Plant Cell Rep (2014) 33:1255–1262
DOI 10.1007/s00299-014-1613-8

ORIGINAL PAPER

Arabidopsis HRE1α, a splicing variant of AtERF73/HRE1, functions as a nuclear transcription activator in hypoxia response and root development

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Received: 17 February 2014 / Revised: 25 March 2014 / Accepted: 31 March 2014 / Published online: 12 April 2014

Abstract
Key message **HRE1α shows transcriptional activation activity in its C-terminal region via GCC box but not DRE/CRT and plays an important role in root development via root meristem cell division regulation.**
Abstract AtERF73/HRE1 protein, a member of the *Arabidopsis* AP2/ERF family, contains a conserved AP2/ERF DNA-binding domain. Here, we studied the molecular function of HRE1α, a splicing variant of AtERF73/HRE1, as well as its role in root development. *HRE1α*-overexpressing transgenic plants (OXs) showed tolerance to submergence. HRE1α showed transcriptional activation activity via GCC box but not DRE/CRT. The 121–211 aa region of HRE1α was responsible for the transcriptional activation activity, and the region was conserved among homologs of other species but was not found in other *Arabidopsis* proteins. *HRE1α* OXs showed increased primary root length due to elevated root cell division. Our results suggest that HRE1α functions as a transcription activator in the nucleus, and plays an important role in root development through regulation of root meristem cell division.

Communicated by Y.-I. Park.

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Electronic supplementary material The online version of this article (doi:10.1007/s00299-014-1613-8) contains supplementary material, which is available to authorized users.

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Keywords *Arabidopsis* · AtERF73/HRE1 · Hypoxia · Root development · Transcription activator

Abbreviations

ABA	Abscisic acid
CaMV	Cauliflower mosaic virus
DAG	Days after germination
DRE/CRT	Dehydration-responsive element/C-repeat
GAPc	Glyceraldehyde-3-phosphate dehydrogenase
MV	Methyl viologen

Introduction

During their life cycle, plants are faced with various environmental stresses. To adjust to such environmental stresses, plants trigger rapid defense responses via a number of signal transduction pathways. A major target of signal transduction is the cell nucleus, where signals lead to the transcriptional activation of numerous genes. Changes in the expression of genes encoding transcriptional regulators can have strong effects on plant stress tolerance (Yi et al. 2004).

AP2/ERF transcription factors, one of the largest plant transcription factor families, contain AP2/ERF DNA-binding domains of 57–66 amino acids in size (Nakano et al. 2006). AP2/ERF genes constitute a large multigene family, including 145 *Arabidopsis* genes, divided into four subfamilies (AP2, RAV, DREB/CBF, and ERF) according to their sequence similarities and numbers of AP2/ERF domains (Sakuma et al. 2002). DREB/CBF and ERF subfamily proteins contain single AP2/ERF domain. DREB/CBF subfamily genes have a function in the resistance of

plants to abiotic stresses by recognizing the DRE/CRT *cis*-acting element, which has a core motif of (A/G)CCGAC (Thomashow 1999; Yamaguchi-Shinozaki and Shinozaki 1994). ERF subfamily is generally involved in the response to biotic stresses by recognizing the *cis*-acting element AGCCGCC, known as GCC box (Hao et al. 1998; Xu et al. 2007). Many ERF subfamily members also bind to DRE/CRT (Hsieh et al. 2013; Wu et al. 2007; Zhang et al. 2009; Zhu et al. 2010).

ERF transcription factors have been identified in various plant species, such as *Arabidopsis thaliana*, tobacco (*Nicotiana tabacum*), and tomato (*Lycopersicon esculentum*) (Fischer and Droge-Laser 2004; Onate-Sanchez and Singh 2002; Tournier et al. 2003). ERF proteins involved in defense responses against pathogen infection have also been extensively documented (Gutterson and Reuber 2004; Park et al. 2001; Shin et al. 2002). Recent studies have revealed a role for some ERF proteins in hormone and abiotic stress responses in plants (Shinozaki et al. 2003). Thus, ERF proteins play an important role not only in pathogen defense responses but also in tolerance to abiotic stresses. However, the molecular functions of ERF subfamily genes have been revealed for only a limited number of genes belonging to the subfamily.

Recently, two *Arabidopsis* ERF transcription factors, AtERF73/HRE1 and AtERF71/HRE2, have been identified to be responsive to hypoxia (Licausi et al. 2010). Expression of both *AtERF73/HRE1* and *AtERF71/HRE2* is increased under hypoxia, and overexpressing transgenic plants show resistance to hypoxia (Licausi et al. 2010). Although both genes are responsive to hypoxia, the response of AtERF73/HRE1 to hypoxia involves both ethylene-dependent and -independent pathways, while the response of AtERF71/HRE2 only involves ethylene-independent pathways (Hess et al. 2011; Licausi et al. 2010; Yang et al. 2011). It was recently reported that *AtERF71/HRE2* is involved in the responses to osmotic stress as well as hypoxia and AtERF71/HRE2 functions as a transcription activator (Park et al. 2011). Single mutants of either AtERF73/HRE1 or AtERF71/HRE2 showed no significant difference compared with wild type (WT), whereas double mutants showed a more sensitive phenotype in response to hypoxic stress conditions (Licausi et al. 2010), suggesting that these two ERF proteins have a partially redundant molecular function in the hypoxic stress response. However, despite these studies, the molecular functions of AtERF73/HRE1 and its role during *Arabidopsis* development have not been reported.

In this study, we characterized the molecular functions of HRE1α, a splicing variant of AtERF73/HRE1, as a nuclear transcription factor having GCC box-dependent transcriptional activation activity in plants. In addition, we found that *HRE1α* is involved in root development through regulation of root meristem cell division.

Materials and methods

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 (Park et al. 2011). Seedlings were grown under short-day (SD) conditions (cycles of 8-h light/16-h dark) or long-day (LD) conditions (cycles of 16-h light/8-h dark) at 22 °C.

Plasmid construction

To generate a plant expression vector overexpressing *HRE1α*, pFGL845, the entire open reading frame (ORF) of *HRE1α* was amplified by PCR and cloned into the *Sal*I-*Bam*HI site of pFGL571 (Park et al. 2011). To construct *HRE1 promoter*::*β-glucuronidase* (*GUS*) vector, the 5′ upstream promoter region (−1,000 to +330) relative to the ATG start codon of *HRE1α* was amplified by PCR and fused with the *GUS* gene. For synthetic Green Fluorescence Protein (sGFP)-fused HRE1α construct, the entire ORF of *sGFP* was amplified by PCR and cloned into pFGL845 in frame with *HRE1α*. To generate constructs for investigation of the transcriptional activation activity of HRE1α, the entire ORF or truncated HRE1α was amplified by PCR. The PCR products were then cloned into pBD-GAL4 (Stratagene) in frame with the GAL4 DNA-binding domain (BD). For the reference plasmid vector expressing *Renilla* luciferase for transactivation analysis, the entire ORF of *Renilla* luciferase was amplified and cloned between modified *CaMV 35S* promoter and *nos* terminator of pFGL1122. For the effector-reporter plasmid vector for transactivation analysis, the core region of *CaMV 35S* promoter, the entire ORF of firefly luciferase and *nos* terminator were amplified and cloned into pBluescriptIIKS, after which four copies of GCC box or DRE/CRT were cloned in front of core *CaMV 35S* promoter, resulting in pFGL1498 and pFGL1499, respectively. The sequences of four copies of GCC box were 5′-CATAAGAGCCGCCACTCATAAGAGCCGCCACTCATAAGAGCCGCCACTCATAAGAGCCGCCACT-3′ and the sequences of four copies of DRE/CRT were 5′-ATTTCATGGCCGACCTGCTTTCATGGCCGACCTGCTTTCATGGCCGACCTGCTTTCATGGCCGACCTGCTT-3′. Then, *CaMV 35S*::*HRE1α*::*nos terminator* in pFGL845 was amplified and cloned into pFGL1498 or pFGL1499.

Plant transformation

Vectors for plant expression were introduced into *Agrobacterium tumefaciens* strain GV3101 (pMP90) using the freeze–thaw method (Höfgen and Willmitzer 1988) and *Arabidopsis* was transformed using the floral-dipping method (Clough and Bent 1998). Transgenic plants were selected on medium containing 25 mg/l of kanamycin.

Stress treatments

For submergence treatment, 4-week-old plants were submerged 5 cm below water level for 10 days, followed by desubmergence for 5 days.

For stress treatments prior to RT-PCR analysis, 10-day-old 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, 8, 12, or 24 h. For hypoxia 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, 6, 8, 12, 18, or 24 h.

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 (Park et al. 2011). The primers used for the PCR reactions are shown in Supplementary Table S1.

GUS activity analysis and transient gene expression in *Arabidopsis* protoplasts

GUS activity was histochemically detected using a protocol adapted from a previous report (Park et al. 2011).

To investigate the subcellular localization of HRE1α in *Arabidopsis* protoplasts, polyethylene glycol (PEG)-mediated protoplast transformations were performed according to the method described by Sheen (2001).

Transcriptional activation activity analysis in yeast

To investigate the transcriptional activation activity of HRE1α in yeast, pBDGAL4-HRE1α constructs were transformed into a yeast strain, YD116, which carries the *GAL1pro*::*URA3* and *UASpro*::*lacZ* reporters. Transformants including BD-fusion vectors were selected on Synthetic Dropout (SD)-Trp. Transcriptional activation activities were confirmed by growth assay on SD-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 (Park et al. 2011), and the unit of β-galactosidase activity was calculated using the formula: $1{,}000 \times OD_{420}/(OD_{600} \times$ assay time in min $\times$ assay volume in ml).

Transactivation analysis in *Arabidopsis* protoplast

For transactivation analysis in *Arabidopsis* protoplasts, reporter plasmid and effector-reporter plasmid harboring GCC box or DRE/CRT were transformed into *Arabidopsis* protoplasts. Reference plasmid was co-transformed for normalization. Firefly luciferase and *Renilla* luciferase activities were determined using the Dual-Luciferase

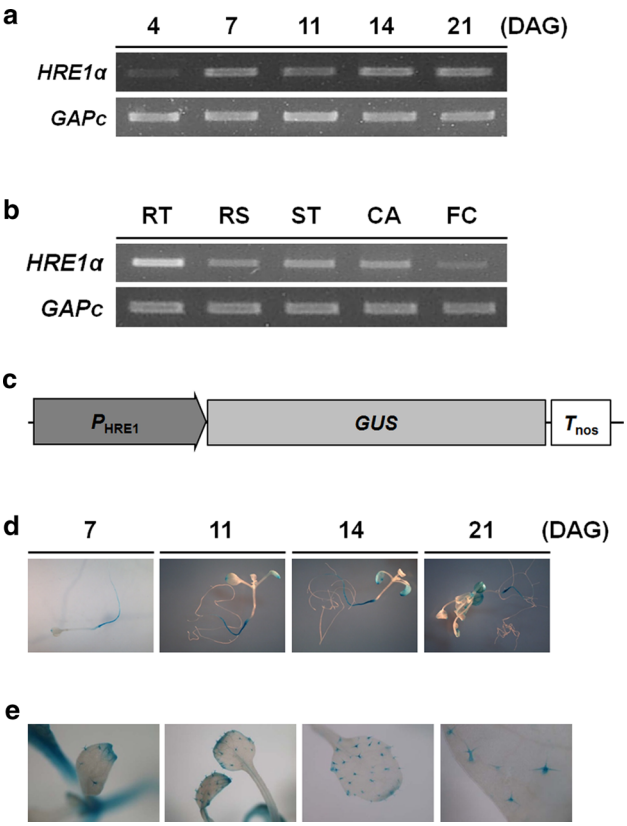

Fig. 1 Temporal and spatial expression patterns of *HRE1α*. **a** Semi-quantitative RT-PCR analysis of *HRE1α* expression in 4-, 7-, 11-, 14-, or 21-day-old WT seedlings grown under SD conditions. **b** Semi-quantitative RT-PCR analysis of *HRE1α* expression in organs of 7-week-old WT plants grown under LD conditions. RT, roots; RS, rosette leaves; ST, stems; CA, cauline leaves; FC, floral clusters. In **a** and **b**, *GAPc* was used as an internal control. Similar results were obtained from at least two biological replicates, with one shown here. **c** Schematic map of *HRE1 promoter*::*GUS* for generating transgenic plants. **d** Histochemical assay of expression in *Arabidopsis* T_2 plants carrying *HRE1 promoter*::*GUS* at 7, 11, 14, and 21 DAG under SD conditions. **e** Histochemical assay of GUS expression in leaves of 21-day-old *HRE1 promoter*::*GUS* T_2 transgenic plants grown under LD conditions. In **d** and **e**, representative staining results are shown

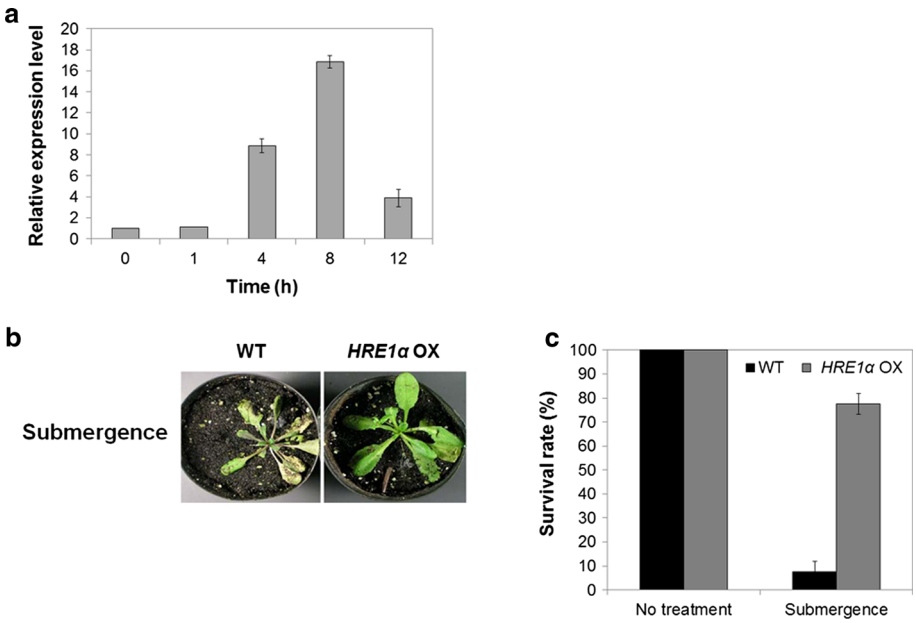

Fig. 2 Submergence response of *HRE1α* OXs. **a** Quantitative RT-PCR analysis of *HRE1α* under 0-, 1-, 4-, 8-, and 12-h hypoxia treatments. *GAPc* was used as an internal control. Transcript level at 0 h was set to 1. Reactions of each technical replicate were performed in triplicate. Two technical replicates were measured for each biological replicate. Data shown are the mean ± SD ($n = 6$). Similar results were obtained from at least two biological replicates, with one shown here. **b** Response of WT and *HRE1α* OX plants to submergence. **c** Survival rates of WT and *HRE1α* OX plants after submergence treatment. *Error bars* represent standard deviation of three independent experiments. Ten plants were analyzed in each experiment

Reporter Assay System (Promega, UK), according to the manufacturer's instructions.

Measurement of root cell length and root meristem size

To determine root cell length and root meristem size, 8-day-old seedlings were used. The roots were excised from the seedlings and stained with 10 μM propidium iodide (PI) for 10 s, rinsed with distilled water, and mounted with distilled water. The specimens were examined with a confocal laser scanning microscope (LSM510NLO, CarlZeiss, Germany). Excitation wavelength of 536 nm was used to visualize the signals of PI staining. The emission wavelength was 617 nm.

Results and discussion

HRE1α is highly expressed in roots and trichomes and its expression is increased by hypoxia treatment

We isolated a cDNA of *AtERF73/HRE1* from seedlings under hypoxia treatment and the cDNA corresponded to gene model 1 (At1g27360.1) of *AtERF73/HRE1* on TAIR website (www.arabidopsis.org). Another splicing variant of *AtERF73/HRE1*, gene model 2 (At1g27360.2), was previously reported (Licausi et al. 2010). We named transcripts of At1g72360.1 and At1g72360.2 *HRE1α* and *HRE1β*, respectively (Supplementary Fig. S1). The predicted HRE1α protein, *Arabidopsis* AP2/ERF transcription factor, contains a conserved 58 aa AP2/ERF DNA-binding domain. Based on sequence homology and its conserved motif, HRE1α belongs to group VII (B-2) (Nakano et al. 2006).

We investigated the spatial and temporal expression patterns of *HRE1α* using semi-quantitative RT-PCR. Transcription of *HRE1α* increased as plants developed (Fig. 1a). In mature plants, *HRE1α* transcripts were highly detected in roots compared with levels in other organs (Fig. 1b). Previously, *HRE1β* was also reported to be highly expressed in roots under hypoxic conditions (Licausi et al. 2010). These results suggested that *HRE1α* might play an important role in root development. Expression patterns of *HRE1α* were also supported by GUS expression analysis using *HRE1 promoter*::*GUS* transgenic plants (Fig. 1c, d). Interestingly, high and specific promoter activity of *HRE1* was observed in trichomes on leaves (Fig. 1e), suggesting that *HRE1α/β* might be involved in development and/or function of trichomes. However, in *HRE1α*-overexpressing transgenic plants (OXs), phenotypes of trichomes did not show any significant difference compared with those in WT plants (data not shown). Further studies will be necessary to elucidate the function of *HRE1α/β* related to its high expression in trichomes.

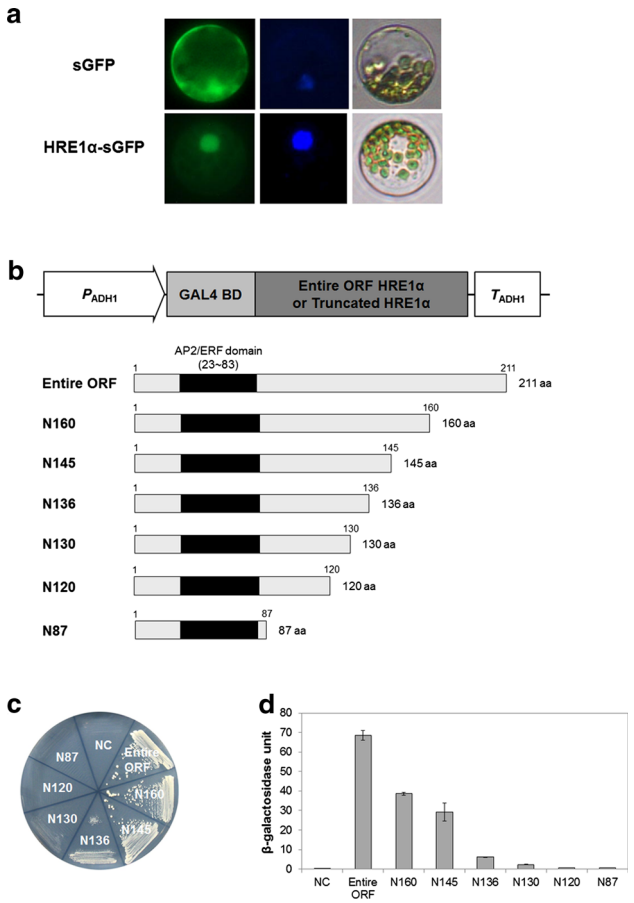

Fig. 3 Transcriptional activation activity of HRE1α. **a** Subcellular localization of HRE1α upon transient expression of HRE1α-sGFP fusion construct in *Arabidopsis* protoplasts. *Left*, GFP signal; *middle*, DAPI (4′,6-diamidino-2-phenylindole) staining; *right*, light microscope pictures. **b** Schematic map of GAL4 BD-fusion HRE1α construct along with the entire ORF and truncated HRE1α used in the analysis of transcriptional activation activity. *Black box* indicates AP2/ERF DNA-binding domain. **c** Growth assay of yeast transformants on SD-Trp-Ura. **d** Quantitative β-galactosidase assay in yeast. Data shown are the mean ± SD ($n = 4$). Similar results were obtained from at least two independent experiments, with one shown here. pBDGAL4 was used as a negative control. *NC* negative control

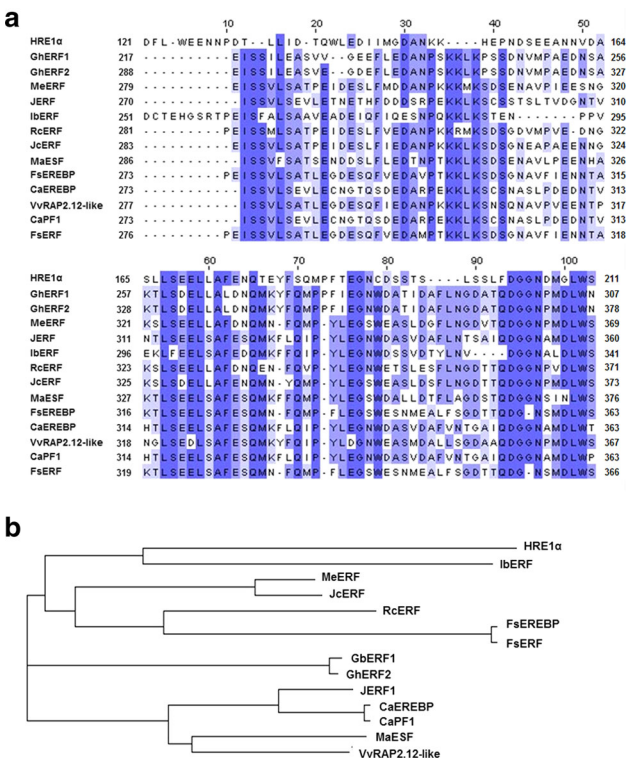

Fig. 4 Multiple alignment and phylogenetic tree of HRE1α. **a** Multiple alignment of 121–211 aa region of HRE1α with corresponding regions of GbERF1, GhERF2, MeERF, JERF, IbERF, RcERF, JcERF, MaESF, FsEREBP, CaEREBP, VvRAP2.12-like, CaPF1, and FsERF. Alignment was made by ClustalW using default parameters. **b** Phylogenetic tree of 121–211 aa region of HRE1α and corresponding regions of GbERF1, GhERF2, MeERF, JERF, IbERF, RcERF, JcERF, MaESF, FsEREBP, CaEREBP, VvRAP2.12-like, CaPF1, and FsERF. Phylogenetic tree was made by ClustalW2-Phylogeny using default parameters

To investigate the expression patterns of *HRE1α* under abiotic stress conditions, we performed quantitative RT-PCR analysis using *HRE1α*-specific primers and 10-day-old WT seedlings treated with hypoxia, NaCl, mannitol, ABA, or MV. Transcription of *HRE1α* increased within 4 h of hypoxia treatment (Fig. 2a), while transcript levels of *HRE1α* were reduced in response to NaCl, mannitol, ABA, or MV treatment (Supplementary Fig. S2). It has been reported that the expression level of *HRE1β* is also increased by hypoxia treatment (Licausi et al. 2010).

HRE1α OXs show tolerance to submergence

It was previously reported that *HRE1β* OXs were more resistant to anoxia than WT (Licausi et al. 2010). It is well known that hypoxic conditions often arise upon water logging or flooding. Since *HRE1α* expression was highly elevated under hypoxic conditions (Fig. 2a), we investigated the response of *HRE1α* OXs to submergence. To do this, we generated *HRE1α* OXs and selected three independent T_1 lines for further analysis (Supplementary Fig. S3). *HRE1α* OXs were more resistant to submergence compared with WT plants (Fig. 2b, c). Both the increase of *HRE1α* transcript level under hypoxia condition and the submergence resistance of *HRE1α* OXs suggest that *HRE1α* is involved in resistance to submergence.

In rice (*Oryza sativa*), *Submergence1* (*Sub1*) locus contains two or three ERF-like genes whose transcripts are regulated by submergence (Perata and Voesenek 2007). In deepwater rice, a pair of ERFs, SNORKEL1 and 2, also plays a key role in the adaptation of rice to submergence (Hattori et al. 2009). In *Arabidopsis*, ERF subfamily factors such as RAP2.2, RAP2.12, and AtERF71/HRE2 also function in the hypoxic response (Licausi et al. 2010). Interestingly, all of these genes (*Sub1*, *SK1*, *SK2*, *RAP2.2*,

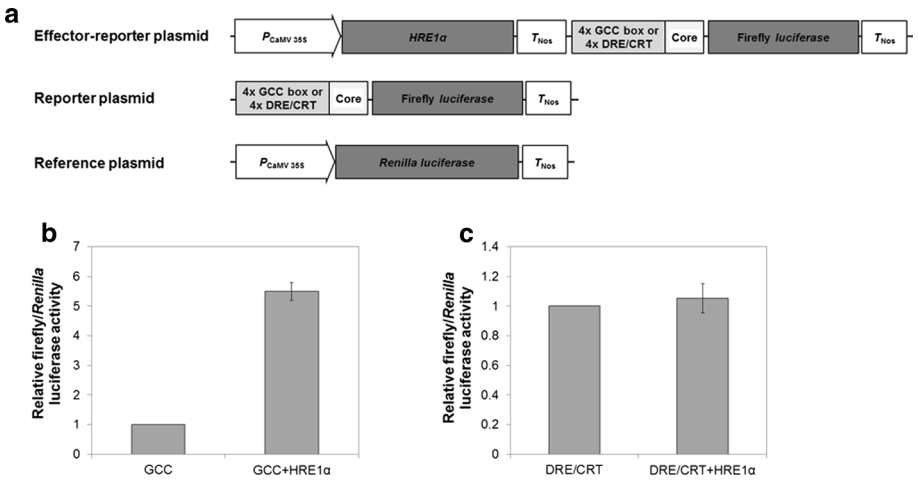

Fig. 5 Transactivation analysis of HRE1α. **a** Schematic maps of the effector-reporter, reporter, and reference plasmids used in transactivation analysis. Relative luciferase activity in transactivation analysis for GCC box (**b**) and DRE/CRT (**c**). In **b** and **c**, firefly luciferase activities of the effector-reporter plasmid and the reporter plasmid were measured in *Arabidopsis* protoplasts. Transformation efficiency was normalized with *Renilla* luciferase activity of the reference plasmid. Relative luciferase activity represents the fold-increase in the normalized luciferase activity of the effector-reporter plasmid compared to that of the reporter plasmid alone. Data shown are the mean ± SD ($n = 5$). Similar results were obtained from at least three independent experiments, with one shown here

RAP2.12, *AtERF71*/*HRE2*, and *AtERF71*/*HRE2*) belong to group VII (B-2) ERFs, suggesting that group VII (B-2) ERFs have a function in the hypoxic response.

HRE1α shows transcriptional activation activity in its C-terminal region

AP2/ERF domain proteins are known to function as transcription factors (Okamuro et al. 1997). However, the molecular functions of HRE1α, including its transcriptional activation activity, have not yet been elucidated. First, we observed that HRE1α was localized in the nucleus using HRE1α-sGFP fusion protein (Fig. 3a) like HRE1β (Licausi et al. 2010).

We investigated whether or not HRE1α functions as a transcriptional activator using a yeast system. The entire ORF of HRE1α was fused to GAL4 BD (Fig. 3b), after which the fusion construct was transformed into yeast cells. Based on the results of growth assay and quantitative β-galactosidase assay, HRE1α showed transcriptional activation activity in yeast (Fig. 3c, d), suggesting that HRE1α could function as a transcriptional activator in plants.

We next identified the domain of HRE1α responsible for the observed transcriptional activation activity. For this, we constructed a set of GAL4 BD-HRE1α vectors, including N160 (1 to 160 aa), N120 (1 to 120 aa), or N87 (1 to 87 aa) (Fig. 3b). In yeast, when the 161–211 aa region of HRE1α was deleted, transcriptional activation activity was significantly reduced compared with that of the entire ORF (Fig. 3c, d). Moreover, when the 121–211 aa region was deleted, truncated HRE1α showed no transcriptional activation activity (Fig. 3c, d). These results indicate that the region between 121 and 211 aa of HRE1α might be responsible for the observed transcriptional activation activity.

Although the 121–211 aa region of HRE1α had no known functional domain (data not shown), it contained conserved amino acid sequences in 13 ERF family proteins of other plant species, including *Gossypium barbadense* GbERF1, *Gossypium hirsutum* GhERF2, *Manihot esculenta* MeERF, *Solanum lycopersicum* JERF1, *Ipomoea batatas* IbERF, *Ricinus communis* RcERF, *Jatropha curcas* JcERF, *Morus alba* MaESF, *Fagus sylvatica* FsEREBP and FsERF, *Capsicum annuum* CaEREBP and CaPF1, and *Vitis vinifera* VvRAP2.12-like (Fig. 4a). These results indicate that the conserved regions might be involved in transcriptional activation activity. In addition, no other *Arabidopsis* protein except for HRE1α itself contained the conserved sequence in the 121–211 aa region (Fig. 4).

HRE1α has GCC box-dependent transcriptional activation activity

It is well known that the ERF subfamily of AP2/ERF transcription factors activates downstream genes via GCC box and/or DRE/CRT (Lee et al. 2005; Park et al. 2001; Tang et al. 2007; Zhang et al. 2004). To determine GCC box and/or DRE/CRT-dependent transcriptional activation activity of HRE1α in *Arabidopsis* protoplasts, we generated reporter, effector-reporter, and reference plasmids (Fig. 5a) and then introduced them into *Arabidopsis* protoplasts. When the GCC box-containing reporter plasmid was introduced together with HRE1α, relative luciferase activity was elevated about fivefold compared to that

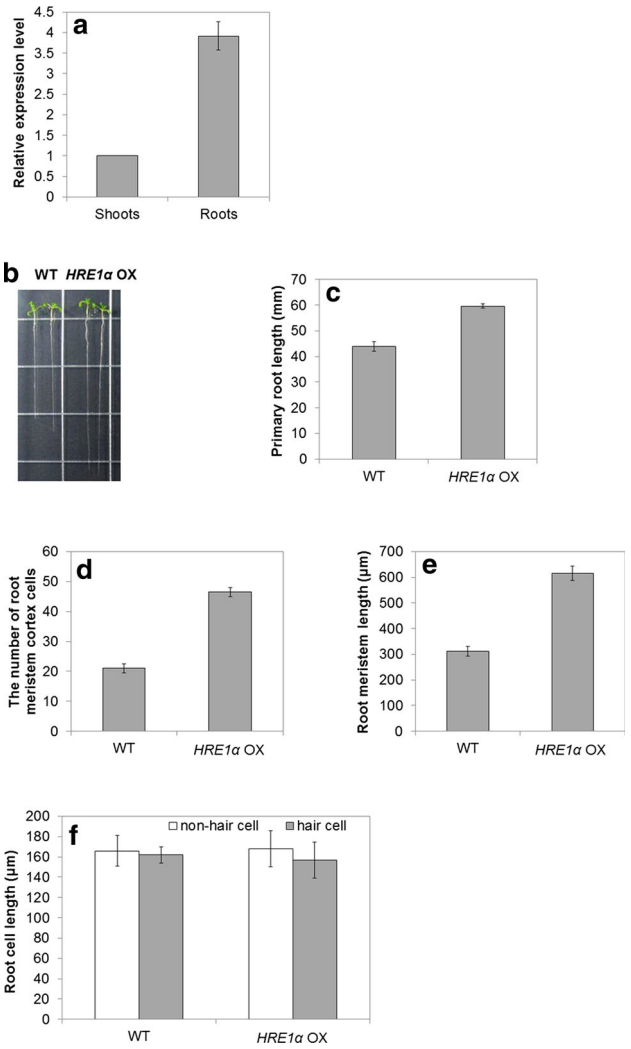

Fig. 6 Root development in *HRE1α* OXs. **a** Quantitative RT-PCR analysis of *HRE1α* expression in shoots and roots of 7-day-old WT plants under SD conditions. *GAPc* was used as an internal control. Transcript level in shoots was set to 1. Reactions of each technical replicate were performed in triplicate. Two technical replicates were measured for each biological replicate. Data shown are the mean ± SD ($n = 6$). Similar results were obtained from at least two biological replicates, with one shown here. **b** Root development of 14-day-old WT and *HRE1α* OX seedlings under SD conditions. **c** Primary root lengths of WT and *HRE1α* OXs. Primary root lengths were measured at 14 DAG under SD conditions. Data shown are the mean ± SD ($n = 21$). The number of root meristem cortex cells (**d**) and root meristem length (**e**), and lengths of hair cells and non-hair cells in roots (**f**) of 8-day-old WT and *HRE1α* OXs under SD conditions. In **d**, **e**, and **f**, data shown are the mean ± SD ($n = 12$)

without HRE1α (Fig. 5b). On the other hand, when the DRE/CRT-containing reporter plasmid was transformed with HRE1α, relative luciferase activity showed no significant differences compared to that without HRE1α (Fig. 5c). These results indicate that HRE1α can activate the firefly *luciferase* gene via GCC box but not DRE/CRT, suggesting that HRE1α might act as a transcriptional activator via GCC box only. However, we could not confirm specific binding of HRE1α to GCC box in vitro, since host *E. coli* cells did not produce adequate amount of recombinant HRE1α protein.

HRE1α is involved in root development

HRE1α was highly expressed in roots compared with other organs of mature plants such as leaves, floral clusters, and stems (Fig. 1b). Therefore, we investigated the involvement of *HRE1α* in root development. Quantitative RT-PCR analysis using *HRE1α*-specific primers revealed that the expression level of *HRE1α* was much higher in roots than in shoots in 7-day-old WT seedlings, similar to mature plants (Fig. 6a). Moreover, we found that the primary root length was longer in *HRE1α* OXs than in WT plants (Fig. 6b, c), suggesting that *HRE1α* might be involved in root development.

The balance between cell division and expansion is important for the maintenance and the size of root meristem and controls the overall rate of root growth (Beemster and Baskin 1998). To determine the role of *HRE1α* in the root development, we analyzed root cells of *HRE1α* OXs. Since cell division activity in roots is reflected by root meristem size, we compared the number of root meristem cortex cells, including cells from the quiescent center to the first elongated cell, as well as root meristem size between WT and *HRE1α* OXs. The number of cortex cells of *HRE1α* OXs was almost twofold higher than that of WT (Fig. 6d), and the root meristem size of *HRE1α* OXs was twofold larger than that of WT (Fig. 6e). These data indicate that cell division activity is higher in root of *HRE1α* OXs compared with WT. We also analyzed root cell expansion by comparing hair cell length as well as non-hair cell length between *HRE1α* OXs and WT. The lengths of hair cells and non-hair cells were not significantly different between *HRE1α* OXs and WT (Fig. 6f), indicating that root cell expansion of *HRE1α* OXs is similar to that of WT. These results suggest that the increased primary root length in *HRE1α* OXs can be attributed to increased root cell division.

Taken together, our results suggest that HRE1α might function as a transcription activator via GCC box in the nucleus. In addition, HRE1α is involved in root development through regulation of root meristem cell division.

Author contribution Most experiments and data analysis were performed by both H.-Y. Seok and V.N. Tarte. S.-Y. Lee contributed to some experimental design and data interpretation. H.-Y Park assisted in the isolation of *HRE1α* and the generation and analysis of *HRE1α* OXs. Y.-H. Moon was responsible for the overall conceptualization and supervision of the experiments and worked on data processing and manuscript preparation.

Acknowledgments This work was supported by a 2-Year Research Grant of Pusan National University.

Conflict of interest The authors have no conflict of interest.

References

Beemster GT, Baskin TI (1998) Analysis of cell division and elongation underlying the developmental acceleration of root growth in *Arabidopsis thaliana*. Plant Physiol 116:1515–1526

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

Fischer U, Droge-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 17:1162–1171

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

Hao DY, Ohme-Takagi M, Sarai A (1998) Unique mode of GCC box recognition by the DNA-binding domain of ethylene-responsive element-binding factor (ERF domain) in plant. 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 460:1026–1031

Hess N, Klode M, Anders M, Sauter M (2011) The hypoxia responsive transcription factor genes ERF71/HRE2 and ERF73/HRE1 of Arabidopsis are differentially regulated by ethylene. Physiol Plant 143:41–49

Höfgen R, Willmitzer L (1988) Storage of competent cells for *Agrobacterium* transformation. Nucleic Acids Res 16:9877

Hsieh EJ, Cheng MC, Lin TP (2013) Functional characterization of an abiotic stress-inducible transcription factor AtERF53 in *Arabidopsis thaliana*. Plant Mol Biol 82:223–237

Lee JH, Kim DM, Lee JH, Kim J, Bang JW, Kim WT, Pai HS (2005) Functional characterization of NtCEF1, an AP2/EREBP-type transcriptional activator highly expressed in tobacco callus. Planta 222:211–224

Licausi F, van Dongen JT, Giuntoli B, Novi G, Santaniello A, Geigenberger P, Perata P (2010) HRE1 and HRE2, two hypoxia-inducible ethylene response factors, affect anaerobic responses in *Arabidopsis thaliana*. Plant J 62:302–315

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

Okamuro JK, Caster B, Villarroel R, Montagu MV, Jofuku KD (1997) The AP2 domain of APETALA2 defines a large new family of DNA binding proteins in Arabidopsis. Proc Natl Acad Sci USA 94:7076–7081

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

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 13:1035–1046

Park HY, Seok HY, Woo DH, Lee SY, Tarte VN, Lee EH, Lee CH, Moon YH (2011) AtERF71/HRE2 transcription factor mediates osmotic stress response as well as hypoxia response in Arabidopsis. Biochem Biophys Res Commun 414:135–141

Perata P, Voesenek LACJ (2007) Submergence tolerance in rice requires Sub1A, an ethylene-response-factor-like gene. Trends Plant Sci 12:43–46

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

Sheen J (2001) Signal transduction in maize and Arabidopsis mesophyll protoplasts. Plant Physiol 127:1466–1475

Shin R, Park JM, An JM, Paek KH (2002) Ectopic expression of Tsi1 in transgenic hot pepper plants enhances host resistance to viral, bacterial, and oomycete pathogens. Mol Plant Microbe Interact 15:983–989

Shinozaki K, Yamaguchi-Shinozaki K, Seki M (2003) Regulatory network of gene expression in the drought and cold stress responses. Curr Opin Plant Biol 6:410–417

Tang M, Sun J, Liu Y, Chen F, Shen S (2007) Isolation and functional characterization of the JcERF gene, a putative AP2/EREBP domain-containing transcription factor, in the woody oil plant *Jatropha curcas*. Plant Mol Biol 63:419–428

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

Tournier B, Sanchez-Ballesta MT, Jones B, Pesquet E, Regad F, Latche A, Pech JC, Bouzayen M (2003) New members of the tomato ERF family show specific expression pattern and diverse DNA binding capacity to the GCC box element. FEBS Lett 550:149–154

Wu L, Chen X, Ren H, Zhang Z, Zhang H, Wang J, Wang XC, Huang R (2007) ERF protein JERF1 that transcriptionally modulates the expression of abscisic acid biosynthesis-related gene enhances the tolerance under salinity and cold in tobacco. Planta 226:815–825

Xu ZS, Xia LQ, Chen M, Cheng XG, Zhang RY, Li LC, Zhao YX, Lu Y, Ni YZ, Liu L, Qiu ZG, Ma YZ (2007) Isolation and molecular characterization of the *Triticum aestivum* L. ethylene-responsive factor 1 (TaERF1) that increases multiple stress tolerance. Plant Mol Biol 65:719–732

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 6:251–264

Yang CY, Hsu FC, Li JP, Wang NN, Shih MC (2011) The AP2/ERF transcription factor AtERF73/HRE1 modulates ethylene responses during hypoxia in Arabidopsis. Plant Physiol 156:202–212

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 136:2862–2874

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 220:262–270

Zhang G, Chen M, Li L, Xu Z, Chen X, Guo J, Ma Y (2009) Overexpression of the soybean GmERF3 gene, an AP2/ERF type transcription factor for increased tolerances to salt, drought, and diseases in transgenic tobacco. J Exp Bot 60:3781–3796

Zhu Q, Zhang J, Gao X, Tong J, Xiao L, Li W, Zhang H (2010) The Arabidopsis AP2/ERF transcription factor RAP2.6 participates in ABA, salt and osmotic stress responses. Gene 457:1–12