Journal of Plant Physiology **165** (2008) 1717—1725

Available online at www.sciencedirect.com
ScienceDirect

JOURNAL OF PLANT PHYSIOLOGY
www.elsevier.de/jplph

Overexpression of *OsERF1*, a novel rice ERF gene, up-regulates ethylene-responsive genes expression besides affects growth and development in *Arabidopsis*

Yibing Hu[a,b], Lifeng Zhao[a], Kang Chong[a], Tai Wang[a,*]

[a]*Research Center for Molecular & Developmental Biology, Key Laboratory of Photosynthesis & Environmental Molecular Physiology, Institute of Botany, Chinese Academy of Sciences, 20 Nanxincun, Xiangshan, Haidianqu, Beijing100093, China*
[b]*Graduate School of Chinese Academy of Sciences, Beijing 100049, China*

Received 17 July 2007; received in revised form 6 December 2007; accepted 7 December 2007

KEYWORDS
Arabidopsis thaliana;
ERF;
Ethylene response;
Oryza sativa;
OsERF1

Summary

Ethylene-responsive factors (ERFs), composing the largest group of AP2/EREBP transcription factors, are involved in diverse functions and some of them have been identified in plants. However, even in model plants *Arabidopsis* and rice, most of the genes in this group are functionally unknown yet. Especially in rice, ERF genes involved in ethylene response have not been reported previously. Here, we report a novel member of ERF group in rice, *OsERF1* (*E*thylene *R*esponse *F*actor gene in *Oryza sativa*). *OsERF1* expressed consistently in different organs of rice and could be up-regulated by ethylene, a plant hormone associated with stress response. Over-expression of *OsERF1* in *Arabidopsis* up-regulated the expression of two known ethylene-responsive genes, *PDF1.2* and *b-chitinase*, and also significantly affected the growth and development of transgenic *Arabidopsis*. These results suggest the involvement of *OsERF1* in ethylene response.

Introduction

AP2/ethylene-responsive element-binding proteins (EREBPs) compose a superfamily of transcription factors that exist extensively in plants and microorganisms (Okamura et al., 1997; Magnani

Abbreviations: ABA, abscisic acid; DREB, dehydration-responsive element-binding protein; EREBP, ethylene-responsive element-binding protein; ERF, ethylene-responsive factor; FITC, fluorescein isothiocyanate; GFP, green fluorescent protein; MeJA, methyl jasmonate; MS, Murashige and Skoog; PEG, polyethylene glycol; SA, salicylic acid; UV, ultraviolet.

*Corresponding author: Tel.: +86 10 62836210; fax: +86 10 62594170.
E-mail address: twang@ibcas.ac.cn (T. Wang).

0176-1617/$ - see front matter © 2007 Elsevier GmbH. All rights reserved.
doi:10.1016/j.jplph.2007.12.006

et al., 2004; Nakano et al., 2006). They are characterized by the presence of the highly conserved AP2/EREBP DNA-binding domain of about 60 amino acids (Weigel, 1995; Okamura et al., 1997). Depending on the number of domains, the superfamily is divided into AP2 and EREBP subfamilies. Members of the AP2 subfamily have two domains in tandem while members of EREBP subfamily contain only one (Weigel, 1995; Okamura et al., 1997; Sakuma et al., 2002). Notably, all of the characterized AP2 subfamily members are found to be development-related (Nakano et al., 2006). In contrast, EREBPs play roles in response to phytohormone, pathogen attack and environmental stresses such as cold, drought and high salt (Liu et al., 1998; Haake et al., 2002; Dubouzet et al., 2003; Gutterson and Reuber, 2004).

Based on their function and conserved amino acids of their AP2/EREBP domain, presently known EREBPs can be further assigned into the ethylene-responsive factor (ERF) or dehydration-responsive element-binding protein (DREB) subgroup (Sakuma et al., 2002). Amino acids 15 and 20 in the domain of DREBs are V (Val) and E (Glu), respectively, in contrast to A (Ala) and D (Asp) in ERFs. This divergence is supposed to explain the functional differences between the two subgroups (Sakuma et al., 2002): DREBs such as *OsDREB* from *Oryza sativa* and *DREB1* from *Arabidopsis* are implicated in responses to abiotic stresses (Liu et al., 1998; Dubouzet et al., 2003; Guo et al., 2004), and ERFs function mainly in biotic stress-resistant responses (Gutterson and Reuber, 2004), examples being *ERF1*, *ERF2*, *ERF3* and *ERF4* from *Arabidopsis thaliana* (Zhou et al., 1997; Solano et al., 1998; Gu et al., 2000; Berrocal-Lobo et al., 2002) and *Pti* from *Solanum tuberosum* (Gu et al., 2000, 2002). In rice, more than 100 genes have been predicted to encode ERF proteins (Nakano et al., 2006). However, functional information are currently available only for a few genes such as *FZP* and *BIERFs*: *FZP* is identified to play a crucial role in establishing floral meristem identity and *BIERFs* show inducible expression to 2,1,3-benzothiadiazole, a plant systematic resistance activator (Cao et al., 2006; Nakano et al., 2006). Further investigations of functional characteristics of more ERFs are essential to extend our understanding of their roles in plants. In *Arabidopsis*, several ERFs such as *AtERF1*, *AtERF3* and *ERF1* are found to be ethylene-responsive (Solano et al., 1998; Fujimoto et al., 2000), but corresponding rice genes involved in ethylene response have not been reported previously. In this study, we cloned a putative rice ERF gene, *OsERF1*, and characterized its function by overexpression of it in *Arabidopsis* since network of gene regulation in *Arabidopsis* is far clearer than in any other plant species.

Materials and methods

Plant materials and nuclear acid extraction

Rice plants (*O. sativa* L. ssp. *japonica* cv. Zhonghua 10) were grown under standard conditions. Leaves were collected from 2-week-old seedlings, and inflorescences were harvested at different stages. Young roots and buds were gathered from seeds germinated on sterile-water soaked filter papers for 3 d.

To detect responses of the gene to plant growth substances or stresses, 2-week-old rice seedlings were transferred into solutions containing 0.1 mM ethrel (purity, 95%), 0.01 mM abscisic acid (ABA) (Invitrogen), 20% polyethylene glycol (PEG) (−4.5 MPa), 1 mM salicylic acid (SA) and 0.1 mM methyl jasmonate (MeJA) with roots immersed completely in these solutions and cultivated at 28 °C for 4 h. For cold treatment, seedlings were exposed to 8 °C with a control at 28 °C for 4 h. To detect detailed responses of *OsERF1* to ethrel inducement, 1 mM ethrel was employed to treat rice seedlings for 1, 2 and 4 h.

Total RNA was isolated by the use of Trizol kit (Invitrogen) according to the manufacturer's protocol and digested by RNase-free DNase (Takara) to remove residual genomic DNA.

Cloning of full-length *OsERF1* cDNA

The full-length cDNA of *OsERF1* was cloned by use of a RACE approach (Invitrogen 5′ RACE kit), with the gene-specific primers synthesized according to the predicted genomic sequence of *OsERF1*. Primers 5′-ATGCGGAGCGGGAAGTTGA-3′ and 5′-AATGTCGACTCCTCCTTCCCGTGC-3′ were used for 5′ RACE; 5′-GAGGTCGGGTCAGCAAGGAA-3′ and 5′-TCCTCAACTTCCCGCTCCGC-3′ for 3′ RACE.

RT-PCR analysis of *OsERF1* mRNA accumulation

An amount of 5 μg of total RNA extracted from a given tissue was reverse-transcribed into first-strand cDNAs by ReverTra Ace (Toyobo). RT-PCR was performed in 50 μL mixture including 5 μL of first-strand cDNA, 20 pmol of each of the gene-specific primers (5′-GGTGCAGGCATGGTACCCC-3′ and 5′-CCCTCACAAACTCACTCGG-3′), 0.4 μM dNTPs, 1 × GC buffer (Takara) and 2.5 U Taq DNA polymerase (5 U/μL, Takara) for 35 cycles. Rice *Tubulin A* cDNA (*Tub A*) was amplified for 25 cycles as a constitutive control (Ding et al., 2002).

Construction of *OsERF1* expression vector

A fragment spanning *OsERF1* ORF was PCR-amplified from genomic DNA by use of the primers 5′-TTTCCATGGCGATGACGGCGCGAAGCATG-3′ and 5′-ACTACTAGTGATGACGAGCTGCTCCACGC-3′, which contained added *Nco*I and

*Spe*I enzyme sites, respectively. After digestion, the amplicon was inserted into the tool vector pCAMBIA1302 (CAMBIA) to produce *OsERF1* expression construct pOsERF1, which contained an *OsERF1:green fluorescent protein (GFP)* fusion under the control of CaMV35S promoter. The resulting *OsERF1* expression construct pOsERF1 and pCAMBIA1302 vector (used as a control) were first introduced into *Agrobacterium tumefaciens* strain EHA105 and then into *A. thaliana* var. Columbia by a floral dip method (http://www.nlh.no/research/narc/protocols/floral_dip.htm).

Subcellular localization of OsERF1 protein

pOsERF1 was introduced into *Allium cepa* (onion) epidermis cells by *A. tumefaciens* transformation (Yang et al., 2000). GFP fluorescence signals were detected under microscopy with a fluorescein isothiocyanate (FITC) filter (Zeiss) after incubation of the transformed cells on the Murashige and Skoog (MS) (1962) medium at 25 °C in the light for 2.5 d. Subcellular localization of OsERF1 was also examined by use of the T3 seedlings of transgenic *Arabidopsis* line L6 expressing OsERF1:GFP.

Screening of transgenic *Arabidopsis* seedlings

Transgenic *Arabidopsis* seeds were germinated and grown on a solid MS medium supplemented with 25 mg/L hygromycin (hyg) in the dark for 3 d. Then, hyg-resistant seedlings were transferred to flower pots and maintained under standard conditions.

Examination of insertion and expression of *OsERF1* in transgenic *Arabidopsis*

Genomic DNAs extracted from *Arabidopsis* wild-type and transgenic plants were digested completely with *Hin*dIII. About 20 μg of the digested DNAs were separated on a 0.8% agarose gel followed by denaturation and neutralization before being blotted onto a Hybond N^+ nylon membrane (Amersham Biosciences). After ultraviolet (UV) cross-linking, hybridization was carried out overnight at 65 °C with an [alpha-^{32}P]CTP-labeled GFP fragment (spanning nucleotides 67–689 of the pCAMBIA1302), which was labeled by use of the primer-a-gene labeling system (Promega).

For RNA gel blot, 25 μg of total RNAs from transgenic and wild-type plants were fractioned on a 1.2% agarose–formaldehyde gel and transferred onto Hybond-N+ nylon membranes, followed by UV cross-linking. Hybridization was performed with the same probe as described for the DNA gel blot, and the resulting membrane was autographed at −80 °C.

mRNA examination of ethylene-responsive genes expression in transgenic *Arabidopsis*

Semi-quantitative RT-PCR was performed to assess transcript accumulation of ethylene-responsive genes in wild-type and transgenic plants. Primers were 5′-TTACAACGCCTTTATCACCG-3′ and 5′-ACCACCAGGATTAACACCAA-3′ for *b-chitinase* gene (At3G12500); 5′-TACATCTATACATTGAAAAC-3′ and 5′-ACAACGGGAAAATAAACA-3′ for *PDF1.2* gene (At5G44420). *ACTIN* 2 (At3G18780) was used as a constitutive control with primers (5′-CTGTGCCAATCTACGAGGGT-3 and 5′-GCTGGAATGTGCTGAGGGA-3′). PCR involved a total of 25 cycles.

Results

OsERF1 is a novel ERF-like gene

Using the known AP2/EREBP DNA-binding domain sequence of EREBP1 as a query, we performed the tblastn search against the TIGR rice genomic database and retrieved several genomic sequences encoding putative ERF genes. One of them, designated as *OsERF1*, was selected for further investigation since sequence analysis indicated that *OsERF1* was a potential ethylene-responsive gene (for details, see description below). We cloned its full-length cDNA of 1589 bp long by a RACE approach (sequence data from this article have been deposited at GenBank under accession no. EF061888). Sequence analysis shows that *OsERF1* has an open-reading frame (OFR) of 957 bp capable of encoding a 318 amino acids long protein. The predicted protein has a calculated molecular mass of 33.1 kD and a p*I* of 4.83, with a relatively hydrophilic feature. By the blastn search against the TIGR rice genomic database with the full-length cDNA, we retrieved only one hit with 100% identity and no other significantly similar ones over the cDNA sequence, suggesting that *OsERF1* exists as a single copy gene. The genomic sequence of *OsERF1* localizes in chromosome 4 (Os04g46220), spans nucleotides 81171–82127 of BAC clone OSJNBa0079A21 (accession no. AL607006), and has no intron.

OsERF1 contains a single AP2/EREBP DNA-binding domain (AAs 151–210) (Figure 1A), an alanine-rich region of 111 amino acids (AAs 119–229) and a serine-rich region of 25 amino acids (AAs 241–265). Moreover, the presence of a nuclear-targeting motif (AAs 266–270) is consistent with the nucleus localization feature of the protein, which was observed experimentally (Figure 2).

Multiple sequence alignment of OsERF1 and other known AP2/EREBP proteins showed their similarity restricted intensively to the AP2/EREBP DNA-binding domain region. Over this region, OsERF1 shared high sequence identity ($\geqslant$80.83%) with other ERFs, relatively low identity ($\leqslant$65.98%) with DREBs and considerably low identity ($\leqslant$37.7%) with APETALA2 proteins (Figure 1A). In addition, the conserved

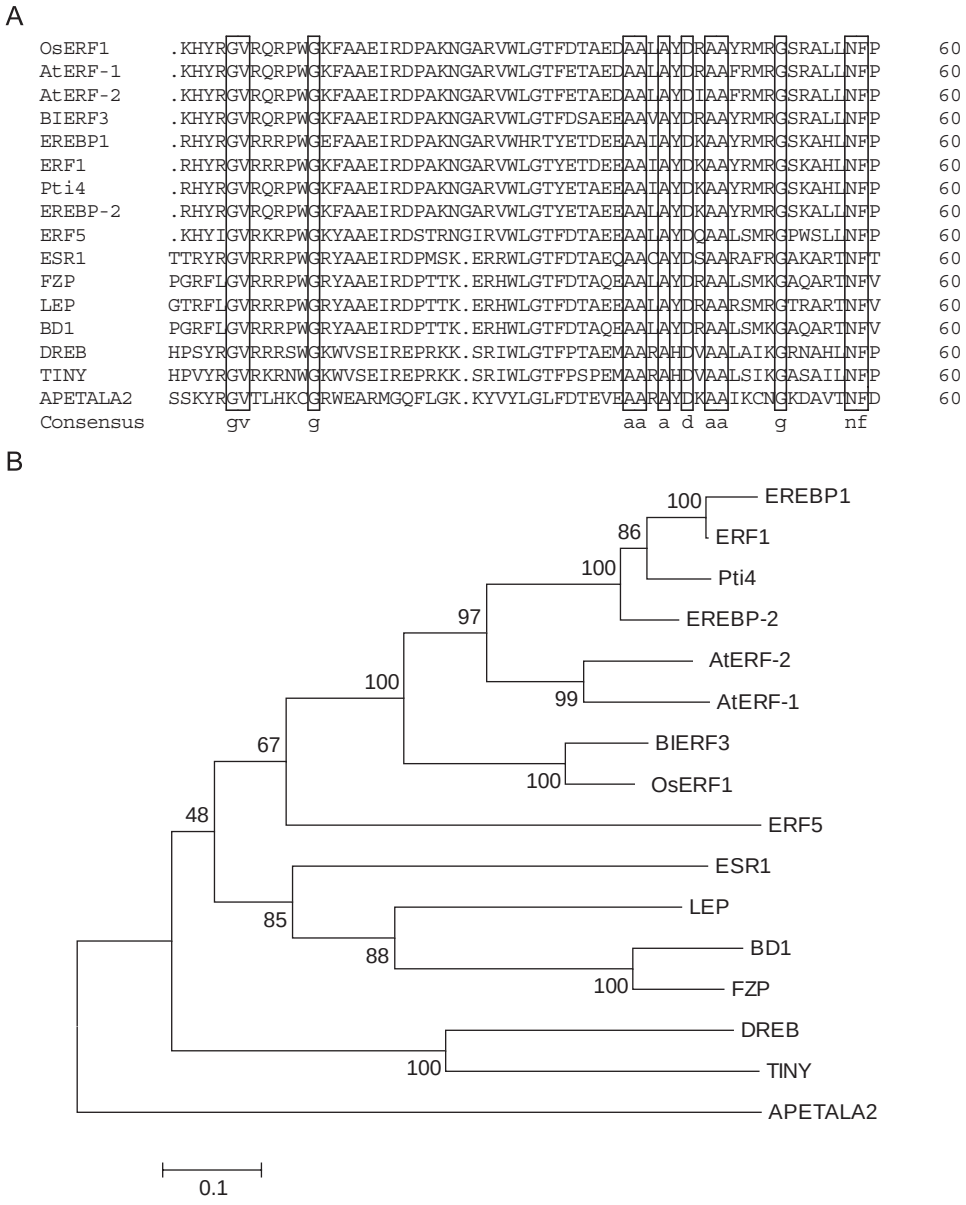

Figure 1. *OsERF1* encodes an ERF-like protein. (A). Multiple sequence alignment of amino acids in the AP2 /EREBP DNA-binding domain region of *OsERF1* and other known AP2/EREBP proteins. The proteins and their accession numbers used for alignment are listed below: APETALA2 (NP_195410, *Arabidopsis thaliana*); EREBP-2 (BAA07324, *Nicotiana tabacum*); EREBP1 (AAC62619 *N. tabacum*); ERF1 (Q40476, *N. tabacum*); ATERF-1 (NP_567530 *A. thaliana*); AtERF2 (NP_199533, *A. thaliana*); *OsERF1* (ABK34954, *Oryza sativa*); DREB (AAO39764, *O. sativa*); ERF5 (AAU81956 *N. tabacum*); Pti4 (AAC50047, *Lycopersicon esculentum*); ESR1 (NP_172758, *A. thaliana*); LEP (NP_196895, *A. thaliana*); BD1 (AAO21119, *Zea mays*); FZP (BAC79264, *O. sativa*); BIERF3 (AAV98702, *O. sativa*); and TINY (CAA64359, *A. thaliana*). (B) A phylogenetic tree constructed by using MEGA version 3.1 (Kumar et al., 2004) based on alignment of the amino acid sequences of the proteins described in ''A'' by Clustal X (version 1.81). Bootstrap values evaluated for 500 bootstrap trails are shown at branch points.

15th A and 20th D in the domain of EFRs, but not DREBs, are present in OsERF1. Phylogenetic analysis based on alignment of full amino acid sequences also revealed that OsERF1 tended to group into the known ERFs but not DREBs (Figure 1B). Consistent with these results, a previous bioinformatic analysis shows the gene (Os04g46220) predicted on the basis of rice genomic sequences belongs to the B-3/IX group of rice ERF gene subfamily (Nakano et al., 2006); and characterized genes from the B-3/IX group such as *ERF1* and *Pti4* are identified to be ethylene-responsive (Solano et al., 1998; Nakano

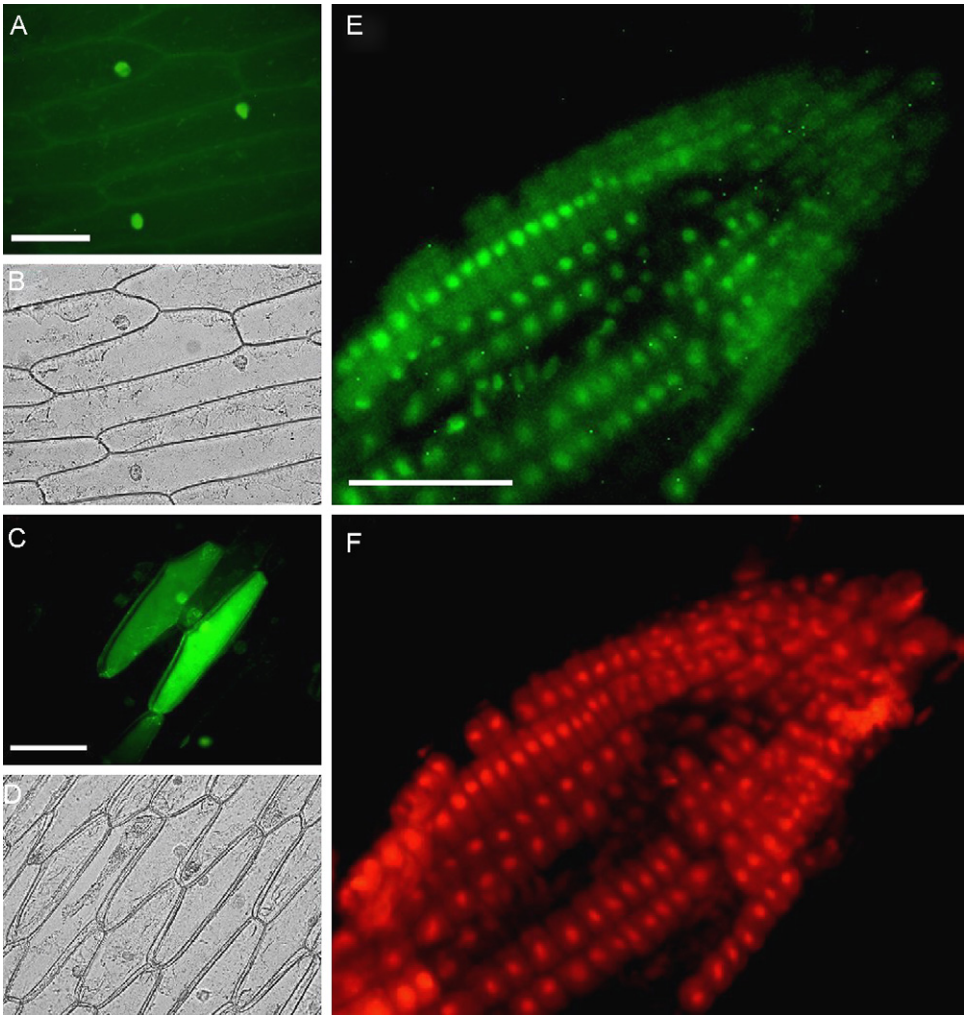

Figure 2. OsERF1 is a nucleus-localizing protein. (A–D). Microscopic observation of onion epidermis transformed with pOsERF1 construct (A, B) or with pCAMBIA1302 vector (control) (C, D). (A, C) were observed with a FITC filter set (Zeiss); (B, D) were observed in bright field. (E) OsERF1:GFP signals in root tip cells from transgenic *Arabidopsis* plants L6 observed under microscopy with a FITC filter (E) or stained by propidium iodide (F). Scale bar = 50 μm in (A) for (A and B), in (C) for (C and D), in (E) for (E and F).

et al., 2006). Together, these data suggest that *OsERF1* is a novel ERF protein-encoding gene and is possibly implicated in ethylene response in rice.

OsERF1 expresses consistently and can be up-regulated by ethylene in a dose-dependent manner

OsERF1 expression pattern was analyzed by semi-quantitative RT-PCR. As shown in Figure 3A, *OsERF1* was expressed almost equally in roots, leaves, inflorescences and buds; in developing flowers its transcripts were accumulated equally from the male meiotic stage to mature pollen stage (Figure 3B).

We analyzed whether the expression of *OsERF1* was affected by stress-associated factors such as ethrel, ABA, cold and water deficit mimicked by PEG solution. RT-PCR results showed that the transcriptional level of *OsERF1* increased (>20%) by 0.1 mM ethrel inducement but seemed not to be affected by other factors tested (Figure 3C). Furthermore, 1 mM ethrel was used to confirm the response of *OsERF1*. Compared with the control, *OsERF1* mRNA accumulation increased obviously (>70%) after 4 h inducement (Figure 3D). We repeated this experiment three times and obtained consistent results, indicating that *OsERF1* is an ethylene-responsive gene.

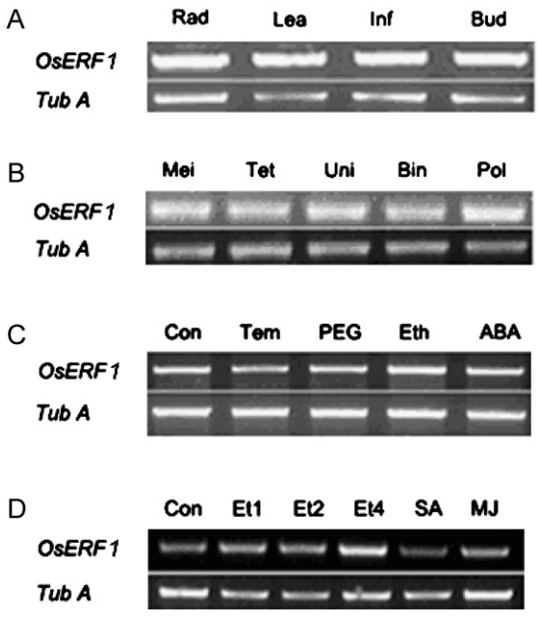

Figure 3. Semi-quantitative RT-PCR analyses of *OsERF1* expression in different tissues (A, B) or under different treatments (C, D). *Tubulin A* was used as a constitutive control. (A) Expression profiles in roots (Rad), leaves (Lea), inflorescences (Inf) and buds (Bud). (B) Expression profiles in developing flowers at male meiosis (Mei), tetrad spore (Tet), uninucleate pollen (Uni), binucleate pollen (Bin) and trinucleate pollen (Pol) stages. (C) Response of *OsERF1* expression to low temperature (8 °C) (Tem), 20% PEG (PEG), Eth (0.1 mM ethrel) and ABA (0.01 mM ABA); Con: control. The treatment time was 4 h. (D). Response of *OsERF1* expression to 1 mM ethrel under 1 h (Et1), 2 h (Et2), 4 h (Et4) inducement or MeJA (0.1 mM), SA (1 mM) for 4 h; Con: control.

Overexpression of *OsERF1* in *Arabidopsis* causes similar phenotypes as in transgenic plants that overexpress known ethylene-responsive factors

The overexpression construct of *OsERF1* and the pCAMBIA1302 vector (control) were introduced into *Arabidopsis*. Among the 25 identified T1 transgenic lines of *OsERF1*, 11 (44%) lines showed dwarf size, dark-green leaves and delayed bolting, and subsequently produced less siliques (L1, L2, L3, L4, L6 produced less seeds and L5 produced few seeds) or no siliques at all (the other five lines); whereas the remainder (such as L11) had no visible aberrances as compared with wild-type plants. In addition, we identified 17 positive lines transformed with empty vector (pCAMBIA1302) and none of them showed aberrant phenotypes, which implied that the aberrant phenotypes were attributable to *OsERF1*.

DNA gel blot revealed two copies of the transgene had been inserted in L1 and L2, one in L3, L4 and L6, three in L11, but none in wild type; and the integration sites of the transgene in these lines were distinct from each other. RNA gel blot (Figure 4) revealed that *OsERF1* expressed at the highest level in L3, to a slightly lesser extent in L2 and L6, and at a relatively low level in L5. No expression was detected in L11. The difference in expression levels paralleled the deviation in phenotypes among these lines: L3 had the most severe aberrant phenotypes, whereas L11 had no observable aberrant phenotypes, which suggested that the transgene was silenced in L11. Moreover, the presence of nucleus-localized OsERF1 in these overexpressed *OsERF1* lines indicated successful translation of the *OsERF1* protein (Figure 2). Together, these facts demonstrate that overexpression of *OsERF1* results in the aberrant phenotypes.

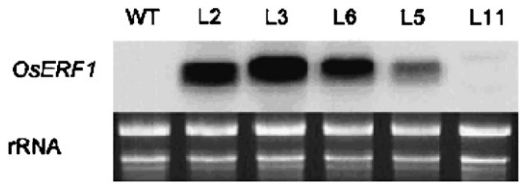

Figure 4. Detection of *OsERF1* expression in transgenic *Arabidopsis* plants by RNA gel blot. Total RNAs were prepared from transgenic plants L2, L3, L5, L6, L11 and wild-type controls (WT). rRNAs were used as a marker to indicate that equal amount of RNAs were loaded in each lane.

Transgenic plants showed dwarf size as compared with wild-type plants, the mean height of L2, L3 and L6 was about 74% of wild type (Figure 5) and their mean bolting time was delayed about 30 d (Figure 6). In addition, the uppermost flowers (usually the first 10) on the inflorescences of the transgenic plants were infertile (Figure 7E). Detailed observation showed that their gynoecium protruded while their pollens were still immature (Figure 7A and B), but their pollens were viable at mature pollen stage (Figure 7C and D). These aberrant phenotypes are very much alike to those of *Arabidopsis* plants that overexpress *ERF1* or *EIN3*, which show constitutive ethylene response (Chao et al., 1997; Solano et al., 1998). It is probable that like *ERF*1, *OsERF1* may be involved in ethylene signaling pathway.

OsERF1 can activate expression of ethylene-responsive genes

To identify this speculation, we first observed the morphologic features of dark-grown *OsERF1*-overexpressed seedlings of L6. As shown in Figure 8A, transgenic seedlings showed short hypocotyls and

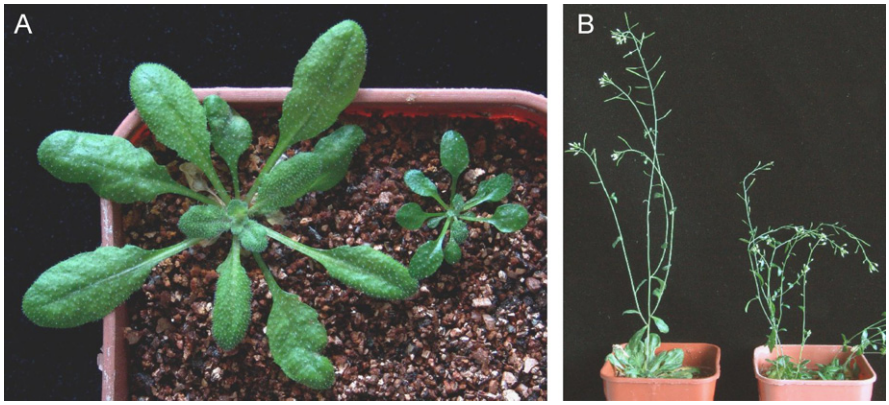

Figure 5. Overall comparison of wild-type and *OsERF1*-overexpressed plants L6. (A) Morphology of *OsERF1*-overexpressed plants (right) and wild type (left) at vegetative growth stage. (B). Morphology of *OsERF1*-overexpressed plants (right) and wild type (left) at reproductive growth stage.

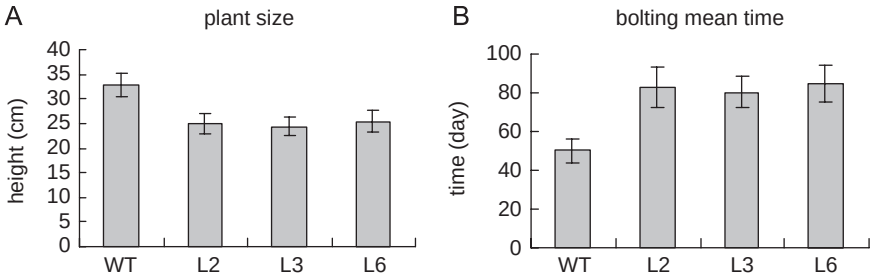

Figure 6. Diagrams of plant size and mean bolting time of wild-type and *OsERF1*-overexpressed *Arabidopsis*. (A) A diagram to show mean height of wild-type and *OsERF1*-overexpressed *Arabidopsis* (L2, L3, L6). (B) A diagram to show mean bolting time of wild-type and *OsERF1*-overexpressed *Arabidopsis* (L2, L3, L6).

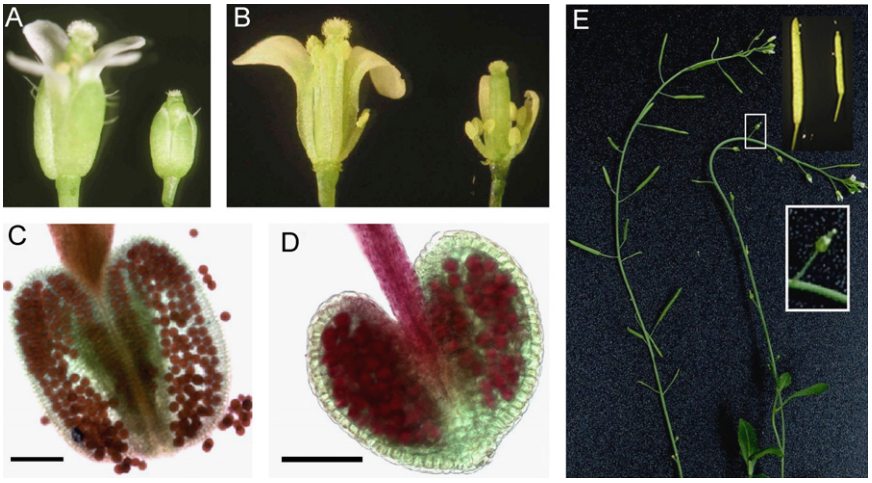

Figure 7. Comparison of flowers from wild-type and *OsERF1*-overexpressed plants L6. (A). Wild-type flower (left) and *OsERF1*-overexpressed infertile flower (right). (B) Dissection structure of wild-type flower (left) and *OsERF1*-overexpressed infertile flower (right). Four sepals and two petals had been stripped off to show the small petals, small and short stamens in *OsERF1*-overexpressed infertile flower. (C, D) Bright-field micrographs of Alexander-stained anthers from wild-type flower (C) and *OsERF1*-overexpressed infertile flower (D). Scale bar = 100 μm. (E) Inflorescences and siliques from wild-type (left) and *OsERF1*-overexpressed plants (right). The magnified part shows *OsERF1*-overexpressed infertile flower.

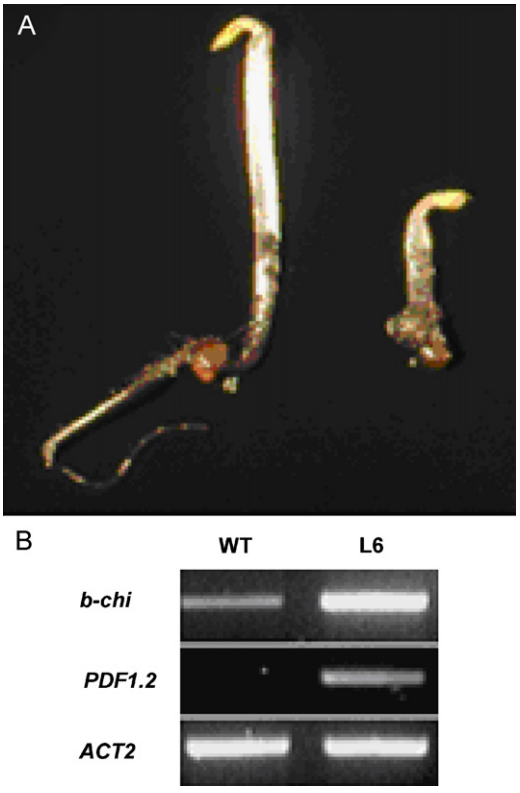

Figure 8. Identification of the ethylene response in wild-type and *OsERF1*-overexpressed *Arabidopsis* L6. (A) Phenotypes of 3-day-old dark-grown wild-type (left) and *OsERF1*-overexpressed (L6, right) seedlings at 23 °C. (B) Semi-quantitative RT-PCR examination of the transcripts of ethylene-responsive genes (*PDF1.2* and *b-chitinase*) in wild-type and *OsERF1*-overexpressed *Arabidopsis*. Total RNAs were prepared from rosette leaves of wild-type (WT) and *OsERF1*-overexpressed (L6) plants at vegetative growth stage. The *ACT2* transcripts were used as a constitutive control. PCR was performed in a total of 25 cycles. Scale bar = 100 μm.

roots but exaggerated hooks were not observed, which was similar to *ERF1*-overexpressed *Arabidopsis* plants. In *OsERF1*-overexpressed *Arabidopsis* plants, examination of the expression of two ethylene-responsive genes *b-chitinase* and *PDF1.2*, which work at the downstream of *ERF1* in *Arabidopsis* (Solano et al., 1998), showed that both of them were up-regulated (Figure 8B).

Discussion

The protein encoded by *OsERF1* in rice is a member of the ERF subgroup. Besides having the AP2/EREBP DNA-binding domain, it contains an alanine-rich and a serine-rich region, which have been proposed to compose a transcriptional activation domain in other transcription factors (Jofuku et al.,1994; Elliott et al., 1996). These features, in combination with its acidic p*I*, relative hydrophilicity and nuclear localization, suggest that *OsERF1* is a potential transcription factor.

OsERF1 is expressed consistently in different organs or tissues of rice and can be up-regulated by ethylene in a dose-dependent manner. Overexpression of *OsERF1* in *Arabidopsis* leads to phenotypes similar to those caused by overexpressing *EIN3* or *ERF1*, two important components of the ethylene signaling pathway in *Arabidopsis* (Chao et al., 1997; Solano et al., 1998). These observations, together with the fact that overexpression of *OsERF1* in *Arabidopsis* up-regulates the expression of *b-chitinase* and *PDF1.2*, suggest the possible involvement of *OsERF1* in ethylene signaling pathway since pervious researches have shown that *b-chitinase* and *PDF1.2* are ethylene-responsive and pathogen-associated genes (Solano et al., 1998). Probably, like other characterized ERF genes such as *ERF1* from *Arabidopsis* (Solano et al., 1998) or *Pti*4, *Pti*5 and *Pti*6 from tomato (Zhou et al., 1997) *OsERF1* may be also involved in this process.

OsERF1 belongs to the B-3/IX group of ERF gene subfamily (Nakano et al., 2006). Functionally characterized members of this group include *AtERF1*, *AtERF3*, *ERF1* from *Arabidopsis*, *Pti4* from tomato (Solano et al., 1998; Fujimoto et al., 2000; Gu et al., 2000), etc. These genes have often been linked to defensive gene expression in response to pathogen infection (Nakano et al., 2006). As identified by Berrocal-Lobo et al. (2002), overexpression of *Arabidopsis ERF1* enhances resistance to necrotic fungi and bacteria. Similarly, *Pti4* overexpression in tomato strengthens its resistance to biotrophic fungi (Gu et al., 2002). In addition, more and more evidences have shown that defense-related phytohormones such as ethylene, jasmonate or SA can induce the expression of genes in B-3/IX group (Gu et al., 2000; Oñate-Sánchez and Singh, 2002; Nakano et al., 2006). Therefore, the fact that ethylene intensified the expression of *OsERF1* in rice and overexpression of *OsERF1* up-regulated the expression of two pathogen-associated genes *b-chitinase* and *PDF1.2* in *Arabidopsis* suggest that *OsERF1* is a possible pathogen-resistant factor. Further investigation focusing on its resistance to pathogen attack may provide us more useful information.

In summary, our findings on *OsERF1* and another newly identified ERF gene *OsRAF* in rice (Hu et al., 2008) show that rice ERF genes are largely conserved both in sequence and in function as in *Arabidopsis* and tobacco. These data, together with

studies on the function of ERFs from other species, suggest that ERF-mediated ethylene response is a basic pattern of plant adaptation to environmental changes during evolution.

Acknowledgment

The research was supported by grants from the Ministry of Science and Technology of China (No. 2007AA021402).

References

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

Cao Y, Song F, Goodman RM, Zheng Z. Molecular characterization of four rice genes encoding ethylene-responsive transcriptional factors and their expressions in response to biotic and abiotic stress. J Plant Physiol 2006;163:1167–78.

Chao Q, Rothenberg M, Solano R, Roman G, Terzaghi W, Ecker JR. Activation of the ethylene gas response pathway in Arabidopsis by the nuclear protein ETHYLENE-INSENSITIVE3 and related proteins. Cell 1997;89:1133–44.

Ding ZJ, Wu XH, Wang T. The rice tapetum-specific gene RA39 encodes a type I ribosome-inactivating protein. Sex Plant Reprod 2002;15:205–12.

Dubouzet JG, Sakuma Y, Ito Y, Kasuga M, Dubouzet EG, Miura S, et al. *OsDREB* genes in rice, *Oryza sativa* L., encode transcription activators that function in drought-, high-salt- and cold-responsive gene expression. Plant J 2003;33:751–63.

Elliott RC, Betzner AS, Huttner E, Oakes MP, Tucker WQJ, Gerentes D, et al. AINTEGUMENTA, an APETALA2-like gene of Arabidopsis with pleiotropic roles in ovule development and floral organ growth. Plant Cell 1996;8:155–68.

Fujimoto SY, Ohta M, Usui A, Shinshi H, Ohem-Takagi M. Arabidopsis ethylene-responsive element binding factors act as transcriptional activation or repressors of GCC box-mediated gene expression. Plant Cell 2000;12:393–404.

Gu YQ, Yang C, Thara VK, Zhou J, Martin GB. *Pti4* is induced by ethylene and salicylic acid, and its product is phosphorylated by the Pto kinase. Plant Cell 2000; 12:771–86.

Gu YQ, Wildermuth MC, Chakravarthy S, Loh YT, Yang C, He X, et al. Tomato transcription factors Pti4, Pti5, and Pti6 activate defense responses when expressed in Arabidopsis. Plant Cell 2002;14:817–31.

Guo ZJ, Chen XJ, Wu XL, Ling JQ, Xu P. Overexpression of the AP2/EREBP transcription factor OPBP1 enhances disease resistance and salt tolerance in tobacco. Plant Mol Biol 2004;55:607–18.

Gutterson N, Reuber TL. Regulation of disease resistance pathways by AP2/ERF transcription factors. Curr Opin Plant Biol 2004;7:465–71.

Haake V, Cook D, Riechmann JL, Pineda O, Thomashow MF, Zhang JZ. Transcription factor CBF4 is a regulator of drought adaptation in Arabidopsis. Plant Physiol 2002;130:639–48.

Hu YB, Chong K, Wang T. *OsRAF* is an ethylene responsive and root abundant factor gene of rice. Plant Growth Regul 2008;54:55–61.

Jofuku KD, den Boer BG, Van Montagu M, Okamuro JK. Control of Arabidopsis flower and seed development by the homeotic gene *APETALA2*. Plant Cell 1994;6: 1211–25.

Kumar S, Tamura K, Nei M. Integrated software for molecular evolutionary genetics analysis and sequence alignment. Brief Bioinform 2004;5:150–63.

Liu Q, Kasuga M, Sakuma Y, Abe H, Miura S, Yamaguchi-Shinozaki K, et al. 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 1998; 10:1391–406.

Magnani E, Sjolander K, Hake S. From endonucleases to transcription factors: evolution of the AP2 DNA binding domain in plants. Plant Cell 2004;16:2265–77.

Murashige T, Skoog F. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant 1962;15:473–97.

Nakano T, Suzuki K, Fujimura T, Shinshi H. Genome-wide analysis of the ERF gene family in Arabidopsis and rice. Plant Physiol 2006;140:411–32.

Okamura JK, Caster B, Villarroel R, Van Montagu M, Jofuku KD. The AP2 domain of *APETALA2* defines a large new family of DNA binding proteins in Arabidopsis. Proc Natl Acad Sci USA 1997;94:7076–81.

Oñate-Sánchez L, Singh KB. Identification of Arabidopsis ethylene-responsive element binding factors with distinct induction kinetics after pathogen infection. Plant Physiol 2002;128:1313–22.

Sakuma Y, Liu Q, Dubouzet JG, Abe H, Shinozaki K, Yamaguchi-Shinozaki K. 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 2002;290:998–1009.

Solano R, Stepanova A, Chao Q, Ecker JR. Nuclear events in ethylene signaling: a transcriptional cascade mediated by ETHYLENE-INSENSITIVE3 and ETHYLENE-RESPONSE-FACTOR1. Genes Dev 1998;12:3703–14.

Weigel D. The APETALA2 domain is related to a novel type of DNA binding domain. Plant Cell 1995;7:388–9.

Yang Y, Li R, Qi M. *In vivo* analysis of plant promoters and transcription factors by agroinfiltration of tobacco leaves. Plant J 2000;22:543–51.

Zhou J, Tang X, Martin GB. The Pto kinase conferring resistance to tomato bacterial speck disease interacts with proteins that bind a cis-element of pathogenesis-related genes. EMBO J 1997;16:3207–18.