

Study on the molecular mechanism of pod wall fiber formation using the bean pod fiber mutant (bfm) of *Phaseolus vulgaris* L

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Abstract

Background and aims: Snap bean is a widely planted legume vegetable crop in the world, with tender pods as its edible organ. In the actual production process, excessive fiber content in bean pods can lead to a deterioration in their taste after cooking, seriously affecting their edible quality. In order to better study the mechanism of fiber formation in bean pod walls, we selected a bean pod fiber mutant (*bfm*) from the bean mutant library.

Methods: Fiber content determination, cytological observation, transcriptome and metabolome analysis were performed on the pod walls of mutant *bfm* and its wild-type at different developmental stages.

Key results: The results showed that the crude fiber content of the *bfm* pod wall tissue was significantly higher than that of the wild type during the mature commercial pod stage, and cellulose may be the main factor causing the increase in fiber content in the bean pod wall. During the mature commercial pod stage, the number of cells in the pod wall tissue of *bfm* increased significantly compared to the wild type, with an increase in phloem fibers and thicker cell walls. It is speculated that this situation led to changes in fiber content in the mutant *bfm*. The combined analysis of transcriptome and metabolome showed that differentially expressed genes and metabolites were enriched in metabolic pathways and secondary metabolite biosynthesis pathways. Metabolites such as glycine, L-glutamine, arabitol (D), and D-ribose were closely related to *Phvul.007G077800* (*CTL*) and *Phvul.003G089600* (*KOR*).

Conclusions: These metabolites and genes mutually regulate and affect or promote precursor substances for cellulose synthesis, providing substrates and energy for cellulose synthesis, thereby promoting cellulose synthesis. This study provides theoretical research on the mechanism of fiber synthesis in bean pod walls, and also to provide some reference for bean breeding improvement and application practice.

1 INTRODUCTION

Snap bean (*Phaseolus vulgaris* L.) has a delicious taste and rich nutrition, and is widely loved by people. The stitching and pod wall fiber content of bean pods are important traits that affect the quality of snap beans. Fiber in snap bean pod belongs to insoluble dietary fiber, namely crude fiber, which is one of the components of cell wall, mainly including cellulose, hemicellulose, lignin and other components.

Cellulose is the most abundant component in plant cell walls, providing the mechanical strength required to resist swelling pressure, while also affecting the size, characteristics, and direction of cell growth and division, thus shaping the morphology of plants. The synthesis of cellulose is the most important biochemical process in plant cells, in which there is an important enzyme - cellulose synthase, including cellulose synthase (Ces) and cellulose like synthase (Csl). Current research indicates that a cellulose synthase gene family has evolved in higher plants, including multiple *CesA* genes, which are divided into PCW (primary cell wall) *CesA* group and SCW (secondary cell wall) *CesA* group. Ten *CESA* proteins have been identified in Arabidopsis, and *CesA* genes have also been identified in crops such as rice and bread wheat. In addition to cellulase, cellulase (*Kor*), sucrose synthase (*SuSy*), and other enzymes may also be involved in cellulose synthesis. Genes involved in starch and sucrose metabolism, as well as signal transduction, also play an important role in cellulose synthesis. Sucrose is the main form of carbohydrate transport in plants and an essential carbon source in the process of cellulose synthesis. Under the catalytic action of key enzymes such as sucrose synthase (*SUS*) and sucrose phosphate synthase (*SPS*), sucrose is broken down into glucose and fructose, which then participate in cellulose synthesis as precursor substances. Research has shown that the expression level of *SUS* genes is closely related to cellulose synthesis. The *GhSUS2* gene in cotton exhibits high expression levels during the rapid fiber elongation stage, providing sufficient substrates for cellulose synthesis. In addition, UDP glucose (UDP Glc), as another important precursor for cellulose synthesis, is mainly synthesized through the breakdown of sucrose to produce glucose-1-phosphate (G1P), which is catalyzed by UDP glucose pyrophosphate (UGPase). In addition to UDP glucose, other sugar nucleotides such as GDP glucose and ADP glucose also participate in the synthesis of cell wall polysaccharides. These sugar nucleotides are

catalyzed by enzymes such as Nucleotide Sugar Pyrophorase to provide substrates for the synthesis of cell wall polysaccharides. Plant chitinase (CTL), as a type of glycoside hydrolase, plays numerous regulatory roles in cell wall metabolism and plant disease resistance. (Wu et al. 2012). conducted in-depth phenotype analysis on the rice bc15 mutant and found that the gene mutation caused thinning of the thick walled cell wall and a significant decrease in cellulose content. Further research revealed that the mutant encodes a chitinase protein, BC15/OsCTL1, providing new clues for analyzing its possible molecular mechanisms in cellulose synthesis and cell wall remodeling pathways.

In the study of bean fiber, (Parker et al. 2021). found in the anatomical analysis of developing pods that genetic changes leading to reduced cracking in dried bean pods may be related to changes in fiber composition or structure. The research results of (Rau et al. 2019). showed that in fiber free snap beans, the lignified wall fiber layer is usually absent. (Parker et al. 2021) observations under an electron microscope further confirmed this conclusion. At present, research on the fiber characteristics of snap bean pods only stays at the physiological level, and there is little research on the molecular mechanisms of pod fiber formation. This study screened a pod fiber mutant *bfm* from the mutant library. Exploring the reasons for the formation of fiber in the pod wall of snap beans through fiber content, cytological observation, and combined analysis of transcriptome and metabolome, providing basic materials for improving snap bean quality and breeding improvement.

2. MATERIALS AND METHODS

2.1 Materials

The wild-type snap bean variety 'Jin Guan' is a high generation inbred line (from the Horticultural Team of Heilongjiang University, China). The bean pod fiber mutant '*bfm*' is a stable genetic mutant strain obtained from dried seeds of 'Jinguan' through EMS mutagenesis treatment. There were no significant differences in plant height and branching between the mutant '*bfm*' and the wild-type 'Jinguan', and the plant type development was basically consistent (Fig. 1). The bean pod hardness and brittleness of '*bfm*' were both higher than those of 'Jinguan'.

2.2 Determination of Fiber Content in Pod Walls and Suture Tissues of Snap Bean

The mutant '*bfm*' and its wild-type 'Jin Guan' were planted in the greenhouse of the Horticultural Experimental Base at Hulan Campus of Heilongjiang University. Bean pods without diseases or pests and of uniform size were collected from '*bfm*' and 'Jinguan' at 6, 10, 14, and 18 days after flowering, respectively. The pod walls and suture tissues of the bean pods were sampled separately, with three replicates set for each treatment. The treated pod walls and suture tissues were placed in an electric thermostatic oven set at 100°C for 30 minutes of blanching treatment, and then transferred to an 80°C electric thermostatic oven for continuous drying for 8–10 hours until constant weight for standby. The contents of crude fiber, cellulose, hemicellulose, and lignin were determined using an automatic fiber analyzer (ANKOM, USA).

2.3 Cytological Observation of Pod Wall Tissues

Bean pods of '*bfm*' and 'Jin Guan' at 6, 10, 14, and 18 days after flowering were collected as observation samples. A 2-cm section from the middle of each pod was taken, excluding the suture and seeds, with three biological replicates set. The sections were cut into approximately 0.5-cm segments and placed into vials containing FAA fixative. The tissues underwent a series of processing steps: fixation - dehydration - clearing - wax infiltration - embedding and sectioning - section spreading and mounting - staining - staining again - coverslipping - microscopic observation.

2.4 Transcriptome and Metabolome Sequencing Analysis

The pod wall tissues of ‘*bfm*’ and ‘Jinguan’ at 6, 10, 14, and 18 days after flowering were used as experimental materials. Name the pod wall samples of mutant ‘*bfm*’ at 6, 10, 14, and 18 days after flowering as M1, M2, M3, and M4, respectively; The pod wall samples of wild-type ‘Jin Guan’ flowers at 6, 10, 14, and 18 days after flowering were named W1, W2, W3, and W4, respectively. For transcriptome analysis, three biological replicates were set with 0.5 g per replicate; for metabolome analysis, six biological replicates were set with 0.1 g per replicate. After sampling, the samples were quickly frozen in liquid nitrogen and then transferred to -80°C for storage. The samples were sent to Hangzhou Lianchuan Biotechnology Co., Ltd (Hangzhou, China) for sequencing. The pod wall samples of the wild-type ‘Jinguan’ at different developmental stages were named WT1, WT2, WT3, and WT4, respectively. The corresponding periods in the mutant ‘*bfm*’ pod wall samples are named M1, M2, M3, and M4.

2.5 Gene expression analysis by qRT-PCR

The cDNA samples from the pod wall tissues of ‘*bfm*’ and ‘Jinguan’ at 6, 10, 14, and 18 days after flowering were obtained as described above, and specific primers for candidate genes were designed by Primer Premier 6.0 (Table 1). A MY17295272 fluorescence quantitative PCR instrument (Agilent Technologies Inc. CA., USA) was used for qPCR experiments. Actin was used as the internal reference gene, the $2^{-\Delta\Delta CT}$ method was used to calculate relative expression levels of genes (Wan et al. 2010), the least significant difference (LSD) test was used for single factor difference analysis, and GraphPad software (<https://www.graphpad.com/scientific-software/prism/>) was used for mapping. The reaction system for qPCR was 10 μ L of 2×Fast qPCR Mix, 0.4 μ L of forward and reverse primer (10 μ M), 2 μ L of cDNA and 7.2 μ L of DEPC-ddH₂O. Thermal cycling included an initial denaturation at 95°C for 30 s, followed by 40 cycles at 95°C for 5 s and 60°C for 15 s. Three technical replicates and three biological replicates were performed.

Table 1
Quantitative genes and primers for qRT-PCR

Markers	Forward (5’-3’)	Reverse (5’-3’)
Phvul.008G256800	ATGGAGATGGACACACAACCACATAAGA	TTATTTGAAGGAAGCAACCAAAAGTGAATTACAGTC
Phvul.002G048400	ATGATCGTGCTAAAATTCTCAAC	CTTGAATGCACACACTCCTAG
Phvul.004G019600	ATGGGGGGAGAGTGGTGGG	TCATAGGAGTGAATGCGAAGCAA
Phvul.010G083900	ATGGCGAATCCTCGATTTC	TTAGATACCAAAATTGCATGCCTG
Phvul.008G268900	ATGGCTTTAACTTTGTGTTACAT	TCAAGAGATGTTGCATTCCGG
Phvul.005G087400	ATGAATGCAGCTTCTTTGCACATAGCAA	TTAGATTATATCACGAAGCTTGTGACAGAAAGCA
Phvul.003G089600	ATGAGTATGTACGGTAGGGATCCGT	TCATGGTTTCCATGGTGCAGGAGGT
Phvul.007G077800	ATGGGCAAGAGCAAAACTCTCGTG	TCACACGCTTCCAATAATTGAAGCACT
Actin	GAAGTTCTCTTCCAACCATCC	TTTCCTTGCTCATTCTGTCCG

3. RESULTS

3.1 Analysis of fiber content in pod wall and suture tissue of mutant and its wild type

Determination of crude fiber, cellulose, hemicellulose, and lignin content in the pod walls and suture tissues of mutant ‘*bfm*’ and wild-type ‘Jin Guan’ at different developmental stages. The results showed that there was no significant difference in the content of various fiber components in the suture tissue of mutant ‘*bfm*’ compared to wild-type ‘Jin Guan’ (Fig. 2). While the crude fiber content of the mutant ‘*bfm*’ pod wall tissue gradually increased with pod development compared to the wild type, showing significant differences in pod wall tissue at 14 and 18 days after flowering (Fig. 3). The content of hemicellulose and lignin in pod wall tissue of ‘*bfm*’ pods is lower than that of wild-type ‘Jin Guan’ at all

developmental stages of the pods. The content of cellulose in pod wall tissue of '*bfm*' pods is not significantly different from that of 'Jin Guan' at 6–14 days after flowering, but is significantly higher than that of 'Jin Guan' at 18 days after flowering (commercial pod stage) (Fig. 3). Therefore, we speculate that the change in cellulose content of the pod wall is the main reason for the difference in fiber content between the pod fiber mutant '*bfm*' and the 'Jin Guan' pod wall.

Figure 2. Comparison of fiber content in suture tissues of '*bfm*' and 'Jin Guan' pods

*Significant difference ($p < 0.05$)

3.2 Cytological observation of pod wall tissue

After observing the longitudinal sections of the pod wall tissues of mutant '*bfm*' and wild-type 'Jin Guan' through paraffin sections (Fig. 4), it was found that cells of different sizes formed a circular structure, from the outside to the inside, consisting of palisade tissue, thin-walled cell layer, cork layer, thick walled cell layer, and thin-walled cell layer. As the pod wall develops, the thin-walled cell layer becomes lignified and thickened into thick walled cells with small or no intercellular gaps, while the innermost thin-walled cell layer is tightly arranged and the intercellular gaps are relatively tight. As the mutant '*bfm*' pods grow and develop, the number of cells increases significantly compared to 'Jin Guan', the number of phloem fibers increases, the cell wall thickens, the intercellular space shortens, and the cell density decreases, resulting in highly fibrotic tissue. It is speculated that this situation is the cause of the change in '*bfm*' fiber content.

3.3 Transcriptome analysis of pod wall tissue at different developmental stages between wild-type and mutant

3.3.1 Illumina Sequencing, DEG Analysis, Functional Annotation and Classification

In this study, we constructed 24 independent sequencing libraries. After Illumina sequencing, a total of 940164398 readings were generated (Supplementary Table 1). To ensure the accuracy of subsequent analysis, quality control analysis is performed on the raw data. Align clean reads to the reference genome of snap beans (<https://phytozome-next.jgi.doe.gov/>). The comparison rate of each sample exceeds 94%, and the sequencing data quality is good, providing good data support for subsequent experimental research.

Compare the sequencing data of the processing group (mutant '*bfm*') and the control group (wild-type 'Jin Guan') at four different stages pairwise to analyze differentially expressed genes. The results showed that there were a total of 500 DEGs in W1 vs. M1, 5640 DEGs in W2 vs., 2713 DEGs in W3 vs. M3, 514 DEGs in W4 vs. M4. Through Venn plot analysis, we found that there were a total of 18 DEGs in the four periods of W1vsM1, W2vsM2, W3vsM3, and W4vsM4 (Supplementary Fig. 1).

Perform GO and KEGG clustering analysis on DEGs in different comparison groups. According to the GO annotation results, it was found that differentially expressed genes were mainly enriched in molecular functions such as hydrolase, pectinase, oxidoreductase, transferase activity, cell wall tissue, and carbohydrate metabolism processes (Supplementary Table 2). This indicates that genes related to cellulose synthesis mainly regulate or affect cellulose synthesis by participating in processes such as cell wall, sugar metabolism, enzyme activity, and biosynthesis of plant secondary metabolites. According to the KEGG annotation results of four comparison groups, it was found that genes related to cellulose synthesis were significantly enriched in secondary metabolite biosynthesis, metabolic pathways, starch and sucrose metabolism, and plant hormone signal transduction, indicating that cellulose synthesis is related to these metabolic pathways (Supplementary Fig. 2).

3.3.2 Analysis of cellulose synthesis related genes

Physiological data indicate that the difference in cellulose content between the pod fiber mutant '*bfm*' and 'Jin Guan' gradually becomes significant with pod development. The cellulose content in the pod wall of '*bfm*' after 18 days of flowering was significantly higher than that of 'Jin Guan'. We analyzed that this may be due to changes in the expression of certain genes during the development of pod wall tissue, which affects the biosynthesis of cellulose in the pod wall tissue. In order to clarify the relationship between changes in the content of cellulose synthesis intermediates and gene regulation expression during the growth and development of snap beans, we studied the expression changes of related genes in the cellulose synthesis pathway during different stages of pod development. Five genes encoding cellulose synthase were found in four periods (Table 2). The expression levels of four genes, Phvul.002G040200, Phvul.002G136300, Phvul.006G08400, and Phvul.007G081700, were higher in '*bfm*' than in 'Jin Guan' as the pods developed. The overall trend of cumulative changes in cellulose content determined in the previous stage is consistent, indicating that changes in the expression of cellulose synthase related genes may be related to the '*bfm*' mutation trait.

Table 2
Cellulose synthase differentially expressed gene

Gene_ID	Pathway	W1vsM1 log2(fc)	W2vsM2 log2(fc)	W3vsM3 log2(fc)	W4vsM4 log2(fc)	Description
Phvul.006G058400	GO:0016760	0.02	2.39	0.42	2.36	Cellulose synthase-like protein E1
Phvul.007G081700	/	-0.53	0.56	0.62	0.01	Cellulose synthase
Phvul.009G242700	GO:0071555 GO:0030244 GO:0016760	0.33	-0.81	-1.98	-0.04	Cellulose synthase A Catalytic Subunit 7 (UDP-forming)
Phvul.002G040200	GO:0071555 GO:0030244 GO:0016760	-0.18	0.01	1.08	0.28	Cellulose synthase-like protein D3
Phvul.002G136300	GO:0071555 GO:0016760	-0.9	2.11	1.18	1.14	Cellulose synthase (UDP-forming)

Through KEGG enrichment analysis, 25 differentially expressed genes related to cellulose synthesis were screened for metabolic pathways, starch and sucrose metabolic pathways, secondary metabolite biosynthesis pathways (Table 3). Compared with the wild-type 'Jin Guan', the mutant '*bfm*' showed no significant changes in the expression levels of these 25 genes in the pod wall of 6–10 days after flowering, which is consistent with the physiological data measured earlier that showed no significant difference in crude fiber content during these two periods. 14 days after flowering, three genes, Phvul.003G089600, Phvul.007G077800, and Phvul.007G218400, were significantly upregulated in the mutant '*bfm*'. Phvul.003G089600 and Phvul.007G077800 were significantly upregulated in the mutant '*bfm*' in the pod wall of 18 days after flowering. In summary, during the period of 14–18 days after flowering, which is the formation period of commercial pods in snap beans, two genes *Phvul.003G089600* (*KOR*) and *Phvul.007G077800* (*CTL*) consistently showed significant upregulation in the mutant '*bfm*'. *KOR* and *CTL* are glycoside hydrolases that play important roles in cell wall synthesis metabolism and cellulose synthesis. Therefore, it is speculated that changes in the expression of these two genes affect or promote precursor substances for cellulose synthesis, leading to changes in cellulose content.

Table 3
6 Analysis of expression levels of 25 genes

Gene_ID	Pathway	W1vsM1 log2(fc)	W2vsM2 log2(fc)	W3vsM3 log2(fc)	W4vsM4 log2(fc)	Description
Phvul.001G209600	pvu01100	1.11	1.90	-0.28	0.22	SUCROSE SYNTHASE 5
Phvul.009G114700	pvu01110 pvu01100	0.95	0.99	0.68	0.44	Sucrose transport protein SUC4
Phvul.003G089600	pvu01100 pvu00500	-0.02	-0.61	7.83	2.13	ENDOGLUCANASE 21
Phvul.006G0049001	/	8.56	-0.56	1.04	-0.10	Endoglucanase 7
Phvul.009G135900	/	-0.16	11.42	-2.62	-0.25	Endoglucanase 25
Phvul.006G133700	/	-0.16	-0.21	-0.06	-0.56	Endoglucanase 25
Phvul.007G218400	pvu01100 pvu00500	-0.89	0.21	2.65	0.93	Endoglucanase 1
Phvul.009G254400	pvu01100 pvu00520	0.60	0.81	-1.86	-0.79	Chitinase-like protein 2
Phvul.008G018100	/	-0.02	-0.75	0.22	0.26	Chitinase-like protein 2
Phvul.007G077800	pvu01100 pvu00520	-4.22	6.55	3.56	12.55	Chitinase
Phvul.011G167000	pvu01100 pvu00520	-0.47	-1.29	1.69	1.28	Chitinase3
Phvul.005G155800	pvu01100 pvu00520	-0.88	6.30	1.40	0.58	CHITINASE-RELATED
Phvul.009G116700	pvu00520	2.12	5.46	-3.54	-0.94	BASIC ENDOCHITINASE B
Phvul.003G158800	pvu01100	-0.70	11.10	0.11	-1.24	Chitinase
Phvul.004G041300	pvu01110	-0.15	-1.34	1.70	0.50	COBRA-like protein
Phvul.009G203100	pvu01110 pvu01100	-0.01	-1.61	0.49	-0.06	COBRA-like protein
Phvul.008G029100	pvu01110	0.27	-0.98	0.44	-0.23	COBRA-like protein
Phvul.008G029200	pvu01110 pvu01100	-0.14	0.65	1.07	-0.08	COBRA-like protein
Phvul.002G075200	pvu01110 pvu01100	-1.13	3.05	-3.68	0.48	UDP-glycosyltransferase 74B1
Phvul.008G208400	pvu01100	-0.17	-0.27	0.96	-1.76	UDP-glycosyltransferase 92A1
Phvul.008G29100	pvu01100	-1.09	0.80	-2.11	-1.61	UDP-glycosyltransferase 82A1

Gene_ID	Pathway	W1vsM1 log2(fc)	W2vsM2 log2(fc)	W3vsM3 log2(fc)	W4vsM4 log2(fc)	Description
Phvul.006G087300	pvu01110 pvu01100	0.18	0.42	1.37	-7.86	SUCROSE SYNTHASE 5
Phvul.004G142800	pvu01100 pvu00500	0.42	0.33	0.29	-2.32	SUCROSE SYNTHASE 5
Phvul.008G241300	pvu01100 pvu00500	0.00	0.06	0.23	-2.10	SUCROSE SYNTHASE 5
Phvul.009G250800	pvu01100 pvu00500	0.00	0.00	-1.36	-7.48	SUCROSE SYNTHASE 5

3.3.3 qRT-PCR validation

To verify the accuracy of RNA-seq sequencing results, 6 DEGs were randomly selected Phvul.008G132200, Phvul.003G147700, Phvul.002G048400, Phvul.008G290300, Phvul.004G019600 and Phvul.005G155800 perform real-time fluorescence quantitative PCR validation. The qRT-PCR results showed that the expression levels of six genes showed the same trend as RNA-seq, indicating that RNA-seq sequencing data has good quality.

3.4 Metabolomic Analysis of Pod Wall Tissues at Different Developmental Stages between Wild-Type and Mutant

3.4.1 Principal component analysis

To validate differences between samples, correlation analysis and principal component analysis (PCA) were performed on the collected samples from two varieties across four growth stages. Correlation analysis revealed that the two principal components (PC1 and PC2) accounted for 31.72% and 94.8% of the variance, respectively. PCA results indicated that samples within each group clustered closely, while samples from different groups showed clear.

3.4.2 differential metabolites analysis

In this study, to identify significantly accumulated metabolites across different comparison groups, we screened for differential metabolites in four comparison sets: W1 vs M1, W2 vs M2, W3 vs M3, and W4 vs M4. A total of 1,030 differential metabolites were identified. Among the four comparison groups (W1 vs M1, W2 vs M2, W3 vs M3, W4 vs M4), 29 differentially expressed metabolites were common across all groups. Key differentially expressed metabolites included amino acids and their derivatives, phenolic acids, nucleotides and their derivatives, trehalose, D-glucose, α -glucose, D-arabinitol, D-ribose, quinic acid, quercetin, corticosterone, caffeic acid, flavonoids, quinones, lignan alkaloids, terpenoids, organic acids, and lipids. Changes in the content of these metabolites are presumed to have caused differences in cellulose content between 'bfm' and 'Jin guang'. KEGG analysis indicated significant enrichment in metabolic pathways (ko01100), starch and sucrose metabolism (ko00500), and biosynthesis of plant secondary metabolites (ko011060) during the comparison of the four periods.

3.5 Integrated Analysis of Transcriptome and Metabolome

To elucidate the correlation between cellulose-related differentially expressed genes and metabolites, KEGG pathway enrichment analysis was performed on the differentially expressed genes and metabolites identified across the four

stages (W1vsM1, W2vsM2, W3vsM3, and W4vsM4). This revealed information about metabolic pathways jointly involved in both groups, with metabolic pathways and secondary metabolite biosynthesis pathways emerging as particularly significant pathways.

3.5.1 Regulatory Network of Metabolic Pathways

Metabolic pathways are crucial for plant growth and development, and they synergistically influence the synthesis of cellulose, the primary component of plant cell walls. Amino acids such as glycine and serine can indirectly affect cellulose synthesis by regulating cell wall structure and the expression of endoglucanases and chitinases. D-ribose can regulate the gene expression of these two enzymes through energy and sugar metabolism, thereby influencing cellulose synthesis. Although D-arabinose does not directly participate in cellulose synthesis, it can indirectly affect cellulose deposition by regulating carbon source allocation to support precursor formation, influencing related enzyme gene expression, or controlling hemicellulose and lignin synthesis.

Within this metabolic pathway, metabolites associated with cellulose synthesis or its regulation were identified: 4-hydroxycinnamic acid, serine, glycine, L-glutamine, arabitol (D), D-ribose, etc. These metabolites were linked to differentially expressed genes *Phvul.003G089600* (*KOR*), *Phvul.005G155800* (*CHI4*), *Phvul.007G077800* (*CTL*), and *Phvul.011G167000* (*CHIT3*). Except for 4-hydroxycinnamic acid, which exhibits negative regulation with other genes, the remaining metabolites all show positive regulation with these genes.

3.5.2 Regulatory Network of Secondary Metabolite Biosynthetic Pathways

The biosynthetic pathways of secondary metabolites are crucial for cellulose synthesis. L-alanine may indirectly regulate sucrose gene expression and glycosyltransferase activity by influencing nitrogen metabolism and energy supply through its association with carbohydrate metabolism, while glycine may do so by participating in cell wall protein synthesis. The Flavonoid secondary metabolite delphinidin-3-glucoside indirectly influences cellulose synthesis by regulating enzyme gene expression through antioxidant effects, secondary metabolism control, and stress response modulation.

In this pathway, metabolites associated with cellulose synthesis or its regulation—L-alanine, glycine, and apigenin-3-glucoside—exhibit positive correlations with genes *Phvul.002G075200* (*UGT74B1*) and *Phvul.006G087300* (*SUS5*).

3.5.3 Key Gene Screening

Through integrated transcriptomic and metabolomic analysis, we identified eight differentially expressed metabolites in the metabolic pathways and secondary metabolic biosynthetic pathways, including 4-hydroxycinnamic acid, serine, glycine, L-glutamine, L-alanine, D-arabinitol, D-ribose, and Delphinidin 3-glucoside were associated with *Phvul.003G089600* (*KOR*), *Phvul.005G155800* (*CHI4*), *Phvul.007G077800* (*CTL*), *Phvul.011G167000* (*CHIT3*), *Phvul.002G075200* (*UGT74B1*), and *Phvul.006G087300* (*SUS5*). Among these, *Phvul.003G089600* (*KOR*) and *Phvul.007G077800* (*CTL*) showed significant correlations with other metabolites. Both genes exhibited consistently significant upregulation during the commercial pod stage (14–18 days after flowering). These genes encode endoglucanase (*KOR*) and chitinase (*CTL*), respectively. Both belong to the glycoside hydrolase family. While they do not directly synthesize sugars or cellulose, they degrade complex polysaccharides into reusable sugar units. These units are then converted into UDP-glucose (a precursor for cellulose synthesis) via glycolysis or gluconeogenesis, thereby indirectly influencing or promoting cellulose synthesis. Therefore, it is inferred that the genes *Phvul.003G089600* (*KOR*) and *Phvul.007G077800* (*CTL*) are associated with the phenotypic traits of the pod fiber mutant.

3.5.4 qRT-PCR Validation of Candidate Genes for Cellulose Synthesis

To verify whether the differential expression patterns of the two genes *Phvul.003G089600* (*KOR*) and *Phvul.007G077800* (*CTL*) align with the cellulose content trend during '*bfm*' growth and development, we performed real-time quantitative PCR analysis (Fig. 11). Results revealed significant differences in both genes during the 14–18 days post-flowering (commercial pod stage) period, consistent with the cellulose content trend observed during '*bfm*' growth and development. This suggests an association with the phenotypic differences in the pod fiber mutant '*bfm*'.

4 DISCUSSION

The causes of plant tissue fibrosis vary across different crops. Previous studies have shown that quality deterioration in fruits such as pears (Cai et al. 2010) and citrus (Wu et al. 2014; Liu et al. 2022) is closely linked to increased lignin synthesis and deposition. Similarly, post-harvest fibrosis in asparagus (Hennion et al. 1992) and bamboo shoots (Luo et al. 2008) is primarily attributed to elevated lignin content. However, fiber composition analysis of the mutant *bfm* and the wild-type 'Golden Crown' in this study revealed no significant differences in hemicellulose or lignin content between the two. Conversely, the cellulose content in *bfm* was significantly higher than that in the wild-type during the marketable pod stage, and this change was consistent with an increase in crude fiber content. This result indicates that the fibrillation phenotype in the fiber wall mutant of common bean is not dominated by lignin but rather caused by abnormal accumulation of cellulose content, consistent with the findings of (Murgia et al. 2017) research. The reason for this discrepancy may be closely related to the specificity of the study material. As a crop whose edible organ is the tender pod, common bean is more sensitive to the impact of cellulose content on texture, and its fibrillation regulatory mechanism may be more inclined toward regulating cellulose metabolism. Furthermore, transcriptomic analysis revealed that differentially expressed genes were predominantly enriched in metabolic pathways related to cellulose synthesis, starch and sucrose metabolism, rather than the phenylpropanoid pathway critical for lignin synthesis. This molecular evidence further substantiates the pivotal role of cellulose in the *bfm* mutant phenotype.

Through transcriptomic and metabolomic analysis, this study identified two core regulatory genes—*Phvul.003G089600* (*KOR*) and *Phvul.007G077800* (*CTL*). Both genes exhibited sustained significant upregulation during the commercial pod formation stage (14–18 days after flowering) and encode glycoside hydrolase family proteins. Previous studies have confirmed that the endo- β -1,4-glucanase encoded by the *KOR* gene is an essential protein for cellulose biosynthesis and cell wall assembly. It promotes cellulose polymer synthesis and recycling by cleaving glucan primers. (Mølholm et al. 2001) research using *Arabidopsis* revealed *KOR* gene involvement in cell wall assembly throughout the entire plant. (von Schaewen et al. 2015) research revealed that *KOR* genes play a central role in maintaining cellulose biosynthesis in both primary and secondary cell walls. Chitinase-like proteins encoded by *CTL* genes play crucial roles in plant cell wall structural organization and cellulose deposition. Their homologs in cotton and *Arabidopsis* have been confirmed to participate in secondary cell wall cellulose synthesis (Kwon et al. 2005). demonstrated that chitinases may contribute to cell wall structural organization and potentially function in *Arabidopsis* (*A. thaliana*) active defense systems. For instance, (Zhang et al. 2004). reported that two homologous cotton chitinase-like proteins (GhCTL1 and GhCTL2) are preferentially expressed during secondary cell wall deposition in cotton fiber cells and are responsible for cellulose biosynthesis during primary and secondary cell wall formation in *Arabidopsis* vascular tissues. (Aktar Hossain et al. 2010). demonstrated that AtCTL2 is essential for proper cell wall biosynthesis in *Arabidopsis* seedlings using a mutant of the chitinase-like protein-encoding gene AtCTL2.

In this study, the abnormal upregulation of these two genes may synergistically regulate the production and conversion of cellulose synthesis precursors through changes in the levels of metabolites such as glycine and D-ribose. This ultimately leads to abnormal accumulation of pod wall cellulose, acting as a key molecular switch driving the fibrillation phenotype.

5 CONCLUSIONS

This study demonstrates that the fiber mutant '*bfm*' in common bean pods exhibits significantly higher fiber content than the wild type during the commercial maturity stage. Cellulose appears to be the primary factor contributing to pod wall thickening. Integrated transcriptomic and metabolomic analyses reveal that differentially expressed genes and metabolites are predominantly enriched in metabolic pathways and secondary metabolite biosynthesis pathways. These include amino acids and their derivatives, arabinol (D), D-ribose, apigenin 3-glucoside, and carbohydrates, closely associated with *Phvul.007G077800* (*CTL*) and *Phvul.003G089600* (*KOR*). These metabolites and genes indirectly influence or regulate precursors involved in cellulose synthesis, providing essential substrates and energy to promote cellulose formation. Additionally, we investigated the key *CTL* and *KOR* genes within the cellulose synthesis regulatory network, briefly elucidating their crucial roles and establishing a theoretical foundation for future studies on the regulation of pod wall cellulose synthesis in *Phaseolus vulgaris*.

In summary, this study confirms that the high cellulose content trait in the bean pod fiber mutant '*bfm*' is not regulated by a single gene or metabolite, but rather results from the synergistic action of a complex regulatory network. These findings provide key candidate genes and metabolic markers for molecular breeding of this fiber bean pod variety.

Declarations

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Ethical approval was not required as the study used only secondary data from publicly available, de-identified sources.

CONSENT FOR PUBLICATION

All authors have read and agreed to the submission and publication of this paper. The content of the paper represents original research results, with no plagiarism, no fabrication, and no duplicate submission. There is no infringement of others' intellectual property rights, no confidential content, and no conflicts of interest. All authors agree to submit this paper to *BMC Plant Biology* for publication and to comply with the relevant publication regulations of the journal.

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Author Contribution

Kanhui Mo: Writing-Original Draft, Writing-Review & Editing, Visualization. Zhuang Sun: Investigation, Data Curation. Guojun Feng, Zhishan Yan: Resources, Supervision. Dajun Liu, Taifeng Zhang: Resources, Software. Xiaoxu Yang*, Chang Liu* (corresponding author): Project administration.

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Data Availability

The raw RNA-seq data generated in this study have been submitted to the NCBI Gene Expression Omnibus (GEO) database under submission ID 2251963@orcid. The submission is currently under review by GEO, and the official accession number will be provided once the review is completed. All data will be made publicly available upon publication.

References

1. Wu B, Zhang B, Dai Y, Zhang L, Shang-Guan K, Peng Y, Zhou Y, Zhu Z. Brittle culm15 encodes a membrane-associated chitinase-like protein required for cellulose biosynthesis in rice. *Plant Physiol.* 2012;159(4):1440–52.
2. Parker TA, Lo S, Gepts P. Pod shattering in grain legumes: emerging genetic and environment-related patterns. *Plant Cell.* 2021;33(2):179–99. 10.1093/plcell/koaa025.
3. Rau D, Murgia ML, Rodriguez M, Bitocchi E, Bellucci E, Fois D, Albani D, Nanni L, Gioia T, Santo D, et al. Genomic dissection of pod shattering in common bean: mutations at non-orthologous loci at the basis of convergent phenotypic evolution under domestication of leguminous species. *Plant J.* 2019;97(4):693–714. 10.1111/tbj.14155.
4. Wan H, Zhao Z, Qian C, Sui Y, Malik AA, Chen J. Selection of appropriate reference genes for gene expression studies by quantitative real-time polymerase chain reaction in cucumber. *Anal Biochem.* 2010;399(2):257–61.
5. Cai Y, Li G, Nie J, Lin Y, Nie F, Zhang J, Xu Y. Study of the structure and biosynthetic pathway of lignin in stone cells of pear. *Sci Hort.* 2010;125(3):374–9. 10.1016/j.scienta.2010.04.029.
6. Wu J-L, Pan T-F, Guo Z-X, Pan D-M. Specific Lignin Accumulation in Granulated Juice Sacs of *Citrus maxima*. *J Agric Food Chem.* 2014;62(50):12082–9. 10.1021/jf5041349.
7. Liu X, Zhang H, Zhang W, Xu W, Li S, Chen X, Chen H. Genome-wide bioinformatics analysis of Cellulose Synthase gene family in common bean (*Phaseolus vulgaris* L.) and the expression in the pod development. *BMC Genomic Data.* 2022;23(1). 10.1186/s12863-022-01026-0.
8. Hennion S, Anthony Little CH, Hartmann C. Activities of enzymes involved in lignification during the postharvest storage of etiolated asparagus spears. *Physiol Plant.* 1992;86(3):474–8. 10.1111/j.1399-3054.1992.tb01346.x.
9. Luo Z, Xu X, Yan B. Accumulation of lignin and involvement of enzymes in bamboo shoot during storage. *Eur Food Res Technol.* 2008;226(4):635–40. 10.1007/s00217-007-0595-y.
10. Murgia ML, Attene G, Rodriguez M, Bitocchi E, Bellucci E, Fois D, Nanni L, Gioia T, Albani DM, Papa R. A comprehensive phenotypic investigation of the pod-shattering syndrome in common bean. *Front Plant Sci.* 2017;8:251.
11. Mølthøj M, Jørgensen B, Ulvskov P, Borkhardt B. Two Arabidopsis thaliana genes, KOR2 and KOR3, which encode membrane-anchored endo-1,4-β-D-glucanases, are differentially expressed in developing leaf trichomes and their support cells. *Plant Mol Biol.* 2001;46(3):263–75. 10.1023/A:1010688726755.
12. von Schaewen A, Rips S, Jeong IS, Koiwa H. Arabidopsis thaliana KORRIGAN1 protein: N-glycan modification, localization, and function in cellulose biosynthesis and osmotic stress responses. *Plant Signal Behav.* 2015;10(5):e1024397. 10.1080/15592324.2015.1024397.
13. Kwon H-K, Yokoyama R, Nishitani K. A Proteomic Approach to Apoplastic Proteins Involved in Cell Wall Regeneration in Protoplasts of Arabidopsis Suspension-cultured Cells. *Plant Cell Physiol.* 2005;46(6):843–57. 10.1093/pcp/pci089.
14. Zhang D, Hrmova M, Wan C-H, Wu C, Balzen J, Cai W, Wang J, Densmore LD, Fincher GB, Zhang H, et al. Members of a New Group of Chitinase-Like Genes are Expressed Preferentially in Cotton Cells with Secondary Walls. *Plant Mol Biol.* 2004;54(3):353–72. 10.1023/B:PLAN.0000036369.55253.dd.

15. Aktar Hossain M, Noh H-N, Kim K-I, Koh E-J, Wi S-G, Bae H-J, Lee H, Hong S-W. Mutation of the chitinase-like protein-encoding *AtCTL2* gene enhances lignin accumulation in dark-grown *Arabidopsis* seedlings. J Plant Physiol. 2010;167(8):650–8. 10.1016/j.jplph.2009.12.001.

Figures

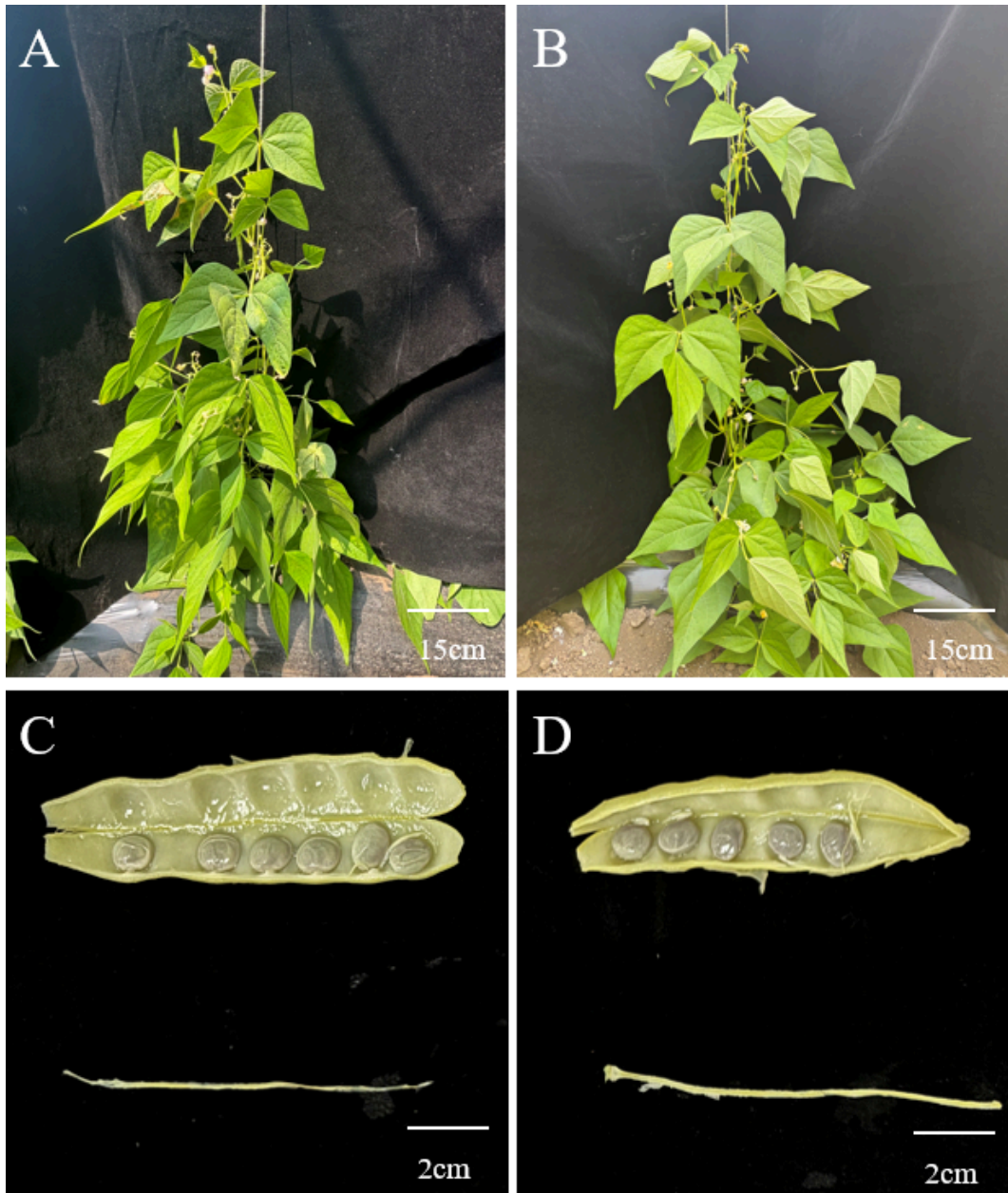


Figure 1

Photos of mutant '*bfm*' and its wild-type 'Jin Guan' plants. A is the wild-type 'Jinguan', B is the mutant '*bfm*'.

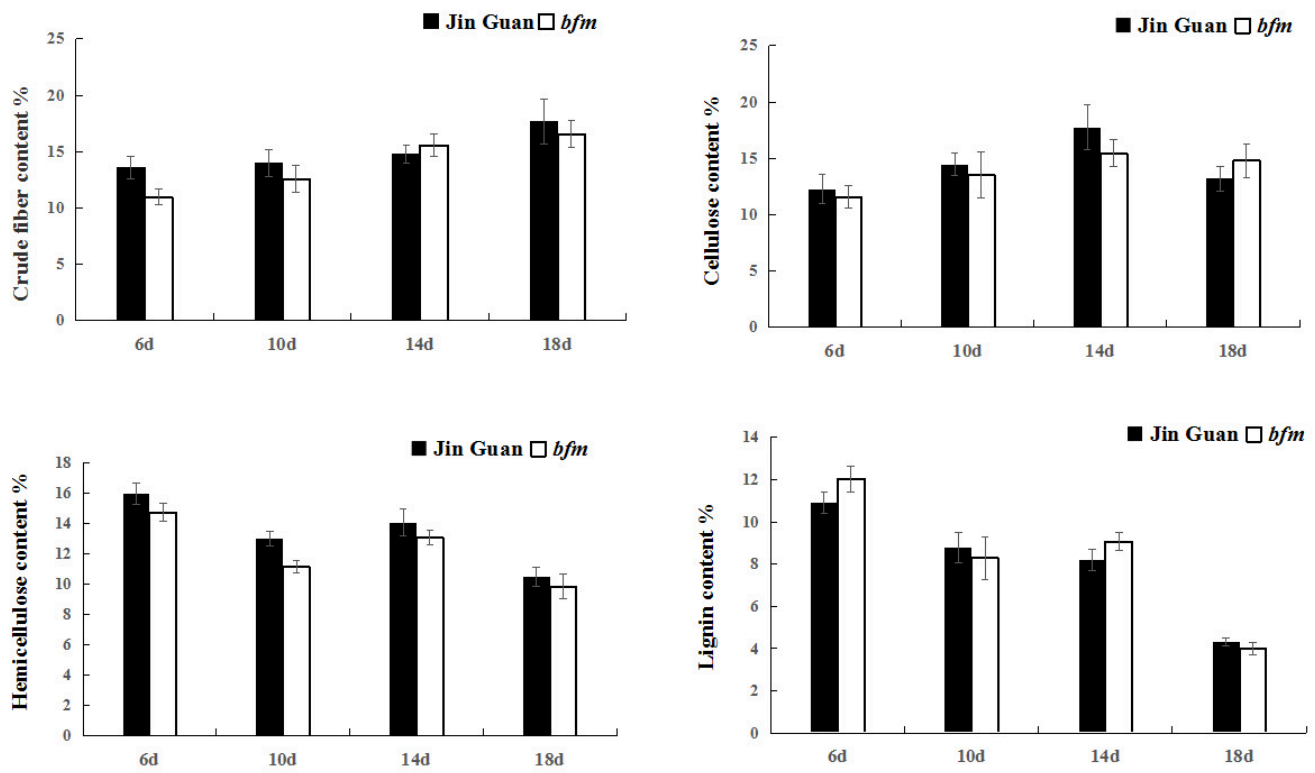


Figure 2

Comparison of fiber content in suture tissues of '*bfm*' and 'Jin Guan' pods

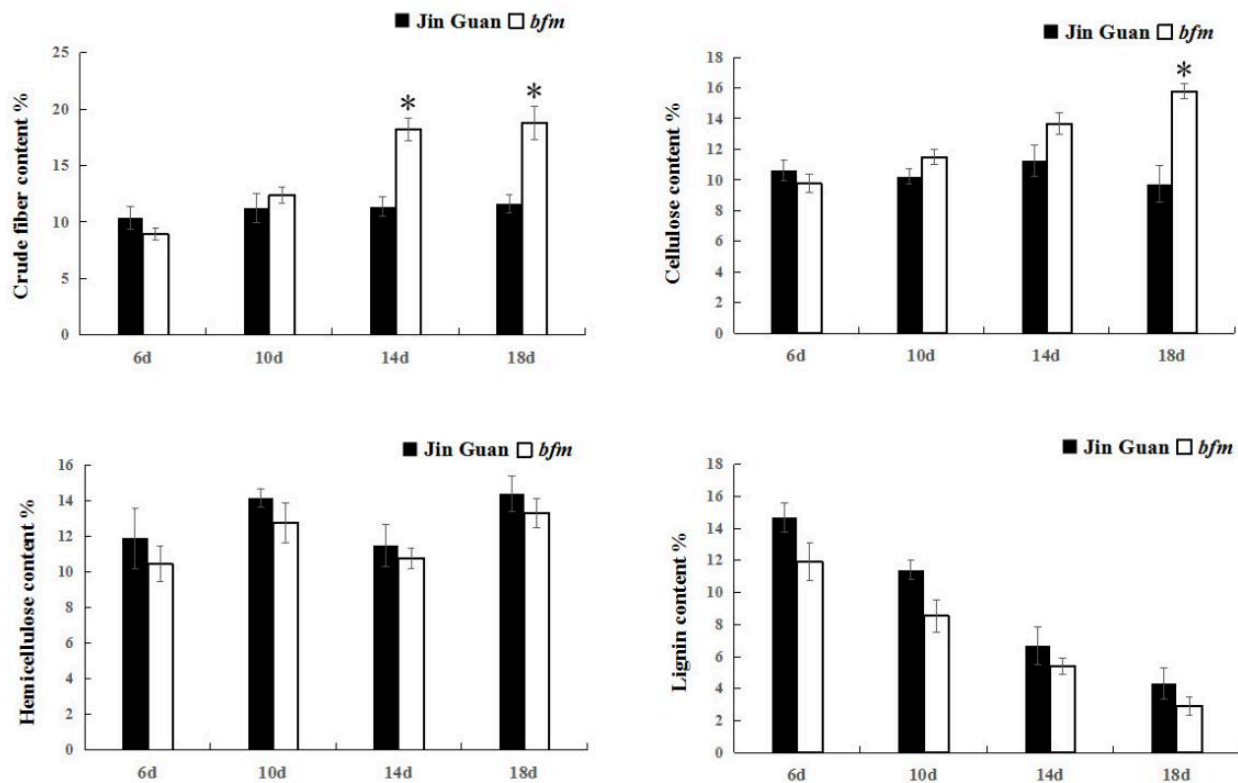


Figure 3

Comparison of fiber content in pod wall of 'bfm' and 'Jin Guan' pods

*Significant difference ($p < 0.05$)

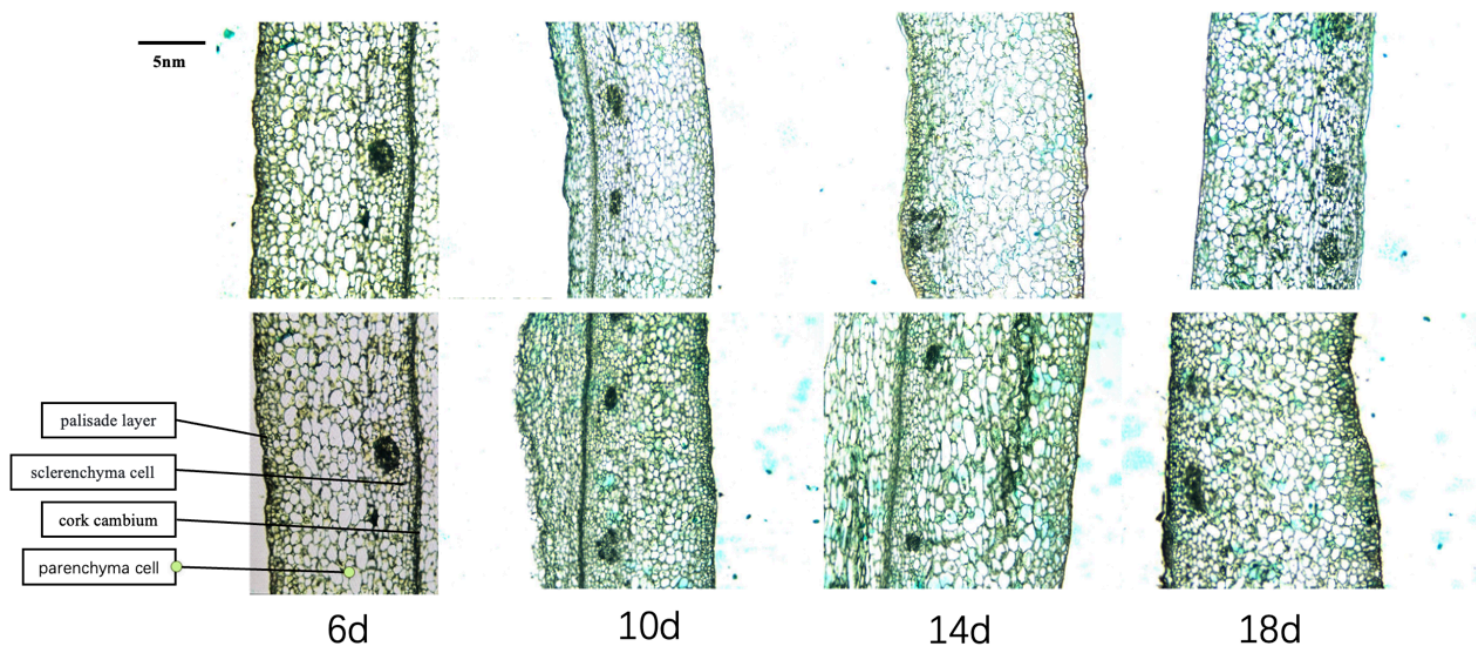


Figure 4

Observation of longitudinal sections of pod wall tissues of the mutant '*bfm*' and the wild-type 'Jin Guan' using paraffin sections

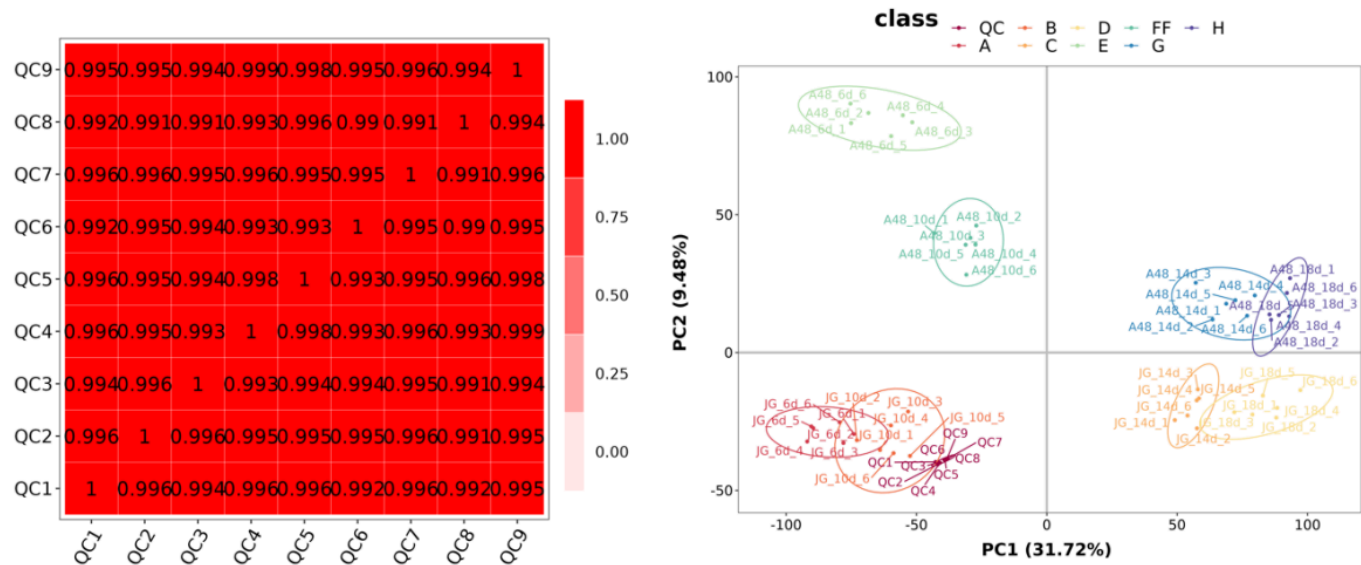


Figure 5

Correlation Analysis and Principal Component Analysis

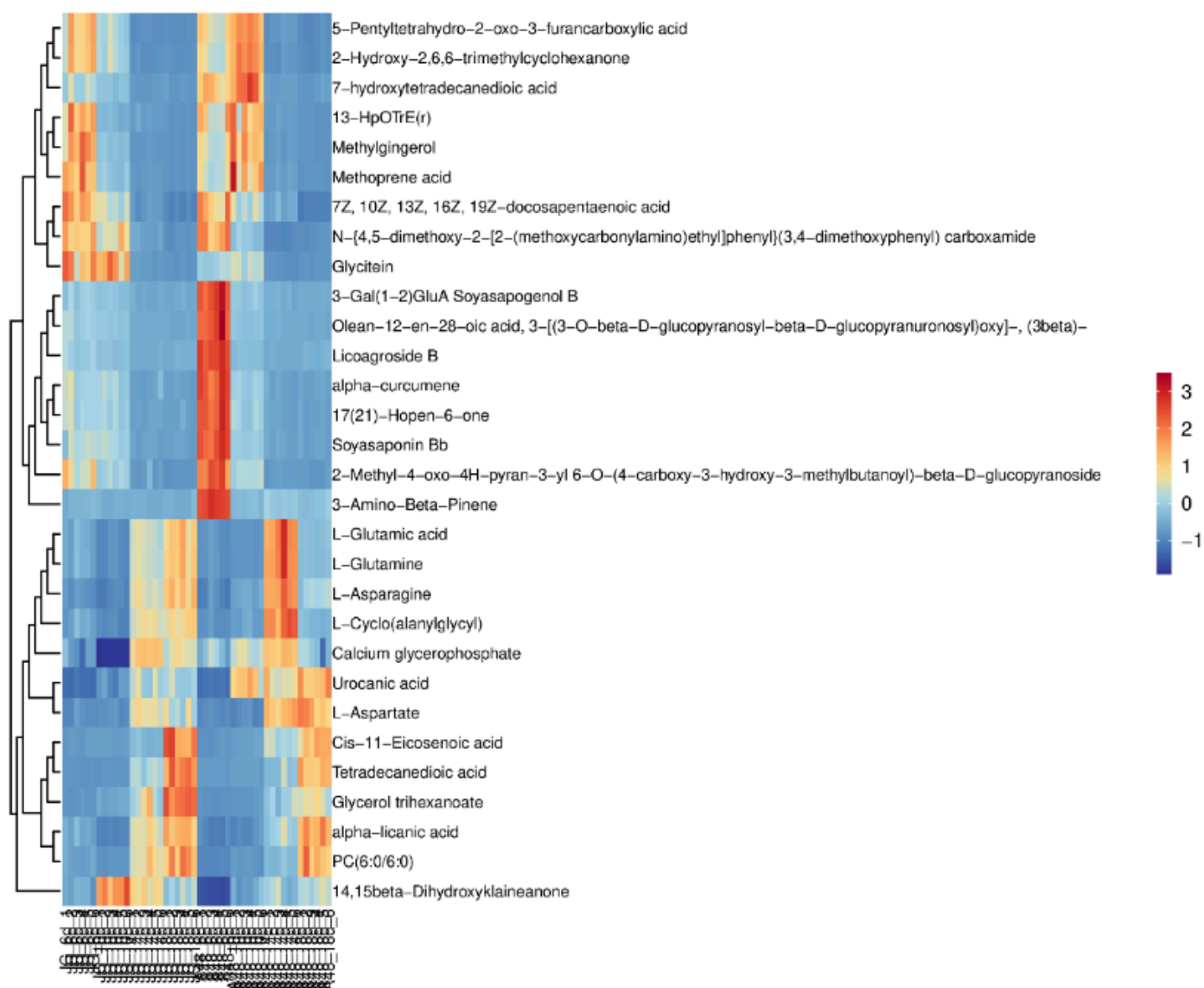


Figure 6

Heatmap of Metabolites Differing Across Four Periods in Fiber Mutant 'bfm' and Golden Crown

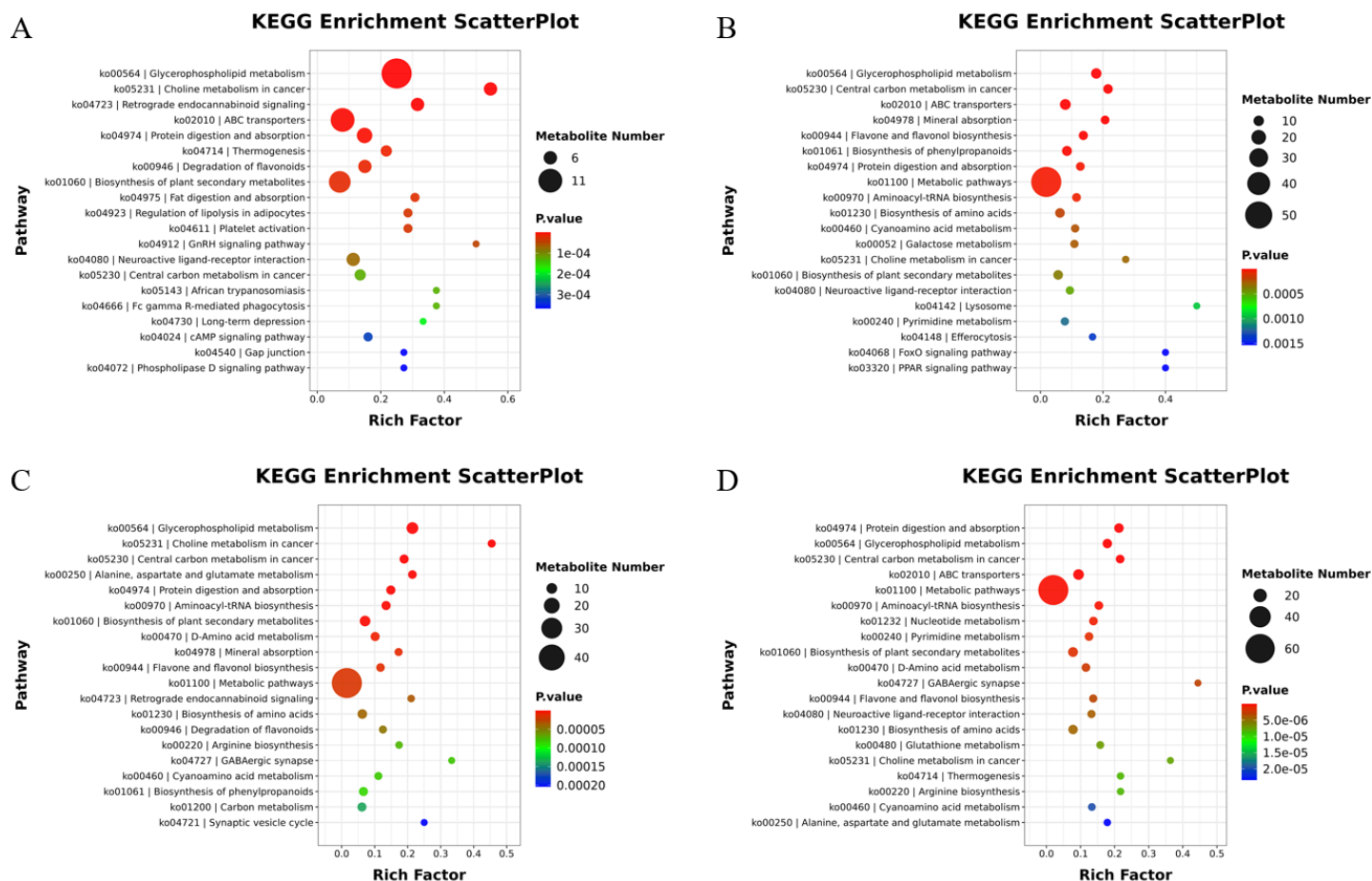
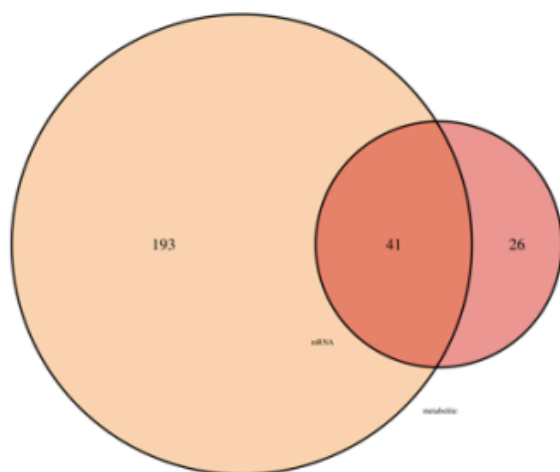


Figure 7

KEGG enrichment analysis ($P \leq 0.05$) of differential metabolites in the four time points of the fiber mutant '*bfm*' and '*jinguan*'. Note: A: KEGG pathways significantly enriched for W1 vs. M1 differential metabolites; B: KEGG pathways significantly enriched for W2 vs. M2 differential metabolites; C: KEGG pathways significantly enriched for W3 vs. M3 differential metabolites; D: KEGG pathways significantly enriched for W4 vs. M4 differential metabolites.

A

Venn diagram of different pathways between genes and metabolites

**B**

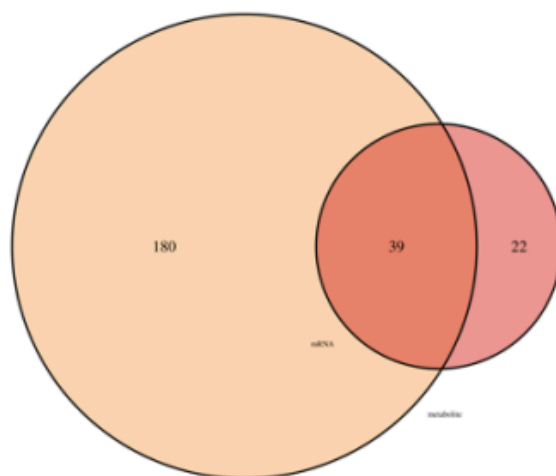
Venn diagram of different pathways between genes and metabolites

**C**

Venn diagram of different pathways between genes and metabolites

**D**

Venn diagram of different pathways between genes and metabolites

**Figure 8**

Venn Diagram of Differentially Expressed Genes and Metabolites Note: A: W1 vs. M1 period; B: W2 vs. M2 period; C: W3 vs. M3 period; D: W4 vs. M4 period

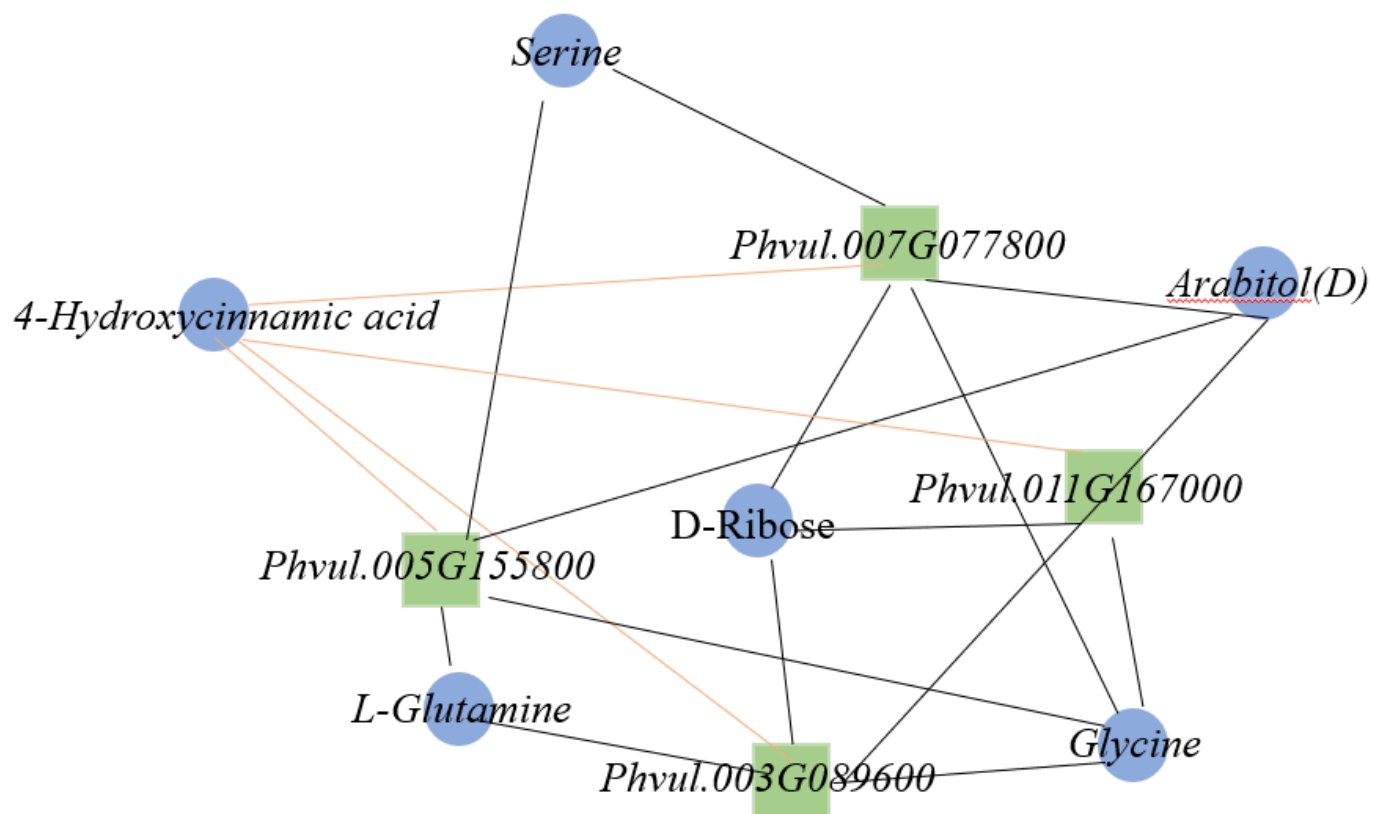


Figure 9

Correlation Network Diagram of Differentially Expressed Genes and Metabolites (ko01100)

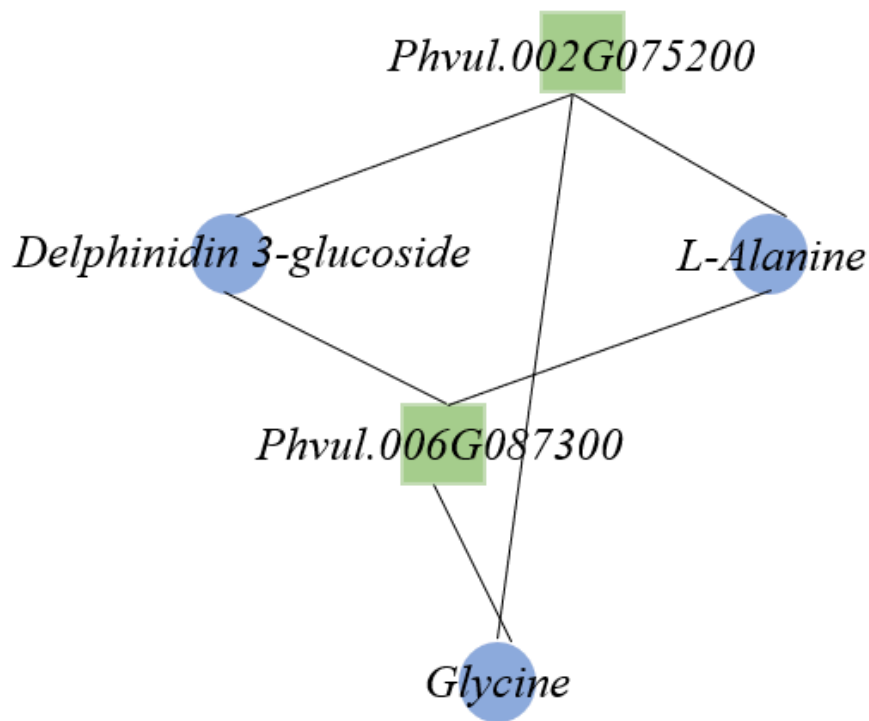


Figure 10

Correlation Network Diagram of Differentially Expressed Genes and Metabolites (ko01110)

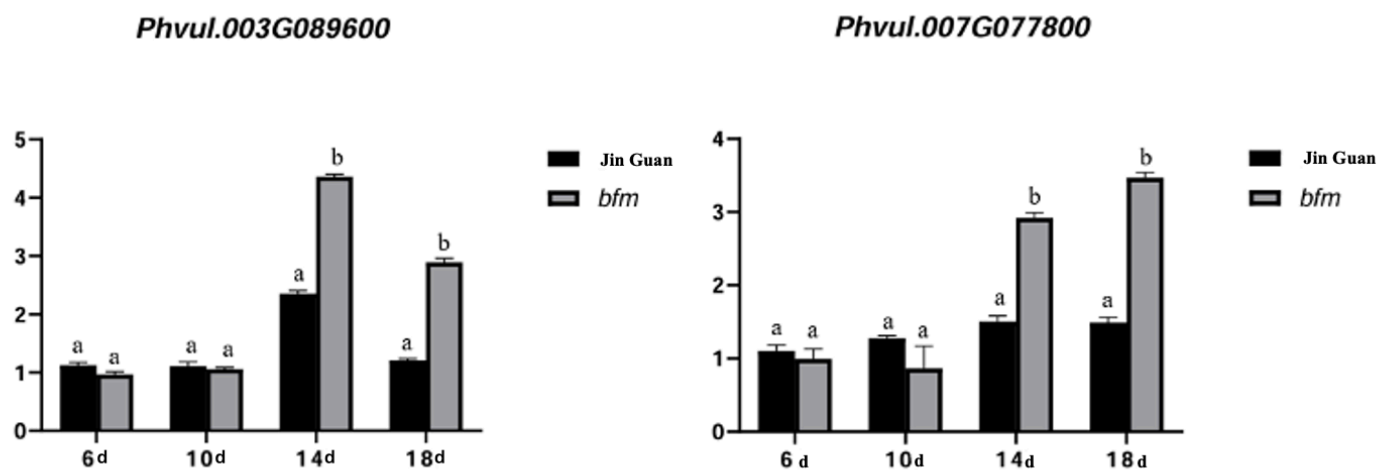


Figure 11

qRT-PCR validation of cellulose synthesis candidate genes. Note: Different letters indicate significant differences between groups (t-test, $p < 0.05$).