Genome-wide identification and characterization of the fibrillin gene family in *Triticum aestivum* (#41538)

First revision

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Genome-wide identification and characterization of the fibrillin gene family in *Triticum aestivum*

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Background. Fibrillin (*FBN*) is a highly conserved family of genes that is widely distributed in the photosynthetic organs of plants. Members of this gene family are widely involved in the growth and development of plants and their response to biotic and abiotic stresses. Wheat (*Triticum aestivum)*, which is an important food crop, has a complex genetic background and little progress in the understanding of its molecular mechanism. **Methods.** In this study, we identified 26 *FBN* genes in the whole genome of *T. aestivum* through bioinformatics. These genes were divided into 11 subgroups and distributed on 11 chromosomes of *T. aestivum*. Interestingly, most of TaFBN genes are located on the chromosomes 2A, 2B and 2D. The gene structure of each subgroup of gene family members and the position and number of motifs were highly similar. **Results.** The evolutionary analysis results indicated that the affinities of *FBNs* in monocots were closer together. Tissue-specific analysis revealed that *TaFBN* genes were expressed in different tissues and developmental stages. In addition, some *TaFBNs* were involved in one or more biotic and abiotic stresses. These results provide a basis for further study of the biological function of the *FBNs*.

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Abstract

Background. Fibrillin (*FBN*) is a highly conserved family of genes that is widely distributed in the photosynthetic organs of plants. Members of this gene family are widely involved in the growth and development of plants and their response to biotic and abiotic stresses. Wheat (*Triticum aestivum)*, which is an important food crop, has a complex genetic background and little progress in the understanding of its molecular mechanism.

Methods. In this study, we identified 26 *FBN* genes in the whole genome of *T. aestivum* through bioinformatics methods. These genes were divided into 11 subgroups and distributed on 11 chromosomes of *T. aestivum*. Interestingly, most of *TaFBN* genes are located on the chromosomes 2A, 2B and 2D. The gene structure of each subgroup of gene family members and the position and number of motifs were highly similar.

Results. The evolutionary analysis results indicated that the affinities of *FBNs* in monocots were closer together. Tissue-specific analysis revealed that *TaFBN* genes were expressed in different

tissues and developmental stages. In addition, some *TaFBNs* were involved in one or more biotic and abiotic stresses. These results provide a basis for further study of the biological function of the *FBNs*.

Subjects: Bioinformatics, Genomics, Plant Science

Keywords: Fibrillin, *Triticum aestivum*, Abiotic stress, Gene duplication, Phylogenetic tree, *Cis*-regulatory elements

Introduction

Fibrillins (FBNs) are named after fibrils because these proteins were first detected in fibrils in the chromoplasts of dog rose (*Rosa rugosa*) and bell pepper (*Capsicum annuum*) fruit (*Newman et al., 1989; Deruère et al., 1994; Kim et al., 2015*). Since then, FBN proteins have been increasingly found in different organelles, including plastoglobules (PGs) in the chloroplasts and algal eyespots. Therefore, members of the FBN protein family have been given many different names, including plastid-lipid associated protein (PAP), plastoglobule (PGL), chloroplastic drought-induced stress protein of 34 kDa (CDSP 34), and chromoplast-specific carotenoid-associated protein (ChrC) (*Pozueta-Romero et al., 1997; Ting et al., 1998; Kim et al., 2015*). FBN proteins are located in the photosynthetic organs of cyanobacteria and some higher plants (*Kim et al., 2015; Kim et al., 2017*). Lundquist *et al*. (2012) identified 14 *FBN* genes in *Arabidopsis* by proteomic analysis, 50% of which were located in PGs and the others are mainly distributed in stroma and thylakoid membranes (*Lundquist et al., 2012; Kim et al., 2015*).

So far, the FBN protein family is mainly composed of 12 subfamilies; 11 of these have been found in higher plants and one has been identified in algae (*Lohscheider and Río Bártulos, 2016; Kim et al., 2017*). The members of these subfamilies were found to have similar hydrophobic structures; however, the biophysical properties of these proteins are quite diverse, including proteins with molecular weights of 20–42 kDa and isoelectric point (pI) values of 4–9 (*Vidi et al., 2006; Lundquist et al., 2012*). These findings suggest that each FBN protein may have specific

biological functions. In *Arabidopsis thaliana*, FBN proteins contain a conserved hydrophobic domain (lipocalin motif 1) in the N-terminus and amino acid residues near the C-terminus, including aspartic acid (*Singh et al., 2010*). Furthermore, Lohscheider and Río Bártulos (2016) predicted that the three-dimensional structure of FBNs is similar to that of lipocalin, with the ability to bind and transport small hydrophobic molecules (*Lohscheider and Río Bártulos, 2016*), which suggests that the FBN family may have similar biological functions (*Singh et al., 2010; Francesc et al., 2015; Kim et al., 2015*).

FBN proteins have a variety of important biological functions, such as participating in photosynthesis, the formation of lipoprotein structures, and responses to abiotic and biotic stresses (*Kim et al., 2015*). Immunogold electron microscopy revealed that fibrillins is located on the outer surface of red pepper chromoplast fibrils (*Deruère et al., 1994*). Furthermore, fibril-like structures can be reconstituted *in vitro* from a mixture of FBN protein, lipids, and bicyclic carotenoids (*Deruère et al., 1994; Kim et al., 2015*). Compared to wild-type plants, RNAi-transgenic tomato plants with suppressed *LeChrC* (FBN1) accumulate 30% less carotenoids (*Leitner-Dagan et al., 2006; Singh et al., 2010*). These results suggest that FBNs regulated the formation of chromoplast fibrils and the accumulation of carotenoids. In addition to structural roles, FBNs are also involved in abiotic stress tolerance, especially oxidative stress (*Youssef et al., 2010*). For example, the knockdown of *FBN4* expression in apple and mutation of *FBN4* in *Arabidopsis* caused plants be sensitivity to ozone. (*Singh et al., 2010*). Interestingly, similar results have been reported in cyanobacteria; in *Synechocystis*, the mutants of *pgl1* and *pgl2* were more sensitive to high light stress than was the wild-type (*Cunningham et al., 2010*). Moreover, when *LeChrC* (*FBN1*), *FBI4* and *AtFBN4* was knocked down in tomato, apple, and *Arabidopsis*, the mutant plants were more susceptible to the phytopathogenic fungus *Botrytis cinerea* and pathogenic bacteria *Erwinia amylovora* and *Pseudomonas syringae* pv. *tomato*, respectively (*Cooper et al., 2003; Leitner-Dagan et al., 2006; Singh et al., 2010*). Meanwhile, the expression of *FBN* is regulated by hormones, including gibberellic acid, jasmonate, and abscisic acid, during plant growth and developmental stages, as well as when plants are subjected to stresses (*Yang et*

al., 2006; Youssef et al., 2010; Kim et al., 2017). The *FBN1* and *FBN2* proteins are involved in the jasmonate biosynthesis pathway in response to light and cold stress (*Youssef et al., 2010*). By contrast, *FBN1* mRNA and protein levels declined in red pepper fruit when treated with gibberellic acid (*Deruère et al., 1994*).

Wheat (*Triticum aestivum* L.) is an important food crop that is widely grown around the world. Approximately 40% of the global population depends on *T. aestivum* as their staple food (*Paux et al., 2008; Han et al., 2019*). Common *T. aestivum* is a heterogenous hexaploid containing A, B, and D genomes; therefore, the genome information is large and complex (*Ling et al., 2013; Glover et al., 2015; Han et al., 2019*). Moreover, owing to the complex genetic background of *T. aestivum*, studies have been limited to the genes cloned for regulated important agronomic traits and molecular breeding. Therefore, the study of *T. aestivum* functional genomics is lagging far behind rice and corn. It must be fortunate that the sequencing of the *T. aestivum* genome has been completed; this will play an important role in elucidating the molecular mechanisms involved in growth and development, resistance, and high yield.

Although there is increasing evidence that *FBN*s play major roles in photosynthetic organisms, to date, they have been identified and characterized in few plant species. In addition, the biological functional study of *T. aestivum FBN*s (*TaFBN*s) is limited in wheat. The identification and functional characterization of the *FBN* family in *T. aestivum* will contribute to elucidating the stress response mechanisms. In this study, we performed a genome-wide survey using the reported FBN protein sequences in the *T. aestivum* database. We identified 26 *FBN* genes in *T. aestivum* and used bioinformatic methods to analyze their biophysical properties, including gene structures and conserved motifs, as well as the chromosome distribution and gene duplication of *FBN* genes. In addition, we analyzed the expression profiles of *TaFBN* genes in different tissues, at different developmental stages, and in response to abiotic and biotic stresses using the *T. aestivum* expression database. These results may provide a basis for studying the biological function of the *FBN* gene in different growth and development stages of *T. aestivum*.

Materials & Methods

Plants material cultivation and treatments

The common *T. aestivum* cultivar "Chinese spring" was used in this study. *Triticum aestivum* seeds were sterilized with 1% NaOCl for 15 min, rinsed thoroughly with distilled water five times, and soaked in distilled water overnight at room temperature. The seeds were transferred to filter paper and germinated for three days. The seedlings were cultured in nutrient solution and grown in a growth chamber with 16 h light (22 °C), 8 h dark (18 °C), and 50% humidity. The nutrient solution was replaced every three days at the growth stage. At 21 days old, seedlings were treated with 20% (m/V) PEG 6000 (Sigma-Aldrich, St. Louis, MO, USA) for 6 h. Untreated seedlings were used as a control, and each treatment contained three independent biological replicates. The roots, shoots, and leaves were collected separately for further analysis at 1 and 6 h after treatment.

Identification of *TaFBN* genes

We used protein sequences of *Arabidopsis thaliana FBN* (*AtFBN*) and *Oryza sativa* FBN (*OsFBN*) genes as queries to perform a BLAST (E-value le^{-10}) search against the *T. aestivum* genome database (genome assembly from IWGSC; http://ensembl.gramene.org/). We obtained a dataset of *TaFBN* sequences and filtered out the redundant sequences. The protein sequences of *AtFBN* and *OsFBN* genes were downloaded from the *Arabidopsis* Information Resource database (https://www.arabidopsis.org/) and the Rice Annotation Project database (https://rapdb.dna.affrc.go.jp/). Since a typical FBN protein is reported to contain a conserved PAP_fibrillin domain (PF04755), the online tools SMART (http://smart.embl-heidelberg.de/) and InterProScan (http://www.ebi.ac.uk/interpro/) were used to predict the functional domains of potential TaFBN proteins. To verify our results, all of the proteins were compared to the PAP_fibrillin domain using the HMMER 3.0 program with the default E-value (E-value<10^{-3}). Proteins without the PAP_fibrillin domain were removed. The biophysical properties of the final TaFBN proteins were calculated using the ExPASy ProtParam tool (https://web.expasy.org), including the theoretical values of pI, relative molecular mass, and the grand average of hydrophobicity (GRAVY). The subcellular localization of *TaFBN*s was analyzed using ProComp

(http://linux1.softberry.com) and WoLF PSORT Ⅱ (https://www.genscript.com/wolf-psort.html). In addition, the signal peptide and chloroplast transit peptides of *TaFBN* genes were predicted using the SignalP 4.1 server (http://www.cbs.dtu.dk/services/SignalP-4.1/) and ChloroP 1.1 server (http://www.cbs.dtu.dk/services/ChloroP/).

Multiple sequence alignments and phylogenetic analysis

Full-length protein sequences of *FBN* gene family members identified in 13 plant species, including eight monocotyledon species and five dicotyledon species, were downloaded from the NCBI database (https://www.ncbi.nlm.nih.gov/), the Ensembl Plants database (genome assembly from IWGSC; http://ensembl.gramene.org/), and the Phytozome v12.1 database (https://phytozome.jgi.doe.gov/pz/portal.html). Full-length protein sequences of these *FBN* genes were aligned using MAFFT software (https://mafft.cbrc.jp/alignment/server/). Based on FASTA files, a neighbor-joining phylogenetic tree was constructed using Molecular Evolutionary Genetics Analysis (MEGA) version 7.0 software with bootstrap values of 1000 replicates.

Analysis of gene structures and conserved motifs

To investigate the structure of *TaFBN* genes, we used the Gene Structure Display Server 2.0 database (http://gsds.cbi.pku.edu.cn/) to analyze the distribution of exons and introns in *TaFBN* genes. Conserved motifs were predicted using the Multiple EM for Motif Elicitation (MEME) database (http://alternate.meme-suite.org/; the number of motifs was set to 10 and the motif width was set to 6–50).

Chromosome distribution of FBN genes in *T. aestivumv*

The *TaFBN* gene distribution map was analyzed based on the sequencing genome information of the *TaFBN* gene family. The information on the position of the *TaFBN* gene family on chromosomes was obtained from the Ensembl Plants database (http://plants.ensembl.org/). The distribution of *FBN* genes on chromosomes and the gene duplication of *TaFBN*s were plotted using TBtools software (https://github.com/CJ-Chen/TBtools) (*Chen et al., 2018*).

Analysis of the *cis*-regulatory element of *FBN* gene promoters

In this study, 2000-bp sequences upstream of translational start sites of *TaFBN* genes were set as promoter sequences. PlantCARE software (http://bioinformatics.psb.ugent.be/webtools/plant care/html/) was used to predict the *cis*-regulatory elements based on these promoter sequences. The distribution of *cis*-regulatory elements in the promoter of the *TaFBN* gene was displayed using TBtools software (https://github.com/CJ-Chen/TBtools) (*Chen et al., 2018*).

Analysis of *TaFBN* gene expression patterns

The expression profile data used in this study were obtained via the Wheat Expression Browser database (http://www.wheat-expression.com/) (*Philippa et al., 2016; Ricardo et al., 2018*). We searched for *FBN* genes on the website using the gene ID as query terms. The expression of *TaFBN*s in different tissues, at different developmental stages, and under different abiotic and biotic stress conditions (including drought, cold, heat, and stripe rust) were analyzed. The results were presented as heatmaps, with different colors representing the absolute signal values. The color scale of the heatmap was given in $\log_2$ ratio values. The cultivar used in the gene expression profiles analysis was "Chinese spring".

Total RNA isolation and real-time PCR analysis

Total RNA from different tissues was extracted using TRIzol Reagent (Invitrogen). The total RNA was treated with RNase-free DNase I for 15 min to remove the remaining genomic DNA. First-strand cDNA was synthesized according to the manufacturer's instructions (TOYOBO, Kita-ku, Osaka, Japan), diluted 20 times, and used as a template for quantitative real-time PCR (qRT-PCR), which was performed using AceQ qPCR SYBR Green Master Mix (Vazyme, Nanjing, China). For an endogenous control, we used the *T. aestivum actin* gene (AB181991). At least three biological replicates, with three technical replicates each, were used for each treatment. Relative expression levels were calculated using the comparative $2^{-\Delta\Delta Ct}$ method (*Willems et al., 2008*). The *TaFBN* primers used for qRT-PCR are listed in Table S1.

Results

Identification and characterization of *FBN* genes in *Triticum aestivum*

In this study, a total of 26 *FBN* genes were identified in *T. aestivum*, which we named *TaFBN-A1–TaFBN-D10* according to their genome location (Table 1). The *TaFBN* characteristics, including the chromosomal position, intron number, gene length, number of amino acids, molecular mass, CDS, subcellular localization, signal peptide, and instability index, are listed in Table 1. The results were as follows: the *TaFBN* protein sequences ranged from 219 to 402 amino acids and the molecular weights ranged from 23.75 to 43.59 kDa. The prediction of subcellular location indicated that 18 *TaFBN*s were located in the chloroplasts and eight were located extracellularly. As we know, the GRAVY values reflect the hydrophobicity of the protein; almost all TaFBN proteins has GRAVY values are less than 0, except for *TaFBN-A1*, *TaFBN-B1*, and *TaFBN-B6*. Meanwhile, the prediction results showed that all TaFBN proteins did not contain signal peptides, but chloroplast transit peptides were found in all TaFBN proteins.

Gene structure analysis of *TaFBN* genes

To gain insight into the evolution of the *TaFBN* gene family, a diagram of the *TaFBN* exon–intron gene structure was constructed based on cDNA and genomic DNA sequence information (Supplementary text 1) using the Gene Structure Display Server (Figure. 1b). A neighbor-joining phylogenetic tree was also constructed to explore the relationship of exon–intron distribution patterns and the phylogenetic classification. Gene structure analyses indicated that homologous genes had similar exon–intron distribution patterns (Fig. 1b). However, the number of introns in different *TaFBN* gene family members varied greatly (ranging from 2 to 10 introns), while there was almost no difference between members of the same subfamily. Interestingly, all *TaFBN* genes had conserved PAP_FBN domains (PF04755), and the distribution of domains was consistent with the genetic homology (Fig. 1c, Supplementary Fig. 2). These results suggested that members of the same subfamily may have similar biological functions. In addition, we used the MEME online tool to analyze the conserved motifs of *TaFBN* genes; the results showed that all *TaFBN* members contained five to nine conserved motifs (Fig. 2). The logo representation of the 10 conserved motifs identified for proteins encoded by *TaFBN*

genes is described in Supplementary Fig. 1. Figure 2 showed that motif 1, motif 2, motif 3, motif 4, and motif 5 were highly conserved and widely distributed in all TaFBN proteins. Motif/domain analysis revealed that motif 1 contained conserved amino acid residues in the C-terminal and motif 3 contained a conserved lipocalin motif (Supplementary Fig. 2). The types and distribution of conserved motifs may be the reason for the functional diversity of *TaFBNs*.

Phylogenetic and evolutionary analysis of *TaFBN*

An unrooted phylogenetic tree was constructed for 179 *FBN* genes from eight monocotyledon species (with 26 *FBNs* from *T. aestivum*, 9 from *Oryza sativa*, 11 from *Zea mays*, 10 from *Sorghum bicolor*, 9 from *Panicum hallii*, 20 from *Panicum virgatum*, 10 from *Setaria italica*, and 4 from *Hordeum vulgare*) and five dicotyledon species (with 14 *FBNs* from *A. thaliana*, 17 from *Brassica oleracea* var. *capitata*, 13 from *Nicotiana tabacum*, 21 from *Glycine max*, and 22 from *Coffea arabica*) to study the evolutionary relationships of *TaFBN* members (Fig. 3). Based on the *FBN* gene characteristics of *A. thaliana*, these *FBN* genes can be classified into 11 subfamilies (Group 1 to Group 11). Interestingly, the members of *TaFBN*s were identified into nine subfamilies, each subfamily containing two or three *FBN* genes. The analysis also revealed that the *FBN* genes in monocots (i.e., *T. aestivum*, *O. sativa*, *Z. mays*, *P. hallii*, and *S. bicolor*) were more closely related than those of the dicots (i.e., *A. thaliana*, *B. oleracea* var. *capitata*, and *N. tabacum*).

Analysis of *TaFBN cis*-regulatory elements

To further identify the *cis*-regulatory elements located upstream of the *TaFBN* genes, 2000-bp sequences upstream from translational start sites of putative *TaFBN* gene families were analyzed using the PlantCARE tool. As shown in Fig. 4, many *cis*-regulatory elements were identified in the promoters of *TaFBN* genes. These *cis*-regulatory elements can be divided into three types: hormone response elements, stress response-related elements, and light response-related elements. The hormone response elements, including the methyl jasmonate (MeJA)-responsive, abscisic acid-responsive, gibberellin-responsive, salicylic acid-responsive, and auxin-responsive elements, were widely distributed in promoters of the *TaFBN*s. The responses

to abiotic stress were the light response-related, low temperature response-related, and drought stress-related response elements, respectively. These results suggested that *TaFBN* genes may be involved in photosynthesis, stress responses, and in maintaining the hormone balance in plants, thereby improving the chances for organisms to escape or better cope with the damaging effects of adverse environmental conditions.

Chromosomal location of *TaFBN* in the *T. aestivum* genome

To clarify the distribution of *TaFBN* genes on *T. aestivum* chromosomes, the 26 *TaFBN* genes were mapped onto *T. aestivum* chromosomes; these *TaFBN* genes were distributed on 11 chromosomes (Fig. 5). As shown in Fig. 5, we found that *TaFBN* genes were not randomly distributed on the chromosome; chromosomes 2A, 2B, and 2D contained six *TaFBN* genes; however, the other chromosomes (i.e., 1A, 1B, 1D, 4A, 4B, 4D, 5A, and 5B) had only one *TaFBN* gene. The unequal distribution of *TaFBN* genes on the chromosomes may be caused by gene duplication.

Tissue special expression patterns of *TaFBN*s at different developmental stages

To explore the tissue-specific expression patterns of *TaFBN* genes at different growth and developmental stages in *T. aestivum*, publicly available expression data sets for 26 *TaFBN*s were analyzed to examine the transcription levels in various *T. aestivum* tissues, including the root, shoot, anther, spikelet, and leaf. Most of the *TaFBN* genes can be detected in at least two or more different tissues. The results suggested that *TaFBN* genes may be widely expressed in wheat tissues (Fig. 6a). However, the expression levels of *TaFBN* genes varied among the different tissues. The expression levels of *TaFBN*s in the tissues with high chlorophyll contents (leaf, shoot, and internode) were significantly higher than those in other tissues. As shown in Fig. 6b, the expression levels of *TaFBN* were notably different at different developmental stages. *TaFBN-A1*, *TaFBN-B1*, *TaFBN-A2*, *TaFBN-B2*, *TaFBN-D2*, *TaFBN-A3*, *TaFBN-A6*, *TaFBN-B6*, and *TaFBN-D6* were highly expressed at all developmental stages. However, the expression levels of *TaFBN-B4*, *TaFBN-D5*, *TaFBN-A9*, *TaFBN-B9*, and *TaFBN-D9* were declined at all developmental stages. The expression levels of other *TaFBN* genes did not change significantly

during any of the developmental stages. These data indicated that *TaFBN* genes have tissue-specific expression patterns, and some *TaFBN* genes play a vital role in the growth and developmental stages of *T. aestivum*.

Expression profiles of *TaFBN* genes in response to abiotic stresses

To further clarify the potential functions of *TaFBN* genes under abiotic stress responses, the expression levels of *TaFBN* genes were analyzed under drought, stripe rust, cold, and heat conditions. Most of the *TaFBN* genes could be shown to be involved in the response to one or more abiotic stresses (Fig. 7). The transcripts of *TaFBN-A1*, *TaFBN-B1*, *TaFBN-A2*, *TaFBN-B2*, *TaFBN-D2*, and *TaFBN-B6* were significantly upregulated by drought, stripe rust, cold, and heat treatments. However, the expression levels of *TaFBN-A5*, *TaFBN-B5*, *TaFBN-D5*, *TaFBN-A9*, *TaFBN-B9*, *TaFBN-D9*, *TaFBN-A10*, *TaFBN-B10*, and *TaFBN-D10* were slightly downregulated under drought, stripe rust, cold, and heat stresses. In addition, other *TaFBN*s can be induced to express under some of the stress conditions. The transcription levels of the tested *TaFBN* genes were significantly downregulated under drought stress conditions. These results indicated that *TaFBN* genes might participate in response to abiotic stresses, especially drought, stripe rust, cold, and heat stress in *T. aestivum*.

Validation of *TaFBN*s by qRT-PCR

To further detect the expression level of *TaFBN* genes in different tissues, we selected nine representative genes from the *TaFBN* gene family (*TaFBN-A1*, *TaFBN-B1*, *TaFBN-A2*, *TaFBN-B2*, *TaFBN-D2*, *TaFBN-B5*, *TaFBN-B6*, *TaFBN-A9*, *TaFBN-B9*, and *TaFBN-D9*) based on the results of the expression profile, and analyzed the expression level using qRT-PCR (Fig. 8a). The results showed that the expression of nine *TaFBN*s in the leaves and shoots was significantly higher than that in the roots. We also analyzed the *TaFBN* gene expression in the leaf under drought stress in *T. aestivum* seedlings (Fig. 8b). The results suggest that the expressions of some *TaFBN* genes, such as *TaFBN-A1*, *TaFBN-B1*, *TaFBN-A2*, *TaFBN-B2*, *TaFBN-D2* and *TaFBN-B5*, were induced at different time points under drought stress. However, *TaFBN-A1*, *TaFBN-B1*, *TaFBN-A2*, and *TaFBN-B2* displayed downregulation after drought treatment. In addition, as the

296 treatment time increased, the expression level was significantly upregulated or downregulated.
297 These results are consistent with the data of the above expression profiles.

298 Discussion

299 In this study, we identified 26 *FBN* genes in the *T. aestivum* genome. These genes were
300 distributed on 11 chromosomes and had molecular masses ranging from 23.75 to 43.59 kDa and
301 pI values ranging from 4.59 to 9.61. This diversity suggests that *TaFBN* genes may have specific
302 biological functions in different metabolic processes. Furthermore, the study results indicate that
303 most of the *TaFBN* genes are located on the chloroplast and contained chloroplast transit
304 peptides. This provides strong evidence that various FBNs might participate in photosynthesis.
305 GRAVY was used to calculate the overall hydrophobicity of the protein sequence, with higher
306 positive GRAVY values indicating a greater level of hydrophobicity (*Faya et al., 2015*). Almost
307 all of the *TaFBN* genes' GRAVY values were less than 0, which means that most of the proteins
308 are hydrophilic. In contrast, previous studies have reported that the FBN family can bind to and
309 transport small hydrophobic molecules in *A. thaliana* (*Singh and McNellis., 2011; Kim et al.,*
310 *2015*). However, the specific spatial structure and the percentage of hydrophobic residues may
311 affect the hydrophobicity of proteins (*Dyson et al., 2004*). Therefore, these different results may
312 reflect the biological function diversity of *TaFBN* genes.

313 The phylogenetic tree analysis showed that the number of *FBN* gene family members in *T.*
314 *aestivum* was higher than those in the other monocots and dicots in this study. The reason for this
315 is that *T. aestivum* is an allohexaploid species with three genomes, A, B, and D. Indeed, some
316 reports have proposed that more than 85% of sequences are repeated in the *T. aestivum* genome
317 (*Ling et al., 2013; Glover et al., 2015; Han et al., 2019*). To analyze the evolutionary
318 relationships of *FBN* genes, we constructed a phylogenetic tree with 179 *FBN*s from *T. aestivum*,
319 *O. sativa*, *S. bicolor*, *Z. mays*, *P. hallii*, *P. virgatum*, *S. italica*, *H. vulgare*, *A. thaliana*, *B.*
320 *oleracea* var. *capitate*, *N. tabacum*, *G. max*, and *C. arabica*. These *FBN* genes were divided into
321 11 subfamilies using the classification method described for *FBN* in *A. thaliana* (*Singh and*
322 *McNellis., 2011*). Interestingly, the exon–intron structures and numbers of conserved motifs were

similar in the same subgroups. This phenomenon suggests that *TaFBN* genes in the same subgroup may have similar functions.

Gene expression levels in different tissues and at different developmental stages may be determined by gene function. Previous studies have shown that *FBNs* are regulated by a variety of biological and environmental factors at different growth and developmental stages (*Singh and McNellis, 2011*). We analyzed the expression patterns of the *TaFBN* gene family during different growth and development stages and under biotic and abiotic stresses in *T. aestivum* though publicly available expression data. In these studies, we obtained 26 *TaFBN* gene expression profiles, which showed that most of the genes were highly expressed in the leaf, shoot, and internode. Similar results have been reported in potato, *Arabidopsis*, and *Brassica rapa* (*Monte et al., 1999; Kim et al., 2001; Yang et al., 2006*). Furthermore, the expression profile data suggested that *TaFBN-A1*, *TaFBN-B1*, *TaFBN-A2*, *TaFBN-B2*, *TaFBN-D2*, and *TaFBN-B6* expressions were strongly induced under drought, stripe rust, cold, and heat stresses, but *TaFBN-A5*, *TaFBN-B5*, *TaFBN-D5*, *TaFBN-A9*, *TaFBN-B9*, *TaFBN-D9*, *TaFBN-A10*, *TaFBN-B10*, and *TaFBN-D10* expressions were slightly inhibited under these stresses. In addition, other *TaFBN*s responded to one or more stresses. As we know, transcription factors participate in various biological processes by regulating the expression of downstream gene *cis*-regulatory elements (*Ning et al., 2017*). In this study, a large number of *cis*-regulatory elements were detected in the promoter sequences of *TaFBN* genes. These elements contained light response-related elements, drought response-related elements, and hormone response elements, such as MeJA, abscisic acid, gibberellic acid, salicylic acid, and auxin. Interestingly, all of the *TaFBN* genes include many light response-related elements. For example, Rey *et al.* (2000) found that overexpressing *FBN1* can promote plant height and flowering under high light levels in tobacco (*Rey et al., 2000; Singh and McNellis., 2011*). Although the expression patterns of *TaFBN* genes were varied and complexed, overall, these genes had similar functions in plant stress resistance and chromoplast development (*Singh and McNellis, 2011*).

Conclusion

In this study, we identified 26 *FBN* genes in *T. aestivum* using a genome-wide screening approach. Based on their phylogenetic relationships, these *FBN* genes were classified into 11 subfamilies. The *TaFBN* gene structures and conserved motifs were highly conserved in the same subgroup. A large number of *cis*-regulatory elements were found in the *TaFBN* gene promoter sequences, which showed that the expression of *TaFBN* genes was regulated by various hormones and environmental factors. Moreover, almost all *TaFBN* genes were highly expressed in the leaf, shoot, and internode. The expression profiling data suggest that *TaFBN-A1*, *TaFBN-B1*, *TaFBN-A2*, *TaFBN-B2*, *TaFBN-D2*, and *TaFBN-B6* were responsive to many biotic and abiotic stresses. These results can help us to clarify the structural and functional relationships among *TaFBN* gene family members.

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470
471

Figure 1

Phylogenetic relationship of the *Triticum aestivum fibrillin* (*TaFBN*) genes, exon–intron *TaFBN* gene structure, and functional domain analysis of TaFBN proteins.

(**a**) A phylogenetic tree inferred using the neighbor-joining method in MEGA7, with bootstrap values of 1000, was constructed to determine whether the exon–intron distribution patterns correlated with the phylogenetic classification of *TaFBN* (the same phylogenetic tree is also shown in Figs. 2 and 4). (**b**) The coding sequences (CDS) of exons are indicated by yellow boxes, introns are represented by lines, and blue boxes indicate untranslated regions (UTRs). (**c**) Conserved domains of TaFBN proteins were identified using the Conserved Domain Database of NCBI against the Pfam v30.0 database (https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi).

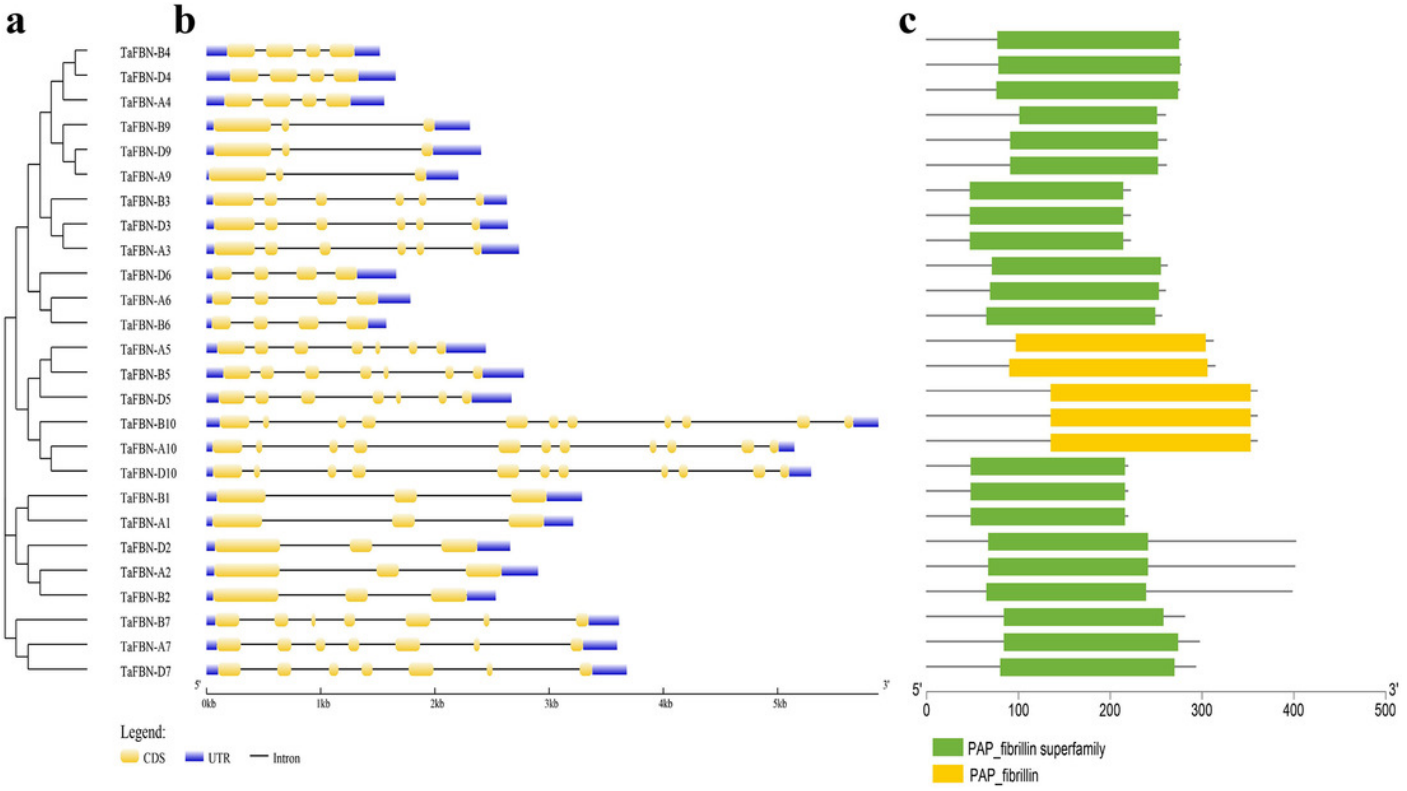

Figure 2

Motif distribution of proteins encoded by *Triticum aestivum fibrillin* (*TaFBN*) genes.

(**a**) The phylogenetic tree of *TaFBN* genes was constructed using the neighbor-joining method in MEGA7, with bootstrap values of 1000. (**b**) Conserved motifs were predicted using Multiple EM for Motif Elicitation (MEME) (http://alternate.meme-suite.org/). Box length indicates the number of amino acids in the motif.

Figure 3

Unrooted phylogenetic tree of all the *Triticum aestivum*, *Oryza sativa*, *Sorghum bicolor*, *Zea mays*, *Panicum hallii*, *Panicum virgatum*, *Setaria italica*□*Hordeum vulgare*□*Arabidopsis thaliana*, [i]Brassica ole

The phylogenetic tree was inferred using the neighbor-joining method in MEGA7, with bootstrap values of 1000. The FBN proteins are clustered into 11 subgroups, which are shown in different colors. Circles and stars indicate dicotyledon and monocotyledon plants, respectively. In addition, different colors represent different species.

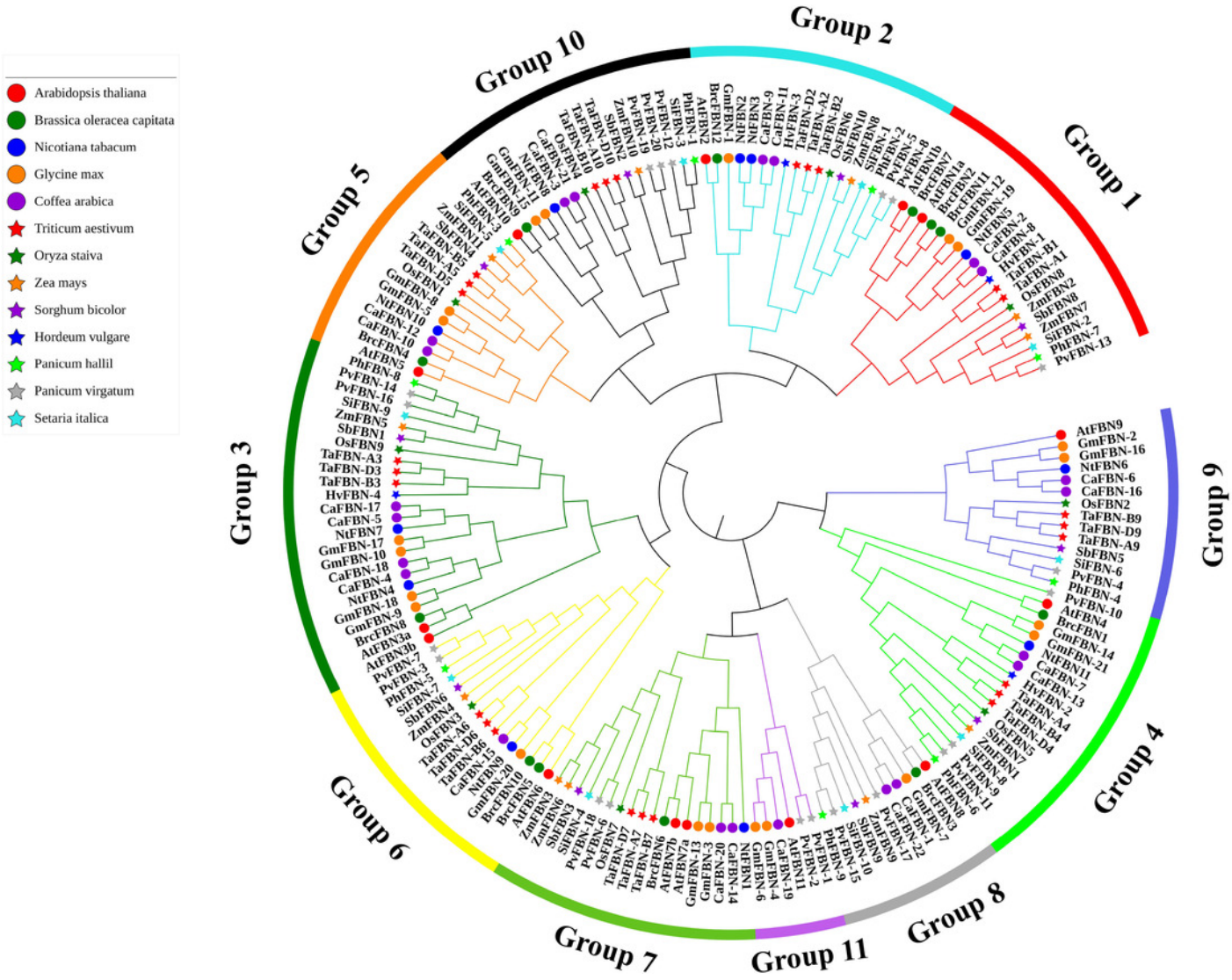

Figure 4

Analysis of *cis*-regulatory elements of *Triticum aestivum fibrillin* (*TaFBN*) gene promoters.

(**a**) A phylogenetic tree inferred using the neighbor-joining method in MEGA7, with bootstrap values of 1000, was constructed to determine whether the exon–intron distribution patterns correlated with the phylogenetic classification of *TaFBN*. (**b**) Promoter sequences (2000-bp) upstream of genes were chosen for *cis*-regulatory element analysis using the PlantCARE online tool (http://www.dna.affrc.go.jp/ PLACE/). Each color indicates a *cis*-regulatory element.

Figure 5

Gene duplication in *Triticum aestivum fibrillin* (*TaFBN*) genes.

A total of 26 *TaFBN* genes were unevenly located on 11 chromosomes.

Figure 6

Expression of *Triticum aestivum fibrillin* (*TaFBN*) in various tissues and developmental stages.

(**a**) Tissue-specific expression of the *TaFBN* gene family in different *Triticum aestivum* tissues; (**b**) expression pattern of the *TaFBN* gene family at different developmental stages; (**c**) a heatmap was created in TBtools software based on the expression data. The color scale represents relative expression levels, with red and green indicating higher and lower levels of expression, respectively.

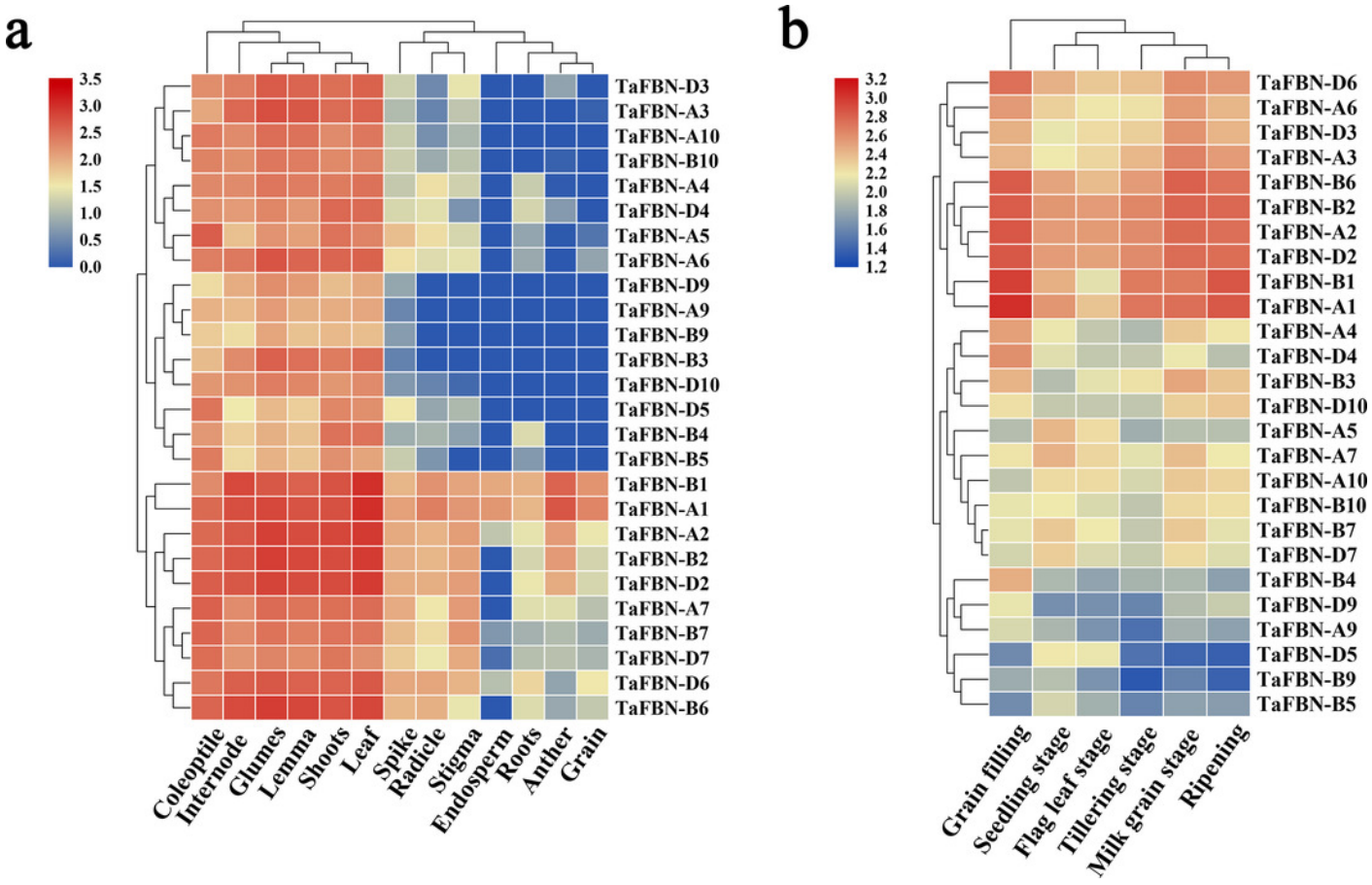

Figure 7

Heat map of expression profiles of *Triticum aestivum fibrillin* (*TaFBN*) genes in *Triticum aestivum* under biotic and abiotic stresses.

Expression levels are indicated in different colors, with red and green indicating higher and lower expression levels, respectively.

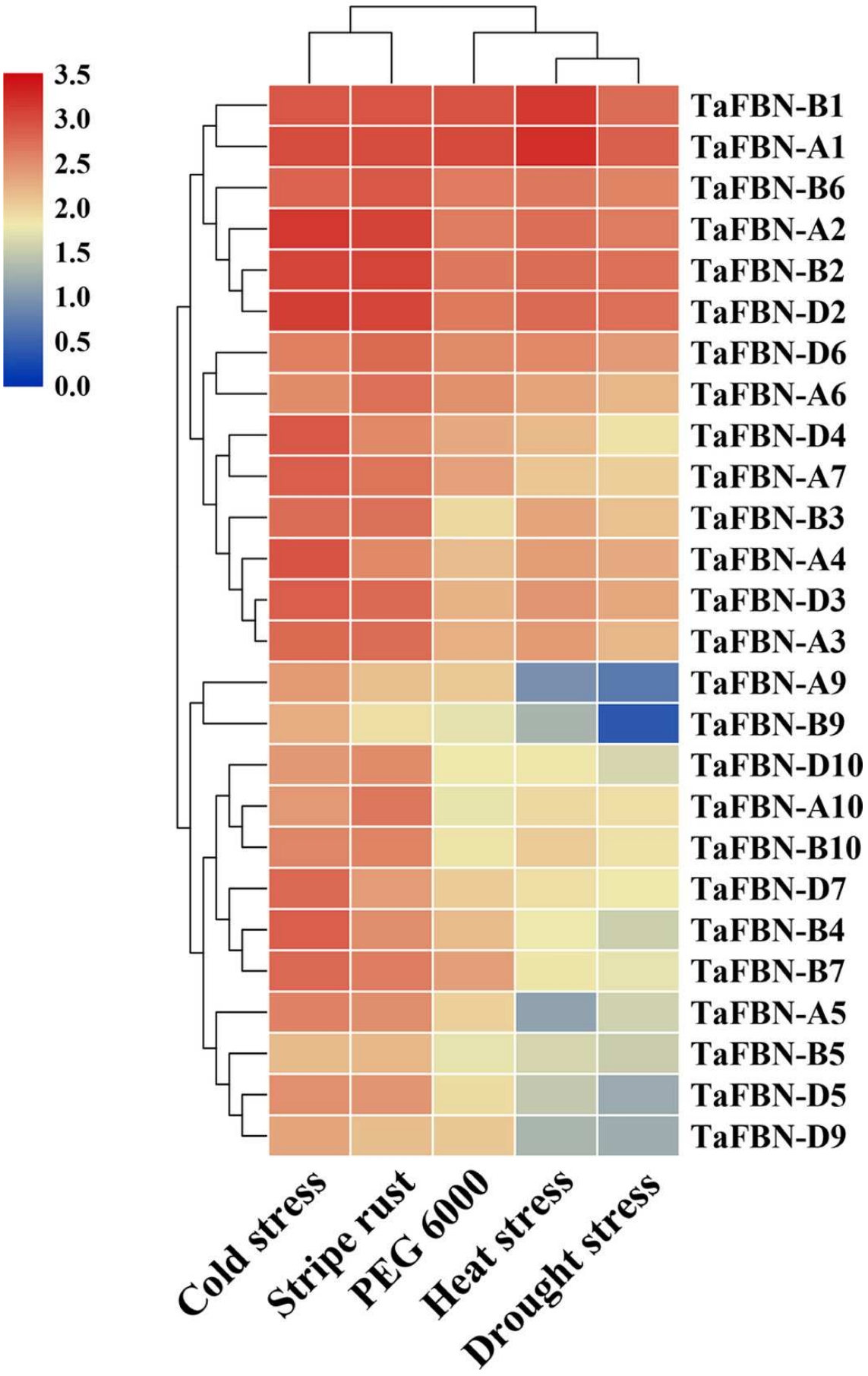
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
TaFBN-B1
TaFBN-A1
TaFBN-B6
TaFBN-A2
TaFBN-B2
TaFBN-D2
TaFBN-D6
TaFBN-A6
TaFBN-D4
TaFBN-A7
TaFBN-B3
TaFBN-A4
TaFBN-D3
TaFBN-A3
TaFBN-A9
TaFBN-B9
TaFBN-D10
TaFBN-A10
TaFBN-B10
TaFBN-D7
TaFBN-B4
TaFBN-B7
TaFBN-A5
TaFBN-B5
TaFBN-D5
TaFBN-D9
Cold stress
Stripe rust
PEG 6000
Heat stress
Drought stress

Figure 8

Expression analysis of *Triticum aestivum fibrillin* (*TaFBN*) genes in different tissues and under drought stress using qRT-PCR.

(a) Relative expression levels of *TaFBN* genes in different tissues. (b) Relative expression levels of *TaFBN* genes in leaves after drought treatment for 1 h and 6 h. 20% (m/v) PEG-6000 was used in this study to simulate drought stress. Each treatment contains three biological replicates. Values and error bars represent mean ± SD values (n = 3, with three technical replicates for each biological replicate). Asterisks (*) indicate significant differences ($P < 0.05$, Student's *t*-test).

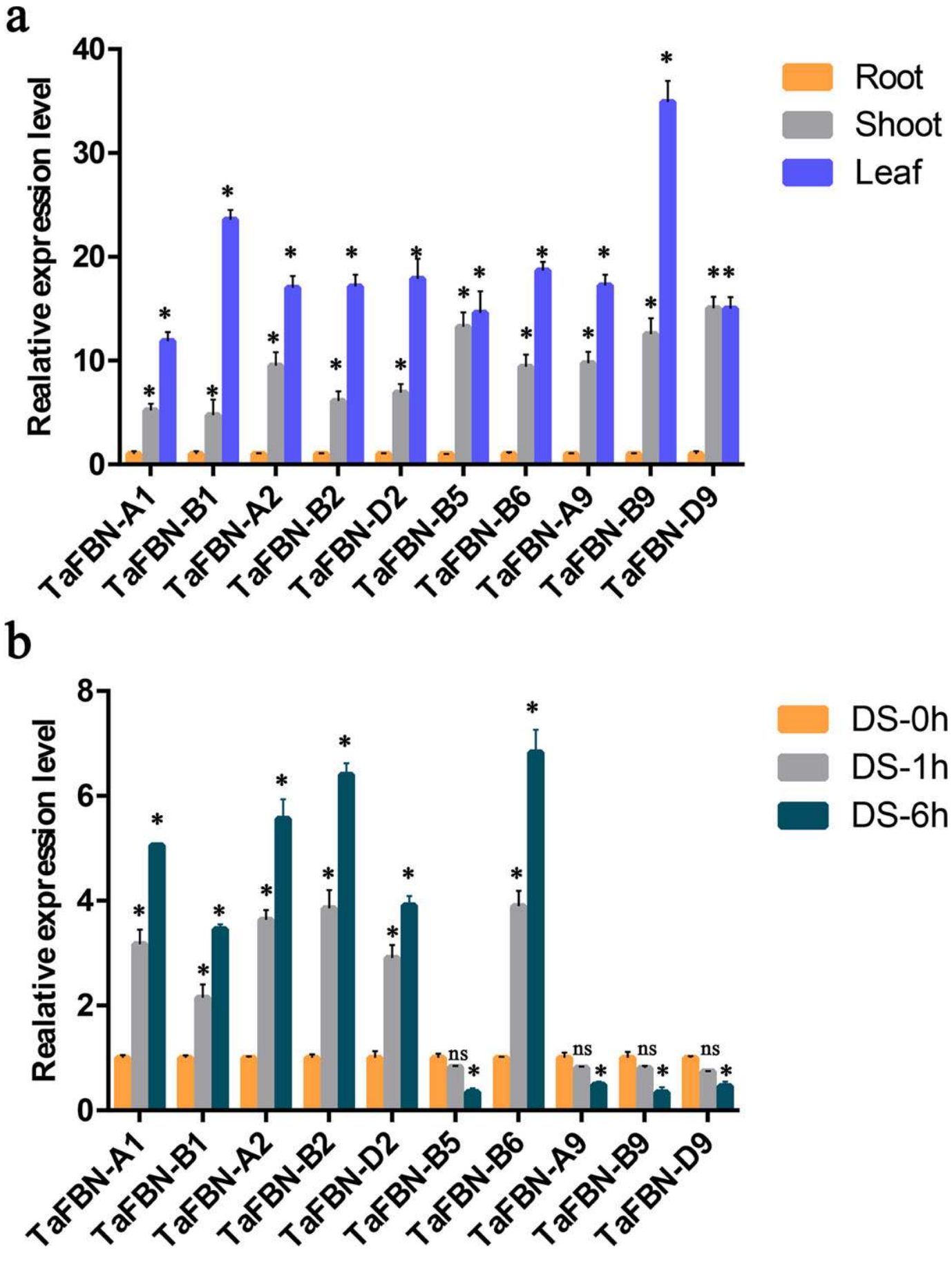
a
Realative expression level
40
30
20
10
0
Root
Shoot
Leaf
TaFBN-A1
TaFBN-B1
TaFBN-A2
TaFBN-B2
TaFBN-D2
TaFBN-B5
TaFBN-B6
TaFBN-A9
TaFBN-B9
TaFBN-D9
b
Realative expression level
8
6
4
2
0
DS-0h
DS-1h
DS-6h
ns
TaFBN-A1
TaFBN-B1
TaFBN-A2
TaFBN-B2
TaFBN-D2
TaFBN-B5
TaFBN-B6
TaFBN-A9
TaFBN-B9
TaFBN-D9

Table 1(on next page)

Fibrillin (*FBN*) gene family in *Triticum aestivum*.

1 Table 1:

Gene name	Sequence ID	Chromosome	Genomic position	Intron number	Gene length (aa)	Molecular weight (kDa)	pI	Predicted pfam domain	Subcellular prediction by PC	Grand average of hydropathicity	Signal peptides	Chloroplast transit peptides
TaFBN-A1	TraesCS5A02G164600.1	Chr5A	353189098-353192310	2	314	33.06	7.77	PAP_fibrillin	Chloroplast	0.039	NA	Y
TaFBN-B1	TraesCS5B02G162100.1	Chr5B	299020240-299020330	2	312	32.94	7.77	PAP_fibrillin	Chloroplast	0.056	NA	Y
TaFBN-A2	TraesCS1A02G193500.1	Chr1A	350749390-350752293	2	360	38.27	4.79	PAP_fibrillin	Chloroplast	-0.261	NA	Y
TaFBN-B2	TraesCS1B02G208500.1	Chr1B	378397002-378399661	2	360	38.33	4.83	PAP_fibrillin	Chloroplast	-0.294	NA	Y
TaFBN-D2	TraesCS1D02G197400.1	Chr1D	278512124-278514657	2	360	38.28	4.79	PAP_fibrillin	Chloroplast	-0.253	NA	Y
TaFBN-A3	TraesCS4A02G272000.1	Chr4A	583754471-583757208	5	261	28.59	9.34	PAP_fibrillin	Extracellular	-0.33	NA	Y
TaFBN-B3	TraesCS4B02G042000.1	Chr4B	28717109-28719740	5	260	28.48	9.61	PAP_fibrillin	Chloroplast	-0.318	NA	Y
TaFBN-D3	TraesCS4D02G039200.1	Chr4D	16799419-16802059	5	261	28.55	9.21	PAP_fibrillin	Chloroplast	-0.325	NA	Y
TaFBN-A4	TraesCS2A02G145900.1	Chr2A	90688741-90690297	3	275	28.99	8.95	PAP_fibrillin	Chloroplast	-0.244	NA	Y
TaFBN-B4	TraesCS2B02G171300.1	Chr2B	144596063-144597581	3	276	29.35	9.51	PAP_fibrillin	Chloroplast	-0.267	NA	Y
TaFBN-D4	TraesCS2D02G150500.1	Chr2D	93046450-93048107	3	277	29.41	9.51	PAP_fibrillin	Chloroplast	-0.277	NA	Y
TaFBN-A5	TraesCS2A02G300200.1	Chr2A	515959001-515961447	6	262	28.96	9.16	PAP_fibrillin	Chloroplast	-0.213	NA	Y
TaFBN-B5	TraesCS2B02G316500.1	Chr2B	451833336-451836114	6	260	28.67	9.28	PAP_fibrillin	Chloroplast	-0.178	NA	Y
TaFBN-D5	TraesCS2D02G298100.1	Chr2D	380429694-380432365	6	256	28.43	9.36	PAP_fibrillin	Chloroplast	-0.2	NA	Y
TaFBN-A6	TraesCS2A02G431000.1	Chr2A	684246511-684248296	3	219	23.78	8.8	PAP_fibrillin	Extracellular	-0.044	NA	Y
TaFBN-B6	TraesCS2B02G452300.1	Chr2B	646214215-646215789	3	219	23.75	8.73	PAP_fibrillin	Chloroplast	0.003	NA	Y
TaFBN-D6	TraesCS2D02G428800.1	Chr2D	540824383-540826044	3	219	23.82	8.74	PAP_fibrillin	Chloroplast	-0.031	NA	Y
TaFBN-A7	TraesCS2A02G487900.1	Chr2A	722519297-722522892	6	297	32.55	5.73	PAP_fibrillin	Chloroplast	-0.231	NA	Y
TaFBN-B7	TraesCS2B02G515500.1	Chr2B	710281451-710285064	6	281	30.92	6.06	PAP_fibrillin	Chloroplast	-0.247	NA	Y

TaFBN-D7	TraesCS2D02G488200.1	Chr2D	587697352-587701032	6	293	32.03	5.35	PAP_fibrillin	Chloroplast	-0.171	NA	Y
TaFBN-A9	TraesCS2A02G413700.1	Chr2A	670791911-670794116	2	222	24.21	6.74	PAP_fibrillin	Extracellular	-0.106	NA	Y
TaFBN-B9	TraesCS2B02G432500.1	Chr2B	621664679-621666987	2	222	24.27	7.9	PAP_fibrillin	Extracellular	-0.123	NA	Y
TaFBN-D9	TraesCS2D02G410900.1	Chr2D	525935293-525937697	2	222	24.24	7.9	PAP_fibrillin	Extracellular	-0.136	NA	Y
TaFBN-A10	TraesCS2A02G434800.1	Chr2A	686874975-686880122	10	401	43.44	9.31	PAP_fibrillin	Extracellular	-0.16	NA	Y
TaFBN-B10	TraesCS2B02G455900.1	Chr2B	650399573-650405456	10	402	43.59	9.19	PAP_fibrillin	Extracellular	-0.182	NA	Y
TaFBN-D10	TraesCS2D02G432600.1	Chr2D	544665056-544670351	10	398	43.12	9.11	PAP_fibrillin	Extracellular	-0.152	NA	Y

2