Biochemical and Biophysical Research Communications 414 (2011) 135–141

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

AtERF71/HRE2 transcription factor mediates osmotic stress response as well as hypoxia response in *Arabidopsis*

Hee-Yeon Park, Hye-Yeon Seok, Dong-Hyuk Woo, Sun-Young Lee, Vaishali N. Tarte, Eun-Hye Lee, Choon-Hwan Lee, Yong-Hwan Moon *

Department of Molecular Biology, Pusan National University, Busan 609-735, Republic of Korea

ARTICLE INFO

Article history:
Received 4 September 2011
Available online 16 September 2011

Keywords:
Arabidopsis
AtERF71/HRE2
AP2/ERF domain
Osmotic stress
Transcription activation

ABSTRACT

Various transcription factors are involved in the response to environmental stresses in plants. In this study, we characterized *AtERF71/HRE2*, a member of the *Arabidopsis* AP2/ERF family, as an important regulator of the osmotic and hypoxic stress responses in plants. Transcript level of *AtERF71/HRE2* was highly increased by anoxia, NaCl, mannitol, ABA, and MV treatments. *aterf71/hre2* loss-of-function mutants displayed higher sensitivity to osmotic stress such as high salt and mannitol, accumulating higher levels of ROS under high salt treatment. In contrast, *AtERF71/HRE2*-overexpressing transgenic plants showed tolerance to salt and mannitol as well as flooding and MV stresses, exhibiting lower levels of ROS under high salt treatment. AtERF71/HRE2 protein was localized in the nucleus, and the C-terminal region of AtERF71/HRE2 was required for transcription activation activity. Taken together, our results suggest that AtERF71/HRE2 might function as a transcription factor involved in the response to osmotic stress as well as hypoxia.

1. Introduction

During their life cycle, plants have to deal with various environmental stress conditions. To adjust to changes in the environment, plants trigger rapid defense responses by regulating the expression of a number of defense genes. As mediators of stress signal transduction, transcription factors modulate the expression of many stress-responsive genes. In *Arabidopsis*, numerous transcription factor families, each containing a distinct type of DNA-binding domain such as AP2/ERF, bZIP/HD-ZIP, MYB, MYC, NAC, and WRKY as well as several classes of zinc-finger domains, have been implicated in plant stress responses [1,2].

AP2/ERF transcription factors belong to one of the largest plant transcription factor families, and are characterized by conserved AP2/ERF DNA-binding domains of 57–66 amino acids in size [3]. In *Arabidopsis*, 145 genes were reported as members of the AP2/ERF superfamily [4]. The AP2/ERF multigene family is divided into four subfamilies named AP2, DREB/CBF, ERF, and RAV based on their sequence similarities and number of AP2/ERF domains [4].

AP2 subfamily proteins contain two AP2/ERF domains, and genes in this subfamily participate in the regulation of developmental processes [5,6]. The RAV subfamily proteins contain one AP2/ERF domain and one B3 domain, and are involved in the ethylene response, brassinosteroid response, and biotic and abiotic stress-responses [7,8]. In contrast to the AP2 and RAV subfamily members, DREB/CBF and ERF subfamily proteins contain only a single AP2/ERF domain [4]. The genes belonging to the DREB/CBF subfamily play a crucial role in the resistance of plants to abiotic stresses by recognizing the dehydration-responsive element/C-repeat (DRE/CRT) [9,10]. The ERF subfamily is involved in the response to both biotic and abiotic stresses by recognizing a *cis*-acting element AGCCGCC, known as the GCC box, and/or DRE/CRT elements [11,12].

In *Arabidopsis*, many ERF subfamily genes are known to be involved in abiotic stress responses. *Arabidopsis ERF1–5* genes are induced by drought, salt, or cold stresses [13]. Among them, *AtERF1*, *AtERF2*, and *AtERF5* function as transcription activators, whereas *AtERF3* and *AtERF4* act as transcription repressors [13]. *AtERF7* and *RAP2.6* have been reported to regulate stomata size or plant responses to drought, high salinity, cold, and ABA stresses via ABA-dependent signaling pathways [14,15]. Recently, *AtERF71/HRE2* and *AtERF73/HRE1*, both *Arabidopsis* ERF subfamily members, were found to function as important regulators in the response to hypoxia [16,17]. Expression of *AtERF71/HRE2* is increased by hypoxia, but it remains basically unaffected by mutations in ethylene

Abbreviations: ABA, abscisic acid; DAG, days after germination; *GAPc*, glyceraldehyde 3-phosphate dehydrogenase; MV, methyl viologen; NBT, nitroblue tetrazolium; ROS, reactive oxygen species.

* Corresponding author. Fax: +82 51 513 9258.
E-mail address: moonyh@pusan.ac.kr (Y.-H. Moon).

0006-291X/$ - see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2011.09.039

signaling, indicating that unlike *AtERF73*/*HRE1*, *AtERF71*/*HRE2* is not related with ethylene signaling [18]. However, despite these studies, the roles of *AtERF71*/*HRE2* in the response to abiotic stresses other than hypoxia have not yet been intensively studied.

In this study, we characterized the role of *AtERF71*/*HRE2* in osmotic stress response as well as hypoxia response in *Arabidopsis*. We found that transcript level of *AtERF71*/*HRE2* was significantly increased by both osmotic stresses and hypoxia, and that *aterf71*/*hre2* loss-of-function mutants as well as *AtERF71*/*HRE2*-overexpressing transgenic plants (OXs) produced aberrant responses to abiotic stress treatments such as NaCl, mannitol, MV, and flooding. Moreover, analysis of the subcellular localization and transcription activation activity of AtERF71/HRE2 demonstrated that it might function as a transcription factor in the nucleus.

2. Materials and methods

2.1. Plant materials and growth conditions

All *Arabidopsis* (*Arabidopsis thaliana*) plants used in this study were Columbia ecotype. *Arabidopsis* seeds were surface-sterilized and germinated on agar plates as previously described [19]. The plates were then placed under short-day (SD) conditions (cycles of 8-h light/16-h dark) at 22 °C. Ten-day-old seedlings were transplanted to soil and grown under long-day (LD) conditions (cycles of 16-h light/8-h dark) at 22 °C.

2.2. Plasmid construction

The vectors for overexpression and RNAi of *AtERF71*/*HRE2*, *AtERF71*/*HRE2* promoter::*GUS* transgenic plants, synthetic Green Fluorescence Protein (sGFP)-fused AtERF71/HRE2, and GAL4 DNA-binding domain (BD)-AtERF71/HRE2 fusion proteins were constructed as described in Supplementary Materials and methods.

2.3. Plant transformation

The constructs for plant expression were transformed into *Agrobacterium tumefaciens* strain GV3101 (pMP90) by the freeze–thaw method [20] and then transformed into *Arabidopsis* using the floral-dipping method [21]. Transgenic plants were selected on medium containing 25 mg/L kanamycin.

2.4. Stress treatments

For NaCl treatment, 7-day-old seedlings grown under SD conditions were transferred to MS-agar medium [22] supplemented with 0, 120, 130, 140, 150, 160, or 170 mM NaCl. Response to NaCl was estimated by measuring the fresh weight (FW) of seedlings after 14 days of treatment. For mannitol treatment, 7-day-old seedlings were transferred to MS medium supplemented with 0, 300, or 400 mM mannitol. Response to mannitol was estimated by measuring primary root elongation after 14 days of treatment. For flooding treatment, 4-week-old plants were dipped in water to a depth of 5 cm for 10 days and recovered for 5 days. For MV treatment, 14-day-old seedlings were transferred to MS medium containing 0, 10, or 15 μM MV and plant phenotypes were observed after 14 days of treatment. For germination ratio analysis, seeds were germinated on MS medium containing 0, 0.1, 0.5, or 1.0 μM MV, and germination ratio was analyzed at 14 days after germination (DAG).

For pathogen treatment, *Pseudomonas syringae* pv. *tomato* (*Pst*) DC3000 was used. *Pst* DC3000 was grown on *Pseudomonas* medium (10% bacto peptone, 10% bacto proteose, 6.1 mM $MgSO_4 \cdot 7H_2O$, 8.6 mM K_2HPO_4, pH 7.0) supplemented with rifampicin (20 μg/mL) at 30 °C. Rosette leaves of 3-week-old plants were sprayed with bacterial suspensions (OD_{600} = 1 in 1 mM $MgCl_2$ with 0.04% silwet-77). After 5 days, plant phenotypes were observed.

For stress treatments prior to RT-PCR analysis, 10-day-old wild-type (WT) seedlings on MS plates were transferred to filter paper saturated with 300 mM NaCl, 300 mM mannitol, 100 μM ABA, or 10 μM MV, followed by incubation for 0, 1, 2, 4, or 8 h. For anoxia treatment, mature rosette leaves detached from 4- to 5-week-old WT plants were floated on water and treated with 99.99% nitrogen gas under dark conditions for 0, 1, 4, 8, or 12 h.

2.5. Semi-quantitative reverse-transcription (RT)-PCR and quantitative RT-PCR

Total RNA was isolated using TRIzol reagent (Molecular Research Center). cDNA synthesis, semi-quantitative RT-PCR, and quantitative RT-PCR were performed as previously described [23]. The primers used for the PCRs are shown in Supplementary Table S1.

For semi-quantitative RT-PCR, PCR of 24 cycles was performed for *GAPc* and 27–31 cycles for other genes. The number of PCR cycles selected was shown to be in the linear range of the amplification reaction. *GAPc* was amplified in the same tube as each gene studied as an internal control.

In quantitative RT-PCR, the normalized amount of the target reflects the relative amount of target transcripts with respect to the endogenous reference gene *GAPc*.

2.6. GUS activity analysis and detection of superoxide production in seedlings

GUS activity was histochemically detected using a protocol adapted from a previous report [23].

Ten-day-old plants grown on MS medium were transferred to MS medium containing 50 or 100 mM NaCl and after 5 h incubation and detection of superoxide production was performed as previously described [24].

2.7. Transient gene expression in Arabidopsis protoplasts

To investigate the subcellular localization of AtERF71/HRE2 in *Arabidopsis* protoplasts, polyethylene glycol (PEG)-mediated protoplast transformations were performed according to the method described by Sheen [25].

2.8. Transcription activation activity analysis in yeast

To investigate the transcription activation activity of AtERF71/HRE2 in yeast, pBDGAL4–AtERF71/HRE2 constructs were transformed into a yeast strain, YD116, which carries the $GAL1_{pro}$::*URA3* and UAS_{pro}::*lacZ* reporters. Transformants including BD fusion vectors were selected on SM-Trp. Transcription activation activities were confirmed by growth assay on SM-Trp-Ura and by quantitative β-galactosidase assay using 2-nitrophenyl-β-D-galacto-pyranoside (ONPG) as a substrate. Quantitative β-galactosidase assay was performed as previously described [23], and the unit of β-galactosidase activity was calculated using the formula: $1000 \times OD_{420}/(OD_{600} \times \text{assay time in min} \times \text{assay volume in mL})$.

3. Results and discussion

3.1. Isolation of AtERF71/HRE2

From the microarray analysis, we selected a rice gene, *Os07g47790*, which was greatly induced under anoxic conditions