UDP-glucose pyrophosphorylase: genome-wide identification, expression and functional analyses in *Gossypium hirsutum*

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**ABSTRACT**

In this study, a total of 66 UDP-glucose pyrophosphorylase (UGP) (EC 2.7.7.9) genes were identified from the genomes of four cotton species, which are the members of Pfam glycosyltransferase family (PF01702) and catalyze the reaction between glucose-1-phosphate and UTP to produce UDPG. The analysis of evolutionary relationship, gene structure, and expression provides the basis for studies on function of UGP genes in cotton. The evolutionary tree and gene structure analysis revealed that the UGP gene family is evolutionarily conserved. Collinearity and Ka/Ks analysis indicated that amplification of UGP genes is due to repetitive crosstalk generating between new family genes, while being under strong selection pressure. The analysis of cis-acting elements exhibited that UGP genes play important role in cotton growth, development, abiotic and hormonal stresses. Six UGP genes that were highly expressed in cotton fiber at 15 DPA were screened by transcriptome data and qRT-PCR analysis. The addition of low concentrations of IAA and GA3 to ovule cultures revealed that energy efficiency promoted the development of ovules and fiber clusters, and qRT-PCR showed that expression of these six UGP genes was differentially increased. These results suggest that the UGP gene may play an important role in fiber development, and provides the opportunity to plant researchers to explore the mechanisms involve in fiber development in cotton.

**INTRODUCTION**

UDP-glucose pyrophosphorylase (UGP) is a member of Pfam glycosyltransferase family (PF01702), an enzyme found in various organisms, including plants, animals and bacteria (*Chen et al., 2007; Johansson et al., 2002; Kleczkowski, 1994; Winter & Huber, 2000*), which catalyzes the reaction between glucose-1-phosphate and UTP to produce UDPG (*Daran et al., 1995*). UDPG is an important molecule in biology, food, biopharmaceutical and
cosmetic chemistry, and an essential glucose donor compound. It is also one of the key precursors for sugar interconversion, disaccharide and polysaccharide formation, and amino and nucleotide sugar metabolism (Lamerz et al., 2006), and is involved in several essential cellular processes, including carbohydrate metabolism, cell wall biosynthesis, and protein glycosylation (Chen et al., 2007; Daran et al., 1995).

Previous studies have shown that UGP genes have diversity of roles in various organisms. For example, in fungi, UDPG is an essential precursor of β-1,3-glucan and β-1,6-glucan, where both are the components of biosynthesis of cell wall (Daran et al., 1995). In yeast, YKL248 cells, UDPG concentration was reduced by 50% when UGP activity was significantly reduced by 10-fold, resulting in the induction of multiple outgrowth phenotypes (Daran, Bell & François, 1997; Daran et al., 1995). It was also reported that antisense repression of UGP genes in plants reduces the content of soluble carbohydrates, starch or sucrose (Borovkov et al., 1996; Spychalla et al., 1994). In Arabidopsis, the AtUGP1/AtUGP2 double-silent mutant showed the decreased concentration of UDPG, growth defects, and male-sterility (Park et al., 2010). Changes in the cell wall structure and number of mycelial meristems in UGP homolog knockout mutants of Ganoderma lucidum (Li et al., 2015b). In rice, silencing of UGP1 by co-repression or double-stranded RNA interference (dsRNAi) affects the callus deposition during meiosis of pollen, resulting in male sterile phenotype (Chen et al., 2007; Woo et al., 2008). The data also suggests that over-expression of native or exotic UGP gene in various plants can the increase plant height, leaf area and leaf-stem biomass ratio or nutritional profile (Coleman et al., 2006; Payyavula et al., 2014).

Cotton is one of the most important sources of fiber in the world. The widely cultivated upland cotton (Gossypium hirsutum) is an allotetraploid originated from two diploid ancestral species, G. arboreum (A-genome) and G. raimondii (D-genome), resulting from natural hybridization and genome doubling over millions of years in natural conditions (Wendel, 1989; Wendel & Cronn, 2003). Amongst the several quality traits, fiber strength is one of the important traits, where cotton fiber is a single-celled seed hair of the ovule epidermis, whose development is accomplished in four different stages (Kim & Triplett, 2004), in which cell elongation determines the primary quality traits of cotton fiber (Deng et al., 2012). Fibroblast elongation is a complex process involving multiple metabolic and regulatory events (Kim & Triplett, 2001). It was reported in literature that various abiotic stresses and hormonal homeostasis play a crucial role in the development and quality of cotton fibers, such as BR (Sun et al., 2005; Yang et al., 2014). Fiber strength is mainly determined by the strength of its cell wall, where main components of cell wall includes cellulose and non-cellulose components. Cellulose is formed by UDPG, and UDPG can be synthesized by employing three enzymes namely, UGP, UDP-sugar-pyrophosphorylase (USP) and sucrose synthase. For the synthesis of UDPG and UGP, USP use monosaccharide-1-phosphate as a substrate, while SuSy catalyzes sucrose cleavage and delivers UDPG directly to the plasma membrane-associated cellulose synthase complex (Amor et al., 1995; Kotake et al., 2004). These findings support the existence and important role of UGP genes in fiber development of cotton, and the sequencing of cotton genome...
has made it possible to analyze various gene families through genome-wide approach (Du et al., 2018; Hu et al., 2019; Paterson et al., 2012; Zhang et al., 2015).

In this study, 19 UGP genes were identified from upland cotton through gene family identification, phylogenetic tree construction, structural analysis, chromosome distribution, analysis of covariance and its Ka/Ks ratio, prediction and analysis of promoter cis-acting elements, transcriptome data analysis, ovule culture and its phenotypic observation, etc. The expression pattern of UGP gene in cotton fiber was analyzed by using qRT-PCR. In addition, we have determined the expression patterns of UGP genes under phytohormone-stimulated conditions to explore the functional role of these genes in cotton. This study provides the foundation for elucidation of evolutionary and functional analysis of UGP genes and provides a molecular and biological basis for a deeper understanding of the association between UGP genes and cotton fiber development.

**MATERIALS AND METHODS**

**Identification of UGP gene family**
The UGP gene was obtained by searching the whole genome (TAIR: http://www.arabidopsis.org) of Arabidopsis thaliana, and Hidden Markov Model (HMM) of UGP (PFAM01704) was obtained by searching the conserved structural domain of proteins through National Center for Biotechnology Information Search database (NCBI), and the HMM was used in the hmmer search program in hmm3.0 software (Finn, Clements & Eddy, 2011). The genomic data of seven species were screened out using the Arabidopsis UDPGP model and downloaded from the CottonFGD (https:/cottonfgd.org/about/download.html) website for G. arboreum (Ga) (Du et al., 2018), G. barbadense (Gb) (Hu et al., 2019), G. hirsutum (Gh) (Hu et al., 2019) and G. raimondii (Gr) (Paterson et al., 2012), Theobroma cacao (Motamayor et al., 2013) and Carica papaya (Ming et al., 2008) were retrieved from ePhytozome 2.1 database6 databases (https://phytozome-next.jgi.doe.gov/) the Arabidopsis thaliana genome sequence (A. thaliana) was retrieved from the TAIR database. The search results were filtered with a threshold value of E-value = 1e−5, and unqualified and duplicate transcripts were discarded after the results were obtained.

**Construction and structural analysis of the phylogenetic tree of UGP gene**
The protein sequences of seven species were compared by using MEGA7.0 “muscle” analysis (Kumar, Stecher & Tamura, 2016), and then neighbor-joining method (NJ) (Saitou & Nei, 1987) was used to generate a phylogenetic tree with a bootstrap of 1000 (Tamura et al., 2013). In addition, the sequences of UGP based from four species of cotton were isolated to construct an evolutionary tree UGP gene, and intron-exon analysis was performed by using TBtools (Chen et al., 2020). The gene motifs were analysed through the MEME website (meme-suite.org/meme/index.html), and a minimum of 10 motifs were designed (Hu et al., 2015). The isoelectric point (PI) and molecular weight (MW) of these genes were calculated separately online through the ExPASy website (http://web.expasy.org/compute_pi).
Chromosome location, collinearity and Ka/Ks analysis
TBtools (Chen et al., 2020) were applied on cotton genome file and GFF3 file to draw the position of gene on the chromosome. Homologous UGP gene pairs were obtained by BLASTP full-pair search (Altschul et al., 1990), and visualization was obtained by TBtools (Chen et al., 2020).

Prediction and analysis of promoter cis-acting elements
The 2,000 bp sequence form upstream of start codon of the UGP gene was extracted from the CottonFGD website, and the cis-regulatory elements were predicted through PlantCARE database (http://bioinformatics.psb.ugent.be/webtools/plantcare/html) (Lescot et al., 2002). The predicted cis-elements were categorized according to the role in transcriptional regulation (Pandey et al., 2016).

Transcriptome data analysis
The transcriptome data of standard genetic line TM-1 was retrieved from NCBI SPA (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA490626) (Hu et al., 2019), and transcriptome data of sGK9708 and 0–153 was obtained from Zhang's research group (Zhang et al., 2020). The date for UGP gene were extracted from three cotton transcriptome databases mentioned above at various time periods for preliminary analysis.

Types of cotton 0–153 and sGK9708 from based at Chinese Academy of Agricultural Sciences, Zhengzhou, China (Zhang et al., 2020). The day to flowering was marked as 0 day post anthesis (0 DPA), and cotton bolls with five, 10, 15, 20, 25 and 30 DPA were taken as materials, and stores in liquid nitrogen for various assays. The RNA extraction calibration of RNA concentration, and reverse synthesis of cDNA was prepared according to the protocols provides by manufacturers, and similar protocols were used previously by Jia et al. (2020) and final results were analysed and plotted by using available TBtools (Chen et al., 2020). In addition, fluorescent quantitative specific primers were designed on Primer3 (bioinfo.ut.ee/primer3-0.4.0/primer3/) and upland cotton GhHistone3 (AF024716) was used as an internal reference gene (Xu et al., 2004) in current studies.

Ovule culture and its phenotypic observation
The response against four growth hormone, namely, indole acidic acid (IAA), gibberellin (GA3), abscisic acid (ABA) and salicylic acid (SA) was determined by 2.4 analysis, and one additional hormone ethylene (ETH) was added for ovule ex vivo culture experiments.

Ovules from cultivars 0–153 were cultured and five hormones, IAA, GA3, ETH, SA and ABA, were added at final concentrations of 0.1 uM, 0.5 uM and 1 uM. Hormone configuration and packaging method; sampling of bolls and ovules, cleaning and sterilization, culture conditions, and fiber cluster area was measured according to methods previously used by Jia et al. (2020). All of experiments were performed in three independent replicates, where, four to five ovules were selected for fiber quality area assessment and were analysed for statistical significance by using a t-test.

Intact ovules cultured for 15 DPA were used as material for RNA extraction and data were summarised by reverse transcription followed by qRT-PCR experiments (methods as above). Data were analysed to observe gene expression.
RESULT

Identification of the UGP gene family
A total of 81 UGP genes were identified in seven species by screening genes containing complete sequences for UGP structural domains, including nine from G. arboreum, 26 from G. barbadense, 19 from G. hirsutum, 12 from G. raimondii, seven in A. thaliana, four in C. papaya, and T. cacao four. Among them, a total of 66 UGP genes were identified from four cotton species (Table S1).

Evolutional analysis of UGP gene family
The UGP gene family was divided into two subclades UGP-I and UGP-II based on the evolutionary tree topology, containing 35 and 46 genes, respectively. Wang et al. (2011) divided the UGP genes into five sub-clades by using an evolutionary tree of UGP genes among 11 species, which were differed from previous studies.

Further analysis of conserved structural domains allowed the division of UGP-I into three subgroups UGP-I-A (Fig. 1A), UGP-I-B (Fig. 1B), and UGP-I-C (Fig. 1B), and UGP-II was further divided into three subgroups UGP-II-D (Fig. 1D), UGP-II-E (Fig. 1E), and UGP-II-F (Fig. 1F). In four cotton species, 66 UGP genes were divided into these six subgroups, of which A contained 15 genes, B contained seven genes, C contained eight genes, D contained 12 genes, E contained six genes, and F contained 18 genes; while none of gene from G. arboreum was found in subgroup C. The number of G. barbadense and G. hirsutum in each sub-population was about twice as compared to other species. The isoelectric points and molecular weights of these 81 genes were counted, and it was found that ~74% of their isoelectric points were between 5.5 and 7.3, and ~72% of their molecular weights were between 48.6 and 80.6 kD (Table S2).

Structural analysis of the UGP gene family
The UGP structural domains were present in all of genes, and UGPs were divided into six sub-groups based on length and composition of structural domains, and the conserved structural domains were similar in each sub-group (Fig. 2A). The gene sequence analysis revealed that each sub-group contained similar types and numbers of motif elements, with sub-population F which contain 9–10 motifs, Sub-population C containing only 2–3 motifs which is minimum as compared to others, while remaining sub-populations contained between four and eight motifs, and majority of genes contained motif1 and motif4 (Fig. 2B). Exon analysis revealed that each UGP gene contained a high number of exons i.e., 12–20, and only sub-group C had 5–12 exons, and exons were similar with reference to position and length, and UTR structure was found only in G. raimondii (Fig. 3).

Chromosome location and collinearity analysis
The UGP genes were evenly distributed on At and Dt sub-genomic chromosomes of upland cotton, where 10 of 19 UGP genes were assigned to eight At sub-genomic and nine to seven Dt sub-genomic chromosomes. Two UGP genes were present on chromosomes A08, D08, A11 and their D11, respectively, while only one UGP gene was contained on A02, A03,
Figure 1  UGP gene phylogenetic tree: A total of 81 UGP genes of seven species, including *G. arboretum*, *G. barbadense*, *G. hirsutum*, *G. raimondii*, *A. thaliana*, *C. papaya*, *T. cacao*.

Because *G. hirsutum* is a four-ploid cotton species formed by natural crosses of two two-ploid cotton species (*G. arboretum* and *G. raimondii*) (Wendel & Cronn, 2003), co-lineage analysis by using 19 UGP genes of upland cotton and *G. arboretum* and *G. raimondii* cotton species showed the presence of 47 homologous pairs of genes. 24 homologous pairs were found in A genome and 23 homologous pairs in D genome of upland cotton (Fig. 4A). Co-lineage analysis revealed 45 homologous gene pairs were part of genome of *G. barbadense* (Fig. 4B). The number of UGP genes in upland cotton was nearly double that of the two-ploid cotton species, and the At and Dt genomes contained essentially the
same number of homologous gene pairs, suggesting that the \textit{UGP} genes were present in cotton before the upland cotton cross.

The Ka/Ks (non-synonymous/synonymous) ratios homologous gene pairs were calculated by TBtools \cite{Chen2020}. It is reported that Ka/Ks = 1.0 represents neutrally selected pseudogenes, Ka/Ks < 1.0 indicates the tendency of purifying selection on replicated genes, and Ka/Ks > 1.0 ratio indicates accelerated evolutionary positive selection \cite{Qanmber2019a}. We found that 18 of 19 Gh/Ga homozygous gene pairs, 19 Gh/Gb homozygous gene pairs and 19 Gh/Gr homozygous gene pairs had Ka/Ks values below 1.0, accounting for about 95%; only one gene pair had a Ka/Ks ratio of > 1.0, and since most Ka/Ks were less than 1.0 (Fig. 4C).

**Cotton transcriptome data analysis**

Two cotton fiber transcriptome datasets were used to study the expression patterns of the \textit{UGP} gene family: one is NCBI SPA database TM-1 dataset (Fig. 5A) and other is raw RNA-Seq dataset of sGK9708 and 0–153 (Fig. 5B) (Table S3). Analysis of expression
data showed that six genes namely GH_A08G0422, GH_D08G0444, GH_A12G0472, GH_D12G0484, GH_A11G1773 and GH_D11G1805 had relatively high PKFM values in all of three genomes of upland cotton, with highest expression of about 15 days of fiber development. The qRT-PCR data of six genes in days 0–30, showed high expression of 0–153 and sGK9708, which was consistent with the data from transcriptome analysis (Fig. 6).

**Cis-acting regulatory elements in promoter region of UGP gene**

Analysis of approximately 2,000 bp sequences upstream of start codon (ATG) of 66 genes in UGP gene family revealed that all of these genes contain several similar elements, including light and stress response elements, growth response elements (endosperm expression elements, meristem expression elements), and various hormone response elements (Fig. 7). Light response elements include GT1-motif, ACE, G-box, GT1-motif, 3-AF1 binding site and Sp1, four hormone response elements include growth hormone
response elements (TGA-element, AuxRR-core), gibberellin response elements (P-box, TATC-box, GARE-motif), salicylic acid response element (TCA-element), and abscisic acid response elements (ABRE), and stress response elements including defense and stress response elements (TC-rich repeats) and low-temperature response elements (LTR). Taken together, the distribution of these different kinds of cis-acting elements responds to the fact that these genes can exert a significant influence on fiber development under light, stress, growth, development, and hormone induction.

Response of UGP genes to different hormones in cotton fibers
Cotton ovules were cultured in three gradients of five hormones, and phenotypic observation and RNA extraction were performed at fiber stage of 15DPA. Six of highly expressed genes (GH_A08G0422, GH_D08G0444, GH_A12G0472, GH_D12G0484, GH_A11G1773, GH_D11G1805) were subjected to qRT-PCR analysis.

Figure 4 Collinearity analysis, Ka/Ks analysis and box plot of UGP homologous pairs of four cotton species. (A) Collinearity analysis of GH and GA, GR (B) GH and GB collinearity analysis (C) GH Comparison of Ka/Ks values with the homologous UGP family of three other cotton species. 
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The supply of IAA and GA3 revealed better growth and development with larger ovule volume and fiber cluster area of 15 DPA ovules than the control, whereas ovules cultures with ABA, ETH and SA grew slowly and had smaller fiber cluster area than the control (Fig. 8A). The area of fiber clusters of ovule culture was counted for each concentration of hormones in a sample of 5–10 ovules, and it was found that fiber cluster development was approximately 10–20% higher than the control with external application of IAA and GA3 hormones, and approximately 20–90% lower under the influence of ETH, SA and ABA hormones (*, P < 0.05; **, P < 0.01) (Fig. 8B). Analysis of qRT-PCR results of ovule fibers at 15 days showed that the expression of GH_A08G0422 and GH_D08G0444 genes were increased differentially with addition of low concentrations of hormones, and were increased to 4–6-fold with addition of IAA. However, the other four genes showed significant increase in expression only when IAA was added (*, P < 0.05; **, P < 0.01) (Fig. 9).

**DISCUSSION**

In the present study, we identified 81 UGP genes in seven different species, a total of 66 UGP genes were identified in four cotton species, which were classified into two subfamilies: UGP-I and UGP-II, containing 46 and 35 genes, respectively, based on topology and conserved structural domains, and the two subfamilies, UGP-I and UGP-II, were divided into three subgroups, respectively. The phylogenetic tree analysis of UGP genes from seven different species revealed a very similar homology of UGP genes among different species. The distribution of each species in the subpopulations was relatively uniform, demonstrating that UGP genes are very conserved across species evolution (Liu et al., 2018; Qanmber et al., 2019a).
Figure 6 Perform qRT-PCR analysis on six UGP genes with high expression of 0–153 and sGK9708. The expression level is shown relative to the internal reference gene GhHis3. Error bars represent the standard deviation of three independent experiments.

The molecular weights of UGP genes are concentrated at 48.6–80.6 kD, and most of isoelectric points are concentrated at 5.5–7.3. The same cis-acting elements were found in most of these genes in the analysis of first 2,000 bp cis-acting elements of the start codon, and hormone response elements have been identified, including growth (Guilfoyle & Hagen, 2007; Hagen & Guilfoyle, 2002) and gibberellin (Wang et al., 2018), abscisic acid (Narusaka et al., 2003; Song et al., 2005) and salicylic acid, and light (Fankhauser & Chory, 1997), drought and low-temperature response elements (Singh, Foley & Oñate Sánchez, 2002). It is tentatively speculated that UGP genes may be involved in growth, development, abiotic and hormonal stresses in cotton (Qanmber et al., 2019a; Qanmber et al., 2019b).

The UGP genes were distributed relatively evenly on the chromosomes, i.e., 10 genes were distributed on At-sub-genic chromosome group and nine genes on Dt-chromosome group. The conserved structural domains, motif analysis and intron analysis revealed that the conserved structural domains were similar in each sub-group with majority of genes containing motif1 and motif4 motif structures, and each UGP gene contained a high number of exons between 12–20, and UTR structure was only found in G. raimondii. From these finding, it is concluded that UGP genes are evolutionarily conserved in cotton (Qanmber et al., 2018).
The number of UGP genes in *G. hirsutum* and *G. barbadense* is about twice as compared to *G. arboreum* and *G. raimondii*, due to polyploidization. Since *G. hirsutum* was evolved 1.5 million years ago by crossing of diploid ancestral species having A and D genomes (Hu et al., 2019; Li et al., 2015a; Schaper & Anisimova, 2015). It is reported that UGP gene was present in parental species of upland cotton. Polyploidy is a common phenomenon in the evolution of plants and is a major mechanism of adaptation and speciation (Ramsey & Schenske, 1998). It is estimated that 47–70% of angiosperms are polyploid in nature (Grant, 1981; Masterson, 1994). Polyploids arises due to involvement of partial or whole-genome duplication (WGD) (Cannon et al., 2004). WGD is also a common phenomenon in evolution, and Arabidopsis thaliana has experienced two WGDs that have resulted in DNA loss and chromosomal rearrangements (Tang et al., 2008). However, gene loss can occur when genes obtain form duplicate amplification after hybridization (Li et al., 2015a; Paterson, Bowers & Chapman, 2004), and homologous of UGP were lost during evolution.
of upland cotton. This indicated that UGP gene is evolutionarily conserved in cotton (Klinghammer & Tenhaken, 2007).

The covariance analysis of 19 UGP genes found in upland cotton and other three cotton species revealed that most of UGP genes were homologous among the four cotton species. Some of homologous genes were prevalent on similar positions, because UGP genes were generated from new genes family through tandem repeats during the doubling of upland cotton (Gě et al., 2020; Jia et al., 2020; Schaper & Anisimova, 2015). By calculating the Ka/Ks ratios of homologous gene pairs, it was found that most of Ka/Ks ratios of upland cotton and other three cotton species were below 1.0. This indicated that UGP genes were under strong purifying selection pressure during evolution (Qanmber et al., 2019a; Qanmber et al., 2018).

Nineteen UGP genes were analyzed for various timelines of DPA by using transcriptome data, including TM-1, 0–153, and sGK9708. Six genes namely GH_A08G0422, GH_D08G0444, GH_A12G0472, GH_D12G0484, GH_A11G1773 and GH_D11G1805 were found to be highly expressed in cotton fiber on 15 DPA. RNA from fiber material of cultivar 0–153 and SGK9708 strain was used for qRT-PCR, which supported with transcriptome data (Zhang et al., 2020), both UGP genes were highly expressed at 15 DPA.

Ovule isolation culture experiments were performed by adding different hormones (Kim et al., 2015) and ovules were observed at 15 days of culture. Ovule growth and development were found to be better under due to IAA and GA3, with larger fiber cluster area, while ovule development was retarded under the influence of ABA, ETH and SA. These results indicate that auxin and gibberellin promote cotton ovule growth, while the other three hormones have opposite effects, which are consistent with previous studies (Wang et al., 2018; Zhang et al., 2011). Ovule samples at 15 days of culture were taken for
RNA extraction, and the expression of six highly expressed genes under the influence of five hormones was measured, and it was found that the expression of UGP genes increased correspondingly under the influence of IAA and GA3, and the expression of GH_A08G0422 and GH_D08G0444 increased 6-fold and 4.5-fold, respectively, and the expression of these two genes increased under the influence of low concentrations of ABA, ETH and SA also increased the expression of these two genes under the influence of low concentrations of ABA, ETH and SA. It showed that expression of UGP genes was influenced by hormones and was more strongly stimulated for growth hormone and gibberellin (Bai et al., 2014; Xiao, Zhao & Zhang, 2019). It is tentatively hypothesized that UGP genes have an indispensable role in cotton fiber development. Its expression is closely related to the activity of pectin pathway in the fiber.

**CONCLUSION**

In this study, a total of 66 UGP genes were identified based on the genomic information of G. raimondii, G. arboreum, G. hirsutum and G. barbadense. Covariance analysis postulated that the amplification of UGP genes was due to repetitive tandem generation of new family
genes. Ka/Ks ratio analysis postulated that UGP genes are under strong purifying selection pressure in cotton. Six highly expressed genes namely GH_A08G0422, GH_D08G0444, GH_A11G1773, GH_D11G1805, GH_A12G0472 and GH_D12G0484, all possessing relatively long UDPGP structural domains, were obtained by qRT-PCR and transcriptome data screening of cotton fiber. The addition of 0.5 mM IAA and GA3 to ovule culture medium promoted the growth of ovule fiber clusters, which showed an increase of about 10% in area and the expression of six UGP genes increased from 1.5-fold to 6-fold. These results suggest that UGP genes may play an important role in the growth and development of cotton fibers, and that the speed of cotton fiber development and level of UGP gene expression are closely related, and that their mechanisms of action in cotton fiber development and fiber quality formation and their effects on pectin synthesis in cotton fiber development deserve in-depth study.

ADDITIONAL INFORMATION AND DECLARATIONS

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**Author Contributions**
- Zhongyang Xu conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the article, and approved the final draft.
- Jiasen He analyzed the data, authored or reviewed drafts of the article, and approved the final draft.
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- Tingting Jia performed the experiments, authored or reviewed drafts of the article, and approved the final draft.
• Haihong Shang conceived and designed the experiments, authored or reviewed drafts of the article, and approved the final draft.
• Youlu Yuan conceived and designed the experiments, authored or reviewed drafts of the article, and approved the final draft.

Data Availability
The following information was supplied regarding data availability:
The raw measurements are available in the Supplementary Files.

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REFERENCES

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. *Journal of Molecular Biology* 215:403–410 DOI 10.1006/jmbi.1990.9999.

Amor Y, Haigler CH, Johnson S, Wainscott M, Delmer DP. 1995. A membrane-associated form of sucrose synthase and its potential role in synthesis of cellulose and callose in plants. *Proceedings of the National Academy of Sciences of the United States of America* 92:9353–9357 DOI 10.1073/pnas.92.20.9353.

Bai WQ, Xiao YH, Zhao J, Song SQ, Hu L, Zeng JY, Li XB, Hou L, Luo M, Li DM, Pei Y. 2014. Gibberellin overproduction promotes sucrose synthase expression and secondary cell wall deposition in cotton fibers. *PLOS ONE* 9:e96537 DOI 10.1371/journal.pone.0096537.

Borovkov AY, McClean PE, Sowokinos JR, Ruud SH, Secor GA. 1996. Effect of expression of UDP-glucose pyrophosphorylase ribozyme and antisense RNAs on the enzyme activity and carbohydrate composition of field-grown transgenic potato plants. *Journal of Plant Physiology* 147:644–652 DOI 10.1016/s0176-1617(11)81473-2.

Cannon SB, Mitra A, Baumgarten A, Young ND, May G. 2004. The roles of segmental and tandem gene duplication in the evolution of large gene families in Arabidopsis thaliana. *BMC Plant Biology* 4:10 DOI 10.1186/1471-2229-4-10.

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* 13:1194–1202 DOI 10.1016/j.molp.2020.06.009.

Chen R, Zhao X, Shao Z, Wei Z, Wang Y, Zhu L, Zhao J, Sun M, He R, He G. 2007. Rice UDP-glucose pyrophosphorylase1 is essential for pollen callose deposition and its cosuppression results in a new type of thermosensitive genic male sterility. *The Plant Cell* 19:847–861 DOI 10.1105/tpc.106.044123.

Coleman HD, Ellis DD, Gilbert M, Mansfield SD. 2006. Up-regulation of sucrose synthase and UDP-glucose pyrophosphorylase impacts plant growth and metabolism. *Plant Biotechnol J* 4:87–101 DOI 10.1111/j.1467-7652.2005.00160.x.
Daran JM, Bell W, François J. 1997. Physiological and morphological effects of genetic alterations leading to a reduced synthesis of UDP-glucose in Saccharomyces cerevisiae. *FEMS Microbiology Letters* 153:89–96 DOI 10.1111/j.1574-6968.1997.tb10468.x.

Daran JM, Dallies N, Thines-Sempoux D, Paquet V, François J. 1995. Genetic and biochemical characterization of the UGP1 gene encoding the UDP-glucose pyrophosphorylase from Saccharomyces cerevisiae. *European Journal of Biochemistry* 233:520–530 DOI 10.1111/j.1432-1033.1995.520_2.x.

Deng F, Tu L, Tan J, Li Y, Nie Y, Zhang X. 2012. GbPDF1 is involved in cotton fiber initiation via the core cis-element HDZIP2ATATHB2. *Plant Physiology* 158:890–904 DOI 10.1104/pp.111.186742.

Du X, Huang G, He S, Yang Z, Sun G, Ma X, Li N, Zhang X, Sun J, Liu M, Jia Y, Pan Z, Gong W, Liu Z, Zhu H, Ma L, Liu F, Yang D, Wang F, Fan W, Gong Q, Peng Z, Wang L, Wang X, Xu S, Shang H, Lu C, Zheng H, Huang S, Lin T, Zhu Y, Li F. 2018. Resequencing of 243 diploid cotton accessions based on an updated A genome identifies the genetic basis of key agronomic traits. *Nature Genetics* 50:796–802 DOI 10.1038/s41588-018-0116-x.

Fankhauser C, Chory J. 1997. Light control of plant development. *Annual Review of Cell and Developmental Biology* 13:203–229 DOI 10.1146/annurev.cellbio.13.1.203.

Finn RD, Clements J, Eddy SR. 2011. HMMER web server: interactive sequence similarity searching. *Nucleic Acids Research* 39:W29–W37 DOI 10.1093/nar/gkr367.

Grant V. 1981. The genetic goal of speciation. *Biologisches Zentralblatt* 100:473–482.

Guilfoyle TJ, Hagen G. 2007. Auxin response factors. *Current Opinion in Plant Biology* 10:453–460 DOI 10.1016/j.pbi.2007.08.014.

Gě Q, Cuí Y, Lú J, Gòng J, Lú Q, Lí P, Shí Y, Shàng H, Liú À, Dèng X, Pán J, Chén Q, Yuán Y, Gòng W. 2020. Disequilibrium evolution of the Fructose-1, 6-Bisphosphatase gene family leads to their functional biodiversity in gossypium species. *BMC Genomics* 21:379 DOI 10.1186/s12864-020-6773-z.

Hagen G, Guilfoyle T. 2002. Auxin-responsive gene expression: genes, promoters and regulatory factors. *Plant Mol Biol* 49:373–385 DOI 10.1023/A:1015207114117.

Hu Y, Chen J, Fang L, Zhang Z, Ma W, Niu Y, Ju L, Deng J, Zhao T, Lian J, Baruch K, Fang D, Liu X, Ruan YL, Rahman MU, Han J, Wang K, Wang Q, Wu H, Mei G, Zang Y, Han Z, Xu C, Shen W, Yang D, Si Z, Dai F, Zou L, Huang F, Bai Y, Zhang Y, Brodt A, Ben-Hamo H, Zhu X, Zhou B, Guan X, Zhu S, Chen X, Zhang T. 2019. Gossypium barbadense and Gossypium hirsutum genomes provide insights into the origin and evolution of allotetraploid cotton. *Nature Genetics* 51:739–748 DOI 10.1038/s41588-019-0371-5.

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

Jia T, Ge Q, Zhang S, Zhang Z, Liu A, Fan S, Jiang X, Feng Y, Zhang L, Niu D, Huang S, Gong W, Yuan Y, Shang H. 2020. UDP-Glucose Dehydrogenases: identification, expression, and function analyses in upland cotton (*Gossypium Hirsutum*). *Front Genet* 11:597890 DOI 10.3389/fgene.2020.597890.
Johansson H, Sterky F, Amini B, Lundeberg J, Kleczkowski LA. 2002. Molecular cloning and characterization of a cDNA encoding poplar UDP-glucose dehydrogenase, a key gene of hemicellulose/pectin formation. *Biochim Biophys Acta* 1576:53–58 DOI 10.1016/s0167-4781(02)00292-0.

Kim HJ, Hinchliffe DJ, Tripelett BA, Chen ZJ, Stelly DM, Yeater KM, Moon HS, Gilbert MK, Thyssen GN, Turley RB, Fang DD. 2015. Phytohormonal networks promote differentiation of fiber initials on pre-anthesis cotton ovules grown in vitro and in planta. *PLOS ONE* 10:e0125046 DOI 10.1371/journal.pone.0125046.

Kim HJ, Tripelett BA. 2001. Cotton fiber growth in planta and in vitro, models for plant cell elongation and cell wall biogenesis. *Plant Physiol* 127:1361–1366 DOI 10.1104/pp.010724.

Kim HJ, Tripelett BA. 2004. Cotton fiber germin-like protein. I. molecular cloning and gene expression. *Planta* 218:516–524 DOI 10.1007/s00425-003-1133-1.

Kleczkowski LA. 1994. Glucose activation and metabolism through UDP-glucose pyrophosphorylase in plants. *Phytochemistry* 37:1507–1515 DOI 10.1016/s0031-9422(00)89568-0.

Klinghammer M, Tenhaken R. 2007. Genome-wide analysis of the UDP-glucose dehydrogenase gene family in Arabidopsis, a key enzyme for matrix polysaccharides in cell walls. *Journal of Experimental Botany* 58:3609–3621 DOI 10.1093/jxb/erm209.

Kotake T, Yamaguchi D, Ohzono H, Hojo S, Kaneko S, Ishida HK, Tsunuraya Y. 2004. UDP-sugar pyrophosphorylase with broad substrate specificity toward various monosaccharide 1-phosphates from pea sprouts. *Journal of Biological Chemistry* 279:45728–45736 DOI 10.1074/jbc.M408716200.

Kumar S, Stecher G, Tamura K. 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution* 33:1870–1874 DOI 10.1093/molbev/msw054.

Lamerz AC, Haselhorst T, Bergfeld AK, von Itzstein M, Gerardy-Schahn R. 2006. Molecular cloning of the Leishmania major UDP-glucose pyrophosphorylase, functional characterization, and ligand binding analyses using NMR spectroscopy. *Journal of Biological Chemistry* 281:16314–16322 DOI 10.1074/jbc.M600076200.

Lescot M, Déhais P, Thijs G, Marchal K, Moreau Y, VandePeer Y, Rouzé P, Rombauts 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* 30:325–327 DOI 10.1093/nar/30.1.325.

Li M, Chen T, Gao T, Miao Z, Jiang A, Shi L, Ren A, Zhao M. 2015b. UDP-glucose pyrophosphorylase influences polysaccharide synthesis, cell wall components, and hyphal branching in ganoderma lucidum via regulation of the balance between Glucose-1-Phosphate and UDP-Glucose. *Fungal Genetics and Biology* 82:251–263 DOI 10.1016/j.fgb.2015.07.012.

Li FG, Fan GY, Lu CR, Xiao GH, Zou CS, Kohel RJ, Ma ZY, Shang HH, Ma XF, Wu JY, Liang XM, Huang G, Percy RG, Liu K, Yang WH, Chen WB, Du XM, Shi CC, Yuan YL, Ye WW, Liu X, Zhang XY, Liu WQ, Wei HL, Wei SJ, Huang GD, Zhang XL, Zhu SJ, Zhang H, Sun FM, Wang XF, Liang J, Wang JH, He Q, Huang LH, Wang J,
Cui JJ, Song GL, Wang KB, Xu X, Yu JZ, Zhu YX, Yu SX. 2015a. Genome sequence of cultivated Upland cotton (Gossypium hirsutum TM-1) provides insights into genome evolution. Nature Biotechnology 33:524–U242 DOI 10.1038/nbt.3208.
Liu Z, Qanmber G, Lu L, Qin W, Liu J, Li J, Ma S, Yang Z, Yang Z. 2018. Genome-wide analysis of BES1 genes in Gossypium revealed their evolutionary conserved roles in brassinosteroid signaling. Science China Life Sciences 61:1566–1582 DOI 10.1007/s11427-018-9412-x.
Masterson J. 1994. Stomatal size in fossil plants: evidence for polyploidy in majority of angiosperms. Science 264:421–424 DOI 10.1126/science.264.5157.421.
Ming R, Hou S, Feng Y, Yu Q, Dionne-Laporte A, Saw JH, Senin P, Wang W, Ly BV, Lewis KL, Salzberg SL, Feng L, Jones MR, Skelton RL, Murray JE, Chen C, Qian W, Shen J, Du P, Eustice M, Tong E, Tang H, Lyons E, Paull RE, Michael TP, Wall K, Rice DW, Albert H, Wang ML, Zhu YJ, Schatz M, Nagarajan N, Acob RA, Guan P, Blas A, Wai CM, Ackerman CM, Ren Y, Liu C, Wang J, Wang J, Na JK, Shakirov EV, Haas B, Thimmmapuram J, Nelson D, Wang X, Bowers JE, Genscheweg AR, Delcher AL, Singh R, Suzuki JY, Tripathi S, Neupane K, Wei H, Irikura B, Paidi M, Jiang N, Zhang W, Presting G, Windsor A, Navajas-Pérez R, Torres MJ, Feltus FA, Porter B, Li Y, Burroughs AM, Luo MC, Liu L, Christopher DA, Mount SM, Moore PH, Sugimura T, Jiang J, Schuler MA, Friedman V, Mitchell-Olds T, Shippen DE, De Pamphilis CW, Palmer JD, Freeling M, Paterson AH, Gonsalves D, Wang L, Alam M. 2008. The draft genome of the transgenic tropical fruit tree papaya (Carica papaya Linnaeaus). Nature 452:991–996 DOI 10.1038/nature06856.
Motamayor JC, Mockaitis K, Schmutz J, Haiminen N, Livingstone 3rd D, Cornejo O, Findley SD, Zheng P, Utro F, Royaert S, Sasaki C, Jenkins J, Podicheti R, Zhao M, Scheffler BE, Stack JC, Feltus FA, Mustiga GM, Amores F, Phillips W, Marelli JP, May GD, Shapiro H, Ma J, Bustamante CD, Schnell RJ, Main D, Gilbert D, Parida L, Kuhn DN. 2013. The genome sequence of the most widely cultivated cacao type and its use to identify candidate genes regulating pod color. Genome Biology 14:r53 DOI 10.1186/gb-2013-14-6-r53.
Narusaka Y, Nakashima K, Shinwari ZK, Sakuma Y, Furihata T, Abe H, Narusaka M, Shinozaki K, Yamaguchi-Shinozaki K. 2003. Interaction between two cis-acting elements, ABRE and DRE, in ABA-Dependent expression of arabidopsis Rd29A gene in response to dehydration and high-salinity stresses. The Plant Journal 34:137–148 DOI 10.1046/j.1365-313x.2003.01708.x.
Pandey A, Misra P, Alok A, Kaur N, Sharma S, Lakhwani D, Asif MH, Tiwari S, Trivedi PK. 2016. Genome-wide identification and expression analysis of homeodomain leucine zipper subfamily IV (HDZ IV) gene family from Musa acuminate. Frontiers in Plant Science 7:20 DOI 10.3389/fpls.2016.00020.
Park JI, Ishimizu T, Suwabe K, Sudo K, Masuko H, Hakozaki H, Nou IS, Suzuki G, Watanabe M. 2010. UDP-glucose pyrophosphorylase is rate limiting in vegetative and reproductive phases in Arabidopsis thaliana. Plant and Cell Physiology 51:981–996 DOI 10.1093/pcp/pcq057.
Paterson AH, Bowers JE, Chapman BA. 2004. Ancient polyploidization predating divergence of the cereals, and its consequences for comparative genomics. *Proceedings of the National Academy of Sciences of the United States of America* 101:9903–9908 DOI 10.1073/pnas.0307901101.

Paterson AH, Wendel JF, Gundlach H, Guo H, Jenkins J, Jin DC, Llewellyn D, Showmaker KC, Shu SQ, Uddall J, Yoo MJ, Byers R, Chen W, Doron-Faigenboim A, Duke MV, Gong L, Grimwood J, Grover C, Grupp K, Hu GJ, Lee TH, Li JP, Lin LF, Liu T, Marler BS, Page JT, Roberts AW, Romanel E, Sanders WS, Szadkowski E, Tan X, Tang HB, Xu CM, Wang JP, Wang ZN, Zhang D, Zhang L, Ashrafi H, Bedon F, Bowers JE, Brubaker CL, Chee PW, Das S, Gingle AR, Haigler CH, Harker D, Hoffmann LV, Hovav R, Jones DC, Lemke C, Mansoor S, Rahman MU, Rainville LN, Rambani A, Reddy UK, Rong JK, Saranga Y, Scheffler BE, Scheffler JA, Stelly DM, Triplette BA, Van Deynze A, Vaslin MFS, Waghmare VN, Walford SA, Wright RJ, Zaki EA, Zhang TZ, Dennis ES, Mayer KFX, Peterson DG, Rokhsar DS, Wang XY, Schmutz J. 2012. Repeated polyploidization of Gossypium genomes and the evolution of spinnable cotton fibres. *Nature* 492:423-+ DOI 10.1038/nature11798.

Payyavula RS, Tschaplinski TJ, Jawdy SS, Sykes RW, Tuskan GA, Kalluri UC. 2014. Metabolic profiling reveals altered sugar and secondary metabolism in response to UGPase overexpression in Populus. *BMC Plant Biology* 14:265 DOI 10.1186/s12870-014-0265-8.

Qanmber G, Ali F, Lu L, Mo H, Ma S, Wang Z, Yang Z. 2019a. Identification of histone H3 (HH3) genes in gossypium hirsutum revealed diverse expression during ovule development and stress responses. *Genes* 10:355 DOI 10.3390/genes10050355.

Qanmber G, Liu J, Yu D, Liu Z, Lu L, Mo H, Ma S, Wang Z, Yang Z. 2019b. Genome-wide identification and characterization of the PERK gene family in gossypium hirsutum reveals gene duplication and functional divergence. *International Journal of Molecular Sciences* 20:1750 DOI 10.3390/ijms20071750.

Qanmber G, Yu DQ, Li J, Wang LL, Ma SY, Lu LL, Yang ZR, Li FG. 2018. Genome-wide identification and expression analysis of Gossypium RING-H2 finger E3 ligase genes revealed their roles in fiber development, and phytohormone and abiotic stress responses. *Journal of Cotton Research* 1:1 DOI 10.1186/s42397-018-0004-z.

Ramsey J, Schemske DW. 1998. Pathways, mechanisms, and rates of polyploid formation in flowering plants. *Annual Review of Ecology and Systematics* 29:467–501 DOI 10.1146/annurev.ecolsys.29.1.467.

Saitou N, Nei M. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution* 4:406–425 DOI 10.1093/oxfordjournals.molbev.a040454.

Schaper E, Anisimova M. 2015. The evolution and function of protein tandem repeats in plants. *New Phytologist* 206:397–410 DOI 10.1111/nph.13184.

Singh K, Foley RC, Oñate Sánchez L. 2002. Transcription factors in plant defense and stress responses. *Current Opinion in Plant Biology* 5:430–436 DOI 10.1016/s1369-5266(02)00289-3.
Song CP, Agarwal M, Ohta M, Guo Y, Halfter U, Wang P, Zhu JK. 2005. Role of an Arabidopsis AP2/EREBP-type transcriptional repressor in abscisic acid and drought stress responses. *The Plant Cell* 17:2384–2396 DOI 10.1105/tpc.105.033043.

Spychalla JP, Scheffler BE, Sowokinos JR, Bevan MW. 1994. Cloning, antisense RNA inhibition, and the coordinated expression of udp-glucose pyrophosphorylase with starch biosynthetic genes in potato tubers. *Journal of Plant Physiology* 144:444–453 DOI 10.1016/s0176-1617(11)82121-8.

Sun Y, Veerabomma S, Abdel-Mageed HA, Fokar M, Asami T, Yoshida S, Allen RD. 2005. Brassinosteroid regulates fiber development on cultured cotton ovules. *Plant and Cell Physiology* 46:1384–1391 DOI 10.1093/pcp/pci150.

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution* 30:2725–2729 DOI 10.1093/molbev/mst197.

Tang H, Wang X, Bowers JE, Ming R, Alam M, Paterson AH. 2008. Unraveling ancient hexaploidy through multiply-aligned angiosperm gene maps. *Genome Research* 18:1944–1954 DOI 10.1101/gr.080978.108.

Wang Y, Wang Y, Zhao J, Huang J, Shi Y, Deng D. 2018. Unveiling gibberellin-responsive coding and long noncoding RNAs in maize. *Plant Molecular Biology* 98:427–438 DOI 10.1007/s11103-018-0788-8.

Wang Q, Zhang X, Li F, Hou Y, Liu X, Zhang X. 2011. Identification of a UDP-glucose pyrophosphorylase from cotton (Gossypium hirsutum L.) involved in cellulose biosynthesis in Arabidopsis thaliana. *Plant Cell Reports* 30:1303–1312 DOI 10.1007/s00299-011-1042-x.

Wendel JF. 1989. New World tetraploid cottons contain Old World cytoplasm. *Proceedings of the National Academy of Sciences of the United States of America* 86:4132–4136 DOI 10.1073/pnas.86.11.4132.

Wendel JF, Cronn RC. 2003. Polyploidy and the evolutionary history of cotton. In: Sparks DL, ed. *Advances in agronomy*. vol. 78. 139–186 DOI 10.1016/S0065-2113(02)78004-8.

Winter H, Huber SC. 2000. Regulation of sucrose metabolism in higher plants: localization and regulation of activity of key enzymes. *Critical Reviews in Biochemistry and Molecular Biology* 35:253–289 DOI 10.1080/10409230008984165.

Woo MO, Ham TH, Ji HS, Choi MS, Jiang W, Chu SH, Piao R, Chin JH, Kim JA, Park BS, Seo HS, Jwa NS, McCouch S, Koh HJ. 2008. Inactivation of the UGPase1 gene causes genic male sterility and endosperm chalkiness in rice (Oryza sativa L.). *The Plant Journal* 54:190–204 DOI 10.1111/j.1365-313X.2008.04305.x.

Xiao G, Zhao P, Zhang Y. 2019. A pivotal role of hormones in regulating cotton fiber development. *Frontiers in Plant Science* 10:87 DOI 10.3389/fpls.2019.00087.

Xu YH, Wang JW, Wang S, Wang JY, Chen XY. 2004. Characterization of GaWRKY1, a cotton transcription factor that regulates the sesquiterpene synthase gene (+)-Delta-cadinene synthase-a. *Plant Physiology* 135:507–515 DOI 10.1104/pp.104.038612.
Yang Z, Zhang C, Yang X, Liu K, Wu Z, Zhang X, Zheng W, Xun Q, Liu C, Lu L, Yang Z, Qian Y, Xu Z, Li C, Li J, Li F. 2014. PAG1, a cotton brassinosteroid catabolism gene, modulates fiber elongation. *New Phytologist* **203**:437–448 DOI 10.1111/nph.12824.

Zhang T, Hu Y, Jiang W, Fang L, Guan X, Chen J, Zhang J, Saski CA, Scheffler BE, Stelly DM, Hulse-Kemp AM, Wan Q, Liu B, Liu C, Wang S, Pan M, Wang Y, Wang D, Ye W, Chang L, Zhang W, Song Q, Kirkbride RC, Chen X, Dennis E, Llewellyn DJ, Peterson DG, Thaxter P, Jones DC, Wang Q, Xu X, Zhang H, Wu H, Zhou L, Mei G, Chen S, Tian Y, Xiang D, Li X, Ding J, Zuo Q, Tao L, Liu Y, Li J, Lin Y, Hui Y, Cao Z, Cai C, Zhu X, Jiang Z, Zhou B, Guo W, Li R, Chen ZJ. 2015. Sequencing of allotetraploid cotton (*Gossypium hirsutum* L. Acc. TM-1) provides a resource for fiber improvement. *Nature Biotechnology* **33**:531–537 DOI 10.1038/nbt.3207.

Zhang Z, Li J, Jamshed M, Shi Y, Liu A, Gong J, Wang S, Zhang J, Sun F, Jia F, Ge Q, Fan L, Zhang Z, Pan J, Fan S, Wang Y, Lu Q, Liu R, Deng X, Zou X, Jiang X, Liu P, Li P, Iqbal MS, Zhang C, Zou J, Chen H, Tian Q, Jia X, Wang B, Ai N, Feng G, Wang Y, Hong M, Li S, Lian W, Wu B, Hua J, Zhang C, Huang J, Xu A, Shang H, Gong W, Yuan Y. 2020. Genome-wide quantitative trait loci reveal the genetic basis of cotton fibre quality and yield-related traits in a *Gossypium hirsutum* recombinant inbred line population. *Plant Biotechnology Journal* **18**:239–253 DOI 10.1111/pbi.13191.

Zhang M, Zheng X, Song S, Zeng Q, Hou L, Li D, Zhao J, Wei Y, Li X, Luo M, Xiao Y, Luo X, Zhang J, Xiang C, Pei Y. 2011. Spatiotemporal manipulation of auxin biosynthesis in cotton ovule epidermal cells enhances fiber yield and quality. *Nature Biotechnology* **29**:453–458 DOI 10.1038/nbt.1843.