

Genome-wide Survey of the Dehydrin Genes in Bread Wheat (*Triticum Aestivum* L.) and its Relatives: Identification, Evolution and Expression Profiling Under Various Abiotic Stresses

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Abstract

Background: Bread wheat (*Triticum aestivum*) is an important and fundamental cereal worldwide. With increasingly severe environmental stress, it is very important to mine stress-resistant genes for wheat breeding. Dehydrin (DHN) genes are primary candidates because they are involved in the response to many stressors.

Results: Here, a genome-wide analysis of this gene family was performed on the genomes of wheat and its three relatives. A total of 55 DHN genes in *Triticum aestivum*, 31 in *Triticum dicoccoides*, 15 in *Triticum urartu*, and 16 in *Aegilops tauschii* were identified. The phylogenetic, synteny, sequence and protein structure analyses showed that the DHN genes were divided into five groups. Genes in the same group share similar conserved motifs, protein structures, and potential functions. The tandem *TaDHN* genes responded strongly to drought, cold and high salinity stresses, while the non-tandem genes were responded weakly to all stress conditions. Further, multiple DHN proteins cooperation maybe an important way to prevent plants from abiotic stress according to the interaction network analysis.

Conclusions: Conserved, duplicated DHN genes may have played an important role in the adaptation of wheat to a variety of conditions, hence, contributing to the distribution of bread wheat as a global staple food. This research illuminates the contributions of DHN genes to abiotic stresses in *Triticeae* species and offers helpful information for further functional study of DHN genes in these crops.

Background

Bread wheat (*Triticum aestivum*) is an important and fundamental cereal that provides about 20% of the dietary protein and calories worldwide [1, 2]. It is comprised of three homologous subgenomes (AABBDD; $2n = 6x = 42$) that originated from two natural hybridization events [3, 4]. First, tetraploidization from the hybridization between *T. urartu* (AA; $2n = 2x = 14$) and an unknown close relative of *Aegilops speltoides* (BB; $2n = 2x = 14$), formed the tetraploid wild emmer wheat (*T. turgidum* ssp. *dicoccoides*, AABB, $2n = 4x = 28$). Wild emmer wheat hybridized with *Ae. tauschii* (DD; $2n = 2x = 14$) to produce hexaploid bread wheat about 8,000 years ago. The yield of bread wheat during the growth period is challenged by various environmental stressors, including abiotic stressors (e.g., drought, salinity, and high and low temperatures) [5–7] and biotic stressors, such as *Fusarium graminearum* (FHB), *Blumeria graminis* (powdery mildew), and *Puccinia striiformis* (stripe rust) [8]. A key approach to face these challenges is to mine the stress-resistant genes available and utilize them for breeding. With the release of the genome assembly and annotation for *T. aestivum* [9], *T. dicoccoides* [10], *T. urartu* [11], and *Ae. tauschii* [12], a genome-wide analysis of all stress-related genes in wheat and its relatives can be realized. Furthermore, large-scale RNA-seq provides a rich resource to analyze the expression patterns of related genes under a variety of stress conditions and at different developmental stages [13].

Dehydrins (DHNs) are a class of highly hydrophilic stress-responsive proteins with a high proportion of charged and polar amino acids [14, 15]. These proteins accumulate during late embryogenesis and are induced in vegetative tissues by several environmental stressors that dehydrate cells, such as drought, salinity, and cold [16]. According to their sequence characteristics, DHNs have been defined as proteins possessing at least one copy of a conserved motif known as the K-segment in their molecules [17, 18]. The K-segment (consensus EKK GIM E/DKI KEK LPG) is a lysine-rich amino acid sequence that forms amphiphilic α -helices located at the C-terminal end of the protein [19, 20]. DHNs also possess other conserved motifs, such as the Y-segment (consensus [T/V]D[E/Q]YGNP), which is a tyrosine-rich region near the N-terminus and the S-segment (consensus LHRSGS4–10(E/D)3), which is serine-rich, and formed by a stretch of 4–10 serine residues [21, 22]. Based on the presence of these conserved motifs (K-, S-, and Y-segment), DHNs have been classified into different categories of YnSKn, YnKn, SKn, KnS, and Kn [17, 18, 23].

DHNs are considered stress proteins involved in plant protective responses to dehydration by binding to metal ions and scavenging reactive oxygen species, binding to DNAs or phospholipids to maintain biological activity, binding to proteins to prevent denaturation, and holding water molecules [24, 25]. DHNs are known as IDPs/IUPs (changes in protein conformation result in changes in protein function; this phenomenon is called “moonlighting”, which is a characteristic of IDPs/IUPs) that may be involved in the formation and stability of the cytoplasmic glassy state when plants suffer dehydration, or may serve as hub proteins and coordinate crosstalk and the cellular signals involved in the response to stress [26, 27]. Many studies have demonstrated that DHNs play crucial roles in abiotic stress tolerance; overexpression of the *Solanum habrochaites* DHN gene enhances transgenic tomato tolerance to multiple abiotic stressors; an oleaster DHN gene called *OesDHN* improves drought tolerance when overexpressed in *Arabidopsis*;

overexpression of four *Prunus mume* DHNs in *Escherichia coli* and tobacco result in increased freezing resistance; *HbDHN1* and *HbDHN2* in *Hevea brasiliensis* significantly increase tolerance to drought, salt, and osmotic stress when overexpressed in *Arabidopsis* [28–31]. These studies indicate that plant DHNs are extensively involved in abiotic stress. Several studies have shown that DHNs might also play important roles in plant development and in the biotic stress response. For example, *Medicago truncatula* Y2K4-type dehydrin (*MtCAS31*) interacts with *AtICE1*, which is essential for stomatal development [32]; several DHNs in drought-tolerant oak species *Quercus ilex* are induced to be expressed by a *Phytophthora cinnamomic* infection [33].

In this study, we identified the DHN genes in bread wheat and its relatives and analyzed their phylogenetic, syntenic, sequence and protein structure relationships. Cis-elements in the putative promoters of the *TaDHN* genes were analyzed. Then, we investigated the expression profiles of the DHN gene family in response to various environmental stressors (including biotic and abiotic stressors) and hormones. Finally, we analyzed the interaction network of DHN genes and verified predicted subcellular location by experimental method. This study provides a comprehensive analysis of the DHN gene family among bread wheat and its relatives, clarifies the important roles of DHN genes in various abiotic stresses, and provides helpful information regarding the function of this significant gene family in bread wheat and its relatives.

Results

Characterization of DHN genes in bread wheat and its relatives

We used HMMER 3.1 and BLASTP to search for DHN genes in the genomes of bread wheat and its relatives using the HMM profile of the DHN domain (PF00257) obtained from the Pfam database as a query. Then, the InterPro and CDD websites were used to verify the predicted sequences. Finally, 117 putative DHN genes were identified. Among them, 55 DHN genes were detected in *T. aestivum*, 31 in *T. dicoccoides*, 15 in *T. urartu*, and 16 in *Ae. tauschii*. These numbers of DHN genes are directly related to the ploidy of the genome. The DHN gene names, locus IDs, and other features are shown in Table 1.

Table 1
The details of DHN genes among bread wheat and its relatives.

Gene Name	Locus ID	Type	Genomic Position	BP	GC (%)	AA	MW (kDa)	pI	Subcellular Localization
<i>TaDHN1-A</i>	<i>TraesCS3A02G254600</i>	YSK2	476563869–476564968(-)	642	68.07	213	21.83	6.75	Cytoplasm Nucleus
<i>TaDHN1-B</i>	<i>TraesCS3B02G286600</i>	YSK2	458398889–458399630(-)	654	67.13	217	22.3	7.5	Cytoplasm Nucleus
<i>TaDHN1-D</i>	<i>TraesCS3D02G255500</i>	YSK2	357146923–357147959(-)	648	67.75	215	22.24	7.19	Cytoplasm Nucleus
<i>TaDHN2-A</i>	<i>TraesCS3A02G396200</i>	YSK3	643459970–643461316(-)	828	67.63	275	27.02	10.13	Cytoplasm
<i>TaDHN2-B</i>	<i>TraesCS3B02G428200</i>	YSK3	667112076–667113352(-)	825	68.12	274	27.19	10.29	Cytoplasm
<i>TaDHN2-D</i>	<i>TraesCS3D02G390200</i>	YSK3	505318572–505319988(-)	828	67.87	275	27.16	10.26	Cytoplasm
<i>TaDHN3-A</i>	<i>TraesCS4A02G250900</i>	Y2SK3	562289788–562291566(-)	1368	70.37	455	43.74	9.28	Cytoplasm
<i>TaDHN3-B</i>	<i>TraesCS4B02G064200</i>	Y2SK3	57136426–57138194(-)	1374	69.92	457	43.89	9.27	Cytoplasm
<i>TaDHN3-D</i>	<i>TraesCS4D02G063100</i>	Y2SK3	39233033–39234840(-)	1293	70.15	430	41.22	9.47	Cytoplasm
<i>TaDHN4-A1</i>	<i>TraesCS5A02G369800</i>	YSK2	569677389–569678193(+)	432	71.53	143	14.57	8.91	Cytoplasm
<i>TaDHN4-A2</i>	<i>TraesCS5A02G369900</i>	YSK2	569682833–569683707(+)	423	70.92	140	14.24	8.91	Cytoplasm
<i>TaDHN4-B1</i>	<i>TraesCS5B02G372100</i>	YSK2	550320429–550321418(+)	432	71.76	143	14.43	8.91	Cytoplasm
<i>TaDHN4-B2</i>	<i>TraesCS5B02G372200</i>	YSK2	550337855–550338611(+)	417	70.5	138	14.22	8.91	Cytoplasm
<i>TaDHN4-D1</i>	<i>TraesCS5D02G379200</i>	YSK2	450373636–450374483(+)	432	71.53	143	14.52	8	Cytoplasm
<i>TaDHN4-D2</i>	<i>TraesCS5D02G379300</i>	YSK2	450379533–450380460(+)	402	69.65	133	13.93	9.44	Cytoplasm
<i>TaDHN5-A1 #</i>	<i>TraesCS5A02G424700</i>	YSK1	610078219–610079136(-)	336	67.26	111	11.48	10.15	Cytoplasm
<i>TaDHN5-A2</i>	<i>TraesCS5A02G424800</i>	YSK2	610184778–610185696(-)	450	65.56	149	15.22	9.96	Cytoplasm
<i>TaDHN5-B1</i>	<i>TraesCS5B02G426700</i>	YSK2	602483279–602484206(-)	453	66	150	15.18	10.16	Cytoplasm
<i>TaDHN5-B2</i>	<i>TraesCS5B02G426800</i>	YSK2	602648556–602649390(-)	453	65.78	150	15.22	9.99	Cytoplasm
<i>TaDHN5-D1</i>	<i>TraesCS5D02G433200</i>	YSK2	489012960–489013838(-)	459	66.67	152	15.35	10.16	Cytoplasm
<i>TaDHN5-D2</i>	<i>TraesCS5D02G433300</i>	YSK2	489166583–489167421(-)	465	66.67	154	15.59	10.16	Cytoplasm
<i>TaDHN6-A</i>	<i>TraesCS6A02G059800</i>	YSK2	31583535–31584464(-)	462	68.4	153	15.51	9.46	Cytoplasm
<i>TaDHN6-B</i>	<i>TraesCSU02G086200</i>	YSK2	76960851–76961538(+)	456	67.76	151	15.29	9.68	Cytoplasm

Gene Name	Locus ID	Type	Genomic Position	BP	GC (%)	AA	MW (kDa)	pI	Subcellular Localization
<i>TaDHN6-D #</i>	<i>TraesCSU02G122200</i>	YSK1	104041218–104042344(-)	450	69.56	149	14.86	7.5	Cytoplasm
<i>TaDHN7-A</i>	<i>TraesCS6A02G253300</i>	SK3	468473627–468475112(-)	807	64.19	268	28.82	5.05	Nucleus
<i>TaDHN7-B</i>	<i>TraesCS6B02G273400</i>	SK3	493352704–493354073(-)	780	62.69	259	27.97	4.98	Nucleus
<i>TaDHN7-D</i>	<i>TraesCS6D02G234700</i>	SK3	329080938–329082415(-)	789	63.88	262	28.16	4.97	Nucleus
<i>TaDHN8-A #</i>	<i>TraesCS6A02G350100</i>	K3	581982926–581983580(+)	573	65.1	190	19.24	7.74	Cytoplasm
<i>TaDHN8-B</i>	<i>TraesCS6B02G383200</i>	K6	658177094–658178907(+)	1218	66.56	405	40.29	7.37	Cell wall Cytoplasm Nucleus
<i>TaDHN8-D #</i>	<i>TraesCS6D02G332500</i>	K3	434811674–434812738(+)	540	66.3	179	17.8	8.23	Cytoplasm
<i>TaDHN9-A</i>	<i>TraesCS6A02G350200</i>	K2	582086438–582087160(+)	282	62.06	93	9.66	7.43	Cytoplasm
<i>TaDHN9-D</i>	<i>TraesCS6D02G332600</i>	K2	435012400–435012681(+)	282	63.12	93	9.66	7.43	Cytoplasm
<i>TaDHN10-A</i>	<i>TraesCS6A02G350300</i>	K14	582092081–582096138(+)	2976	62.96	991	101.61	6.33	Cytoplasm
<i>TaDHN10-B #</i>	<i>TraesCS6B02G695200LC</i>	K8	658234035–658241687(+)	1671	61.88	556	57.44	6.67	Cytoplasm
<i>TaDHN10-D</i>	<i>TraesCS6D02G332700</i>	K12	435072895–435075633(+)	2739	63.08	912	93.49	6.45	Cell wall Cytoplasm Nucleus
<i>TaDHN11-A</i>	<i>TraesCS6A02G350500</i>	YSK2	582264726–582266027(+)	666	68.92	221	22.05	9.45	Cell wall Cytoplasm Nucleus
<i>TaDHN11-B</i>	<i>TraesCS6B02G383500</i>	YSK2	658402976–658404180(+)	690	69.13	229	23.01	9.79	Cell wall Cytoplasm Nucleus
<i>TaDHN11-D</i>	<i>TraesCS6D02G332900</i>	YSK2	435351033–435352218(+)	651	69.12	216	21.62	9.5	Cell wall Cytoplasm Nucleus
<i>TaDHN12-A1</i>	<i>TraesCS6A02G350600</i>	YSK2	582511276–582512221(+)	489	66.87	162	16.28	9.88	Cytoplasm
<i>TaDHN12-A2</i>	<i>TraesCS6A02G350700</i>	YSK2	582516436–582517359(+)	459	64.49	152	15.52	8.05	Cytoplasm
<i>TaDHN12-A3 *</i>	<i>TraesCS6A02G350800</i>	SK2	582630751–582631295(-)	432	63.66	143	14.82	9.88	Cytoplasm
<i>TaDHN12-A4 *</i>	<i>TraesCS6A02G350900</i>	YSK1	582638141–582638862(-)	573	64.92	190	20.14	11.34	Cytoplasm
<i>TaDHN12-B1</i>	<i>TraesCS6B02G695700LC</i>	YSK2	658477430–658478020(+)	489	63.27	162	16.13	9.79	Cytoplasm
<i>TaDHN12-B2</i>	<i>TraesCS6B02G695800LC</i>	YSK2	658496215–658496805(+)	489	63.27	162	16.13	9.79	Cytoplasm
<i>TaDHN12-B3</i>	<i>TraesCS6B02G695900LC</i>	YSK2	658515366–658515956(+)	489	63.27	162	16.1	9.79	Cytoplasm

Gene Name	Locus ID	Type	Genomic Position	BP	GC (%)	AA	MW (kDa)	pI	Subcellular Localization
<i>TaDHN12-B4</i>	<i>TraesCS6B02G383600</i>	YSK2	658530539–658531483(+)	477	66.46	158	15.84	9.69	Cytoplasm
<i>TaDHN12-B5</i>	<i>TraesCS6B02G383800</i>	YSK2	658577562–658578499(-)	501	65.67	166	16.7	8.89	Cytoplasm
<i>TaDHN12-D1</i>	<i>TraesCS6D02G333000</i>	YSK2	435711668–435712623(+)	489	66.87	162	16.2	8.93	Cytoplasm
<i>TaDHN12-D2</i>	<i>TraesCS6D02G333100</i>	YSK2	435749803–435750719(+)	435	65.06	144	14.51	9.79	Cytoplasm
<i>TaDHN12-D3</i>	<i>TraesCS6D02G333200</i>	YSK2	435763700–435764598(+)	468	65.6	155	15.73	9.41	Cytoplasm
<i>TaDHN12-D4</i>	<i>TraesCS6D02G333300</i>	YSK2	435831506–435832386(-)	483	66.87	160	16.26	8.94	Cytoplasm
<i>TaDHN12-D5</i>	<i>TraesCS6D02G333600</i>	YSK2	435962275–435963310(-)	504	66.27	167	16.71	8.05	Cytoplasm
<i>TaDHN13-A</i>	<i>TraesCS7A02G560000</i>	K3	731882428–731883023(+)	375	61.87	124	12.83	8.08	Cytoplasm
<i>TaDHN13-B</i>	<i>TraesCS7B02G484900</i>	K3	741668510–741668887(+)	378	64.02	125	12.67	7.04	Cytoplasm
<i>TaDHN13-D</i>	<i>TraesCS7D02G549900</i>	K2	634439606–634440272(-)	339	63.13	112	11.53	6.8	Cytoplasm
<i>TdDHN1-A</i>	<i>TRIDC3AG038190</i>	YSK2	483446623–483451982(-)	660	67.42	219	22.53	6.93	Cytoplasm Nucleus
<i>TdDHN1-B</i>	<i>TRIDC3BG042940</i>	YSK2	468417850–468427038(-)	654	66.97	217	22.37	7.19	Cytoplasm Nucleus
<i>TdDHN2-A #</i>	<i>TRIDC3AG056410</i>	YSK2	639664417–639665447(-)	390	64.36	129	13.24	10.56	Cytoplasm Nucleus
<i>TdDHN2-B #</i>	<i>TRIDC3BG063150</i>	YSK2	677652490–677653501(-)	405	65.43	134	13.87	10.69	Cytoplasm
<i>TdDHN3-A #</i>	<i>TRIDC4AG039320</i>	SK3	555522541–555523348(-)	552	66.67	183	18.37	10.19	Cytoplasm
<i>TdDHN3-B #</i>	<i>TRIDC4BG009930</i>	Y2SK3	55083350–55084815(-)	543	65.38	180	18.02	10.19	Cytoplasm
<i>TdDHN4-A1</i>	<i>TRIDC5AG054100</i>	YSK2	564921473–564922000(+)	432	71.76	143	14.56	8.91	Cytoplasm
<i>TdDHN4-A2</i>	<i>TRIDC5AG054110</i>	YSK2	564926944–564927492(+)	423	70.92	140	14.27	8.87	Cytoplasm
<i>TdDHN4-B1</i>	<i>TRIDC5BG058060</i>	YSK2	556085190–556085719(+)	432	71.76	143	14.43	8.91	Cytoplasm
<i>TdDHN4-B2</i>	<i>TRIDC5BG058080</i>	YSK2	556101420–556101927(+)	417	70.26	138	14.25	8.91	Cytoplasm
<i>TdDHN5-A1 #</i>	<i>TRIDC5AG061380</i>	SK1	605402094–605402742(-)	219	63.01	72	7.61	10.81	Nucleus
<i>TdDHN5-A2 *</i>	<i>TRIDC5AG061420</i>	YSK2	605513222–605513988(-)	522	63.03	173	17.79	10.35	Cytoplasm
<i>TdDHN5-B</i>	<i>TRIDC5BG065560</i>	YSK2	608742508–608743380(-)	456	65.79	151	15.37	10.13	Cytoplasm

Gene Name	Locus ID	Type	Genomic Position	BP	GC (%)	AA	MW (kDa)	pI	Subcellular Localization
<i>TdDHN6-A</i>	<i>TRIDC6AG007480</i>	YSK2	31000232–31000891(-)	462	67.97	153	15.44	8.93	Cytoplasm
<i>TdDHN6-B</i>	<i>TRIDC6BG010780</i>	YSK2	55591718–55592361(-)	456	67.76	151	15.29	9.68	Cytoplasm
<i>TdDHN7-A</i>	<i>TRIDC6AG039020</i>	SK3	470140072–470141622(-)	807	64.19	268	28.82	5.05	Nucleus
<i>TdDHN7-B</i>	<i>TRIDC6BG045690</i>	SK3	486136098–486141800(+)	780	62.56	259	27.94	4.98	Nucleus
<i>TdDHN8-A</i> #	<i>TRIDC6AG052540</i>	K4	582467279–582468478(+)	792	68.06	263	26.21	7.44	Cytoplasm
<i>TdDHN8-B</i> *	<i>TRIDC6BG061300</i>	K7	642747123–642748606(+)	1410	66.67	470	47.07	7.63	Cell wall Cytoplasm Nucleus
<i>TdDHN9-A</i>	<i>TRIDC6AG052550</i>	K2	582534354–582534931(+)	282	62.06	93	9.66	7.43	Cytoplasm
<i>TdDHN9-B</i> *	<i>TRIDC6BG061310</i>	K2	642748977–642749569(+)	354	65.82	117	11.67	7.54	Cytoplasm
<i>TdDHN10-A</i> #	<i>TRIDC6AG052570</i>	K8	582541438–582543392(+)	1698	61.37	565	58.87	6.45	Cytoplasm
<i>TdDHN11-A</i> #	<i>TRIDC6AG052590</i>	YSK2	582698877–582699685(+)	489	67.28	162	16.66	9.6	Cell wall Cytoplasm Nucleus
<i>TdDHN11-B</i>	<i>TRIDC6BG061340</i>	YSK2	643041875–643042827(+)	696	69.25	231	23.13	9.45	Cell wall Cytoplasm Nucleus
<i>TdDHN12-A1</i>	<i>TRIDC6AG052630</i>	YSK2	582893775–582898592(+)	489	66.87	162	16.22	9.13	Cytoplasm
<i>TdDHN12-A2</i>	<i>TRIDC6AG052640</i>	YSK2	582992994–582993790(-)	474	64.14	157	16.14	8.08	Cytoplasm
<i>TdDHN12-A3</i>	<i>TRIDC6AG052650</i>	YSK2	583001187–583001807(-)	441	65.76	146	14.75	9.79	Cytoplasm
<i>TdDHN12-B1</i>	<i>TRIDC6BG061350</i>	YSK2	643116359–643132143(+)	477	66.67	158	15.87	9.71	Cytoplasm
<i>TdDHN12-B2</i>	<i>TRIDC6BG061380</i>	YSK2	643190550–643191397(-)	501	65.87	166	16.65	8.89	Cytoplasm
<i>TdDHN13-A</i>	<i>TRIDC7AG077740</i>	K3	723743247–723743983(-)	375	61.87	124	12.83	8.08	Cytoplasm
<i>TdDHN13-B</i>	<i>TRIDC7BG076250</i>	K3	750614280–750614913(+)	381	63.78	126	12.8	7.38	Cytoplasm
<i>TuDHN1</i>	<i>TuG1812G0300003021.01</i>	YSK2	477045519–477046650(-)	660	67.42	219	22.57	7.14	Cytoplasm Nucleus
<i>TuDHN3</i>	<i>TuG1812G0400000583.01</i>	Y2SK3	41901253–41903075(-)	1392	70.55	463	44.55	9.28	Cytoplasm
<i>TuDHN4-1</i>	<i>TuG1812G0500003981.01</i>	YSK2	535938718–535939515(+)	432	71.53	143	14.55	8.03	Cytoplasm
<i>TuDHN4-2</i>	<i>TuG1812G0500003982.01</i>	YSK2	535944152–535945001(+)	423	71.16	140	14.24	8.91	Cytoplasm

Gene Name	Locus ID	Type	Genomic Position	BP	GC (%)	AA	MW (kDa)	pI	Subcellular Localization
<i>TuDHN5-1 #</i>	<i>TuG1812G0500004492.01</i>	YSK1	574218374–574219129(-)	336	67.26	111	11.46	9.8	Cytoplasm
<i>TuDHN5-2 #</i>	<i>TuG1812S0001634800.01</i>	YSK1	1-593(-)	414	67.87	138	13.91	7.97	Cytoplasm
<i>TuDHN6</i>	<i>TuG1812G0600000621.01</i>	YSK2	31890359–31891122(-)	462	68.18	153	15.4	8.1	Cytoplasm
<i>TuDHN7</i>	<i>TuG1812G0600002817.01</i>	SK3	439323348–439324718(-)	807	64.06	268	28.85	5.05	Nucleus
<i>TuDHN8</i>	<i>TuG1812G0600003768.01</i>	K6	539540401–539541995(+)	1176	68.54	391	38.82	7.48	Cell wall Cytoplasm Nucleus
<i>TuDHN9</i>	<i>TuG1812G0600003769.01</i>	K2	539598064–539599229(+)	282	62.06	93	9.66	7.43	Cytoplasm
<i>TuDHN11</i>	<i>TuG1812G0600003775.01</i>	YSK2	539946480–539947767(+)	756	68.65	251	24.98	9.12	Cell wall Cytoplasm Nucleus
<i>TuDHN12-1 *</i>	<i>TuG1812S0003423700.01</i>	YSK1	1979–2944(+)	537	67.6	178	17.93	10.89	Cytoplasm
<i>TuDHN12-2</i>	<i>TuG1812S0003424100.01</i>	YSK2	5679–6605(+)	459	64.27	152	15.64	8.89	Cytoplasm
<i>TuDHN12-3</i>	<i>TuG1812G0600003779.01</i>	YSK2	540160098–540161020(-)	510	67.06	169	16.94	8.89	Cytoplasm
<i>TuDHN13</i>	<i>TuG1812G0700005988.01</i>	K3	712117702–712118431(+)	375	61.87	124	12.74	7.47	Cytoplasm
<i>AetDHN1</i>	<i>AET3Gv20620600</i>	YSK2	364603011–364610005(-)	648	67.75	215	22.24	7.19	Cytoplasm Nucleus
<i>AetDHN2 #</i>	<i>AET3Gv20881700</i>	SK2	513201759–513203320(-)	396	66.41	131	13.27	10.87	Cytoplasm
<i>AetDHN3</i>	<i>AET4Gv20132600</i>	Y2SK3	41638766–41640754(-)	1383	67.97	460	44.58	9.03	Cytoplasm
<i>AetDHN4-1</i>	<i>AET5Gv20866700</i>	YSK2	458683120–458684124(+)	432	71.53	143	14.52	8	Cytoplasm
<i>AetDHN4-2</i>	<i>AET5Gv20866800</i>	YSK2	458689123–458689989(+)	402	69.65	133	13.93	9.44	Cytoplasm
<i>AetDHN5</i>	<i>AET5Gv20990000</i>	YSK2	498787518–499029693(-)	465	67.1	154	15.56	10.16	Cytoplasm
<i>AetDHN6 #</i>	<i>AET6Gv20153700</i>	K1	34801689–34803209(-)	270	66.3	89	8.86	9.64	Cytoplasm
<i>AetDHN7</i>	<i>AET6Gv20653900</i>	SK3	352066646–352068329(-)	789	63.88	262	28.16	4.97	Nucleus
<i>AetDHN8</i>	<i>AET6Gv20864100</i>	K6	458864577–458866768(+)	1176	66.75	391	38.89	7.59	Cell wall Cytoplasm Nucleus
<i>AetDHN9</i>	<i>AET6Gv20864400</i>	K2	459070823–459071706(+)	282	63.12	93	9.66	7.43	Cytoplasm
<i>AetDHN10 #</i>	<i>AET6Gv20864500</i>	K9	459131190–459134316(+)	2190	63.56	729	75.05	6.35	Cytoplasm

Gene Name	Locus ID	Type	Genomic Position	BP	GC (%)	AA	MW (kDa)	pI	Subcellular Localization
<i>AetDHN11</i> *	<i>AET6Gv20864900</i>	YSK1	459422062– 459423545(+)	444	67.79	148	16.32	10.99	Cytoplasm Nucleus
<i>AetDHN12-1</i>	<i>AET6Gv20865700</i>	YSK2	459787474– 459841184(+)	468	65.6	155	15.73	9.41	Cytoplasm
<i>AetDHN12-2</i>	<i>AET6Gv20866000</i>	YSK2	459889128– 459890166(-)	483	66.87	160	16.26	8.94	Cytoplasm
<i>AetDHN12-3</i>	<i>AET6Gv20866400</i>	YSK2	460020381– 460021566(-)	504	66.27	167	16.71	8.05	Cytoplasm
<i>AetDHN13</i>	<i>AET7Gv21347200</i>	K2	641274370– 641275124(+)	339	63.13	112	11.53	6.8	Cytoplasm
Ta: <i>T.aestivum</i> , Td: <i>T.dicoccoides</i> , Tu: <i>T.urartu</i> , Aet: <i>Ae. tauschii</i> ; DHN: Dehydrin; "#": truncated genes, "*": potential misannotated genes; BP: coding sequence length, AA: amino sequence length, MW: molecular weight, pI: isoelectric point.									

To study the phylogenetic relationships of the DHN family, an unrooted phylogenetic tree was constructed using the 117 protein sequences of bread wheat and its relatives (Fig. 1). The DHN genes clustered into five major groups; group I contained *DHN8/9/10/13*, which all encode Kn type DHNs (Table 1). The K-segment copies varied from 2 to 14. Group II contained *DHN11/12*, which encode YnSKn type DHNs (except *TaDHN12-A3* encodes SK2 type DHNs), mainly the YSK2 type (Table 1). Group III contained *DHN2/3/5/6*, which encode YnSKn type DHNs (except *TdDHN3-A*, *TdDHN5-A1*, *AetDHN2*, and *AetDHN6*). Group IV contained the *DHN4* genes, which encode YSK2 type DHNs. The remaining clade was group V, containing *DHN1* and *DHN7*, which encode YSK2 and SK3 type DHNs, respectively (Fig. 1 and Table 1).

We analyzed all DHN protein sequences in wheat and its relatives, and three types (YnSKn, SKn, and Kn) of DHNs were identified. YnSKn was the most frequent type, with 81 among 117 DHNs, followed by Kn with 26, and only 10 DHN genes encoded SKn type proteins (Table 1).

Chromosomal distribution, synteny analysis of the DHN genes

To analyze the synteny of the relationships in the DHN genes between bread wheat and its relatives, we identified the orthologous genes among these four released species genomes. There were 18 *TaDHNs*, 17 *TdDHNs*, and 15 *TuDHNs* in the A subgenome; 18 *TaDHNs* and 14 *TdDHNs* in the B subgenome; and 19 *TaDHNs* and 16 *AetDHNs* in the D subgenome. The gene pairs of *Ta/Td/Tu-A*, *Ta/Td-B*, and *Ta/Aet-D* were identified and mapped to corresponding genomic chromosomes (Fig. 2 and **Table S1**). The five genes *TaDHN6B/D*, *TuDHN5-2*, and *TuDHN12-1/2* were not assigned to chromosomes in the genome annotation file we used. We re-assigned these genes to the corresponding chromosomes based on the homologous and phylogenetic relationships (**Table S1** and Fig. 1) and genomic location information (Table 1) of all DHN genes between the different diploid subgenomes (Fig. 2). The DHN genes were distributed in the third to seventh homologous groups of bread wheat and its relatives, of which the fourth and seventh homologous groups had only one gene copy (except *T. urartu-3A*, with a missing gene), and the sixth homologous group had the most DHN genes, ranging from 7 to 14. Although genes were missing from some regions of the genome, homologous relationships were clear among the DHN genes, which were located in different diploid subgenomes, indicating that this gene family has been conserved during evolution.

Sequence analysis and re-annotation of the DHN gene family

We collected the gene structural information of all DHN genes in the annotation file and visualized it using the GSDS web tool. The results of the structural analysis showed that the number of exons varied between one and four. Then, the conserved domains of DHN genes were analyzed, according to their protein sequences. All DHN proteins contained one dehydrin core motif (K-segment), but different numbers of Y-/S-segments (Fig. 3B and 3C). The remaining motif are shown in **Figure S1**. We manually checked the coding sequences and amino sequences of all DHN genes among bread wheat and its relatives and combined the results with those of the phylogenetic (Fig. 1), synteny (Fig. 2), and sequence structural (Fig. 3) analyses. The truncated genes and potential misannotated genes are identified and marked in Table 1, and the identified missing genes are shown in **Table S1**.

According to the ploidy of bread wheat and its relatives, we speculate that the theoretical numbers of DHN genes should be 17 in *Ae. tauschii*, 17 in *T. urartu*, 34 in *T. dicoccoides*, and 52 in *T. aestivum*. However, *TaDHN9-B*, *TdDHN5-B2/10-B/12-B3*, *TuDHN2/10*, and

AetDHN5-2 were missing genes, and tandem duplication events occurred in *TaDHN12-A/B/D*, so the actual gene number deviated slightly from the theoretical gene number (**Table S1**). In the phylogenetic analysis (Fig. 1), genes belonging to the same group occasionally had the DHN type encoded by individual genes that was different from most of the genes in the group (e.g., group II genes mostly encoded YSK2 type DHNs, but *TaDHN12-A3* encoded SK2 type DHNs). After manually checking the sequence, we found that this occurred due to sequence truncation or potential misannotation. The first 28 amino acids of *TaDHN12-A3* were misannotated, resulting in loss of a Y-segment.

Analysis of cis-acting elements in the promoter regions and three-dimensional modeling of *TaDHN* Genes

DHN genes play important roles in the response to various stressors. *Cis*-acting elements control the expression of target genes by interacting with transcription factors [34, 35]. Hence, identifying the *cis*-acting elements will help to understand the potential regulatory mechanism. The putative *cis*-acting elements of all *TaDHN* genes involved in the response to stress within the 1,500 bp region upstream of the start codon (ATG) were analyzed. Eight different types of *cis*-acting elements were identified (Fig. 4). Among them, the abscisic acid (ABA) responsive element (ABRE), the methyl jasmonate (MeJA) responsive element (MeJA-RE), and the TCA-element are involved in hormone signaling, whereas the drought responsive element (DRE1/DRE core), low temperature responsive (LTR), TC-rich, and MYB binding site (MBS) are involved in the stress response. The results show differences in the distribution and abundance of the *cis*-acting elements in the 55 DHN promoters (Fig. 4). ABRE elements broadly involving ABA signaling and osmotic stress [36–38], it was appeared in all DHN gene promoter regions. DRE1/DRE core appears in 45 DHN promoter regions, and the abundance of these *cis*-acting elements ensured that gene expression is regulated in response to drought stress [39]. MeJA-RE appeared in the promoter regions of 41 DHN genes, followed by TC-rich repeats and LTR, with a number of 21. These three types of *cis*-acting elements also play critical roles in the response to abiotic or biotic stress [40]. The MBS *cis*-acting element appeared in 16 DHN genes, and it is important for the stress response (particularly the response to drought) and ABA signaling [25]. Taken together, the wide distribution of various hormone and stress responsive elements in the promoter regions demonstrates the potential role of DHN genes in the response to environmental stress in plants.

All DHN proteins have a small molecular weight and a relatively simple structure (Fig. 5). Each group contains at least one α -helices structure. Group I and group IV contain one α -helices, and group I/III/V contain two α -helices structures. In general, different group DHN proteins had similar three-dimensional structures, indicating the conservation of this gene family.

Expression profile of DHN genes in different tissues and in response to various biotic stressors

The RNA-seq data of five tissues/organs (roots, leaves, stems, spikes, and seeds) were analyzed to characterize expression of the bread wheat DHN genes. Of the 55 *TaDHN* genes, 65% ($n = 36$) were expressed in at least one tissue, with a wide expression level range of 1–204 tpm (tpm_{max}) (Fig. 6A and **Table S2**). The remaining 35% of the wheat DHN genes showed no or very low expression with a tpm_{max} < 1, such as *DHN6* and *DHN13* (Fig. 6A and **Table S2**). About 48% ($n = 26$) of the DHN genes were expressed in roots; *DHN2/4/5* and some *DHN8/10/12* genes were expressed specifically in roots. Twelve DHN genes were expressed in seeds and leaves, and the *DHN1/3* genes were expressed specifically in seeds. Few DHN genes were expressed in stems ($n = 3$) or spikes ($n = 4$) (Fig. 6A and **Table S2**).

The RNA-seq data of bread wheat inoculated with *F. graminearum*, *B. graminis*, or *P. striiformis* were analyzed to investigate the functions of the *TaDHN* genes in response to biotic stress. About 55% ($n = 30$) of the DHN genes were expressed [expression level range of 1–671 tpm (tpm_{max})] (Fig. 6A and **Table S2**). These expressed DHN genes were *DHN4/5/7/8/9/10/12/13*. To accurately understand the response of the DHN genes to different biotic stressors, we divided them into three levels according to gene expression (tpm): 0–1, 1–10, and > 10, corresponding to no or low, medium, and high expression, respectively. Most of the DHN genes showed no or low expression in response to the different biotic stressors with tpm < 1, and DHN genes with medium expression also accounted for a large portion (Fig. 6B). Although the number of highly expressed (tpm > 10) genes changed by increasing the inoculation time, the DHN gene family did not respond strongly when bread wheat was inoculated with *F. graminearum*, *B. graminis*, or *P. striiformis* (Fig. 6B).

We defined DHN genes with tpm fold change > 1 (treatment vs. control) and tpm value change > 10 as up- and down-regulated genes (URs and DRs) to further understand the changes in DHN family gene expression under different biotic stressors. No URs or DRs were

detected after 24 h in bread wheat inoculated with *F. graminearum*. There was one DR and one UR at 48 h and one DR and four URs at 72 h (Fig. 6C). *TaDHN5-D1* was upregulated at 48 and 72 h, and *TaDHN5-A1/B2* and *TaDHN12-B5* were upregulated at 72 h (Fig. 6A and **Table S2**). Similarly, no URs occurred in bread wheat inoculated with *B. graminis* or *P. striiformis*, and most genes were down-regulated or had a very low tpm value (Fig. 6C).

Expression profile of DHN genes in response to various abiotic stressors

The RNA-seq data of bread wheat under cold, drought, heat, and salt conditions were analyzed to understand the functions of the *TaDHN* genes in response to abiotic stress. Of the 55 *TaDHN* genes, 78% (n = 43) were expressed, and genes from *DHN1/2/3* showed no or very low expression (Fig. 7A and **Table S2**). We analyzed the expression levels of the DHN genes under the four different abiotic stressors. Ten DHN genes were expressed at the medium level in response to cold stress ($1 < \text{tpm} < 10$) and 15 were highly expressed ($\text{tpm} > 10$) (Fig. 7B). Twelve and 15 genes were medium and highly expressed at 1 h in response to drought stress, and seven were medium and 35 were highly expressed at 6 h. When bread wheat was subjected to heat stress, only five DHN genes were highly expressed at 1 and 6 h and eight and five were expressed at medium levels at 1 and 6 h, respectively (Fig. 7B). The DHN genes were not sensitive to 100 or 200 mM NaCl, as only three DHN genes were highly expressed, and two and nine were expressed at medium levels, respectively. However, 10 and 16 DHN genes were highly expressed and expressed at a medium level when bread wheat was subjected to 300 mM NaCl (Fig. 7B).

Then, we analyzed the DRs and URs of the DHN family genes in response to the four abiotic stressors. No DRs appeared in response to the cold or drought stressors, but 13 DHN genes were up-regulated by cold stress. Fifteen and 35 URs responded to drought stress at 1 and 6 h, respectively (Fig. 7C), and most genes were highly expressed (Fig. 7A and **Table S2**). In summary, the DHN genes were not sensitive to heat or the 100/200 mM NaCl stressors, as only a few DRs or URs appeared in response to these stress conditions. Six URs were detected under the 300 mM NaCl stress, indicating that DHN genes were sensitive to high salinity. The group I genes encode Kn type proteins, which were mainly expressed under cold and drought stress, and most were URs (Fig. 7A and 7C). In contrast, although some of the group I DHN genes were expressed with high tpm values under the *B. graminis* and *P. striiformis* inoculation, most were down-regulated (Fig. 6C).

Response of DHN genes under various hormone treatments

To understand the roles of the *TaDHN* genes in response to hormones, six *TaDHN* genes with higher expression levels under various stress conditions were selected to analyze their expression profiles. All selected genes had strong responses to the ABA treatment (Fig. 8). Among them, *TaDHN4-D1* was significantly down-regulated during the treatment period, and the other genes were up-regulated. In contrast, the expression of DHN genes was weakly induced or inhibited by the GA treatment. *TaDHN7-B* and *TaDHN9-A* were significantly up-regulated during the entire SA treatment, while the other genes were down-regulated or not induced (Fig. 8). All selected genes were significantly induced by the MeJA treatment; *TaDHN4-D1* and *TaDHN13-A* peaked at 6 h, *TaDHN9-A* and *TaDHN12-B5* peaked at 12 h, and *TaDHN7-B* and *TaDHN8-D* were up-regulated during the entire MeJA treatment period (Fig. 8). In summary, the DHN genes revealed various expression patterns under different hormone treatments. All selected DHN genes were very sensitive to the ABA treatment (particularly *TaDHN9-A* and *TaDHN12-B5* with strikingly high expression). ABA signaling is very important in the regulation of plant stress response [36]. The enrichment of ABA-related *cis*-acting elements (Fig. 4) and the strong response of the DHN genes to the ABA treatment reflected the crucial role of the DHN gene family in various stress conditions.

Interaction network and subcellular localization

In order to further understand the function of the DHN protein which strongly induced by abiotic stress, we used the STRING database to annotate the protein sequence encoded by the DHN genes of wheat and the homologous genes of *Aegilops tauschii* (**Table S3**). Then, the well-studied *AetDHNs* were used to construct interaction network (Fig. 9). We found that these DHN proteins were closely connected (except EMT32858 and EMT15121, which are annotated as cold shock protein). The Function of these genes covered many aspects of abiotic stresses to wheat, such as *DHN8/9/10/14* are encode cold shock protein, *DHN4/12/13* are encode salt induced protein (Fig. 9 and **Table S3**), EMT-25371/30993/24840 are encoded by *DHN5/4 - 1/12* and interacted with EMT106830, which is a heat shock protein (Fig. 9 and **Table S3**). Above all, we speculate that DHN proteins can cooperate with each other when plants are under abiotic stress.

We used bioinformatics methods to predict the subcellular location of the DHN protein to understand where the DHN protein might function (Table 1). In order to determine the accuracy of the prediction, two genes (*TaDHN12-A1* and *TaDHN7-A1*) were selected for

experimental verification. The protein products of *TaDHN12-A1* and *TaDHN7-A1* are located in the cytoplasm and nucleus, respectively (Fig. 10). The results are consistent with bioinformatics prediction, which confirmed the accuracy of this analysis.

Discussion

Identification of DHN genes in bread wheat and its relatives

Many DHN genes play key roles protecting plants from various environmental stressors; therefore, they are potential targets for crop breeding and improvement. In this study, we identified 55 DHN genes in hexaploid bread wheat (*T. aestivum*), 31 in tetraploid durum wheat (*T. dicoccoides*), 15 in diploid *T. urartu*, and 16 in diploid *Ae. tauschii*, using a comprehensive approach. Based on the highly homologous relationships among the four species, we manually checked the missing, truncated, and potential misannotated genes. The gene families in different subgenomes of polyploid plants, such as bread wheat, particularly small or medium-sized families, are conserved in number or sequence. Using the sequence similarity of the gene family between subgenomes can help to accurately identify the target genes. Furthermore, as automated annotation produces truncated and misannotated genes, a manual check is necessary for identification.

Evolution and expansion of the DHN genes among bread wheat and its relatives

Gene duplication can occur in different patterns, including whole genome duplication, segmental duplication, and single-gene duplication (including tandem and dispersed duplications) events. Duplication events play important roles in expansion of a gene family [41–43]. According to the chromosomal distribution and syntenic relationships between bread wheat and its relatives, allopolyploid events were the main driving force for expansion of the DHN family in hexaploid wheat. The *DHN4/5/12* genes have undergone tandem duplication events. Three *DHN12* genes occur in the diploid genome of wheat ancestors (one gene is missing in *Td-B*), while the number of *DHN12* has changed in the three subgenomes (A, B, and D) of wheat. The A subgenome of bread wheat has one more copy than the A genome of the ancestors. The B and D subgenomes have two more copies than the B and D genomes of the ancestors, respectively (**Table S1**). We analyzed the *TaDHN12* genes as tandems. Therefore, we speculate that the DHN gene family in bread wheat has undergone tandem duplication events after polyploidization, which led to more bread wheat DHN genes in the family than in its diploid donors.

The DHN family is a small community in many plant species, such as seven members in *Oryza sativa* [44], ten in *Arabidopsis* [45, 46], seven in *Pyrus pyrifoli* [47], and four each in *Vitis vinifera* and *V. yeshanensis* [15]. The 55 DHN genes in bread wheat are the largest DHN gene count among the plants mentioned above. Even its diploid ancestors *T. urartu* and *Ae. tauschii*, have 15 and 17 DHN genes, respectively, far more than these diploid plants. The number of DHN genes in bread wheat is >7.8 times higher than that in rice. This phenomenon cannot generally be explained by their ploidy. We hypothesize that the expansion of DHN genes may facilitate rapid adaptation of *Triticeae* crops to different stress conditions, particularly water-related stressors, such as drought, therefore, contributing to the global distribution of bread wheat and its relatives. Whether copy number variations in the DHN genes can be detected in different wheat varieties is an interesting issue with the recent release of the wheat pan-genomic data [48].

In the present study, tandem duplications occurred in linkage groups five and six, and tandem duplication genes (*DHN4/5/12*) appeared in clusters at corresponding chromosomes. The genes were combined with the expression profile results. Interestingly, these tandem genes were mostly up-regulated under various abiotic stressors. In contrast, non-tandem genes, such as *DHN1/2/3/6*, had no or very low expression (low tpm values), indicating that these genes are not sensitive to these stressors. Notably, the *DHN 8/9/10/11* genes were also up-regulated with high tpm values under drought and cold (Fig. 7A). These genes and the *DHN12* genes exist in the form of gene clusters and are continuous in position (Fig. 2 and Table 1). These findings combined with the sequence characteristics infer that these genes (*DHN8/9/10/11/12*) may have originated from tandem duplications of an ancestral gene. They evolved into the Kn and YSK2 groups during subsequent evolution, due to the need for ecological adaptability. Adaptive evolution may be the driving force behind tandem duplication events in the DHN gene family. Tandem duplication events are the main reason for expansion of the DHN gene family in bread wheat diploid donors.

Expression analysis of the *TaDHN* genes

We analyzed the expression profiles of the DHN genes in bread wheat under various biotic and abiotic stress conditions. *TaDHN1/2/3/6* were not sensitive to any of the stress conditions analyzed in this study with non or very low expression in all six tissues (Fig. 6A and Fig. 7A). The *TaDHN4* genes were mainly induced by drought and salt and were specifically expressed in roots (Fig. 6A). Previous study has demonstrated that the *TaDHN5* genes and their homologs contribute to drought and salt tolerance [49, 50]. This is consistent with the results of our expression profile analysis that the *TaDHN5* genes were induced by drought and salt stress (Fig. 7A). Some *TaDHN5* genes can also be induced by biotic stressors (inoculation with *F. graminearum*), indicating that these genes may play functional roles in resistance to *F. graminearum*.

The *TaDHN7* genes were generally highly expressed (high tpm values) under all stress conditions (except *DHN7-A* in cold stress) and were constitutively expressed in all tissues (Fig. 6A and Fig. 7A). Among all DHN genes in bread wheat, *TaDHN7-A/B/D* are the only three genes that encode SK3 type proteins (Table 1). Many SK3-type DHN genes were identified in a previous study, and have important functions under various abiotic stress conditions. For example, overexpression of *ShDHN* in tomato increases tolerance to drought and cold stress and improves seedling growth under osmotic and salt stress [28]. Overexpression of *MusaDHN-1* in banana improves tolerance to drought and salt stress [51]. A functional analysis demonstrated that *SpDHN1* in *Stipa purpurea* plays important roles in resistance to drought stress [52]. These studies of SK3-type DHN proteins reveal that *TaDHN7* genes may also have important functions in bread wheat suffering from various abiotic stressors. Taken together, *TaDHN* genes mainly responded cold, drought and high salinity stressors, but were not sensitive to heat, low or medium salinity, or most biotic stressors.

Conclusions

We comprehensively analyzed the DHN gene family, including a molecular characterization, phylogenetic classification, chromosomal distributions, gene structure, conserved motifs, and missing, truncated, and misannotated genes, as well as cis-acting elements. Based on six RNA-seq datasets, the DHN genes exhibited distinct tissue-specific expression patterns, and the induced genes were identified under different stress conditions. Conserved, duplicated DHN genes may have played an important role in the adaptation of wheat to a variety of conditions, hence, contributing to the distribution of bread wheat as a global staple food. Multiple DHNs cooperation maybe an important way to prevent plants from abiotic stress. These results will provide helpful information for further study of the stress resistance mechanism of the DHN gene family, and facilitate wheat breeding by fine-tuning important traits.

Methods

Plant materials

The bread wheat variety “Chinese Spring” and *N. benthamiana* were used for RT-PCR and subcellular localization, respectively. And these materials are presented from State Key Laboratory of Crop Biology, College of Agronomy, Shandong Agricultural University (Taian, China).

Sequence search, identification, and naming of the DHN genes

The genome sequences and gene annotations of bread wheat (*T. aestivum*) and wild emmer wheat (*T. dicoccoides*) were obtained from the Ensemble Plants website (<http://plants.ensembl.org/>). The genome files for *T. urartu* and *Ae. tauschii* were obtained from the (<http://www.mbkbase.org/Tu/>) and (<http://aegilops.wheat.ucdavis.edu/ATGSP/annotation/>) websites, respectively (Table S4).

To identify the DHN genes in bread wheat and its relatives, HMMER 3.1 (<http://www.hmmer.org/>) with default parameter settings and the BLAST algorithm for proteins (BLASTP) with the threshold expectation value set to 1E-20 were performed using the hidden Markov model (HMM) (version 3.0) profiles of the dehydrin domain (PF00257) obtained from the Pfam database (<http://pfam.xfam.org/>) as the query. We merged all hits obtained and removed the redundant hits. All non-redundant protein sequences were further analyzed with the NCBI conserved domain database (CDD, <https://www.ncbi.nlm.nih.gov/cdd>) and InterPro (<http://www.ebi.ac.uk/interpro/>) to confirm the conserved domain of the DHN protein in each candidate sequence. Tandem genes were screened by a custom Perl script, according to the following standards: (i) length of alignable sequence covers > 70% of longer gene; (ii) similarity of aligned regions > 70%; (iii) The physical distance between the align genes on the chromosome < 500kb.

We suggest a consistent naming pattern for all DHN genes of bread wheat and its relatives, considering the genomic location and phylogenetic and syntenic relationships of the DHN genes between different diploid subgenomes (*Ta/Td/Tu-A*, *Ta/Td-B*, and *Ta/Aet-D*). (i) Each DHN gene name starts with an abbreviation for the species name. For example, *T. aestivum* (Ta), followed by the abbreviation of dehydrin gene family: DHN; (ii) the gene names include an A, B, or D, indicating the subgenome where they are located. For example, *TaDHN1-A*; (iii) putative homologs between subgenomes have identical gene names except for the subgenome identifier or species name (e.g., *TaDHN7-A*, *TaDHN7-B*, *TaDHN7-D*, *TdDHN7-A*, *TdDHN7-B*, *TuDHN7*, and *AetDHN7*); (iv) tandem genes are consecutively numbered (e.g., *TaDHN4-A1* and *TaDHN4-A2*).

Phylogenetic and syteny analysis

All identified GST protein sequences were aligned using the MUSCLE [53] program with default parameters. Phylogenetic trees were constructed using MEGA X software with the neighbor joining method and the following parameters: bootstrap (1,000 replicates) and the Jones-Taylor-Thornton substitution model [54].

All identified DHN genes in wheat and its relatives were located on pseudo-chromosomes based on the physical location information acquired from the genomic database. To understand the relationship between the DHN genes identified in wheat and its relatives at the genomic level, the hexploid bread wheat (*Ta*) and tetraploid durum wheat (*Td*) genomes were split into three and two diploid subgenomes (AA, BB, DD and AA, BB), respectively. A collinear analysis was performed using the five subgenomes with diploid *T. urartu* and *Ae. tauschii* genomes and JCVI software (<https://github.com/tanghaibao/jcvi/wiki>). The results were visualized by Circos [55].

Analysis of DHN gene characteristics

Isoelectric points and molecular weights were determined using ExPASy (<https://web.expasy.org/protparam/>). Subcellular localization of all DHN genes was predicted using the Cell-PLoc (version 2.0) website (<http://www.csbio.sjtu.edu.cn/bioinf/Cell-PLoc-2/>) [56]. Exon-intron structures of the DHN genes in bread wheat and its relatives were displayed using the Gene Structure Display Server (GSDS, <http://gsds.gao-lab.org/index.php>) [57]. The promoter sequences (1,500-bp upstream of the ATG translation start codon) of the DHN genes were extracted from the bread wheat genome sequence (IWGSC v1.0). *Cis*-acting elements were predicted in the PlantCARE database (<http://bioinformatics.psb.ugent.be/webtools/plantcare/html/>) [34], and the promoter sequences are listed in Table S5.

Expression profiles of the DHN genes in RNA-seq

To understand the expression profiles of the DHN genes in different tissues and under different stress conditions, six transcriptome datasets were downloaded from the NCBI (<https://www.ncbi.nlm.nih.gov/>) with accession numbers SRP043554, SRP045409, SRP300360, SRP041017, ERP013829, and ERP107574.

The RNA-seq data accession numbers SRP043554, SRP300360 and ERP013829 involved cold, salt and FHB infections. The SRP045409 data involved drought and heat stress. The SRP041017 data involved stripe rust and powdery mildew. The ERP107574 data were collected from various bread wheat tissues. The expression levels of the DHN genes were quantified as transcripts per kilobase million (TPM). The tpm value was calculated using Kallisto software [58].

Plant cultivation, RNA isolation and RT-PCR

To investigate the expression patterns of the DHN genes in wheat under different hormone treatments, *T. aestivum* cv. Chinese Spring was used for the reverse transcription-polymerase chain reaction (RT-PCR) analysis. Bread wheat was planted in a growth chamber at 23°C under a 16 h/8 h (light/dark) photoperiod. Then, 2-week-old seedlings were transferred to a hormone treatment solution containing 100 µM ABA, 50 µM GA, 100 µM SA, or 100 µM MeJA. The leaf tissues were harvested at 0, 3, 6, and 12 h and stored at -80°C after being frozen in liquid nitrogen. Total RNA of all samples was extracted using the RNAprep Pure Plant Kit (TIANGEN, Beijing, China) according to the manufacturer's instructions. cDNA was generated with a one-step reverse transcription kit

(TIANGEN). The Lightcycler 96 system (Roche, Mannheim, Germany) was used for the RT-PCR assay with the SYBR qPCR Master Mix (Vazyme, Nanjing, China); three technical replicates were carried out. Primer information is shown in Table S6.

Three-dimensional modeling and interaction network construction

The I-TASSER program (v5.1) was used to predict the three-dimensional structures of selected DHN proteins [59]. STRING website (<https://string-db.org/>) was used to analyze the interaction of DHN proteins with a confidence parameter set at 0.4 threshold.

Subcellular localization

Gene-specific primers were designed to amplify the coding sequences of the two selected *TaDHN* genes (Table S7). Amplified fragments were ligated in-frame to the 5'-terminus with the expression vector pEGAD-GFP. Then, Constructed plasmids were infiltrated into abaxial air space of six-week-old *N. benthamiana* leaves using the transformed *Agrobacterium* strain *GV3101*. Infiltrated parts of the leaves were marked and fluorescence was observed under the confocal laser scanning microscope (Leica, German) after 48 h of infiltration.

Declarations

Availability of data and materials

The datasets used during the current study are available from NCBI (<https://www.ncbi.nlm.nih.gov/>) with accession numbers SRP043554, SRP045409, SRP300360, SRP041017, ERP013829, and ERP107574.

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Contributions

HWW designed the research; YCH analyzed the data. MH performed the experiments; YCH wrote the manuscript, HWW revised the manuscript. All authors have read and approved the final version of the manuscript.

Ethics declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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Figures

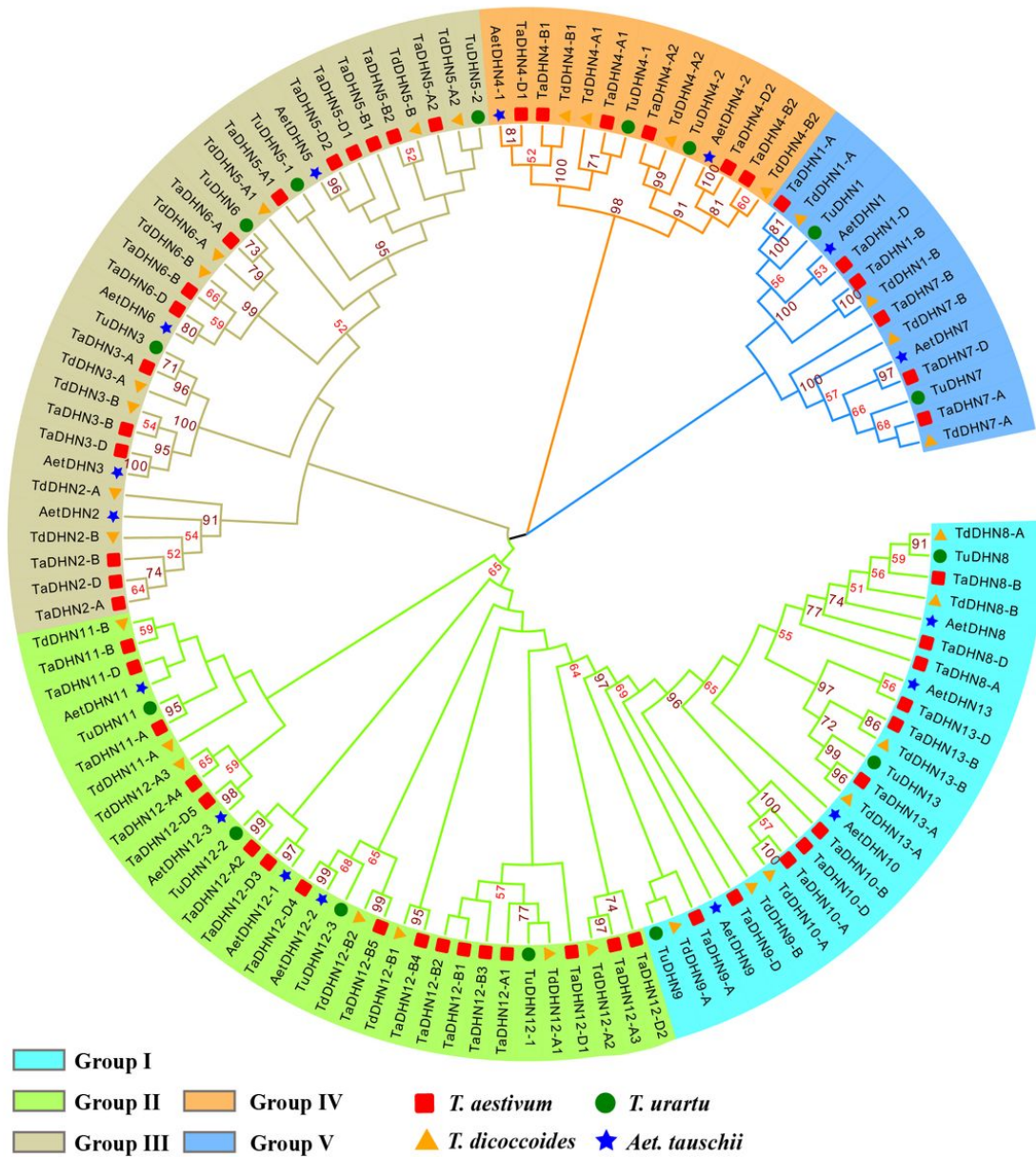


Figure 1

Phylogenetic analysis of the DHN genes in *Triticum aestivum* and its relatives. The phylogenetic tree was constructed using the neighbor-joining method and MEGA-X software; bootstrap scores > 50% are displayed; Different symbols represent different species, and different background colors represent different groups.

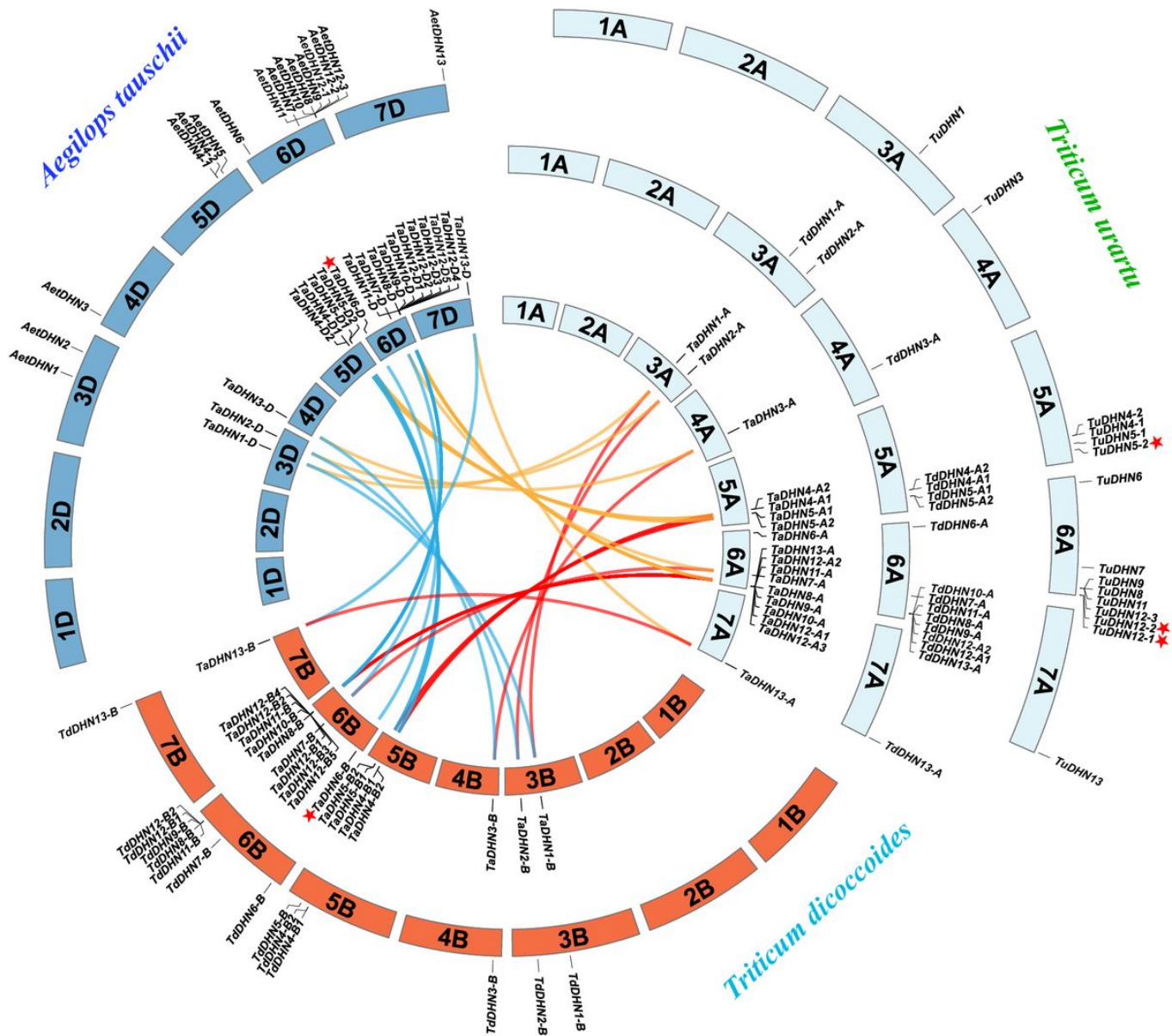


Figure 2

Syntenic analysis and chromosomal distribution of DHN genes in bread wheat and its relatives. The hexaploid bread wheat (Ta) and tetraploid durum wheat (Td) genomes were split into three and two diploid subgenomes, respectively. The A, B, and D genomes are represented by different colors. The DHN genes were assigned to the corresponding chromosome, according to their genome annotation file, except TaDHN6B/D, TuDHN5-2, and TuDHN12-1/2, which are labeled with a red star, and their positions are predicted by their homologs. Syntenic DHN gene pairs belonging to the same linkage groups between A and B, A and D, and B and D are linked with orange, red, and blue lines, respectively.

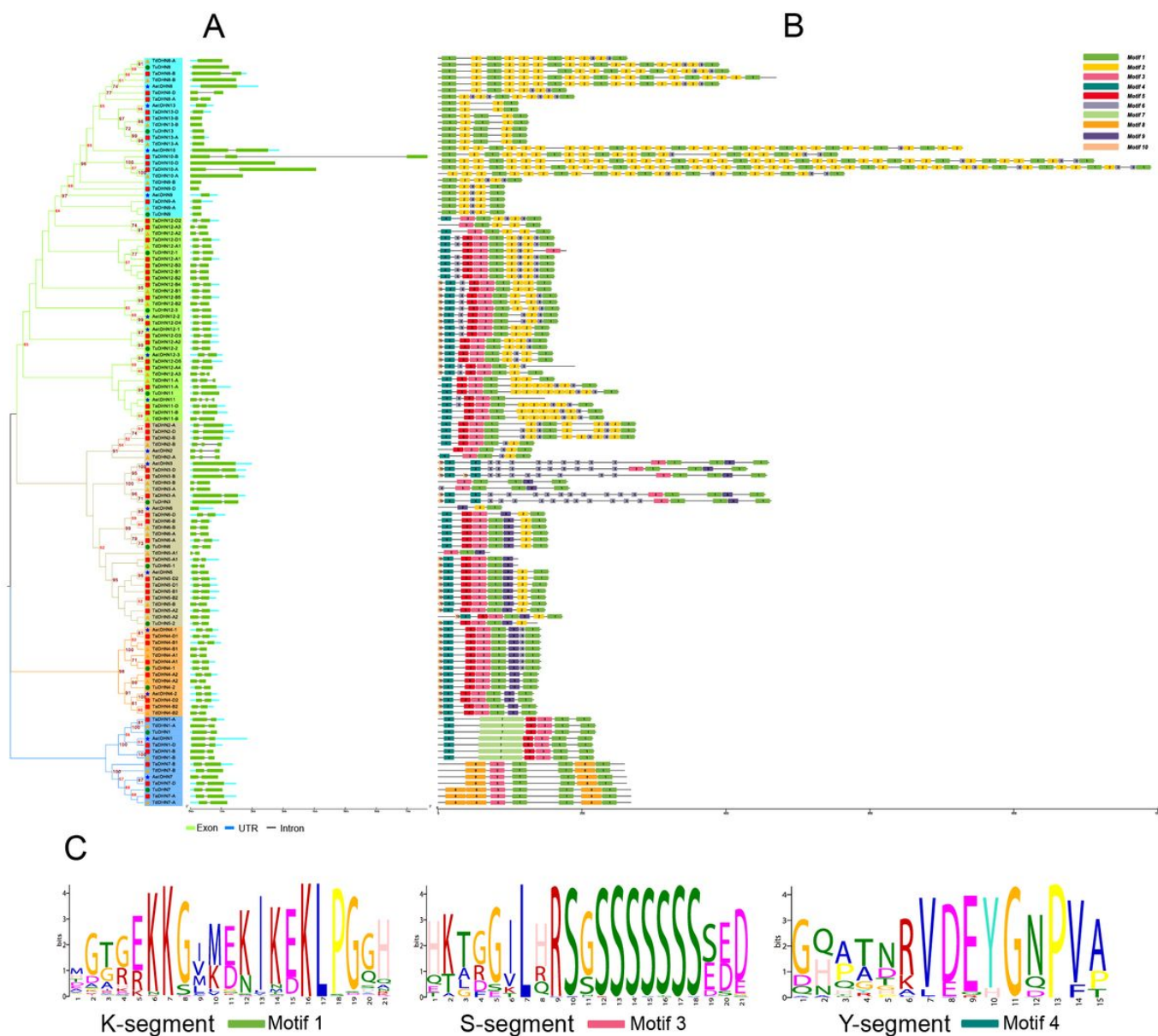


Figure 3

Sequence information of the DHN genes among bread wheat and its relatives. (A) Exon-intron structures. Green boxes, light blue boxes, and black horizontal lines indicate exons, UTRs, and introns, respectively. (B) Conserved domain composition. Dehydrin core domain (K-segment) and the other two dehydrin-related domains (S-segment and Y-segment) are shown in different colors. (C) Logos of the dehydrin-conserved domains.

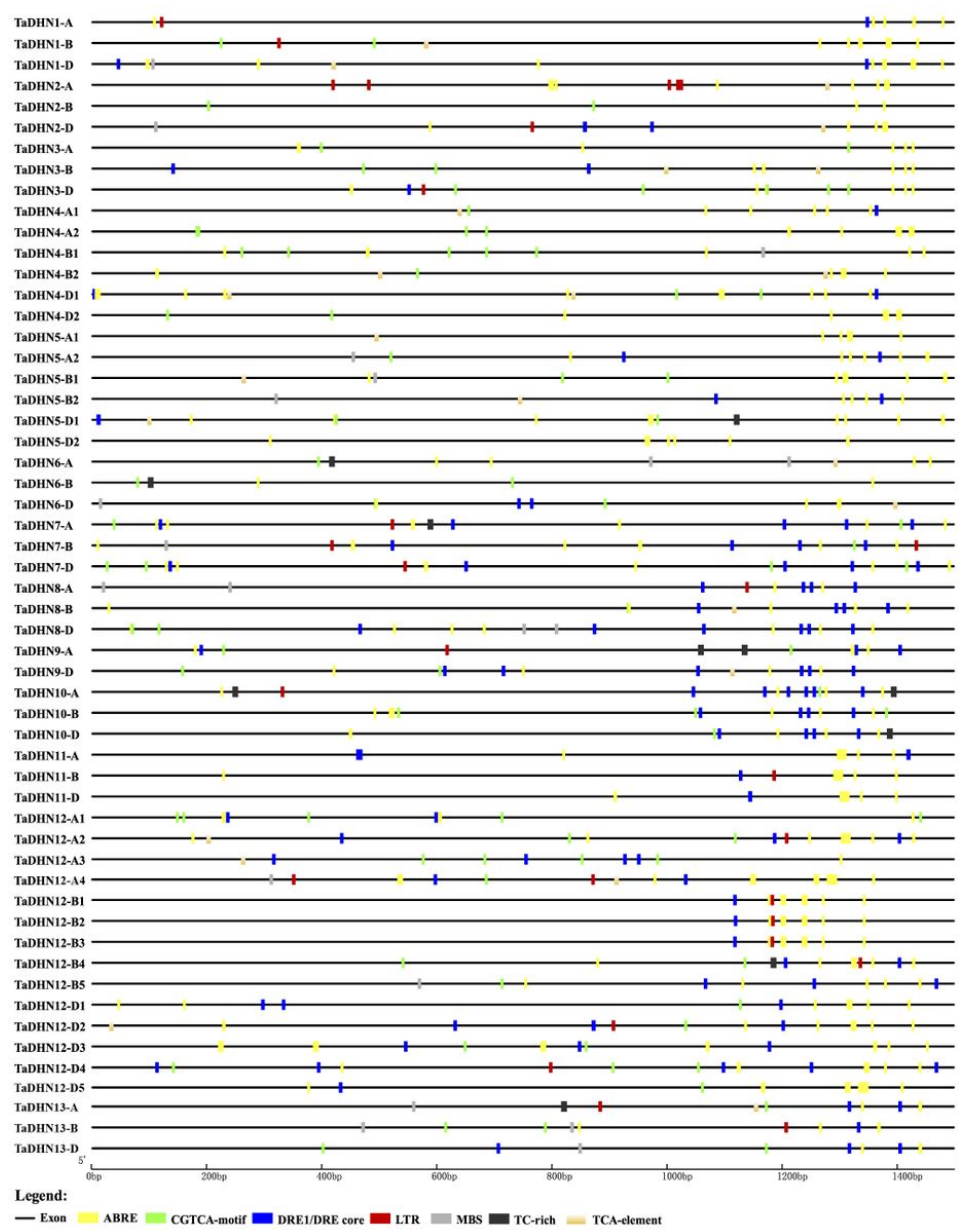
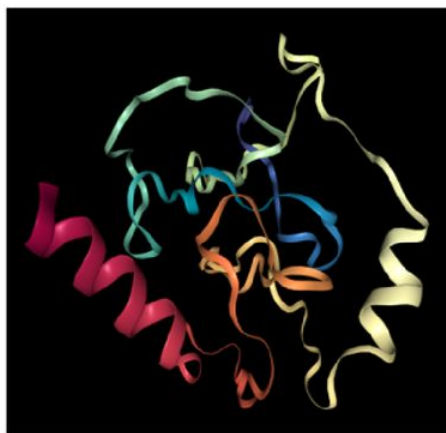
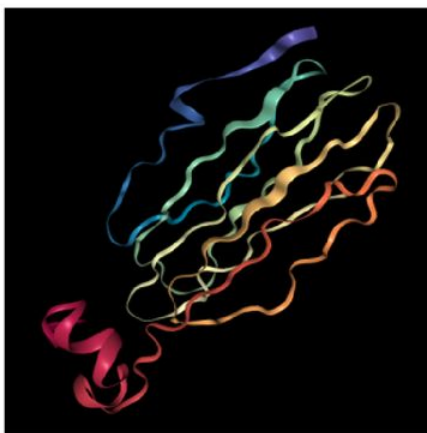


Figure 4

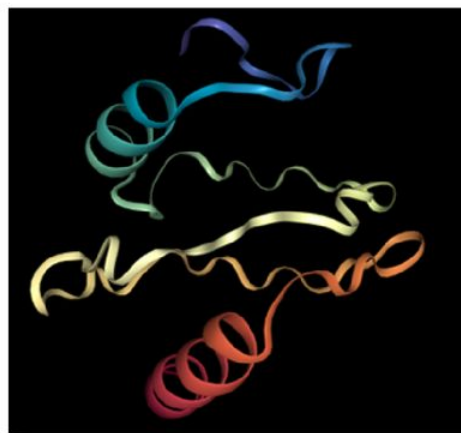
Potential cis-acting elements in the TaDHN gene promoter regions. Eight stress-related cis-acting elements in the TaDHN gene promoter regions were analyzed. Different cis-acting elements are represented by boxes with different colors.



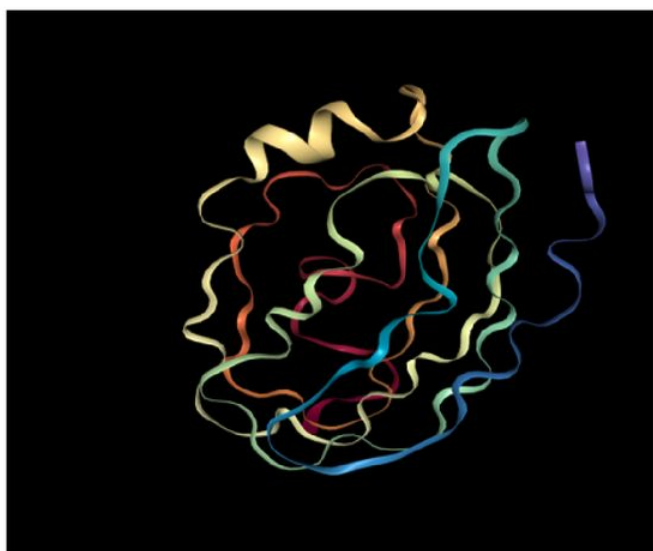
Group I



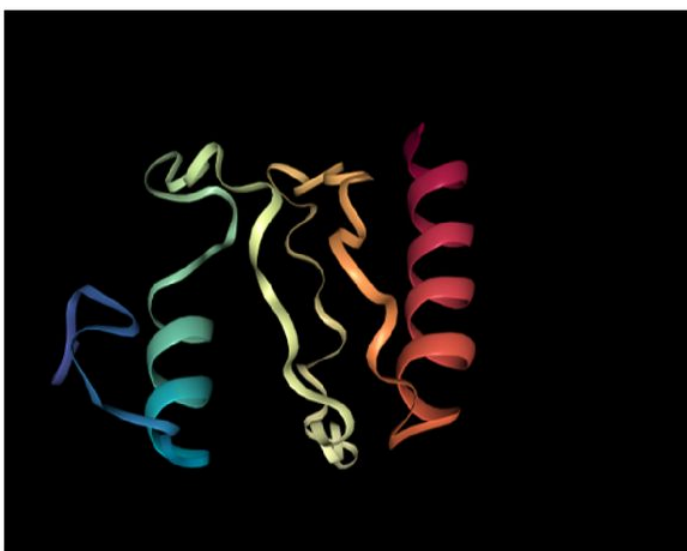
Group II



Group III



Group IV



Group V

Figure 5

Three-dimensional modeling of DHN proteins in wheat. The DHN proteins were selected from 5 groups for structure prediction and display. The displayed DHN proteins are TaDHN13-A, TaDHN12-A1, TaDHN5-A1, TaDHN4-A1 and TaDHN7-A.

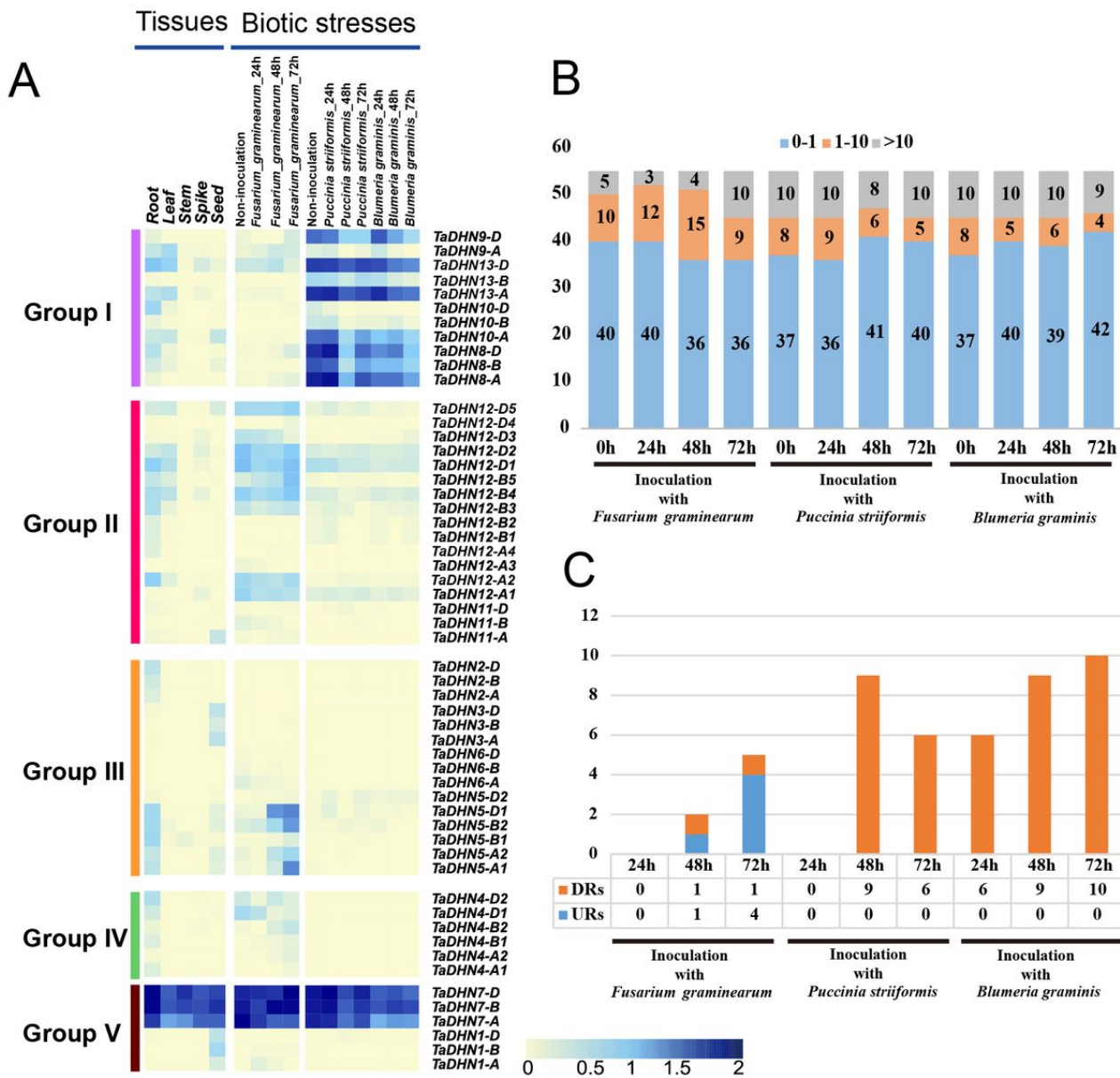


Figure 6

Expression analysis of TaDHN genes in various tissues and stress treatments. (A) The expression profiles of the TaDHN genes in different tissues under biotic stress. The expression data of 55 TaDHN genes were involved in roots, leaves, stems, spikes, seeds, and pathogen infection (*Fusarium graminearum*, Fusarium head blight (FHB); *Blumeria graminis*, powdery mildew; *Puccinia striiformis*, stripe rust) under different treatment times. (B) The number of TaDHN genes corresponding to the three expression levels (tpm: 0–1, 1–10, and >10) under different pathogen infection times. The three expression levels are represented by different colors. (C) The number of up/down-regulated (URs or DRs) genes under different pathogen infection times. The DRs and URs are represented by orange and blue, respectively.

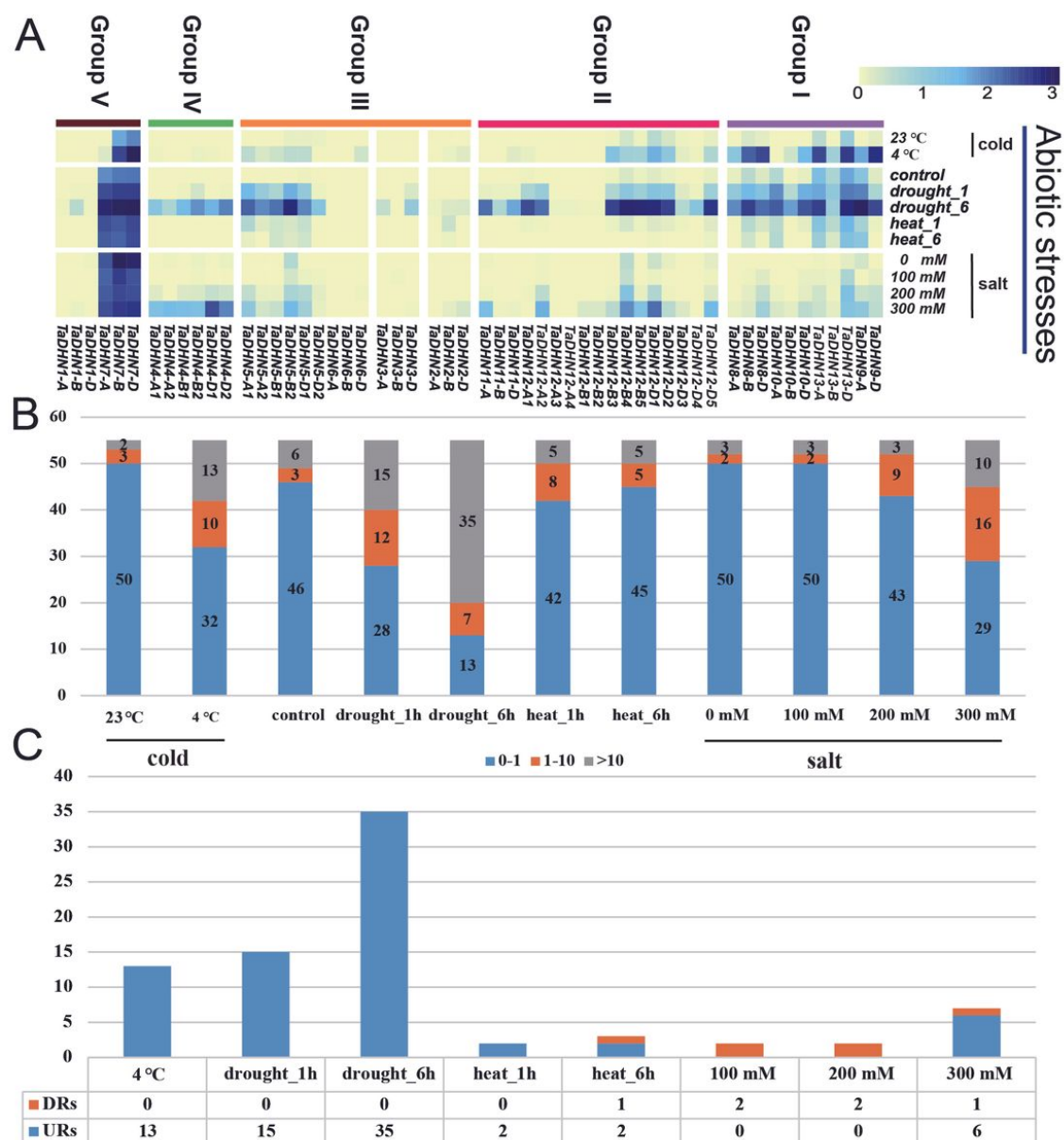


Figure 7

Expression analysis of the TaDHN genes under various abiotic stressors. (A) The expression profiles of the TaDHN genes under the abiotic stress treatments. The expression levels of 55 TaDHN genes changed in response to cold, drought, heat, and salt under different treatment times and concentrations. (B) The numbers of TaDHN genes corresponding to the three expression levels (tpm: 0–1, 1–10, and >10) under different abiotic treatment times. The three expression levels are represented by different colors. (C) The number of up/down-regulated (URs or DRs) genes under different abiotic treatment times. The DRs and URs are represented by orange and blue, respectively.

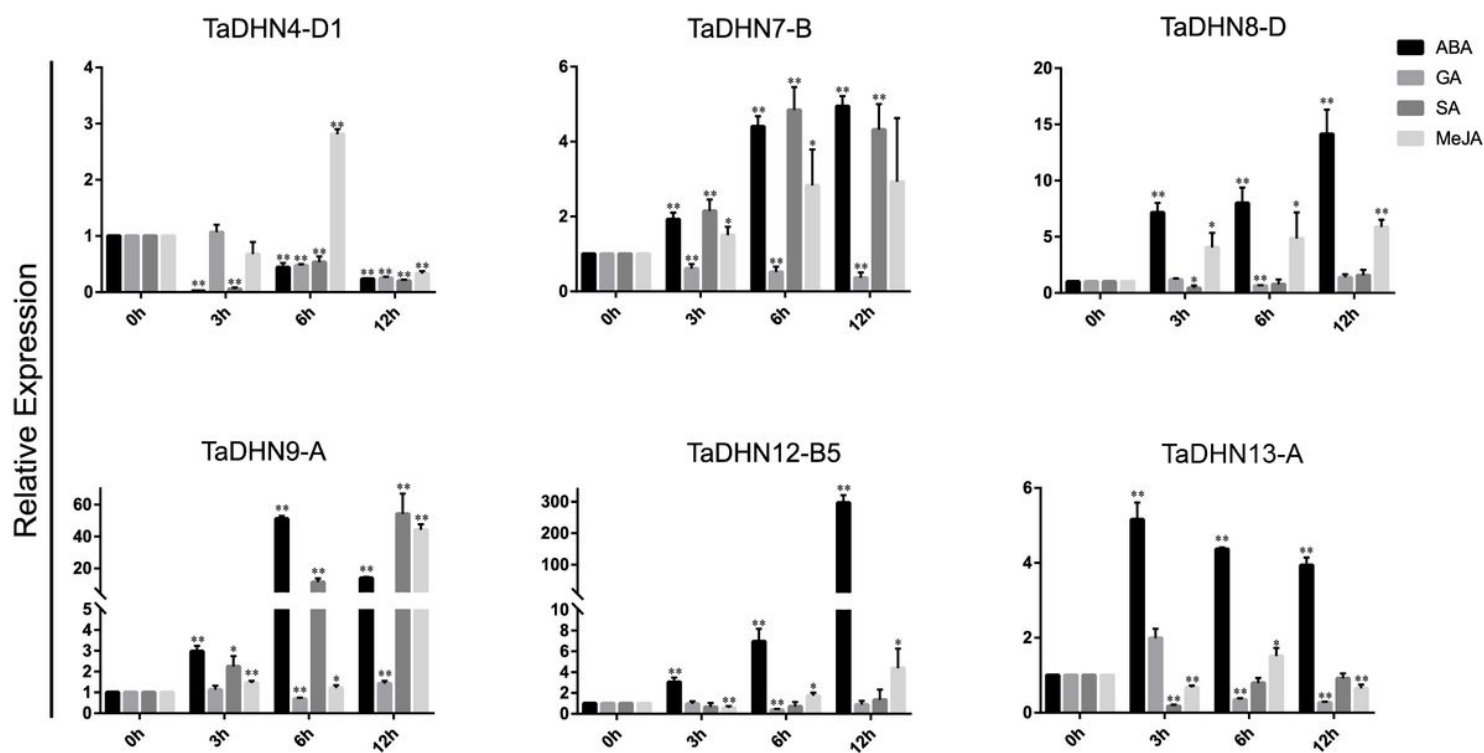


Figure 8

Expression analysis of six selected TaDHN genes under different hormone treatments. Expression profiles of six selected TaDHN genes (including TaDHN4-D1, TaDHN7-B, TaDHN8-D, TaDHN9-A, TaDHN12-B5, and TaDHN13-A) were analyzed under ABA (100 μ M), GA (50 μ M), SA (100 μ M), and MeJA (100 μ M) treatments. Error bars represent standard deviations (SDs) calculated from three independent biological replicates. P < 0.05 (*) and P < 0.01 (**) by Student's t-test.

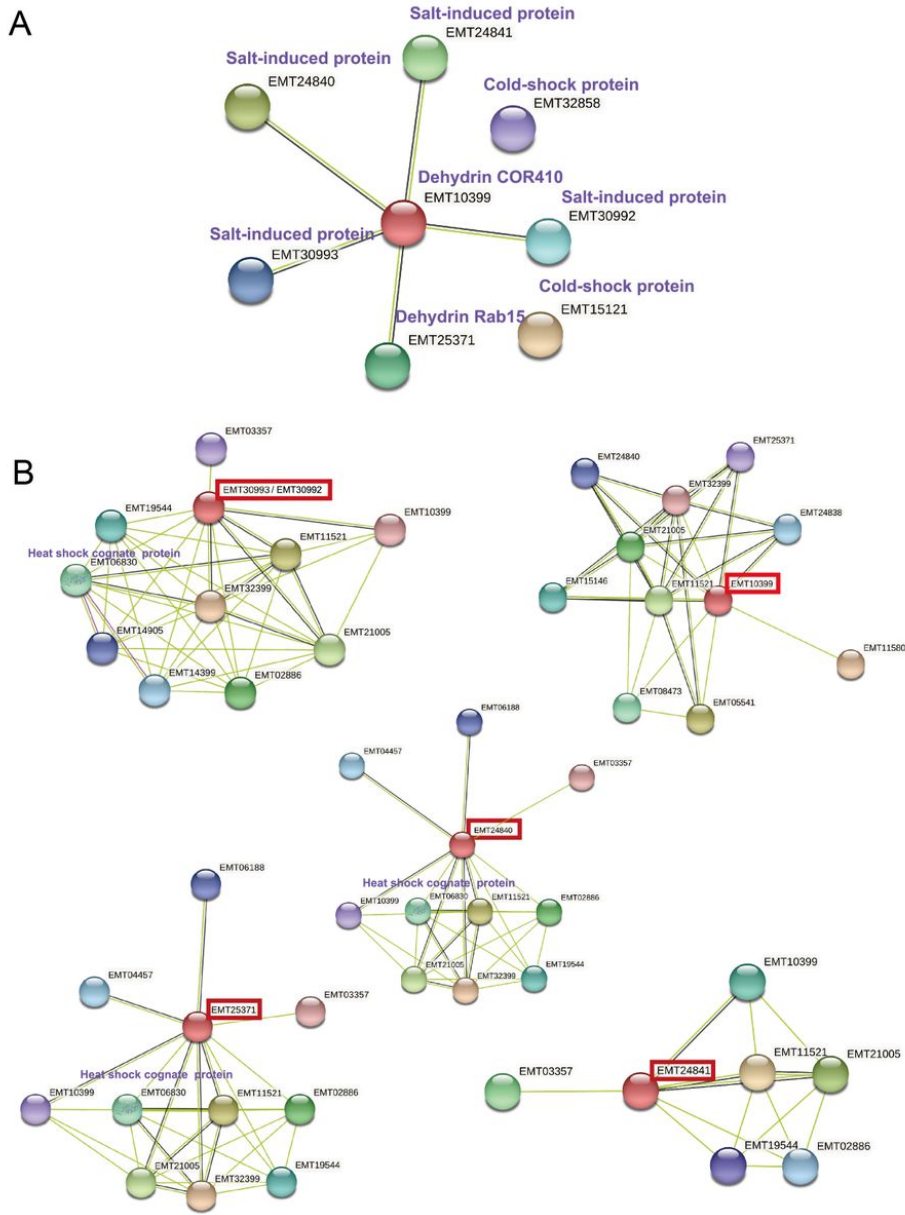


Figure 9

Interaction network of DHN proteins. (A) Interaction network of selected DHN proteins. The blue letters represent the annotation of these proteins. (B) The interaction networks of different DHN proteins. The selected DHN proteins were surrounded by red box, the blue letters represent the annotation of the protein which interacted with DHNs.

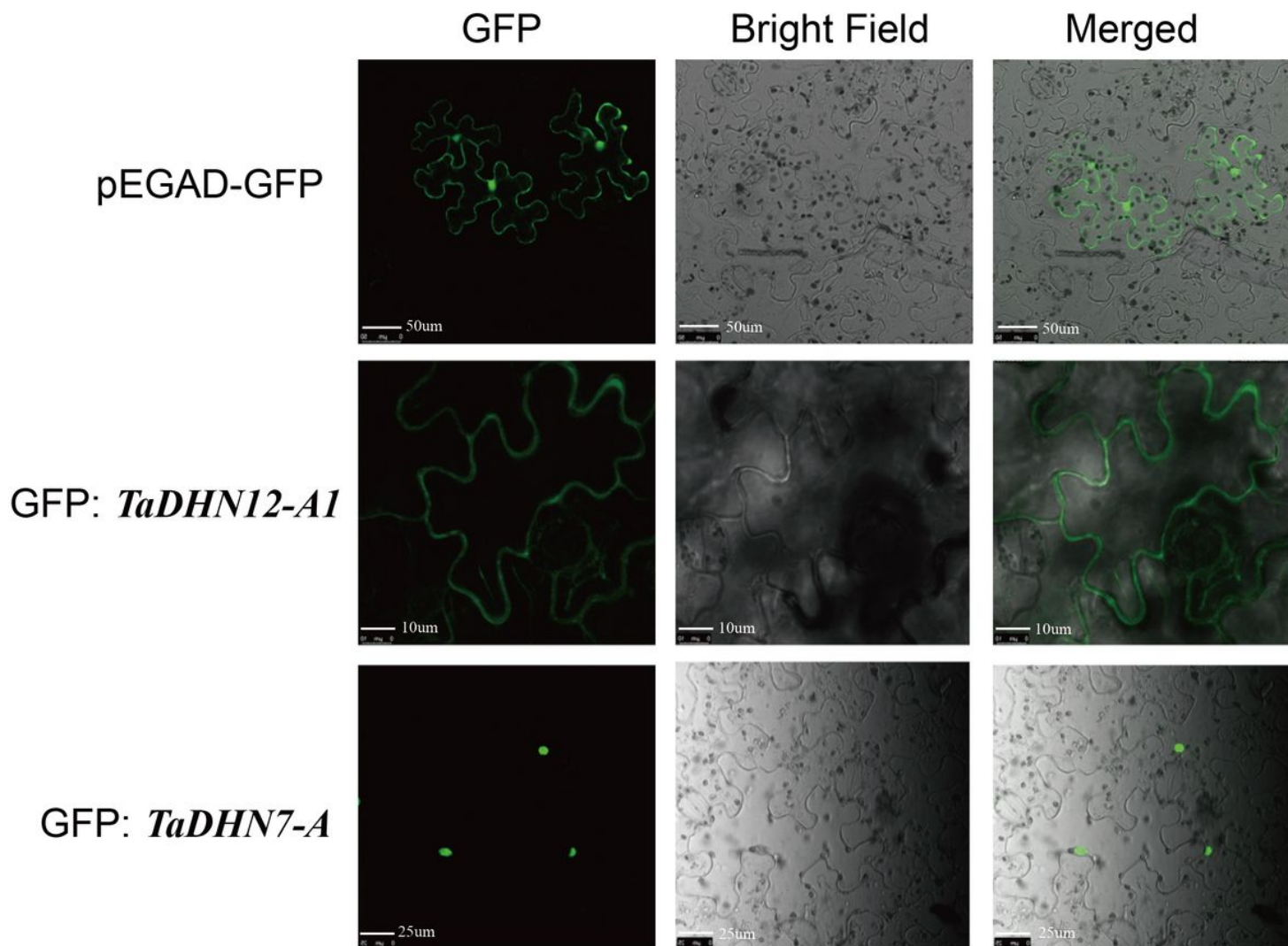


Figure 10

Subcellular localization of selected TaDHN-GFP fusion proteins in *N. benthamiana* leaf epidermal cells.. TaDHN12-A1 and TaDHN7-A were localized to the cytoplasm and nucleus, respectively. pEGAD-GFP was used as a positive control.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryMaterial.zip](#)