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A novel DREB transcription factor from *Halimodendron halodendron* leads to enhance drought and salt tolerance in *Arabidopsis*

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Abstract

A new member of the APETALA2/ethylene responsive element binding protein (AP2/EREBP) transcription factor family, HhDREB2, was isolated from *Halimodendron halodendron*. Based on the similarity of the AP2/ERF domain, HhDREB2 was classified into A-5 group of the DREB subfamily. The expression of *HhDREB2* gene was induced by drought, high salinity, and low temperature, but not by exogenous plant hormones. Trans-activity assay demonstrated that *HhDREB2* gene encodes a transcription activator. Furthermore, over-expression of *HhDREB2* gene under the stress-inducible *rd29A* promotor in *Arabidopsis* resulted in enhanced tolerance to salt and drought stresses. The overall results reveal that HhDREB2 functioned as important transcription factor in regulation of stress-responsive signaling in plants and may be used for improving plant tolerance to abiotic stresses.

Additional key words: abiotic stress, abscisic acid, auxin, cytokinin, gibberelic acid, expression pattern, transcriptional regulation, transgenic plant.

Introduction

Plants are often subjected to many environmental stresses which negatively influence their survival, biomass production, and yields around the world. Plants respond and adapt to these stresses with a series of biochemical and physiological processes involving numerous stress responsive genes. Transcription factors, like AP2/EREBP, bHLH, NAC, ZFHD, Zinc finger, bZIP, MYB, and Homeodomain, can regulate these genes by interacting with *cis*-elements present in promoter regions (Yamaguchi-Shinozaki and Shinozaki 2006, Zhao *et al.* 2007).

APETALA2/ethylene responsive element binding protein (AP2/EREBP) transcription factors are characterrized by a conserved AP2/ERF domain of approximately 60 amino acids for DNA binding proteins. Dehydration responsive element binding (DREB) proteins, as subfamily of AP2/EREBP transcription factors with only one AP2/ERF domain, were identified in various plant species (Gutha and Reddy 2008). According to the structure characteristics, the DREB transcription factors (DREBs) can be further divided into six subgroups from A-1 to A-6 (Zhou *et al.* 2010). DREBs specifically interact with a dehydration-responsive element/C-repeat sequence (DRE/CRT; A/GCCGAC) and play a crucial role in enhancing plant stress tolerance by regulating the expression of stress-related genes in response to a wide spectrum of abiotic stresses (Zhou *et al.* 2010). Constitutive expression of DREBs in transgenic plants enhances tolerance to cold, drought, and salinity (Dubouzet *et al.* 2003, Tang *et al.* 2011, Bouaziz *et al.* 2012, Li *et al.* 2012, Sazegari and Niazi 2013). However, the over-expression of DREBs driven by the cauliflower mosaic virus (CaMV) 35S promoter causes severe growth retardation under normal growth conditions. To minimize the negative effect on plant growth, the stress-inducible responsive to dehydration 29A (rd29A) promoter can be used instead of the constitutive CaMV 35S promoter for genes over-expression (Behnam *et al.* 2007, Chen *et al.* 2009, Zhou *et al.* 2012).

Plant response to abiotic stimuli is a complex process

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Abbreviations: ABA - abscisic acid; BA - 6-benzylaminopurine; CaMV - cauliflower mosaic virus; DREB - dehydration responsive element binding; GA_3 - gibberellic acid; GFP - green fluorescent protein; MS - Murashige and Skoog; NAA - 1-naphthaleneacetic acid; RACE - rapid amplification of cDNA ends; TBA - thiobarbituric acid; TCA - trichloroacetic acid.
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which might be affected also by phytohormones (Santner and Estelle 2009, Zhou *et al.* 2010). Some DREBs can integrate input stimuli from abiotic factors and exogenous phytohormones, *e.g*., abscisic acid (ABA; Huang and Liu 2006, Chen *et al.* 2007, Xu *et al.* 2009, Yang *et al*. 2011, Bouaziz *et al.* 2012, Roychoudbury *et al.* 2013). In our previous research, we isolated a gene from *Populus euphratica* named *PeDREB2a* which is not only induced by salinity, drought, and cold, but also by 1-naphthaleneacetic acid (NAA), gibberellic acid (GA_3), and 6-benzylaminopurine (BA; Zhou *et al.* 2012). This shows that DREBs may play different roles in the complex regulatory networks or on the sites of cross-talk between different signaling pathways.

Halimodendron halodendron is a typical deciduous shrub growing well on alkaline, sandy, and saline soils in China and Russia. *H. halodendron* is highly tolerant to drought and salt stresses, but its molecular mechanism of stresses tolerance is not clear yet. In this study, we isolated and characterized a novel DREB homologous gene, designating it as *HhDREB2*, from salt-treated *H. halodendron* seedlings. The subcellular localization, trans-activating ability and expression pattern of *HhDREB2* in different plant organs were investigated. Furthermore, we also generated transgenic *Arabidopsis* plants over-expressing *HhDREB2* to evaluate their response to stress conditions.

Materials and methods

Plants and treatments: *Halimodendron halodendron* (Pall.) Druce seeds were collected from Lop Nur, Xinjiang, China, and seedlings were grown in loam soil in a growth chamber with a 16-h photoperiod, an irradiance of 400 μmol m^{-2} s^{-1}, a temperature of 28 °C, and a relative humidity of 50 %. The 4-week-old seedlings growing in mixed peat, *Vermiculite*, and *Perlite* (1:1:1) were irrigated with 250 mM NaCl, 20 % (m/v) PEG 6000, 100 μM ABA, 100 μM GA_3, 100 μM BA, or 100 μM NAA. PEG 6000 was used to simulate a drought stress. A cold treatment was conducted by transferring the seedlings to a growth cabinet setting at 4 °C. The above treatments lasted 0, 1, 3, 6, 12, and 24 h. During the treatments, the plants were grown at a temperature of 22 °C, a relative humidity of 50 %, a 16-h photoperiod, and an irradiance of 120 μmol m^{-2} s^{-1}. For observation of the root development under the salt stress, *Arabidopsis* seedlings were grown on a Murashige and Skoog (MS) medium in a growth chamber for one week before transferring the seedlings onto MS media with 0, 50, 100, 150, and 200 mM NaCl. To detect the developmental phenotypes and stress tolerance, the control and transgenic plants were treated for 3 weeks with 200 mM NaCl or deprivedof water after growing for 4 weeks in soil.

Isolation of *HhDREB2* gene from *H. halodendron*: To isolate a DREB gene from *H. halodendron*, homologous regions, the AP2/ERF domains, of the reported AP2/EREBP genes in other plants were used to design a pair of degenerate primers. The primers were SP1, 5'-C(A/C)(G/A)(A/T/C)GG(A/C/G)ATAAGGATG(A/C) GGAAGTG-3' and ASP2, 5'-(G/A)(T/C)(T/C)TGGAG (C/A)GCGTCGACT-3'. Total RNA was extracted from leaves of *H. halodendron* treated with 250 mM NaCl for 6 h with a plant *RNAout* column according to the instruction (*Tiandz*, Beijing, China), and then specific primers were designed for the rapid amplification of cDNA ends (RACE). For 3' RACE, two antisense gene-specific primers (GSP) were designed, 3HDGSP1, 5'-TAT TCTACCTCAGAGGTCCTTCGGC-3', and 3HDGSP2, 5'-TACCTCAGAGGTCCTTCGGCTC-3'. Primers for 5' RACE were 5HDGSP1, 5'-GAACCCAACCAAATC CTTGACCT-3', and 5HDGSP2, 5'-AAATTGAGGCG AGCCGAAGGAC-3'. The RACE reactions were performed according to the manufacturer's protocol (*Invitrogen*, Carlsbad, CA, USA). The full-length of *HhDREB2* cDNA was obtained by combining three fragments: 5' RACE, conserved cDNA, and 3' RACE. A pair of primers was then designed as HDREB1 (5'-GTTTTGAAGAAAGATTATCAC-3') and HDREB2 (5'-CGATTATCTAGGCTCAGGCA-3') from the putative 3'- and 5'- untranslated regions (UTRs) of the full-length cDNA sequence. The amplified products were gel purified, cloned in a pMD19-T vector (*TaKaRa*, Shiga, Japan) and sequenced.

Transcriptional activity assay and subcellular localization: Full-length cDNA of *HhDREB2* was cloned into *EcoR* I and *Sal* I restriction sites of yeast expression vectors, pBD (*Clontech*, Palo Alto, CA, USA), without the activation domain (AD) of GAL4. Plasmid pBD-*HhDREB2* was introduced into yeast strain AH109 with reporter genes *His3* and *LacZ* following the manufacturer's instructions (*Clontech*). The colony-lift filter β-galactosidase assay was carried out according to the Yeast Protocols Handbook (*Clontech*). The β-galactosidase activity was expressed in Miller units (1 unit of β-galactosidase hydrolyzes 1 μmol of ONPG to *o*-nitrophenol and D-galactose per cell per min). Yeast cells containing pGAL4 and pBD were used as positive and negative controls, respectively.

To get the fusion protein HhDREB2-GFP, the full length open reading frame of *HhDREB2* without the termination codon was cloned into *Sal*I and *BamH*I sites of vector p163-GFP (Zhou *et al.* 2012). The fusion construct (*35S::HhDREB2-GFP*) and the control (*35S::GFP*) were respectively bombarded into onion epidermis cells. Plasmid DNA (4 μg) was used to coat 300 μg gold particles as described previously (Kinkema *et al.* 2000). Transformed cells were incubated on a MS

medium at 25 °C in the dark for 24 h, and green fluorescence was visualized with a laser confocal scanning microscope (*Leica TCS SP2*, Germany).

Generation of transgenic *Arabidopsis* plants and semi-quantitative RT-PCR: To express *HhDREB2* in *Arabidopsis thaliana* L. (ecotype Columbia-0), *HhDREB2* cDNA was ligated into pCAMBIA1302-*rda9A* vector between sites of *BamH* I and *BstE* II. pCAMBIA1302-*rda9A* vector was constructed by inserting *rd29A* promoter into pCAMBIA1302 between sites of *Kpn* I and *BamH* I. The gene driven by stress-inducible promoter *rd29A* was introduced into *Arabidopsis* by the *Agrobacterium*-mediated floral-dip method (Clough and Bent 1998). Seeds were screened on a MS selection medium containing 50 μg cm^{-3} hygromycin for 14 d. The homozygous lines were used for further analyses.

The gene-specific primers of *HhDREB2* for a RT-PCR analysis were HD-RT (F), 5'-GGCGGTTATGATGGAAGAAGA-3', and HD-RT (R), 5'-GAAGAGCGGTTTGGATAGCAT-3'. As control, RT-PCR to detect an internal control gene, *actin*, was run at the same time. To normalize the amount of templates, primers were designed as *actin*-F, 5'-CAACCTTAATCTTCATGCTG-3', and *actin*-R, 5'-AGCAACTGGGATGACATGGAG-3'.

Determination of physiological and biochemical properties: Leaves were harvested and the malondialdehyde (MDA) content was determined by a modified method of Cui and Wang (2006). Leaf powder (0.5 g) was transferred into 2 cm^3 of 10 % (m/v) trichloroacetic acid (TCA). After centrifugation at 12 000 *g* for 10 min, the supernatant was mixed with an equal volume of 0.6 % (m/v) thiobarbituric acid (TBA) comprising 10 % TCA. The mixture was heated in a boiling water for 30 min and then quickly cooled in an ice bath. After another centrifugation at 10 000 *g* for 10 min, the absorbances of the supernatant was measured at 450, 532, and 600 nm with a spectrometer *Unicam UV-330*

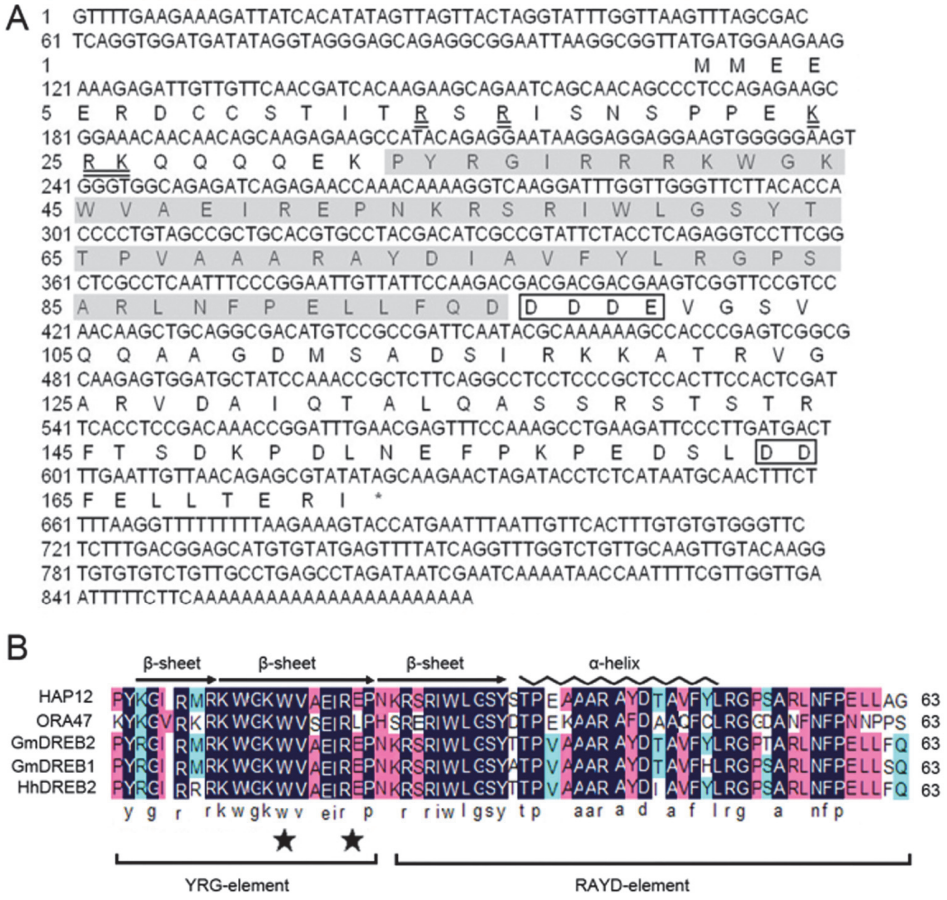

Fig. 1. The sequence analysis of HhDREB2 and other AP2/ERF-related proteins from different species. *A* - The nucleotide and deduced amino acid sequence of HhDREB2 analysed by *DNAMAN* software. The structural and function domains of HhDREB2 were analysed by *SMART* and *cNLS Mapper*. The AP2/ERF domain is in the *shaded region*; basic amino acids that potentially act as nuclear localization signals (NLS) in the N-terminal region are *double underlined* and the acidic C-terminal region that might act as transcriptional activation domain is *boxed*. *B* - The alignment of AP2/ERF domain of HhDREB2 and other closely related DREB proteins. HAP12 and ORA47 were taken from *Arabidopsis* information resource *TAIR*. The accession numbers of other proteins are as follows: GmDREB1, AAP47161.1; GmDREB2, ABB36645.1; HhDREB2, ACJ66376.1. The highly conserved amino acid 14 V and 19 E have the *asterisks*. Three β-sheets and α-helix structures are marked above corresponding sequences.

(Cambridge, UK). The MDA content was calculated according to the formula $[6.45 \times (A_{532} - A_{600})] - 0.56 \times A_{450}$. Soluble sugars were determined by thiobarbi-turic acid method (Li *et al.* (2008) and their content was calculated as $11.71 \times A_{450}$. The water content of leaves was calculated as [(fresh mass - dry mass)/fresh mass] × 100.

Statistics: All experiments were repeated at least three times with five biological replicates. Data were examined using the one-way analysis of variance (*ANOVA*). The Student-Newman-Keuls test was used to identify statistically different means.

Results

A full-length cDNA sequence of the putative *DREB* gene was isolated by RACE-PCR and designated as *HhDREB2* (Genbank accession No. EU872018). The full-length of cDNA was 873 bp including a complete open reading frame of 519 bp with 5'-UTR of 107 bp and 3'-UTR of 247 bp (Fig. 1*A*). The predicted protein of HhDREB2 was composed of 172 amino acids with a calculated molecular mass of 19.7 kDa and a theoretical isoelectric point of 8.95. The genomic PCR products revealed that *HhDREB2* had no intron interrupting its coding region (data not shown).

A bioinformatics analysis revealed that the deduced amino acid sequence of HhDREB2 contained a typical AP2/ERF domain which included 64 amino acids, from 33 to 96, and shared a very high amino acid identity with DREBs from other plants. HhDREB2 had the same 14th Val (V) and 19th Glu (E) in the AP2/ERF domain as other DREBs. A putative nuclear localization signal sequence and an acidic region that might act as transcriptional activation domain were found at the N-terminal and C-terminal regions, respectively. Two important elements, YRG and RAYD, were also found within the AP2/ERF domain (Fig. 1*B*). These features suggest that *HhDREB2* encodes a possible DREB protein. Furthermore, a phylogenic analysis of the conserved AP2/ERF domains from various plants confirmed that HhDREB2 belongs to the A-5 group of the DREB subfamily (Fig. 2).

To confirm the transcriptional activation of HhDREB2 protein, the yeast one-hybrid system was employed. A yeast strain containing pBD-*HhDREB2* or pGAL4 grew well on a synthetic dropout (SD) medium lacking histidine and tryptophan (SD/-His-Trp), whereas the negative control pBD strain could not grow on it. Besides, the β-galactosidase activity assay suggests that pBD-*HhDREB2* and pGAL4 strains could activate

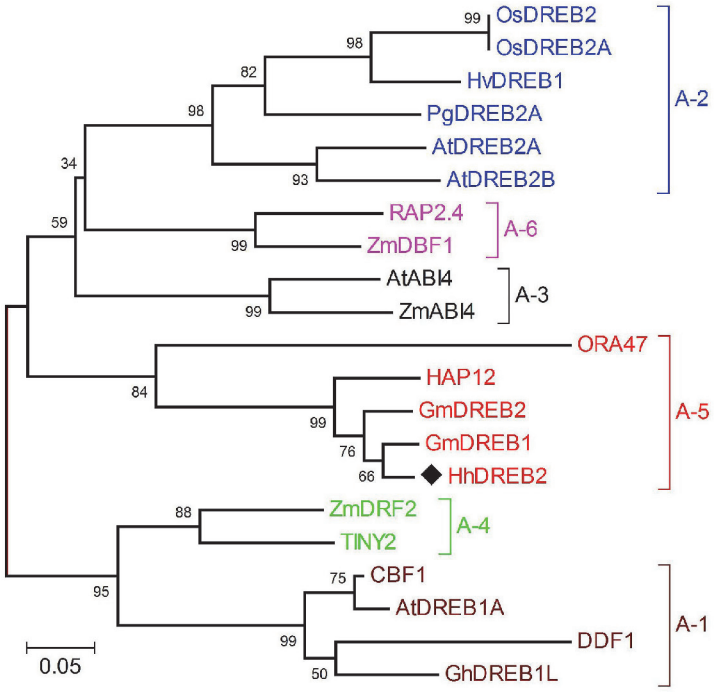

Fig. 2. A phylogenetic tree of AP2/ERF domains of *HhDREB2* and other higher plants constructed by the *MEGA 4.1* program. The branch numbers represent percentages of bootstrap values in 10 000 replicates, and the scale indicates branch lengths. Sequence information of *Arabidopsis* DREB was taken from *TAIR*. The accession numbers of other plant proteins are: GhDREB1L, ABD65473.1; OsDREB2, AAN02487.2; OsDREB2A, AAN02487.2; HvDREB1, AAY25517.1; PgDREB2A, AAV90624.1; ZmABI4, AAM95247.1; ZmDRF2, AAM80485.1; GmDREB1, AAP47161.1; GmDREB2, ABB36645.1; HhDREB2, ACJ66376.1.

reporter genes *his3* and *LacZ* and made the colony-lift filters showing a clear blue (Fig. 3*A*). These results confirm the transcriptional activity of the HhDREB2 protein. A fused construct *35S::HhDREB2-GFP* was introduced into onion epidermal cells by particle bombardment. The fusion proteins *HhDREB2-GFP* were exclusively localized in the nucleus with a strong fluorescence signal, whereas the control GFP protein was distributed throughout the onion cell (Fig. 3*B*). These results suggest the nucleus-localized HhDREB2 protein.

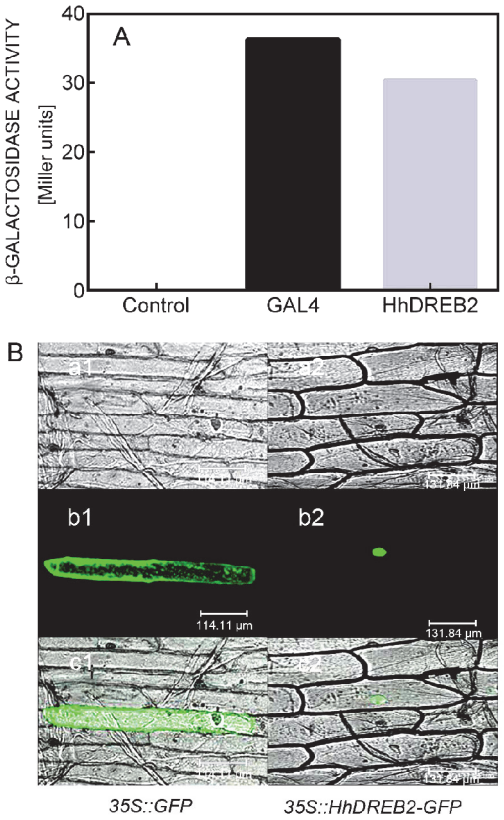

Fig. 3. The transcriptional activity and subcellular localization of HhDREB2 protein. *A* - The analysis of DNA-binding activity of HhDREB2 protein by the yeast one-hybrid assay. *B* - The subcellular localization of HhDREB2-GFP fusion proteins. The fusion construct for HhDREB2-GFP (1) and the GFP control plasmid (2) were introduced into onion epidermal cells by particle bombardment. Nuclear localization was investigated by confocal microscopy: *a* - a bright field image; *b* - a fluorescence image; *c* - conformity for both.

To further investigate the role of *HhDREB2* gene in plant tolerance to abiotic stresses, the gene under the control of *rd29A* promoter was transformed into *Arabidopsis* plants. We confirmed nine transgenic lines by PCR (Fig. 5*A*) and randomly selected one (HhDREB2OE#3) as research object. Compared with the wild-type plants (WT), the transgenic plants (T_2) showed no detectable phenotype defects in growth and flowering.

After treatments with 200 mM NaCl and drought for 3 weeks, the plants over-expressing *HhDREB2* gene showed higher survival rates compared with the wild-type plants. The transgenic line even could flower and set fruits under drought (Fig. 5*B*,*C*). There were no differences in root lengths between T_2 and WT on the normal MS medium. In the presence of 50 mM NaCl, the transgenic plants showed a less severe inhibition on primary root growth and plant height than the WT plants. However, the primary root growth of the transgenic plants was dramatically retarded compared with WT when subjected to 100, 150, and 200 mM NaCl, but not for plant height (Fig. 5*D*,*E*).

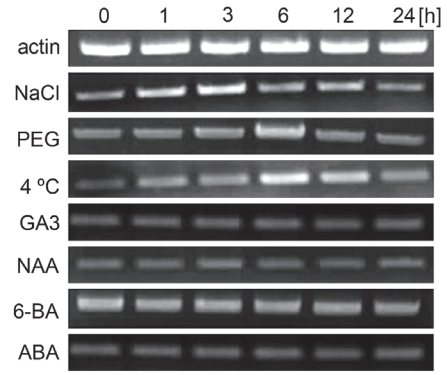

Fig. 4. The semi-quantitative RT-PCR analyses of *HhDREB2* in response to various stresses. *Halimodendron halodendron* seedlings were subjected to 250 mM NaCl, 20 % PEG 6000, 4 °C, 100 µM GA_3, 100 µM NAA, 100 µM 6-BA, and 100 µM ABA for the indicated times. An *actin* gene was amplified as template control.

In order to clarify the potential function of HhDREB2 in response to different stimuli, the expression pattern of the *HhDREB2* gene was analysed by semi-quantitative RT-PCR under various stress conditions. Under the NaCl stress, *HhDREB2* mRNA accumulated quickly and reached its maximal level at 3 h and then decreased to its basic level at 24 h. In contrast, the expression of *HhDREB2* peaked within 6 h by the 20 % PEG 6000 or 4 °C treatment. Interestingly, *HhDREB2* mRNA still maintained a high expression between 6 - 12 h under the cold treatment. Thus, the RT-PCR analysis revealed that the expression of *HhDREB2* was induced by salinity, drought, and low temperature, however, it was not affected by GA_3, NAA, BA, or ABA (Fig. 4).

To evaluate physiological changes of the transgenic plants, the content of water, soluble sugars, and MDA in WT and T_2 were compared after the drought and salt treatments. These parameters were similar in the WT and T_2 plants before the application of the stresses. After 14 d, the water content under the drought stress decreased much more in WT than in T2. The salinity hardly affected the water content except for the 200 mM NaCl treatment (Fig. 6). After the treatment with 100 mM NaCl, the content of soluble sugars in T_2 was about 2.8-fold higher than that under the normal conditions, whereas it was only 1.7-fold higher in WT (Fig. 6). After 14 d of the drought stress or 200 mM NaCl, the content of MDA in T_2 was much lower than that in the WT plants (Fig. 6).

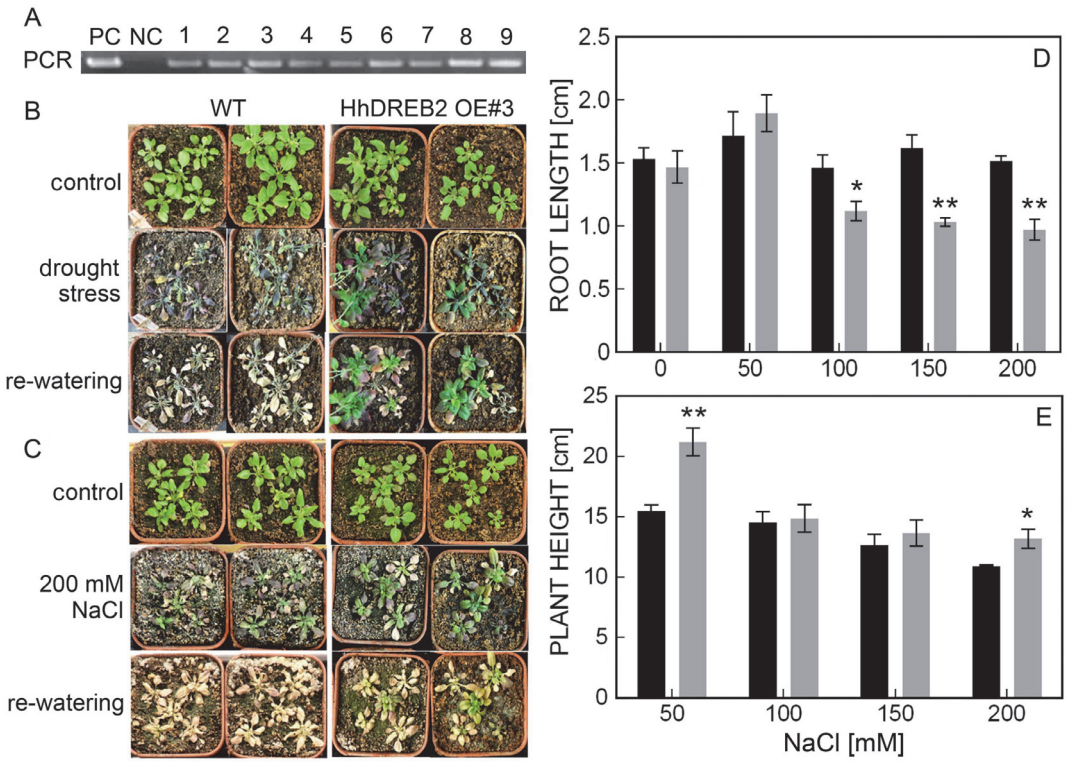

Fig. 5. The growth analysis of transgenic lines HhDREB2OE#3 and WT *Arabidopsis* seedlings under stress treatments. *A* - PCR to identify positive transgenic lines. PC - positive control, NC - negative control. *B* and *C* - Performances of transgenic lines with drought-inducible expression of *HhDREB2* during a survival test (drought or 200 mM NaCl for three weeks, and then re-watering for one week). *D* - Root length under normal conditions, or treatments with 50, 100, 150, and 200 mM NaCl for 3 weeks. *E* - Plant height after treatments with 50, 100, 150, and 200 mM NaCl for 3 weeks. Each experiment was repeated three times. The *grey bars* - transgenic lines, the *black bars* – WT. Data were obtained from three independent experiments. Means ± SE, $n = 5$, significant differences: * - $P \leq 0.05$, ** - $P \leq 0.01$.

Discussion

After the discovery of DREB protein, accumulating evidence has revealed that DREBs act as important regulators in plant abiotic stress responses (Dubouzet *et al.* 2003, Xu *et al.* 2009, Zhou *et al.* 2010). In this study, a new member of the AP2/EREBP transcription factors, HhDREB2, was identified in the 250 mM NaCl-treated *H. halodendron* seedlings. Detailed analyses show that HhDREB2 had many typical features of other DREBs. YRG and RAYD as two important elements binding with the promoter sequence, and some other interacting proteins were also found within the AP2/ERF domain of HhDREB2. The 14th valine and the 19th glutamic acid in the YRG element are highly conserved in diverse plant species and considered to play an important role in the binding to the DRE *cis*-elements (Zhou *et al.* 2010). The results suggest that conserved elements may play an important role in DNA-binding specificity during the evolution of higher plants.

Earlier studies showed that DREB2-type proteins contain a conserved Ser/Thr-rich region (a putative phosphorylation site) adjacent to or far away from the AP2/ERF domain. The over-expression of DREB2-type proteins in plants was not sufficient for the induction of target stress-responsive genes, although corresponding mRNA accumulates even under unstressed conditions (Liu *et al.* 1998, Dubouzet *et al.* 2003). Sakuma *et al.* (2006) found that deletion of the Ser/Thr-rich motif lead to a constitutively active protein form. The DNA binding of phosphoproteins is negatively influenced by phosphorylation which gets reversed by a dephosphorylating treatment (Agarwal *et al.* 2007). Therefore, phosphorylation sites in DREBs may act as negative regulation motifs. However, recent reports suggest that some DREB2 homologues, like ZmDREB2A and HvDREB1, can function as transcriptional activators without any post-translational modification (Qin *et al.* 2007, Xu *et al.* 2009). In this work, a Ser/Thr-rich region was found in the C-terminal region, but we did not observe any negative effect on the transgenic lines as described by Liu *et al.* (2007). It appears that the Ser/Thr-rich region perhaps serves dissimilar biological function in DREBs from different plant species.

Transcriptional regulatory networks in plants in response to abiotic stresses involve a large number of

genes. Signaling cross-talks exists in the regulation of different DREB groups. Earlier studies found that DREB2-type genes are induced mainly by drought and salinity, whereas *CBF/DREB1* genes are induced mainly by low temperature (Liu *et al.* 1998, Sakuma *et al.* 2006, Agarwal *et al.* 2007, Xu *et al.* 2008). It is interesting that *CBF4/DREB1D*, as CBF/DREB1-type gene, is induced by osmotic stress, but *DDF1/DREB1F* and *DDF2/DREB1E* are induced by salinity (Yamaguchi-Shinozaki and Shinozaki 2006). In this study, *HhDREB2* (A-5) gene was induced by the drought, salinity, and low temperature, which is similar with the earlier studies with *PpDBF1* (A-5) (Liu *et al.* 2007), *GhDREB1L* (A-1) (Huang *et al.* 2007), *TaAIDFa* (A-2) (Xu *et al.* 2008), and *GhDBP3* (A-4) (Huang and Liu 2006).

ABA as signaling molecule plays a key role in many aspects of abiotic stress responses by regulating gene expression including transcription factor genes. However, not all abiotic responses appear to be mediated by ABA in plants. The signaling pathways can be divided into ABA-dependent and ABA-independent (Yamaguchi-Shinozaki and Shinozaki 2006). The expression patterns of *HhDREB2* (Fig. 4) show that *HhDREB2* was not induced by ABA, but it was very sensitive to the abiotic stresses, especially NaCl. The data presented in our study also show that the water content of leaves decreased when the plants were subjected to the stresses. Plants accumulate osmolytes to adjust the intracellular osmotic potential, which was evidenced by determining the soluble sugar content of the *HhDREB2* transgenics. Similar results were shown in *OsDREB2A* and *PeDREB2a* transgenic plants that maintain a higher soluble sugar content than control plants under stress conditions (Cui *et al.* 2011, Zhou *et al.* 2012). MDA is the final decomposition product of membrane lipid peroxidation, so the MDA content is considered as characteristics of damage of cellular membranes (Lei *et al.* 2007). A lower content of MDA in T_2 than in WT after the treatments suggest that *HhDREB2* improved stress tolerance of the plants by increasing the amount of osmolytes *via* controlling the expression of some related genes in the ABA-independent pathway.

In conclusion, it is postulated that *HhDREB2* activated the expression of downstream genes that regulate inherent complex physiological and biochemical processes and improve tolerance to drought and salt stresses. However, limited knowledge in relationships between physiological metabolism and stress-tolerance

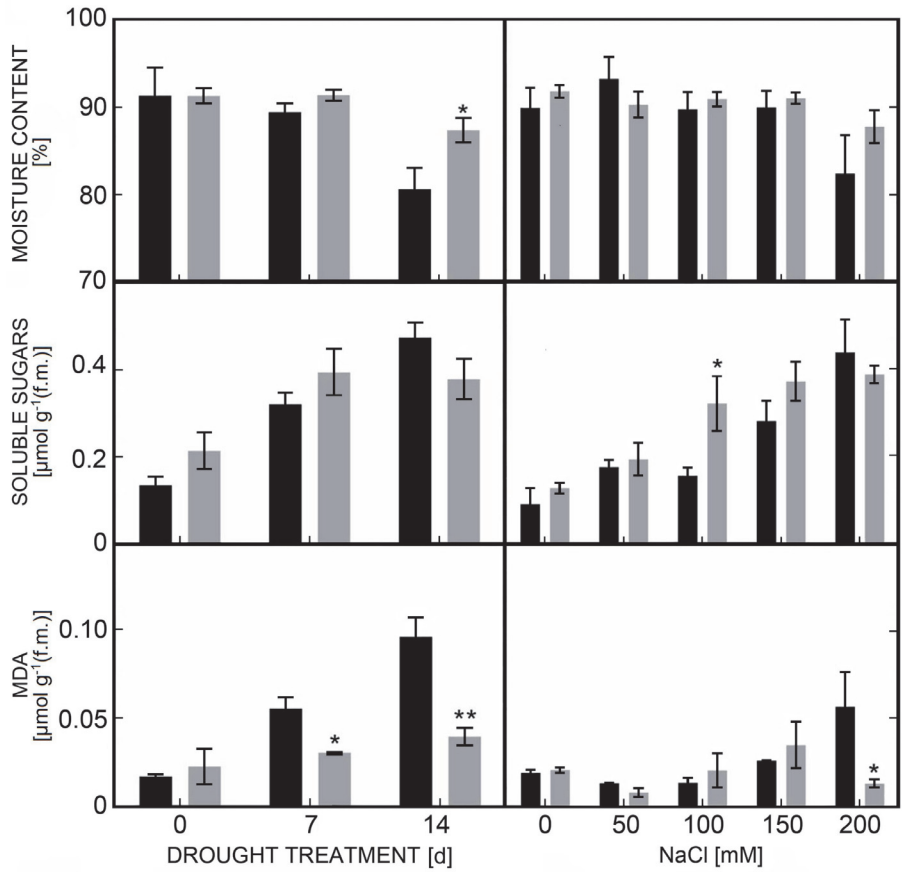

Fig. 6. The changes of physiological characteristics of wild-type and transgenic line HhDREB2OE#3 of *Arabidopsis*. The moisture content, soluble sugars, and MDA content were determined under the normal conditions (0 d), drought stress (7 or 14 d), and 0 - 200 mM NaCl. The *black bars* - WT, the *grey bars* - transgenic plants. Data were obtained from three independent experiments. Means ± SE, $n = 5$, significant differences: * - $P \leq 0.05$, ** - $P \leq 0.01$.

genes, and the cross points between stress signaling pathways are the barriers for us to understand regulation mechanisms of plants under stress conditions. To identify more genes related to stress tolerance and study their expression in other plants will help us to reveal the network in future.

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