Phenylpropanoid metabolism and pigmentation show divergent patterns between brown color and green color cottons as revealed by metabolic and gene expression analyses

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Abstract

Background

Naturally colored cotton has become increasingly popular because of their natural properties of color, UV protection, flame retardant, antibacterial activity and anti-mildew. But poor fiber quality and restricted color choices are two key issues that have limited the cultivation of naturally colored cotton. To identify the possible pathways participating in fiber pigmentation in naturally colored cottons, three differently colored cottons (with green, brown and white colored fiber) were chosen for a comprehensive analysis of phenylpropanoid metabolism during fiber development.

Results

The expression levels of flavonoid biosynthesis pathway genes in brown cotton fibers were significantly higher than in white and green cotton fibers. Total flavonoids and proanthocyanidin (PA) were high in brown cotton fibers but low in white and green cotton fibers, which suggested that flavonoid biosynthesis pathway might not participate in the pigmentation of green cotton fibers. Further expression analysis indicated that genes encoding enzymes for the synthesis of caffeic acid derivatives, lignin and lignan were activated in 10 DPA (days post-anthesis) and 15 DPA fibers of green cottons.

Conclusions

Our results strengthen the understanding of phenylpropanoid metabolism and pigmentation in different colored cottons, and may provide strategies for improving the breeding of green and brown cottons.

1. Background

Cotton as the world’s most important natural textile crop shares more than one third of the world textile fiber market, playing a significant role in the world economy (Ma et al. 2018). Naturally colored cottons (NCC) refer to the cotton varieties that have natural color and can be directly used for colored products processing (Günaydin et al. 2019; Matusiak and Frydrych, 2014; Rathinamoorthy and Parthiban, 2017). It is also called “5C cotton” (cotton, color, charming, certification, and care) (Zhang et al. 2011). As a peculiar type of cotton varieties, colored cotton has the characteristics of UV protection (Crews and Hustvedt, 2005), flame retardancy (Hinchliffe et al. 2016), antibacterial activity (Chen and Cluver, 2010). It requires fewer dyeing in the textile production process, satisfying consumers' advocation of natural and health-conscious consumer products. The International Committee on Organic Agriculture predicts that 30% of the total global cotton production will be replaced by colored cotton and organic cotton in the next 30 years, and NCC fiber will be a valuable commodity in the textile market (Günaydin et al. 2019; Hinchliffe et al. 2016; Rathinamoorthy and Parthiban, 2017).

Accompanied by the growing demands for NCC products, there has been no corresponding increase in its cultivation because of the tight association between natural color and poor fiber quality and low yield (Chen and Cluver, 2010; Feng et al. 2015; Semïzer-Cumıng et al. 2015; Tu et al. 2014). Since brown and green are the major two fiber colors in the NCCs production, the corresponding limited color choices for consumers has been another major problem inhibiting the large-scale commercialization of NCC products (Blas-Sevillano et al. 2018). Therefore, the chemical basis underpinning NCC colors and the control of the biosynthesis of associated pigments have become key issues in NCC research.

Over the past ten years, many studies have focused on the metabolic and transcriptional analyses and QTL mapping of brown cotton fibers. Flavonoids are detected in the extractions of brown cotton fibers (Hua et al. 2007), and the flavonoid biosynthesis pathway, especially proanthocyanidin (PA) biosynthesis, is activated during fiber development of brown cottons (Feng et al. 2013; Liu et al. 2018; Tan et al. 2013; Xiao et al. 2014). QTL mapping found six genetic loci (Lc1, Lc2, Lc3, Lc4, Lc5 and Lc6) which are associated with fiber colors of brown cottons, and further studies showed that *GhTT2-3A*(*Gh_A07G2341*), a gene controlling PA biosynthesis, is a candidate gene that was confirmed by transgenic analysis to control fiber pigmentation of brown cotton (Hinchliffe et al. 2016; Wen et al. 2018; Yan et al. 2018).

Therefore both metabolic and gene expression analyses show that the pigments in brown cotton fibers are PA or PA derivatives (Feng et al. 2014; Xiao et al. 2014; Yan et al. 2018), while the pigments in green cotton fibers remain uncertain. Some transcriptional and metabolic analyses support the view that flavonoids and their derivatives are the dominant pigments in green cotton fibers (Hua et al. 2007; Liu et al. 2018; Yuan et al. 2012), but other analyses suggest that caffeoyl residues are related to pigmentation in these fibers (Feng et al. 2017; Ma et al. 2015). Proteomics-based analysis of green cotton fibers found the phenylocumaran benzylic ether reductase (PCBER), a key enzyme in lignan biosynthesis, is specifically expressed in green cotton fibers, and the total lignan contents in green cotton fibers are significantly higher than in white cotton fibers (Li et al. 2018). Although the pigments in green cotton fibers have not been definitively identified, it is certain that phenylpropanoid metabolism plays a key role.

To date, no studies have compared the entire phenylpropanoid metabolism in green and brown cotton fibers to confirm the associated pigmentation pathways. In this study, both brown and green colored cottons were compared with white cotton as a control. The expression of phenylpropanoid pathway genes and the contents of flavonoids and PAs in these 3 kinds of cotton fibers were analyzed. Our data may shed some light on the molecular pathways underlying the differences between the fiber coloration of green and brown cottons.

2. Materials And Methods

2.1. Plant materials and growth conditions

Three different types of fiber color and five cotton genotypes were used in this study, and all these accessions belong to *Gossypium hirsutum*. These include one accession with white fiber (YZ1), one accession with brown fiber (T586/T, dark brown) and three accessions (G1, G2, G3) with green fiber. G1, G2, G3 were developed by crossing of green cotton accessions with one white cotton accession. Plants were grown in parallel in a controlled greenhouse (Wuhan, China) at a constant temperature of 28°C to 32°C under a 14 h day/10 h night photoperiod with identical management practice. Cotton bolls were tagged on the day of flowering as 0 day post anthesis (0 DPA). Bolls were harvested at 5-d intervals during the course of fiber development (0 DPA, 5 DPA, 10 DPA, 15 DPA, 20 DPA), and frozen in liquid nitrogen immediately after removing the cotton shells. All samples were collected from 9:00-11:00am to minimize potential variability associated with circadian rhythms. For 0 DPA and 5 DPA ovule samples, whole ovules were ground into powder in liquid nitrogen. For 10 DPA, 15 DPA and 20 DPA, fibers were gently knocked off ovules in liquid nitrogen, and seeds were removed with forceps. Then fibers were ground into powder and stored at −80 °C until RNA and metabolite extraction.

2.2. Length measurement of cotton fibers

Mature cotton bolls from similar fruit-bearing positions of individual plants were collected at the same time. The middle two mature seeds from each ovary were specifically chosen for fiber length measurement and color observation. The fiber length was measured with a ruler according to a previous report (Tang et al. 2014). For each accession, at least 15 seeds were measured. Error bars represent the standard deviation (SD) of the mean.

2.3. Retrieval and identification of phenylpropanoid metabolism genes from the cotton genome

The coding sequences (CDS) of phenylpropanoid metabolism genes from Arabidopsis (Table S1) were used as BLAST queries against the *Gossypium hirsutum* L. TM-1 genome to identify all homologues to the query genes using the COTTONGEN database (https://www.cottongen.org/blast/nucleotide/nucleotide) (Wang et al. 2019; Yu et al. 2014). These sequences were then selected according to the annotation information and Fragments Per Kilobase of transcript per Million fragments mapped (FPKM) values of these genes were downloaded. Genesis software (Sturn et al. 2002) was used to generate heatmaps from the expression values.

2.4. RNA extraction and qRT-PCR analysis

Total RNA of cotton fiber samples (0 DPA, 5 DPA, 10 DPA, 15 DPA) was extracted using RNAprep Pure Plant Kit (TIANGEN Biotech). For each sample, 2 mg of total RNA was reverse transcribed into cDNA using M-MLV reverse transcriptase (Promega). For qRT-PCR analysis, 15 μL reactions for each sample were performed using SYBR Green (Applied Biosystems) as fluorescent dye on an ABI 7500 Real-Time PCR System (Applied Biosystems) (Guo et al. 2017). *GhUB7* (GenBank: DQ116441.1) was used as the reference gene to normalize gene expression levels. Primers were designed according to previous studies (Hu et al. 2018; Tan et al. 2013) and are listed in Table S2. Three technical replicates were performed, and the error bars represented the standard deviations.

2.5. Determination of the total flavonoid content

The total flavonoid content was determined based on a previous method (Hu et al. 2018). In brief, approximately 100 mg fiber (precise weight recorded) for each sample was placed in 2 mL centrifuge tube. 1 mL of 80% (v/v) methanol was added to extract the metabolites on a shaker at 4°C overnight. The supernatant was collected after centrifugation at 12 000 r·min $^{-1}$, and the residual pellet was re-extracted with 1 mL of 80% (v/v) methanol. The supernatants were combined and mixed thoroughly. 0.2 mL of the extract was mixed with 0.4 mL 0.1 mol·L^{-1} aluminum chloride ($AlCl_3$) solution in a test tube, to which was added 0.6 mL 1 mol·L^{-1} potassium acetate (KAc) solution. Then the mixture was diluted to 2 mL with 0.8 mL 80% (v/v) methanol and mixed thoroughly. After standing for 30 min, the absorbance was immediately measured at 420 nm using a Multimode Plate Reader (PerkinElmer). Rutin standard solutions were prepared as shown in Table S3 to make a standard curve.

2.6. Determination of the proanthocyanidin (PA) content

4-dimethylaminocinnamaldehyde (DMACA) was used to visualize PA in cotton fibers (Xiao et al. 2007). Mature seeds with fibers were immersed in 6 mol·L^{-1} HCl/95% ethanol (v/v) containing 0.1% (w/v) DMACA solution for 10 min. Seeds immersed in 6 mol·L^{-1} HCl/95% ethanol (v/v) solution were used as control.

The PA content was measured according to a previously reported method with some modifications (Tan et al. 2013). Approximately 100 mg samples were extracted with 500 mL of 80% methanol, then shaken at 4°C for 12 h. The residues were extracted with 500 mL of 80% (v/v) methanol again, and the two supernatants were combined as the extract solution. 600 mL of 3 mol·L^{-1} HCl/80% methanol (v/v) containing 0.1% (w/v) DMACA solution were

added to 40 mL extract solutions and mixed thoroughly. Place at room temperature for 20 min in the dark. The absorbance was measured at 643 nm using a Multimode Plate Reader (PerkinElmer).

3. Results

3.1. Fiber phenotypic characterization of brown and green cottons

We collected four colored cotton accessions, including three accessions of green colored cottons (G1, G2, G3) and one accession of brown colored cotton (T586/T). YZ1, one accession of white colored cotton was used as control. The fiber color and length of these materials are shown in **Fig. 1**. The fiber color of the three green cotton accessions is yellow-green, and similar to each other. Another feature of these three green cotton fibers is the uneven coloration. The regions of the fibers near the base of the seed coat, which were wrapped inside, are dark green, but fibers exposed to the outside exhibit a light green or even white. The brown cotton T586 (T) fiber colored uniformly (**Fig. 1A-B**).

The fiber lengths of these three different colored cottons were measured, and white cotton YZ1 has the longest fiber, with an average length of 27.9 mm. The length of three accessions of green cotton (G1, G2, G3) was similar to each other but shorter than white cotton YZ1, with a length of about 24 mm. The brown cotton T586 (T) had significantly shorter fibers than white and green cottons, at a mean of 15.8 mm (**Fig. 1B-C**).

3.2. Expression analysis of flavonoid biosynthesis genes in brown and green cotton fibers.

Flavonoids are thought to be involved in the formation of NCC fiber pigments (Feng et al. 2014; Hua et al. 2007; Liu et al. 2018; Tan et al. 2013; Yan et al. 2018). To investigate expression patterns, annotated flavonoid biosynthesis genes in *G. hirsutum* genome were selected, and a heatmap for these genes in white cotton fiber was constructed to illustrate transcriptional changes during fiber development (**Fig. 2**). All genes in the flavonoid biosynthesis pathway had only one or two copies in each subgenome except *CHS*, which has 9 copies in the Dt subgenome and 8 copies in the At subgenome. Most genes of flavonoid biosynthesis pathway had a similar expression pattern, being highly expressed at the fiber initiation stage (0 DPA, 5 DPA), and the expression levels decreased during fiber development.

To reveal the relationship between endogenous flavonoid biosynthesis gene expression and fiber colors, the expression levels of flavonoid biosynthesis pathway genes were analysed by qRT-PCR in NCC accessions (**Fig. 3**). *F3H* was the most abundantly expressed gene in brown cotton fiber, and its expression gradually increased during fiber development, with the highest expression level at 15 DPA. The stage with the highest *F3H* expression in green cottons was 10 DPA and in white cotton was 5 DPA. Moreover, the expression of *F3H* in brown cotton fibers was 10 times higher than in white and green cotton fibers.

As the first key enzyme in the flavonoid biosynthesis pathway, CHS plays an extremely important role in flavonoid metabolism. The expression of *CHS* in brown cotton fibers was the highest in 10 DPA fiber. Like *F3H*, the expression level peak in green cottons was in 10 DPA and white cotton was 5 DPA. ANR can catalyze the synthesis of PAs, and the corresponding gene was also found to be highly expressed in brown cotton fiber. *ANR* shows the highest expression at 10 DPA in the brown fibers, and its expression level was 4-5 times higher than that in white and green fibers.

ANS is the downstream gene of flavonoid metabolism, catalyzing the synthesis of anthocyanins. *ANS* was highly expressed in brown cotton fibers to a level of 4-5 times higher than that of white and green cotton fibers. Whether anthocyanins are involved in brown fiber pigmentation remains to be explored. Nevertheless, the expression level of flavonoid biosynthetic genes in brown cotton fibers was significantly higher than in green and white cotton fibers.

3.3. Endogenous flavonoid contents in brown and green cotton fibers

We measured the total flavonoid contents (TFCs) in the ovules and fibers from 5 DPA to 20 DPA to determine whether they changed during the development of different colored fibers (**Fig. 4A**). White cotton and green cotton fibers had similar trends in TFCs during different developmental stages. In these accessions, the TFCs accumulated to the highest levels in 5 DPA ovule samples, about 8mg/g. In fibers, TFCs were the highest in 15 DPA fibers. TFCs of all fiber samples were significantly lower than in 5 DPA ovule samples. In contrast for brown fibers, the total flavonoid contents in 10 DPA, 15 DPA and 20 DPA fibers were markedly higher than in 5 DPA ovule samples. The TFCs concnetrations in 10 DPA fibers were the highest (35mg/g), and at 15 DPA fiber the levels decreased sharply, but then increased again in 20 DPA fibers. Overall, the TFC contents in brown fibers were significantly higher than those of white and green fibers.

3.4. Proathocyanidin (PA) contents in brown and green cotton fibers.

To further investigate whether PA plays the same role in the pigmentation of green and brown cotton fibers, the PA contents were measured. 4-dimethylaminocinnamaldehyde (DMACA) staining method, which gives a blue coloration in the presence of PA, was employed to visualize PA in mature fibers. Brown cotton fibers showed the presence of PA while white and green fibers showed no difference with controls (**Fig. 5**). These results suggested that PA accumulated in mature brown cotton fibers, but not detectably in mature fibers of white and green cottons.

We also checked the PA contents in immature fibers (**Fig. 4B-C**). Like the results found for the total flavonoids, the PA contents at different developmental stages in white and green cotton fibers were similar, but significantly lower than those in brown fiber samples. The highest PA content in brown cotton fibers was at 10 DPA, and PA contents decreased slightly at 15 DPA and 20 DPA. In summary, significantly higher level of PA was accumulated in brown cotton than in green and white cottons.

3.5. Expression analysis of lignin and lignan biosynthesis pathway genes in brown and green cotton fibers.

The presence of caffeoyl and caffeoyl glycerides in extracts of green cotton fibers have been studied (Feng et al. 2017; Ma et al. 2015). Caffeic acid and caffeoyl-CoA are intermediate metabolites of lignin and lignan metabolism (Davin and Lewis, 2000). Therefore, qRT-PCR was used to detect the expression levels of lignin and lignan biosynthetic pathway genes in these three types of cotton fibers (**Fig. 6**).

PAL, *C4H* and *4CL* are the most upstream genes in phenylpropanoid metabolism, and are involved in the synthesis of not only flavonoids, but also lignin and lignan. The expression levels of *PAL* and *C4H* in brown fibers were significantly higher than those in white and green fibers. *PAL* and *C4H* transcripts accumulated to the highest levels in 5 DPA samples of white cotton (**Fig. 6, Table S4**), but in 10 DPA fibers of green cottons. The expression levels of these two genes in green fibers were slightly higher than in white fibers. The expression of *4CL* in brown fibers was higher than in ovules at 0 DPA and 5 DPA and than in white and green cotton fibers, but lower than in green fibers at 10 DPA and 15 DPA.

HCT is the first key enzyme in the lignin synthesis pathway. The expression of this gene in white and green fibers was higher than in brown fibers, and the expression levels in G1 fibers were significantly higher than in white fibers. *CCoAOMT* and *COMT* are downstream genes in lignin metabolism, and influence the biosynthesis of monolignols, which are further used to synthesize lignin or lignan. The green fibers showed a slightly higher expression of this gene than brown or white fibers at 10 DPA and 15 DPA (**Fig. 6**). *PCBER* encodes a key enzyme in the metabolism of lignan, and is a novel candidate gene that potentially is responsible for pigmentation in green cotton fibers (Li et al. 2018). Analysis of its expression in fibers showed that, similar to *HCT*, the expression levels of this gene were higher in white cotton and green cotton fibers than in brown fibers (**Fig. 7**).

4. Discussion

4.1. Flavonoid accumulation is not a key determinant in green cotton fiber pigmentation

Green and brown cotton are the major two NCC types grown around the world. Determining the pigment components of colored fibers is a key first step in breeding improved colored cotton cultivars. Most research has been carried out on the pigmentation of brown cotton fiber, with little known about green fiber pigments. We therefore chose 3 green cotton accessions, 1 brown cotton accession and 1 white cotton accession to study the differences between these 3 type cottons. Unlike brown cotton, green cotton fibers colored unevenly (**Fig. 1**). Green but not white or brown cotton fiber color changed when treated with 6 mol·L^{-1} HCl/95% ethanol solution (**Fig. 5**), likely due to the instability of the green fiber pigments. Previous studies have shown the color of green cotton fiber was easily changed by oxidants, reductants, metallic ions, alkalis, UV exposure and high temperature (Günaydin et al. 2019; Zhang and Hu, 2003). All these results suggested that the fiber pigment components of brown and green cotton are different.

Flavonoids are one of the three major plant pigments, including six major subgroups such as chalcones, anthocyanins and proanthocyanins. Intensive biochemical and transcriptomics analyses have indicated that flavonoid biosynthesis, and especially PAs biosynthesis and accumulation, plays a key role in the coloration of brown cotton fibers (Feng et al. 2014; Gong et al. 2014; Li et al. 2013; Yan et al. 2018). In agreement with previous studies, we found that flavonoid metabolism was transcriptionally activated in brown cotton fibers, and high levels of flavonoids were synthesized during fiber development (**Fig. 2 - Fig. 5**).

The relationship between green fiber pigmentation and flavonoids is still controversial. Flavonoids have been found to be the dominant pigment in green cotton fibers by measuring the flavonoids content during fiber development in previous work (Hua et al. 2007; Yuan et al. 2016). Further study found PAs were not the pigments in green cotton fibers based on DMACA staining (Li et al. 2018). But a recent study about transcriptomic and transgenic analyses of green and brown cotton suggested that the flavonoid biosynthetic pathway controls green fiber pigmentation (Liu et al. 2018).

Our results found that differences in the flavonoid metabolism between green and white fibers were not as significant as between brown and white fibers (**Fig. 2 - Fig. 5**). The expression levels of flavonoid metabolism genes in green fibers were similar to those in white fibers and significantly lower than in brown fibers (**Fig. 3**), which was consistent with the measurement of flavonoid contents (**Fig. 4 - Fig. 5**). The measurement of PA contents and DMACA staining of green fibers also indicated that PA was not the accumulated pigment in green fibers. These results suggest that flavonoids are not the key determinant of pigmentation in green cotton fibers.

4.2. Lignin and lignan biosynthesis pathways were slightly activated at the transcriptional level during the development and coloration of green cotton fibers.

Caffeic acid is a key intermediate in the biosynthesis of lignin and lignan (Davin and Lewis, 2000)and caffeic-acid derivatives have been detected in green cotton fibers (Feng et al. 2017; Ma et al. 2015; Schmutz et al. 1993; Schmutz et al. 1994). Furthermore, colored cotton fibers have been found to contain more lignin and lignan than white cotton fibers (Ioelovich and Leykin, 2008; Li et al. 2018). However, the comparison of lignin contents in green cotton and brown cotton fibers depends on the variety tested. Some brown cotton fibers contained higher total lignin contents than green cotton fibers (Morais Teixeira et al. 2010), but some are exactly the opposite (Ioelovich and Leykin, 2008).

We checked the expression levels of six key genes involved in caffeic acid and lignin biosynthesis to gain insights into whether this pathway participates in green fiber development. The phenylpropanoid pathway was significantly up regulated in brown fibers compared with white and green fibers (**Fig. 6**), consistent with previous reports. The expression levels of *PAL* and *C4H* in brown fibers were markedly higher than in white and green fibers. However, the expression of genes for the metabolic flux to lignin biosynthesis was similar or slightly lower than in white and green fibers, implying that a large amount of phenylpropanoid metabolism is directed to flavonoids in brown fibers.

Although most of the caffeic-acid and lignin and lignan biosynthesis genes in green fibers did not exhibit noticeably increased expression compared with white and brown fibers, they did have a slightly higher expressions during some stages of fiber development. C4H, 4CL, HCT are the enzymes directly responsible for caffeic acid and caffeoyl-CoA synthesis (Vanholme et al. 2012). At 10 DPA and 15 DPA, the expression levels of *C4H*, *4CL* in green fibers were higher than in white fibers, and the green accession G1 had a significantly higher expression of *HCT* at 10 DPA and 15 DPA (**Fig. 6**). A similar situation also was seen for lignan metabolism. 15 DPA is the point of secondary cell wall biosynthesis, and also an important stage for the initiation of pigmentation in colored cotton fibers (Kim, 2015; Yuan et al. 2012). Our results indicate that caffeic acid derivatives, and lignin and lignan biosynthesis pathways are activated during the development and coloration of green fibers, which may explain why green fibers have a higher lignan and caffeic acid derivatives contents than white fibers. Detailed biochemical and transcriptional systems biology analyses should be carried out to investigate the precise roles of the caffeic acid derivatives, lignin and lignan in the pigmentation of green cotton fibers.

Suberin is an analogous biopolymer of cutin found in some specialized plant cell walls(Cohen et al. 2017; Graca, 2015) . It is composed of very long chain aliphatic acid derivatives, glycerol, and linked with phenolics and embedded waxes. Typically, the phenolic components are ferulic acid, caffeic acid, coumaric acid and monolignol derivatives (Cohen et al. 2017; Vishwanath et al. 2015), which are derivatives of phenylpropanoid metabolism.

Interestingly, electron microscope thin sections of cotton fibers revealed that suberin lamellae are only found in the cell wall of green cotton fibers (Ryser et al. 1983). Caffeic acid and glycerol have been detected in extracts of green fibers, and presence of these two chemicals in the suberin of green fiber has been confirmed in subsequent studies, leading to the proposal that they may be the pigments in green fibers (Schmutz et al. 1996). By comparing the previous studies on the location of pigments and suberin lamellae in green cotton fibers and surprisingly, we found both are deposited in alternating layers with cellulose in the secondary cell walls of fibers (Ryser et al. 1983; Zhang et al. 2011). Suberin lamellae must therefore be a key feature of green cotton fibers that is involved in fiber coloration.

Since some caffeic acid derivatives have a yellow-green color and have been detected in extracts of green fibers (Feng et al. 2017), caffeic acid derivatives are likely to be some of the pigments in green fibers. Monolignol derivatives and lignan might act as structural components of suberin. So far, few studies have focused on this particular cell wall structure as compared with other components in plant cell walls. More effort is needed in this area and on the relationship between the suberin lamellae and lignin and lignan. A comprehensive research effort on suberin lamellae will greatly assist in understanding the control of green cotton pigmentation and inform fiber quality breeding in green cotton cultivars.

5. Conclusions

For the first time, a comprehensive analysis of phenylpropanoid metabolism during three differently colored cottons (three with green, one with brown and one with white colored fiber) fiber development have been carried out in this work. The expression levels of flavonoid structural genes were significant higher, and the endogenous total flavonoids and PA were highly accumulated in brown cotton fibers than white cotton fibers during the fiber development, but not in green cotton fibers. So, flavonoid is not a key determinant in green cotton fiber pigmentation. Compared with white cotton fibers, the lignin and lignan biosynthesis were activated in 10DPA and 15DPA fibers of green cottons.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

Not applicable.

Competing interests

The authors declare no conflict of interest.

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Author's contributions

You CY, Tu LL and Zhang XL conceived and designed the project. You CY provided green cotton. Li ZH and Li JY managed and collected fiber samples and performed experiments. Li ZH, Su Q and Xu MQ analysed the qRT-PCR results. You JQ modified the heatmap. KHAN AQ contributed to project discussion. Li ZH wrote the manuscript draft, Tu LL and Zhang XL revised the manuscript.

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Figures

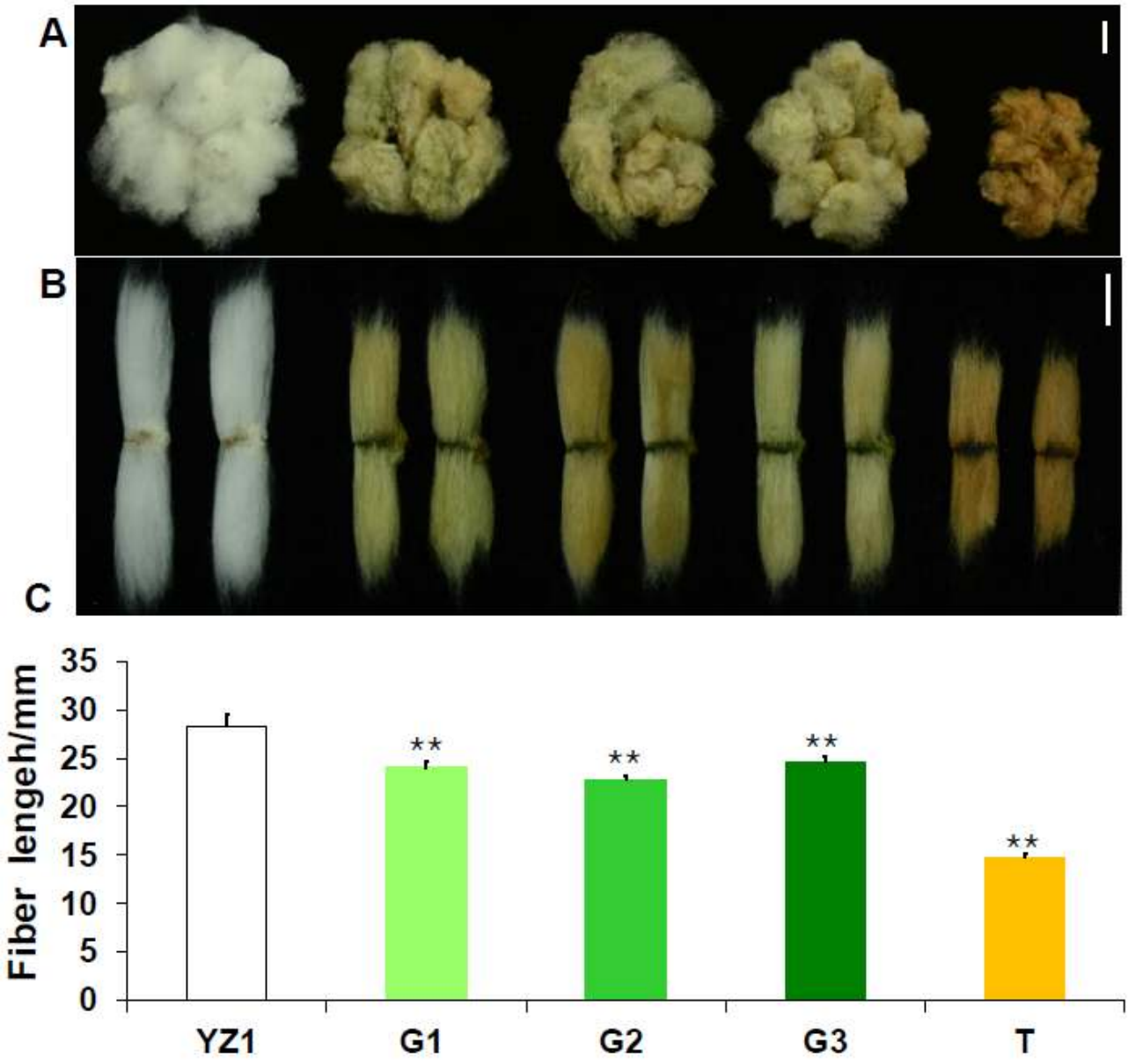

Figure 1

Fiber color and length of brown and green cottons. (A) The typical phenotype of ten mature seeds of white (YZ1), green (G1, G2, G3) and brown (T) colored cottons. All these accessions belong to Gossypium hirsutum; (B) Images of fibers from YZ1, G1, G2, G3, T; Bars = 1 cm; (C) The fiber length of YZ1, G1, G2, G3, T. Error bars represent the standard deviations. ** represent $P < 0.01$ based on Student's t test.

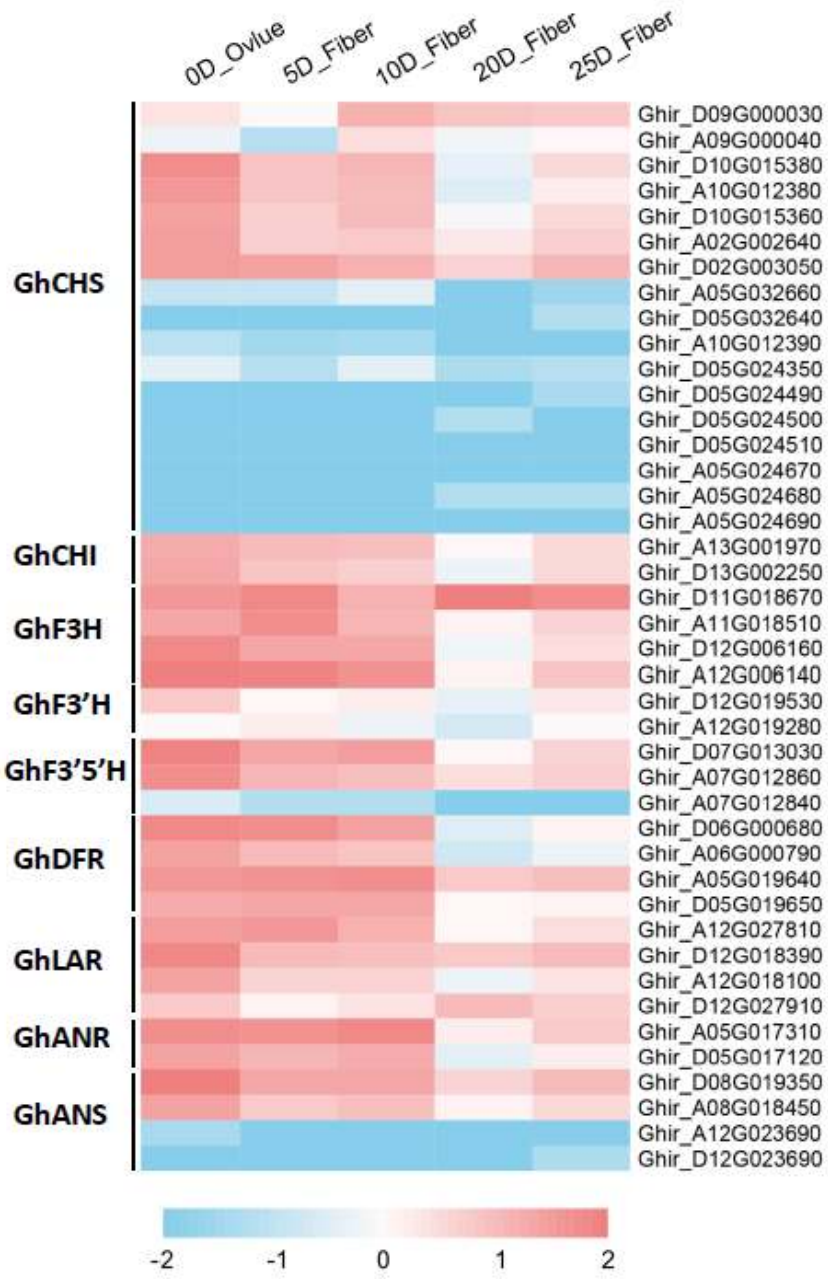

Figure 2

Expression patterns of flavonoid biosynthesis genes in white cotton fibers. The color scale at the bottom represents log2 expression values, with blue indicating low levels and red indicating high levels of transcript abundance. FPKM values of these genes were downloaded from the G.hirsutum accession Texas Marker-1 (TM-1) transcriptome.

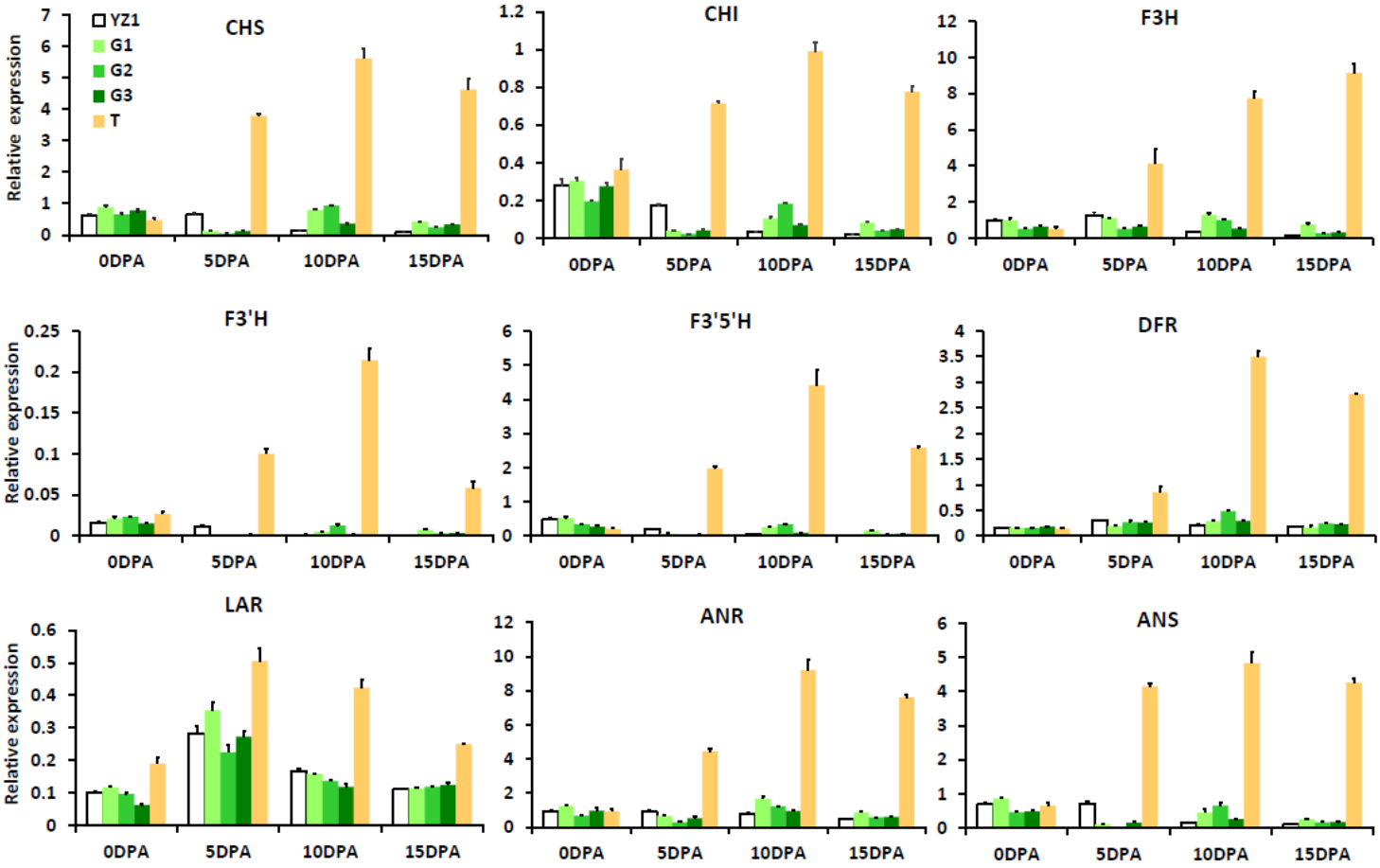

Figure 3

qRT-PCR analysis of flavonoid biosynthesis genes in brown (T) and green (G1-3) cotton fibers. CHS: CHALCONE SYNTHASE; CHI: CHALCONE ISOMERASE; F3H: FLAVANONE 3-HYDROXYLASE; F3'H: FLAVONOID 3'-HYDROXYLASE; F3'5'H: FLAVONOID- 3'5'-HYDROXYLASE; DFR: DIHYDROFLAVONOL 4-REDUCTASE; LAR: LEUCOANTHOCYANIDIN REDUCTASE; ANR: ANTHOCYANIN REDUCTASE; ANS: ANTHOCYANIDIN SYNTHASE. Error bars represent the standard error of 3 biological replicates.

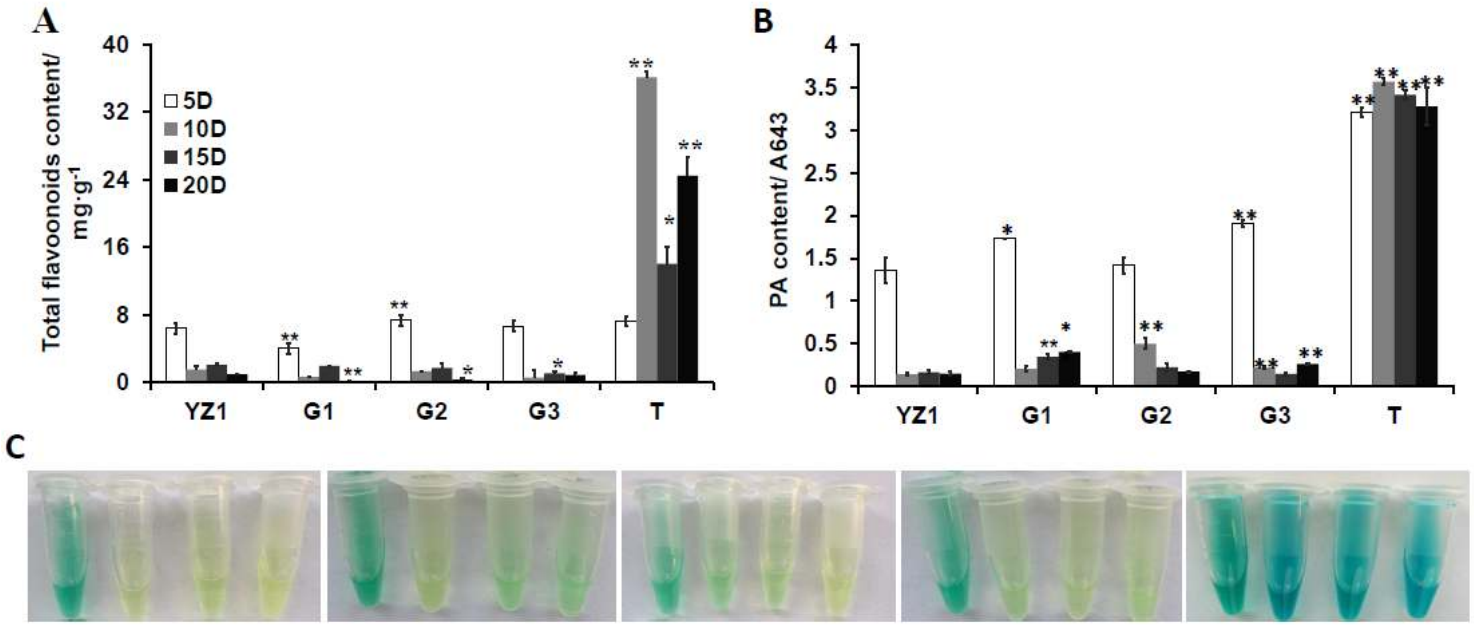

Figure 4

Total flavonoid and PA contents in white (YZ1), brown (T) and green (G1-3) cotton fibers. (A) Total flavonoid contents and (B) PA contents in white and natural colored cottons; 5D: ovules from 5 DPA; 10D, 15D, 20D: fibers from 10, 15, 20 DPA. Error bars represent the standard error of 3 biological replicates. * and ** represent $P < 0.05$ and $P < 0.01$ based on Student's t test, respectively; (C) Images of 4-dimethylaminocinnamaldehyde (DMACA) reaction solutions. The five small pictures from left to right correspond to the DMACA reaction solutions of YZ1, G1, G2, G3 and T. For each small picture from left to right correspond to 5DPA, 10DPA, 15DPA, and 20DPA samples.

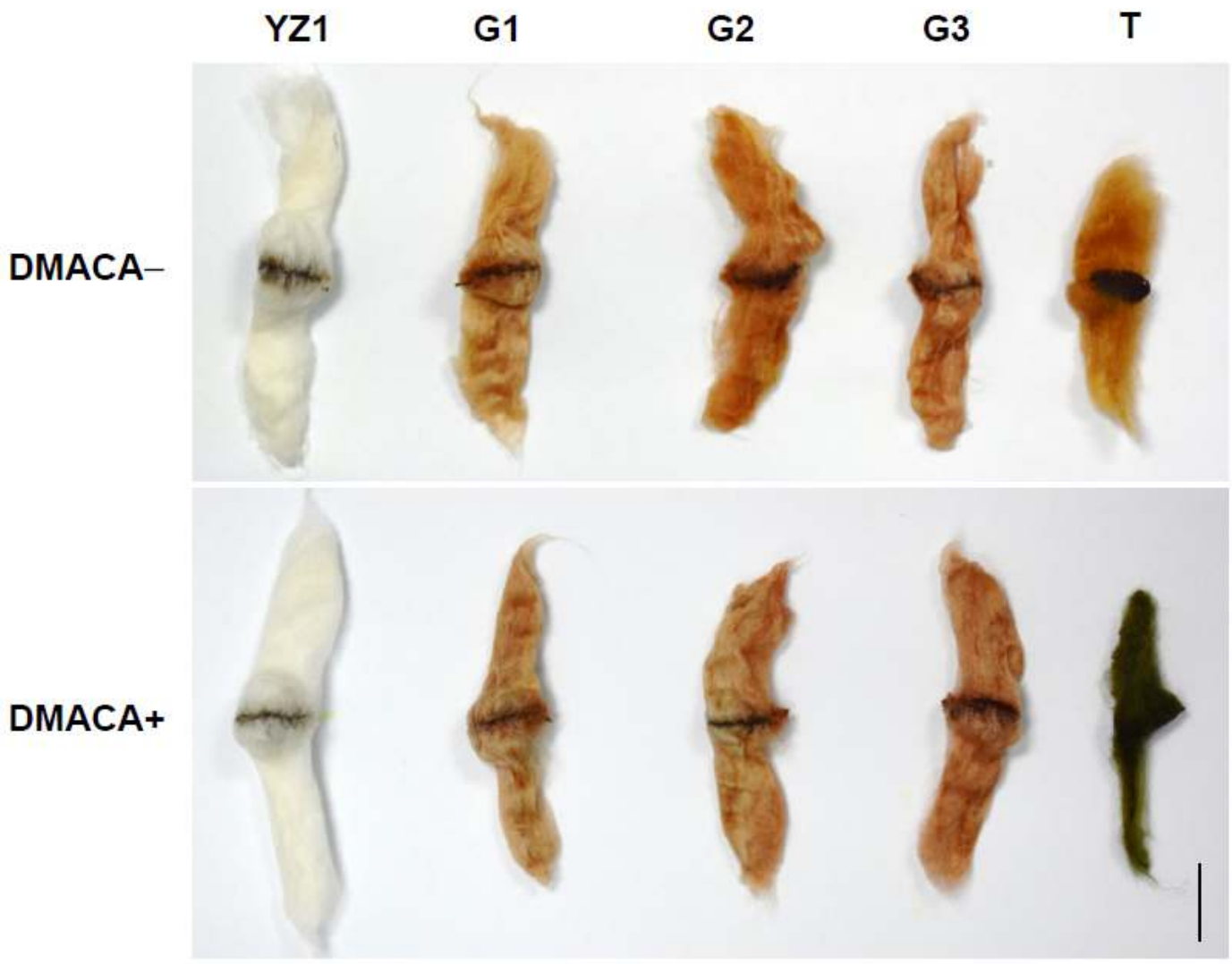

Figure 5

DMACA staining of mature fibers of brown (T) and green (G1-3) cottons. DMACA-: Mature fibers treated with 6 M HCl/95% ethanol (v/v); DMACA +: Mature fibers treated with 6 M HCl/95% ethanol (v/v) containing 0.1% (w/v) DMACA. Bars = 1 cm.

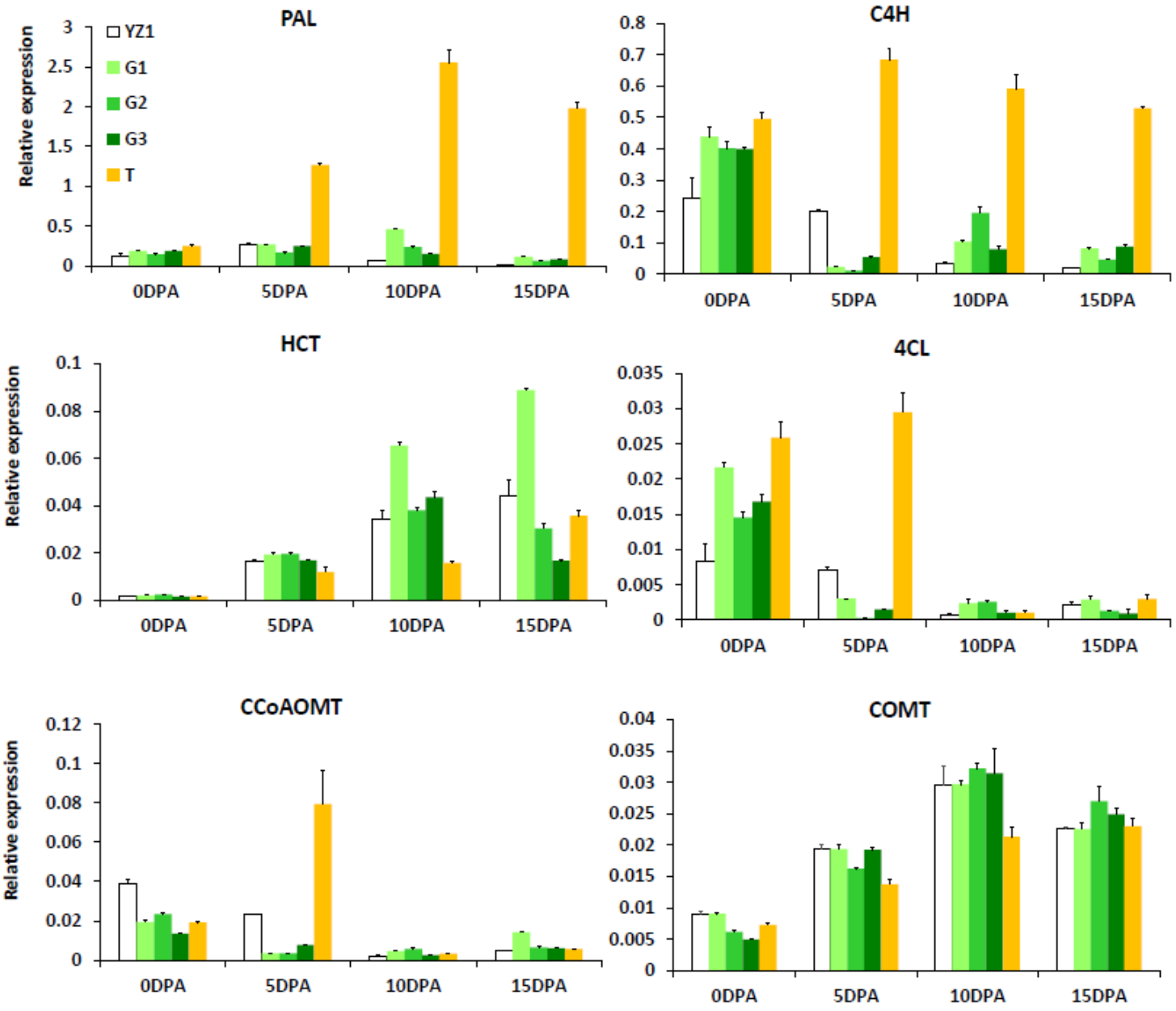

Figure 6

qRT-PCR analysis of lignin biosynthesis structural genes in brown (T) and green (G1-3) cotton fibers. PAL: PHENYLALANINE AMMONIA LYASE; C4H: CINNAMATE 4-HYDROXYLASE; 4CL: 4-COUMAROYL: COA LIGASE; HCT: HYDROXYCINNAMOYL TRANSFERASE; CCOAOMT: CAFFEOYL-COA O-METHYLTRANSFERASE; COMT: CAFFEIC ACID O-METHYLTRANSFERASE. Error bars represent the standard error of 3 biological replicates.

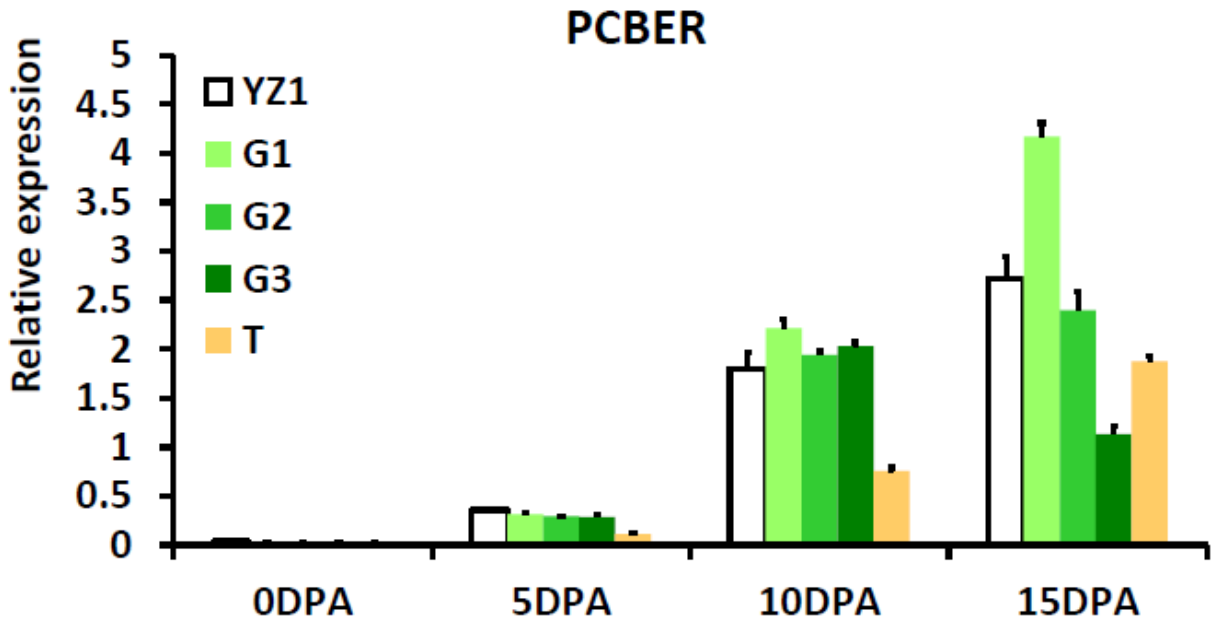

Figure 7

qRT-PCR analysis of PCBER in brown (T) and green (G1-3) cotton fibers. PCBER: PHENYLOCUMARAN BENZYLIC ETHER REDUCTASE. Error bars represent the standard error of 3 biological replicates.

Supplementary Files

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

- SupplementaryTableS1S4.xlsx