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ORIGINAL ARTICLE

Identification and transcriptional analysis of dehydrin gene family in cucumber (*Cucumis sativus*)

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Abstract

Dehydrins (DHNs) are a group II late embryogenesis abundant (LEA) proteins that play essential roles in plant growth, development and responses to diverse environmental stimuli. Here, four DHNs in cucumber genome were identified using bioinformatics-based methods according to the highly conserved K-, Y- and S-segments, including 1 Y_nK_n-type, 2 Y_nSK_n-type, and 1 SK_n-type DHNs. All of them are intrinsically disordered proteins (IDPs) and possess a large number of disorder-promoting amino acids. Secondary structure prediction revealed that each of them is composed of high proportion of alpha helix and random coil. Gene structure and phylogenetic analyses with DHNs from cucumber and several other species revealed that some closely related *DHN* genes had similar gene structures. A number of *cis*-elements involved in stress responses and phytohormones were found in each *CsDHN* promoter. The tissue expression profiles suggested that the *CsDHN* genes have overlapping, but different expression patterns. qRT-PCR results showed that three selected *CsDHN* genes could respond to heat, cold, osmotic and salt stresses, as well as to signaling molecules such as H_2O_2 and ABA. These results lay a solid foundation for future functional investigation of the cucumber dehydrin gene family in tissue development and stress responses in plants.

Keywords Cucumber · Dehydrin (DHN) · Gene family · Intrinsically disordered protein (IDP) · Expression pattern · Abiotic stress

Introduction

Plants are frequently challenged by abiotic stresses such as heat, cold, drought, and high salinity, which cause impaired growth and development of plants. To deal with these stresses, numerous stress-related genes were induced in plants, leading to physiological and metabolic changes that improve stress tolerance (Shinozaki et al. 2003; Zhou et al. 2017b). Among them, late embryogenesis abundant (LEA) proteins, together with osmotin, chaperones, and detoxification enzymes, play vital roles in abiotic stress tolerance (Cao et al. 2017; Chen et al. 2015).

Dehydrins (DHNs) are a group of highly hydrophilic proteins that belong to the group II of LEA with low molecular weights from 10 to 70 kDa, and possess high contents of Gly, Ala and Ser, and large net charge relative to non-polar hydrophobic residues (Charfeddine et al. 2017). Due to their unified and outstanding characteristics, DHNs are universally categorized as a structural family of proteins which are known as intrinsically disordered proteins (IDPs) (Tompa 2002) without a defined 3D structure, and are highly dynamic to form a lot of different structures (Malik et al. 2017). DHNs have three categories of conserved motifs named as K-, Y-, and S-segments. Usually, the K-segment is located near the C-terminus with a typical sequence EKKGIMDIKEKLPG, which was predicted to form an

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amphipathic helix and to be associated with the functions dehydrin, and it is a common feature of all DHNs (Hara et al. 2017; Koag et al. 2009) except for several members (Perdiguero et al. 2014; Verma et al. 2017). Different DHNs may occasionally have some single nucleotide substitutions or structural modifications in the K-segment (Graether and Boddington 2014). The other two conserved motifs have been termed as the Y-segment and S-segment, which consist of the sequences of DEYGNP and LHRSGSSSSSSSEDD (or related sequences), respectively (Graether and Boddington 2014; Malik et al. 2017). Unlike K-segment, Y-segment and S-segment are not necessarily present in all DHNs. According to the presence and number of Y-, S- and K-segments, DHNs can be divided into five major types: Y_nSK_n, K_n, SK_n, Y_nK_n, and K_nS (Close 1996; Lv et al. 2017), and an intermediate type S_nKS (Abedini et al. 2017).

Many studies have reported that various abiotic stresses can induce the expression of *DHN* genes, such as cold, drought, and salinity (Abedini et al. 2017; Charfeddine et al. 2017; Jing et al. 2016; Verma et al. 2017). It is worth noting that many *DHN* genes are only responsive to one or several abiotic stresses, suggesting that different mechanisms have been developed in plants to adapt to low temperature, drought, and salinity (Malik et al. 2017). For example, in apple, *MdDHN1, MdDHN2, MdDHN4, MdDHN5*, and *MdDHN6* were obviously induced under drought conditions, while *MdDHN2, MdDHN4*, and *MdDHN6* were also significantly up-regulated by low temperature (Liang et al. 2012). Many studies showed that the expression levels of SK_n-, Y_nK_n-, and K_nS-type *DHN* genes are mainly up-regulated by low temperature, while drought or salt exposure promotes the expression of Y_nSK_n-type *DHN* genes (Graether and Boddington 2014; Rorat 2006). These findings suggest that DHN proteins and abiotic stress tolerance are positively correlated.

A number of studies have revealed that DHNs can protect cells from dehydration or cryoprotection and participate in responses to various abiotic stresses, and their overexpression can enhance stress tolerance of plants. For example, transgenic *Arabidopsis* plants overexpressing *DHN* genes from *Arabidopsis* or other plant species showed tolerance to various abiotic stresses, such as cold (Aguayo et al. 2016; Peng et al. 2008), salinity (Brini et al. 2007; Saibi et al. 2015), and drought (Chiappetta et al. 2015), or multiple stresses (Cao et al. 2017; Munoz-Mayor et al. 2012). *Arabidopsis* plants expressing *Rab17* from maize (Figueras et al. 2004), *DHN-5* from wheat (Brini et al. 2007), *HbDHN1* and *HbDHN2* from *Hevea brasiliensis* (Cao et al. 2017) also exhibited higher osmotic stress tolerance. Improved stress tolerance was also observed in cucumber plants with overexpression of *DHN24* from *Solanum sogarandinum* (Yin et al. 2006), strawberry plants with overexpression of wheat *Wcor410a* (Houde et al. 2004), banana plants with constitutive overexpression of *MusaDHN-1* (Shekhawat et al. 2011), rice plants overexpressing *OsDhn1* (Kumar et al. 2014), tobacco plants with overexpression of *CarDHN* (Hill et al. 2016), *SiDHN* (Guo et al. 2017), *CdDHN4* (Lv et al. 2017), and *SbDhn1* (Halder et al. 2017), and so on. These results indicate the potential roles of DHNs in stress tolerance.

A previous research that analyzed cucumber *CsLEA* gene family suggested that one *DHN* gene named as *CsLEA54* can be induced under drought stress conditions in leaf and root tissues (Altunoglu et al. 2016). Another *DHN* gene named as *CsLEA11* could be induced by heat and cold stress, and heterologous expression of *CsLEA11* in *E. coli* displayed an enhanced tolerance to heat and cold stress (Zhou et al. 2017a). However, these results are not enough for understanding the roles of DHNs in the response to various abiotic stresses in cucumber. In this study, four putative DHN family members were identified from cucumber, and their phylogenetic relationships, protein motifs, gene structures, expression profiles in different tissues and under various abiotic stresses were systematically examined. The results lay a foundation for further functional study of *CsDHN* genes, and provide new information for the use of them in future breeding of stress tolerance in cucumber.

Materials and methods

Identification of cucumber *DHN* genes

To predict the *DHN* gene family members in cucumber, BLAST analysis was conducted using the protein sequences of the *DHN* genes of *Arabidopsis* (Hundertmark and Hincha 2008), rice (Verma et al. 2017), and tomato (Cao and Li 2015) as the query sequences against the cucumber genome database from Cucumber Genome Initiative (CuGI, http://cucumber.genomics.org.cn) (Huang et al. 2009). All redundant putative DHN sequences were excluded by aligning to each other, and further confirmation was performed using the Pfam (http://pfam.xfam.org/) (Finn et al. 2016) and Simple Modular Architecture Research Tool (SMART) (http://smart.embl-heidelberg.de) (Letunic and Bork 2018) databases for the existence of a typical dehydrin domain (PF00257) in their protein structures.

Characterization of CsDHN proteins

The disordered regions of the deduced CsDHN proteins were predicted using the protein disorder prediction system (PrDOS) (http://prdos.hgc.jp/cgi-bin/top.cgi) (Ishida and Kinoshita 2007). The theoretical isoelectric point (pI), molecular weight (MW) and the grand average of hydropathy index (GRAVY) of the deduced CsDHN proteins were

calculated using the ProtParam tool (http://web.expasy.org/protparam) (Gasteiger et al. 2005). The secondary structure prediction of each CsDHN protein was carried out by SOPMA tool (Geourjon and Deléage 1995).

Multiple sequence alignment and phylogenetic analysis

The multiple sequence alignments of the full-length protein sequences of CsDHNs and DHN proteins from *Arabidopsis*, tomato, and rice were carried out by ClustalW with default parameters (Larkin et al. 2007). A phylogenetic tree based on the alignment was then constructed using the MEGA 5.0 software by employing the neighbor-joining (NJ) method with 1000 replicates of bootstrap analysis (Tamura et al. 2011).

Chromosomal location, gene duplication, gene structure and promoter region analysis

The chromosomal locations of the *CsDHN* genes were determined with the MapInspect tool as previously described (Hu et al. 2016). Segmental and tandem duplication events were investigated according to the criteria in previous studies (Kong et al. 2013; Liu et al. 2012). The gene structures of *CsDHN* genes were analyzed with the Gene Structure Display Server (http://gsds.cbi.pku.edu.cn) (Hu et al. 2015) by comparing the coding sequence (CDS) with their corresponding genomic DNA (gDNA) sequences retrieved from the cucumber genome database. The putative promoter region (1.0-kb sequence upstream of the ATG start codon) of each *CsDHN* gene was analyzed for the identification of the *cis*-elements using the PlantCARE database (http://bioinformatics.psb.ugent.be/webtools/plantcare/html) (Lescot et al. 2002).

Plant materials and treatments

The seeds of a cucumber inbred line (*Cucumis sativus* L. cv. Chinese long No. 9930) were germinated and grown in the field of Jiangxi Agricultural University as previously described (Zhou et al. 2017c). Different tissues including root, stem, leaf, flower, and fruit were sampled and snap-frozen in liquid nitrogen for storage at − 80 °C before isolation of total RNA.

Two-week-old cucumber seedlings were subjected to different abiotic stresses including heat, salt, cold, abscisic acid (ABA), H_2O_2 and PEG treatments, which were described previously (Zhou et al. 2017b, c). The leaf tissues were harvested at 0, 3 and 12 h after treatment, frozen immediately in liquid nitrogen, and stored at − 80 °C before isolation of total RNA.

RNA extraction, RT-PCR and quantitative RT-PCR (qRT-PCR)

Total RNA of the harvested samples was extracted using Trizol reagent (Tiangen Biotech Co., Ltd. Beijing, China) according to the manufacturer's instruction, and RNA integrity was visualized on a 1% agarose gel. The first-strand cDNA was synthesized according to the manufacturer's instructions (Tiangen Biotech Co., Ltd. Beijing, China). RT-PCR was performed with Taq DNA Polymerase (TaKaRa, Japan), and the thermal cycling procedure was conducted under the following conditions: 94 °C for 5 min, followed by 28–30 cycles of 58 °C for 30 s, 72 °C for 30 s, and 72 °C for 5 min. qRT-PCR was performed in triplicate with SYBR Green Master Mix (Tiangen Biotech Co., Ltd. Beijing, China) on the Light Cycler LC480 system as described previously (Zhou et al. 2017b). The relative expression level of each target gene was determined based on the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen 2001), using an endogenous *actin* gene (*CsActin*) as the reference gene (Zhou et al. 2017a). The primers used for RT-PCR and qRT-PCR are designed and provided in Table S1.

Results

Genome-wide identification of *CsDHNs* in cucumber

To identify the DHN members in cucumber, the sequences of DHN proteins from other species including *Arabidopsis*, tomato, and rice were used to conduct an extensive search of the cucumber database (http://cucumber.genomics.org.cn). The resulting candidate members were further checked for the presence of dehydrin domain using Pfam and SMART tools. As a result, a total of four candidate *DHN* genes were identified (Table 1), which were named as *CsDHN1–CsDHN4* according to their chromosome locations (Table 1). Among them, *CsDHN2, CsDHN3* and *CsDHN4* genes were previously designated as *CsLEA31, CsLEA54*, and *CsLEA11*, respectively (Altunoglu et al. 2016).

Characterization and comparison of deduced DHN proteins in cucumber

CsDHN genes exhibited gDNA lengths varying from 836 bp (*CsDHN2*) to 3175 bp (*CsDHN1*), and encode putative polypeptides with lengths of 162–255 amino acids. CsDHN4 and CsDHN1 showed the smallest (17.47 kD) and largest (29.32 kD) molecular weight of the encoded proteins. GRAVY analysis revealed that the predicted CsDHN proteins were highly hydrophilic,

Table 1 Basic information of four *CsDHN* genes and their encoded proteins in cucumber

Isoforms	Gene ID	Chr location	CuGI (5′–3′)	gDNA (bp)	Intron number	Length (aa)	MW (kDa)	pI	GRAVY	Alpha helix (%)	Extended strand (%)	Beta turn (%)	Random coil (%)
CsDHN1	Csa016162	4	6312049–6315223	3175	1	255	29.32	8.50	–0.982	40.39	12.94	7.84	38.82
CsDHN2	Csa016163	4	6316948–6317783	836	1	177	19.18	7.26	–1.383	17.51	13.56	12.99	55.93
CsDHN3	Csa013074	6	14084808–14085996	1189	1	236	26.63	5.17	–1.525	54.24	3.39	5.51	36.86
CsDHN4	Csa020767	scaffold000176	103088–101475	1614	1	162	17.47	8.11	–1.230	13.58	16.67	11.73	58.02

with GRAVY values ranging from − 1.525 (CsDHN3) to − 0.982 (CsDHN1). Among the four proteins, CsDHN1, CsDHN2 and CsDHN4 were basic proteins, and CsDHN3 was an acidic protein (Table 1).

DHNs are rich of glycine (Gly) and polar amino acids, but are lack of cysteine (Cys) and tryptophan (Trp) (Jing et al. 2016). Similarly, four CsDHNs also show such characteristics, except for CsDHN1 and CsDHN3. CsDHN1 possesses 1 Cys (0.4%), 1 Trp (0.4%), 16 Gly (6.3%), 37 negatively charged residues including Asp (6.3%) and Glu (8.2%), 39 positively charged residues including Arg (7.8%) and Lys (7.5%). CsDHN2 harbors no Cys or Trp, with much higher Gly (20.3%), and high percentages of charged and polar amino acids such as Asp (4.5%), Glu (8.5%), Arg (6.2%), Lys (6.8%), Thr (9.6%), and Ser (6.8%). One Cys (0.4%) was also found in the CsDHN4 protein sequence, and the CsDHN3 protein was rich of polar amino acids including Glu (24.2%), Lys (18.2%), Ala (7.2%), Gly (6.8%), Ser (5.9%) and Asp (4.2%). No Cys or Trp was detected in the CsDHN4 protein sequence, and this protein possessed high percentages of polar amino acids such as Thr (13.0%), Gly (10.5%), Ser (9.3%), and Glu (8.6%). CsDHNs had a large number of disorder-promoting amino acids such as Glu, Gly, Lys, Ala, Thr and Ser (Fig. 1), suggesting that they are IDPs.

The DHN proteins occurring as IDPs in aqueous solutions could form randomly coiled unordered structures, which can maintain the large amount of hydrogen bonds with water molecules and the small percentage of intramolecular hydrogen bonds (Banerjee and Roychoudhury 2016; Tompa et al. 2005). In addition, K-segments have been proposed to resemble a class "A" amphipathic alpha helix, which thus enhances their amphipathic character in the interactions between protein and protein or protein and biomembrane (Abedini et al. 2017; Hanin et al. 2011; Koag et al. 2009). We then analyzed the secondary structure of CsDHN proteins by SOPMA tool. The CsDHN1 protein was composed by 40.39% of alpha helix, 12.94% of extended strand, 7.84% of beta turn, and 38.82% of random coil (Table 1; Fig. 2). Compared with CsDHN1, CsDHN3 had a larger amount of alpha helices (54.24%) and a relatively smaller amount of random coils (36.86%). The amounts of random coils in CsDHN2 and CsDHN4 were significantly larger than those in CsDHN1 and CsDHN3, while the amount of alpha helices was much smaller.

To further characterize the CsDHNs, the full-length deduced amino acid sequences were comparatively analyzed by ClustalW. The results revealed that the four DHN proteins belong to three subgroups: Y_nK_n (CsDHN1), SK_n (CsDHN3) and Y_nSK_n (CsDHN2 and CsDHN4) (Fig. 3). The RRKK motif, which is considered as the nuclear localization signal (NLS), was present in 2 DHN proteins (CsDHN2 and CsDHN4). In addition, CsDHN2 and CsDHN4 contained a novel conserved motif EDDGXGG$(R)_{1-3}$ or GXGGRRKK

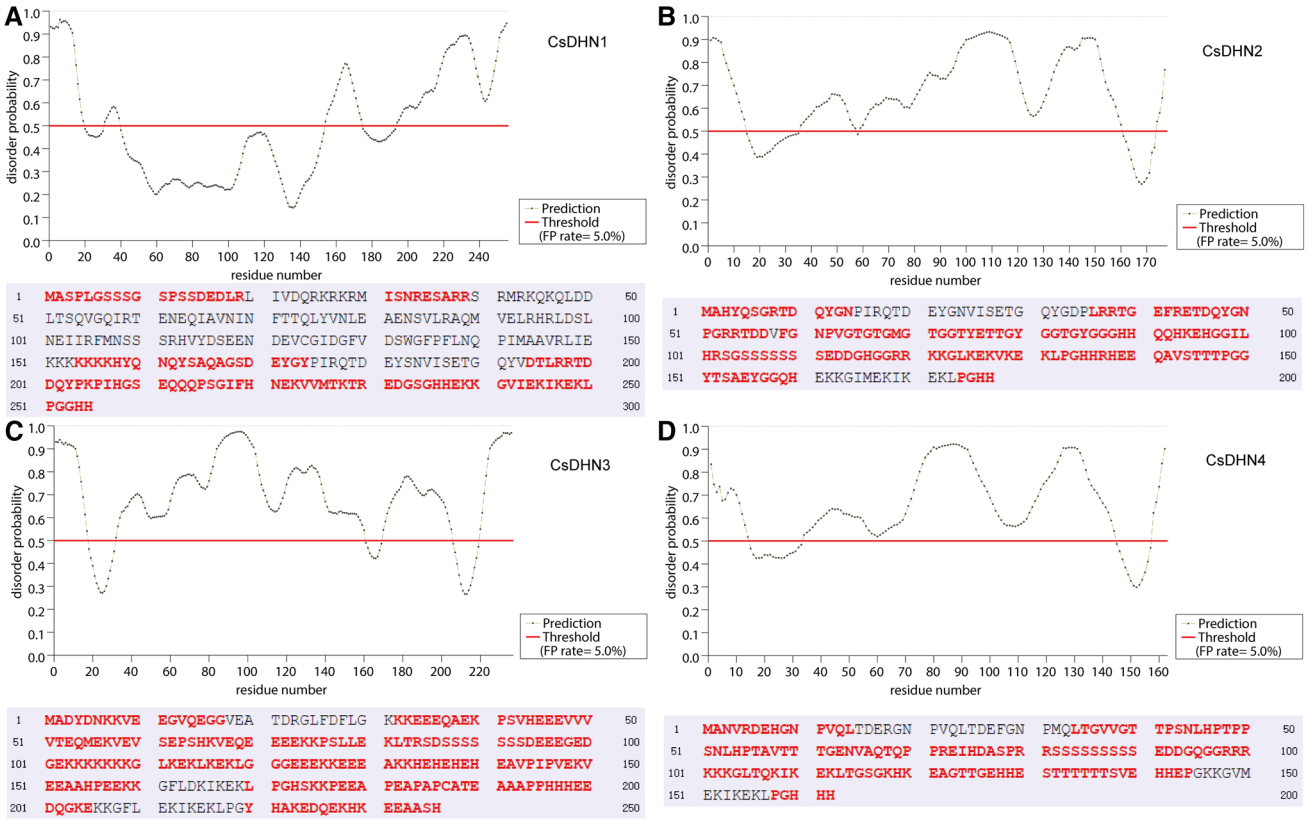

Fig. 1 Prediction of intrinsically disordered residues of CsDHN protein sequences via PrDOS program. Disorder probability above 0.5 represents the disordered amino acid residues. Red: disordered residues. Black: ordered residues

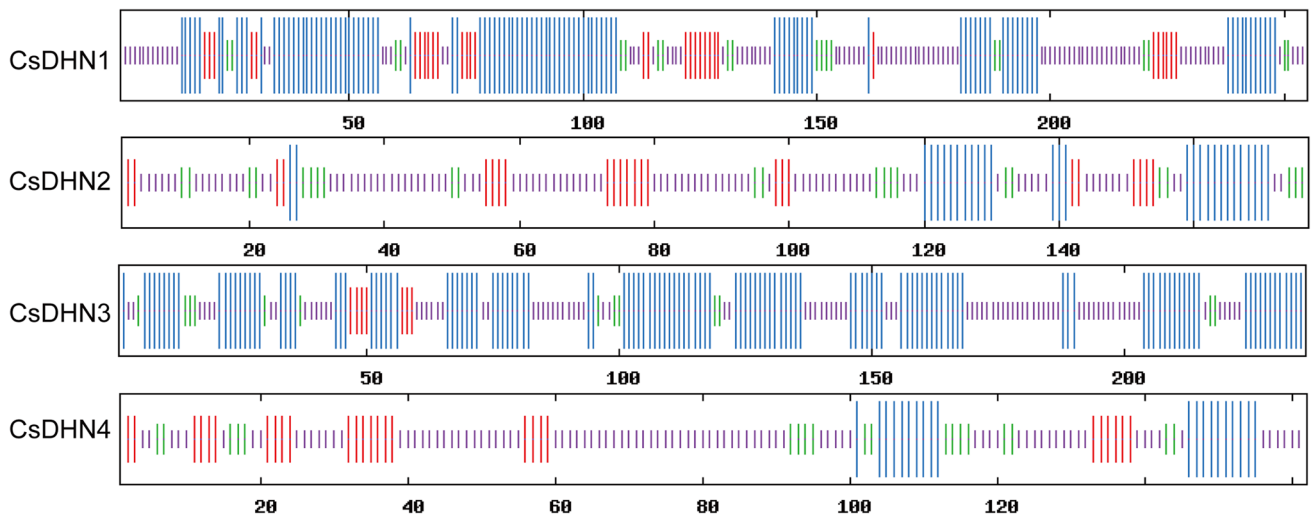

Fig. 2 Protein secondary structural analysis of CsDHN proteins. The blue longest vertical lines represents alpha helical regions, the red vertical lines represent extended strand regions, the green vertical lines represent beta turn regions, and the purple shortest vertical lines represent random coil regions

between the S-segment and the first K-segment (Fig. 2) as described previously (Abedini et al. 2017; Malik et al. 2017). Moreover, CsDHN2 harbored an additional dehydrin conserved motif (LXRXXS) which is phosphorylated by an Snf1-related kinase (SnRK2-10) (Vlad et al. 2008). This phenomenon was also found in some DHNs in other plant

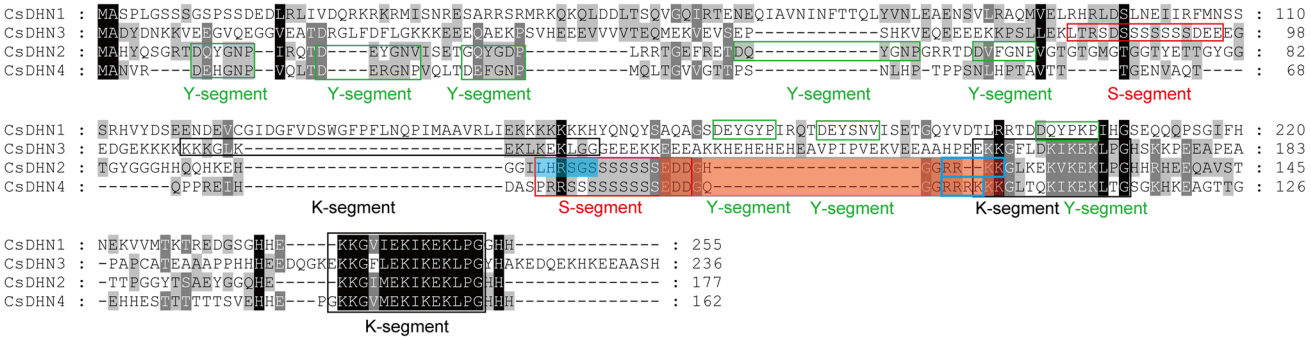

Fig. 3 Protein sequence alignment of CsDHN protein sequences. Y-, S- and K-segments are boxed by green, red and black, respectively. The conserved motif EDDGXGG(R)$_{1-3}$ or GXGGRRKK and SnRK-10 sites are highlighted in red and blue, respectively. The NLS motifs (RRKK) are boxed by blue

species including grape (Yang et al. 2012), pepper (Jing et al. 2016), and barley (Abedini et al. 2017).

Phylogenetic analysis and gene structure of *DHN* gene family between cucumber and other plant species

To investigate the phylogenetic relationships of *DHN* genes in plants, a neighbor-joining phylogenetic tree was generated according to the alignment of DHN protein sequences from cucumber, *Arabidopsis*, tomato, and rice. These DHN proteins could be classified into five groups that correspond to the types of Y_nSK_n, K_n, SK_n, Y_nK_n, and K_nS (Fig. 4a). CsDHN1 protein clustered with AtLEA45 in Y_nK_n group, while tomato and rice were lack of this type DHNs. CsDHN4 was closely related to AtLEA45, but it clustered together with CsDHN2 in Y_nSK_n group. CsDHN3 shared a 51.7% identity in deduced amino acid sequence with SlLEA10, and clustered with the K_nS-type DHN proteins (Fig. 4a). Interestingly, no K_n- and K_nS-type DHN proteins were detected in

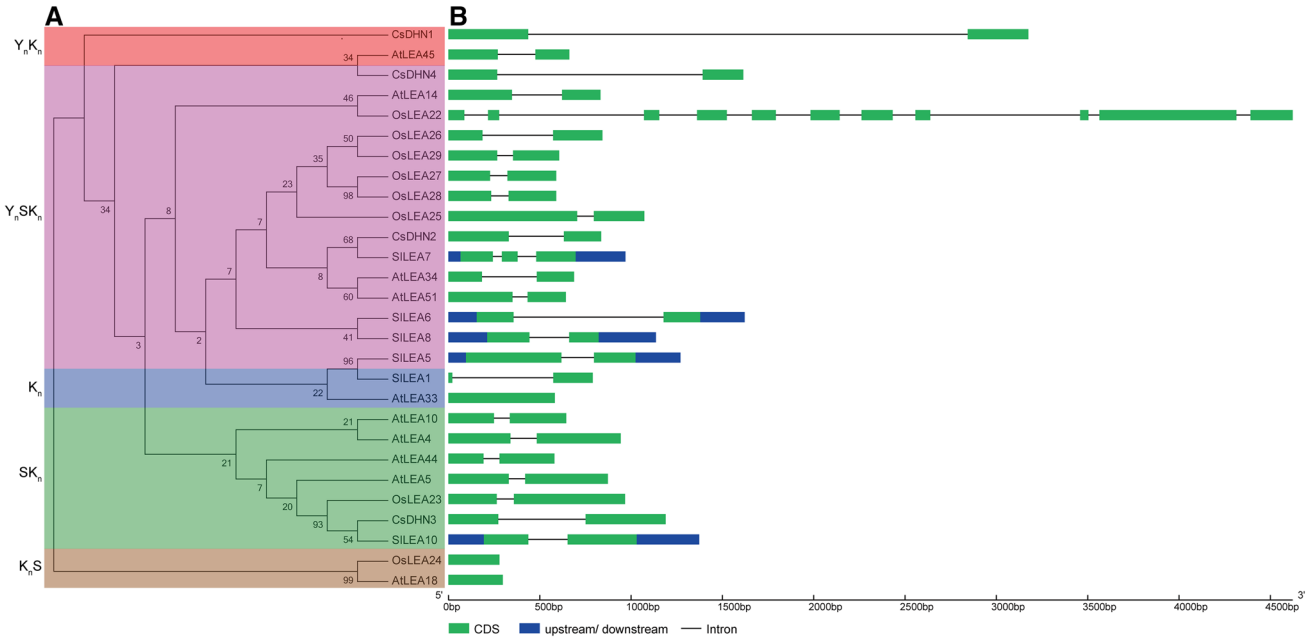

Fig. 4 Phylogenetic relationships and gene structure of *DHN* gene family from cucumber, *Arabidopsis*, tomato, and rice. **a** The phylogenetic tree of cucumber, *Arabidopsis*, tomato, and rice DHN proteins was constructed from a complete alignment of DHN proteins using MEGA 5.0 by the neighbor-joining method with 1000 bootstrap replicates. The groups are marked by colorful backgrounds. The protein names and corresponding accession numbers used to build the phylogenic tree are listed in Table S2. **b** Gene structure analyses were carried out by GSDS software. The blue boxes indicate upstream/downstream, the green boxes indicate CDSs, and the black lines indicate introns

cucumber and tomato. It could be observed that CsDHN2 and CsDHN3 in cucumber had close relationships with SlLEA7 and SlLEA10 in tomato, respectively, suggesting that these two pairs of proteins may be functionally similar.

To study the gene structures of these *DHN* genes, we used GSDS software to compare the CDS sequences with the corresponding gDNA sequences of the *DHN* genes in cucumber, *Arabidopsis*, tomato, and rice. Most of these genes had only one intron (23 out of 28) (Fig. 4b). Among these genes, three had two introns (*SlLEA7*), one had ten introns (*OsLEA22*), and three had no intron (*AtLEA33, OsLEA24, AtLEA18*). The intronless genes were clustered in the K_n and K_nS groups. In addition, some closely related *DHN* genes showed similar gene structures, such as *OsLEA27* and *OsLEA28, OsLEA24* and *AtLEA18*.

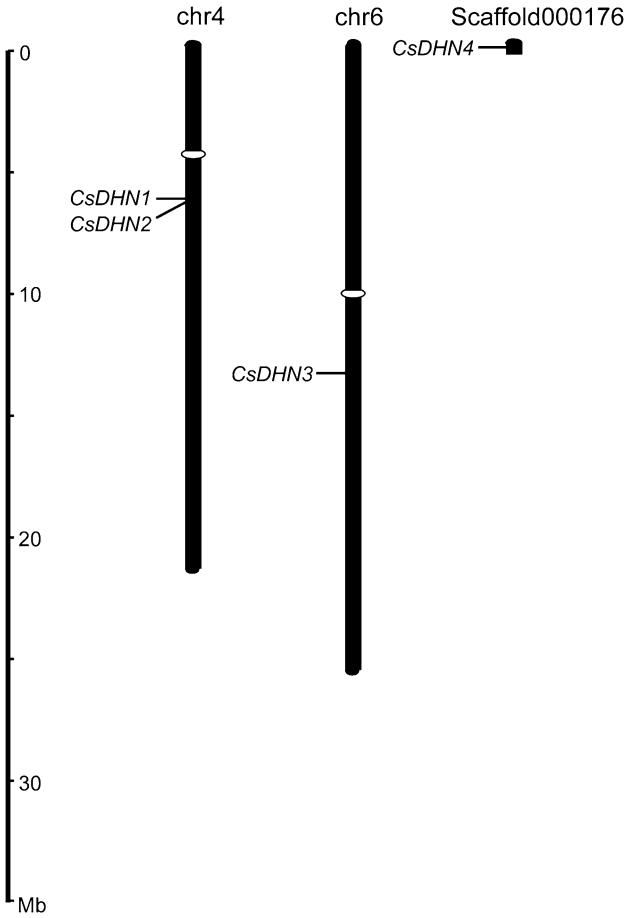

Fig. 5 Positions of *CsDHN* genes on cucumber chromosomes. Scale represents a 10 Mb chromosomal distance

Chromosomal location and duplication of *CsDHN* genes

These four *DHN* genes were distributed on two cucumber chromosomes, except for one, which was found on an unassembled sequence scaffold000176 (Table 1; Fig. 5). Segmental and tandem duplication events are recognized as the primary reasons for *DHN* gene family expansion, and have been found in some plant species, such as *Arabidopsis* (Hundertmark and Hincha 2008), poplar (Liu et al. 2012), apple (Liang et al. 2012), barley (Abedini et al. 2017), and rice (Verma et al. 2017; Wang et al. 2007). In the current study, no segmental duplication event was identified. However, a pair of genes (*CsDHN1* and *CsDHN2*) were arranged in tandem repeats on chromosome 4, implying that they may be tandemly duplicated genes.

Analysis of *CsDHNs* promoters

To study the potential gene function, 1.0-kb promoter region of each *CsDHN* gene was downloaded from CuGI and analyzed by PlantCARE. The results showed that the *cis*-acting regulatory elements in *CsDHNs* promoters could be mainly categorized into hormone-responsive and stress-responsive elements (Table S3; Table 2). Five kinds of stress-responsive elements were found, and all of the *CsDHN* promoter sequences contained LTR motif (low temperature) and anaerobic induction element (ARE), which reflect plant responses to low temperature and anaerobic induction, respectively. Three other stress-related elements, including MBS and TC-rich repeats, were also present in a series of members. Meanwhile, we observed the wide presence of nine kinds of hormone-related *cis*-elements in the promoter regions of *CsDHNs* (Table S3; Table 2). These results

Table 2 Putative stress-responsive and hormone-responsive *cis*-elements in the promoter regions of *CsDHN* genes

	Stress-responsive *cis*-element	Hormone-responsive *cis*-element
CsDHN1	HSE [2], MBS [1], TC-rich repeats [4], LTR [1], ARE [1]	GARE-motif [3], TATC-box [1], TCA-element [1]
CsDHN2	MBS [1], LTR [1], ARE [2]	ABRE [6], CGTCA-motif [1]
CsDHN3	HSE [1], LTR [2], ARE [2]	ABRE [2], CGTCA-motif [1], ERE [1], AuxRR-core [2]
CsDHN4	MBS [1], TC-rich repeats [2], LTR [1], ARE [3]	ABRE [2], CE3 [1], ERE [1], TCA-element [3], TGA-element [1]

Numbers in brackets refer to the number of the *cis*-element in the promoter regions of *CsDHN* genes

suggested that *CsDHNs* are related to the responses to various abiotic stresses and hormones.

Additionally, several *cis*-acting regulatory elements involved in developmental processes, such as flavonoid biosynthetic genes regulation (MBSI), seed-specific regulation (RY-element), zein metabolism regulation (O2-site), circadian control (circadian), and endosperm-specific element (Skn-1 motif), were also found in several *CsDHNs* promoters (Table S3), implying that *CsDHNs* may have tissue-specific expression.

Tissue-specific expression of *CsDHN* genes

To understand the functions of *CsDHNs*, their expression profiles in cucumber tissues were determined by RT-PCR. As shown in Fig. 6, *CsDHN2* and *CsDHN3* had similar expression patterns, and their expression was detected in all five tissues, with higher expression levels in stem, flower and fruit, and significantly lower expression levels in root and leaf. Interestingly, *CsDHN4* was only expressed in leaf at a relatively low level, and the expression of *CsDHN1* was not observed in all five tissues (Fig. 6). The overlapping but different expression patterns of the *CsDHN* genes indicate that they may play various important roles in cucumber.

Expression profiles of *CsDHN* gene under various abiotic stresses and ABA treatment

To determine the effect of environmental stresses on the expression of *CsDHN* genes, the transcripts of *CsDHN* genes under various abiotic stresses and ABA treatment were examined by qRT-PCR. Most *CsDHN* genes showed altered expression under these treatments, except for *CsDHN1*, whose expression was not detected (Fig. 7), implying that it is a pseudogene. All of the remaining three *CsDHN* genes were gradually up-regulated under heat stress, and their maximum transcripts were found at 12 h (10.99- to 757.74-fold) (Fig. 7a). Similar results were also observed under cold stress, except for *CsDHN4*, whose expression was down-regulated at 3 h, and subsequently reached to the maximum (12.72-fold) at 12 h (Fig. 7b). Upon NaCl treatment, the *CsDHN* genes showed diverse expression profiles (Fig. 7c). The transcription of *CsDHN2* sharply increased at 3 h (527.44-fold), and continued to increase after 12 h treatment (898.08-fold). The expression of *CsDHN3* was induced at 3 h (46.05-fold), followed by a decrease at 12 h (37.31-fold). The transcription of *CsDHN4* was slightly induced at 3 h and became the highest (11.50-fold) at 12 h. All of the three *CsDHN* genes were induced at 3 h by PEG treatment, and the expression of *CsDHN2* dramatically increased at 12 h (335.29-fold), while that of *CsDHN3* and *CsDHN4* slightly decreased at 12 h (Fig. 7d). Under H_2O_2 treatment, the expression of *CsDHN2* was induced at 3 h and dramatically increased at 12 h (260.46-fold) (Fig. 7e). The transcription of *CsDHN3* and *CsDHN4* also reached the highest level at 12 h, while that of *CsDHN3* was unchanged at 6 h, and *CsDHN4* was sharply induced at 6 h (Fig. 7e). Under ABA treatment, all of the three *CsDHN* genes were notably induced at 3 h, followed by an observable decrease at 12 h (Fig. 7f).

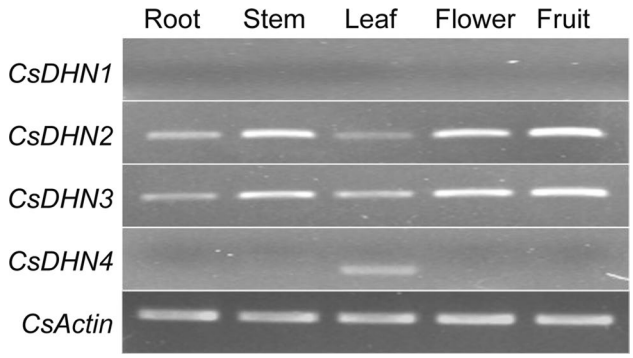

Fig. 6 RT-PCR analysis of *CsDHN* genes in different tissues of cucumber. RNA was isolated from different tissues including root, stem, leaf, flower, and fruit

Discussion

In previous studies, genome-wide identification and characterization of *DHN* family have been performed in various plant species, such as *Arabidopsis* (10 members) (Hundertmark and Hincha 2008), *Populus trichocarpa* (11 members) (Liu et al. 2012), *Vitis vinifera* (4 members) (Yang et al. 2012), *Malus domestica* (12 members) (Liang et al. 2012), *Hordeum vulgare* (13 members) (Abedini et al. 2017; Karami et al. 2013), *Solanum lycopersicum* (6 members) (Cao and Li 2015), *Pyrus pyrifolia* (7 members) (Hussain et al. 2015), *Capsicum annuum* (7 members) (Jing et al. 2016), *Oryza sativa* ssp. *japonica* (8 members) (Verma et al. 2017), and *Solanum tuberosum* (5 members) (Charfeddine et al. 2017). In the current study, a total of four *DHN* family genes were identified from cucumber (Table 1). The number of *DHN* genes is not proportional to the size of genomes in the above mentioned plant species. This phenomenon might be due to gene duplication, which plays an important role in the evolution of gene families in different plants (Flagel and Wendel 2009). In addition, one tandem duplication event (*CsDHN1* and *CsDHN2*) was found on chromosome 4 (Fig. 5), which might have made contribution to the expansion of *CsDHN* gene family in cucumber.

The four CsDHN proteins, respectively, consisted of 255, 177, 236, and 162 amino acids, and showed common features of DHN proteins. For example, they were rich of glycine (Gly) and polar amino acids, and were strongly

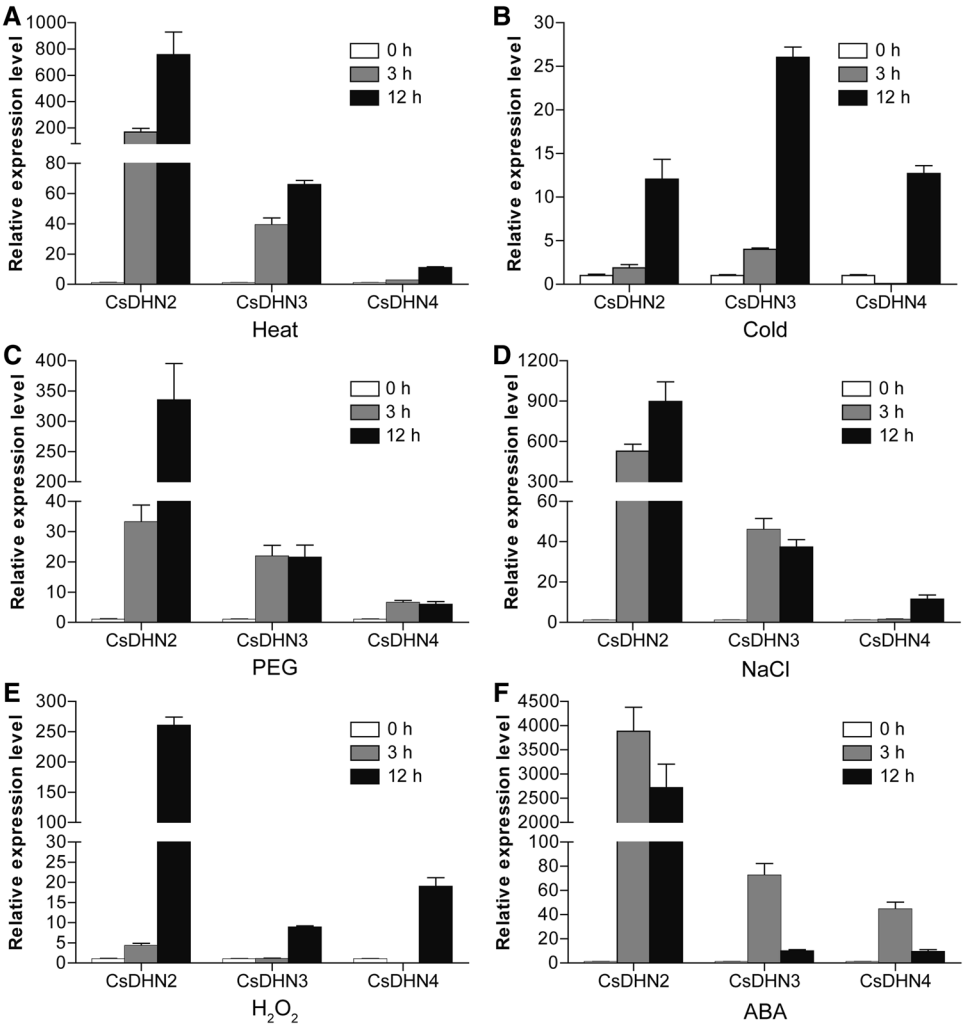

Fig. 7 qRT-PCR analysis of cucumber *DHN* genes in response to various abiotic stresses including heat (**a**), cold (**b**), PEG (**c**), NaCl (**d**), H_2O_2 (**e**) and ABA (**f**). Error bars indicate SD based on three biological replicates

hydrophilic (Table 1). In addition, the distribution of disorder residues in CsDHN protein sequences implied that they are IDPs (Fig. 1). Protein secondary structure analysis by SOPMA tool revealed that CsDHN proteins possess a high percentage of random coils (Table 1; Fig. 2), which can maintain a large amount of hydrogen bonds with water molecules and a small amount of intramolecular hydrogen bonds. These results further confirm that CsDHNs are IDPs, which is in accordance with DHN protein characteristics (Charfeddine et al. 2017).

Among the five types of DHNs, only three types were found in cucumber, including two Y_nSK_n-type (CsDHN2 and CsDHN4), one Y_nK_n-type (CsDHN1), and one SK_n-type (CsDHN3) DHNs, while K_nS- and K_n-type DHNs were not detected (Figs. 3, 4a). CsDHN2 and CsDHN4 are Y_nSK_n-type DHNs, and contain the RRKK motif and a novel conserved motif between the S-segment and the first K-segment, which was previously identified as a phosphorylation site (Lv et al. 2014). In addition, CsDHN2 harbors an extra conserved motif (LXRXXS) that can be phosphorylated by an Snf1-related kinase (Vlad et al. 2008). Phosphorylation of DHNs was suggested to play an important role in functionally regulating stressed plant cells and modulating the membrane binding of DHNs (Eriksson et al. 2011; Yang et al. 2012). Moreover, the Y_nSK_n-type CsDHNs are basic in character, and were suggested to combine to negatively charged membrane with a high affinity (Jing et al. 2016; Koag et al. 2009).

The four CsDHNs (except for CsDHN1) show closer phylogenetic relationships with the DHNs in *Arabidopsis* and tomato than with those in rice (Fig. 4a). Gene structure analysis revealed that each of the four *CsDHNs* harbors one intron (Fig. 4b), which is in agreement with the previous reports in pepper (Jing et al. 2016). The *DHN* genes contain very few introns, with most of them harboring one intron. This phenomenon may be caused by a conservative evolution pattern, and the intron length can affect the functional divergence in plants (Guo et al. 2014). A previous study has also reported that plants tend to retain more genes with no introns or short introns (Mattick and Gagen 2001). In

addition, some closely related *DHN* genes exhibit similar gene organization patterns and demonstrate a conserved evolutionary pattern, implying that they may have similar functions.

Gene expression patterns are important for understanding gene functions, and the expression of *DHN* genes has been reported to be at high levels in all vegetative tissues of many plant species, including apple (Liang et al. 2012), pepper (Jing et al. 2016), and rice (Verma et al. 2017). In this study, *CsDHN2* and *CsDHN3* were expressed in all five detected tissues (Fig. 6), which is consistent with previous studies of other plant species, indicating that they may be involved in the growth and development of cucumber. Notably, *CsDHN4* was only expressed in leaf, implying that it may play a particular role in leaf. However, the expression of *CsDHN1* was not detectable in any tissue under the experimental conditions of this study (Fig. 6), suggesting that it may be a pseudogene. Such results were also obtained in some other plant species, such as *VvDHN3* (Yang et al. 2012) and *CaDHN6* (Jing et al. 2016). The spatial variations in the expression levels of *CsDHN* genes in different organs suggest that *CsDHNs* may participate in various processes of cucumber growth and development.

Many studies have reported the induction of *DHN* gene expression by various abiotic stresses in plants, suggesting that DHNs may play a positive role in plant response and adaptation to abiotic stresses (Banerjee and Roychoudhury 2016; Charfeddine et al. 2017; Jing et al. 2016; Rodziewicz et al. 2014; Verma et al. 2017). In this study, the expression of all four *CsDHN* genes except for *CsDHN1* was induced in response to stresses such as heat, PEG, salt and cold (Fig. 7), which is in agreement with the findings in previous studies. Phylogenetic analysis showed that *CsDHN3* and *SlLEA10* were in the same cluster of the SK_n subgroup (Fig. 4a), and were both up-regulated under salt and drought treatments (Fig. 7) (Cao and Li 2015). In addition, *CsDHN* genes can be induced in response to stresses, but they may have different expression patterns. For example, under cold treatment, the transcription of *CsDHN4* sharply decreased at 3 h, followed by an increase at 24 h, while that of *CsDHN2* and *CsDHN3* gradually increased (Fig. 7b). Similar expression patterns were also observed under H_2O_2 treatment (Fig. 7e). Upon NaCl treatment, the transcription of *CsDHN4* had no significant change at 3 h, while that of *CsDHN2* and *CsDHN3* was significantly up-regulated; the expression of *CsDHN2* and *CsDHN4* remarkably increased at 12 h, while that of *CsDHN3* decreased (Fig. 7d). Under PEG treatment, the expression of *CsDHN2* was continuously up-regulated at 12 h, while that of *CsDHN3* and *CsDHN4* had no significant change (Fig. 7c). These findings indicate that they play various important roles in cucumber, and different mechanisms might have been formed in cucumber to adapt to various abiotic stresses. The diverse expression patterns could be owing to the presence of *cis*-elements related to stress in *CsDHN* promoters. Obvious differences were found in the abundance and distribution of stress-responsive *cis*-elements in the promoters of four *CsDHNs* (Table 2). For example, all the *CsDHN* promoters had up to two cold-responsive elements (LTR), which are related to low-temperature response. *CsDHN2* and *CsDHN4* possessed four and two TC-rich repeats, respectively (Table 2). Each of *CsDHN1, CsDHN2* and *CsDHN4* promoters contains one MBS, while *CsDHN3* promoter does not contain any MBS. *CsDHN1* promoter has two HSE, and *CsDHN1* promoter includes one HSE (Table 2); however, *CsDHN2* and *CsDHN4* were remarkably induced by heat stress (Fig. 7a), implying that some other heat-responsive elements are present in their promoter regions. Further research is required to determine whether and how the *cis*-elements of *CsDHNs* regulate the response to various abiotic stresses.

Previous studies have revealed that most of *DHN* genes are induced under ABA treatment, and several members were proven to play important roles in plant resistance to stress via ABA-dependent pathway (Cao et al. 2017; Lv et al. 2017). In this study, the expression of *CsDHN2, CsDHN3* and *CsDHN4* was up-regulated by ABA, and they harbored 2–6 ABA-responsive elements (ABREs) (Table 2). The number of ABREs was correlated with the transcripts of *DHN* genes in response to ABA (Fig. 7f). In addition, SnRK2-10, which positively regulates ABA-mediated signaling, may be related to the phosphorylation of S-segments in DHNs (Vlad et al. 2008). These results reveal that *CsDHNs* are regulated through an ABA-dependent signal pathway.

Conclusions

In this study, a complete analysis of the *DHN* genes in cucumber was carried out, including genome organization, gene structure, phylogenetic relationship, and expression pattern analyses. Our results would help the functional characterization of *CsDHN* genes, and facilitate future study of their response to various abiotic stresses.

Author contribution statement YZ, LH, and SL conceived and designed the experiments. YZ, LH, SX, LJ, and SL carried out the experiments. YZ, LH, and SL analyzed the data. YZ and SL wrote the paper. SL edited the manuscript, secured the funds to support this research.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing financial interests.

References

Abedini R, GhaneGolmohammadi F, PishkamRad R, Pourabed E, Jafarnezhad A, Shobbar ZS et al (2017) Plant dehydrins: shedding light on structure and expression patterns of dehydrin gene family in barley. J Plant Res 130:747–763

Aguayo P, Sanhueza J, Noriega F, Ochoa M, Lefeuvre R, Navarrete D et al (2016) Overexpression of an SK_n-dehydrin gene from *Eucalyptus globulus* and *Eucalyptus nitens* enhances tolerance to freezing stress in *Arabidopsis*. Trees 30:1785–1797

Altunoglu YC, Baloglu P, Yer EN, Pekol S, Baloglu MC (2016) Identification and expression analysis of *LEA* gene family members in cucumber genome. Plant Growth Regul 80:225–241

Banerjee A, Roychoudhury A (2016) Group II late embryogenesis abundant (LEA) proteins: structural and functional aspects in plant abiotic stress. Plant Growth Regul 79:1–17

Brini F, Hanin M, Lumbreras V, Amara I, Khoudi H, Hassairi A et al (2007) Overexpression of wheat dehydrin DHN-5 enhances tolerance to salt and osmotic stress in *Arabidopsis thaliana*. Plant Cell Rep 26:2017–2026

Cao J, Li X (2015) Identification and phylogenetic analysis of late embryogenesis abundant proteins family in tomato (*Solanum lycopersicum*). Planta 241:757–772

Cao Y, Xiang X, Geng M, You Q, Huang X (2017) Effect of *HbDHN1* and *HbDHN2* genes on abiotic stress responses in *Arabidopsis*. Front Plant Sci 8:470

Charfeddine S, Charfeddine M, Saïdi MN, Jbir R, Bouzid RG (2017) Potato dehydrins present high intrinsic disorder and are differentially expressed under ABA and abiotic stresses. Plant Cell Tiss Organ Cult 128:423–435

Chen RG, Jing H, Guo WL, Wang SB, Ma F, Pan BG et al (2015) Silencing of dehydrin *CaDHN1* diminishes tolerance to multiple abiotic stresses in *Capsicum annuum* L. Plant Cell Rep 34:2189–2200

Chiappetta A, Muto A, Bruno L, Woloszynska M, Van Lijsebettens M, Bitonti MB (2015) A dehydrin gene isolated from feral olive enhances drought tolerance in *Arabidopsis* transgenic plants. Front Plant Sci 6:392

Close TJ (1996) Dehydrins: emergence of a biochemical role of a family of plant dehydration proteins. Physiol Plant 97:795–803

Eriksson SK, Kutzer M, Procek J, Grobner G, Harryson P (2011) Tunable membrane binding of the intrinsically disordered dehydrin Lti30, a cold-induced plant stress protein. Plant Cell 23:2391–2404

Figueras M, Pujal J, Saleh A, Save R, Goday A (2004) Maize Rab17 overexpression in *Arabidopsis* plants promotes osmotic stress tolerance. Ann Appl Biol 144:251–257

Finn RD, Coggill P, Eberhardt RY, Eddy SR, Mistry J, Mitchell AL et al (2016) The Pfam protein families database: towards a more sustainable future. Nucleic Acids Res 44:D279–D285

Flagel LE, Wendel JF (2009) Gene duplication and evolutionary novelty in plants. New Phytol 183:557–564

Gasteiger E, Hoogland C, Gattiker A, Duvaud SE, Wilkins MR, Appel RD et al (2005) Protein identification and analysis tools on the ExPASy server. In: Walker JM (ed) The proteomics protocols handbook. Humana Press, Totowa, pp 571–607

Geourjon C, Deléage G (1995) SOPMA: significant improvements in protein secondary structure prediction by consensus prediction from multiple alignments. Bioinformatics 11:681–684

Graether SP, Boddington KF (2014) Disorder and function: a review of the dehydrin protein family. Front Plant Sci 5:576

Guo M, Yin YX, Ji JJ, Ma BP, Lu MH, Gong ZH (2014) Cloning and expression analysis of heat-shock transcription factor gene CaHsfA2 from pepper (*Capsicum annuum* L.). Genet Mol Res 13:1865–1875

Guo X, Zhang L, Zhu J, Liu H, Wang A (2017) Cloning and characterization of *SiDHN*, a novel dehydrin gene from *Saussurea involucrata* Kar. et Kir. that enhances cold and drought tolerance in tobacco. Plant Sci 256:160–169

Halder T, Upadhyaya G, Ray S (2017) YSK_2 type dehydrin (*SbDhn1*) from *Sorghum bicolor* showed improved protection under high temperature and osmotic stress condition. Front Plant Sci 8:918

Hanin M, Brini F, Ebel C, Toda Y, Takeda S, Masmoudi K (2011) Plant dehydrins and stress tolerance: versatile proteins for complex mechanisms. Plant Signal Behav 6:1503–1509

Hara M, Endo T, Kamiya K, Kameyama A (2017) The role of hydrophobic amino acids of K-segments in the cryoprotection of lactate dehydrogenase by dehydrins. J Plant Physiol 210:18–23

Hill W, Jin XL, Zhang XH (2016) Expression of an arctic chickweed dehydrin, CarDHN, enhances tolerance to abiotic stress in tobacco plants. Plant Growth Regul 80:323–334

Houde M, Dallaire S, N'Dong D, Sarhan F (2004) Overexpression of the acidic dehydrin WCOR410 improves freezing tolerance in transgenic strawberry leaves. Plant Biotechnol J 2:381–387

Hu B, Jin J, Guo AY, Zhang H, Luo J, Gao G (2015) GSDS 2.0: an upgraded gene feature visualization server. Bioinformatics 31:1296–1297

Hu L, Yang Y, Jiang L, Liu S (2016) The catalase gene family in cucumber: genome-wide identification and organization. Genet Mol Biol 39:408–415

Huang S, Li R, Zhang Z, Li L, Gu X, Fan W et al (2009) The genome of the cucumber, *Cucumis sativus* L. Nat Genet 41:1275–1281

Hundertmark M, Hincha DK (2008) LEA (late embryogenesis abundant) proteins and their encoding genes in *Arabidopsis thaliana*. BMC Genom 9:118

Hussain S, Niu Q, Qian M, Bai S, Teng Y (2015) Genome-wide identification, characterization, and expression analysis of the dehydrin gene family in Asian pear (*Pyrus pyrifolia*). Tree Genet Genomes 11:110

Ishida T, Kinoshita K (2007) PrDOS: prediction of disordered protein regions from amino acid sequence. Nucleic Acids Res 35:W460–W464

Jing H, Li C, Ma F, Ma JH, Khan A, Wang X et al (2016) Genome-wide identification, expression diversication of dehydrin gene family and characterization of *CaDHN3* in pepper (*Capsicum annuum* L.). PLoS One 11:e0161073

Karami A, Shahbazi M, Niknam V, Shobbar ZS, Tafreshi RS, Abedini R et al (2013) Expression analysis of dehydrin multigene family across tolerant and susceptible barley (*Hordeum vulgare* L.) genotypes in response to terminal drought stress. Acta Physiol Plant 35:2289–2297

Koag MC, Wilkens S, Fenton RD, Resnik J, Vo E, Close TJ (2009) The K-segment of maize DHN1 mediates binding to anionic phospholipid vesicles and concomitant structural changes. Plant Physiol 150:1503–1514

Kong X, Lv W, Jiang S, Zhang D, Cai G, Pan J et al (2013) Genome-wide identification and expression analysis of calcium-dependent protein kinase in maize. BMC Genom 14:433

Kumar M, Lee SC, Kim JY, Kim SJ, Kim SR (2014) Over-expression of dehydrin gene, *OsDhn1*, improves drought and salt stress tolerance through scavenging of reactive oxygen species in rice (*Oryza sativa* L.). J Plant Biol 57:383–393

Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H et al (2007) Clustal W and Clustal X version 2.0. Bioinformatics 23:2947–2948

Lescot M, Dehais P, Thijs G, Marchal K, Moreau Y, Van de Peer Y et al (2002) PlantCARE, a database of plant *cis*-acting regulatory elements and a portal to tools for in silico analysis of promoter sequences. Nucleic Acids Res 30:325–327

Letunic I, Bork P (2018) 20 years of the SMART protein domain annotation resource. Nucleic Acids Res 46:D493–D496

Liang D, Xia H, Wu S, Ma F (2012) Genome-wide identification and expression profiling of dehydrin gene family in *Malus domestica*. Mol Biol Rep 39:10759–10768

Liu CC, Li CM, Liu BG, Ge SJ, Dong XM, Li W et al (2012) Genome-wide identification and characterization of a dehydrin gene family in poplar (*Populus trichocarpa*). Plant Mol Biol Rep 30:848–859

Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. Methods 25:402–408

Lv DW, Subburaj S, Cao M, Yan X, Li X, Appels R et al (2014) Proteome and phosphoproteome characterization reveals new response and defense mechanisms of *Brachypodium distachyon* leaves under salt stress. Mol Cell Proteom 13:632–652

Lv A, Fan N, Xie J, Yuan S, An Y, Zhou P (2017) Expression of *CdDHN4*, a novel YSK_2-type dehydrin gene from Bermudagrass, responses to drought stress through the ABA-dependent signal pathway. Front Plant Sci 8:748

Malik AA, Veltri M, Boddington KF, Singh KK, Graether SP (2017) Genome analysis of conserved dehydrin motifs in vascular plants. Front Plant Sci 8:709

Mattick JS, Gagen MJ (2001) The evolution of controlled multitasked gene networks: the role of introns and other noncoding RNAs in the development of complex organisms. Mol Biol Evol 18:1611–1630

Munoz-Mayor A, Pineda B, Garcia-Abellan JO, Anton T, Garcia-Sogo B, Sanchez-Bel P et al (2012) Overexpression of dehydrin *tas14* gene improves the osmotic stress imposed by drought and salinity in tomato. J Plant Physiol 169:459–468

Peng Y, Reyes JL, Wei H, Yang Y, Karlson D, Covarrubias AA et al (2008) RcDhn5, a cold acclimation-responsive dehydrin from *Rhododendron catawbiense* rescues enzyme activity from dehydration effects in vitro and enhances freezing tolerance in *RcDhn5*-overexpressing *Arabidopsis* plants. Physiol Plant 134:583–597

Perdiguero P, Collada C, Soto A (2014) Novel dehydrins lacking complete K-segments in Pinaceae. The exception rather than the rule. Front Plant Sci 5:682

Rodziewicz P, Swarcewicz B, Chmielewska K, Wojakowska A, Stobiecki M (2014) Influence of abiotic stresses on plant proteome and metabolome changes. Acta Physiol Plant 36:1–19

Rorat T (2006) Plant dehydrins—tissue location, structure and function. Cell Mol Biol Lett 11:536–556

Saibi W, Feki K, Ben Mahmoud R, Brini F (2015) Durum wheat dehydrin (DHN-5) confers salinity tolerance to transgenic *Arabidopsis* plants through the regulation of proline metabolism and ROS scavenging system. Planta 242:1187–1194

Shekhawat UKS, Srinivas L, Ganapathi TR (2011) *MusaDHN-1*, a novel multiple stress-inducible SK_3-type dehydrin gene, contributes affirmatively to drought- and salt-stress tolerance in banana. Planta 234:915

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

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol 28:2731–2739

Tompa P (2002) Intrinsically unstructured proteins. Trends Biochem Sci 27:527–533

Tompa P, Szasz C, Buday L (2005) Structural disorder throws new light on moonlighting. Trends Biochem Sci 30:484–489

Verma G, Dhar YV, Srivastava D, Kidwai M, Chauhan PS, Bag SK et al (2017) Genome-wide analysis of rice dehydrin gene family: its evolutionary conservedness and expression pattern in response to PEG induced dehydration stress. PLoS One 12:e0176399

Vlad F, Turk BE, Peynot P, Leung J, Merlot S (2008) A versatile strategy to define the phosphorylation preferences of plant protein kinases and screen for putative substrates. Plant J 55:104–117

Wang XS, Zhu HB, Jin GL, Liu HL, Wu WR, Zhu J (2007) Genome-scale identification and analysis of *LEA* genes in rice (*Oryza sativa* L.). Plant Sci 172:414–420

Yang Y, He M, Zhu Z, Li S, Xu Y, Zhang C et al (2012) Identification of the dehydrin gene family from grapevine species and analysis of their responsiveness to various forms of abiotic and biotic stress. BMC Plant Biol 12:140

Yin Z, Rorat T, Szabala BM, Ziołkowska A, Malepszy S (2006) Expression of a *Solanum sogarandinum* SK_3-type dehydrin enhances cold tolerance in transgenic cucumber seedlings. Plant Sci 170:1164–1172

Zhou Y, He P, Xu Y, Liu Q, Yang Y, Liu S (2017a) Overexpression of *CsLEA11*, a Y_3SK_2-type dehydrin gene from cucumber (*Cucumis sativus*), enhances tolerance to heat and cold in *Escherichia coli*. AMB Express 7:182

Zhou Y, Hu L, Jiang L, Liu H, Liu S (2017b) Molecular cloning and characterization of an *ASR* gene from *Cucumis sativus*. Plant Cell Tiss Organ Cult 130:553–565

Zhou Y, Hu L, Wu H, Jiang L, Liu S (2017c) Genome-wide identification and transcriptional expression analysis of cucumber superoxide dismutase (SOD) family in response to various abiotic stresses. Int J Genom 2017:7243973