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

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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.

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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

(Supplementary Fig. S1). By BlastP analysis, we isolated *AtERF71*, an *Arabidopsis* homolog of *Os07g47790* [26]. During preparation of this manuscript, *AtERF71* was also reported as *HRE2* [16]. AtERF71/HRE2 showed high homology with Os07g47790, especially in the N-terminal region and AP2/ERF domain (Supplementary Fig. S2).

The predicted AtERF71/HRE2 protein contains a conserved DNA-binding domain, AP2/ERF domain, of 58 amino acids and a conserved N-terminal motif of unknown function, MCGGAII/L (Supplementary Fig. S2). Tournier et al. [27] identified a novel class IV ERF, characterized by the N-terminal signature sequence MCGGAII/L. On the basis of sequence homology and its conserved motif, AtERF71/HRE2 belongs to Class IV ERF.

3.2. Expression analysis of AtERF71/HRE2 under anoxia, NaCl, mannitol, ABA, or MV treatment

To investigate the expression patterns of *AtERF71/HRE2* under abiotic stress conditions, we performed quantitative RT-PCR analysis using 10-day-old WT seedlings treated with anoxia, NaCl, mannitol, ABA, or MV. Transcript level of *AtERF71/HRE2* increased within 1 h of treatment of all stresses, except MV treatment (Fig. 1). Among the stresses investigated, *AtERF71/HRE2* showed the highest increase under anoxia treatment (Fig. 1A). Previously, the increased expression of *AtERF71/HRE2* under hypoxia was reported [16,17]. Under MV treatment, transcript level of *AtERF71/HRE2* increased within 8 h of treatment (Fig. 1E). Induction of *AtERF71/HRE2* expression by anoxia and NaCl was also confirmed in *AtERF71/HRE2* promoter::*GUS* transgenic plants (Fig. 1F and G). Interestingly, activity of the *AtERF71/HRE2* promoter was greatly increased in cotyledons by anoxia and NaCl treatment (Fig. 1F and G). These results suggest that *AtERF71/HRE2* might be involved in the response to osmotic stress as well as hypoxia.

Semi-quantitative RT-PCR analysis also showed very similar results to those of quantitative RT-PCR in which transcript level of *AtERF71/HRE2* was significantly increased by NaCl, mannitol, ABA, and MV treatments as well as anoxia treatment (Supplementary Fig. S3).

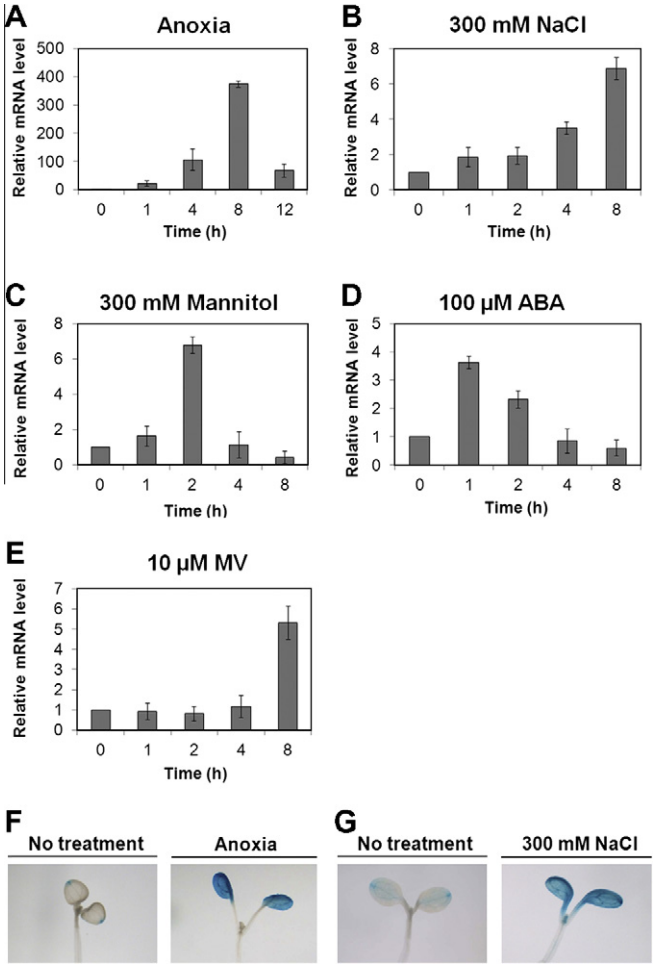

Fig. 1. Expression analysis of *AtERF71/HRE2* under abiotic stress conditions. Quantitative RT-PCR analysis of *AtERF71/HRE2* under anoxia treatment (A), 300 mM NaCl (B), 300 mM mannitol (C), 100 μM ABA (D), or 10 μM MV (E) for the indicated times. *GAPc* was used as an internal control. The transcript levels at 0 h were set to 1. Reactions of each technical replicate were performed in triplicate. Two technical replicates were measured for each biological replicate. Data shown are the means ± SD ($n = 6$). Similar results were obtained from at least two biological replicates, with one shown here. (F, G) Histochemical assay of *GUS* expression in *Arabidopsis* T_2 plants carrying *AtERF71/HRE2* promoter::*GUS* under anoxic conditions (99.99% nitrogen gas) for 6 h (F) and under 300 mM NaCl treatment for 6 h (G). Representative GUS staining results are shown here.

3.3. Response of aterf71/hre2 mutants to osmotic stress

To investigate biological function of *AtERF71/HRE2* in osmotic stress-response, *aterf71/hre2* T-DNA insertional mutant was analyzed for its response to osmotic stress such as high concentration of NaCl and mannitol. For this analysis, the seeds of a T-DNA insertional mutant (SALK_052858) of *AtERF71/HRE2* were obtained from the SIGnAL Collection at the Salk Institute [28], and homozygous mutant lines were selected (Fig. 2A and B). *aterf71/hre2* mutants displayed a salt-sensitive phenotype and a reduction in FW compared with WT plants under NaCl treatment (Fig. 2C and D). Under mannitol treatment, *aterf71/hre2* mutants showed reduced primary root elongation compared with WT plants (Fig. 2E and F). These results suggest that *aterf71/hre2* mutants are sensitive to osmotic stress.

Most abiotic stresses cause enhanced production of ROS in plants [29]. We therefore measured the generation of superoxide in *aterf71/hre2* mutants. After NaCl treatment, superoxide production in *aterf71/hre2* mutants was higher than that in WT plants (Fig. 2F). These results are consistent with the previous conclusion that *aterf71/hre2* mutants are sensitive to osmotic stress.

To confirm the osmotic stress response of *aterf71/hre2* mutants, we generated and selected RNAi transgenic plants suppressing *AtERF71/HRE2* expression (Supplementary Fig. S4A). The RNAi transgenic plants also showed similar sensitivity to NaCl and mannitol treatments compared with WT plants (Supplementary Fig. S4).

3.4. Response of transgenic plants overexpressing AtERF71/HRE2 to osmotic stress, MV, flooding, or pathogen

To confirm the role of *AtERF71/HRE2* in the osmotic stress response, we generated *AtERF71/HRE2* OXs in which *AtERF71/HRE2* was overexpressed under the control of the *cauliflower mosaic virus* (*CaMV*) *35S* promoter. We selected *AtERF71/HRE2* OX T_1 lines (Supplementary Fig. S5). T_2 and T_3 OXs of the selected *AtERF71/HRE2* OX T_1 lines were resistant to 150, 160, or 170 mM NaCl (Fig. 3A), and their FWs were also heavier than those of WT plants (Fig. 3B). Additionally, *AtERF71/HRE2* OXs showed enhanced primary root elongation compared with WT plants under 300 or 400 mM mannitol treatment (Fig. 3C and D). These results, together with the sensitive response of *aterf71/hre2* mutants to osmotic stress, indicate that *AtERF71/HRE2* is involved in osmotic stress response.

Hypoxic conditions often arise upon water logging or flooding [18]. Since *AtERF71/HRE2* expression was highly increased under anoxic conditions (Fig. 1A), we investigated the response of

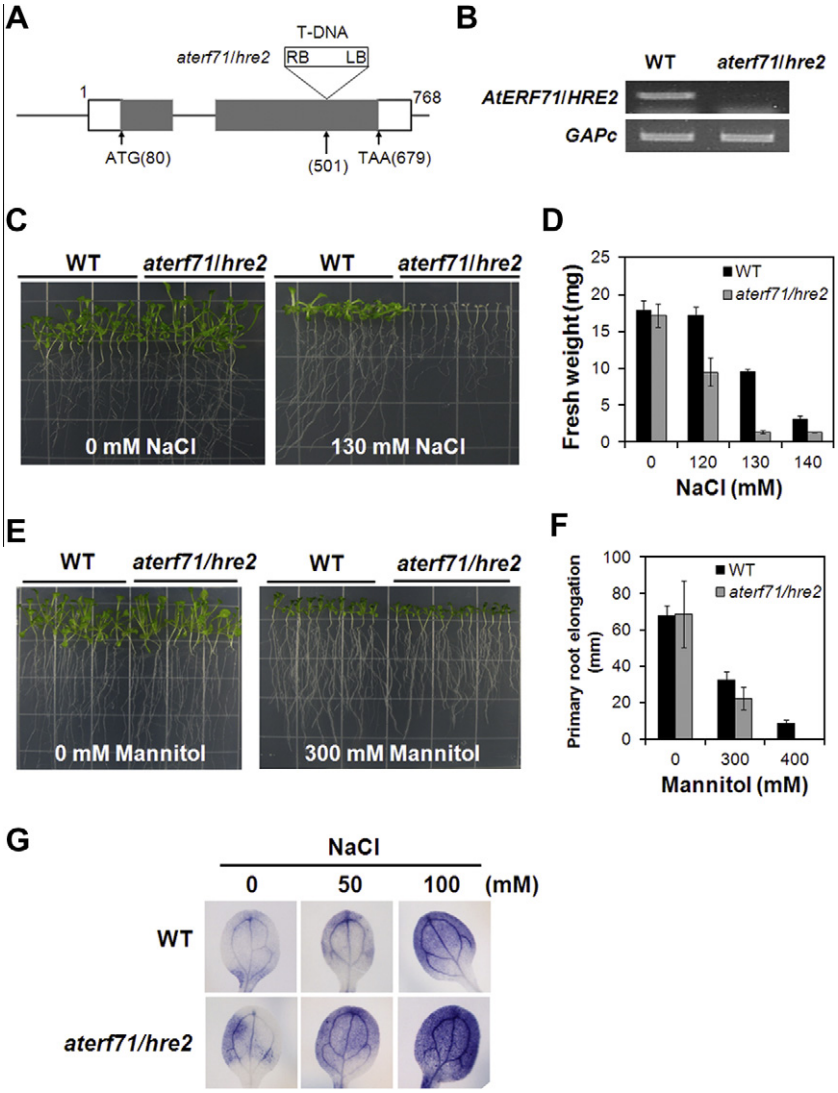

Fig. 2. Osmotic stress response of *aterf71/hre2* mutants. (A) Genomic structure of *AtERF71/HRE2* locus and the position of T-DNA insertion within *AtERF71/HRE2* in SALK_124398 line. Boxes represent exons; gray boxes and white boxes represent coding regions and untranslated regions, respectively. (B) Semi-quantitative RT-PCR analysis of *AtERF71/HRE2* transcript level in 4-week-old WT and *aterf71/hre2* mutant. *GAPc* was used as an internal control. (C) Response of WT and *aterf71/hre2* seedlings to 130 mM NaCl. (D) Fresh weights measured after NaCl treatments. (E) Response of WT and *aterf71/hre2* seedlings to 300 mM mannitol. (F) Primary root elongation measured after mannitol treatments. In (D) and (F), error bars represent standard deviation ($n = 24$ plants). (G) Superoxide accumulation in cotyledons of 10-day-old WT and *aterf71/hre2* seedlings under NaCl treatments for 5 h.

AtERF71/HRE2 OXs to flooding. *AtERF71/HRE2* OXs were more resistant to flooding (Fig. 3E and F). This result is consistent with a previous report, in which *AtERF71/HRE2* OX seedlings were found to be more tolerant under anaerobic conditions [16]. Moreover, *AtERF71/HRE2* OXs were resistant to MV treatment (Fig. 3G), and seeds of *AtERF71/HRE2* OXs showed higher germination ratio than those of WT plants (Fig. 3H). All together, these results suggest that *AtERF71/HRE2* might be involved in the response to various abiotic stresses such as osmotic stress, hypoxia, and oxidative stress.

Superoxide accumulation in *AtERF71/HRE2* OXs was lower than that in WT plants under NaCl treatment (Fig. 3I), which is consistent with previous results that *AtERF71/HRE2* OXs are more tolerant to high salt stress.

ERF subfamily genes involved in defense responses against pathogen infection have also been extensively documented [12,30]. We therefore investigated the response of *aterf71/hre2* mutants and *AtERF71/HRE2* OXs to *Pseudomonas* DC3000. Both *aterf71/hre2* mutants and *AtERF71/HRE2* OXs showed similar *Pseudomonas* DC3000 responses as that of WT plants (Supplementary Fig. S6A). In addition, transcript levels of defense-responsive genes such as *PDF1.2*, *PR-4*, *PR-5*, *CAX8*, and *GST6* were similar between WT plants and *AtERF71/HRE2* OXs (Supplementary Fig. S6B). These results suggest that *AtERF71/HRE2* might not be involved in the response to pathogens.

3.5. Spatial and temporal expression patterns of AtERF71/HRE2

We investigated the spatial and temporal expression patterns of *AtERF71/HRE2* by semi-quantitative RT-PCR. Transcript level of *AtERF71/HRE2* increased as plants developed (Supplementary Fig. S7A). In 7-day-old seedlings, *AtERF71/HRE2* expression was much higher in roots than in shoots (Supplementary Fig. S7B). In mature plants, *AtERF71/HRE2* transcripts were also highly detected in roots compared with that in other organs (Supplementary Fig. S7C). Previously, *AtERF71/HRE2* was reported to be highly expressed in roots under hypoxic conditions [16,17]. These results suggest that *AtERF71/HRE2* might play an important role in roots. The expression patterns of *AtERF71/HRE2* were also supported by

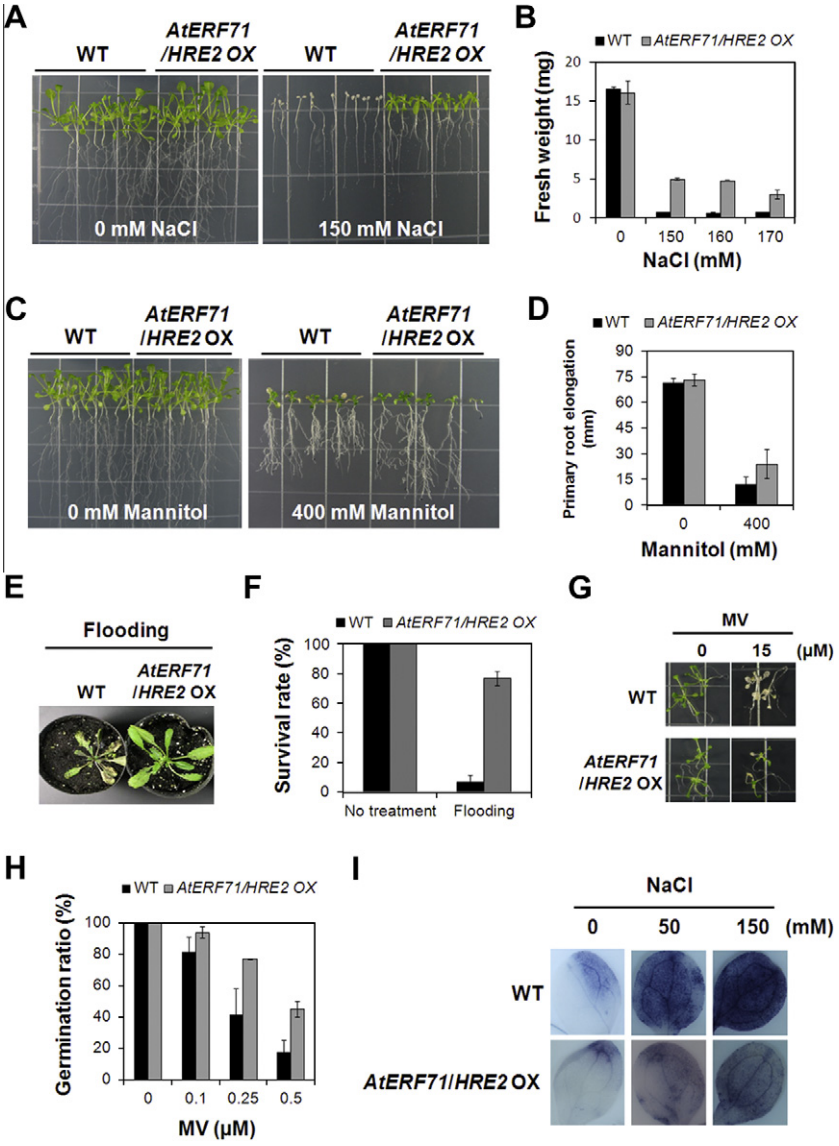

Fig. 3. Abiotic stress response of *AtERF71/HRE2* OXs. (A) Response of WT and *AtERF71/HRE2* OX seedlings to 150 mM NaCl. (B) Fresh weights of WT and *AtERF71/HRE2* OXs measured after NaCl treatments. (C) Response of WT and *AtERF71/HRE2* OX seedlings to 400 mM mannitol. (D) Primary root elongation of WT and *AtERF71/HRE2* OXs measured after mannitol treatment. In (B) and (D), error bars represent standard deviation ($n = 24$ plants). (E) Response of WT and *AtERF71/HRE2* OX plants to flooding. (F) Survival rate of WT and *AtERF71/HRE2* OX plants after flooding treatment. Error bars represent standard deviation of three independent experiments. Ten plants were analyzed in each experiment. (G) Response of WT and *AtERF71/HRE2* OX plants to 15 μM MV. (H) Germination ratio of WT and *AtERF71/HRE2* OXs on MS media containing MV. (G) Superoxide accumulation in cotyledons of 10-day-old WT and *AtERF71/HRE2* OX seedlings under NaCl treatments for 5 h.

GUS expression analysis in *AtERF71/HRE2* promoter::*GUS* transgenic plants (Supplementary Fig. S7D).

3.6. Subcellular localization and transcription activation activity of AtERF71/HRE2

Most AP2/ERF domain proteins are known to function as transcription factors [4]. AP2/ERF domain is involved in DNA-binding [3], and many AP2/ERF domain proteins possess transcription activation activity [4,8,13]. The N-terminal region of AtERF71/HRE2 contains two putative basic amino acid regions, K29KRK and R44ERK, which potentially act as NLSs. We studied the subcellular localization of AtERF71/HRE2 using sGFP-AtERF71/HRE2 fusion protein. As a result, we observed that AtERF71/HRE2 was localized in the nucleus (Fig. 4A), indicating that AtERF71/HRE2 functions in the nucleus.

It has been speculated that AtERF71/HRE2 lacks a transcription activation domain and requires interaction with a partner having *trans*-activating activity [16]. We investigated whether or not AtERF71/HRE2 could act as a transcription activator using a yeast system. The entire open reading frame (ORF) of AtERF71/HRE2 was fused to GAL4 BD (Fig. 4B), and the fusion construct was transformed into yeast cells. Based on the results of growth assay and quantitative β-galactosidase assay, AtERF71/HRE2 showed transcription activation activity in yeast (Fig. 4C and D), suggesting that AtERF71/HRE2 could act as a transcription activator in plants.

We next identified the domain of AtERF71/HRE2 responsible for transcription activation activity. We constructed a set of GAL4 BD-AtERF71/HRE2 vectors, including NM120 (1–120 aa), NM112 (1–112 aa), C71 (101–171 aa), or C51 (120–171 aa) (Fig. 4B). Yeast assay showed that the C-terminal region (120–171 aa) of AtERF71/HRE2 was responsible for the transcription activation activity (Fig. 4C and D). Although the C-terminal region does not include a known functional domain, it is conserved among class IV ERF proteins of other plant species such as *Cucumis melo* CMe-ERF2, *Prunus salicina* Ps-ERF2b, and soybean GmEBP (Fig. 4E) [31,32], suggesting that the

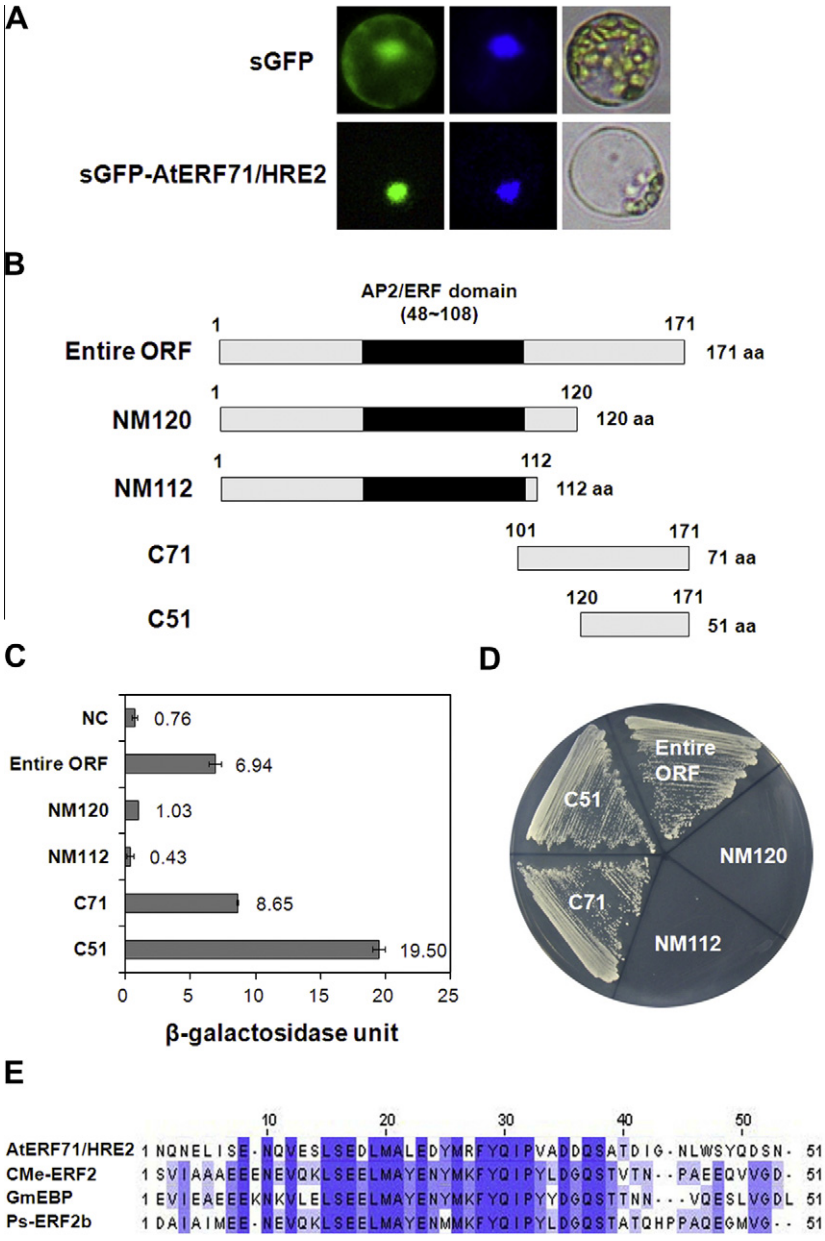

Fig. 4. Subcellular localization and transcription activation activity of AtERF71/HRE2. (A) The subcellular localization of AtERF71/HRE2 by transient expression of sGFP-AtERF71/HRE2 fusion construct in *Arabidopsis* protoplast. Left, GFP signal; middle, DAPI (4′,6-diamidino-2-phenylindole) staining; right, light microscope pictures. (B) Entire protein and fragments of AtERF71/HRE2 used in the analysis of transcription activation activity. Black box indicates AP2/ERF DNA-binding domain. (C) Quantitative β-galactosidase assay in yeast. Data shown are the means ± SD ($n = 4$). pBDGAL4 was used as a negative control. NC, negative control. (D) Growth assay of yeast transformants on SM-Trp-Ura. (E) Multiple alignment of 120–171 aa region of AtERF71/HRE2 with corresponding regions of CMe-ERF2, Ps-ERF2b, and GmEBP. Alignment was made by ClustalW using the default parameters.

conserved domain might be involved in transcription activation activity.

3.7. Expression analysis of ABA-dependent and ABA-independent stress-responsive genes in aterf71/hre2 mutants and AtERF71/HRE2 OXs

To determine whether or not the abiotic stress response of *AtERF71/HRE2* includes an ABA-dependent or ABA-independent pathway, we investigated transcript levels of ABA-dependent and ABA-independent stress-responsive genes in *aterf71/hre2* mutants and *AtERF71/HRE2* OXs under NaCl treatment. Transcript levels of ABA-dependent stress-responsive genes such as *NCED3*, *RD29A*, *Rab18*, and *COR15A* in *aterf71/hre2* mutants and *AtERF71/HRE2* OXs were similar to those in WT plants (Supplementary Fig. S8). In addition, transcript levels of ABA-independent stress-responsive genes such as *DREB2A* and *DREB2B* in *aterf71/hre2* mutants and *AtERF71/HRE2* OXs were also similar to those in WT plants (Supplementary Fig. S8). These results suggest that other ABA-independent stress-responsive genes might be involved in *AtERF71/HRE2*-mediated abiotic stress signal transduction.

Taken together, our results suggest that AtERF71/HRE2 might be involved in the response to osmotic stress as well as hypoxia, functioning as a transcription factor.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2011.09.039.

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