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ORIGINAL PAPER

Expression analysis of dehydrin multigene family across tolerant and susceptible barley (*Hordeum vulgare* L.) genotypes in response to terminal drought stress

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Abstract Dehydrins are one of the characteristic families of plant proteins that usually accumulate in response to drought. In the present study, gene expressions of dehydrin multigene family (13 genes) were examined in flag leaves of tolerant (Yousef) and susceptible (Moroco) barley varieties under terminal drought to characterize the involvement of dehydrins in the adaptive processes. The stomatal conductance, RWC, and Chl *a, b* contents had more reduction in Moroco than the Yousef which has more elevated osmotic adjustment. Drought stress increased significantly MDA and electrolyte leakage levels, but greater in Moroco, indicating a poor protection of cell and cytoplasmic membrane in this variety. Yousef variety had no reduction in grain yield under drought condition. Five genes (*Dhn1*, *Dhn3*, *Dhn5*, *Dhn7* and *Dhn9*) were exclusively induced in Yousef under drought stress. In the stress condition, relative gene expression of *Dhn3*, *Dhn9* had the direct correlations ($P < 0.05$) with Chl *a*, *b* contents, osmotic adjustment, stomatal conductance, plant biomass and grain yield, and the negative correlations ($P < 0.05$) with MDA and electrolyte leakage levels. The results supported the impending functional roles of dehydrin K_n and particularly Y_nSK_n types in dehydration tolerance of barley during the reproductive stage.

Keywords Barley · Dehydrin · Gene expression · Osmotic adjustment · Terminal drought stress

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Introduction

Among the environmental stresses limiting the growth and yield of plants, drought is the most important stresses throughout the world (Mahajan and Tuteja 2005) including Iran which is located in an arid and semi-arid region. Iran receives less than one-third of global average precipitation with an average annual precipitation of 250 mm. Terminal drought represents a significant problem especially in temperate regions and water stress at the reproductive stage may cause an important reduction on grain yield (Ceccarelli et al. 2004). Understanding the basic biological mechanisms underlying plant responses to dehydration can be very helpful to increase crop yield under this condition. Drought tolerance is under complex genetic control, and many genes are involved in plant response to water deficit (Cattivelli et al. 2008).

To alleviate water and/or osmotic stress, plants have adopted strategies by several physiological and biochemical alterations reflected at the level of gene expression. Plants normally present an increased expression of protective proteins, such as chaperons, late embryogenesis abundant (LEA) proteins and several proteins implicating in osmotic adjustment (OA) and repair systems (Brini et al. 2007).

Dehydrins (DHNs; LEA D11 protein family) are part of a large family of highly hydrophilic proteins known as LEA. DHNs accumulate to high levels throughout late stages of seed development and in vegetative tissues subjected to water shortage, salinity, low temperature, or abscisic acid (ABA) treatment. These typical stress-induced proteins in plants are suggested to play an important role in plant stress tolerance (Svensson et al. 2002).

Dehydrins are present in almost all vegetative tissues through normal and certain stress conditions (Rorat et al. 2006; Rodríguez et al. 2005). The differences in their temporal and local expression suggest that individual members of *Dhn* multigene family have fairly diverse biological functions (Koag et al. 2009). However, the overexpression of a single DHN protein has not generally been adequate to give stress tolerance (Puhakainen et al. 2004). Dehydrins enclose several special sequence motifs including a conserved, Lysine-rich 15-amino acid domain, EKKGIMDKEKLPG, named the K-segment, which is usually present near the C-terminus. Other distinctive dehydrin elements include a track of Ser residues (the S-segment), a consensus motif, T/VDEYGNP (the Y-segment), situated close to the N-terminus and less conserved regions, usually rich in polar amino acids (the Φ-segments) (Close 2006). On the basis of amino acid sequence similarity, as well as the number and order of the Y-, S- and K-segments, DHNs can be divided into five sub-classes; namely, Y_nSK_n, Y_nK_n, SK_n, K_n, and K_nS (Rorat et al. 2006). Based on the protein isoelectric point, dehydrins are divided into acidic and basic types. The *Dhn* genes that encode YSK_2-type dehydrins are commonly basic and upregulated by dehydration and ABA, but not by low temperature (Choi et al. 1999; Tommasini et al. 2008).

Barley (*Hordeum vulgare* L.) is one of the most important crops in arid and semiarid regions, as it is relatively resistant to drought and salinity and requires less water as compared to wheat and corn. Barley is also a major crop in local cropping systems in Iran. About 1.7 million hectare of the farming areas in Iran is under barley cultivation that contributes 56 % of rain fed area (Bannayan et al. 2010). Furthermore, barley is considered as a model species for genetic and physiological studies. In fact, 13 *Dhn* genes have been identified in barley and their expressions have been investigated in response to drought, salinity and low temperature (Choi et al. 1999; Tommasini et al. 2008). Three genes encoding low-molecular-weight DHNs (*Dhn1*, *Dhn2* and *Dhn9*) were found within barley chromosome 5H overlapping a quantitative trait locus for winter hardiness. Other *Dhn* genes encoding low and high-molecular-weight DHNs are located on chromosome 3H, 4H and 6H (Choi et al. 1999). However, comparative differential expressions of dehydrin genes at the reproductive stage between drought tolerant and susceptible barley genotypes under drought stress have not been examined. The data could be important in determining the candidate genes for drought tolerance used as a marker for crop breeding.

Materials and methods

Plant materials and experimental treatment

Seeds of spring barley (*H. vulgare* L.) cv Yousef and Moroco 9–75 were obtained from Seed and Plant Improvement Institute of Iran (SPII) and from Dr. Stefania Grando (International Center for Agricultural Research in the Dry Area, ICARDA), respectively. The experiment conducted in randomized complete block design with two treatments (well-watered and drought-stressed) and three replications under field conditions in a greenhouse as shelter at the Agricultural Biotechnology Research Institute of Iran (ABRII) in Karaj. The seeds were planted in two plots with 5 cm distance in line and 10 cm between the lines. Each genotype was planted in three lines giving a total of 120 plants for each replication. Drip irrigation was performed for both plots. Plants were grown in the same conditions until anthesis, under well-watered conditions. The drought treatment was started by withholding water at the anthesis. The soil moisture for the plots of the well-watered (at soil field capacity, FC) and drought-stressed conditions was maintained with the required amounts of water by digital moisture meter daily. Twenty-eight days after anthesis (when the soil moisture for the plots reached at 0.4 of FC with a water potential of −1.5 MPa), the flag leaves were sampled, rapidly frozen in liquid nitrogen, and stored at −80 °C until use.

Measurement of physiological traits, grain yield and biomass

Flag leaves were removed from the main stem of the stressed and control plants, and physiological traits were measured in these leaves. The grain yield and the number of seeds were determined and analyzed when grains were mature in both drought–stress and control conditions. The total biomass for each genotype was measured with weighing the 15 plants in replications.

Leaf relative water content (RWC) and stomatal conductivity measurement

Relative water content was calculated using the following formula:

$$RWC\% = (FW - DW) / (TW - DW) \times 100$$

where FW is fresh leaf weight, TW is turgid leaf weight and DW is dry leaf weight, respectively.

Stomatal conductivity of four flag leaves was determined using a Porometer (Delta-T AP4, Delta-T Devices, Cambridge, UK) between 8 and 9 a.m.

Chlorophylls a, b content, malondialdehyde content and electrolyte leakage determination

For determining chlorophylls, leaves were homogenized in ice-cold 80 % (v/v%) acetone, and extracted. Samples were centrifuged at 5,000*g* for 15 min at 4 °C. The pellet was extracted again with 80 % (v/v%) acetone. The supernatants were collected after centrifugation (5,000*g*, 15 min, 4 °C). The pigment composition was measured by a double-beam spectrophotometer (UV-Visible Spectrophotometer CARY 300 Scan).

Malondialdehyde (MDA) formation was assayed using a thiobarbituric acid method (Ederli et al. 1997). About 100 mg of fresh tissue were ground in 1 ml of chilled reagent [0.25 % (w/v) thiobarbituric acid in 10 % (w/v) TCA]. After incubation at 95 °C for 20 min, extracts were cooled at room temperature and then centrifuged. Thiobarbituric acid reactivity was determined in the supernatant by measuring the A_{532}. Nonspecific absorbance was determined at 600 nm.

For electrolyte leakage, out of several leaves, 15 leaf discs (6 mm in diameter) were cut off. The leaf discs were transferred to the tubes containing 15 ml of distilled water in room temperature. After 24 h, the electrical conductivity was measured ($r1$, representing the ion leakage from the leaf discs). Then, the solutions were autoclaved (to achieve the total ions) and after cooling down the liquid, the electrical conductivity was measured ($r2$). Electrolyte leakage is represented as the percentage of total ions released ($\frac{r1}{r2} \times 100$) (Habibi 2012).

Osmotic adjustment (OA) determination

Detached leaves were rehydrated in distilled water for 24 h, wrapped in polyethylene strips and then frozen at −20 °C. After thawing at room temperature (≈15 min), the cell sap was extracted and the osmotic potential (Ψs) of the extracted sap was determined using a vapor pressure osmometer (model 5520 XR, Wescor, Inc., Logan, UT, USA), as osmotic potential at full turgor (Ψs100). OA was assumed to be the difference between Ψs100 of stressed and unstressed plants (Fischer et al. 2005).

RNA extraction and cDNA synthesis

Total RNA was extracted from the barley genotypes flag leaves using TRIzole reagent following the manufacturer's protocol (Invitrogen, Karlsruhe, Germany) and quantified spectrophotometrically. Three biological replicates were performed. To remove contaminating DNA, RNA samples were treated with DNase1 (RQ1 RNase-free DNase) Kit (Promega Corporation, Madison, WI). The cDNA was synthesized using iScript cDNA Synthesis Kit (Bio-Rad Laboratories Inc., Hercules, California, USA).

Real-time PCR

The specific primer pairs were designed for barley β-actin gene (as an internal control) and the 13 *Dhn* genes based on the derived sequences from NCBI (Table 1). Primers were selected using Oligo v.5.1 software and were prepared commercially (MWG, Ebersberg, Germany). The real time-PCR reaction was performed in 25 μL containing 12.5 μL 2 × SYBR Green Mastermix (Applied Biosystems), 0.5 μL of each primer and 1 μL of cDNA. The PCR reactions were run in iCycler iQ Real Time PCR Detection System (BioRad). Three biological replicates were examined. β-actin, as constitutively expressed gene, was used as internal control for normalization. Quantifying the relative changes in gene expression was performed using the $2^{-\Delta\Delta CT}$ method (Pfaffl 2001).

Phylogenetic trees analysis

The protein sequences of the 13 barley *Dhn* genes were driven from NCBI. Sequence alignments and tree drawings were conducted by CLUSTALW and MEGA 5.1 (beta #3) software (Arizona State University, Tempe, USA). *Dhn* sequences downloaded from the National Center for Biotechnology Information (NCBI). The nucleotide sequences of barley *Dhn* genes and corresponding peptide sequences which used for analysis are presented in FASTA format files (S1, S2).

Statistical analysis

The analysis of variance (ANOVA) and the Duncan's multiple range test (DMRT) at $P \leq 0.05$ were performed by SAS and MSTATC software to determine the significance of the variations on the groups under different treatments.

Table 1 Primers used in quantitative real-time PCR

Target gene	Locus or accession no.	Forward primer	Reverse primer
Dhn1	AF043087	5' GCACTTCTCTCCGTCGCAGT 3'	5' CATTGTGGTGCTACGAAGTAC 3'
Dhn2	AF043088	5' CACACTTACACAGCCATACAC 3'	5' CCGTAGTCCTCCACCTTGTC 3'
Dhn3	AF043089	5' GCAACCAAGATCAACACCACC 3'	5' CTTGTGCTCCTCCCTCGTGG 3'
Dhn4	AF043090	5' AGGGACAGCAGCAGCAGCA 3'	5' TCTGGTGCTCGTCCCTCATG 3'
Dhn5	AF043096	5' TACGGGCAGCAGGCACAG 3'	5' GCAGCTTGTCCTTGATCTTG 3'
Dhn6	AF043091	5' GGCATCCGCTTGACATTGAC 3'	5' GCCATCTCTTCGCTTCACGG 3'
Dhn7	AF043092	5' TTACACAACACAGCCACCAGT 3'	5' TGCTGCTGTCCCTGGTACTC 3'
Dhn8	AF043093	5' GCTTGGGCACCTTCATCATTCA 3'	5' GTATGACTGGGTGCTCCTCTC 3'
Dhn9	AF043094	5' GTAGCAGGTAAGATGGAGTTC 3'	5' GTCTTGTGCTCCTCCCTGC 3'
Dhn10	AF043095	5' GAGGCAGCAAGATGGAATACC 3'	5' GGTCTTGTGCTCCTCCCTGC 3'
Dhn11	AF043086	5' CAACCAAAAGCACGAGGAGCA 3'	5' ATACCCTTCTTCTCGTGCGTC 3'
Dhn12	AF155129	5' GGAGACTACGGGCAGCAAG 3'	5' CTTGATCTTGTCCACGACTC 3'
Dhn13	AY681974	5' CCATTTCTTCAGACACCCTC 3'	5' GCTAATGGGATGGGATGATG 3'

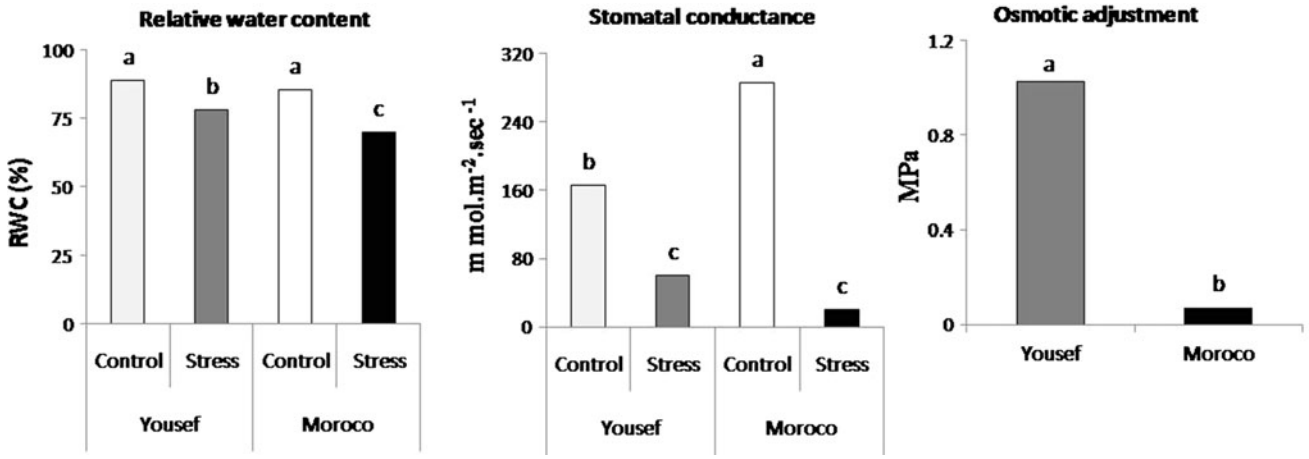

Fig. 1 The effects of water stress on stomatal conductivity, RWC and osmotic adjustment of tolerant (*Yousef*) and susceptible (*Moroco*) barley varieties under well-watered condition (soil water at field capacity) and terminal drought conditions on 28th day after stress. The drought treatment was started by withholding water at the anthesis. *Means* in each *column* followed by *similar letter(s)* are not significantly different at $P \leq 0.05$, using DMRT test

Results

Physiological responses of the two barley species to stresses

The leaf RWC, stomatal conductance, Chl *a*, *b* contents, total biomass and grain yield decreased under stress conditions (Figs. 1, 2). The RWC, stomatal conductance and Chl *a, b* contents had more reduction in Moroco variety as compared to the tolerant Yousef variety with elevated OA. On day 28 of drought stress, Moroco showed a sharp drop in stomatal conductance as compared to the irrigated plants (286–20 mmol m^{-2} s^{-1}), whereas in Yousef, stomatal conductance decreased only 165–60 mmol m^{-2} s^{-1} (Fig. 1).

However, the changing ratios of these physiological parameters in Moroco were always higher than those in Yousef genotype, suggesting a better dehydration tolerance in Yousef. As it will be discussed later, this can be attributed to the fact that a reduction in relative leaf water content may result in loss of turgor, which leads to reduced CO_2 uptake, hence an increase in oxidative stress (Acar et al. 2001).

Drought stress increased MDA and electrolyte leakage levels, but the increase was more pronounced in Moroco genotype (Fig. 2), indicating that cells of this variety damaged more than Yousef genotype. Ion leakage occurs well before programmed cell death is visible (Brini et al. 2007).

In Moroco, some differences were observed in grain yield (Fig. 2) and seed number per spike per m^2 (data not shown) in well-watered conditions and under water stress (Fig. S1), but Yousef showed no significant difference in

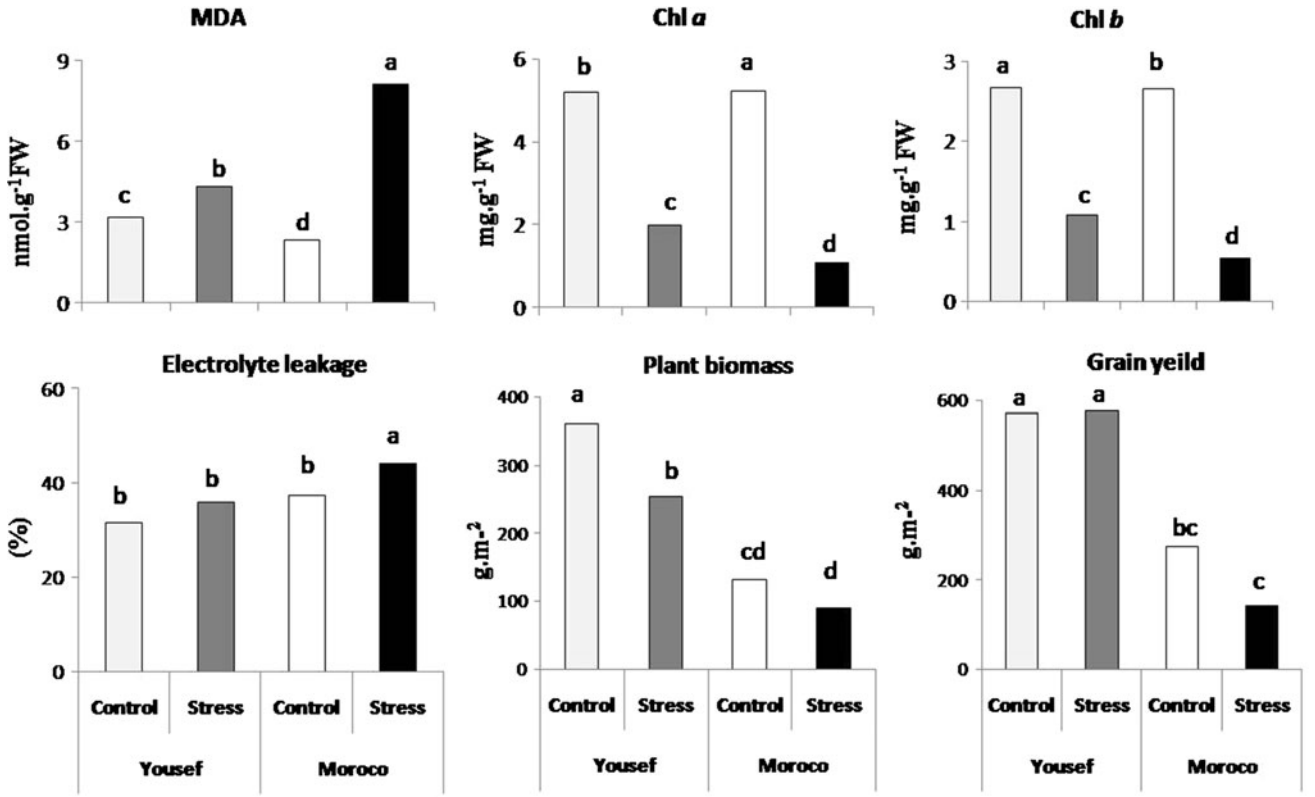

Fig. 2 The effects of water stress on chlorophyll *a* and *b* content, lipid peroxidation as MDA level, electrolyte leakage on 28th day after stress, and grain yield, and total biomass of tolerant (*Yousef*) and susceptible (*Moroco*) barley varieties under well-watered condition (soil water at field capacity) and terminal drought conditions. The drought treatment was started by withholding water at the anthesis. *Means* in each *column* followed by *similar letter*(s) are not significantly different at $P \leq 0.05$, using DMRT test

these two traits in both well-watered conditions and under water stress (Figs. 2, S1, S2). Both of the varieties had the same total biomass reduction in drought condition (Fig. 2).

Comparison of dehydrin expression in flag leaves under water stress

To determine the putative roles of dehydrin genes in drought stress tolerance of barley, differential expression of this multigene family (13 members) was investigated in tolerant and susceptible barley varieties under terminal drought stress. Barley cv Yousef is improved and cultivated in temperate zones of Iran and is well adapted to drought stress (Abedini et al. 2012) and Moroco 9–75 is considered to be susceptible to drought stress (Ceccarelli et al. 2004).

Flag leaves were sampled 28 days after anthesis and withholding water. Among the 13 genes, five genes (*Dhn1*, *Dhn3*, *Dhn5*, *Dhn7* and *Dhn9*) were exclusively induced in Yousef under drought stress (Fig. 3). The other genes of *Dhn* multigene family did not show significant changes at this point of time (e.g., *Dhn13,* as shown in Fig. 3); they may not be directly responsible for drought tolerance. It is worthy to mention that, among 13 barley dehydrin genes, only two genes (*Dhn3* and *Dhn5*) were induced in the tolerant genotype earlier and just 21 days after anthesis and withholding water (data not shown).

In the stress condition, significant direct correlations ($P < 0.05$) were found (Table 2) between relative gene expression of *Dhn3*, *Dhn9* and Chl *a* (+0.91 and +0.90, respectively), *b* contents (+0.86 and +0.85, respectively), osmotic adjustment (+0.97 and +0.96, respectively), stomatal conductance (+0.86 and +0.88, respectively), plant biomass (+0.93 and +0.87, respectively), and grain yield (+0.91 and +0.86, respectively). On the other hand, significant negative correlations ($P < 0.05$) were obtained between relative gene expression of *Dhn3*, *Dhn9* and MDA −0.87 and −0.88, respectively), and electrolyte leakage levels (−0.87 and −0.81, respectively; Table 2). In addition, there were significant positive correlations ($P < 0.05$) between relative gene expression of *Dhn1* and OA (+0.84), stomatal conductivity (+0.87) and Chl *a* contents (+0.81). Significant negative correlations ($P < 0.05$) were obtained between relative gene expression of *Dhn1* and MDA (−0.85; Table 2).

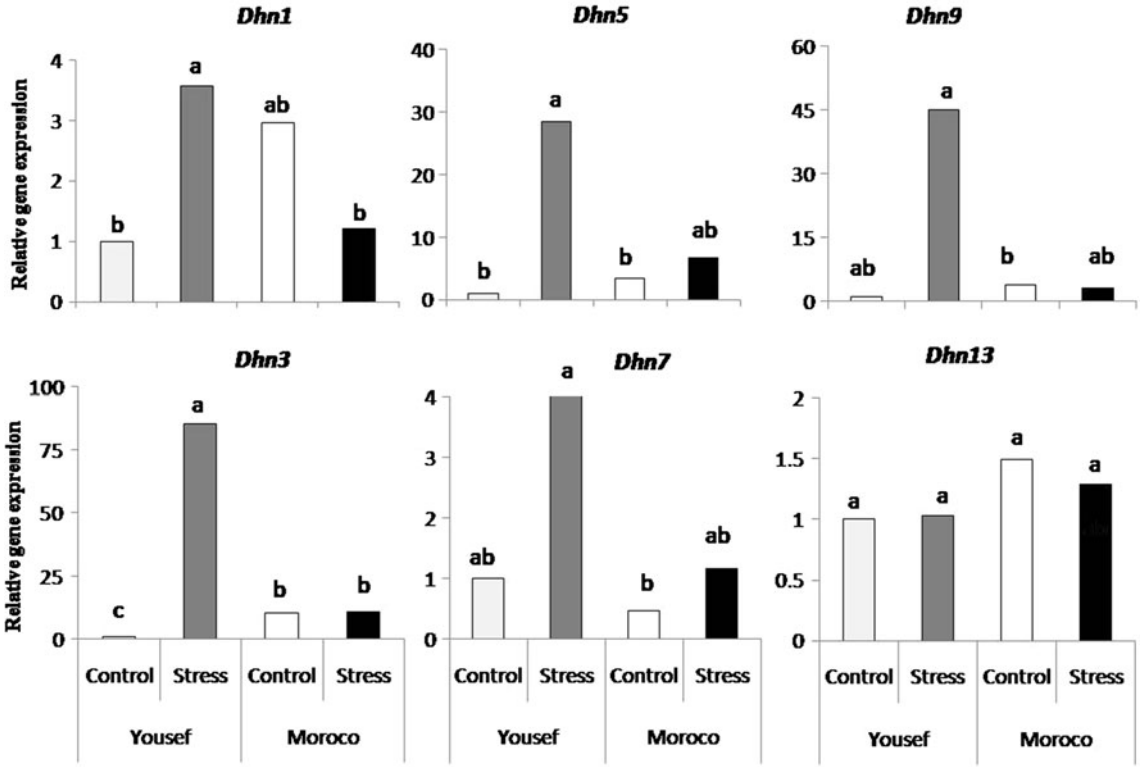

Fig. 3 Quantitative real-time PCR analysis of *Dhn1*, *Dhn3*, *Dhn5*, *Dhn7, Dhn9* and *Dhn13* transcripts in tolerant (*Yousef*) and susceptible (*Moroco*) barley genotypes under well-watered condition (soil water at field capacity) and terminal drought conditions. Using actin as a reference gene, the gene expression ratios, which were calculated relative to the expression in Yousef control condition. *Means* in each *column* followed by *similar letter*(s) are not significantly different at $P \leq 0.05$, using DMRT test

Table 2 Correlations between physiological traits, total plant biomass, grain yield and relative expression of the genes (*Dhn1, Dhn3, Dhn5, Dhn7* and *Dhn9*) under stress conditions

Gene	RWC	gs	OA	EL	MDA	Chl *a*	Chl *b*	PB	GY
Dhn1	0.669	0.868*	0.842*	−0.540	−0.851*	0.809*	0.786	0.653	0.697
Dhn3	0.773	0.864*	0.973**	−0.874*	−0.873*	0.904*	0.863*	0.930**	0.907*
Dhn5	0.277	0.650	0.541	−0.161	−0.561	0.596	0.609	0.281	0.387
Dhn7	0.210	0.624	0.463	−0.031	−0.505	0.586	0.624	0.195	0.349
Dhn9	0.755*	0.876*	0.959**	−0.812*	−0.882*	0.886*	0.845*	0.874*	0.861*

RWC relative water content; *gs* stomatal conductance; *OA* osmotic adjustment; *EL* electrolyte leakage; *MDA* malondialdehyde; *Chl* chlorophyll; *PB* plant biomass; *GY* grain yield

* $P < 0.05$ and ** $P < 0.01$, respectively

Phylogenetic tree and protein sequence analysis

Phylogenetic and protein sequence analysis of barley dehydrins revealed that they can be categorized in four subclasses out of five plant DHN subclasses and of 13 barley DHNs, ten DHNs belong to subclass Y_nSK_n (Fig. 4).

Under drought stress, among the 13 genes, five *Dhn* genes were solely induced in drought-tolerant variety, Yousef, whose corresponding proteins are fit in two subclasses YSK_2 (*Dhn1*, *Dhn3*, *Dhn7* and *Dhn9*) and K_9 (*Dhn5*) (Fig. 3).

Discussion

Dehydrins, group 2 of LEA proteins that are suggested to play an important role in plant stress tolerance, accumulate in many plant species in response to abiotic stresses, particularly drought, salinity and low temperature (Hu et al. 2010). There are many evidences that drought tolerance in barley is highly correlated with the expression of *H. vulgare* aleurone 1 (HVA1) which encodes another LEA protein. However, it seems to be possible that in drought tolerant genotypes, HVA1 are not only more expressed

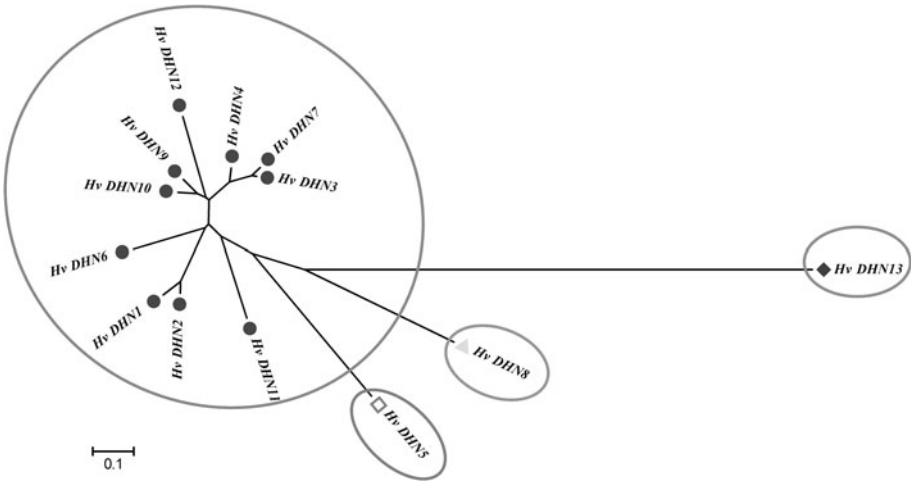

Fig. 4 Sequence analysis and similarity tree showing the relationships between barley dehydrins. Alignments of the deduced amino acid sequences were performed by CLUSTALW. The tree was generated by the neighbor-joining method with Poisson correction using Mega 5 software

during drought but tolerant genotypes are more sensitive to various unidentified internal signals confirming environmental water deficit (Wójcik-Jagła et al. 2012). The HVA1 gene from barley was used to obtain transgenic plants of rice, wheat and oat, also confirming their increased drought tolerance under field conditions (Wójcik-Jagła et al. 2012).

Comparative studies about expression of 5 dehydrins in Arabidopsis (Nylander et al. 2001) and transcriptome-based analysis of barley cv. Morex (Tommasini et al. 2008) under environmental stresses have been well performed. The accurate function of dehydrins *in planta* has not been well known, but in vitro findings have shown that each dehydrin type could have a specific function (Brini et al. 2010; Choi et al. 1999).

In this study, we examined the differential expressions of dehydrin genes at the reproductive stage between drought tolerant (Yousef) and susceptible (Moroco) barley genotypes under drought stress and normal condition in a controlled environment. Amongst five genes which were exclusively and strongly induced in Yousef (Fig. 3), *Dhn1*, *Dhn3*, *Dhn7* and *Dhn9* (YSK_n type) are drought/ABA-inducible genes and *Dhn5* (K_n type) is a drought/cold/ABA-inducible gene (Choi et al. 1999). These genes were considerably induced in association with induction of drought tolerance in Yousef interpreted as higher RWC and stomatal conductivity and better OA (Fig. 1). These results are consistent with those of (Nylander et al. 2001), who concluded DHNs may act as water attractants in cells with low water potential, having a role in osmotic potential regulation in Arabidopsis. Brini et al. (2007) reported the overexpression of *Dhn5* in Arabidopsis resulted in a faster recovery than wild type to drought stress which was attributed to an increase in endogenous proline. Also here, the level of osmotic potential regulation of Yousef was higher (Fig. 1) and proline, an osmolyte that is known to contribute to OA, has a higher accumulation than in Moroco (data not shown). The physiological function of DHN-5 at a molecular level has not been identified, but the drought stress tolerance mechanisms could be due to the developed OA (Brini et al. 2007). However, another important role of *Dhn5* in tolerance to cold stress was shown in wheat (reviewed in (Brini et al. 2010). Dehydrin type Kn, represented by barley DHN-5 (K_9) (Choi et al. 1999) and the wheat WCS120 family (K_6), which is an ortholog of barley *Dhn5* (Brini et al. 2010) was confirmed to be accumulated mainly in response to cold stress. There is some evidence showing that DHN-5 is able to preserve enzyme activities in vitro from adverse effects induced by heating (Brini et al. 2010).

Our results revealed that the cells of Moroco received more damages than Yousef, i.e., MDA and electrolyte leakage levels increased more in Moroco (Fig. 2). In addition, Chl *a, b* contents had more reduction in this variety (Fig. 2). Another proposed function of DHNs is stabilizing protein and membrane structures by surfactant or chaperon-like activities (Du et al. 2011; Rorat et al. 2006). In vitro experiments showed that DHNs YSKn- type (maize YSK_2 DHN1) bind to lipid vesicles that contain acidic phospholipids, and the binding is more favorable to vesicles of smaller diameter prepared from negatively charged phospholipids. Upon binding to PA-derived lipid vesicles, the DHN1 adopts an α-helical structure. The two K-segments present in the protein might be involved in membrane binding. The increase in α-helicity of the DHN1 when bound to phospholipid vesicles in vitro may suggest that the DHN1 also takes on α-helical structures when associated with vesicles in vivo. The preference of DHN1 to bind PA-derived phospholipids is intriguing, as PA is a minor lipid fraction in plant cells (1–2 % of the total lipids), but its levels typically increase with the activation of phospholipase D activity in response to abiotic stresses, including drought (Koag et al. 2009).

The measurement of MDA level as a secondary end product of the oxidation of polyunsaturated fatty acids is

routinely used as an index of lipid peroxidation under stress conditions (Sun et al. 2006). MDA levels increased more significantly in Moroco, as a susceptible barley variety in comparison to Yousef (Fig. 2). It was demonstrated an antioxidative activity for citrus DHN proteins (CuCOR19), and such antioxidative activity of dehydrin is crucial for plants to adapt to a wide range of abiotic stresses such as cold, drought or salinity (Hara et al. 2004).

In the present study, four of drought induced *Dhn* genes in drought-tolerant variety under drought stress, belonged to DHN YSKn-type. Ten out of 13 barley DHN proteins belong to subclass Y_nSK_n (Fig. 4), and as barley is one the most drought tolerant crops, this suggests an important role of these DHNs in dehydration tolerance. Based on our results, the key role of YSK_n DHN type in stability of plant membrane is very prominent. Terminal drought did not induce dehydrin genes expression in Moroco as a susceptible barley cultivar. Moreover, negative and significant correlations ($P < 0.05$) were obtained between relative gene expression of *Dhn3* and *Dhn9* genes and MDA and electrolyte leakage levels. The results support the impending functional roles of these genes in dehydration tolerance in barley during the reproductive stage.

Author contribution Conceived and designed the experiments: M. Shahbazi, V. Niknam, Z. S. Shobbar, H. Ebrahimzadeh Mabood. Performed the experiments: M. Shahbazi, A. Karami, R. S. Tafreshi, R. Abedini. Analyzed the data: M. Shahbazi, A. Karami, R. S. Tafreshi, Z. S. Shobbar. Wrote and revised paper: M. Shahbazi, Z. S. Shobbar and A. Karami.

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