*Pak. J. Bot.*, 56(6): DOI: http://dx.doi.org/10.30848/PJB2024-6(13)

# GENOME-WIDE IDENTIFICATION AND EXPRESSION PATTERN ANALYSIS OF *DHN* FAMILY GENES IN MAIZE

**CHUNYAN GAO, XIAOHAN CAO, JIAWEI SHEN, XV WANG, XINYUE JIAO, YANTING GONG, XINYUE PENG AND LIPING REN***

*Biology and Food Engineering College, Fuyang Normal University, Fuyang 236037, Anhui, China*
**Corresponding author's email: renliping@fynu.edu.cn*

**Abstract**

Plant dehydrin (DHN) is a hydrophilic protein widely present in organisms, and it is accumulated when plants encounter adverse environments. Based on the sequence information of *DHN* family genes in *Arabidopsis thaliana*, 13 *DHN* genes were found in maize using the homology sequence alignment method, in which five *DHN* genes were located on chromosome 8. The maize *DHN* gene family can be classified into five branches by evolutionary analysis. The DHN proteins contain five subclasses which are $K_n$, $SK_n$, $K_nS$, $Y_nSK_n$ and $FSK_n$. Analysis of cis-acting elements showed that the promoters of *DHN* gene family harbor cis-acting elements mainly responsive to stress and plant hormones. Tissue expression patterns revealed that almost all *ZmDHN* genes are expressed in the embryo and aleurone layer. DHN proteins were mainly distributed in the nucleus and cytoplasm by subcellular localization prediction. The present findings allow insight into understanding maize *DHN* family genes' functions and facilitate the molecular breeding of *ZmDHN*s in coping with abiotic stress.

**Key words**: Maize, *DHN* gene family, Phylogenetic analysis, Expression patterns.

## Introduction

As one of the world's three major grain crops, maize (*Zea mays* L.) was ranked first in the grain yield worldwide. Except as food and feed, maize could be processed into industrial products such as adhesives, industrial alcohol and fuel ethanol, which made it one of the most significant crops in the world (Jiao *et al*., 2022). A range of environmental factors influence the yield and quality of maize, so mining and identifying stress-responsive genes are urgent and effective for molecular breeding to cope with increasingly severe environmental challenges. Late embryogenesis abundant proteins (LEA proteins) are vitally involved in plant stress which act as protective agents against cell dehydration. The LEA proteins keep the cells glassy and prevent excessive seed dehydration mainly by binding to intracellular sugars (Jia *et al*., 2023). Secondly, LEA proteins can buffer cell ion concentration, regulate the osmotic pressure inside and outside cells, thus stabilize protein structure, and prevent other proteins and cell membranes from aggregating during drought and low-temperature stress (Magwanga *et al*., 2018). LEA proteins are divided into seven subfamilies, and DHN proteins belong to Group 2 LEA family members (Wang *et al*., 2021). They are also known as LEAD II proteins and abscisic acid-responsive (RAB) proteins or water stress proteins (WSP) (Szlachtowska & Rurek, 2023). DHN proteins are hydrophilic proteins widely present in plants. Generally, they consist of 82-575 amino acids, and the molecular weight ranges from 9-200 kD. The primary structure of DHN proteins includes three highly conserved domains: K, S and Y segments. The K segment, an essential structure of DHN proteins, is capable of forming amphipathic α helices by binding to phospholipid vesicles, thereby maintaining the stability of cell membranes. The K segment is located at the C-terminus with abundant lysine (Jia *et al*., 2022). The Y segment contains conserved sequences rich in tyrosine at the N-terminus. The S segment, mainly composed of serine, functions through phosphorylation by kinase. Besides, DHN proteins contain a poorly conserved φ segment with abundant glycine and polar amino acids. Based on the presence and distribution of K, S and Y fragments, DHNs can be classified into five types: $K_n$, $SK_n$, $K_nS$, $Y_nK_n$, and $Y_nSK_n$. These types of structures vary among different plants (Chen *et al*., 2023).

DHN was reported to protect cells from low-temperature and dehydration damage by preserving enzyme activity, reducing the phase transition temperature of the membrane, removing free radicals and reactive oxygen species from cells, and binding heavy metal ions (Shi *et al*., 2020). During oxidative stress, the SbDHN1 protein, a $YSK_2$ dehydrating protein in *Sorghum bicolor*, protected transgenic tobacco from oxidative damage by directly eliminating free radicals and protecting reactive oxygen species scavenging enzymes. As a result, the plants reduced the accumulation of $H_2O_2$ significantly, increased proline content and other antioxidant levels (Halder *et al*., 2018). The *AtHIRD11* gene, a KS-type *DHN* in *Arabidopsis thaliana*, bound to free $Cu^{2+}$ and alleviated the physiological damage caused by heavy metal stress, and then restored the activity of lactate dehydrogenase that had been denatured by $Cu^{2+}$ (Hara *et al*., 2016). The KS-type DHN protein GmERD14 accumulated significantly under low temperature in soybeans (*Glycine max*) (Guo *et al*., 2023). When barley (*Hordeum vulgare*) was subjected to low temperature and freezing, the expression level of DHN13, a KS-type DHN protein, increased significantly (Rodríguez *et al*., 2005). When *Brassica napus* was subjected to cold stress, the content of three types of DHN proteins, which were $SK_n$ (BnLEA10 and BnLEA18), $Y_nK_n$ (BnLEA90), and $Y_nSK_n$ (BnLEA104), increased significantly in cold tolerant varieties (Maryan *et al*., 2019). Moreover, the homologous or heterologous expression of *DHN* genes could also improve the plant's ability to resist abiotic stress. The *AtLTI30* gene in *A. thaliana* positively regulated catalase activity and endogenous proline content, thereby reduced $H_2O_2$ accumulation caused by drought stress and improved the drought tolerance in transgenic *A. thaliana* (Shi *et al*., 2015). The overexpression of *ZmDHN13* in tobacco, induced by $H_2O_2$, resulted in a reduction in the generation of free radicals under oxidative stress, thereby enhanced the oxidative tolerance of the

transgenic tobacco (Liu *et al*., 2017). Overexpression of the soybean *GmDHN9* enhanced drought tolerance in *A. thaliana* by keeping the homeostasis of ROS (Fan *et al*., 2024). Under drought stress, overexpression of the dehydrin gene *MtCAS31* in *Medicago truncatula* promoted autophagic degradation of the plasma membrane intrinsic protein 2;7 (PIP2;7), lessened root hydraulic conductivity and thereby decreased water loss and enhanced drought resistance (Li *et al*., 2020). By knocking out two *DHN* genes in cotton, *GhDHN_03* and *GhDHN_04*, the antioxidant pathways were affected, resulted in a significantly increase in $H_2O_2$ and MDA content. Besides, the expression of stress response genes *GhLEA2, CDKF4* (cyclin-dependent kinase F-4), and *GPCR* (G protein-coupled receptor) were also considerably downregulated. As a result, the plant's tolerance to salt stress is significantly reduced, indicated that the *DHN* genes *GhDHN_03* and *GhDHN_04* were capable of enhancing the cotton's tolerance to salt stress (Kirungu *et al*., 2020).

Maize is widely utilized in genetics and evolutionary research. Although some *DHN* genes have been identified in maize, a systematic analysis of DHN gene family at the whole genome level has not yet been conducted with the improvement and integration of genome databases. In this study, we used *AtDHN* family genes as a reference to identify 13 members of the maize *DHN* gene family in the Zm-B73-REFERENCE-NAM-5.0 genome. The physico-chemical properties, functional domains, phylogenetic relationships of homologous genes, chromosomal localization, promoter cis-acting elements (CREs), collinearity analysis, expression patterns and other aspects of Zm*DHNs* were studied. Our findings provided a foundation for function research in the maize *DHN* gene family.

## Material and Methods

**Identification of *DHN* genes in maize:** The maize Zm-B73-REFERENCE-NAM-5.0 genome, CDS, protein sequence, and annotation files were acquired from the maizeGDB database (https://www.maizegdb.org) , and the *A. thaliana* genome sequences and protein sequences from TAIR10 (https://www.arabidopsis.org/) database. Blast and Hmmer searches were performed by screening in maizeGDB and UniProt and using the *A. thaliana* DHN family protein as a reference (parameters evaluated<1e-5) in the maize protein sequence. After taking the intersection and removing duplicate genes, the selected maize *DHN* gene family members were analyzed using the reference genome's T001 transcript as a reference.

**Analysis of physicochemical properties of ZmDHN family proteins:** The basic protein characteristics of ZmDHN family members, such as the number of amino acids, molecular weight, theoretical isoelectric point, etc., were analyzed by using TBtools software (Chen *et al*., 2020). The lengths of the coding region, exons/introns, were obtained by searching in the Ensembl database (https://plants.ensembl.org/). Subcellular localization was predicted by using Cell-PLoc online software (http://www.csbio.sjtu.edu.cn/bioinf/plant-multi/).

**Prediction of secondary structure and subclass of ZmDHN family protein:** The secondary structure of ZmDHN proteins was analyzed using SOPMA software online (https://npsa-prabi.ibcp.fr/cgi-bin/npsa_automat.pl?page =npsa%20_sopma.html). The subclass structure was classified according to the sequence features of K, Y (or F), and S domains based on the complete amino acid sequences.

**Phylogenetic analysis of ZmDHN family proteins:** Using ClustalX software, the ZmDHN protein sequences were aligned with the DHN protein sequences of *A. thaliana*, *Triticum aestivum*, and *Oryza sativa*. Next, the evolutionary tree of the four species was constructed using the Neighbor-Joining method of MEGAX (parameters: Bootstrap, 1000 iterations), and finally, the evolutionary tree was beautified using iTOL (https://itol.embl.de/).

**Conserved motif, domain, and gene structure analysis of ZmDHNs:** The ZmDHN protein sequences were aligned by using ClustalX software, and then an evolutionary tree was constructed using MEGAX's Neighbor-Joining method (Bootstrap 1000). The protein sequences of ZmDHN were submitted to the MEME Suite website to obtain information on conserved motifs. The ZmDHN protein sequences were submitted to the NCBI CDD website (https://www.ncbi.nlm.nih.gov/Structure/cdd) to obtain prediction information on protein domains. The gene structure information for the *ZmDHN* gene family was extracted from the gene annotation file downloaded from maizeGDB, and the data was visually analyzed using TBtools software.

**Chromosome distribution and synteny analysis in the *ZmDHN* gene family:** The *ZmDHN* genes' chromosomal location information was obtained from the TBtools software. The interspecies collinearity analysis of *DHN* gene families among maize, *A. thaliana*, rice, and within maize was performed using the TBtools software.

**Cis-acting elements analysis in promoter region:** Using TBtools software, the DNA sequence of the first 2000 bp of the initiation codon (ATG) of *DHN* gene family members was extracted. The cis-elements were predicted using PlantCare (https://bioinformatics. psb.ugent.be/ webtools/ plantcare/html/), and visualized (Lescot *et al*., 2002).

**Expression profiling of *ZmDHN*s:** Based on the published transcriptome data (Walley *et al*., 2016), the expression patterns were screened of the *ZmDHN* genes for 23 tissues and organs in different developmental stages. The fragments per kilobase of transcript per million mapped (FPKM) values were transformed and used to draw a heat map by TBtools. If the FPKM showed "NA" (not available), it was replaced by FPKM = 0, and all FPKM values were transformed into $\log_2$ (FPKM+1) to make the heatmap.

## Results and Analysis

**Characterization of *DHN* genes in maize:** By running Blast and Hmmer searches against the protein sequences of

10 *DHN* family genes in *A. thaliana,* a total of 13 *ZmDHN* genes were identified and named *ZmDHN1-ZmDHN7, ZmDHN13-ZmDHN18* in maize (Table 1).

Tables 1 and 2 show that the *DHN* gene family has 1-6 exons. The different number of exons in each phylogenetic group may be caused by alternative splicing, leading to structural differences in different genes. The length of the proteins encoded by the *DHN* gene family in maize ranges from 100 to 326 amino acids, with an isoelectric point between 5.51 and 9.52 and a protein molecular weight of 10423.37-31770.4 Da. Most are hydrophilic and stable, but *ZmDHN3* and *ZmDHN15* are less stable. The *DHN* gene family in maize is unevenly distributed on 7 of its 10 chromosomes, with most (five *DHN* genes) on chromosome 8. Chromosomes 3 and 5 have two genes each, and the remaining four chromosomes have one gene. This indicates the diversity of physicochemical properties of the proteins encoded by this family. Subcellular localization prediction found that 7 of the 13 DHN proteins have nuclear localization signals, and six have cytoplasmic localization signals. ZmDHN6 and ZmDHN7 proteins are also localized in chloroplasts, but ZmDHN6 may also be localized in the cell membrane. ZmDHN6 and ZmDHN7 proteins have three transmembrane domains (Tables 1 and 2).

**Secondary structure and subclass analysis of ZmDHN family proteins:** Previous research has found that the K-segment rich in lysine, is consisted of 15 amino acids (conserved motif: XKXGXX(D/E)KIK(D/E)KXPG) in all dehydrin proteins, located at the C-terminus, and contained highly conserved charged and nonpolar residues. The Y-segment, rich in T/VDEYGNP motifs and located at the N-terminus, is rich in tyrosine and typically has one to three closely spaced copies. The S-segment is a tandem repeat sequence of 3-13 serine residues (with a conserved sequence of SSSSSSED), often followed by a residue of D, E, or K (Chen *et al*., 2023). Many SKn-type dehydrins possess a conserved sequence known as the F-segment, located at the N-terminus, which can form a short amphipathic helix and may have membrane or protein binding properties similar to the K-segment. They also typically contain a conserved motif of D(D/E/Q)(Y/H/F)GNP (Strimbeck G.R. 2017). In light of this, we observed that ZmDHNs encompass all types except for the YnKn-type motif, including Kn (ZmDHN16 and ZmDHN17), SK (ZmDHN6 and ZmDHN7), K2S (ZmDHN13 and ZmDHN14), YnSKn (ZmDHN2, ZmDHN5, and ZmDHN4, ZmDHN1, and ZmDHN18), and FSKn (ZmDHN3 and ZmDHN15). This is consistent with the phylogenetic relationships of ZmDHN proteins. Although the function of dehydrins is still to be determined, each structural type of DHN may be associated with specific functions. The secondary structure analysis displayed α helices, and irregular coils comprised the highest proportion: 11 ZmDHN proteins were irregular coils, while the ZmDHN6 and ZmDHN7 displayed α helices (Table 3).

**Table 1. The sequence information and physicochemical properties of ZmDHNs.**

| Gene ID | Gene name | Location | Number of amino acid | CDS length | Molecular weight |
|---|---|---|---|---|---|
| Zm00001eb285360 | *ZmDHN1* | chr6:148134643-148135795 | 168 | 504 | 17075.48 |
| Zm00001eb376710 | *ZmDHN2* | chr9:20829686-20831292 | 326 | 978 | 31690.09 |
| Zm00001eb187010 | *ZmDHN3* | chr4:159677441-159678859 | 289 | 867 | 31466.47 |
| Zm00001eb348040 | *ZmDHN5* | chr8: 99203907-99204981 | 323 | 969 | 31588.08 |
| Zm00001eb153940 | *ZmDHN6* | chr3:205353689-205358204 | 257 | 771 | 28656.46 |
| Zm00001eb371250 | *ZmDHN7* | chr8: 181883182-181920704 | 253 | 759 | 28296.99 |
| Zm00001eb052790 | *ZmDHN13* | chr1:265841055-265842318 | 100 | 300 | 11312.28 |
| Zm00001eb218350 | *ZmDHN14* | chr5:16294978-16295737 | 108 | 324 | 12199.09 |
| Zm00001eb250120 | *ZmDHN15* | chr5: 198232923-198234455 | 290 | 870 | 31440.76 |
| Zm00001eb348010 | *ZmDHN16* | chr8: 99135743-99136383 | 180 | 540 | 19058.54 |
| Zm00001eb348190 | *ZmDHN4* | chr8: 99899524-99900604 | 325 | 975 | 31770.4 |
| Zm00001eb347970 | *ZmDHN17* | chr8: 99044661-99044986 | 102 | 306 | 10423.37 |
| Zm00001eb155620 | *ZmDHN18* | chr3: 210644563-210645769 | 236 | 708 | 24810.07 |

**Table 2. The sequence information and physicochemical properties of ZmDHNs.**

| Gene name | Exon/intron | Theoretical isoelectric point | Instability index | Aliphatic index | Hydrophilic index | Subcellular localization |
|---|---|---|---|---|---|---|
| *ZmDHN1* | 2/1 | 8.78 | 24.26 | 31.43 | -1.144 | Cytoplasm |
| *ZmDHN2* | 2/1 | 7.37 | 24.17 | 36.04 | -0.798 | Cytoplasm |
| *ZmDHN3* | 2/1 | 5.51 | 57.06 | 54.78 | -1.3 | Nucleus |
| *ZmDHN5* | 2/1 | 7.38 | 24.08 | 39.38 | -0.766 | Cytoplasm. Nucleus |
| *ZmDHN6* | 5/4 | 8.16 | 42.78 | 102.45 | 0.053 | Cell membrane. Chloroplast |
| *ZmDHN7* | 6/5 | 8.45 | 42.39 | 100.2 | 0.031 | Chloroplast. Mitochondrion. Nucleus |
| *ZmDHN13* | 2/1 | 6.59 | 49.45 | 31.2 | -2.075 | Nucleus |
| *ZmDHN14* | 1/0 | 6.22 | 29.71 | 31.57 | -2.158 | Nucleus |
| *ZmDHN15* | 2/1 | 6.05 | 54.2 | 55.93 | -1.25 | Nucleus |
| *ZmDHN16* | 1/0 | 9.52 | 41.08 | 55.94 | -0.711 | Nucleus |
| *ZmDHN4* | 2/1 | 8.85 | 22.61 | 41.23 | -0.735 | Cytoplasm |
| *ZmDHN17* | 1/0 | 7.99 | 33.41 | 27.84 | -1.138 | Cytoplasm |
| *ZmDHN18* | 2/1 | 5.99 | 44.47 | 44.75 | -0.893 | Cytoplasm |

**Table 3. Secondary structure and subclass of ZmDHN family proteins.**

| Gene name | Subclass | α helix (%) | Extended strand (%) | β-turn (%) | Random coil (%) |
|---|---|---|---|---|---|
| *ZmDHN1* | $YSK_2$ | 12.5 | 14.29 | 17.86 | 55.36 |
| *ZmDHN2* | $YSK_3$ | 13.8 | 13.8 | 11.96 | 60.43 |
| *ZmDHN3* | $FSK_2$ | 41.52 | 6.92 | 4.5 | 47.06 |
| *ZmDHN5* | $YSK_3$ | 13.62 | 14.55 | 12.07 | 59.75 |
| *ZmDHN6* | SK | 66.15 | 8.17 | 2.72 | 22.96 |
| *ZmDHN7* | SK | 65.22 | 6.32 | 3.56 | 24.9 |
| *ZmDHN13* | $K_2S$ | 36 | 4 | 6 | 54 |
| *ZmDHN14* | $K_2S$ | 34.26 | 3.7 | 3.7 | 58.33 |
| *ZmDHN15* | $FSK_3$ | 43.79 | 3.1 | 5.17 | 47.93 |
| *ZmDHN16* | $K_3$ | 21.67 | 17.22 | 10 | 51.11 |
| *ZmDHN4* | $YSK_3$ | 13.85 | 15.69 | 16.62 | 53.85 |
| *ZmDHN17* | $K_2$ | 13.73 | 15.69 | 12.75 | 57.84 |
| *ZmDHN18* | $Y_2SK_2$ | 19.49 | 7.2 | 8.47 | 64.83 |

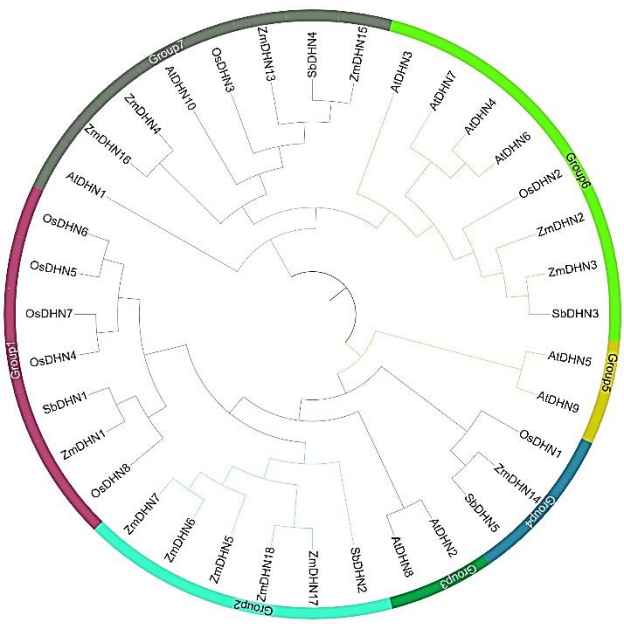


Fig. 1. Phylogenetic tree of DHNs in *Z. mays*, *A. thaliana*, *O. sativa,* and *S. bicolor*.

**Phylogenetic analysis of ZmDHN family proteins:** To gain a deeper understanding of the evolutionary patterns of the *DHN* gene across various species, we constructed a phylogenetic tree (Fig. 1) using the protein sequence data of the *DHN* family genes from *Zea mays*, *Arabidopsis thaliana*, *Oryza sativa*, and *Sorghum bicolor*. The phylogenetic tree divides the *DHN* family genes from different plants into seven groups. Closer branching of two genes indicates higher homology between them. Groups 3 and 5 contain only two *A. thaliana* DHN proteins, while no DHN proteins are found in the other three species. Group 2 includes five maize *DHN* genes and one *Sorghum bicolor DHN2* gene. In groups 1, 4, 6, and 7, maize and sorghum, as monocotyledonous plants, have the closest homology in their DHN proteins. This suggests that the domains of the *DHN* gene remain stable and conserved during plant evolution.

**Conserved motifs, domain, and gene structure analysis of ZmDHNs:** The conservative motifs, domains, and gene structures of the amino acid sequences encoded by the corn *DHN* gene family were analyzed by using TBtools (Fig. 2). Among all ZmDHNs, a lysine-rich K segment, comprising 15 amino acids (EKKGIMDKIKEKLPG), clusters at the C-terminus and contains highly conserved charged and nonpolar amino acid residues. The Y segment (T/VDEYGNP), located at the N-terminus, is rich in tyrosine and has one to three closely spaced copies. The S segment comprises a tandem sequence of 3-13 serine residues. Within the corn *DHN* gene family, the conserved motifs predicted within the same developmental group exhibit homogeneity. For example, *ZmDHN2, ZmDHN4,* and *ZmDHN5* contain eight conserved motifs. The conserved motifs in other developmental groups are consistent or similar. The domains of most *DHN* family genes comprise DHN proteins, but *ZmDHN6* and *ZmDHN7* each contain a reticulon domain, consistent with transmembrane three times and membrane or chloroplast localizations. The composition of introns and exons within genes is an essential basis for determining gene function. Having the same or a similar number of exons in the same developmental group indicates that the amino acid residue evolution of the *DHN* gene family is relatively conserved.

**Chromosomal distributions and synteny analysis in *DHN* gene family:** The gene density (per 1000 kb) is calculated using the starting and ending positions of genes in the gene annotation files. The maize *DHN* gene family is distributed on seven of the ten chromosomes, with five genes on chromosome 8. Except for chromosomes 2, 7, and 10, other chromosomes contain 1-2 genes (Fig. 3). During plant evolution, duplication events play a significant role in amplifying gene family members (Zhou *et al*., 2022). If the spacing between two homologous genes is less than five genes, it is referred to as tandem duplication, and if the spacing is greater than five genes, segmental duplication. We observed that *ZmDHN17* is separated by only three genes (*Zm00001eb347980, Zm00001eb347990,* and *Zm00001eb348000*) from ZmDHN16, and *ZmDHN16* is separated by only two genes (*Zm00001eb348020* and *Zm00001eb348030*) from *ZmDHN5*, indicating that *ZmDHN17, ZmDHN16,* and *ZmDHN5* belong to tandemly duplicated genes.

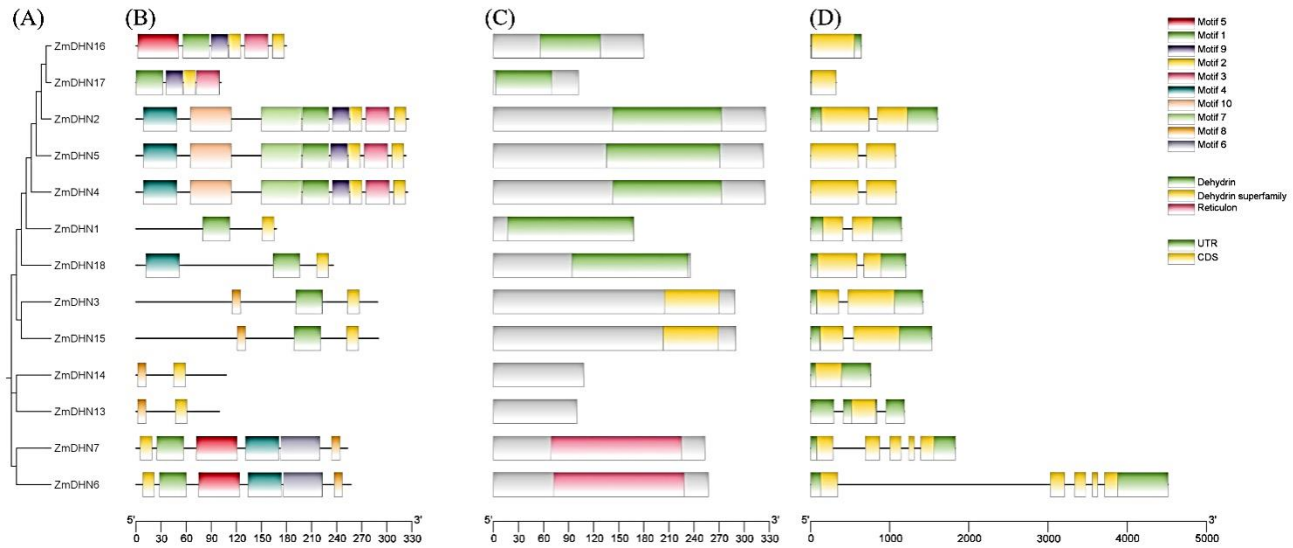


Fig. 2. The phylogenetics (A), conserved motifs (B), domains (C), and gene structure (D) of the *ZmDHN* gene family.

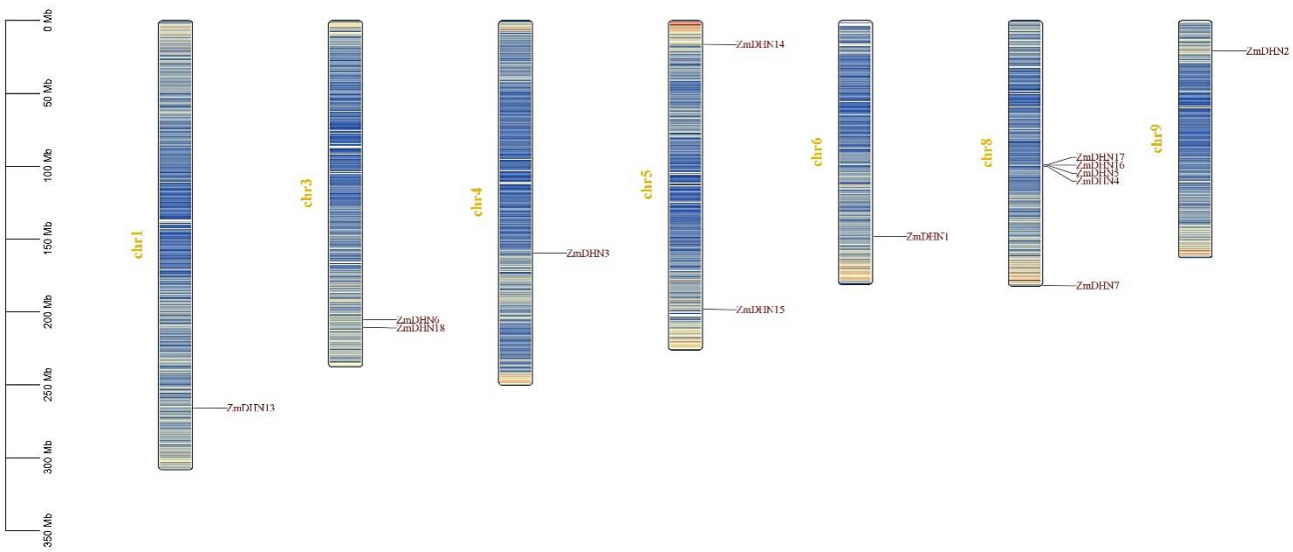


Fig. 3. The distribution of the *DHN* gene family on maize chromosomes.

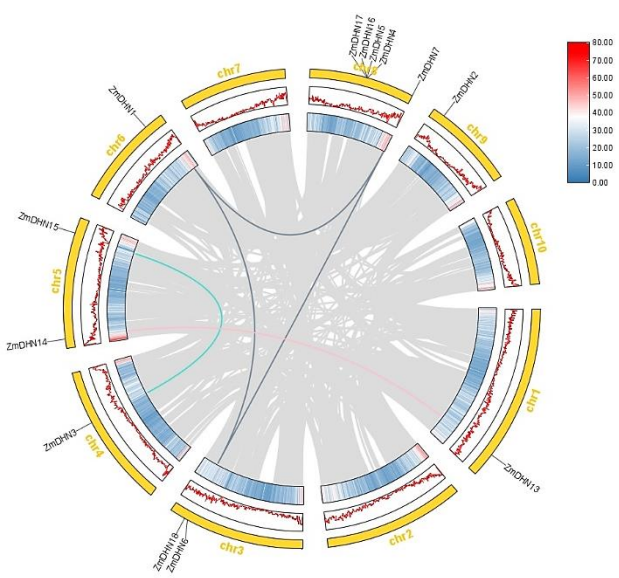


Fig. 4. Synteny analysis in *DHN* gene family of maize.

The collinearity of 13 genes within the *DHN* gene family was analyzed. The lines in the innermost loop in Fig. 4 represent the genes' collinearity, showing five segmental duplications. The first loop represents a heatmap of chromosome gene density, and the second is a line graph of chromosome gene density. The outermost loop displays the chromosome markers (Fig. 4). Through collinearity analysis of the *DHN* gene families in maize, rice, sorghum and Arabidopsis, no maize collinear *DHN* genes were detected in Arabidopsis, while eight pairs were detected in rice and six pairs in sorghum. This suggests that the *DHN* family is highly conserved during the evolution of Poaceae crops (Fig. 5).

**Cis-acting element analysis in promoter region:** Analysis of CREs within promoters provides a foundation for the functional study of genes. Previous studies indicated a certain correlation between the CREs and the specific response of genes (Abdullah *et al*., 2018). Within the promoter regions of the *DHN* gene family, multiple CREs relating to stress, plant

hormones, and growth and development regulation were identified (Fig. 6). Elements responsive to stress include drought-responsive elements (DREs), light-responsive elements, anaerobic induction elements, low-temperature responsive elements (LTRs), and stress-responsive elements. The *DHN* genes may be involved in various stress responses. Among the promoter elements of the maize *DHN* gene family, light-responsive elements are the most numerous and are distributed among all the family members, with an average of 13.2 per gene. Elements responsive to plant hormones include methyl jasmonate (MeJA), gibberellic acid (GA), abscisic acid (ABA), salicylic acid (SA), and auxin (IAA). Some maize *DHN* genes also possess regulatory elements involved in plant growth and development, including seed-specific regulation, meristem expression, endosperm expression, and other elements.

**Expression profiling of *ZmDHN*s:** To investigate the functions of *ZmDHN*s, we analyzed the expression patterns of 13 *ZmDHNs* across 23 different tissues and organs using publicly available transcriptomic data (Walley *et al*., 2016). Based on the gene expression levels within the family, the 13 *ZmDHNs* can be divided into two groups. The first group consists of seven members (*ZmDHN2, ZmDHN18, ZmDHN17, ZmDHN16, ZmDHN4, ZmDHN5,* and *ZmDHN14*) that exhibit low expression levels in most tissues. The second group has relatively higher expression levels and includes *ZmDHN13, ZmDHN3, ZmDHN6, ZmDHN7, ZmDHN15,* and *ZmDHN1*. However, *ZmDHN14* is highly expressed only in maize B73 mature pollen, while four closely related genes (*ZmDHN4, ZmDHN5, ZmDHN16,* and *ZmDHN17*) are not expressed or exhibit very low expression levels across all stages of tissue. Interestingly, almost all *ZmDHN*s are expressed in the embryo (Embryo) and the pericarp/aleurone layer (Pericarp/Aleurone). Some *ZmDHN*s exhibit similar expression patterns, reflecting their close genetic relationship. This is particularly evident for *ZmDHN6* and *ZmDHN7*, suggesting that they may have similar functions in plant growth and development (Fig. 7).

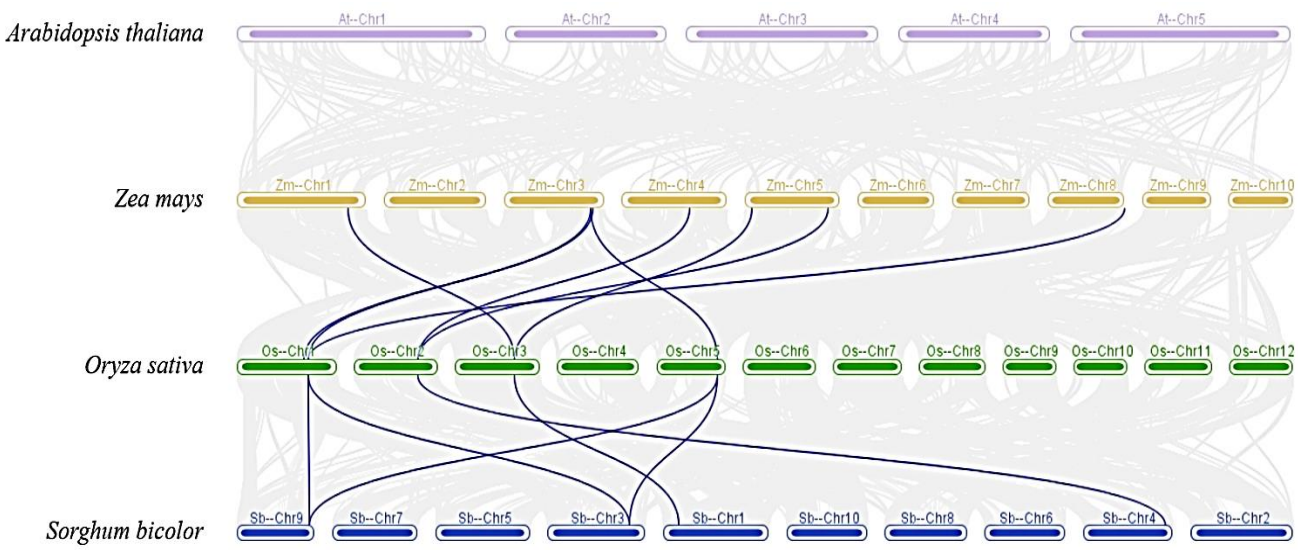


Fig. 5. Synteny analysis in the *DHN* gene family of *Z. mays*, *S. bicolor*, *A. thaliana* and *O. sativa*.

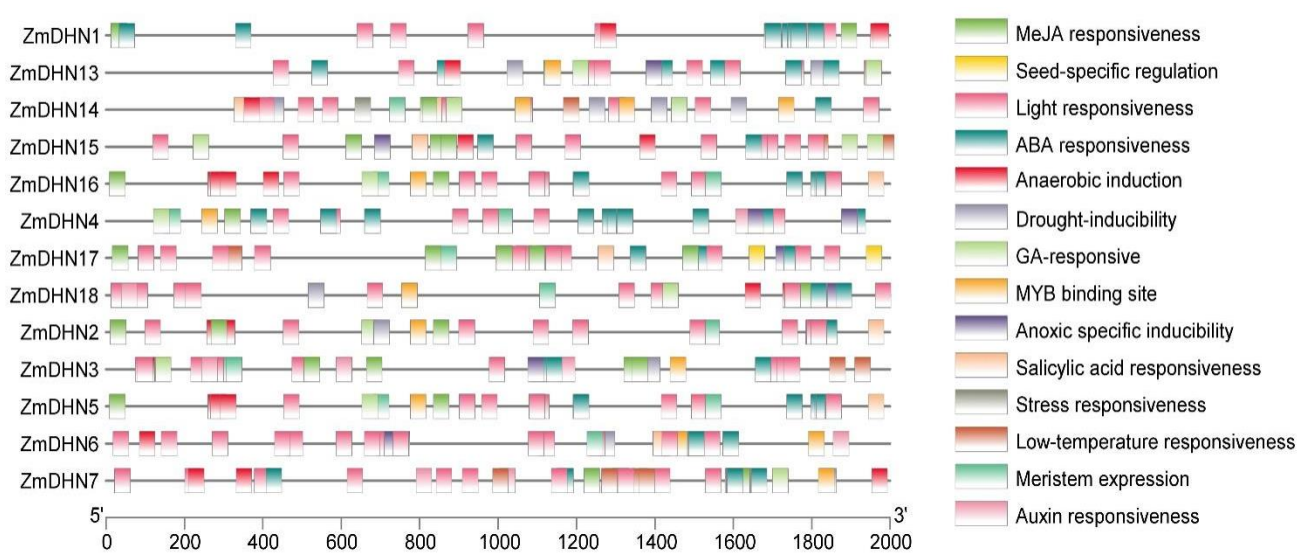


Fig. 6. Cis-acting elements analysis in the promoter region of the *ZmDHN* gene family.
Note: The horizontal axis represents the nucleotide length of the gene promoter; the bar's color represents the different cis-elements in the promoter region.

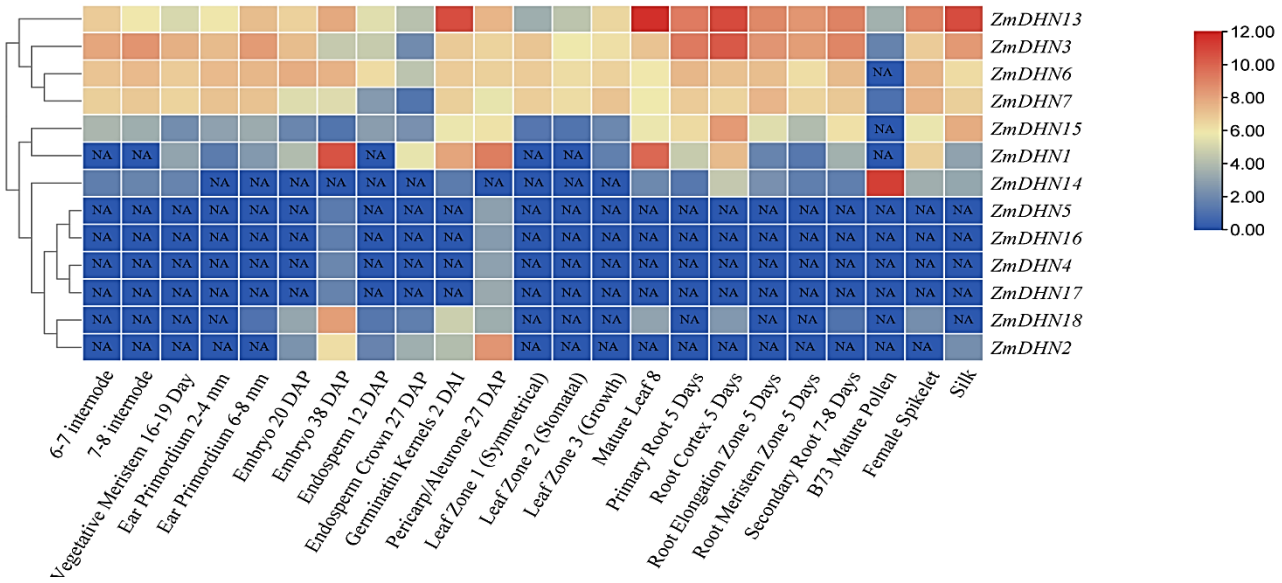


Fig. 7. Silicon expression pattern analysis of the *ZmDHN* genes in different tissues and organs.

## Discussion

DHN proteins have characteristic irregular structures and can effectively resist adverse external environments. Under low water potential in cells, they adsorb water molecules and act as osmoregulators (Bao *et al*., 2017). Studies have shown that *DHN* genes play a crucial role in maize in responding to different abiotic stress conditions. *ZmDHN13* reduces the formation of reactive oxygen species by binding to metal ions and positively regulating the tolerance of transgenic yeast and tobacco to copper stress (Liu *et al*., 2019). Low temperature induces the expression of *ZmDHN2b* in maize, and overexpression of *ZmDHN2b* enhances tobacco's cold tolerance (Zhang *et al*., 2023). Overexpression of *ZmDHN11* could improve the tolerance of transgenic yeast and tobacco to osmotic stress (Ju *et al*., 2021).

With the improvement of genomic data, some *DHN* genes have been discovered in maize, but a systematic analysis of this family is still to be conducted. Given the high accuracy and integrity of the B73 reference genome version 5.0, this study identified 13 maize *DHN* genes, which is similar to the numbers in other species (such as 10 in *Arabidopsis thaliana*, 8 in rice, and 5 in sorghum). *ZmDHN*s have high hydrophilicity, and the hydroxyl group within the molecule forms hydrogen bonds with the polar bonds in cell membrane phospholipid molecules, replacing the position of water, leaving the membrane lipids in a similar non-dehydrated state, thus maintaining the stability of the cell membrane (Jia *et al*., 2023).

Previous studies have shown that different types of dehydrins respond differently to ABA signaling. The expression of KnS, Kn, and SKn type dehydrins is both ABA-dependent and ABA-independent, while the expression of YnSKn and YnKn type dehydrins is ABA-dependent only. The expression of ABA-dependent *DHN* genes is regulated by ABA signaling. These *DHN* genes have ABA-responsive elements (ABRE) or coupling elements (CE) in their promoters, and ABA targets these elements to regulate gene expression (Maruyama *et al*., 2012). On this basis, we speculate that *ZmDHN2*, *ZmDHN5, ZmDHN4*, *ZmDHN1,* and *ZmDHN18* belong to ABA-dependent genes.

Constructing a multi-species phylogenetic tree allowed us to compare the phylogenetic evolution of *DHN* genes in maize, rice, sorghum, and *A. thaliana*. We found that the phylogenetic relationship between maize *DHN* genes and those in rice and sorghum was relatively close, indicating the conservation of monocots in evolution. Conservation domain analysis revealed that *ZmDHN*s contain typical conserved domains of the Y fragment (N-terminal of the protein sequence), S fragment, and K fragment (C-terminal) in the protein sequence. Cis-acting element analysis of promoter sequences revealed multiple functional elements, including LTR, DRE, MYB, G-box, and ABRE, which are associated with light, drought, cold, hormone, and other stress responses. Heterologous expression of *AmDHN200* localized to the cell membrane, *AmDHN154* to the nucleus, and *AmDHN132* to both the cytoplasm and nucleus in *A. thaliana* increased plant cold tolerance, with *AmDHN132* being the most significant (Cui *et al*., 2020). At the same time, we observed diverse tissue expression patterns and subcellular localization of *ZmDHN*s. Previous studies have shown that *ZmDHN*s could maintain fresh weight and improve photosynthesis under drought stress by reducing stomatal density, increasing chlorophyll content, protecting photosystem II and the electron transport chain, maintaining the stability of thylakoid and chloroplast membranes, and protecting plant cells (Xie *et al*., 2012). Therefore, different structural types of *ZmDHN*s suggest that they play specific functions. These findings lay an essential foundation for further studying the evolution of the *ZmDHN* gene family in maize and provide reference information for identifying key *ZmDHN* genes under abiotic stress.

## Acknowledgements

This work was supported by the Fuyang Normal University doctoral talent introduction project (2023KYQD0028), the Biological and Medical Sciences of Applied Summit Nurturing Disciplines in Anhui Province (Anhui Education Secretary Department [2023]13) and the key Supporting Program for Excellent Young Talents of Fuyang Normal University (rcxm202309). We thank Fuyang Hongpeng Cooperation -Fuyang Normal University's horizontal cooperative research project (hx2023081000).

**References**


Abdullah, M., X. Cheng, Y. Cao, X. Su, M.A. Manzoor, J. Gao, Y. Cai and Y. Lin. 2018. Zinc finger-homeodomain transcriptional factors (ZHDs) in upland cotton (*Gossypium hirsutum*): Genome-wide identification and expression analysis in fiber development. *Front Genet*., 9: 357.

Bao, F., D. Du, Y. An, W. Yang, J. Wang, T. Cheng and Q. Zhang. 2017. Overexpression of *Prunus mume* dehydrin genes in tobacco enhances tolerance to cold and drought. *Front Plant Sci*., 8: 151.

Chen, C., H. Chen, Y. Zhang, H.R. Thomas, M.H. Frank, Y. He and R. Xia. 2020. TBtools: An integrative toolkit developed for interactive analyses of big biological data. *Mol Plant*, 13(8): 1194-1202.

Chen, N.; X. Fan, C. Wang, P. Jiao, Z. Jiang, Y. Ma, S. Guan and S. Liu. 2023. Overexpression of ZmDHN15 enhances cold tolerance in Yeast and Arabidopsis. *Int. J. Mol. Sci*., 24(1): 480.

Cui, H., Y. Wang, T. Yu, S. Chen, Y. Chen and C. Lu. 2020. Heterologous expression of three *Ammopiptanthus mongolicus* dehydrin genes confers abiotic stress tolerance in *Arabidopsis thaliana*. *Plants (Basel)*., 9(2): 193.

Fan, J., Y. Zhang, H. Sun, R. Duan, Y. Jiang, X. Wang, Y. Sun, Z. Luo, P. Wang, S. Guan, S. Liu, X. Fan, P. Jiao, Y. Wang, J. Yang, Z. Zhang and H. Yu. 2024. Overexpression of soybean *GmDHN9* gene enhances drought resistance of transgenic Arabidopsis. *GM Crops Food*, 15(1): 118-129.

Guo, B., J. Zhang, C. Yang, L. Dong, H. Ye, B. Valliyodan, H.T. Nguyen and L. Song. 2023. The late embryogenesis abundant proteins in soybean: identification, expression analysis, and the roles of GmLEA4_19 in drought stress. *Int. J. Mol. Sci*., 24(19): 14834.

Halder, T., G. Upadhyaya, C. Basak, A. Das, C. Chakraborty and S. Ray. 2018. Dehydrins impart protection against oxidative stress in transgenic tobacco plants. *Front Plant Sci*., 9: 136.

Hara, M., S. Monna, T. Murata, T. Nakano, S. Amano, M. Nachbar and H. Wätzig. 2016. The Arabidopsis KS-type dehydrin recovers lactate dehydrogenase activity inhibited by copper with the contribution of His residues. *Plant Sci*., 245: 135-142.

Jia, C., B. Guo, B. Wang, X. Li, T. Yang, N. Li, J. Wang and Q. Yu. 2022. The LEA gene family in tomato and its wild relatives: genome-wide identification, structural characterization, expression profiling, and role of SlLEA6 in drought stress. *BMC Plant Biol*., 22(1): 596.

Jia, J.S., N. Ge, Q.Y. Wang, L.T. Zhao, C. Chen and J.W. Chen. 2023. Genome-wide identification and characterization of members of the LEA gene family in *Panax notoginseng* and their transcriptional responses to dehydration of recalcitrant seeds. *BMC Genom*., 24(1): 126.

Jiao, P., Z. Jiang, X. Wei, S. Liu, J. Qu, S. Guan and Y. Ma. 2022. Overexpression of the homeobox-leucine zipper protein ATHB-6 improves the drought tolerance of maize (*Zea mays* L.). *Plant Sci*., 316: 111159.

Ju, H., D. Li, D. Li, X. Yang and Y. Liu. 2021. Overexpression of *ZmDHN11* could enhance transgenic yeast and tobacco tolerance to osmotic stress. *Plant Cell Rep*., 40(9): 1723-1733.

Kirungu, J.N., R.O. Magwanga, L. Pu, X. Cai, Y. Xu, Y. Hou, Y. Zhou, Y. Cai, F. Hao, Z. Zhou, K. Wang and F. Liu. 2020. Knockdown of *Gh_A05G1554* (*GhDHN_03*) and *Gh_D05G1729* (*GhDHN_04*) Dehydrin genes, reveals their potential role in enhancing osmotic and salt tolerance in cotton. *Genomics*, 112(2): 1902-1915.

Lescot, M., P. Déhais, G. Thijs, K. Marchal, Y. Moreau, Y. Van de Peer, P. Rouzé and S. Rombauts. 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(1): 325-327.

Li, X., Q. Liu, H. Feng, J. Deng, R. Zhang, J. Wen, J. Dong and T. Wang. 2020. Dehydrin MtCAS31 promotes autophagic degradation under drought stress. *Autophagy*., 16(5): 862-877.

Liu, Y., D. Li, Q. Song, T. Zhang, D. Li and X. Yang. 2019. The maize late embryogenesis abundant protein ZmDHN13 positively regulates copper tolerance in transgenic yeast and tobacco. *Crop J*., 7: 403-410.

Liu, Y., L. Wang, T. Zhang, X. Yang and D. Li. 2017. Functional characterization of KS-type dehydrin ZmDHN13 and its related conserved domains under oxidative stress. *Sci Rep*., 7(1): 7361.

Magwanga, R.O., P. Lu, J.N. Kirungu, H. Lu, X. Wang, X. Cai, Z. Zhou, Z. Zhang, H. Salih, K. Wang and F. Liu. 2018. Characterization of the late embryogenesis abundant (LEA) proteins family and their role in drought stress tolerance in upland cotton. *BMC Genet*., 19(1): 6.

Maruyama, K., D. Todaka, J. Mizoi, T. Yoshida, S. Kidokoro, S. Matsukura, H. Takasaki, T. Sakurai, Y.Y. Yamamoto, K. Yoshiwara, M. Kojima, H. Sakakibara, K. Shinozaki and K. Yamaguchi-Shinozaki. 2012. Identification of cis-acting promoter elements in cold- and dehydration-induced transcriptional pathways in Arabidopsis, rice and soybean. *DNA Res*., 19(1): 37-49.

Maryan, K.E., H.S. Lahiji, N. Farrokhi and H. Hasani Komeleh. 2019. Analysis of *Brassica napus* dehydrins and their Co-Expression regulatory networks in relation to cold stress. *Gene Exp. Patterns*., 31-17.

Rodríguez, E.M., J.T. Svensson, M. Malatrasi, D.W. Choi and T.J. Close. 2005. Barley *Dhn13* encodes a KS-type dehydrin with constitutive and stress responsive expression. *Theor. Appl. Genet*., 110(5): 852-858.

Shi, H., X. He, Y. Zhao, S. Lu and Z. Guo. 2020. Constitutive expression of a group 3 LEA protein from *Medicago falcata* (MfLEA3) increases cold and drought tolerance in transgenic tobacco. *Plant Cell Rep*., 39: 851-860.

Shi, H.,Y. Chen, Y. Qian and Z. Chan. 2015. Low Temperature-Induced 30 (LTI30) positively regulates drought stress resistance in Arabidopsis: effect on abscisic acid sensitivity and hydrogen peroxide accumulation. *Front Plant Sci*., 6: 893.

Strimbeck, G.R. 2017. Hiding in plain sight: the F segment and other conserved features of seed plant SKn dehydrins. *Planta*, 245(5): 1061-1066.

Szlachtowska., Z. and M. Rurek. 2023. Plant dehydrins and dehydrin-like proteins: characterization and participation in abiotic stress response. *Front. Plant Sci*., 14: 1213188.

Walley, J.W., R.C. Sartor, Z. Shen, R.J. Schmitz, K.J. Wu, M.A .Urich, J.R. Nery, L.G. Smith, J.C. Schnable, J.R. Ecker and S.P. Briggs. 2016. Integration of omic networks in a developmental atlas of maize. *Science*, 353(6301): 814-818.

Wang, X., M. Zhang, B. Xie, X. Jiang and Y. Gai. 2021. Functional characteristics analysis of dehydrins in *Larix kaempferi* under osmotic stress. *Int. J. Mol. Sci*., 22(4): 1715.

Xie, C., R. Zhang, Y. Qu, Z. Miao, Y. Zhang, X. Shen, T. Wang and J. Dong. 2012. Overexpression of *MtCAS31* enhances drought tolerance in transgenic Arabidopsis by reducing stomatal density. *New Phytol*., 195(1): 124-135.

Zhang, Y., X. Zhang, L. Zhu, L. Wang, H. Zhang, X. Zhang, S. Xu and J. Xue. 2023. Identification of the maize LEA gene family and its relationship with kernel dehydration. *Plants (Basel)*., 12(21): 3674.

Zhou, H., D. Hwarari, H. Ma, H. Xu, L. Yang and Y. Luo. 2022. Genomic survey of TCP transcription factors in plants: phylogenomics, evolution and their biology. *Front. Genet*., 13: 1060546.


(Received for publication 10 September 2023)