Genome-wide identification and expression analysis of fibrillin (FBN) gene family in tomato (Solanum lycopersicum L.)

Huiru Sun1,2, Min Ren1 and Jianing Zhang1

1 College of Life Sciences, Yan’an University, Yan’an, Shaanxi Province, China
2 Shaanxi Key Laboratory of Chinese Jujube, Yan’an University, Yan’an, Shaanxi Province, China

ABSTRACT

Background: Fibrillin (FBN) proteins are widely distributed in the photosynthetic organs. The members of FBN gene family play important roles in plant growth and development, and response to hormone and stresses. Tomato is a vegetable crop with significantly economic value and model plant commonly used in research. However, the FBN family has not been systematical studied in tomato.

Methods: In this study, 14 FBN genes were identified in tomato genome by Pfam and Hmmer 3.0 software. ExPASy, MEGA 6.0, MEME, GSDDS, TBtools, PlantCARE and so on were used for physical and chemical properties analysis, phylogenetic analysis, gene structure and conserved motifs analysis, collinearity analysis and cis-acting element analysis of FBN family genes in tomato. Expression characteristics of SlFBNs in different tissues, fruit shape near isogenic lines (NILs), Pst DC3000 and ABA treatments were analyzed based on transcriptome data and quantitative Real-time qPCR (qRT-PCR) analysis.

Results: The SlFBN family was divided into 11 subgroups. There were 8 FBN homologous gene pairs between tomato and Arabidopsis. All the members of SlFBN family contained PAP conserved domain, but their gene structure and conserved motifs showed apparent differences. The cis-acting elements of light and hormone (especially ethylene, methyl jasmonate (MeJA) and abscisic acid (ABA)) were widely distributed in the SlFBN promoter regions. The expression analysis found that most of SlFBNs were predominantly expressed in leaves of Heinz and S. pimpinellifolium LA1589, and showed higher expressions in mature or senescent leaves than in young leaves. Expression analysis of different tissues and fruit shape NILs indicated SlFBN1, SlFBN2b and SlFBN7a might play important roles during tomato fruit differentiation. All of the SlFBNs responded to Pst DC3000 and ABA treatments. The results of this study contribute to exploring the functions and molecular mechanisms of SlFBNs in leaf development, fruit differentiation, stress and hormone responses.

INTRODUCTION

Plastoglobules (PGs) are lipoprotein particles in plastid, which are involved in plant growth and development, and stress resistance. Fibrillin (FBN) proteins are plastid...
lipid-associated and highly conserved, which encoded by nuclear genes and are the most abundant in chloroplast PGs (Singh & McNellis, 2011). FBNs showed important regulatory roles in plastid stability, plant growth and development, and stress response (Rey et al., 2010; Simkin et al., 2007; Yousef et al., 2010; Ytterberg, Peltier & van Wijk, 2006).

FBN proteins contain a conserved plastid-lipids associated protein (PAP) domain and are widely exist in photosynthetic organisms from cyanobacteria to plants (Cunningham et al., 2010; Lohscheider & Bártulos, 2016; Simkin et al., 2007). FBN proteins were early found in the chromoplasts of red rose (Rosa rugosa) (Wuttke, 1976) and bell pepper (Capsicum annuum) fruit (Deruère et al., 1994). Subsequently, the similar proteins were isolated from thylakoids of potato (Solanum tuberosum) leaves (Pruvot et al., 1996), chromoplast in cucumber (Cucumis sativus) petals (Vishnevetsky et al., 1996), chloroplast PG of Arabidopsis thaliana leaves (Vidi et al., 2006) etc, which have been named PGL, PAP and FBN. Ultimately, these similar proteins are collectively referred to as FBN (Singh & McNellis, 2011). So far, the FBN families in different species have been divided into 12 groups (FBN1–FBN12) (Kim et al., 2018). The FBN12 group is only present in lower algal fungi (Lohscheider & Bártulos, 2016). Besides the unique PAP domain, FBN11 group also contain a protein kinase C domain (PKC), which indicates that members of this group might have other functions that need to be studied other than lipoprotein-related functions (Li et al., 2020). Moreover, the isoelectric point and molecular weight ranges of FBN family are wide, and they are distributed in different plastids, including chloroplasts, elaioplasts, chromoplasts and etioplasts, which might be related to the diversified functions (Kim et al., 2018).

The FBN family genes have showed important roles in various processes, such as plastid structure stabilization, organ development, stress response and hormone signal transmission (Kim, Lee & Kim, 2015). FBN1 protein was detected in the chromoplasts of ripe pepper fruits, and overexpression of this gene promoted the increase and aggregation of PGs in chromoplasts (Jotham et al., 2006). In addition, GUS activity of FBN1 promoter increased with tomato (Solanum lycopersicum) leaf aging (Georg et al., 2001). The expression of Pap2 (FBN1b) in Brassica rapa decreases with the aging of leaves (Kim, Wu & Huang, 2001). The content of FBN1 protein in bell pepper gradually increased with fruit ripening and reached the peak at the full fruit ripening stage. C40.4 (FBN1) in potato was expressed in leaves and multiple flower organs, and inhibition of this gene expression leaded to plant growth retardation and smaller tuber size (Monte, Ludevid & Prat, 2010). Some FBN family genes in rice (Oryza sativa) were respond to ABA and extreme temperature treatments (Lee et al., 2007; Li et al., 2020). CsaFBN1, CsaFBN6 and CsaFBN11 in cucumber were induced and up-regulated expressed under high-light and low temperature stress (Kim et al., 2018). The RNAi of LeChrC (FBN1) and mutants of fbi4 in apple (Malus domestica) and Arabidopsis were more sensitive to Botrytis cinerea, Erwinia amylovora and Pseudomonas syringae, respectively (Leitner-Dagan et al., 2006; Singh et al., 2010). The fnb6 mutant of Arabidopsis showed resistance to cadmium and high-light stresses (Lee et al., 2020). The expression of jasmonic acid (JA) synthesis related genes in fnb5 mutants were inhibited under high-light stress (Otsubo et al., 2018). Under drought stress, FBN1 showed significantly decreased expression in tomato flacca.
mutant with deficient in ABA synthesis (Gillet et al., 1998). FBN1 and FBN2 responded to light and cold stresses through JA synthesis pathway (Youssef et al., 2010). Some FBN family members in wheat (Triticum aestivum) were involved in the response to drought, cold, heat and stripe rust stresses (Jiang et al., 2020).

Tomato has rich nutritional value and is widely cultivated in the world as an important vegetable crop (Albacete et al., 2008). According to the Food and Agriculture Organization of the United Nations (FAO) statistics, the tomato cultivated area in the world has exceeded five million hectares, and the production has reached 187 million tons in 2020 (http://www.fao.org/faostat/en/#data/QC). The FBN family genes play key regulatory roles in many biological processes such as plant development and stress response. However, the vast majority of FBN family genes in tomato are unknown, and no comprehensive analysis of this family in tomato has been reported. In this study, bioinformatics methods were used to identify and analyze the phylogenetic relationship, conserved domain, collinearity, cis-acting element in promoter regions of SlFBN gene family. The expression characteristics of SlFBNs in different tissues, Pst DC3000 and ABA treatments were analyzed using transcriptome data and qRT-PCR, which provided a theoretical foundation for exploring the potential functions of SlFBNs.

**MATERIALS AND METHODS**

**Tomato material and treatments**

The tomato used in this study was Micro-Tom. The tomato seedlings grew under normal temperature conditions (26 °C/16 h in light condition and 18 °C/8 h in dark condition). When the seedlings grew to 6-leaf stage, the young leaves, mature leaves and aging leaves (the yellowing part less than 5% of the whole leaf) were collected. The 6-leaf seedlings with similar growth were treated with 100 μM ABA, and water as a control group. The leaves were collected at 0, 6, 12 and 24 h after treatments. The samples were immediately frozen in liquid nitrogen and stored at −80 °C. All samples were tested with three independent biological replicates.

**Identification of FBNs in tomato**

Genomic data of tomato was downloaded from Sol Genomics Network (SL4.0, http://solgenomics.net/). The genomes of Arabidopsis, rice, maize (Zea mays), sorghum (Sorghum bicolor), cucumber and pepper were downloaded from TAIR (http://www.arabidopsis.org/), phytozome (https://phytozome.net/) and PGP (https://db.cngb.org/search/assembly/GCF_000710875.1/) databases, respectively. The PAP domain model of FBN family was obtained from Pfam database (http://pfam.xfam.org). FBN genes in tomato genome were screened by HMMER 3.0 based on the model (the threshold set as E < 1e−4). The normal mode of SMART (http://smart.embl-heidelberg.de/) and CD–search (https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi) were used to further verify SlFBN family genes. Finally, members of FBN family in tomato were determined. The lengths, molecular weights (Mws), isoelectric points (pIs) and hydrophilicities of SlFBN proteins were predicted by ExPASy website (http://web.expasy.org/protparam/)
The subcellular localizations of tomato FBN proteins were predicated using WoLF PSORT (https://wolfpsort.hgc.jp/) (Paul et al., 2007).

**Phylogenetic tree, gene structure and conserved domain analysis**

The FBN protein sequences from tomato, Arabidopsis, rice, maize, sorghum, cucumber and pepper were aligned and constructed phylogenetic tree using MEGA 6.0 by the neighbor-joining (NJ) with the p-distance model, and bootstrap was set to 1,000 replications.

The gene structures of SlFBNs were drawn in GSDS 2.0 (http://gsds.cbi.pku.edu.cn/) based on the introns-exon position information (Hu et al., 2015). The PAP domains of SlFBN proteins were analyzed using SMART website. The conserved motifs of SlFBN proteins were identified by MEME website (https://meme-suite.org/meme/). The maximum number of motif findings was set to 10, and other parameters were set to default values (Bailey et al., 2009).

**Chromosome location and collinearity analysis**

The chromosome distribution of SlFBNs was drawn by TBtools according to the location information from tomato genome database (Chen et al., 2020). MCScanx software was used to analyze the collinearity of FBN genes among Arabidopsis, rice and tomato (Wang et al., 2012), and then the collinearity diagram was shown through the Basic Circos module of TBtools.

**Cis-acting elements in FBN promoter regions**

The 1.5 kb sequences located upstream of the start codon of tomato FBN family genes were extracted to analyze the cis-acting elements on the PlantCARE website (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/) (Lescot et al., 2002).

**Transcriptome analysis of SlFBNs in different tissues and under Pst DC3000 treatment**

The transcriptome data of SlFBNs in different tissues of Heinz and S. pimpinellifolium LA1589, under Pst DC3000 treatment in tomato varieties with different resistances (RG-PtoR: resistant, RG-prf3: sensitive and RG-prf9: sensitive) and in flower meristems at different developmental stages of LA1589 and three fruit shape near isogenic lines (NILs) in LA1589 background (WT, sun, ovate and fs8.1) (Wang et al., 2019) were obtained from TFGD (http://ted.bti.cornell.edu/cgi-bin/TFGD/digital/home.cgi). The expression heat maps of SlFBNs were drawn using TBtools.

**RNA extraction and quantitative real-time PCR (qRT-PCR)**

Total RNA was extracted from the collected samples using the Total RNA Kit (TIANGEN, Beijing, China). The cDNA was synthesized from 1 μg using the StarScript II First-strand cDNA Synthesis Mix kit (GenStar, Beijing, China) according to the manufacturer’s instructions. Then qRT-PCR was carried out with an Applied Biosystems StepOnePlus using RealStar Green Fast Mixture with ROX (2x) (GenStar, Beijing, China). The gene-specific primers used for qRT-PCR were designed by Primer 5.0 (Table S1).
The qRT-PCR was performed as follows: step 1: 95 °C for 2 min; step 2: 40 cycles of 95 °C for 15 s, 60 °C for 30 s; and step 3: melting curve analysis. Three biological replicates and the $2^{-\Delta\Delta CT}$ method were used to calculate the relative expression level (Livak & Schmittgen, 2001). The tomato *ACTIN* gene (Solyc04g011500.3.1) was used as internal reference gene (Liu et al., 2022). The t-test was used to analyze the significance of the difference.

**RESULTS**

Identification of *FBN* members in tomato

In our study, a total of 14 putative *FBN* genes were identified in tomato genome through screening using PAP domain (PF04755) and validating in SMART database. According to the homology with *FBNs* in *Arabidopsis*, *SlFBNs* were named as *SlFBN1–SlFBN11* (Table S2). The physical and chemical properties of *SlFBN* proteins were analyzed. The result showed that the lengths of *SlFBN* proteins ranged from 206 amino acids (aa) (*SlFBN9*) to 541 aa (*SlFBN11*). The predicated Mw and pI ranged from 23.43 kDa (*SlFBN9*)–61.35 kDa (*SlFBN11*), and 4.65 (*SlFBN2b*)–9.72 (*SlFBN3a*). The hydrophobicities of *SlFBNs* were less than zero, indicating that they were all hydrophilic proteins. Most (11/14) of *SlFBNs* were located in chloroplasts, except for *SlFBN3* (nucleus), *SlFBN11* (nucleus) and *SlFBN9* (mitochondria) by subcellar location predication.

Phylogenetic analysis of *SlFBNs*

To detect the evolutionary relationships of *SlFBN* family, total 85 FBN proteins were collected from rice (10), maize (13), sorghum (11), *Arabidopsis* (14), cucumber (10), pepper (13) and tomato (14) to construct the phylogenetic tree (Fig. 1). These FBN proteins were divided into 11 groups (Group 1–Group 11) which distributed FBN members from mono-and dicotyledons suggesting that the differences of FBN groups were completed before the separation of mono-and dicotyledons. In some species, group 1, 2, 3 and 7 were amplified. FBN members of tomato and pepper, which belong to Solanaceae, were firstly cluster in the same branch, while the FBN members from mono- and dicotyledons were clustered distantly. These results suggested that FBN family members in mono- and dicotyledons showed different evolutionary characteristics.

Chromosomal location and collinearity analysis of *FBN* genes

According to analysis of the chromosome positions, the 14 tomato *FBN* genes were unevenly distributed on seven chromosomes (Chr.01, Chr.02, Chr.03, Chr.08, Chr.09, Chr.10 and Chr.11). Among them, four *SlFBNs* were located on Chr.08, and 1 to 2 *SlFBNs* were located on the other six chromosomes (Fig. 2). The analysis of gene duplication event showed that there was no *FBN* gene duplication in tomato. To understand the collinearity relationship of *FBN* genes, the *FBN* homologous gene pairs between tomato and other plant species (*Arabidopsis* and rice) were found. There were eight collinear gene pairs between six *SlFBNs* and seven *AtFBNs*. *SlFBN1* and *SlFBN7b* were collinear with two *AtFBN* genes (*AtFBN 1a–AtFBN1b* and *AtFBN 7a–AtFBN7b*), respectively. *AtFBN2* was collinear with two *SlFBN* genes (*SlFBN2a–SlFBN2b*). *SlFBN4* and *SlFBN5* were collinear...
with one AtFBN gene, respectively (Fig. 3 and Table S3). There were no FBN homologous gene pairs between tomato and rice.

**Gene structure and conserved domain of SIFBNs**

To further explore the conservation and diversification of SIFBNs, the exon-intron structures and conserved motifs were analyzed. The gene structure analysis showed that the intron numbers of SIFBNs were various, ranging from 2 to 12 (Fig. 4A). Among them, SIFBN1, SIFBN2a, SIFBN2b, SIFBN9 contain two introns, which might be due to intron loss. SIFBN10 and SIFBN11 contained the 10 and 12 introns, respectively, which might be related to intron increase. Similar results were also found in FBN family genes in rice and Arabidopsis (Li et al., 2020).

Analysis of the conserved domains of SIFBN family proteins revealed that all SIFBNs contained the PAP domain, which was unique to this family (Fig. 4B). SIFBN11 also contained a protein kinase C domain (PKC) besides PAP domain.

The 10 conserved motifs of SIFBN family members were analyzed to further analyze the characteristics of this family proteins. The sequences of the 10 conserved motifs were listed in Table S4. The results showed that all SIFBN proteins contained Motif2. Motif1 and Motif3 existed in most of SIFBNs, and the rest motifs existed in only individual SIFBN
members (Fig. 4C). SlFBN11 only contained Motif2, which indicated that SlFBN11 might show different functions from other group members, combined with the analysis of gene structure and conserved domain.

**Cis-element analysis of SlFBN promoter regions**

The cis-acting element in promoter region can partly reflect the characteristic of gene expression. The cis-acting elements in the promoter regions of SlFBNs were analyzed and the result showed that all of the SlFBN promoters contained two to 15 light response elements (Fig. 5), which indicated that the functions of SlFBNs might be related with light response liking photosynthesis. The hormone responsive elements, especially ethylene, MeJA and ABA, were widely distributed in the SlFBN promotes. The promoters of SlFBN10, SlFBN6 and SlFBN4 contained 11, nine and five ABA response elements (ABRE), respectively, suggesting that these genes might be directly or indirectly involved in ABA response pathway. In addition, auxin, gibberellin and salicylic acid response elements were distributed in some SlFBN promoters. Drought response element (MBS), pathogen response element (W-box), trauma response element (WUN-Motif) and defense and stress response element (TC-rich repeats) were distributed in the 6, 5, 3 and 3 SlFBN gene promoters, respectively. In addition to the above elements, individual SlFBN promoters also distributed endosperm expression elements (GCN4-Motif), meristematic expression regulation elements (CAT-box) and circadian control elements (circadian).

**Expression patterns of SlFBNs in different tissues**

In order to explore the biological functions of SlFBNs in tomato growth and development, the expression patterns of SlFBNs in different tissues of tomato cultivar Heinz (Fig. 6A) and wild species *S. pimpinellifolium* LA1589 (Fig. 6B) were analyzed using published
transcriptome data. Similar expression characteristics, which was that most of *SlFBNs* were preferentially expressed in leaves, were observed in Heinz and LA1589. Meanwhile, In Heinz, four *SlFBNs* (*SlFBN1, SlFBN2b, SlFBN3a* and *SlFBN7a*) were highly expressed in flowers. In LA1589, except *SlFBN2b*, the other three *SlFBNs* were also highly expressed in flowers. In addition, the expressions of some *SlFBNs* (*SlFBN4, SlFBN6, SlFBN8, SlFBN9* and *SlFBN11*) were increased with fruit ripening both in Heinz and LA1589, suggesting that these genes might be involved in regulating tomato fruit ripening.

The *SlFBN* expressions in flower meristems at 4, 6, 8, 10, 13 and 16 days post-initiation of floral meristem (DPI) of LA1589 and three fruit shape NILs (*sun, ovate* and *fs8.1*) were analyzed to further analyze their expression characteristics at different flower development stages (Fig. 6C). It was found that there was no significant difference in the expressions of 11 *SlFBNs* at different flower meristem stages. Notably, *SlFBN1, SlFBN7a* and *SlFBN2b* with high expressional levels in Heinz and LA1589 flower showed significantly higher expressions in three NILs than wild type at 16 DPI. The result indicated that *SlFBN1, SlFBN7a* and *SlFBN2b* might play important roles in tomato fruit early differentiation.
Tissue expression analysis showed that SlFBN family genes were mostly expressed preferentially in leaves. The expressions of SlFBNs at different development stages of tomato leaf were analyzed using qRT-PCR to explore the possible functions in tomato leaf development (Fig. 7 and Table S5). The expressions of 11 SlFBNs (except SlFBN2b, SlFBN7a and SlFBN7b) in young leaves were significantly different from those in mature or senescent leaves. These SlFBNs showed up-regulated expressions with leaf development except SlFBN11, which showed opposite trend. The results indicated that SlFBN family genes generally perform functions during leaf development. The study of FBN1 in bell pepper and tomato confirmed this result (Deruère et al., 1994).

**Expression profiles of SlFBNs under Pst DC3000 treatment**

Previous studies showed that FBN family genes played important regulatory roles in stress response. The published transcriptomic data were used to analyze the expression...
characteristics of *SlFBN* family genes in tomato varieties with different resistances (RG-PtoR: resistant, RG-prf3: sensitive and RG-prf9: sensitive) under *Pst* DC3000 treatment (Fig. 8). The result showed that all of *SlFBN* family genes could respond to *Pst* DC3000 treatment, and 12 *SlFBNs* showed higher expression levels in resistant varieties than in sensitive varieties. The expressions of *SlFBN1* and *SlFBN11* in resistant varieties were higher than in sensitive varieties at 4 h, while the opposite trends were observed at 6 h under *Pst* DC3000 treatment.

**Expression profiles of *SlFBNs* under ABA treatment**

The ABA response elements were generally distributed in *SlFBN* promoter regions. The expression levels of *SlFBNs* under ABA treatment were analyzed by qRT-PCR (Fig. 9 and Table S6). It was found that all of *SlFBNs* had significantly differences compared to the control. Compared to the 0 h, the expressions of *SlFBN1*, *SlFBN2a*, *SlFBN2b*, *SlFBN3a* and *SlFBN5* were up-regulated, and the expressions of *SlFBN3b*, *SlFBN4*, *SlFBN7b*, *SlFBN8* and *SlFBN9* were down-regulated. The expression of *SlFBN7a* and *SlFBN10* were increased firstly and then decreased. *SlFBN6* and *SlFBN11* showed early down-regulation followed by up-regulation. Notably, *SlFBN11* showed the most significant response to ABA, and its expression increased 27.0 times at 12 h and 9.7 times at 24 h compared with the 0 h, suggesting that this gene was likely to involved in the ABA signal pathway.
Figure 6 Expressions of SIFBNs in different organs. (A) Expression profile of SIFBNs in different organs of cultivated tomato cultivar Heniz; (B) expression profile of SIFBNs in different organs of wild species S. pimpinellifolium LA1589; (C) expression profile of SIFBNs in LA1589, sun, ovate and fs8.1. The three red asterisks represent the differentially expressed genes between LA1589 and the three fruit-shape NILs (sun, ovate and fs8.1). Scale bars in A, B and C represent log2-transformed FPKM values.
DISCUSSION

FBN family proteins were early discovered in fibrils of chromoplasts (Deruère et al., 1994) and were involved in the various plant tissues growth and development, stress and hormone signal response (Jiang et al., 2020; Liu et al., 2018). In recent years, FBN gene families have been identified in several plants, such as rice (Li et al., 2020), cucumber (Kim et al., 2018) and wheat (Jiang et al., 2020). In this study, 14 FBN family genes were identified in tomato genome (Fig. 1 and Table S2) and unevenly distributed in the chromosomes (Fig. 2). The similar numbers of FBN family were found in diploid plants such as rice (Li et al., 2020) and Arabidopsis (Singh & McNellis, 2011), but less than that in heterogenous hexaploid wheat, which might be related to the genome sizes and gene duplication events. Subcellular localization prediction found that most (11/14) of SlFBNs were located in chloroplasts (Table S2), and similar results were found in the studies of FBN family in wheat (Jiang et al., 2020) and rice (Li et al., 2020), suggesting that FBN family gene might be involved in controlling chloroplast structure. Phylogenetic analysis showed that SlFBN family genes were divided into 11 groups (Fig. 1). SlFBN11 belonging to group11 were significantly different from other group members in gene structure and conserved domains, suggesting that the gene might undergo new functionalization.

The gene structures of SlFBNs were distant with 2 to 12 introns (Fig. 3A), but the members in the same group showed similar intron numbers and the conserved motif

Figure 7  Expressions of SlFBNs at different development stages of tomato leaf. The expression levels of SlFBNs were tested by qRT-PCR and estimated by the $2^{-\Delta\Delta C_{T}}$ method. Leaf-1, Leaf-2 and Leaf-3 represent young, mature and senescent leaves, respectively; The error bars show the standard error (SE) of three biological replicates. The $p$ value was calculated through t-test. Asterisk indicate the significant difference compared with control (Leaf-1). * and ** indicate $p < 0.05$ and $p < 0.01$, respectively.
The tissue expression analysis was carried out to explore the biological functions of tomato FBN family genes, it was found that the majority of SIFBNs were preferentially...
expressed in leaves (Figs. 6A and 6B). Further analysis of the expressions of \textit{SIFBNs} in different development stages of leaf showed that most (11/14) of \textit{SIFBNs} showed significantly increased expression trends with leaf maturation or aging (Fig. 7). This result was consistent with the study of subcellular localization and the GUS activity characteristics of \textit{SlFBN1} in process of tomato leaf senescence (Georg et al., 2001), which suggested that \textit{SlFBN1} might be involved in leaf maturation or senescence by regulating chloroplast structure. Previous studies have found that \textit{FBN} genes were involved in regulating the fruit development of bell pepper (Kilcrease et al., 2015), satsuma mandarin (\textit{Citrus unshiu} Marc.) (Moriguchi et al., 1998), sweet orange (\textit{Citrus sinensis}) (Muccilli et al., 2009) and so on. In our study, \textit{SlFBN1}, \textit{SlFBN7a} and \textit{SlFBN2b} were highly expressed in flowers, and the expression levels in flower meristem of three fruit shape NILs (sun, ovate and fs8.1) at 16 DPI were higher than those in LA1589, and the most significant differences were in \textit{sun} (Fig. 6C). These results indicated that \textit{SlFBN1}, \textit{SlFBN7a} and

\textbf{Figure 9} Expressions of \textit{SIFBNs} under ABA treatment. The expression levels of \textit{SIFBNs} were tested by qRT-PCR and estimated by the $2^{-\Delta\Delta CT}$ method. 0, 6, 12 and 24 h represent 0, 6, 12 and 24 h under ABA treatment, respectively. The error bars show the standard error (SE) of three biological replicates. The $p$ value was calculated through $t$-test. Asterisk indicate the significant difference compared with control (0 h). Asterisks (* and **) indicate $p < 0.05$ and $p < 0.01$, respectively.

DOI: 10.7717/peerj.13414/fig-9
SlFBN2b might be involved in regulating tomato fruit early differentiation and might be related with sun, ovate or fs8.1 gene loci. The studies of LeChrC in tomato (Leitner-Dagan et al., 2006), FBI4b in apple and AtFBN4 in Arabidopsis (Singh et al., 2010) showed that FBN genes could respond to pathogen infection. Through analysis of the transcriptomic data, all of SlFBNs could respond to Pst DC3000 treatment and most (12/14) of them showed higher expressions in resistant varieties than in sensitive variety (Fig. 8), which verified the previous studies. Analysis of the response of SlFBN family genes to ABA treatment showed that all of FBNs in tomato showed significant response to ABA treatment, especially SlFBN11 (Fig. 9), suggesting that this gene might play certain roles in the ABA signal pathway. This result was different from the FBN family study in rice (Jiang et al., 2020) indicated that functional differentiation was occurred in FBN family between mono-and dicotyledons. Together, most of SlFBN family genes were involved in leaf development and all of them could respond to Pst DC3000 and ABA treatments. SlFBN1, SlFBN7a and SlFBN2b might play roles in regulating tomato fruit differentiation. In addition, SlFBN11 might show different functions from other SlFBNs.

CONCLUSIONS

We identified 14 FBN genes in tomato genome, which were divided into 11 groups and unevenly distributed on seven chromosomes. There were eight FBN homologous gene pairs between tomato and Arabidopsis and no homologous gene pairs between tomato and rice. The FBN gene structures were divergent. The analysis of cis-acting elements found that hormone responce elements were extensive discovered in SlFBN promoter regions. The results of expression analysis were found that SlFBN family genes might show certain functions in leaf development, fruit differentiation, stress and hormone responses. These results could provide relevant information for further study on the biological functions of FBN family genes.

ADDITIONAL INFORMATION AND DECLARATIONS

Funding

This work was supported by the Specialized Research Fund for the Doctoral Program of Yan’an University (No. YDBK2019-42), the Natural Science Basic Research Plan of Shaanxi Province, China (No. 2022JQ-159); and the Special Scientific Research Project of Education Department of Shaanxi Province, China (No. 21JK0993). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Grant Disclosures

The following grant information was disclosed by the authors:
Specialized Research Fund for the Doctoral Program of Yan’an University: YDBK2019-42.
Natural Science Basic Research Plan of Shaanxi Province, China: 2022JQ-159.
Special Scientific Research Project of Education Department of Shaanxi Province, China: 21JK0993.
Competing Interests
The authors declare that they have no competing interests.

Author Contributions
- Huiru Sun conceived and designed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft.
- Min Ren analyzed the data, authored or reviewed drafts of the paper, and approved the final draft.
- Jianing Zhang performed the experiments, authored or reviewed drafts of the paper, and approved the final draft.

Data Availability
The following information was supplied regarding data availability:

The raw data are available in the Supplemental Files.

Supplemental Information
Supplemental information for this article can be found online at http://dx.doi.org/10.7717/peerj.13414#supplemental-information.

REFERENCES
Albacete A, Ghanem ME, Martínez-Andújar C, Acosta M, Pérez-Alfocea F. 2008. Hormonal changes in relation to biomass partitioning and shoot growth impairment in salinized tomato (Solanum lycopersicum L.) plants. Journal of Experimental Botany 59(15):4119–4131 DOI 10.1093/jxb/ern251.

Artimo P, Jonnalagedda M, Arnold K, Baratin D, Csardi G, de Castro E, Duvaud S, Flegel V, Fortier A, Gasteiger E, Grosdidier A, Hernandez C, Ioannidis V, Kuznetsov D, Liechti R, Moretti S, Mostaguir K, Redaschi N, Rossier G, Xenarios I, Stockinger H. 2012. ExPASy: SIB bioinformatics resource portal. Nucleic Acids Research 40(W1):W597–W603 DOI 10.1093/nar/gks400.

Bailey TL, Mikael B, Buske FA, Martin F, Grant CE, Luca C, Jingyuan R, Li WW, Noble WS. 2009. MEME SUITE: tools for motif discovery and searching. Nucleic Acids Research 37:W202–W208 DOI 10.1093/nar/gkp335.

Chen C, Chen H, Zhang Y, Thomas HR, Frank MH, He Y, Xia R. 2020. TBtools: an integrative toolkit developed for interactive analyses of big biological data. Molecular Plant 13(8):1194–1202 DOI 10.1016/j.molp.2020.06.009.

Cunningham FX, Tice AB, Pham C, Gantt E. 2010. Inactivation of genes encoding plastoglobulin-like proteins in synechocystis sp. PCC, 6803 leads to a light-sensitive phenotype. Journal of Bacteriology 192(6):13 DOI 10.1128/JB.01434-09.

Deruère J, Römer S, d’Harlingue A, Backhaus RA, Kuntz M, Camara B. 1994. Fibril assembly and carotenoid overaccumulation in chromoplasts: a model for supramolecular lipoprotein structures. The Plant Cell 6(1):119–133 DOI 10.2307/3869680.

Georg L, Nathalie MH, Mélanie B, Stephan C, Noëlle B, Marcel K, Pascal R. 2001. Accumulation of plastid lipid-associated proteins (fibrillin/CDSP34) upon oxidative stress, ageing and biotic stress in Solanaceae and in response to drought in other species. Journal of Experimental Botany 52(360):1545–1554 DOI 10.1093/jexbot/52.360.1545.
Gillet B, Beyly A, Peltier G, Rey P. 1998. Molecular characterization of CDSP 34, a chloroplastic protein induced by water deficit in Solanum tuberosum L. plants, and regulation of CDSP 34 expression by ABA and high illumination. The Plant Journal 16(2):257–262 DOI 10.1046/j.1365-313x.1998.00292.x.

Hu B, Jin J, Guo AY, Zhang H, Luo J, Gao G. 2015. GSDD 2.0: an upgraded gene feature visualization server. Bioinformatics 31(8):1296–1297 DOI 10.1093/bioinformatics/btu817.

Jiang Y, Hu H, Ma Y, Zhou J. 2020. Genome-wide identification and characterization of the fibrillin gene family in Triticum aestivum. PeerJ 8(8):e9225 DOI 10.7717/peerj.9225.

Jotham II, Frost E, Vidi PA, Staehelin F. 2006. Plastoglobules are lipoprotein subcompartments of the chloroplast that are permanently coupled to thylakoid membranes and contain biosynthetic enzymes. The Plant Cell 18(7):1693–1703 DOI 10.1105/tpc.105.039859.

Kilcrease J, Rodriguez-Uribe L, Richins RD, Arcos JM, Victorino J, O’Connell MA. 2015. Correlations of carotenoid content and transcript abundances for fibrillin and carotenogenic enzymes in Capsicum annum fruit pericarp. Plant Science 232:57–66 DOI 10.1016/j.plantsci.2014.12.014.

Kim EH, Lee Y, Kim HU. 2015. Fibrillin 5 is essential for plastoquinone-9 biosynthesis by binding to solanesyl diphosphate synthases in Arabidopsis. The Plant Cell 27(10):2956–2971 DOI 10.1105/tpc.15.00707.

Kim I, Lee SC, Kim EH, Song K, Yang TJ, Kim HU. 2018. Genome-wide identification and expression analyses of the fibrillin family genes reveal their involvement in the photoprotection in Cucumber. Plants (Basel) 7(3):50–65 DOI 10.20944/preprints201805.0330.v1.

Kim HU, Wu SS, Huang AH. 2001. Brassica rapa has three genes that encode proteins associated with different neutral lipids in plastids of specific tissues. Plant Physiology 126(1):330–341 DOI 10.1104/pp.126.1.330.

Lee DG, Ahsan N, Lee SH, Kang KY, Lee BH. 2007. An approach to identify cold-induced low-abundant proteins in rice leaf. Comptes Rendus Biologies 330(3):215–225 DOI 10.1016/j.crvi.2007.01.001.

Lee K, Gehmann M, Paul MV, Wang L, Luckner M, Wanner G, Geigenberger P, Leister D, Kleine T. 2020. Lack of FIBRILLIN6 in Arabidopsis thaliana affects light acclimation and sulfate metabolism. The New Phytologist 225(4):1715–1731 DOI 10.1111/nph.16246.

Leitner-Dagan Y, Ovadis M, Shklarman E, Elad Y, Rav David D, Vainstein A. 2006. Expression and functional analyses of the plastid lipid-associated protein CHRC suggest its role in chromoplastogenesis and stress. Plant Physiology 142(1):233–244 DOI 10.1104/pp.106.082404.

Lescot M, Déhais P, Thijs G, Marchal K, Moreau Y, Van de Peer Y, Rouzé P, Rombaerts S. 2002. PlantCARE, a database of plant cis-acting regulatory elements and a portal to tools for in silico analysis of promoter sequences. Nucleic Acids Research 1:1 DOI 10.1093/nar/30.1.325.

Li J, Li X, Khatab AA, Xie G. 2020. Phylogeny, structural diversity and genome-wide expression analysis of fibrillin family genes in rice. Phytochemistry 175(10):112377 DOI 10.1016/j.phytochem.2020.112377.

Liu Z, Pan X, Wang C, Yun F, Huang D, Yao Y, Gao R, Ye F, Liu X, Liao W. 2022. Genome-wide identification and expression analysis of serine hydroxymethyltransferase (SHMT) gene family in tomato (Solanum lycopersicum). PeerJ 10(2):e12943 DOI 10.7717/peerj.12943.

Liu W, Qin Z, Xin M, Zhou X, Yang J, Wang C. 2018. Analysis of CsPAP-fib regulation of cucumber female differentiation in response to low night temperature conditions. Scientia Horticulturae 240:81–88 DOI 10.1016/j.scienta.2018.05.011.
Livak KJ, Schmittgen TD. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. *Methods* 25(4):402–408 DOI 10.1006/meth.2001.1262.

Lohscheider JN, Bártulos CR. 2016. Plastoglobules in algae: a comprehensive comparative study of the presence of major structural and functional components in complex plastids. *Marine Genomics* 28(Pt 23):127–136 DOI 10.1016/j.margen.2016.06.005.

Monte E, Ludevid D, Prat S. 2010. Leaf C40.4: a carotenoid-associated protein involved in the modulation of photosynthetic efficiency? *The Plant Journal* 19(4):399–410 DOI 10.1046/j.1365-313X.1999.00537.x.

Moriguchi T, Kita M, Endo-Inagaki T, Ikoma Y, Omura M. 1998. Characterization of a cDNA homologous to carotenoid-associated protein in citrus fruits. *Biochimica et Biophysica Acta-Gene Structure and Expression* 1442(2–3):334–338 DOI 10.1016/S0167-4781(98)00182-1.

Muccilli V, Licciardello C, Fontanini D, Russo MP, Foti S. 2009. Proteome analysis of *Citrus sinensis* L. (Osbeck) flesh at ripening time. *Journal of Proteomics* 73(1):134–152 DOI 10.1016/j.jprot.2009.09.005.

Otsubo M, Ikoma C, Ueda M, Ishii Y, Tamura N. 2018. Functional role of fibrillin5 in acclimation to photooxidative stress. *Plant & Cell Physiology* 59(8):1670–1682 DOI 10.1093/pcp/pcy093.

Paul H, Keun-Joon P, Takeshi O, Naoya F, Hajime H, Adams-Collier CJ, Kenta N. 2007. WoLF PSORT: protein localization predictor. *Nucleic Acids Research* 35:585–587 DOI 10.1093/nar/gkm259.

Pruvot G, Cuiné S, Peltier G, Rey P. 1996. Characterization of a novel drought-induced 34-kDa protein located in the thylakoids of *Solanum tuberosum* L. plants. *Planta* 198(3):471–479 DOI 10.1007/BF00620065.

Rey P, Gillet B, Römer S, Eymery F, Kuntz M. 2010. Over-expression of a pepper plastid lipid-associated protein in tobacco leads to changes in plastid ultrastructure and plant development upon stress. *The Plant Journal* 21(5):483–494 DOI 10.1046/j.1365-313x.2000.00699.x.

Simkin AJ, Gaffe J, Alcaraz JP, Carde JP, Bramley PM, Fraser PD, Kuntz M. 2007. Fibrillin influence on plastid ultrastructure and pigment content in tomato fruit. *Phytochemistry* 68(11):1545–1556 DOI 10.1016/j.phytochem.2007.03.014.

Singh DK, Maximova SN, Jensen PJ, Lehman BL, Ngugi HK, McNellis TW. 2010. FIBRILLIN4 is required for plastoglobule development and stress resistance in apple and *Arabidopsis*. *Plant Physiology* 154(3):1281–1293 DOI 10.1104/pp.110.164095.

Singh DK, McNellis TW. 2011. Fibrillin protein function: the tip of the iceberg? *Trends in Plant Science* 16(8):432–441 DOI 10.1016/j.tplants.2011.03.014.

Vidi PA, Kanwischer M, Baginsky S, Austin JR, Csucs G, Dormann P, Kessler F, Brehelin C. 2006. Tocopherol cyclase (VTE1) localization and vitamin E accumulation in chloroplast plastoglobule lipoprotein particles. *Journal of Biological Chemistry* 281(16):11225–11234 DOI 10.1074/jbc.M511939200.

Vishnevetsky M, Ovadis M, Itzhaki H, Levy M, Libal-Weksler Y, Adam Z, Vainstein A. 1996. Molecular cloning of a carotenoid-associated protein from *Cucumis sativus* corollas: homologous genes involved in carotenoid sequestration in chromoplasts. *The Plant Journal* 10(6):1111–1118 DOI 10.1046/j.1365-313X.1996.10061111.x.

Wang Y, Clevenger JP, Illa-Berenguer E, Meulia T, van der Knaap E, Sun L. 2019. A comparison of *sun*, *ovate*, fs8.1 and auxin application on tomato fruit shape and gene expression. *Plant & Cell Physiology* 60(5):1067–1081 DOI 10.1093/pcp/pcz024.
Wang Y, Tang H, Debarry JD, Tan X, Li J, Wang X, Tae-Ho L, Jin H, Barry M, Guo H. 2012. MCScanX: a toolkit for detection and evolutionary analysis of gene synteny and collinearity. *Nucleic Acids Research* 40(7):e49 DOI 10.1093/nar/gkr1293.

Wuttke HG. 1976. Chromoplasts in *Rosa rugosa*: development and chemical characterization of tubular elements. *Zeitschrift Für Naturforschung C* 31(7–8):456–460 DOI 10.1515/znc-1976-7-821.

Youssef A, Laizet Y, Block MA, Maréchal E, Alcaraz JP, Larson TR, Pontier D, Gaffé J, Kuntz M. 2010. Plant lipid-associated fibrillin proteins condition jasmonate production under photosynthetic stress. *The Plant Journal* 61(3):436–445 DOI 10.1111/j.1365-313X.2009.04067.x.

Ytterberg AJ, Peltier JB, van Wijk KJ. 2006. Protein profiling of plastoglobules in chloroplasts and chromoplasts. A surprising site for differential accumulation of metabolic enzymes. *Plant Physiology* 140(3):984–997 DOI 10.1104/pp.105.076083.