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Original Article

Novel Zinc Finger-Homeodomain Gene from Barley (*HvZFHD1*) is Differentially Regulated During Spike Development and under Hormonal Treatments and Abiotic Stresses

Saeid ABU-ROMMAN[1]*, Khaldoun J. AL-HADID[2]

[1]*Al-Balqa' Applied University, Faculty of Agricultural Technology, Department of Biotechnology, Al-Salt 19117, Jordan; ssadroman@yahoo.com (*corresponding author)*

[2]*The University of Jordan, Faculty of Science, Department of Biological Sciences, Amman 11942, Jordan; kalhadid@ju.edu.jo*

Abstract

Plant zinc finger-homeodomains (ZFHDs) are transcriptional factors that play an important role in regulating plant growth and development. Several ZFHD genes were cloned and characterized in many plant species. In the present study, a full-length cDNA sequence of *ZFHD* gene was cloned from barley (termed as *HvZFHD1*) using reverse transcription polymerase chain reaction (RT-PCR). The sequence analysis showed that the *HvZFHD1* was 1477 bp in length, and contained a complete open reading frame (ORF) of 1161 bp. The deduced protein is composed of 386 amino acids, with a predicted molecular weight of 40.46 kDa and a theoretical isoelectric point of 8.5. Multiple sequence alignment indicated that HvZFHD1 protein shared high identity with ZFHD proteins from wheat, maize, and rice. The predicted HvZFHD1 protein contained the characteristic putative zinc finger domain in the N-terminus and a DNA binding homeodomain in the C-terminus. The expression level of *HvZFHD1* was investigated using qRT-PCR during spike development and in response to exogenous phytohormones and abiotic stresses. The results showed that the expression level of *HvZFHD1* was fluctuated during spike development with higher expression during anthesis, medium milk, late milk, and early dough stages. The expression of barley *ZFHD1* was strongly responsive to abscisic acid treatment and was up-regulated in seedlings treated with methyl jasmonate, salicylic acid, and ethephone. In addition, the expression levels of *HvZFHD1* were increased by dehydration, salinity, and heat stress, but not affected by cold stress. The expression patterns of *HvZFHD1* suggest that it might play a role in flowering and flower development and is involved in plant responses to abiotic stresses.

Keywords: abiotic stresses, barley, HvZFHD1, phytohormones, spike development, transcription factor, zinc finger homeodomain

Introduction

Zinc finger" is a term applied to a diverse set of protein motifs that commonly binds to zinc ions (Klug and Schwabe, 1995). These motifs were called "zinc fingers" because a zinc atom is coordinated by cysteines and/or histidines to form the finger-like structures required for their specific functions (Takatsuji, 1998). Therefore, the zinc ion works as stabilizer of the zinc finger structure (Klug and Schwabe, 1995). Zinc fingers are classified into different types according to the nature, spacing pattern, and number of zinc-binding residues. For instance, C2H2, C2C2 and C3H zinc fingers interact with one zinc ion, whereas the RING, PHD, and LIM zinc fingers interact with two zinc ions (Hu et al., 2008). About 176 zinc finger proteins of C2H2-type were reported in the Arabidopsis genome, many of which of this type of zinc finger protein function as transcriptional factors (Fujita *et al.,* 2011). Zinc fingers have broad range of functions in metabolism, signaling, transcription, translation, replication, repair, proliferation and apoptosis (Krishna *et al.,* 2003). Moreover, zinc fingers can bind to a variety of compounds, such as proteins, nucleic acid, and small molecules (Krishna *et al.,* 2003).

The homeodomain (HD) is a 60-amino acid DNA-binding motif that is common in many transcription factors (Wolberger, 1996). These transcriptional factors are encoded by homeobox genes, which play important roles in the development of diverse organisms like humans, insects, and plants (Wolberger, 1996). Homeodomain proteins act as transcription factors because they have the ability to bind to promoter sequences and act to enhance or repress mRNA synthesis (Biggin and Tjian, 1989). The typical structure of a homeodomain consists of three α helices and a flexible N-terminal arm (Wolberger, 1996).

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Zinc finger-homeodomains (ZFHDs) are considered transcriptional factors that regulate plant growth and development. ZFHDs contain several cysteine and histidine residues that shape the zinc finger structure of N-terminal conserved domain (Park *et al.,* 2010). The ZFHD is ideal for DNA binding because it can easily insert itself into the major groove of DNA with minimal entropic cost, which enables to recognize and bind a specific DNA sequence (Klug and Schwabe, 1995).

ZFHDs have been identified and characterized in many plants, such as *Arabidopsis thaliana*, *Oryza sativa*, *Glycine max*, and *Triticum aestivum* (Park *et al*., 2007; Hong *et al.,* 2011; Figueiredo *et al.,* 2012; Abu-Romman, 2014). The Arabidopsis genome has 14 ZFHD genes that have unique biochemical activities. This gene family of ZFHD in Arabidopsis is believed to play collectively an overlapping and important role in regulating floral development (Tan and Irish, 2006; Hu *et al.,* 2008). Moreover, ZFHD1 in Arabidopsis was reported to be induced under abiotic stresses such as drought and salt conditions (Tan and Irish, 2006). In fact, ZFHD1 binds specifically to the promoter of a drought-responsive gene called "EARLY RESPONSIVE TO DEHYDRATION STRESS 1" (Tan and Irish, 2006). Moreover, Arabidopsis ZFHD can be regulated by other genes. For instance, the function of ZFHD (ZHD5) as a transcription factor that regulates leaf development and floral architecture, is inhibited by binding with Arabidopsis MIFI (mini zinc finger 1) (Hong *et al*., 2011).

In soybean, ZFHDs bind to the promoter of the gene encoding calmodulin isoform 4 (*GmCaM4*) that is its transcription is induced in response to pathogen infection and plant stress (Park *et al.,* 2007). In *Flaveria*, ZFHDs bind and regulate the gene encoding phosphoenolpyruvate carboxylase (PEPCase) (Windhövel *et al.,* 2001). In grapes (*Vitis vinifera* L.), 13 ZFHDs, termed *VvZHD*, were identified and classified into seven groups. These ZFHDs were differentially regulated under certain hormone treatment and stress conditions (Wang *et al.,* 2014).

In cereals, 17 ZFHD genes were identified in maize (Wang *et al.,* 2014). In fact, ZFHDs in maize are plant-specific zinc fingers characterized by a highly conserved domain called ID, which includes three different types of zinc fingers separated by a long spacer from a fourth C2H2 finger. These maize ID domain proteins have unique DNA binding properties and novel zinc finger configurations (Kozaki *et al.,* 2004). In rice, 14 ZFHDs were found mediating homo- and hetero-dimerization in protein–protein interaction. Two more rice ZFHDs (Os02g05450 and Os06g12400) had Cys4-His-Cys3-type zinc-finger domains (Jain *et al.,* 2008). Moreover, seven novel zinc fingers bind to the promoter of OsDREB1B gene, which is induced by low temperatures, drought, and mechanical stress. Four of the seven zinc-fingers were characterized ZFHD that form homo- and heterodimers during protein-protein interaction while the other three zinc fingers are C2H2-type. These transcriptional factors are transcribed differentially under different abiotic stress. This indicates the role of ZFHD in abiotic stress. Moreover, a zinc finger transcription factor called "DROUGHT AND SALT TOLERANCE" (DST) regulates the expression of *OsCKX2* that encodes cytokinin oxidase. OsCKX2 regulates cytokinin accumulation in rice shoot apical meristem and, consequently, the number of the reproductive organ (Li *et al.,* 2013). In addition, a novel zinc finger protein of CCCH type (OsDOS) was reported to have a role in delaying leaf senescence in rice (Kong *et al.,* 2006). Recently, a novel rice ZFHD was identified and termed OsZHD1. The artificial overexpression of *OsZHD1* induced the baxially curled leaf phenotype in rice (Xu *et al.,* 2014). In wheat (*Triticum aestivum*), transcriptional profile analysis of seven RING zinc finger genes showed differential transcription in the different plant organs as well as during aging and leaf development (Kam *et al.,* 2007). Moreover, zinc factors of type C2H2 were reported to be predominantly expressed in roots in wheat (Kam *et al.,* 2008). Recently, *Triticum aestivum* ZFHD (*TaZFHD1) was identified* and isolated (Abu-Romman, 2014). The *TaZFHD1* transcriptional profile was analyzed during wheat spike development. *TaZFHD1* was preferentially transcribed during "half emerged," completely emerged," and "half anthesis" stages of wheat spike development. Moreover, *TaZFHD1* was found to be up-regulated under the treatment of methyl jasmonate, abscisic acid, and ethylene (Abu-Romman, 2014). The aim of this study was to identify a barley ZFHD homologue to the wheat *(TaZFHD1)* to investigate its role in barley spike development, and under abiotic stresses and different phytohormone treatments.

Materials and Methods

Plant materials and treatments

Barley (*Hordeum vulgare*) cv. 'Rum' seeds were planted under greenhouse conditions as described by Abu-Romman *et al*. (2011). Ten-day-old seedlings were subjected to different abiotic stresses and hormonal treatments. Dehydration treatment was performed according to Abu-Romman (2012) in which primary leaves of barley seedlings were cut off and left on the lab bench at room temperature (22 ± 2 °C) and under light. For salinity stress, seedlings were irrigated with 250 mM NaCl. Cold stress and heat stress were induced by transferring seedlings to growth chambers set to 4 °C and 40 °C, respectively. For phytohormone treatment, seedlings were sprayed with 75 µM methyl jasmonate (MeJA), 1 mM salicylic acid (SA), 100 µM ethephone (ET) and 150 µM abcissic acid (ABA). In all stress and hormone treatments, tissue samples were collected at 0, 3, 6, 9, 12, and 24 h post-treatment.

Barley spikes were collected at 16 developmental stages ranging from Zadoks growth stage (GS) 45-87 (Zadoks *et al*., 1974): boots swollen (GS 45); flag leaf sheath opening (GS 47); awns emerging (GS 49); one-fourth of spike emerged (GS 53); spike half emerged (GS 55); three-fourth of spike emerged (GS 57); spike completely emerged (GS 59); half anthesis (GS 65); anthesis completed (GS 69); kernels at watery ripe (GS 71); kernels at early milk (GS 73); kernels at medium milk (GS 75); kernels at late milk (GS 77); kernels at early dough (GS 83); kernels at soft-dough (GS 85) and kernels at hard dough (GS 87).

All tissue samples were promptly frozen in liquid nitrogen and stored at −70 °C for RNA extraction. Three biological replications were conducted for each treatment.

Total RNA isolation

Frozen plant materials were ground in liquid nitrogen, and about 60 mg fresh material were used for total RNA extraction using the Total RNA Purification Kit (Jena Bioscience, Germany) according to the manufacturer's instructions. RNA concentration and purity were determined using a spectrophotometer.

Cloning of full-length HvZFHD1 cDNA

Candidate cDNA contigs were selected that showed high sequence identity with wheat *ZFHD1* (*TaZFHD1*, GenBank accession No. KF697362) by blast *TaZFHD1* mRNA sequence against the Triticeae Full Length CDS database (Mochida *et al*., 2009). Sequence alignment of these cDNA contigs was conducted to design cloning primers for barley *ZFHD1* (Table 1). First-strand cDNA was synthesized using PrimeScript™ RT Master Mix (TaKaRa, Japan) from a pool of mRNAs isolated from different spike developmental stages and leaf tissues of seedlings exposed to the above mentioned treatments. This cDNA was used as a template in a PCR reaction to amplify full-length barley *ZFHD1* clone using iNtRON *i*-MAX II Kit (iNtRON, Korea). The PCR program was 2 min at 94 °C with 32 cycles of 30 s at 95 °C, 40 s at 57 °C, and 2 min at 72 °C, then a final extension for 10 min at 72 °C. The PCR product was sequenced after cloning into the pGEM-T Easy Vector (Promega, Madison, USA).

Protein sequence and phylogenetic analysis

The ProtParam tool of the ExPASy proteomics server (http://web.expasy.org/protparam/) was used to analyze the amino acid sequence of HvZFHD. Protein similarity analysis and multiple sequence alignment were performed using the Clustal-Omega program (Sievers *et al*., 2011). The prediction of HvZFHD domains was performed by searching NCBI Conserved Domain Database (http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi) (Marchler-Bauer *et al*., 2009) and PROSITE database (prosite.expasy.org). To explore the evolutionary relationship between HvZFHD and other ZFHD proteins, a phylogenetic tree was constructed by the neighbor-joining method with 1000 bootstrap replicates using MEGA 5.0 software (Tamura *et al*., 2011).

qRT-PCR analysis of HvZFD1 expression

Quantitative real-time PCR (qRT-PCR) was conducted in triplicate with a CFX96 Real-Time PCR Detection System (Bio-Rad) using KAPA SYBR® FAST qPCR Kit (KAPA BIO, USA) according to the manufacturer's instructions. The relative expression level of *HvZFHD1* was normalized against the expression of the barley *α–Tubulin* (GenBank accession No. U40042) according to the $2^{-\Delta\Delta Ct}$ method (Schmittgen and Livak, 2008). Primers used in qRT-PCR are listed in Table 1.

Table 1. Primer sequences used for cloning and expression analysis of *HvZFHD1*

Primer	Primer sequence (5'–3')	Description
HvZFHD1-F	CCTGTAGTTTTCCCTCACTCCC	Gene cloning
HvZFHD1-R	CTAAATCGCAAGCTTCCATCGG	Gene cloning
HvZFHD1-qF	TCCTGAGACGCCTGATGAC	qRT-PCR
HvZFHD1-qR	CTGGAGTTCAGGGAGAGCAG	qRT-PCR
α–Tubulin-qF	AATGCTGTTGGAGGTGGAAC	qRT-PCR
α–Tubulin-qR	GAGTGGGTGGACAGGACACT	qRT-PCR

Results and Discussion

A zinc finger-homeodomain gene was cloned from barley. The clone was sequenced and submitted to GenBank (accession No. KT361919) and named *HvZFHD1* (Fig. 1). The full length cDNA of *HvZFHD* is 1477 bp with an Open Reading Frame (ORF) of 1161 bp that has a 5′ untranslated region (UTR) of 114 bp and 3′ UTR of 202 bp. The ORF encodes a zinc finger-homeodomain protein of 386 amino acids with a deduced molecular weight of 40.46 kDa and a theoretical isoelectric point of 8.5 (Fig. 1A).

HvZFHD1 protein exhibited high sequence identity with ZFHD proteins from some monocot plants. As expected, the amino acid sequence of HvZFHD1 had 91.45% identity with wheat TaZFHD1. However, the amino acid sequence of HvZFHD1 had 59% identity with both rice OsZFHD1 and maize ZmZFHD3 proteins (Fig. 1B). In fact, this result matches many reports in the literature that illustrate the evolutionary relationship between wheat and barley. For instance, Mena *et al*. (1998) reported a striking sequence identity and similar DNA-binding properties of a wheat transcriptional factor with another barley transcriptional factor (BPBF) of DOF class transcriptional factors.

Two conservative putative zinc finger domains at the N-terminus and a DNA binding homeodomain at the C-terminus were detected in the deduced HvZFHD1 protein (Fig. 1B). This proves that the cloned sequence is a zinc finger-homeodomain gene, in which the zinc finger and the DNA-binding domains have 100% sequence identity with other zinc finger and DNA-binding domain of other plants, such as maize, rice, and wheat.

A phylogenetic tree was constructed between HvZFHD1 and ZFHD of some plants, based on their amino acid sequences. HvZFHD1 is closely related to wheat TaZFHD1 (Fig. 2). The phylogenetic relationship of ZFHD of different monocot and dicot plants can be divided into four clades (Fig. 2). The first clade can be divided into six subclades (Fig. 2). HvZFHD1 is among the first subgroup, which contains both monocot and dicot plants. However, monocot ZFHDs are classified together including HvZFHD1. Moreover, HvZFHD1 and TaZFHD1 have a very close phylogenetic relationship with both maize and rice ZFHD. This probably is explained by an evolutionary relationship of wheat and barley on one side and maize and rice on the other.

The transcriptional profile of *HvZFHD1* was measured during spike development using qRT-PCR technique (Fig. 3). *HvZFHD1* was differentially expressed during spike development. *HvZFHD1* is transcribed at a relatively low level in "boots swollen", "sheath opening", "awns emergence", and "¼ of head emergence". *HvZFHD1* transcripts increased significantly during "½ head emergence", "¾ of head emergence" and "complete head emergence". The highest transcriptional level of *HvZFHD1* (23.3-fold) was measured at the "half anthesis" stage. *HvZFHD1* transcription decreases significantly from the "anthesis complete" stage to "water ripe" stage. The transcriptional level of *HvZFHD1* gradually

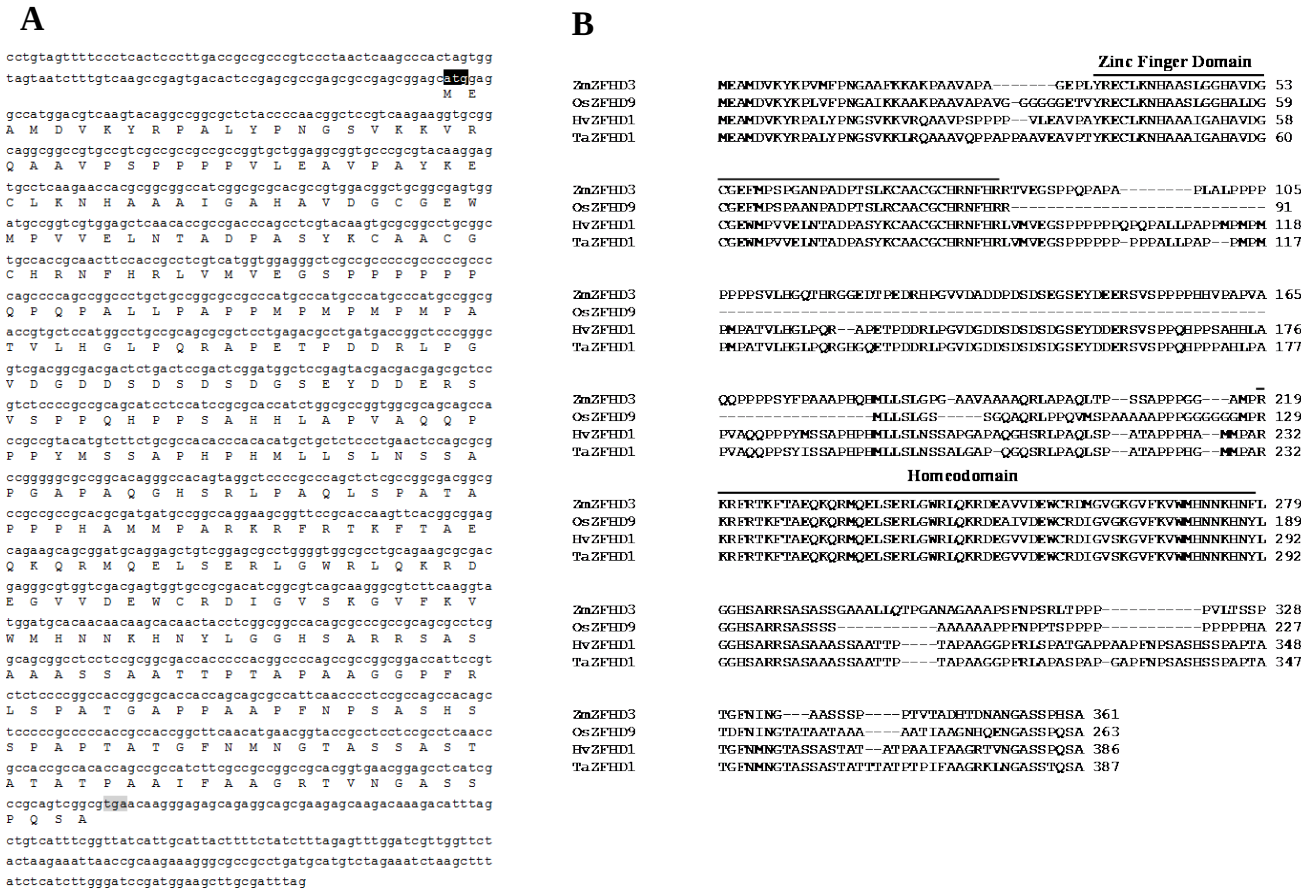

Fig. 1. Sequence analysis of barley *HvZFHD1* gene and protein. (A) Nucleotide sequence of HvZFHD1 and its deduced amino acid sequence. The deduced amino acid sequence is shown in single letter code below the nucleotide sequence. The black box indicates the start codon and the gray box indicates a stop codon. (B) Comparison of putative amino acids sequence of HvZFHD1 (KT361919) with the most closely related ZFHD from wheat (TaZFHD1, KF697362), rice (OsZFHD9, BAD28899) and maize (ZmZFHD3, ACG40010). The zinc finger domain and the homeodomain are indicated by a line over the domain

increased during the milk development stages to "early dough" stage. In the late stages of the spike development, *HvZFHD1* transcripts decreased significantly until it was almost as much as the early stages of the spike development. The correlation of transcription of zinc fingers and spike development in cereals has been reported in wheat and rice. *ZFHD* was induced in wheat spikes particularly during early stages of anthesis (Abu-Romman, 2014). In rice, C2H2-type zinc finger (*ZFP15*) was highly transcribed in the flowering spike, but much less transcribed in the immature spike (Huang *et al*., 2005). This indicates that some zinc fingers in plants could play a regulatory role in spike development.

The qRT-PCR analysis showed that the relative mRNA expression level of *HvZFHD1* reached the highest level of 7.5-fold 3 h post applying MeJA. The transcriptional level of *HvZFHD1* decreased from 3-h to 24-h post applying MeJA. This means that MeJA enhance *HvZFHD1* transcriptional level in a very short time; however, this up-regulation does not last long in barley leaves. In fact, the transcriptional level of *HvZFHD1* went down after 24-hour of applying MeJA to almost the transcriptional level of *HvZFHD1* of the untreated plants (Fig. 4). In fact, the link between zinc fingers and jasmonate was established. For instance, a zinc finger (OsDOS) is believed to delay leaf senescence in rice, likely, at least in part, by interfering with the jasmonate pathway (Kong *et al*., 2006).

The transcriptional profile of *HvZFHD1* post SA treatment showed a bell-shaped pattern with the greatest fold increase at 6-h post SA treatment (4.2-fold). The treatment of barley seedlings with SA increased the relative expression level of *HvZFHD1* 2.3-fold at 3 h to its highest level at 6 h (Fig. 4).

ABA treatment showed significant and highest up-regulation of *HvZFHD1* with a many fold increase of *HvZFHD1* transcripts comparing MeJA, SA, and ET treatment for 6-, 9-, 12-, and 24-h time points. In fact, the number of fold increase of *HvZFHD1* transcript at the 3-h point almost equals the highest fold increase of *HvZFHD1* transcript of any of the other hormone treatments. Therefore, the magnitude of *HvZFHD1* expression in response to ABA was dramatically higher than the other phytohormones magnitude. The results of the qRT-PCR showed that the relative expression level of *HvZFHD1* increased to reach 18.4-fold at 6 h, and then gradually decreased to 11.7-fold at 24 h. This shows a strong relationship of the gene regulation mechanism between ABA and *HvZFHD1*. This also indicates probably that the role of HvZFHD1 is probably linked to the role of ABA physiologically. ABA plays a key role in generating a broad physiological response to stress in plants, particularly dehydration (Cutler *et al*., 2010). ABA increases endogenously

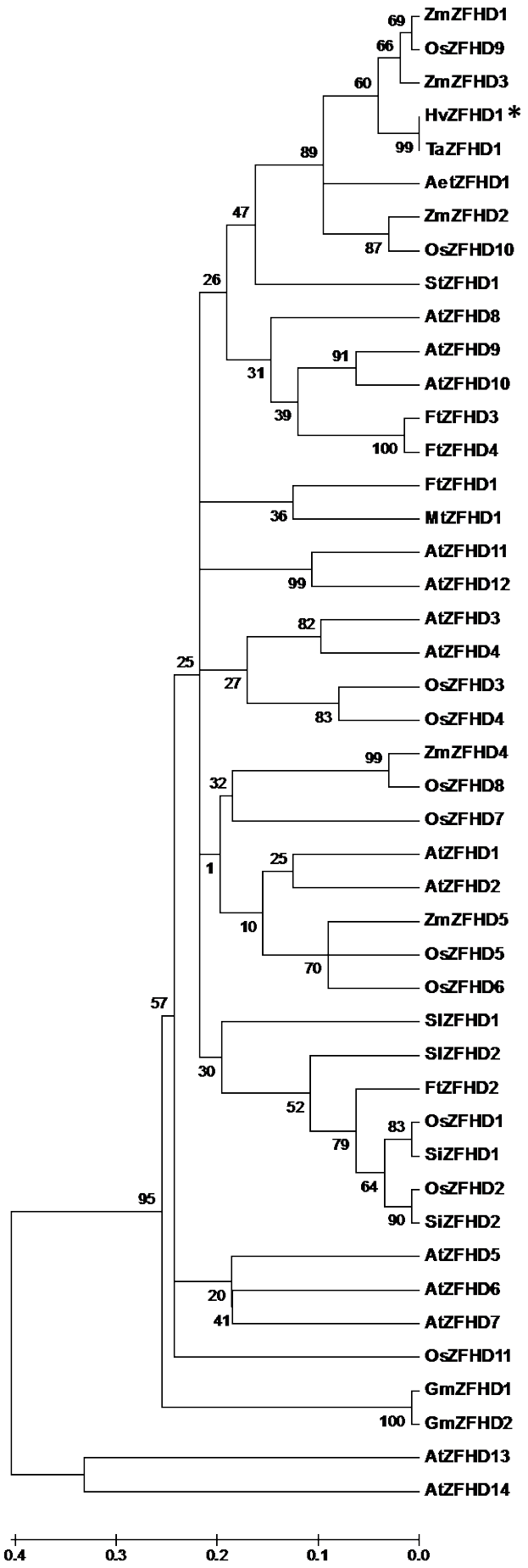

Fig. 2. Phylogenetic tree of the amino acid sequences of ZFHD of different plant species constructed by the neighbor-joining method on MEGA 5 program. GenBank accession numbers: *Hordeum vulgare* HvZFHD1 (KT361919) *Triticum aestivum* TaZFHD1 (KF697362); *Arabidopsis thaliana* AtZFHD1 (AAP13412), AtZFHD2 (AEE84939), AtZFHD3 (AEC05592), AtZFHD4 (AEE29164), AtZFHD5 (ABI49441), AtZFHD6 (AEC06760), AtZFHD7 (AEE78722), AtZFHD8 (AAS76682), AtZFHD9 (AEE77508), AtZFHD10 (AED94472), AtZFHD11 (AEE34954), AtZFHD12 (AED97335), AtZFHD13 (AED94862), and AtZFHD14 (AEE29206); *Oryza sativa* OsZFHD1 (BAD17515), OsZFHD2 (BAD09750), OsZFHD3 (ABA96146), OsZFHD4 (AAX95984), OsZFHD5 (BAD69443), OsZFHD6 (BAG99227), OsZFHD7 (BAD08049), OsZFHD8 (CAE01709), OsZFHD9 (BAD28899), OsZFHD10 (BAD09869), and OsZFHD11 (ABF98569); *Zea mays* ZmZFHD1 (ACG35864), ZmZFHD2 (ACG36188), ZmZFHD3 (ACG40010), ZmZFHD4 (ACG45353), and ZmZFHD5 (ACG46677); *Flaveria trinervia* FtZFHD1 (CAC34413), FtZFHD2 (CAC34447), FtZFHD3 (CAC34409), and FtZFHD4 (CAC34410); *Setaria italica* SiZFHD1 (XP_004957068) and SiZFHD2 (XP_004973708); *Glycine max* GmZFHD1 (AAW22594) and GmZFHD2 (AAW22595); *Solanum lycopersicum* SlZFHD1 (XP_004236700) and SlZFHD2 (XP_004237971); *Solanum tuberosum* StZFHD1 (ABO92969); *Medicago truncatula* MtZFHD1 (ABN05735); *Aegilops tauschii* AeZFHD1 (EMT26453). HvZFHD1 is marked with an asterisk

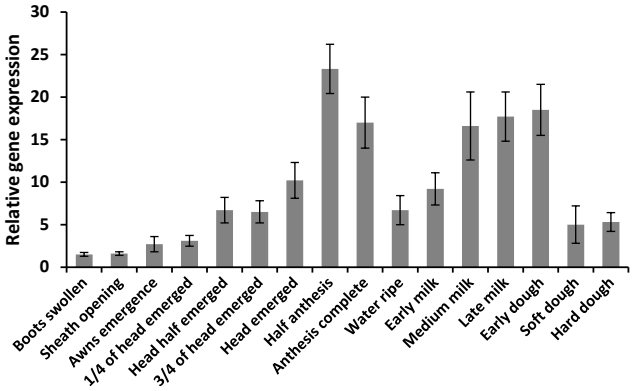

Fig. 3. qRT-PCR analysis of *HvZFHD1* expression during spike development of barley. The expression level of *HvZFHD1* was normalized to the *α–Tubulin* reference gene. Each value represents the mean ± standard error of three biological replicates

when plants are subjected to dehydration stress. One reasoning for this increase in ABA under dehydration stress is its role in regulating dehydration-responsive genes. Therefore, the increase of *HvZFHD1* transcripts after ABA treatment is probably due to the regulatory link between ABA and *HvZFHD1*. It was reported that overexpression of zinc finger

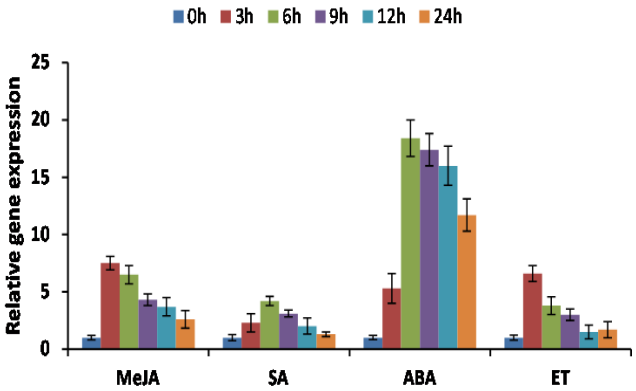

Fig. 4. qRT-PCR analysis of *HvZFHD1* expression in response to treatment with selected phytohormones. The expression level of *HvZFHD1* was normalized to the *α–Tubulin* reference gene and expressed as a ratio relative to the control seedlings (0 h), which was set at 1. Each value represents the mean ± standard error of three biological replicates

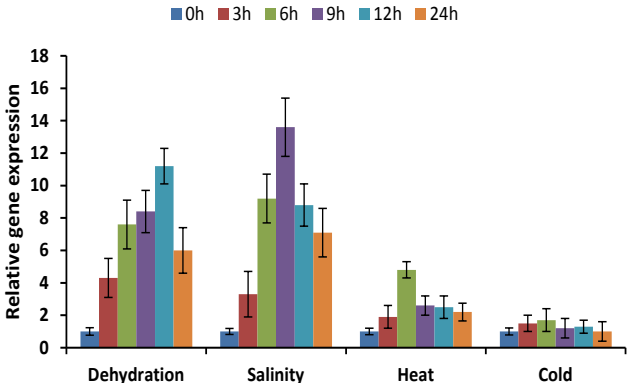

Fig. 5. qRT-PCR analysis of *HvZFHD1* expression in response to different abiotic stresses. The expression level of *HvZFHD1* was normalized to the *α–Tubulin* reference gene and expressed as a ratio relative to the control seedlings (0 h), which was set at 1. Each value represents the mean ± standard error of three biological replicates

genes (RING) up-regulated a key ABA-biosynthesis gene (Ko *et al*., 2006). This shows the relationship with some zinc finger protein and the accumulation ABA as a molecular and physiological response to dehydration stress.

In response to ET treatment, the relative expression level of *HvZFHD1* increased and reached the highest level at 3-h point (6.6 fold). However, the *HvZFHD1* transcript decreased thereafter to 1.7-fold at 24-h point (Fig. 4). In fact, the transcriptional profile of *HvZFHD1* in response to ET is much similar to the transcriptional profile of *HvZFHD1* in response to MeJA. This indicates that similar gene regulation mechanism of *HvZFHD1* probably is in common in response to MeJA and ET. Moreover, our results indicate a matching of transcriptional profile of *HvZFHD1* as response to ET and MeJA more than the transcriptional profile of *HvZFHD1* in response of SA. The transcriptional regulatory role of *HvZFHD1* in response to MeJA and ET might be more similar than that of SA. This matches the report that JA and ET have a distinct response mechanism than SA in disease resistance (Dong, 1998).

In response to dehydration condition, the relative expression level of *HvZFHD1* in barley seedlings increased 4.3-fold at the time point of 3 h. The transcripts of *HvZFHD1* kept increasing while the plant suffer from dehydration for 12 h, in which *HvZFHD1* transcripts reached the highest level of 11.2-fold. However, the transcripts of *HvZFHD1* decreased dramatically between 12 h and 24 h post the dehydration treatment (Fig. 5). This could mean that *HvZFHD1* plays an initial role in response to drought stress in plant. It might trigger other drought-related genes that play roles in drought resistance in the long-term. In Arabidopsis, *ZFHD1* was shown to bind the ZFHD recognition sequence in the promoter of *EARLY RESPONSIVE TO DEHYDRATION STRESS 1* (*ERD1*) and thereby function as a transcriptional activator in response to drought stress (Tran *et al*., 2007).

The transcriptional profile of *HvZFHD1* with response to dehydration treatment is much similar to the transcriptional profile of *HvZFHD1* in response to salinity. Salinity increased the relative transcriptional level of *HvZFHD1* 3.3-fold at the 3-h point. The transcripts of *HvZFHD1* kept increasing from the 3-h point until its highest level at 9-h. However, *HvZFHD1* transcripts decreased at 12-h and decreased further at 24-h post treatment (Fig. 5). This result of up-regulation of a zinc finger with salinity treatment agrees with up-regulation of rice C2H-type zinc finger (*ZFP179*) under salinity treatment (Sun *et al*., 2010). It is interesting to find out this salt-responsive zinc finger gene is highly expressed in the immature spike in rice. Another zinc finger gene (*GmZFP1*) was reported to be highly expressed in the reproductive organ of soybean (Huang *et al*., 2006). This probably indicates that a relationship at molecular level, at least, exists between the role of zinc finger genes as a response to salinity stress and their role in spike and seed development.

High temperature treatment (40 °C) increased the expression level of *HvZFHD1* by 4.8-fold at 6 h. However, the transcripts of *HvZFHD1* leveled back at 9 h, 12 h, and 24 h with the initial amount at 0 h and 3 h. Low temperature treatment (4 °C) had no significant change of *HvZFHD1* transcripts (Fig. 5). In fact, both high and low temperature had much lower change in *HvZFHD1* transcripts comparing dehydration and salinity treatment. This means that HvZFHD1's regulatory role could be involved in dehydration and salinity more than heat and cold stress. Our results confirm the fact that the regulation of *ZFHD* transcription is associated with stress conditions. For instance, zinc finger of type C2H2 in Arabidopsis has been reported to play a responsive role to drought, high-salinity, and cold conditions as transcription repressors (Sakamoto *et al*., 2004).

Here we report identification and characterization of a novel ZFHD from barley termed *HvZFHD1*. The transcriptional analysis of *HvZFHD1* showed that it is differentially regulated during spike development and up-regulated with hormonal treatments of ABA, MeJA, SA, and ET. Moreover, *HvZFHD1* is up-regulated under drought, high salinity, and high temperature conditions. This correlation of up-regulation of *HvZFHD1* under hormonal, abiotic stress conditions, and spike development indicates that *HvZFHD1* has multiple regulatory roles in barley.

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