Blancaflor EB, Sledge MK, Sumner LW, Wang ZY (2005) Overexpression of *WXP1*, a putative Medicago truncatula AP2 domain-containing transcription factor gene, increases cuticular wax accumulation and enhances drought tolerance in transgenic alfalfa (*Medicago sativa*). Plant J 42(5):689–707

Zhang JY, Broeckling CD, Sumner LW, Wang ZY (2007) Heterologous expression of two Medicago truncatula putative ERF transcription factor genes, *WXP1* and *WXP2*, in *Arabidopsis* led to increased leaf wax accumulation and improved drought tolerance, but differential response in freezing tolerance. Plant Mol Biol 64(3):265–278

Zhang G, Chen M, Chen X, Xu Z, Guan S, Li LC, Li A, Guo J, Mao L, Ma Y (2008) Phylogeny, gene structures, and expression patterns of the ERF gene family in soybean (*Glycine max* L.). J Exp Bot 59(15):4095–4107

Zhang Z, Zhang H, Quan R, Wang XC, Huang R (2009) Transcriptional regulation of the ethylene response factor LeERF2 in the expression of ethylene biosynthesis genes controls ethylene production in tomato and tobacco. Plant Physiol 150(1):365–377

Zhu Z, Shi J, Xu W, Li H, He M, Xu Y, Xu T, Yang Y, Cao J, Wang Y (2013) Three ERF transcription factors from Chinese wild grapevine *Vitis pseudoreticulata* participate in different biotic and abiotic stress-responsive pathways. J Plant Physiol 170(10):923–933

Zhuang J, Cai B, Peng RH, Zhu B, Jin XF, Xue Y, Gao F, Fu XY, Tian YS, Zhao W, Qiao YS, Zhang Z, Xiong AS, Yao QH (2008) Genome-wide analysis of the AP2/ERF gene family in *populous trichocarpa*. Biochem Biophys Res Commun 371(3):468–474

Zhuang J, Chen JM, Yao QH, Xiong F, Sun CC, Zhou XR, Zhang J, Xiong AS (2011) Discovery and expression profile analysis of AP2/ERF family genes from *Triticum aestivum*. Mol Biol Rep 38(2):745–753