Journal of Cotton Research

RESEARCH Open Access

Identification and expression analysis of *Tubulin* gene family in upland cotton

CHEN Baojun†, ZHAO Junjie†, FU Guoyong, PEI Xinxin, PAN Zhaoe, LI Hongge, AHMED Haris, HE Shoupu* and DU Xiongming*

Abstract

Background: Cotton fibers are single-celled extensions of the seed epidermis, a model tissue for studying cytoskeleton. *Tubulin* genes play a critical role in synthesizing the microtubules (MT) as a core element of the cytoskeleton. However, there is a lack of studies concerning the systematic characterization of the tubulin gene family in cotton. Therefore, the identification and portrayal of *G. hirsutum tubulin* genes can provide key targets for molecular manipulation in cotton breeding.

Result: In this study, we investigated all *tubulin* genes from different plant species and identified 98 *tubulin* genes in *G. hirsutum*. Phylogenetic analysis showed that *tubulin* family genes were classified into three subfamilies. The protein motifs and gene structure of α-, β-*tubulin* genes are more conserved compared with γ-*tubulin* genes. Most *tubulin* genes are located at the proximate ends of the chromosomes. Spatiotemporal expression pattern by transcriptome and qRT-PCR analysis revealed that 12 α-*tubulin* and 7 β-*tubulin* genes are specifically expressed during different fiber development stages. However, *Gh.A03G027200*, *Gh.D03G169300,* and *Gh.A11G258900* had differential expression patterns at distinct stages of fiber development in varieties J02508 and ZRI015.

Conclusion: In this study, the evolutionary analysis showed that the *tubulin* genes were divided into three clades. The genetic structures and molecular functions were highly conserved in different plants. Three candidate genes, *Gh.A03G027200*, *Gh.D03G169300,* and *Gh.A11G258900* may play a key role during fiber development complementing fiber length and strength.

Keywords: Upland cotton, Fiber quality, Cytoskeleton, Microtubules (MT), Tubulin

Introduction

The production of upland cotton (*G. hirsutum*) accounts for a significant proportion of global cotton production. Although upland cotton is a highly yielding fiber source, it is pertinent to improve the fiber quality to meet the industrial demand (Su et al. 2018). Therefore, current cotton breeding programs are also focused on fiber quality improvement in upland cotton. Wild progenitors are sources carrying excellent genetic characteristics for developing desirable variation in plants (Nazir et al. 2020). It is pertinent to explore genetic variation among different species of *Gossypium* and further to utilize in breeding programs.

Fiber length and strength are essential indicators in assessing fiber quality (Gao et al. 2019). The development of cotton fiber can be delineated into four distinct overlapping stages: fiber initiation, elongation, secondary wall synthesis, and maturation. It is a highly revised, basic biological process (Gao et al. 2007; Wang et al. 2017). Microtubules (MTs) are the cytoskeleton's core element and act a pivotal function in cellular migration, mitosis, mechanical stress, cell polarity, intracellular transport, cell division, and cell morphogenesis (Nieuwenhuis and Brummelkamp 2019). Many studies have shown that the *actin* and *tubulin* genes play a critical role in the cytoskeleton synthesis and cotton fiber elongation (Pydiura et al. 2019; Li et al. 2005). *Tubulin*

* Correspondence: heshoupu@caas.cn; duxiongming@caas.cn
†Baojun CHEN and Junjie ZHAO contributed equally to this work.
State Key Laboratory of Cotton Biology, Institute of Cotton Research, Chinese Academy of Agricultural Sciences (ICR, CAAS), Anyang 455000, China

genes which are highly conserved in structure and function from alpha, beta, and gamma-*tubulin* families were usually used as reference genes for qRT-PCR (Findeisen et al. 2014; Zhao et al. 2014; Jayaswal et al. 2019; Ray and Johnson 2014). Most *tubulin* genes have different configurations merely due to few amino acid substitutions, which have two conserved domains Tubulin and Tubulin_C (Mubeen et al. 2016). Superfamilies or subfamilies of *Tubulin* genes were already reported in some species, such as flax (*Linum usitatissimum*) (Gavazzi et al. 2017; Pydiura et al. 2019), fungal (Zhao et al. 2014), *Salix arbutifolia* (Rao et al. 2016).

Microtubules are chiefly built-up heterodimers of α-tubulin and β-tubulin, which are closely related to cellulose and microfiber deposition and also play a vital role in the secondary cell wall development in all plants (Seaguii 1992). At the same time, γ-tubulin may only mediate microtubule nucleation. The reduction of α-tubulin can disrupt the microtubule structure and further result in deviant cell expansion (Rao et al. 2016). The tubulin proteins can be easily purified from cell suspension cultures of tobacco and *Arabidopsis* (Hotta et al. 2016), which may intuitively study its structure and function for laying a good foundation for *in vitro* research. Individual *tubulin* family members showed organizational differences in various organisms (Rao et al. 2016). The γ-*tubulin* gene *GCP6* is a key for spindle morphogenesis but not necessary for microtubule reorganization in *Arabidopsis* (Miao et al. 2019). *TUA1* had specific expression in leaves in poplars but abnormally low in xylem, which indicated that high expression levels of tubulin protein were not tolerated in wood-forming tissues. As in leaves, the relative transcriptional abundance of the *TUB* is lower than that of the *TUA* in transgenic poplars (Swamy et al. 2015). Interfering with tubulin proteins affects behavioral changes in guard cells, to delay stomatal closure under stress and light-induced leaf stomatal opening (Swamy et al. 2015), which emphasized tubulin involvement in non-cellulosic polysaccharides assembly during cell wall biogenesis (Swamy et al. 2015).

Although the significant role of the *tubulin* gene family has been reported previously in other species, it is less understood in cotton. Our study systematically explored the *tubulin* gene family and further characterized phylogeny relationships, gene structures, chromosomal locations, expression patterns in cotton. The yielded information can be further utilized as a reference for fiber length and strength.

Materials and methods

Plant materials and nucleic acid extraction

Two upland cotton varieties (J02508 and ZRI015), with significant differences in cotton fiber length and strength, were grown in the field station of Institute of Cotton Research (CAAS), Anyang, China. Cotton fibers at 0, 1, 3, 5, 10, 15, 20, 25 days to post-anthesis (DPA), and root, stem, and leaves were collected to extract RNA with three biological replicates. All samples were kept in liquid nitrogen for transportation and stored at − 80 °C. Total RNA was extracted using the RNAprep Pure Plant Plus Kit (TIAN GEN). Electrophoresis and spectrophotometric detection were adopted to detect the quality and quantity of the nucleic acids. Then, using the RNA as the template, every sample contained 1 μg RNA. The cDNA was then reverse-transcribed using a First Strand cDNA Synthesis Kit (TAKARA).

Sequence retrieval and phylogenetic analysis

G. hirsutum genome sequence was obtained from the CottonGen (https://www.cottongen.org). To identify *tubulin* genes in the genome of cotton, *Arabidopsis*, flax, and rice *tubulin* gene families were identified through a genomewide sequence and downloaded from Phytozome (https://phytozome.jgi.doe.gov/pz/portal.html) (Swarbreck et al. 2008; Pydiura et al. 2019). BLASTP with default parameters was used to identify the tubulin proteins in cotton with *Arabidopsis* and flax tubulin sequences as the queries based on homology search. The selected cotton tubulin proteins were used for further identification by searching the cotton database again. These identified sequence domains were performed to make clear whether all protein sequences contain tubulin-conserved domains using InterProScan (http://www.ebi.ac.uk/Tools/pfa/iprscan). Subsequently, HMMER software with default parameters and conserved tubulin domains was used to search for the above sequences. Using the same method, we obtained other three predicted cotton *tubulin* genes from *G. barbadense*, *G. arboreum,* and *G. raimondii*. Multiple sequence alignment of all identified tubulin proteins was performed using ClustalX with default parameters (Thompson et al. 1997). Phylogenetic trees were constructed using bootstrap Neighbor-Joining algorithm with bootstrap analysis of 1 000 replicates in MEGA7 v7.0.26 (Kumar et al. 2016).

Gene structure, chromosomal mapping and collinearity analysis

The Gene Structure Display Server Program (http://gsds.cbi.pku.edu.cn/) was employed to derive exon-intron structure representations based on basic coding information as described above (Ali et al. 2019). All motifs were identified by MEME software (http://meme-suite.org/). Relational gene chromosomal position information for all *G. hirsutum tubulin* sequences was obtained from annotation files downloaded from the CottonGen website and visualized by tool CIRCOS to illustrate the chromosomal distribution. CIRCOS was used to draw the distribution

(Zou et al. 2013). The collinearity pairs of the *tubulin* family were mapped adopting CIRCOS software (Krzywinski et al. 2009).

Gene expression analysis

The RNA-seq data of five distinct tissues from two upland cotton varieties J02508 and ZRI015 were already uploaded to the NCBI Gene Expression repository (PRJNA634606). The reference genome sequences for *G. hirsutum* were downloaded (Yang et al. 2019). The FPKM values of *tubulin* genes were calculated using the Cufflinks program. The gene expression data (FPKM) were divided by the mean of all values, then were normalized with the $\log_2$ (FPKM+ 1) method to calculate the expression levels. Gene expression patterns between different fiber developments were visualized with heat maps using the TBtools software package (Chen et al. 2020).

The gene-specific primers used for qRT-PCR were designed with a primer database (http://biodb.swu.edu.cn/qprimerdb). All primers are listed in Table S1. We used the *GhUBQ* (*GhA10G005800*) gene as an internal control, and a total volume of 20 μL that contained 0.4 μL of each primer (10 μmol·L^{-1}), 2 μL cDNA, 10 μL SYBR Premix Ex Taq (2×), and ddH_2O to make up the volume used to perform qRT-PCR with three biological and technological replicates on ABI 7500 fast real-time PCR system using SYBR Premix Ex Taq (TAKARA). The gene relative expression levels were calculated using the $2^{-\triangle\triangle C}{}_T$ method.

Result

Phylogenetic analysis of the tubulin protein family

To investigate the evolutionary relationships of *tubulin* genes, the tubulin protein sequences from *O. sativa*, *Arabidopsis*, *L. usitatissimum*, *G. arboreum*, *G. barbadense*, *G. raimondii*, and *G. hirsutum* were used to

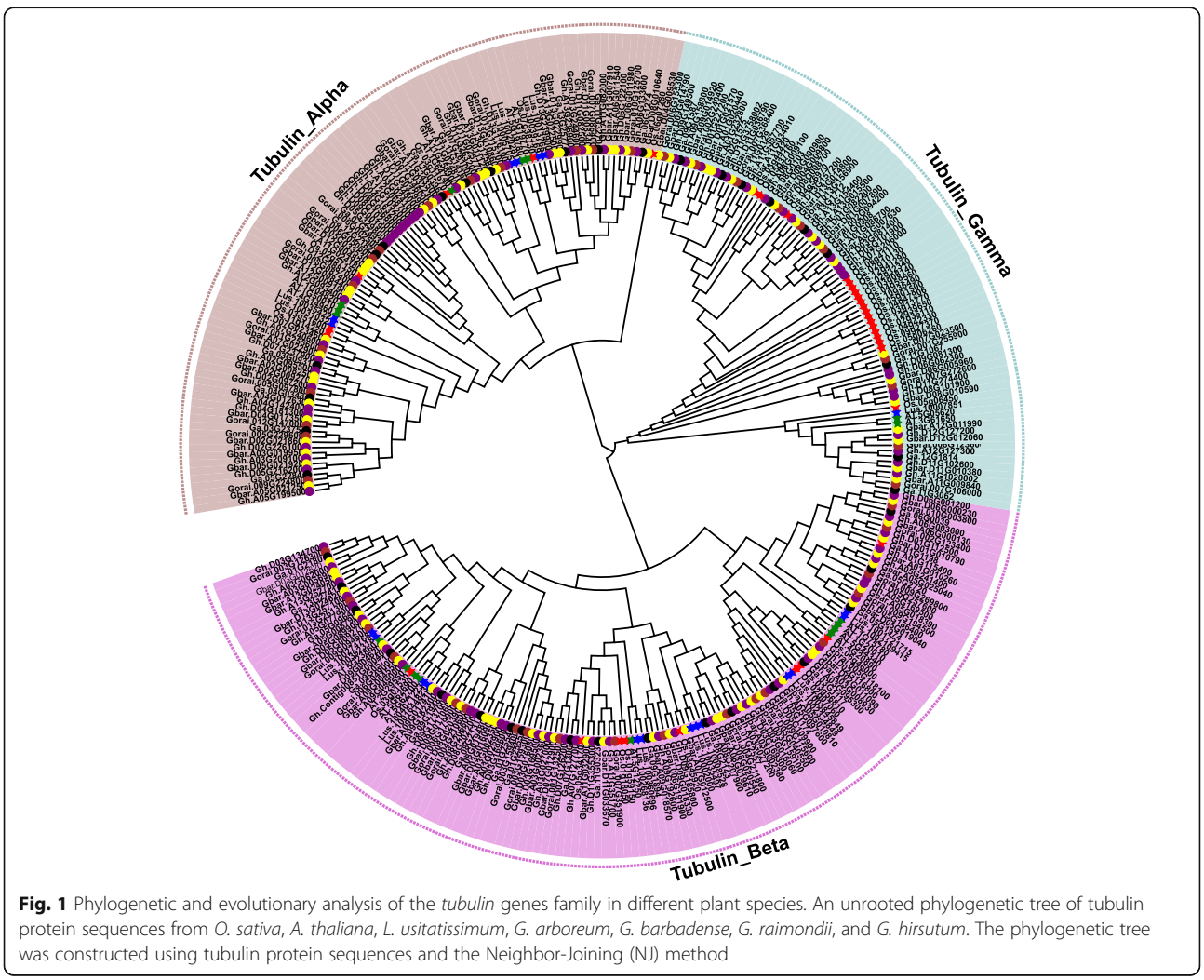

Fig. 1 Phylogenetic and evolutionary analysis of the *tubulin* genes family in different plant species. An unrooted phylogenetic tree of tubulin protein sequences from *O. sativa*, *A. thaliana*, *L. usitatissimum*, *G. arboreum*, *G. barbadense*, *G. raimondii*, and *G. hirsutum*. The phylogenetic tree was constructed using tubulin protein sequences and the Neighbor-Joining (NJ) method

generate an unrooted phylogenetic tree using various sequence alignments (Fig. 1). The tubulin proteins were mainly classified into three groups, viz., α-, β-, and γ-tubulins, which revealed similar results to previous studies concerning different plants (Jayaswal et al. 2019). Most of the orthologous genes between the allotetraploid and the corresponding diploid cotton were clustered into the same clade based on phylogenetic analysis with tubulin protein sequences, which indicated that the *tubulin* genes had been differentiated into three different subfamilies. In each group, most tubulin proteins from the four cotton species were relatively farther than tubulin proteins from *O. sativa*, *Arabidopsis*, *L. usitatissimum*, but a few had a particular case, including some genes in *O. sativa*, which were closely related to cotton species. γ-tubulins were abundant in *O. sativa*, which may play a vital role in the development of rice. However, γ-tubulins were scarce in *Arabidopsis,* and only two genes, viz., *AT.5G05620* and *AT.3G61650* were from this group. Interestingly, the α- and β-*tubulins* subfamilies are expended, but the γ-*tubulin* subfamily is relatively minor in cotton. The above results depicted that *tubulin* genes are either lost or expanded during evolution, according to plant growth needs.

Gene structure and conserved motifs analysis

Exon-intron distribution is generally associated with biological functions and is helpful to understand the evolutionary relationship among different gene families. Therefore, we analyzed the conserved motif and structural characterizations to investigate the phylogenetic relationships among different members of the *tubulin* family of *G. hirsutum* (Fig. 2). Members of α-, β-*tubulin* genes are quite conserved. However, γ-*tubulin* genes were not retained in protein motifs and gene structure analysis. The exon/intron structural variation of the *tubulin* gene family was then compared, yielding a comprehensive illustration of their relative lengths. We found a variable structural pattern of exon-intron in *tubulin* family genes. Closely related genes (sharing the same phylogenetic branch) depicted similar structural patterns; however, a considerable distinction was identified among genes from different branches. The majority of α-*tubulin* subfamily genes were identified with four introns, while the β-*tubulin* subfamily consists of three introns. In contrast, five to eleven introns were found in the γ-*tubulin* subfamily.

Further, we identified ten conserved motifs utilizing MEME program. There are almost all conserved sites in α-, β-*tubulin* subfamilies. However, the genes *Gh.D05G257500*, *Gh.D12G088700* and *Gh.D04G181300* may have lost one or two motifs 4/5/8 in α-, β-tubulin subfamily. Most γ-*tubulin* genes are poorly conserved compared with the members of α-, β-*tubulin* genes. Surprisingly, *Gh.A11G102000*, *Gh.D11G102600*, and *Gh.A12G127300* contain ten conserved motifs in the γ-*tubulin* subfamily. The above results showed that most members from the same subfamily have similar motif features and exon-intron structure, supporting close evolutionary relationships.

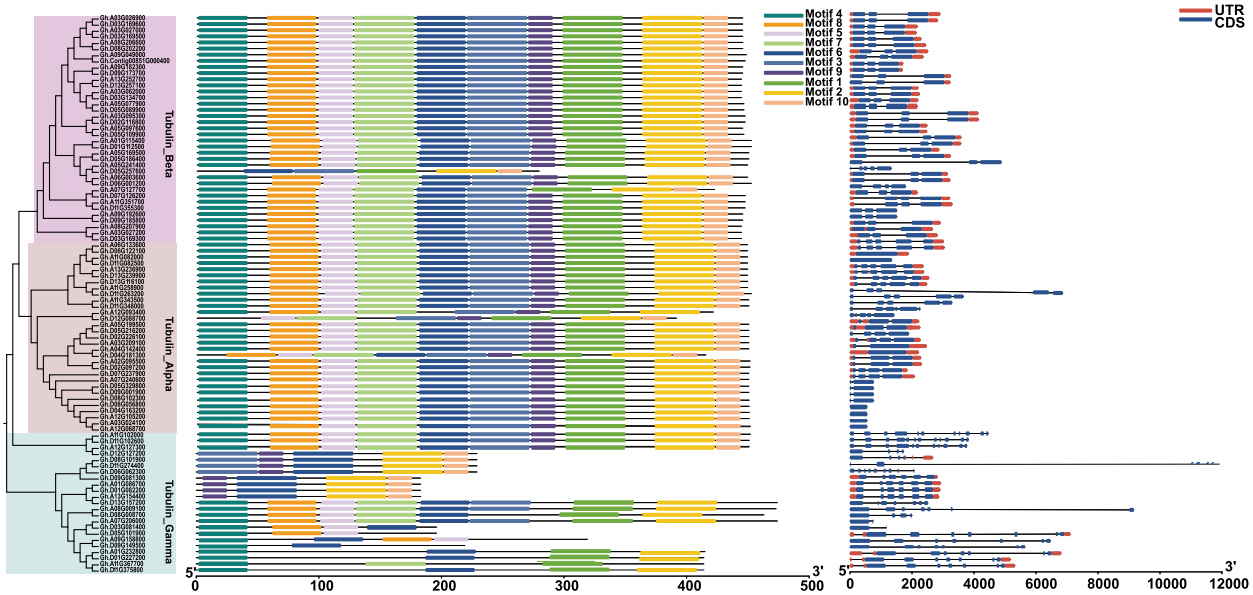

Fig. 2 Phylogenetic relationship, conserved motif, and gene structural characterizations analysis of *tubulin* genes in *G. hirsutum*. A neighbor-joining phylogenetic tree was created using the MEGA7 program, conversed protein motifs, and gene structure analysis

As depicted in Table S2 and Fig. S1, the results showed that the most α-, β-tubulin proteins have conserved domains with Tubulin and Tubulin_C; a theoretical isoelectric point of the most tubulin proteins was weakly acidic; however, when the proteins lose Tubulin_C, the pIs of proteins were alkaline. Conserved site analysis of *tubulin* gene family suggested that some amino acids are very conserved (Fig. S2). The BaCelLo (Pierleoni et al. 2006), EpiLoc (http://epiloc.cs.queensu.ca/) and Plant-mPLoc (Chou and Shen 2010) predicted that the subcellular localization of all tubulin proteins is Cytoplasmic/Chloroplast. TMHMM2.0 (http://www.cbs.dtu.dk/services/TMHMM/) and TOPCONS (Tsirigos et al. 2015) predicted that all tubulin proteins are located outside the membrane. These results indicated that the *tubulin* genes might synthesize the cytoskeleton in the plant cell cytoplasm.

Chromosomal location, gene duplication, and collinearity relationships

We constructed chromosomal distribution maps of *tubulin* genes to understand the genomic distribution and structural variation of the *tubulin* gene family in *Gossypium* species. An uneven distribution pattern of these genes, on sub-genomic (A sub-genome and D sub-genome) and chromosomal level (24 chromosomes) was observed, mainly distributed towards proximate ends of the chromosomes except for At10 and Dt10, which showed nonexistence of *tubulin* genes (Fig. 3). The highest number of *tubulin* genes (7) were present on At03 and Dt05. In contrast, At02, At04, At06, Dt04, Dt07, and Dt12 of *G. hirsutum* included only one or two *tubulin* genes. Based on the uneven distribution pattern on sub-genomes, we speculated that this variation might have resulted from the evolutionary process.

We further analyzed the collinearity relationships for all *tubulin* genes on the chromosomes At and Dt of *G. hirsutum* with other *Gossypium* species. Among 42 *tubulin* genes on the chromosomes At of *G. hirsutum*, 33 had intergenomic homologous genes on chromosomes Dt of *G. hirsutum* (Fig. 4); 40 *tubulin* homologous genes in *G. arboretum* (Fig. S3); all genes on the chromosomes Dt of *G. hirsutum* had intergenomic homologous genes in *G. raimondii* (Fig. S4). Among all the 91 *tubulin* genes of *G. hirsutum*, 77 genes *tubulin* had intergenomic homologous genes in *G. barbadense* (Fig. S5), 91 *tubulin* homologous genes in *G. darwinii* and *G. tomentosum* (Fig. S6/7), respectively. The above results emphasized an independent evolutionary process for *tubulin* gene quantity in At subgroup and Dt subgroup after the formation of allotetraploid, while afterward same directional evolution for different allotetraploid cotton species.

Expression patterns of *tubulin* genes in different upland cotton

Although most *tubulin* superfamily genes have redundant functionality, they have similar functions to promote cell elongation (Segami et al. 2012; Blume et al. 2013). The expression patterns analyses were performed to understand the role of *tubulin* genes during fiber elongation and development. Firstly, RNA-sequencing data were used to detect the expression patterns of 98 *tubulin* genes in root, stem, leaf, ovule, and fiber tissues of *G. hirsutum* (J02–508 and ZRI-015) using a heatmap.

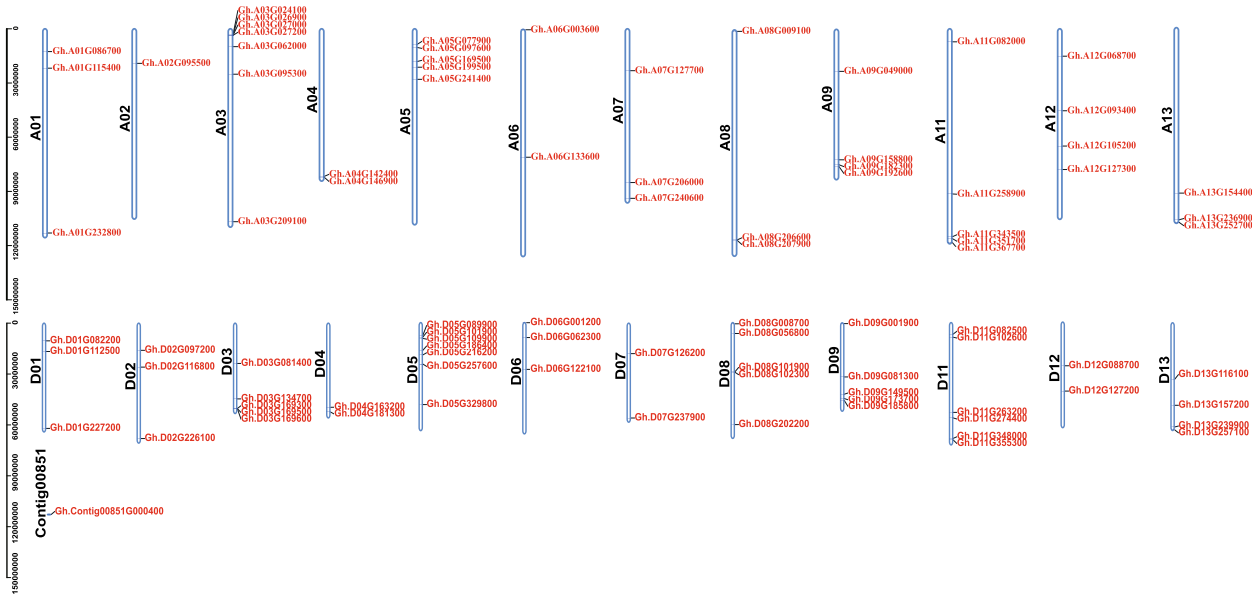

Fig. 3 Chromosomal locations of *tubulin* genes in *G. hirsutum*

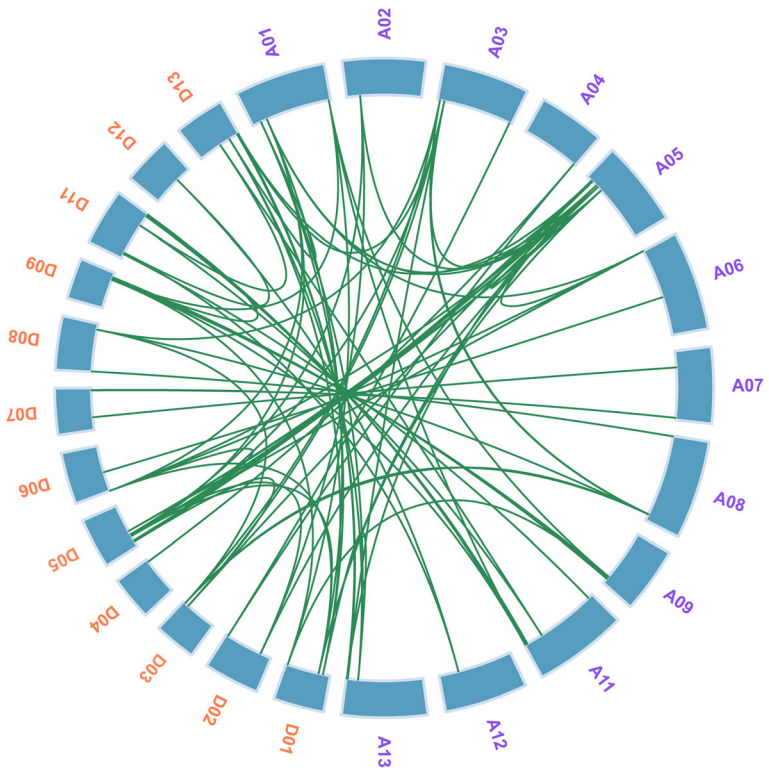

Fig. 4 The chromosome distribution and the synteny of *tubulin* genes in *G. hirsutum*

The results showed that most α-, β-*tubulin* genes had higher expression levels relative to γ-*tubulin* genes in all tissues, and the expression levels of most α-, β-*tubulin* gene subfamilies were very obvious tissue specificity of fiber development stages (Fig. 5). Secondly, the expression levels of *tubulin* genes in the fiber development stage had higher levels than other tissues, e.g. *Gh.A08G206600*, *Gh.D08G202200*, *Gh.A11G351700*, *Gh.D11G355300*, *Gh.A08G207900*, *Gh.A03G027200*, *Gh.D03G169300*, *Gh.A11G082000*, *Gh.D11G082500*, *Gh.A13G236900*, *Gh.A11G258900*, *Gh.A05G199500*, *Gh.D05G216200*, *Gh.D02G226100*, *Gh.A03G209100*, *Gh.A04G142400*, *Gh.D04G181300*, *Gh.A02G095500* and *Gh.D02G097200*, which may contribute to fiber development. Among them, only *Gh.A03G027200*, *Gh.D03G169300*, and *Gh.A11G258900* had differential expression patterns at distinct stages of fiber development in the two varieties, and they were significantly different at 15 DPA, which is a critical time for fiber length and strength in J02–508 compared with ZRI-015. The above results indicated the three genes might inhibit fiber length and strength.

The expression levels of *Gh.A03G027200*, *Gh.D03G169300*, and *Gh.A11G258900* in different fiber development stages for J02508 and ZRI015 were detected using qRT-PCR. As shown in Fig. 6, the relative expression data revealed that the expression levels of *Gh.A03G027200* and *Gh.D03G169300* were higher from 10 to 25 DPA in J02508 and ZRI015, and the expression levels of *Gh.A11G258900* from 3 to 15 DPA in ZRI015 was twice as much as that of J02508.

Discussion

Cotton fibers are single-celled trichomes, which are the essential textile raw material. The tubulin proteins belong to microtubule proteins, which play an important role in synthesizing the cytoskeleton (He et al. 2008). Microtubules of all eukaryotic cells are formed by α- and β-tubulin heterodimers (Schwarzerová et al. 2019). Microtubules are the core element of the cytoskeleton and act a pivotal function in cellular migration, mitosis, mechanical stress, cell polarity, intracellular transport, cell division, and cell morphogenesis (He et al. 2008; Nieuwenhuis and Brummelkamp 2019; Meiring et al. 2020). Based on the genome-wide characterization of the *tubulin* gene family, this study determined 40 *tubulin* genes in A genome, 43 *tubulin* genes in D genome, 84 *tubulin* genes in AD_2, and 98 *tubulin* genes in AD_1 genome. The results showed that the loss of *tubulin* genes did not happen in the allotetraploid *G. hirsutum* and *G. barbadense*, and some *tubulin* genes had been extended in *G. hirsutum*. The number of genes in the allotetraploid cotton is twice as high as that of diploid cotton, contrasting with high rates of gene loss in

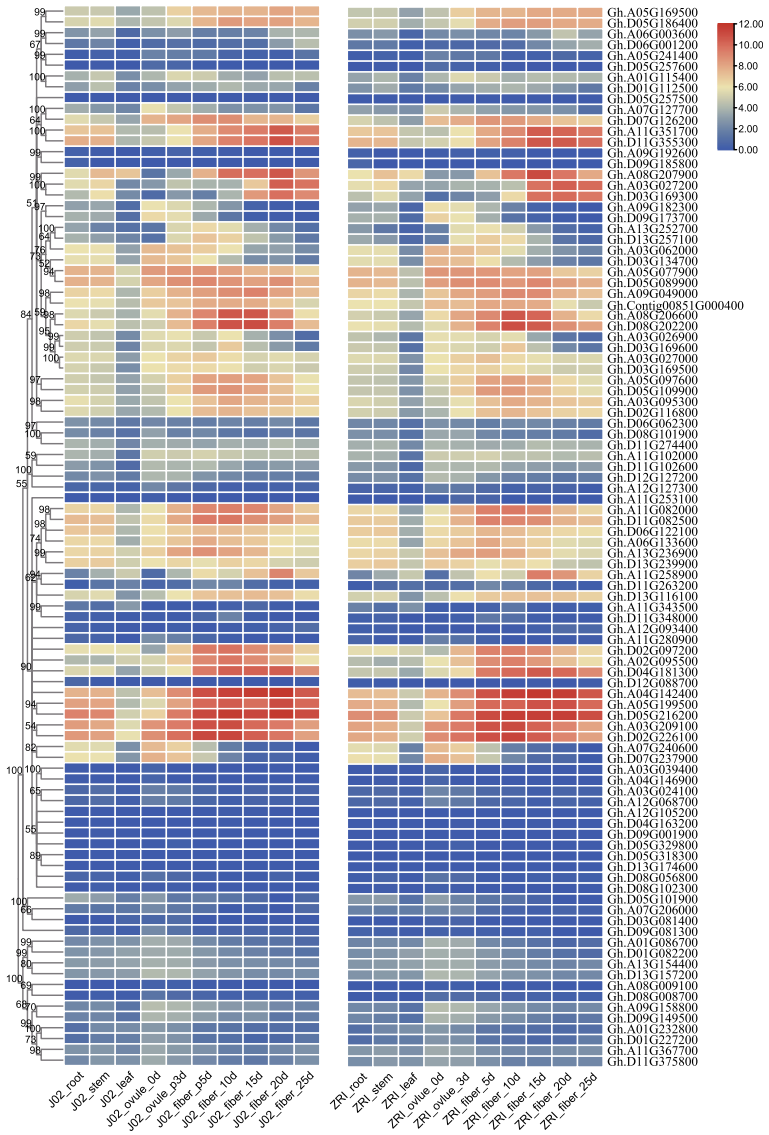

Fig. 5 Expression analysis of *tubulin* family genes in different tissues. A neighbor-joining phylogenetic tree was created using the MEGA7 program and expression level in different tissues of J02–505 and ZRI-015

the allotetraploid plants (Zhang et al. 2015). Based on the previous report about *tubulin* families in different plants (Pydiura et al. 2019), *tubulin* genes had been classified into 3 subfamilies, viz., α-, β- and γ-*tubulins*, our results were consistent with this report (Pydiura et al. 2019). The previous reports suggested that the structure and function of α-, β-*tubulins* were quite similar but the γ-*tubulins* had significant differences (Pydiura et al. 2019; Rao et al. 2016). In flax, expression analysis of *tubulin* genes showed that specific β-*tubulin* members could uphold various microtubule functions such as cell elongation, pollen tube development, and cell wall thickening (Gavazzi et al. 2017; Rao et al. 2016). The amino acid sequences of eukaryotic tubulins are well conserved but their function still display considerable differences (Hotta et al. 2016). The difference in the number of conserved motifs and exons of genes showed that the functional diversity of *tubulin* might closely related to evolution. Furthermore, we identified *tubulin* gene family members that play critical roles in fiber development.

To date, few *tubulin* genes have been functionally characterized in plants, especially in fiber-rich cotton. Combining the transcriptome expression of *tubulin* genes in J02508 and ZRI015, we find that some α-, β-*tubulins* were explicitly expressed in fiber development stages, while the γ-*tubulin* genes showed low expression levels in all tissues. Previous researches showed that some *TUB* genes were highly expressed at 10-DPA in cotton (Li et al. 2002; He

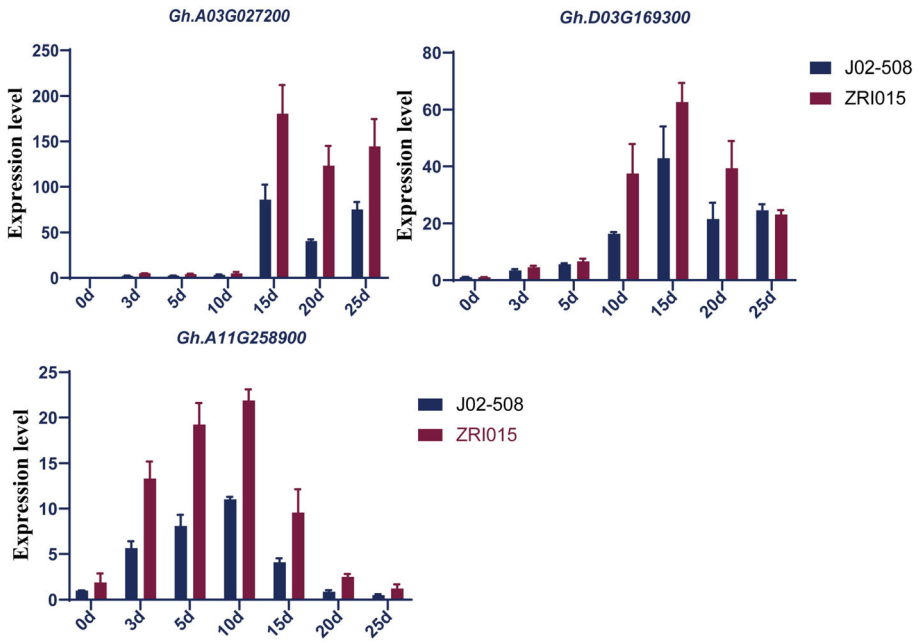

Fig. 6 RT-PCR analysis of expression levels of three key *tubulin* genes in different stages of ovules (0d, 3d) and fibers(5d, 10d, 15d, 20d, 25d)

et al. 2008). Some *α-tubulins* had a specific expression model for developing cotton fibers (Whittaker and Triplett 1999). Interestingly, *Gh.A03G027200* and *Gh.D03G169300* (both belong to β-t*ubulins*), *Gh.A11G258900* (belongs to α-*tubulins*) had significant variable expression profiles during fiber development stages in J02508 and ZRI015. Significant differential expressions were observed at 15-DPA, a critical stage for fiber development (Liu et al. 2006). We further speculated that the expression of these genes might inhibit fiber length and strength during fiber development. Both *Gh.A03G027200* and *Gh.D03G169300* are homologous genes located in At and Dt of *G. hirsutum,* respectively, which showed that they perform the same function together. However, the expression level of the homologous gene of *Gh.A11G258900* was very low in all tissues of J02508 and ZRI015.

Conclusion

Tubulin genes family plays a key role in cotton fiber development. Therefore, following a comprehensive approach including the phylogenetic tree, chromosomal location, collinearity, gene structure, and expression profile, genome-wide characterization of the *tubulin* gene family were performed. The *tubulin* family genes were classified into three clades in the phylogenetic tree. The α-, β-*tubulin* genes are highly conserved; however, γ-*tubulin* genes are less conservative among cotton and other plant species. The high expression levels of some α- and β-*tubulin* genes emphasized that the *tubulin* family plays a significant role in the cotton fiber development stage. The *Gh.A03G027200*, *Gh.D03G169300,* and *Gh.A11G258900* had significant variation at a critical time for fiber length and strength in J02–508 compared with ZRI-015, indicating that three genes may inhibit fiber length and strength during fiber development. Our study's results built the foundation for excavating important functional genes and further studying the fiber length and strength mechanism of cotton.

Abbreviations

MT: Microtubules; BLASTP: Basic Local Alignment Search Tool for Protein; *FPKM*: Fragments per kilobase million; DPA: Day(s) post-anthesis

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s42397-021-00097-1.

Additional file 1: Table S1. Primers used in qRT-PCR detection of three key *Tubulin* genes from *G. hirsutum*. **Table S2.** Basic characteristic of tubulin family genes in cotton genome.

Additional file 2: Fig. S1. Conserved domain analysis of *tubulin* gene family.

Additional file 3: Fig. S2. Conserved site analysis of *tubulin* gene family.

Additional file 4: Fig. S3. Collinearity of *tubulin* genes in *Goppypium arboretum* and *Goppysium hisutum* species.

Additional file 5: Fig. S4. Collinearity of *tubulin* genes in *Goppypium raimondii* and *Goppysium hisutum* species.

Additional file 6: Fig. S5. Collinearity of *tubulin* genes in *Goppysium hisutum* and *Goppysium bartadense* species.

Additional file 8: Fig. S7. Collinearity of *tubulin* genes in *Goppysium hisutum* and *Goppysium darwinii* species.

Additional file 7: Fig. S6. Collinearity of *tubulin* genes in *Goppysium hisutum* and *Goppysium tomentosum* species.

Acknowledgments
Nazir MF edit the language of this paper.

Authors' contributions
Chen BJ, Du XM, and He SP conceived and designed the experiments; Zhao JJ, Fu GY, Pei XX, Pan ZE, and Li HG performed the experiments and collected the data; Chen BJ obtained funding and Chen BJ, Ahmed H and Zhao JJ contributed reagents/materials/analysis tools; Chen BJ, Zhao JJ, Du XM, and He SP revised the paper. All authors read and approved the final manuscript.

Funding
This work was supported by grants from the Foundation for Innovative Research Groups of the National Natural Science Foundation of China (Grant No. 31621005).

Availability of data and materials
Not applicable.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Received: 1 December 2020 Accepted: 23 June 2021
Published online: 13 July 2021

References

Ali F, Qanmber G, Li YH, et al. Genome-wide identification of *Gossypium INDETERMINATE DOMAIN* genes and their expression profiles in ovule development and abiotic stress responses. J Cotton Res. 2019;2(1):3. https://doi.org/10.1186/s42397-019-0021-6.

Blume YB, Krasylenko YA, Demchuk OM, Yemets AI. *Tubulin* tyrosine nitration regulates microtubule organization in plant cells. Front Plant Sci. 2013;4:530. https://doi.org/10.3389/fpls.2013.00530.

Chen CJ, Chen H, Zhang Y, et al. TBtools - an integrative toolkit developed for interactive analyses of big biological data. Mol Plant. 2020;13(8):1194–202. https://doi.org/10.1101/289660.

Chou KC, Shen HB. Plant-mPLoc: a top-down strategy to augment the power for predicting plant protein subcellular localization. PLoS One. 2010;5(6):e11335. https://doi.org/10.1371/journal.pone.0011335.

Findeisen P, Mühlhausen S, Dempewolf S, et al. Six subgroups and extensive recent duplications characterize the evolution of the eukaryotic tubulin protein family. Genome Biol Evol. 2014;6(9):2274–88. https://doi.org/10.1093/gbe/evu187.

Gao P, Zhao PM, Wang J, et al. Identification of genes preferentially expressed in cotton fibers: a possible role of calcium signaling in cotton fiber elongation. Plant Sci. 2007;173(1):61–9. https://doi.org/10.1016/j.plantsci.2007.04.008.

Gao ZY, Sun WJ, Wang J, et al. *GhbHLH18* negatively regulates fiber strength and length by enhancing lignin biosynthesis in cotton fibers. Plant Sci. 2019;286:7–16. https://doi.org/10.1016/j.plantsci.2019.05.020.

Gavazzi F, Pigna G, Braglia L, et al. Evolutionary characterization and transcript profiling of β-*tubulin* genes in flax (*Linum usitatissimum* L.) during plant development. BMC Plant Biol. 2017;17(1):237. https://doi.org/10.1186/s12870-017-1186-0.

He XC, Qin YM, Xu Y, et al. Molecular cloning, expression profiling, and yeast complementation of 19 β-*tubulin* cDNAs from developing cotton ovules. J Exp Bot. 2008;59(10):2687–95. https://doi.org/10.1093/jxb/ern127.

Hotta T, Fujita S, Uchimura S, et al. Affinity purification and characterization of functional tubulin from cell suspension cultures of *Arabidopsis* and tobacco. Plant Physiol. 2016;170(3):1189–205. https://doi.org/10.1104/pp.15.01173.

Jayaswal PK, Shanker A, Singh NK. Phylogeny of *actin* and *tubulin* gene homologs in diverse eukaryotic species. Indian J Genet. 2019;79(1):284–91. https://doi.org/10.31742/IJGPB.79S.1.20.jxb/eru452.

Krzywinski M, Schein J, Birol I, et al. Circos: an information aesthetic for comparative genomics. Genome Res. 2009;19(9):1639–45. https://doi.org/10.1101/gr.092759.109.

Kumar S, Stecher G, Tamura K. MEGA7: molecular evolutionary genetics analysis. Mol Biol Evol. 2016;33(7):1870–4. https://doi.org/10.1093/molbev/msw054.

Li XB, Cai L, Cheng NH, Liu JW. Molecular characterization of the cotton *GhTUB1* gene that is preferentially expressed in fiber. Plant Physiol. 2002;130(2):666–74. https://doi.org/10.1104/pp.005538.

Li XB, Fan XP, Wang XL, et al. The cotton *ACTIN1* gene is functionally expressed in fibers and participates in fiber elongation. Plant Cell. 2005;17:3. https://doi.org/10.1105/tpc.104.029629.

Liu D, Zhang X, Tu L, et al. Isolation by suppression-subtractive hybridization of genes preferentially expressed during early and late fiber development stages in cotton. Mol Biol. 2006;40(5):825–34. https://doi.org/10.1134/S0026893306050086.

Meiring JCM, Shneyer BI, Akhmanova A. Generation and regulation of microtubule network asymmetry to drive cell polarity. Curr Opin Cell Biol. 2020;62:86–95. https://doi.org/10.1016/j.ceb.2019.10.004.

Miao H, Guo R, Chen J, et al. The γ-tubulin complex protein GCP6 is crucial for spindle morphogenesis but not essential for microtubule reorganization in *Arabidopsis*. Proc Natl Acad Sci U S A. 2019;116(52):27115–23. https://doi.org/10.1073/pnas.1912240116.

Mubeen H, Shoaib MW, Raza S. In silico analysis and comparison of alpha *tubulin* gene with other *tubulin* families. JABB. 2016;7(4):1–7. https://doi.org/10.9734/JABB/2016/26641.

Nazir MF, He S, Ahmed H, et al. Genomic insight into biogeographic history, divergence, and adaptive potential of *G. purpurascens*, a forgotten landrace of *G. hirsutum*. bioRxiv. 2020. https://doi.org/10.1101/2020.09.03.280800.

Nieuwenhuis J, Brummelkamp TR. The tubulin detyrosination cycle: function and enzymes. Trends Cell Biol. 2019;29(1):80–92. https://doi.org/10.1016/j.tcb.2018.08.003.

Pierleoni A, Martelli PL, Fariselli P, Casadio R. BaCelLo: a balanced subcellular localization predictor. Bioinformatics. 2006;22(14):e408–16. https://doi.org/10.1093/bioinformatics/btl222.

Pydiura N, Pirko Y, Galinousky D, et al. Genome-wide identification, phylogenetic classification, and exon-intron structure characterization of the *tubulin* and *actin* genes in flax (*Linum usitatissimum*). Cell Biol Int. 2019;43(9):1010–9. https://doi.org/10.1002/cbin.11001.

Rao G, Zeng Y, He C, Zhang J. Characterization and putative post-translational regulation of α- and β-*tubulin* gene families in *Salix arbutifolia*. Sci Rep. 2016;6(1):19258. https://doi.org/10.1038/srep19258.

Ray DL, Johnson JC. Validation of reference genes for gene expression analysis in olive (*Olea europaea*) mesocarp tissue by quantitative real-time RT-PCR. BMC Res Notes. 2014;7(1):304. https://doi.org/10.1186/1756-0500-7-304.

Schwarzerová K, Bellinvia E, Martinek J, et al. Tubulin is actively exported from the nucleus through the Exportin1/CRM1 pathway. Sci Rep. 2019;9:5725. https://doi.org/10.1038/s41598-019-42056-6.

Seaguii RW. A quantitative electron microscopic study of changes in microtubule arrays and wall microfibril orientation during in vitro cotton fiber development. J Cell Sci. 1992;101(3):561–77. https://doi.org/10.1242/jcs.101.3.561.

Segami S, Kono I, Ando T, et al. Small and round seed 5 gene encodes alpha-*tubulin* regulating seed cell elongation in rice. Rice. 2012;5(1):4. https://doi.org/10.1186/1939-8433-5-4.

Su JJ, Ma Q, Hao FS, et al. Multi-locus genome-wide association studies of fiber-quality related traits in Chinese early-maturity upland cotton. Front Plant Sci. 2018;9:1169. https://doi.org/10.3389/fpls.2018.01169.

Swamy PS, Hu H, Pattathil S, et al. Tubulin perturbation leads to unexpected cell wall modifications and affects stomatal behaviour in *Populus*. J Exp Bot. 2015;66(20):6507–18. https://doi.org/10.1093/jxb/erv383.

Swarbreck D, Wilks C, Lamesch P, et al. The *Arabidopsis* information resource (TAIR): gene structure and function annotation. Nucleic Acids Res. 2008;36(Database issue):D1009–14. https://doi.org/10.1093/nar/gkm965.

Thompson JD, Gibson TJ, Plewniak F, et al. The CLUSTAL X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. 1997;25(24):4876–82. https://doi.org/10.1093/nar/25.24.4876.

Tsirigos KD, Peters C, Shu N, et al. The TOPCONS web server for consensus prediction of membrane protein topology and signal peptides. Nucleic Acids Res. 2015;43(W1):W401–7. https://doi.org/10.1093/nar/gkv485.

Wang M, Sun RR, Li C, et al. MicroRNA expression profiles during cotton (*Gossypium hirsutum* L) fiber early development. Sci Rep. 2017;7(1):44454. https://doi.org/10.1038/srep44454.

Whittaker DJ, Triplett BA. Gene-specific changes in *α-Tubulin* transcript accumulation in developing cotton fibers. Plant Physiol. 1999;121(1):181–8. https://doi.org/10.1104/pp.121.1.181.

Yang ZE, Ge XY, Yang ZR, et al. Extensive intraspecific gene order and gene structural variations in upland cotton cultivars. Nat Commun. 2019;10(1):2989. https://doi.org/10.1038/s41467-019-10820-x.

Zhang TZ, Hu Y, Jiang WK, et al. Sequencing of allotetraploid cotton (*Gossypium hirsutum* L. acc. TM-1) provides a resource for fiber improvement. Nat Biotechnol. 2015;33(5):531–7. https://doi.org/10.1038/nbt.3207.

Zhao ZT, Liu HQ, Luo YP, et al. Molecular evolution and functional divergence of *tubulin* superfamily in the fungal tree of life. Sci Rep. 2014;4(1):6746. https://doi.org/10.1038/srep06746.

Zou C, Lu C, Shang H, et al. Genome-wide analysis of the *Sus* gene family in cotton. J Integr Plant Biol. 2013;55(7):643–53. https://doi.org/10.1111/jipb.12068.