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The ABRE-binding bZIP transcription factor OsABF2 is a positive regulator of abiotic stress and ABA signaling in rice

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ABSTRACT

Abscisic acid (ABA) is an important phytohormone involved in abiotic stress tolerance in plants. The group A bZIP transcription factors play important roles in the ABA signaling pathway in *Arabidopsis* but little is known about their functions in rice. In our current study, we have isolated and characterized a group A bZIP transcription factor in rice, OsABF2 (*Oryza sativa* ABA-responsive element binding factor 2). It was found to be expressed in various tissues in rice and induced by different types of abiotic stress treatments such as drought, salinity, cold, oxidative stress, and ABA. Subcellular localization analysis in maize protoplasts using a *GFP* fusion vector indicated that OsABF2 is a nuclear protein. In yeast experiments, OsABF2 was shown to bind to ABA-responsive elements (ABREs) and its N-terminal region found to be necessary to transactivate a downstream reporter gene. A homozygous T-DNA insertional mutant of *OsABF2* is more sensitive to salinity, drought, and oxidative stress compared with wild type plants. In addition, this *Osabf2* mutant showed a significantly decreased sensitivity to high levels of ABA at germination and post-germination. Collectively, our present results indicate that OsABF2 functions as a transcriptional regulator that modulates the expression of abiotic stress-responsive genes through an ABA-dependent pathway.

1. Introduction

Abiotic stresses constitute a major constraint for agricultural production worldwide, particularly in developing countries where crop growth and productivity can be significantly reduced by these stimuli. Improvements in the abiotic stress tolerance capacity of plants through biotechnology thus holds great promise for increasing food production in regions with limited resources but this requires a better understanding of the molecular mechanisms underlying abiotic stress adaptation in plants (Xiong et al., 2002; Zhu, 2002; Yamaguchi-Shinozaki and Shinozaki, 2006; Seki et al., 2007).

The phytohormone abscisic acid (ABA) controls various aspects of plant growth and development (Finkelstein et al., 2002; Himmelbach et al., 2003). During vegetative growth, one of the major roles of ABA is to mediate adaptive responses to various environmental stresses, such as drought, high salinity, low temperature, oxidative stress, and mechanical wounding. The pathways leading to stress adaptation are categorized as ABA-dependent and ABA-independent. In addition, underlying the ABA-mediated stress responses is the transcriptional regulation of stress-responsive gene expression (Shinozaki and Yamaguchi-Shinozaki, 2000; Xiong et al., 2002; Zhu, 2002; Finkelstein et al., 2002; Jakoby et al., 2002; Himmelbach et al., 2003).

During the response and adaptation of plants to diverse abiotic stresses, many stress-related genes are induced and the levels of a variety of stress resistance-related functional proteins accumulate. Numerous genes have also been reported to be upregulated under stress conditions in vegetative tissues (Zhu, 2002; Seki et al., 2002). As a trigger of gene expression, transcription factors play important regulatory roles in every aspect of the biology of plants, including growth, development, and the response to abi-

Abbreviations: ABA, abscisic acid; ABRE, abscisic acid responsive element; ABF, abscisic acid responsive element binding factor; bZIP, basic leucine zipper; OsABF2, *Oryza sativa* ABA-responsive element binding factor 2.

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otic and biotic stress. The basic leucine zipper (bZIP) transcription factor family is one of the largest such families in plants and its members have diverse roles, particularly in plant stress-responsive and hormone signal transduction (Jakoby et al., 2002; Uno et al., 2000; Rodriguez-Uribe and O'Connell, 2006). To activate downstream gene expression, the bZIP transcription factors interact with ABA-responsive elements (ABREs). These are *cis*-acting elements containing the PyACGTGGC core sequence and are present in the promoter region of ABA-inducible genes. Hence, these bZIP transcription factors are designated as ABRE-binding factors (ABFs) or ABRE-binding proteins (AREBs) (Niu et al., 1999; Kim et al., 1997; Yamaguchi-Shinozaki and Shinozaki, 2005). In transient experiments using *Arabidopsis* mesophyll protoplasts, the transcription of a reporter gene driven by ABRE has been demonstrated to be activated by several ABFs such as AREB1 and AREB2 (Uno et al., 2000; Nakashima et al., 2006).

In *Arabidopsis*, the 75 identified bZIP transcription factors can be classified into 10 subfamilies based on sequence homology. Thirteen bZIPs belong to the A group which contains the ABF genes (Jakoby et al., 2002). These bZIP ABF genes, ABF2/AREB1, ABF4/AREB2, and ABF3, are upregulated by ABA, dehydration, and salinity stress in vegetative tissues (Choi et al., 2000; Uno et al., 2000). The constitutive overexpression of ABF3 in *Arabidopsis* and rice also results in enhanced drought tolerance (Kang et al., 2002; Oh et al., 2005). Moreover, in rice, overexpression of the positive regulators of ABA signaling, OsbZIP23 and OsbZIP72, enhances abiotic stress tolerance (Xiang et al., 2008; Lu et al., 2008) and mutants of *OsABF1*, which is a positive regulator of ABA signaling, are more sensitive to drought and salinity (Hossain et al., 2010). Besides abiotic stress tolerance, ABF proteins also play an important role in plant growth and development. For example, the *Arabidopsis abi5* mutant shows decreased sensitivity to ABA inhibition of seed germination and also an altered ABA-regulated gene expression profile, indicating that AtABI5 links ABA signal transduction with seed-specific gene expressions (Finkelstein and Lynch, 2000). In monocots, a bZIP transcription factor, OsABI5, is involved in rice fertility and stress tolerance (Zou et al., 2008). Furthermore, the AtABI5 homologs, TRAB1 and HvABI5 in rice and barley, respectively, physically interact with their corresponding AtABI3 homologs, OsVP1 and HvVP1, and regulate seed maturation and dormancy by activating ABA-responsive genes (Hobo et al., 1999; Nakamura et al., 2001; Casaretto and Ho, 2003). These previous data indicate that ABF proteins function in a conserved ABA signal transduction pathway in both dicot and monocot plant species.

Rice is one of the most important stable crops globally and also a model monocot species for molecular research. About 89 bZIP transcription factors are predicted in the rice genome belonging to 11 groups based on their DNA-binding specificity and amino acid sequences within the basic and hinge regions. Among these, 14 belong to group VI (equivalent to group A in *Arabidopsis*) which contains the ABF genes (Nijhawan et al., 2008). However, despite the biotechnological potential of utilizing the group A *AtbZIP* orthologs in rice, relatively few of these factors have been functionally studied thus far. In our present study, we have identified and functionally characterized OsABF2, an abiotic stress-inducible bZIP transcription factor in rice. The tissue-specific expression of *OsABF2* was examined and its expression in response to several environmental stresses and plant hormones was also evaluated. The ABRE-binding and transactivation ability of OsABF2 was then tested using a yeast system. To investigate the *in vivo* function of OsABF2, a T-DNA insertional mutant of this gene was analyzed under salinity, drought, and oxidative stress conditions. We also compared the ABA sensitivity of mutant plants and wild type plants at the germination and post-germination stages. Our results suggest that OsABF2 is a positive regulator of the abiotic stresses response and ABA-dependent signaling transduction pathway in rice.

2. Materials and methods

2.1. Phylogenetic analysis

The ClustalW-EBI program was used for multiple sequence alignments (http://www.ebi.ac.uk/clustalW). The phylogenetic tree was constructed using MEGA software version 4.0 via the neighbor-joining method (Tamura et al., 2007). Bootstrap analysis was performed with 1000 replicates and bootstrap values are shown as percentages.

2.2. Plant materials, growth conditions and stress treatments

Wild type rice (*Oryza sativa* L. cultivar Dongjin) seeds were surface sterilized with 70% ethanol and immersed in distilled water for 1 day at 4 °C in the dark and then grown in a plant growth chamber (27 ± 1 °C, 80% relative humidity and 14/10 h day/night photoperiod). Two-week old seedlings were subjected to different stress treatments including drought (10% polyethylene glycol (PEG)), salinity (250 mM NaCl), oxidative stress (10 μM Methyl Viologen (MV)), cold (4 °C), and ABA (100 μM) for 0, 3, 6, 12, 24, and 48 h. A T-DNA insertional mutant line of *OsABF2* was identified from the rice T-DNA Insertion Sequence Database (Jeong et al., 2006; http://www.postech.ac.kr/life/pfg/risd/index.html). A homozygous *Osabf2* line was isolated by PCR screening using *OsABF2* gene-specific and T-DNA specific primers.

2.3. cDNA cloning

The full length cDNA sequence of *OsABF2* was isolated from drought treated shoots of rice seedlings (*O. sativa* cv. Dongjin) by RT-PCR using the forward primer 5′-AAGCTTATGGAGTTGCCGGCGGATGGG-3′ and the reverse primer 5′-GAATTCTCAGCATGGACCAGTCAGTGT-3′. The RT-PCR products were subsequently inserted into the pLUG-TA vector (iNTRON Biotechnology, Seoul, Korea), and sequenced. The confirmed full length cDNA sequence has been deposited into NCBI GenBank under the accession number GU552783.

2.4. RT-PCR

Total RNAs were isolated from the shoot, root, stem, mature leaves, flag leaves, and panicle of rice plants under normal growth condition and also from stress-treated seedling shoots using Trizol reagent (Gibco-BRL, Grand Island, NY). First strand cDNA was synthesized with 2 μg of purified total RNA using M-Mulu^{TM} Reverse Transcriptase (New England Biolabs, Beverly, MA) and an oligo (dT) primer in a total volume of 25 μl. The RT reaction was incubated at 37 °C overnight. The gene-specific primers 5′-ATCAAGAACAGGGAGTCCGC-3′ and 5′-GAGCCATCACCATTCACCAA-3′ were used in the RT-PCR and the rice *actin1* gene (accession number NP_001051086) was amplified as an internal control to quantify the relative amounts of cDNA (McElroy et al., 1990).

2.5. Subcellular localization of GFP fusion proteins

The *OsABF2* gene was amplified by PCR using the primer pairs: forward primer, 5′-CACCATGGAGTTGCCGGCGGATGGGAGC-3′ and either a reverse primer for an N-terminal GFP fusion, 5′-TCAGCATGGACCAGTCAGTGTTCG-3′ or C-terminal GFP fusion, 5′-GCATGGACCAGTCAGTGTTCGTCG-3′. The PCR products were then subcloned into the pENTR/D-TOPO vector (Invitrogen, Carlsbad, CA). Validated inserts were then subcloned into the respective destination vectors p2FGW7 for N-terminal GFP fusion and p2GWF7 for C-terminal GFP fusion (Karimi et al., 2002), using