Article

Genome-Wide Study of the GATL Gene Family in Gossypium hirsutum L. Reveals that GhGATL Genes Act on Pectin Synthesis to Regulate Plant Growth and Fiber Elongation

Lei Zheng 1,2, Huanhuan Wu 1, Ghulam Qanmber 1, Faiza Ali 1, Lingling Wang 1, Zhao Liu 1, Daoqian Yu 1, Qian Wang 1, Aixia Xu 2 and Zuoren Yang 1,*

1 State Key Laboratory of Cotton Biology, Institute of Cotton Research of the Chinese Academy of Agricultural Sciences, Anyang 455000, China; zhengleiwangyi@126.com (L.Z.); wuhuan621@163.com (H.W.); gqkhan12@gmail.com (G.Q.); faizabiochemist2017@gmail.com (F.A.); wll_198927@126.com (L.W.); liuzhaocaas@163.com (Z.L.); yudaqian88@163.com (D.Y.); 15211177047@163.com (Q.W.)
2 College of Agronomy, Northwest A & F University, Yangling 712100, China; xuaixia2013@163.com
* Correspondence: yangzuoren4012@163.com; Tel.: +86-377-5591-2760

Received: 16 December 2019; Accepted: 3 January 2020; Published: 6 January 2020

Abstract: Pectin is a major polysaccharide component that promotes plant growth and fiber elongation in cotton. In previous studies, the galacturonosyltransferase-like (GATL) gene family has been shown to be involved in pectin synthesis. However, few studies have been performed on cotton GATL genes. Here, a total of 33, 17, and 16 GATL genes were respectively identified in Gossypium hirsutum, Gossypium raimondii, and Gossypium arboreum. In multiple plant species, phylogenetic analysis divided GATL genes into five groups named GATL-a to GATL-e, and the number of groups was found to gradually change over evolution. Whole genome duplication (WGD) and segmental duplication played a significant role in the expansion of the GATL gene family in G. hirsutum. Selection pressure analyses revealed that GATL-a and GATL-b groups underwent a great positive selection pressure during evolution. Moreover, the expression patterns revealed that most of highly expressed GhGATL genes belong to GATL-a and GATL-b groups, which have more segmental duplications and larger positive selection value, suggesting that these genes may play an important role in the evolution of cotton plants. We overexpressed GhGATL2, GhGATL9, GhGATL12, and GhGATL15 in Arabidopsis and silenced the GhGATL15 gene in cotton through a virus induced gene silencing assay (VIGS). The transgenic and VIGS lines showed significant differences in stem diameter, epidermal hair length, stamen length, seed size, and fiber length than the control plant. In addition, the pectin content test proved that the pectin was significantly increased in the transgenic lines and reduced in VIGS plants, demonstrating that GhGATL genes have similar functions and act on the pectin synthesis to regulate plant growth and fiber elongation. In summary, we performed a comprehensive analysis of GhGATL genes in G. hirsutum including evolution, structure and function, in order to better understand GhGATL genes in cotton for further studies.

Keywords: GhGATL gene family; Gossypium hirsutum L.; evolution; pectin; stem diameter; fiber

1. Introduction

The plant cell wall is a complex macromolecular structure mostly composed of polysaccharides. It has important roles in plants, including defending against pathogens, providing structural support to cells, and regulating cell to cell communication [1,2]. Pectin is an essential polysaccharides within the cell wall that makes connections between cellulose, hemicelluloses, and proteoglycans
in the plant [3]. The presence of pectin promotes cell wall deposition and assembly, which can regulate cell expansion [4,5]. Homogalacturonan (HG), a linear homopolymer of 1,4-linked-D-galactopyranosyluronic acid (GalA) [6,7], is the most abundant pectic domain and an important polysaccharide that contributes to the structure and mechanical strength of the plant cell wall [8].

The glycosyltransferases (GT) family of carbohydrate-active enzymes (CAZy) constitute a large family of enzymes that are involved in the biosynthesis of oligosaccharides, polysaccharides, and glycoconjugates [9]. Recently, GT family 8 (GT8) was confirmed to catalyze the transfer of diverse sugars onto lipo-oligosaccharide, protein, inositol, oligosaccharide or polysaccharide acceptors, involved in the synthesis of the cell wall pectic polysaccharide in higher plants [10,11]. Galacturonosyltransferase (GAUT) is the largest family of the GT8, a kind of alpha-1,4-galacturonosyltransferase (GalAT) that can transfer galacturonic acid onto the pectic polysaccharide and synthesizes HG [12]. In Arabidopsis, the Arabidopsis thaliana GAUT1-related (AtGAUT1-related) gene family was divided into four related clades of GAUT and GAUT-like genes by sequence alignment and phylogenetic analysis, and those genes are distinct from the other members of GT8 [10,13].

The function of some GAUT genes has been reported in Arabidopsis. GAUT1 was the first gene verified to be involved in pectin synthesis, and it was confirmed to catalyze the transfer of GalA onto HG and exhibited the characteristic of Golgi-localized type-II membrane protein [12]. The GAUT1 anchor in the Golgi requires association with GAUT7 to form a GAUT1:GAUT7 heteromeric enzyme complex, and the GAUT1:GAUT7 complex shows soluble properties and catalyzes elongation of HG products in vitro [14,15]. Further, mutation in GAUT8 (QUAI) showed a dwarf phenotype and reduced in cell adhesion, and GAUT8 gene had been identified to affect HG and xylan biosynthesis [16–19]. The GAUT12 is abundantly expressed in xylem vessels and interfascicular fiber cells. The irx8/gaut12 mutants showed collapse of xylem vessels and the xylan and HG significantly decreased in the vascular tissues [20,21]. The transcription of GAUT13 and GAUT14 is strongest in pollen tubes, which are essential for pollen tube growth and possibly participate in pectin biosynthesis of the pollen tube wall [22,23]. In addition, Caftall et al. systematically analyzed the composition of somatic cell wall glycosyl residues in 26 homozygous Transfer DNA (T-DNA) insertion mutants for 13 of the 15 Arabidopsis GAUT genes. The results showed that GAUT6, 8, 9, 10, 11, 12, 13, 14 mutants significantly changed the composition of the glycosyl residues in cell walls compared to wild type, suggesting that mutations of these GAUT genes affect the biosynthesis of pectin and xylan [24].

The galacturonosyltransferase-like (GATL) gene family was identified as closely related to the GAUT family based on the conservation of amino acid motifs [10]. In Arabidopsis, AtGATL5 is expressed in all plant tissues and the T-DNA insertion mutant of AtGATL5 caused seed coat epidermal cell defects by affecting mucilage synthesis and cell adhesion [25]. In woody plants, PdGATL1.1 and PdGATL1.2 were shown to function in xylan synthesis, and may be involved in the synthesis of other cell wall polymers [26]. The GATL genes family has been systematically analyzed in Arabidopsis, in terms of gene structure, genomic organization, protein topology, phylogeny, evolutionary history, and expression pattern, and three AtGATL genes have been identified to encode proteins involved in cell wall biosynthesis [27]. In addition, seven members of the GATL gene family have been identified in the rice and their preliminary evolutionary and structural analyses have been carried out [28]. Nevertheless, the detailed biological functions of most of the GATL genes still remain elusive.

Cotton is the most important fiber crop and cotton fiber is an important raw material for the textile industry. In cotton fiber, cell walls not only define plant morphogenesis, but also define the industrially important fiber quality parameters. Pectin can provide structural support in primary walls, and influence secondary wall formation in fibers and woody tissues [29,30]. In previous studies, the cotton GT8 gene was found highly express in the zero days post-anthesis (DPA) ovules and its transcripts were regulated by the GbPDF1 gene, which was confirmed to participate in fiber initiation and early elongation [31]. The cotton GT8 gene was highly identical to Arabidopsis AtGATL1 [32], demonstrating that the GATL family proteins may also be related to fiber growth in cotton. However, a comprehensive analysis of the GATL gene family in cotton remains elusive. Thereby, it was imperative to identify
GATL family members and analyze their functions in cotton. In this study, we identified GATL genes in three cotton species and 12 other plant species in order to determine their phylogenetic relationships. Furthermore, gene structure, chromosome location, segmental duplication, and expression patterns of the GhGATL gene family were analyzed. Moreover, for functional analysis we overexpressed the GhGATL genes in Arabidopsis, and silenced them by virus induced gene silencing (VIGS) in cotton.

2. Materials and Methods

2.1. Identification of GATL Genes

The genome sequences of Gossypium hirsutum (NAU, v1.1 and HAU, v1.1), Gossypium raimondii (JGI, v1.0), and Gossypium arboreum (BJI, v1.0) were downloaded from COTTONGEN (www.cottongen.org). The amino acid sequences of GATLs from Arabidopsis thaliana were acquired from The Arabidopsis Information Resource, version 10 (TAIR 10) (http://www.arabidopsis.org). The genome sequences of grape (Vitis vinifera Genoscope v2.1), poplar (Populus trichocarpa v3.1), cacao (Theobroma cacao v1.1), peach (Prunus persica v2.1), maize (Zea mays v4), apple (Malus domestica v1.1), rice (Oryza sativa v7.0), sorghum (Sorghum bicolor v3.1), soybean (Glycine max v1.1), and two types of moss (Sphagnum fallax v3.3, Physcomitrella patens v3.3) were obtained from JGI (https://phytozome.jgi.doe.gov). We downloaded the Hidden Markov Model (HMM) of the Glyco_transf_8 (PF01501) domain from Pfam (http://pfam.xfam.org) and used it to build a new HMM with Arabidopsis GATL protein sequences as template with the hmmbuild program (http://hmmer.org). Then we used the new HMM of GATL as a query to search the G. hirsutum protein database for candidate sequences, employing the hmmsearch program (http://hmmer.org). The GATL gene families in G. arboreum, G. raimondii, and other species were analyzed as described above. The blastp program was also used to search GATL genes and compared with the result of hmmsearch.

2.2. Conserved Sequence and Phylogenetic Analysis

The ClustalW program was used for multiple-sequence alignments (build-in MEGA 7.0) [33]. First, we aligned the full length GATL protein sequences of all 15 plant species, and the multiple sequence alignment results were used to construct a phylogenetic tree by neighbor-joining (NJ). The best substitution model was tested with the method of maximum likelihood (ML) (build-in MEGA 7.0). The minimum-evolution method (ME) was also used to construct a phylogenetic tree for validating the NJ tree. Methods and parameters were used as in the Jones–Taylor–Thornton (JTT) model, gamma distributed rates (G) and gamma parameter 1. The bootstrap method was used with 1000 replicates.

2.3. Chromosomal Location and Synteny Analysis

The GhGATL gene loci were extracted from the annotated gff3 file using a Perl script, and their locations were displayed on the chromosome using MapChart [34]. For synteny analysis, Basic Local Alignment Search Tool (BLAST) was used to align the entire protein sequences of G. hirsutum with each other by an e-value of $1 \times 10^{-5}$. The MCSCAN software was used to analyze the results of BLASTP, identify collinearity blocks across the whole genome and classify the duplicate type of GhGATL genes [35]. The collinearity pairs belonging to the GhGATL gene family were extracted and used to draw a synteny map by CIRCOS software [36].

2.4. Gene Structure Analysis and Protein Motif Analysis

The Clustal W program was used for protein sequences of G. hirsutum, and NJ was used to construct the phylogenetic tree with the method and the parameters as described above. The conserved motifs of the GhGATL gene family were determined by the online program MEME (http://meme-suite.org) [37] and displayed by TBtools [38]. The online tool GSDS 2.0 was used to display the exon positions acquired from the gff3 file using a Perl script [39].
2.5. Selective Pressure Analysis

The Clustal ×2.0 was used to align amino acid sequences from GATL homologous genes and the results were converted to PAML format using the EasyCodeML convertor program [40]. The MEGA 7.0 was used to build the tree file from the alignment result and formed a Newick format file. The selective pressure was estimated using the EasyCodeML. A branch model was used in this current study, the free-ratio model and two-ratio model were used to determine the ratio of nonsynonymous to synonymous substitution rates (ω) among branches of the tree. The adaptability of these two models was assessed by likelihood ratio test (LRT).

2.6. Transcriptome Data Analysis and Gene Expression Heatmap

The raw RNA-Seq data of G. hirsutum TM-1 were downloaded from the National Center for Biotechnology Information (NCBI) Gene Expression repository under the accession number PRJNA248163 (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA248163/). TopHat and cufflinks were used to map reads and analyze gene expression levels, FPKM (fragments per kilobase million values) were used to normalize gene expression levels. The expression pattern of GhGATLs were visualized using MeV_4.9.0 software (open source genomic analysis software; www.tm4.org).

2.7. Plasmid Construction and Plant Transformation

To generate over expression plant lines, the coding regions of four GhGATL genes were amplified from complementary DNA (cDNA) of the CRI24 accession obtained from the Institute of Cotton Research of the Chinese Academy of Agricultural Sciences and inserted into the vector pCambia2300 containing the CAULIFLOWER MOSAIC VIRUS (CaMV) 35S constitutive promoter. The fusion genes were introduced into Arabidopsis wild-type plants (Columbia-0 ecotype) via the floral dip method [41] with Agrobacterium tumefaciens (strain GV3101). Positive lines were selected on 1/2 MS medium plates containing kanamycin (50 mg/L), kept at 4 °C for 3 days in darkness to break seed dormancy and then shifted to a growth incubator at 22 °C under 16 h light and 8 h dark cycle. The positive lines were further verified by using PCR and the same method was used until Transgenic 4 (T4) homozygous generations. Collected phenotypic data of the transgenic and wild type (WT) plants at different developmental stages.

2.8. Pectin Staining

Paraffin sections were made from the stem of Arabidopsis, and the sections were placed on a glass slide. After dewaxing and rehydration, sections were stained with 0.02% ruthenium red for 4 min, then decolorized with water for 30 min at room temperature [42]. Staining was observed under microscope (LEICA DM6B).

2.9. Pectin Content Determination

Pectin content was determined using the pectin content kit from Sino Best Biological Technology Company. The sample and distilled water were mixed at a ratio of 1:5 (mass:volume), fully ground and the supernatant was collected directly after centrifugation (8000x g for 10 min) at 37 °C. The carbazole reagent and standard reagent were preheated at 37 °C, then the supernatant, carbazole reagent, standard reagent and concentrated sulfuric acid were mixed in the following proportions: A1 (blank tube), 100 µL ddH2O, 100 µL carbazole reagent, and 800 µL concentrated sulfuric acid; A2 (standard tube), 100 µL standard reagent, 100 µL carbazole reagent, and 800 µL concentrated sulfuric acid; A3 (control tube), 100 µL supernatant, 100 µL ddH2O, and 800 µL concentrated sulfuric acid; A4 (measuring tube), 100 µL supernatant, 100 µL carbazole reagent, and 800 µL concentrated sulfuric acid. The mixtures were put in a 95 °C water bath for 5 min and detected with a microplate reader. The pectin content was calculated using the following formula:

\[
\text{Pectin content (mg/g)} = \left( \frac{C \times V1 \times (A4 - A3)}{(A2 - A1)} \right) \times W \times \text{dilution multiple},
\]
where C is the concentration of the standard tube (0.05 mg/mL), V1 is sample volume, and W is the fresh weight of the sample (g) [43].

2.10. Virus Induced Gene Silencing Assay

Virus induced gene silencing (VIGS) was used to mute GATL gene function in plants. An approximately 300-bp coding region of GhGATL15 was amplified by using CRI24 cDNA as a template and inserted into the pCLCtVA vector. The fusion GhGATL15 and empty vector (negative control) transferred into Agrobacterium tumefaciens (strain GV3101) and respectively mixed with the pCLCtVB (helper vector) strain in ratio of 1:1 (OD600 = 1.5), injected into cotyledons of CRI24 wild-type plants. The plants first were dark cultured for 24 h, then shifted to a growth environment of 22 °C with a 16 h light/8 h dark cycle. For qRT-PCR analysis, samples were collected from at least six uniformly injected plants. Phenotypic variations in the plants were observed at different developmental stages [44].

3. Results

3.1. Identification of GATL Genes

Ten Arabidopsis GATL protein sequences were obtained from TAIR (http://www.arabidopsis.org) and used to build a new GATL HMM with the HMM of the Glyco_transf_8 (PF01501) domain from Pfam (http://pfam.xfam.org/). Thereafter, we queried the GATL HMM to search for the GATL proteins among the obtained protein database, and 33, 17, 16, 7, 10, 6, and 5 genes were confirmed as GATL genes in G. hirsutum, G. raimondii, G. arboreum, rice, maize, cacao, grape, poplar, peach, sorghum, apple, Sphagnum fallax and Physcomitrella patens, respectively (Table S1). The numbers of GATL genes in our search results were the same as that identified in rice and Arabidopsis in previous studies [27,28].

The number of GATL genes in G. hirsutum was the sum of GATL genes present in G. arboreum (AA) and G. raimondii (DD), consistent with the established fact that G. hirsutum was derived from hybridization of G. arboreum (A2) and G. raimondii (D5) and their subsequent polyploidization [45,46]. In addition, the Dt-subgenome had one more gene (GhGATL2) than the At-subgenome in G. hirsutum, consistent with the fact that G. raimondii had one more gene (GrGATL2) than G. arboreum.

To verify the previous results of GhGATL genes obtained from the G. hirsutum NAU (v1.1) database, the HMM of GATL was used as a query to identify the GhGATL genes from the G. hirsutum HAU (v1.1) database. We found a slight difference between the two versions (NAU and HAU) of G. hirsutum database: in D05 and A05 chromosomes, G. hirsutum NAU had one more GhGATL gene than G. hirsutum HAU, and in D07 chromosome G. hirsutum HAU had one more GhGATL gene than G. hirsutum NAU. We amplified these three GhGATL genes using CRI24 cDNA as a template, and the sequencing results showed that these three GhGATLs were present in the cotton genome (Figure S1). Besides this, there were two scaffolds which contained GhGATL genes that have not been assembled into the chromosome in G. hirsutum NAU; we confirmed that one was the same as the GhGATL gene in the A02 chromosome of G. hirsutum HAU and the other was just a repeat sequence segment in G. hirsutum NAU. Finally, the GhGATL genes were grouped according to the Arabidopsis homologous gene, and sorted the genes in each group based on positions on the chromosome, then given the proposed genes names GhGATL1 to GhGATL17 (Table S2), and the same numbers were given to their orthologs in G. raimondii and G. arboreum (Table S3).

3.2. Phylogenetic Analysis of GATL Genes

To investigate the phylogenetic relationships of GATL genes among three cotton species (G. hirsutum, G. raimondii, and G. arboreum) and the 12 other species, we constructed a NJ tree using MEGA 7.0 [33]. Like the previous phylogenetic analysis of GATL genes in Arabidopsis, GATL genes from all 15 plant species in this study were divided into five groups from GATL-a to GATL-e (Figure 1) [27]. The ME tree was constructed using MEGA 7.0 to evaluate the accuracy of the NJ tree (Figure S2). Both
Genes 2020, 11, x FOR PEER REVIEW 7 of 19

NJ and ME trees showed almost the same topology, indicating the NJ tree could be used for further analysis. Group GATL-d contained the maximum number of GATL genes, group GATL-c contained the minimum number of GATL genes, and we found that group GATL-c contained at least one gene from each dicot plant, indicating that group GATL-c has not undergone expansion and the members of this group may have similar biological functions. Only groups GATL-a and GATL-e contained GATL genes from mosses of Physcomitrella patens and Sphagnum fallax; monocots species such as maize, rice, and sorghum had GATL genes in only three groups (GATL-b, GATL-d, and GATL-e); and other dicotyledons contained GATL genes from four or five groups. These findings suggested that the number of GATL groups may have changed gradually during the evolutionary process.

Figure 1. Phylogenetic tree of GATL genes from 15 species indicating that GATL genes can be divided into five groups. The outer colored circles represent the GATL gene family groups from GATL-a to GATL-e. The prefixes Gh, Ga, Gr, At, Os, Sobic, Zm, Thecc, VIT, Prupe, Potri, MD, Glyma, Pp3c, and Sphfalx, represent Gossypium hirsutum, Gossypium arboreum, Gossypium raimondii, Arabidopsis thaliana, Oryza sativa, Sorghum bicolor, Zea mays, Theobroma cacao, Vitis vinifera Genoscope, Prunus persica, Populus trichocarpa, Malus domestica, Glycine max, Physcomitrella patens, and Sphagnum fallax, respectively. The “At” and “Dt” indicate the A-and D-subgenomes in G. hirsutum respectively. The reliability value of nodes was provided using bootstrapping.
In addition, cotton GATL genes showed a close relationship with cacao GATL genes, as their GATL genes clustered together with each other in different groups; this is consistent with the fact that the cacao genome is the closest relative of cotton sequenced so far. There are seven to twelve GATL genes in most diploid species such as grape, poplar, cacao, maize, sorghum, rice, and apple. Soybean is a diploid crop derived from an ancient tetraploid that has two times the number of GATL genes compared to other diploid species [47]. G. hirsutum has four times the number of GATL genes compared to other diploid species, because G. hirsutum is a typical allotetraploid composed from G. arboresum and G. raimondii [48]. These results showed that the number of GATL genes were stable over evolution, but the genes have different duplications or doubling in each subgroup; whole genome duplication (WGD) is the major impetus of GATL genes expansion in evolution.

3.3. Gene Expansion and Synteny Analysis

G. hirsutum is a typical allotetraploid, which is an ideal material for studying genome polyploidy and its effects [48]. The synten analysis in G. hirsutum was performed to analyze the collinearity relationship of orthologs between the At and Dt subgenomes. The incomplete sequencing in G. hirsutum NAU (v1.1) meant that GhGATL3_At and GhGATL12_Dt were not mapped to a chromosome. Therefore, we localized them in the chromosomes map based on the G. hirsutum HAU (v1.1) gff3 file. The synteny analysis results indicated that most of the GhGATL loci were significantly conserved between the At and Dt subgenomes. A total of 33 GhGATL genes were distributed unevenly among 19 chromosomes and no GhGATL gene was found on chromosomes A04, A08, D08, A09, and D09. Two homologous gene pairs of GhGATL3 (At/Dt) and GhGATL15 (At/Dt) were located on the same two non-homologous chromosomes of A02/D02 and A03/D03, which showed that translocation might occur between two non-homologous chromosomes after allotetraploid formation. GhGATL16_Dt on the D04 chromosomes is homologous with GhGATL16_At on the A05 chromosomes and there was not any other GhGATL gene on the A04 chromosomes, which might result from possible chromosome fragment translocation from the A05 to D04 (Figure 2).

Duplication is the major impetus underlying gene expansion during evolution. Five types of gene duplication may occur in evolution, including singleton, dispersed, tandem, proximal, and segmental duplication. We used MCSCAN to determine the duplicate gene type and collinearity blocks across the whole genome [35]. During our analysis, as GhGATL12_Dt could not be found in G. hirsutum NAU (v1.1) database, the duplication type was not identified. Many members of the GhGATL genes were duplicated in collinearity regions. In particular, chromosome 5 contained four GhGATL genes and an abundant collinearity replication relationship with other chromosomes, suggesting that these genes were active during evolution. GhGATL9_At/Dt did not have segmental duplication, and GhGATL9_At/Dt belonged to the GATL-c group which had the fewest GATL genes based on phylogenetic analysis. Among the GhGATL genes, there were no tandem or proximal duplications (in the nearby chromosomal region, not in the adjacent region) (Figure 3, Table S4).

In evolution, function mutations may occur after genes duplication, some genes may retain original function, lose their functions, or acquire some novel function [49]. In order to determine the significance of these duplicated genes during the long history of evolution, we used EasyCodeML to identify genes based on different selective pressures, including purifying selection, positive selection, and negative selection [40]. The selection pressure was estimated using a branch model test among different branches of the phylogenetic tree (Table 1). As shown in Table 1, the mean \( \omega \) values of GATL-a and GATL-b branches were all 999.0, which were significantly larger than 1.0 and background values, suggesting that the branches of GATL-a and GATL-b underwent major positive selection. The mean \( \omega \) value of GATL-e branches was 2.0, suggesting that it underwent less positive selection. Moreover, the other two branches had the mean \( \omega \) values smaller than 1.0, suggesting that they underwent purifying selection.
The synteny analysis results indicated that most of the *GhGATL* loci were significantly conserved between the At and Dt subgenomes. A total of 33 *GhGATL* genes were distributed unevenly among 19 chromosomes and no *GhGATL* gene was found on chromosomes A04, A08, D08, A09, and D09. Two homologous gene pairs of *GhGATL3 (At/Dt)* and *GhGATL15 (At/Dt)* were located on the same two non-homologous chromosomes of A02/D02 and A03/D03, which showed that translocation might occur between two non-homologous chromosomes after allotetraploid formation. *GhGATL16_Dt* on the D04 chromosomes is homologous with *GhGATL16_At* on the A05 chromosomes and there was not any other *GhGATL* gene on the A04 chromosomes, which might result from possible chromosome fragment translocation from the A05 to D04 (Figure 2).

**Figure 2.** Chromosomal distribution of *GhGATL* genes in *G. hirsutum*. The number represents the median bases number of *GhGATL* genes in the splicing sequence of the chromosome. The red dotted lines link the orthologs genes on the At and Dt subgenomes.
blocks across the whole genome [35]. During our analysis, as GhGATL12_Dt could not be found in G. hirsutum NAU (v1.1) database, the duplication type was not identified. Many members of the GhGATL genes were duplicated in collinearity regions. In particular, chromosome 5 contained four GhGATL genes and an abundant collinearity replication relationship with other chromosomes, suggesting that these genes were active during evolution. GhGATL9_At/Dt did not have segmental duplication, and GhGATL9_At/Dt belonged to the GATL-c group which had the fewest GATL genes based on phylogenetic analysis. Among the GhGATL genes, there were no tandem or proximal duplications (in the nearby chromosomal region, not in the adjacent region) (Figure 3, Table S4).

Figure 3. Synteny analyses of GhGATL genes in G. hirsutum. The orange lines link the orthologous genes from the At and Dt subgenomes. The blue lines link paralogous pairs derived from segmental duplication. The green lines link paralogous pairs derived from segmental duplication in the same chromosomes.

Table 1. Analysis of natural selection patterns using PAML.

| Group   | Model          | LnL            | Estimates of Parameters | LRT     | ω for Branch |
|---------|----------------|----------------|-------------------------|---------|--------------|
|         |                | Background (ω) | Foreground (ω)          | p-Value |              |
| GATL-a  | Two ratio Model 2 | -17,512.215008 | 0.07026 | 999      | 0.046449     |
|         | Model 0        | -17,514.197630 | 0.07048 |           |              |
| GATL-b  | Two ratio Model 2 | -17,511.041741 | 0.06975 | 999      | 0.011994     |
|         | Model 0        | -17,514.197630 | 0.07048 |           |              |
| GATL-c  | Two ratio Model 2 | -17,509.850784 | 0.06975 | 0.57126  | 0.003193     |
|         | Model 0        | -17,514.197630 | 0.07048 |           |              |
| GATL-d  | Two ratio Model 2 | -17,514.197593 | 0.07048 | 1.82286  | 0.993136     |
|         | Model 0        | -17,514.197630 | 0.07048 |           |              |
| GATL-e  | Two ratio Model 2 | -17,511.329004 | 0.06997 | 2.01258  | 0.016494     |
|         | Model 0        | -17,514.197630 | 0.07048 |           |              |
3.4. Gene Structure and Motif Analysis

The GATL gene family was identified as closely related to the GAUT family, but GATL protein sequences had some significant differences from GAUTs. For instance, GATL protein sequences were smaller than GAUTs, an identifiable transmembrane domain that is found in almost all GAUTs was lacking in GATL proteins, and most of GATL genes lacked introns [11,50]. To further study the structure of GhGATL genes in cotton, we analyzed gene structure and conserved domains of GhGATL genes. Most of the GhGATL genes lacked introns except GhGATL1 Dt, GhGATL16 At, and GhGATL16 Dt (Figure 4). The GhGATL16 At and GhGATL16 Dt are homologous genes, and both had an 85 base pair (bp) intron in the N-terminal region. Similarly, GhGATL1 Dt had a 1901bp intron behind the 7 bp exon in the N-terminal region (Figure 4b). In order to better understand the phylogenetic relationships and structure of GhGATL genes, we used MEME to discover the possible motifs within GhGATL gene sequences (Table S5). A total of seven motifs were identified in the Glyco_transf_8 (PF01501) domain of GhGATL genes except GhGATL1 Dt and GhGATL8 At which lacked two and one motifs in the N-terminal region of Glyco_transf_8 domain, respectively (Figure 4c). We found that the missing motif did not affect the phylogenetic relationship of GATL genes.

![Figure 4.](image)

**Figure 4.** Comparison of the gene structures and motif distribution pattern of GhGATL gene in *G. hirsutum*. (a) The neighbor-joining (NJ) tree of *G. hirsutum* GhGATL genes. (b) The position of exons and introns within GhGATL genes. Exons and introns are shown by blue boxes and orange lines, respectively. (c) The distribution pattern of predicted motifs in the GhGATL genes.

3.5. Expression Profiles of GhGATL Gene in Different Tissues under Multiple Stresses

The GhGATL gene expression profiles were investigated in different tissues and under various stresses, because the reference genome of the transcriptome is the *G. hirsutum* NAU (v1.1) genome, the transcription information of GhGATL12 Dt is lacking. The results showed that GhGATL genes were significantly different in each vegetative tissue and reproductive tissue, include roots, stems, leaves, torus, petal, stamen, pistil, and calycle. In particular, GhGATL10 At/Dt and GhGATL11 At/Dt were specifically expressed in stamens, suggesting that these genes may function in the growth and development of stamens (Figure 5a). Cotton fiber regulation is an important part of cotton research, there was no gene specifically expressed in stages of ovule development (−3, −1, 0, 1, 3, 5, 10, 20,
25, and 35 days) or stages of fiber development (5, 10, 20, and 25 DPA). However, GhGATL15_At/Dt expression was found to increase gradually along with fiber growth, suggesting that GhGATL15_At/Dt may contribute to the fiber elongation process. In addition, there were six pairs of GhGATL genes including GhGATL9_At/Dt, GhGATL12_At, GhGATL13_At/Dt, GhGATL14_At/Dt, GhGATL15_At/Dt, and GhGATL16_At/Dt that were highly expressed in almost all tissues, however, with no significant tissue specificity. Among them, the GhGATL9_At/Dt belonged to the GATL-c subgroup, which was the smallest group and contained at least one gene from each dicot species and GhGATL12_At, GhGATL13_At/Dt, GhGATL14_At/Dt, GhGATL15_At/Dt, and GhGATL16_At/Dt belonged to GATL-a and GATL-b groups which had a large positive selection value (Figure 5a). Additionally, most of the GhGATL gene expression patterns did not exhibit significant differences under cold, heat, PEG (polyethylene glycol), and NaCl treatments, indicating that the expression of GhGATL genes was less affected by stress. We only found GhGATL3_At/Dt displayed down-regulated patterns under cold treatments, indicating that GhGATL3_At/Dt might be a negative regulator under exposure to cold stress (Figure 5b).

Figure 5. Expression profiles of GhGATL genes in different tissues (a), and under different stresses including cold, heat, NaCl, and PEG (polyethylene glycol) (b). Levels of gene expression are depicted in different colors on the scale, red represents high expression and blue represents low expression. DPA is an acronym for days post-anthesis.

3.6. Overexpression of the GhGATL Genes in Arabidopsis

To investigate the function of the GhGATL gene family in plants, four genes were selected including GhGATL2, GhGATL9, GhGATL12, and GhGATL15 from GATL-a, GATL-b, GATL-c, and GATL-d groups to generate overexpression lines, respectively. The GhGATL2 was unique in the D subgenome, GhGATL9 belonged to the GATL-c subgroup which contained at least one gene from each dicot plant, GhGATL12, and GhGATL15 were the most highly expressed genes in subgroup GATL-a and GATL-b which had the highest positive selection during evolution. We generated overexpression lines under the control of CAULIFLOWER MOSAIC VIRUS (CaMV) 35S promoter in Arabidopsis (Columbia-0). Two representative overexpression lines were selected in each of the overexpressed
GATL gene lines in the T3 generation and detected gene expression by RT-PCR, the T4 generation was obtained by self-cross. The T4 generation was grown under normal growth conditions and 20 plants were selected from each inbred line to collect phenotypic data. We observed that all transgenic lines of these genes depicted diverse phenotypes including larger stamens, longer epidermal hair, and especially thicker and stronger stem as compared to wild type (WT) (Figure 6, S3).

Figure 6. Phenotypic observation and pectin content detection between overexpressing GhGATL genes plants and wild type. (a) The middle part of the stem between the rosette leaves and the first leaf after 35 days of growth. Bar = 1 mm. (b) Measurement of stem diameter and (c) epidermal hair after 35 days of growth. (d) The cross-section of the stem was stained with ruthenium red after 35 days of growth. Bar = 150 μm. (e) Determination of pectin content in stem by carbazole reagent reaction. Significant differences compared with wild type (WT) (Student’s t test): *** p < 0.001.
The GATL gene family has been reported as closely related to the GAUT gene family and might be involved in plant pectin synthesis. We determined the pectin content in Arabidopsis transgenic lines by staining the stem sections of transgenic lines and wild type with ruthenium red. As in a previous report, the pectin will turn red after staining and the difference in pectin content under different treatments can be directly observed [51]. We observed that the transgenic lines had a significantly deeper staining effect than wild type on the section of the stem (Figure 6d). The open source software image J was used to convert the degree of staining into gray values to quantify the effect of staining, the staining area and total gray value significantly increased in transgenic lines (Table S6). After that, we measured the pectin content of the stem by the pectin content kit. In order to eliminate the effects of different pectin contents in different structures, we removed the stem epidermis and only took the pith to measure the pectin content, and the results were consistent with the staining results (Figure 6a). Therefore, we speculated that GhGATL genes had a similar function and overexpressing GhGATL genes could increase the pectin content in Arabidopsis stems, which is likely to be the reason for the apparent thickening of transgenic Arabidopsis stems.

3.7. Silencing of GhGATL15 in Cotton via VIGS

Fiber growth regulation is an important part of cotton research. In previous reports, the GATL gene was considered to be possibly involved in the fiber growth regulation of cotton. In addition, we found the expression of GhGATL15 gradually increased with the elongation of fiber. To further identify the functional role of GhGATL15 genes, a VIGS assay was performed in cotton and the expression of GhGATL15 was tested by qRT-PCR to evaluate the gene silencing effects. First, two weeks after VIGS when the third leaf was grown, the stem of the third leaf from the control plants (CLCrVA) and GhGATL15 silencing plants (VI-GhGATL15) were tested for GhGATL15 gene expression level and pectin content. The results showed that pectin content was significantly down-regulated with the decrease of GhGATL15 gene expression, indicating that the GhGATL15 gene is related to the production of pectin in cotton (Figure S4). Following this, three two-month-older cotton plants were selected to collect phenotypic data. The VI-GhGATL15 showed shorter plants compared with CLCrVA (Figure 7a) and these results were consistent with the expression of GhGATL15 tested by qRT-PCR (Figure 7b). In addition, the cotton fiber length tended to become slightly shorter (Figure 7c,d) in VI-GhGATL15 plants suggesting that the GhGATL15_At/Dt gene might function in cotton fiber elongation. Moreover, the seed size of VI-GhGATL15 was significantly smaller than control plants, especially the width of the seeds (Figure S5).

Figure 7. Cont.
was the major impetus underlying the expansion of GATL genes from monocots, suggesting it might have an indispensable function in dicots. The GATL-a, GATL2 (Figure 3). Therefore, we hypothesized that the GATL2 (Figure 1), and synteny analysis showed a collinear relationship between the GATL gene family has been studied in model plants such as Arabidopsis. The GATL gene family has been identified as closely related to the GAUT family which has been found to be involved in pectin synthesis [11]. The GATL gene family has been studied in model plants such as Arabidopsis and rice [27,28], but few studies of GATL genes have been performed in cotton. In this study, the evolution, structure, and function of the GhGATL gene family in G. hirsutum were systematically analyzed, in order to better understand their roles in plant growth and development for further studies.

4. Discussion

Pectin is a major polysaccharide component in the plant cell wall [3] and is an important compound that promotes plant growth and fiber elongation in cotton [52]. The GATL gene family was identified due to more GATL gene groups necessitates further study. Moreover, we found that GATL-c was the smallest group, but it contained at least one gene from each dicot species and did not have any GATL genes from monocots, suggesting it might have an indispensable function in dicots. The GATL-a,
GATL-b, and GATL-d groups have undergone abundant expansion compared to GATL-c and GATL-e groups, and selective stress analysis showed strong positive selection pressure in GATL-a and GATL-b groups compared with the other three groups, indicating that the GATL genes from GATL-a and GATL-b groups might have more important functions in the plant during evolution.

4.2. GhGATL Genes Were Highly Conserved during Evolution

In our study, we found that GhGATL genes all have a conserved Glyco_transf_8 (PF01501) domain and most lack introns consistent with previous reports [11]. In the motif analysis, GhGATL1_Dt and GhGATL8_At lacked two and one motifs, respectively (Figure 4C), but the missing motifs only appeared in the N-terminal of the Glyco_transf_8 (PF01501) domain, the main domain was not changed. In addition, we found that the lack of motif did not affect its classification in the evolutionary tree, which also showed that the missing motif did not affect the integrity of the conserved sequence. Although there were significant differences in protein sequences between GATLs and GAUTs, the Glyco_transf_8 domain in the C-terminal domains of both GAUTs and GATLs were relatively conserved. The results of structural analysis showed that the GATL genes were highly conserved during cotton evolution and may have similar functions as GAUT genes, but there was no detailed information on the function of GATL genes in cotton.

4.3. Diverse Expression Patterns of GhGATL Genes in G. hirsutum

Based on gene expression patterns, six pairs of GhGATL genes were highly expressed in most tissues: GhGATL9_At/Dt, GhGATL12_At, GhGATL13_At/Dt, GhGATL14_At/Dt, GhGATL15_At/Dt, and GhGATL16_At/Dt, without significant tissue specificity (Figure 5). In these GhGATL genes, GhGATL9_At/Dt belonged to the GATL-c subgroup, which was the smallest group and contained at least one gene from each dicot species, indicating that the genes in group GATL-c have not undergone expansion but might have stable biological functions in plants. GhGATL12_At, GhGATL13_At/Dt, GhGATL14_At/Dt, GhGATL15_At/Dt, and GhGATL16_At/Dt belonged to GATL-a and GATL-b subgroups, which had the largest positive selection values. As in previous reports, gene duplication had significant effects on stabilizing selection via perturbation of gene expression [54,55]. For these highly expressed GhGATL genes, we found that the gene expression pattern was associated with the results of gene duplication analysis and selective pressure detection, suggesting that these genes may play an important role in the evolution of cotton and affect growth and development.

4.4. GhGATL Genes Have Similar Function and Control Fiber Growth in Cotton

In our study, we respectively selected GhGATL2, GhGATL9, GhGATL12, and GhGATL15 from GATL-a, GATL-b, GATL-c, and GATL-d subgroups, and generated overexpression lines. The transgenic lines showed that GhGATL genes have similar functions in plant growth and the pectin content test also proved that the pectin was all significantly increased in the transgenic lines, the similar functions might be caused by the highly conserved structure of the GhGATL gene family. On the other hand, as the stem of transgenic Arabidopsis became thicker, the epidermal hair became longer than the wild type (Figure 6c). In a previous report, the trichomes of Arabidopsis and cotton fibers were shown to have a similar formation mechanism: the polar elongation of a single epidermal cell [56]. Then, we demonstrated that GhGATL15 regulated cotton fibers through a virus induced gene silencing assay (Figure 7b,d). And a cotton GT8 gene was been reported highly expressed in the 0-DPA ovules and regulated by GbPDF1, which was confirmed to participate in fiber initiation and early elongation [31], the cotton GT8 gene was the GhGATL4_Dt we identified in this study. Therefore, we predicted that the GhGATL genes may be involved in the pathway of regulating plant pectin content, and may play an important role in fiber elongation in cotton (Table S7).
Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4425/11/1/64/s1.

Figure S1: Sequencing and comparing differential GhGATL genes between the two versions of G. hirsutum (NAU, v 1.1) and G. hirsutum (HAU, v 1.1) databases, Figure S2: Phylogenetic tree of GATL genes constructed by MEGA software version 7.0 using minimum evolution (ME) method, Figure S3: Detection of GhGATL genes expression and stamen phenotype in overexpressing GhGATL genes lines and WT, Figure S4: Determination of pectin content between control plant and VI-GhATL15 plants, Table S1: Protein sequences used in this study, Table S2: The GhGATL gene family between G. hirsutum (NAU, version 1.1) and G. hirsutum (HAU, version 1.1) database, Table S3: The GATL genes in G. raimondii and G. arboreum, Table S4: The GhGATL genes duplication types in G. hirsutum, Table S5: The motifs consensus identified by MEME, Table S6: The degree of staining of Arabidopsis stem sections converted into gray values, Table S7: Sequences of primers.

Author Contributions: Conceptualization, L.Z., A.X., and Z.Y.; data curation, L.W., Z.L., and Q.W.; formal analysis, L.Z.; investigation, H.W.; methodology, Z.Y.; software, L.W.; visualization, D.Y.; writing—original draft, L.Z.; writing—review and editing, G.Q. and F.A. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the Major Research Plan of National Natural Science Foundation of China (NO.31690093), National Science and Technology Major Project of China (2016YFD0101006).

Acknowledgments: We would like to thank Peng Huo, Xin Li (Zhengzhou Research Center, Institute of Cotton Research of CAAS, Zhengzhou, China) for technical assistance.

Conflicts of Interest: The authors declare that they have no competing interests.

References

1. Keegstra, K. Plant cell walls. Plant Physiol. 2010, 154, 483–486. [CrossRef] [PubMed]
2. Wilder, B.M.; Albersheim, P. The Structure of Plant Cell Walls. Plant Physiol. 1973, 51, 327–370. [CrossRef] [PubMed]
3. Caffall, K.H.; Mohnen, D. The structure, function, and biosynthesis of plant cell wall pectic polysaccharides. Carbohydr. Res. 2009, 344, 1879–1900. [CrossRef] [PubMed]
4. Tan, L.; Eberhard, S.; Pattathil, S.; Warder, C.; Glushka, J.; Yuan, C.; Hao, Z.; Zhu, X.; Avci, U.; Miller, J.S.; et al. An Arabidopsis cell wall proteoglycan consists of pectin and arabinoxylan covalently linked to an arabinogalactan protein. Plant Cell 2013, 25, 270–287. [CrossRef] [PubMed]
5. Willats, W.G.T.; Mccartney, L.; Mackie, W.; Knox, J.P. Pectin: Cell biology and prospects for functional analysis. Plant Mol. Biol. 2001, 47, 9–27. [CrossRef]
6. Atmodjo, M.A.; Hao, Z.; Mohnen, D. Evolving views of pectin biosynthesis. Annu. Rev. Plant Biol. 2013, 64, 747–779. [CrossRef]
7. Mohnen, D. Pectin structure and biosynthesis. Curr. Opin. Plant Biol. 2008, 11, 266–277. [CrossRef]
8. Lionetti, V.; Francocci, F.; Ferrari, S.; Volpi, C.; Bellincampi, D.; Galletti, R.; D’Ovidio, R.; De Lorenzo, G.; Cervone, F. Engineering the cell wall by reducing de-methyl-esterified homogalacturonan improves saccharification of plant tissues for bioconversion. Proc. Natl. Acad. Sci. USA 2010, 107, 616–621. [CrossRef]
9. Cantarel, B.L.; Coutinho, P.M.; Rancurel, C.; Bernard, T.; Lombard, V.; Henrissat, B. The Carbohydrate-Active EnZymes database (CAZy): An expert resource for Glycogenomics. Nucleic Acids Res. 2009, 37, D233–D238. [CrossRef]
10. Lairson, L.L.; Henrissat, B.; Davies, G.J.; Withers, S.G. Glycosyltransferases: Structures, functions, and mechanisms. Annu. Rev. Biochem. 2008, 77, 521–555. [CrossRef]
11. Yin, Y.; Mohnen, D.; Gelineo-Albersheim, I.; Xu, Y.; Hahn, M.G. Glycosyltransferases of the GT8 Family. Annu. Plant Rev. 2010, 41, 167–211. [CrossRef]
12. Sterling, J.D.; Atmodjo, M.A.; Inwood, S.E.; Kolli, V.K.; Quigley, H.F.; Hahn, M.G.; Debra, M. Functional identification of an Arabidopsis pectin biosynthetic homogalacturonan galacturonosyltransferase. Proc. Natl. Acad. Sci. USA 2006, 103, 5236–5241. [CrossRef] [PubMed]
13. Yonekura-Sakakibara, K.; Hanada, K. An evolutionary view of functional diversity in family 1 glycosyltransferases. Plant J. 2011, 66, 182–193. [CrossRef] [PubMed]
14. Amos, R.A.; Pattathil, S.; Yang, J.Y.; Atmodjo, M.A.; Urbanowicz, B.R.; Moremen, K.W.; Mohnen, D. A two-phase model for the non-processive biosynthesis of homogalacturonan polysaccharides by the GAUT1-GAUT7 complex. *J. Biol. Chem.* 2018, 293, 19047–19063. [CrossRef] [PubMed]

15. Atmodjo, M.A.; Sakuragi, Y.; Zhu, X.; Burrell, A.J.; Mohanty, S.S.; Atwood, J.A.; Orlando, R.; Scheller, H.V.; Mohnen, D. Galacturonosyltransferase (GAUT)1 and GAUT7 are the core of a plant cell wall pectin biosynthetic homogalacturonan:galacturonosyltransferase complex. *Proc. Natl. Acad. Sci. USA* 2011, 108, 20225–20230. [CrossRef]

16. Bouton, S. QUASIMODO1 Encodes a Putative Membrane-Bound Glycosyltransferase Required for Normal Pectin Synthesis and Cell Adhesion in Arabidopsis. *Plant Cell Online* 2002, 14, 2577–2590. [CrossRef]

17. Durand, C.; Vicre-Gibouin, M.; Follet-Gueye, M.L.; Duponchel, L.; Moreau, M.; Lerouge, P.; Driouich, A. The organization pattern of root border-like cells of Arabidopsis is dependent on cell wall homogalacturonan. *Plant Physiol.* 2009, 150, 1411–1421. [CrossRef]

18. Leboeuf, E.; Guillou, F.; Thoiron, S.; Lahaye, M. Biochemical and immunohistochemical analysis of pectic polysaccharides in the cell walls of Arabidopsis mutant QUASIMODO 1 suspension-cultured cells: Implications for cell adhesion. *J. Exp. Bot.* 2005, 56, 3171–3182. [CrossRef]

19. Orflia, C.; Sorensen, S.O.; Harholt, J.; Geshi, N.; Crombie, H.; Truong, H.N.; Reid, J.S.; Knox, J.P.; Scheller, H.V. QUASIMODO1 is expressed in vascular tissue of Arabidopsis thaliana inflorescence stems, and affects homogalacturonan and xylan biosynthesis. *Planta* 2005, 222, 613–622. [CrossRef]

20. Pena, M.J.; Zhong, R.; Zhou, G.K.; Richardson, E.A.; O’Neill, M.A.; Darvill, A.G.; York, W.S.; Ye, Z.H. Arabidopsis irregular xylem8 and irregular xylem9: Implications for the complexity of glucuronoxylan biosynthesis. *Plant Cell* 2007, 19, 549–563. [CrossRef]

21. Persson, S.; Caffall, K.H.; Feshour, G.; Hilley, M.T.; Bauer, S.; Poindexter, P.; Hahn, M.G.; Mohnen, D.; Somerville, C. The Arabidopsis irregular xylem8 mutant is deficient in glucuronoxylan and homogalacturonan, which are essential for secondary cell wall integrity. *Plant Cell* 2007, 19, 237–255. [CrossRef] [PubMed]

22. Mollet, J.C.; Leroux, C.; Dardelle, F.; Lehner, A. Cell Wall Composition, Biosynthesis and Remodeling during Pollen Tube Growth. *Plants (Basel)* 2013, 2, 107–147. [CrossRef] [PubMed]

23. Wang, L.; Wang, W.; Wang, Y.Q.; Liu, Y.Y.; Wang, J.X.; Zhang, X.Q.; Ye, D.; Chen, L.Q. Arabidopsis galacturonosyltransferase (GAUT) 13 and GAUT14 have redundant functions in pollen tube growth. *Mol. Plant* 2013, 6, 1131–1148. [CrossRef] [PubMed]

24. Caffall, K.H.; Pattathil, S.; Phillips, S.E.; Hahn, M.G.; Mohnen, D. Arabidopsis thaliana T-DNA mutants implicate GAUT genes in the biosynthesis of pectin and xylan in cell walls and seed testa. *Mol. Plant* 2009, 2, 1000–1014. [CrossRef]

25. Kong, Y.; Zhou, G.; Abdeen, A.A.; Schafhauser, J.; Richardson, B.; Atmodjo, M.A.; Jung, J.; Wicker, L.; Mohnen, D.; Western, T.; et al. GALACTURONOSYLTRANSFERASE-LIKEs is involved in the production of Arabidopsis seed coat mucilage. *Plant Physiol.* 2013, 163, 1203–1217. [CrossRef] [PubMed]

26. Kong, Y.; Zhou, G.; Auci, U.; Gu, X.; Jones, C.; Yin, Y.; Xu, Y.; Hahn, M.G. Two poplar glycosyltransferase genes, PdGATL1.1 and PdGATL1.2, are functional orthologs to PARVUS/AtGATL1 in Arabidopsis. *Mol. Plant* 2009, 2, 1040–1050. [CrossRef]

27. Kong, Y.; Zhou, G.; Yin, Y.; Xu, Y.; Pattathil, S.; Hahn, M.G. Molecular analysis of a family of Arabidopsis genes related to galacturonosyltransferase-like (GATL) proteins in rice. *Genes Genom.* 2016, 38, 917–929. [CrossRef]

28. Al-Ghazii, Y.; Bourot, S.; Arioli, T.; Dennis, E.S.; Llewellyn, D.J. Transcript profiling during fiber development identifies pathways in secondary metabolism and cell wall structure that may contribute to cotton fiber quality. *Plant Cell Physiol.* 2009, 50, 1364–1381. [CrossRef]

29. Haigler, C.H.; Betancur, L.; Stiffler, M.R.; Tuttle, J.R. Cotton fiber: A powerful single-cell model for cell wall and cellulose research. *Front. Plant Sci.* 2012, 3, 104. [CrossRef]

30. Gou, J.Y.; Wang, L.J.; Chen, S.P.; Hu, W.L.; Chen, X.Y. Gene expression and metabolite profiles of cotton fiber during cell elongation and secondary cell wall synthesis. *Cell Res.* 2007, 17, 422–434. [CrossRef] [PubMed]
33. Kumar, S.; Stecher, G.; Tamura, K. MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. *Mol. Biol. Evol.* 2016, 33, 1870–1874. [CrossRef] [PubMed]

34. Voorrips, R.E. MapChart: Software for the graphical presentation of linkage maps and QTLs. *J. Hered.* 2002, 93, 77–78. [CrossRef]

35. Wang, Y.; Tang, H.; Debarry, J.D.; Tan, X.; Li, J.; Wang, X.; Lee, T.H.; Jin, H.; Marler, B.; Guo, H.; et al. MCSamX: A toolkit for detection and evolutionary analysis of gene synten and collinearity. *Nucleic Acids Res.* 2012, 40, e49. [CrossRef]

36. Krzywinski, M.; Schein, J.I. Circos: An information aesthetic for comparative genomics. *Genome Res.* 2009, 19, 1639–1645. [CrossRef]

37. Bailey, T.L.; Williams, N.; Misleh, C.; Li, W.W. MEME: Discovering and analyzing DNA and protein sequence motifs. *Nucleic Acids Res.* 2006, 34, W369–W373. [CrossRef]

38. Chen, C.; Xia, R.; Chen, H.; He, Y. TBtools, a toolkit for biologists integrating various HTS-data handling tools with a user-friendly interface. *bioRxiv* 2018. [CrossRef]

39. Hu, B.; Jin, J.; Guo, A.Y.; Zhang, H.; Luo, J.; Gao, G. GSDS 2.0: An upgraded gene feature visualization server. *Bioinformatics* 2012, 40, e49. [CrossRef]

40. Gao, F.; Chen, C.; Arab, D.A.; Du, Z.; He, Y.; Ho, S.Y.W. Easycodeml: A visual tool for analysis of selection using codeML. *Ecol. Evol.* 2019, 9, 3891–3898. [CrossRef]

41. Clough, S.J.; Bent, A.F. Floral dip: A simplified method for Agrobacterium-mediated transformation of Arabidopsis thaliana. *Plant J.* 1998, 16, 735–743. [CrossRef] [PubMed]

42. Hanke, D.E.; Northcote, D.H. Molecular visualization of pectin and DNA by ruthenium red. *Biopolymers* 2010, 14, 1–17. [CrossRef]

43. Kyriakidis, N.B.; Psoma, E. Hydrocolloid interferences in the determination of pectin by the carboxazole method. *J. AOAC Int.* 2001, 84, 1947–1949. [PubMed]

44. Tuttle, J.R.; Idris, A.M.; Brown, J.K.; Haigler, C.H.; Robertson, D. Geminivirus-mediated gene silencing from Cotton leaf crumple virus is enhanced by low temperature in cotton. *Plant Physiol.* 2008, 148, 41–50. [CrossRef] [PubMed]

45. Senchina, D.S.; Alvarez, I.; Cronn, R.C.; Liu, B.; Rong, J.; Noyes, R.D.; Paterson, A.H.; Wing, R.A.; Wilkins, T.A.; Wendel, J.F. Rate Variation Among Nuclear Genes and the Age of Polyploidy in Gossypium. *Mol. Biol. Evol.* 2003, 20, 633–643. [CrossRef]

46. Wendel, J.F. New World tetraploid cottons contain Old World cytoplasm. *Proc. Natl. Acad. Sci. USA* 1989, 86, 4132–4136. [CrossRef]

47. Gupta, S.K. Volume 1 Breeding. In *Technological Innovations in Major World Oil Crops*; Springer: New York, NY, USA, 2011.

48. Paterson, A.H.; Wendel, J.F.; Gundlach, H.; Guo, H.; Jenkins, J.; Jin, D.; Llewellyn, D.; Showmaker, K.C.; Shu, S.; Udall, J.; et al. Repeated polyploidization of Gossypium genomes and the evolution of spinnable cotton fibres. *Nature* 2012, 492, 423–427. [CrossRef]

49. Prince, V.E.; Pickett, F.B. Splitting pairs: The diverging fates of duplicated genes. *Nat. Rev. Genet.* 2002, 3, 827–837. [CrossRef]

50. Coutinho, P.M.; Deleury, E.; Davies, G.J.; Henriessat, B. An Evolving Hierarchical Family Classification for Glycosyltransferases. *J. Mol. Biol.* 2003, 328, 307–317. [CrossRef]

51. Qu, L.; Wu, C.; Zhang, F.; Wu, Y.; Fang, C.; Jin, C.; Liu, X.; Luo, J. Rice putative methyltransferase gene OsTSD2 is required for root development involving pectin modification. *J. Exp. Bot.* 2016, 67, 5349–5362. [CrossRef]

52. Liu, Q.; Talbot, M.; Llewellyn, D.J. Pectin methylesterase and pectin remodelling differ in the fibre walls of two gossypium species with very different fibre properties. *PLoS ONE* 2013, 8, e65131. [CrossRef] [PubMed]

53. Fraser, J.A.; Huang, J.C.; Pukkila-Worley, R.; Alspaugh, J.A.; Mitchell, T.G.; Heitman, J. Chromosomal translocation and segmental duplication in Cryptococcus neoformans. *Eukaryot. Cell* 2005, 4, 401–406. [CrossRef] [PubMed]

54. Klipstein, D.J. A role for gene duplication and natural variation of gene expression in the evolution of metabolism. *PLoS ONE* 2008, 3, e1838. [CrossRef] [PubMed]
55. Ohta, T. Evolution by Gene Duplication Revisited: Differentiation of Regulatory Elements Versus Proteins. *Genetica* **2003**, *118*, 209–216. [CrossRef] [PubMed]

56. Kim, H.J.; Triplett, B.A. Cotton Fiber Growth in Planta and in Vitro. Models for Plant Cell Elongation and Cell Wall Biogenesis. *Plant Physiol.* **2001**, *127*, 1361–1366. [CrossRef] [PubMed]

© 2020 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/).