African Journal of Biotechnology Vol. 8 (19), pp. 4755-4764, 5 October, 2009
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2009 Academic Journals

Full Length Research Paper

Isolation and molecular characterization of a novel homogalacturonan galacturonosyl- transferase gene (*GbGAUT1*) from *Gossypium barbadense*

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Accepted 8 May, 2009

A novel gene, designated as *GbGAUT1* (*Gossypium barbadense* homogalacturonan galacturonosyl-transferase gene 1), was successfully cloned from *G. barbadense* (GenBank accession No. EF552432) by bioinformatics methods, RT-PCR and RACE. The open reading frame (ORF) was 1797 bp long and putatively encoded a polypeptide of 598 amino acids, with a predicted molecular mass of 69 kDa. *GbGAUT1* could be considered as a homogalacturonan (HG) galacturonosyltransferase protein since the conserved GAUT1-related supperfamily motif had been found and it was clustered into GAUT-A group in the phylogenetic tree. Semi quantitative RT-PCR showed that *GbGAUT1* preferentially expressed in the fiber secondary cell wall thickening, especially in the fibers of 35 day post anthesis (DPA). These results suggested that the novel cotton *GbGAUT1* might play an important role in fiber development.

Key words: *Gossypium barbadense*, fiber development, cDNA cloning, pectin polysaccharide, homogalacturonan galacturonosyltransferase.

INTRODUCTION

Cell walls of dicotyledonous plants were mainly composed of cellulose, hemicellulose and pectin etc. When viewed by electron microscopy, cell wall appears to be a network of extended polysaccharides with high molecular weights. In higher plants, the visually dominate structural features were cellulose microfibrils with diameters of ~3 nm, which appeared to wrap around the cell and were cross-linked by single-chain polysaccharides such as pectins (Sterling et al., 2006). Pectins are structurally complex plant cell wall polysaccharides comprised of domains of HG, rhamnogalacturonanI (RG-I), and rhamnogalacturonanII (RG-II) (Willats et al., 2001; Somerville et al., 2004).Pectins have been proposed to be important for control of cell wall porosity, for adhesion of adjoining cells and in controlling the ionic environment of the cell wall (McCann et al., 1990; Baron-Epel et al., 1998). Compared with cellulose and hemicellulose, little was known about the synthesis and assembley of pectin.

The biosynthesis of the cell wall carbohydrate polymers played very important roles in secondary cell wall formation. More than 50 glycosyltransferases (GTs) were predicted to be required for pectin synthesis(Coutinho et al., 2003), but until now, only 3 genes for putative pectin biosynthetic GTs, *QUA1, NpGUT1* and *GAUT1*, had been identified (Sterling et al., 2006; Bouton et al., 2002; Iwai et al., 2002). 2 *QUA1* allelic mutants for a putative glycosyltransferase of GT family 8 were identified firstly. The mutants were dwarf and their cell walls showed a reduction in both GalA and pectin content. The results suggested that the encoded enzyme may be involved in the synthesis of pectin polysaccharides (Bouton et al., 2002). Furthermore, the research also reported that the presumed orthologs of *QUA1* from *G. arboreum* and *G. hirsutum* were expressed in elongating fibers at the time

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Abbreviations: GAUT, galacturonosyltransferase; **RACE**, rapid amplification of cDNA ends; **ORF,** open reading frame; **DPA**, day post anthesis, **HG,** homogalacturonan; **RG-I,** rhamnogalacturonanI; **RG-II**, rhamnogalacturonanII; **GTs**, glycosyltransferases; **GATL,** GAUT-like.

when pectins were synthesized actively (7 to 10 DPA), consistent with a possible role for this protein in pectin synthesis (Bouton et al., 2002). *NpGUT1* encoded a unique glucuronyltransferase, which was essential for intercellular attachment in plant meristems and tissues and the mutation caused defects in the glucuronic acid of RG-II of pectin (Iwai et al., 2002). A homogalacturonan galacturonosyltransferase gene, *GAUT1*, was the first functional identification of an *Arabidopsis* pectin HG using biochemical and functional genomic approaches (Sterling et al., 2006). *GAUT1* has 2 highly conserved domains. One is D-[DHS]-DxxxxD motif and the other is the conserved GAUT1-related superfamily motif [H-[FWY]-[DNS]-G-x(2)K-P-W-x(2)-[ILM]-[ADGS] (Sterling et al., 2006). It was also identified that there was a specifically discrimination between the GAUT and GATL subclasses within the GAUT1-related superfamily from 15 *AtGAUT* genes and their orthologoues GT8 proteins in other plants (Sterling et al., 2006). Some researches also identified *irx8* (At5g54690), GT8 family, which was co-expressed with secondary cell wall CESA genes by regression analysis, expression profiling and reverse genetics methods(Persson et al., 2005; Brown et al., 2005). So the pectin biosynthesis genes in GT8 family played an important role in secondary cell wall morphogenesis.

The cell wall biosynthesis was a major synthetic activity in fiber cell primary wall elongation and secondary wall deposition (Wilkins and Jernstedt, 1999). Many researches on cell wall of some plants, such as *Arabidopsis*, could reveal some important information to help us make a thorough inquiry in cotton fiber development. Compared with the *Arabidopsis* trichome, little work had been done on the isolation and characterization of homogalacturonan galacturonosyltransferase genes involved in the synthesis of cross-linked plyaccharides in plant cell walls.

The sea island cotton (*Gossypium barbadense* L.) has been highly valued in many fiber quality traits including fiber length, strength and fineness. In order to understand the fiber development mechanism of the sea island cotton and to improve fiber quality of upland cotton (*G. hirsutum*) by use of transgenic breeding, some researches had been done in the isolation and identification of genes that were expressed in the development of sea island cotton fibers (Wu et al., 2005; Pan et al., 2007; Chi et al., 2008). In this research, *GbGAUT1*, the homogalacturonan galacturonosyltransferase genes of GT8 family, for pectin biosynthesis, was firstly cloned and characterized in the secondary cell wall formation of *G. barbadense* fiber. The results suggested that *GbGAUT1* might play a pivotal role in cotton fiber development.

MATERIALS AND METHODS

Cotton materials

The sea island cotton (cv. Pima 90 - 53) was planted in a field. Developing bolls were tagged from the starting day post anthesis (DPA). All developing fibers of 10 - 45 DPA were removed from the seeds of the tagged bolls. Cotyledons, hypocotyls and roots were collected from 2-week-old young seedlings. The fibers and the collected tissues were immediately frozen in liquid nitrogen for extraction of nucleic acids.

Isolation and purification of cotton gDNA and total RNA

The genomic DNA (gDNA) of Pima 90-53 was extracted from plantlets with 2 cotyledons according to the published protocol (Paterson et al., 1993). Total RNA was extracted from fibers of 10-45 DPA, roots, cotyledons and hypocotyls with PlantRNA Kit (Tiangen) according to the manufacture's instruction. The isolated RNA was treated by RNase-free *DNase* I according to manufacture's protocol to remove any potential gDNA contamination. The purity and concentration of the total RNA were determined using gel electrophoresis and Beckman spectrophotometer.

Amplification of *GbGAUT1* fragment

To clone the internal conservative fragment, primers, G1 and G2 (Table 1), were designed based on the conserved amino-acid and nucleotide sequences between PttGT8D and At5g54690. PCR and RT-PCR were carried out as such 1 cycle of 1 min at 95℃, 30 cycles of 1 min at 94℃, 1 min at 58℃, 1.5 min at 72℃ and a final extension of 10 min at 72℃, then held at 10℃. The *Taqplus* DNA polymerase (Tiangen) was used for fidelity. The products from RT-PCR of cDNA and PCR of gDNA were cloned by using vector pMD18-T (TaKaRa) and *Escherichia coli* strain DH5α and then sequenced.

The cloned internal fragment was blasted in NCBI cotton EST database and *in silico* cloning method was employed to assemble a contig. And primers G3 and G4 (Table 1) were designed to extend the fragment. RT-PCR was carried out as such, 1 cycle of 1 min at 95℃, 30 cycles of 1 min at 94℃, 1 min at 58℃, 1.5 min at 72℃ and a final extension of 10 min at 72℃, then held at 10℃. The *Taqplus* DNA polymerase (Tiangen) was used for fidelity. The products from RT-PCR of cDNA and PCR of gDNA were cloned into the pMD18-T (TaKaRa), transformed into *E. coli* strain DH5α and then sequenced.

3′- and 5′-RACE (rapid amplification of cDNA ends) of *GbGAUT1*

According to the sequence of the cloned internal contig, specific primer 3′GSP1 and the nested specific primer 3′GSP2 (Table 1) were designed and synthesized for 3′-RACE according to the kit manual (SMART™ RACE cDNA synthesis Kit, Clontech). The amplified fragment was cloned into the pMD18-T (TaKaRa) and then sequenced.

For the 5′-end of cDNA cloning, GeneRacer™ RACE Ready cDNA Kit (Invitrogen) was used. Primer 5′GSP1 and the nested specific primer 5′GSP2 (Table 1) were designed based on the sequenced contig. The products were cloned into the pMD18-T (TaKaRa) and then sequenced.

Full-length cDNA amplification of *GbGAUT1*

Compared the above 3 partial fragments with the DNAMAN software, the full-length cDNA of *GbGAUT1* was deduced. The primers for ORF amplification were specific primer Gof and Gor (Table 1). The full-length cDNA was cloned into the pMD18-T (TaKaRa) and then sequenced.

Table1. Primers used for cloning full-length cDNA and genomic sequence of *GbGAUT1*.

Primer	Sequence	Description
G1	5'-ACGTNGGACACNGAGGNCGTT-3′	Degenerate primer, forward
G2	5'- TGGACNTGNCCNTGGAAAGC-3′	Degenerate primer, reverse
G3	5′- GACGAGCCCATATTGAGCAGACTATC-3′	Specific primer, forward
G4	5′- TACATCCTCGGAACCTACCGGC-3′	Specific primer, reverse
3′GSP1	5′- TTGAACCACCAGGTAGTAACTCTCGC-3	3′-RACE forward primer, outer
3′GSP2	5′-GCTCGAATCAGAGAGTTGGAACGA-3′	3′-RACE forward primer, nested
5′GSP1	5' -GTTTTGTAGCCATCGCAGAGCAGTCA-3'	3′-RACE forward primer, outer
3′GSP2	5′-TTTTCCATTCCCATTGGTTTCGTTT-3′	5′-RACE forward primer, nested
Gof	5′-GATGGTACCGAATTCATGAAGAAGTG TCACCGATGGC-3′	ORF forward primer
Gor	5′-TACGGATCCGCGGCCGCTCACTCGTG GATATTGCACTGTTG-3′	ORF reverse primer
Ubif	5′-CTGAATCTTCGCTTTCACGTTATC-3′	Forward primer of the internal control
Ubir	5′-GGGATGCAAATCTTCGTGAAAAC-3′	Reverse primer of the internal control

Bioinformatics analysis

Several clones were randomly selected and confirmed by sequence analysis at Sangon (Shanghai) and Sino Genomax Company Limited (Beijing). The DNA sequences were analyzed with DNAstar software and the BLAST program (http://ncbi.nlm.nih.gov). SignalP 3.0 server was used to predict the presence and location of signal peptide cleavage site in amino acid sequence. Conserved domain, PROSITE motifs and CAM (calmodulin) binding site, were analyzed with NCBI cds Blast (http://www.ncbi.nlm.nih.gov/structure/), ProDom (http://motif.genome.jp/motif-bin/) and CTDB (http://calcium.uhnres.utoronto.ca), respectively. Homology modeling was completed by SWISS MODEL WORKSPACE at http://swissmodel.expasy.org/workspace/ and the structure was viewed with Rasmol software. The GAUT and GATL protein sequences of different species used for the construction of a phylogenetic tree were downloaded from GenBank. Homological comparison and phylogenetic tree were made using ClustalW software.

Gene expression analysis

First-strand cDNA was synthesized from 5 µg of total RNA using the StrataScript Kit (Stratagen). The congruent first strand cDNA was used for PCR amplification of *GbGAUT1* ORF with gene-specific primers with *Kpn*I and *Bam*HI enzyme sites, Gof and Gor. The parameters were: 95℃ for 1 min, followed by 5 cycles of 94℃ for 1 min, 55℃ for 1 min, 72℃ for 2 min, followed by 25 cycles of 94℃ for 1 min, 68℃ for 1 min, 72℃ for 2 min and 72℃ for 10 min. The RT-PCR reactions were repeated 3 times in order to obtain the identical results. Moreover, the primers, Ubif and Ubir (Table 1), were used to amplify the ubiquitin cDNA fragments using parameters as: 95℃ for 1 min, followed by 19 cycles of 94℃ for 1 min, 58℃ for 1 min, 72℃ for 1.5 min and 72℃ for 10 min (Zhang et al., 2007). The products were then separated on a 1.0% (w/v) agarose gel. The expression of a ubiquitin gene was used as an internal control for determining the RT-PCR amplification efficiency among different samlpes.

RESULTS

Full-length cDNA cloning and sequence character of *GbGAUT1*

With the degenerate primers designed from conserved motif, an 880 bp gDNA fragment of *GbGAUT1* was obtained from *G. barbadense*. The nucleotide sequence from 120 bp to 440 bp showed a high homology to AtGAUT6. 1921 bp contig was attained based on the 320 bp EST using *in silico* cloning by blasting EST database. Then special primers were designed according to the 1921 bp contig and about 1800 bp internal sequence was amplified. To clone the full-length cDNA of the *GbGAUT1*, a cloning strategy of rapid amplification of cDNA ends was employed. A fragment of approx.1600 bp at 3′-end and a fragment of approx. 750 bp at 5′-end were amplified, respectively. Finally, the full-length cDNA sequence of *GbGAUT1* was deduced and ORF was amplified through RT-PCR using the specific primers. The nucleotide sequence and deduced amino-acid sequence of this gene were shown in Figure 1. The sequence analysis indicated that the cloned fragment was 2528 bp in length and contained an ORF of 1797 bp encoding a polypeptide of 598 amino acids with a predicted molecular mass of 69 kDa and pI of 8.35. There were a 269 bp 5′-end UTR and a 462 bp 3′-end UTR (Figure 1).

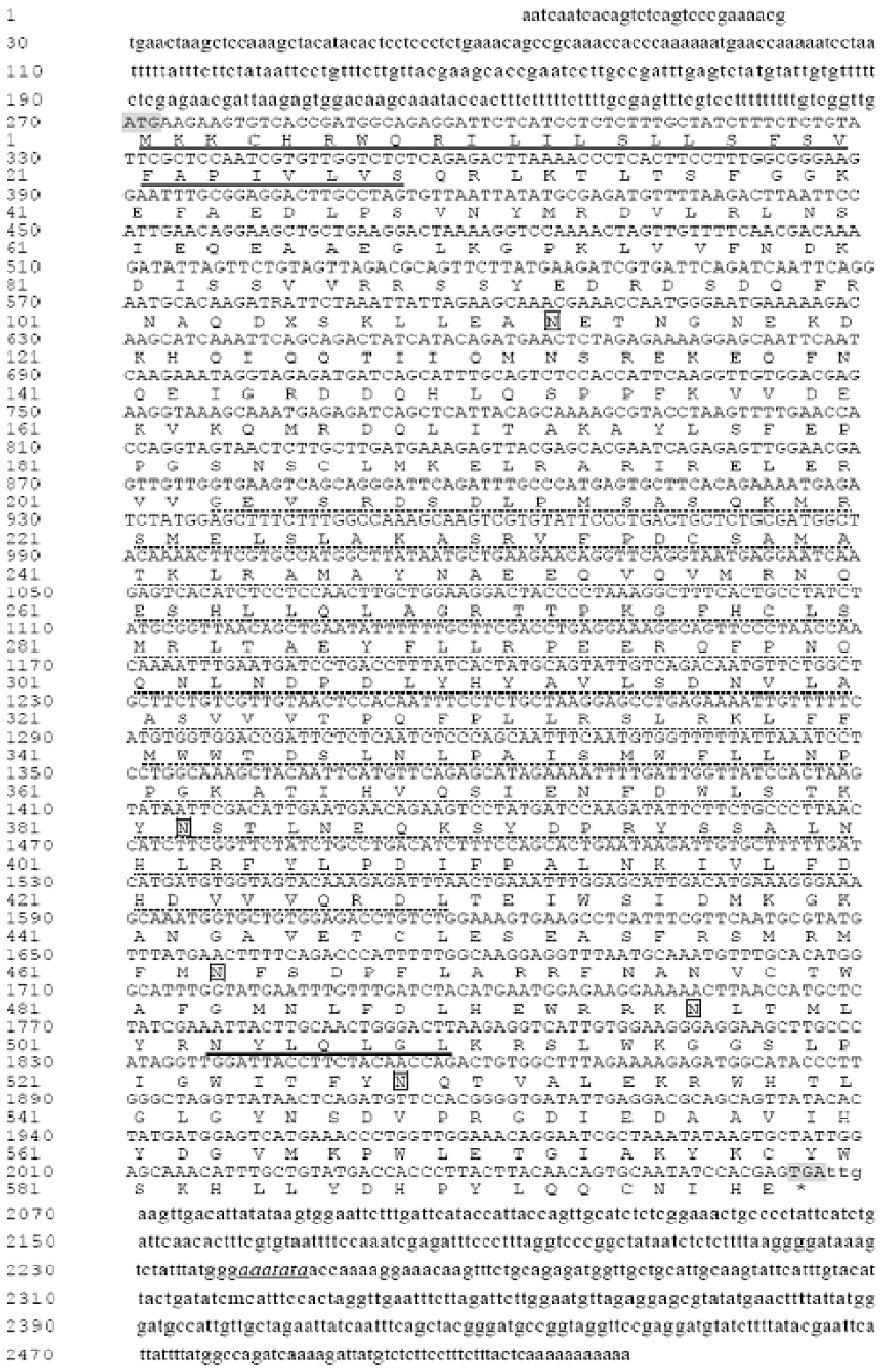

Figure 1. Nucleotide sequence of *GbGAUT1* from cDNA and its deduced amino acid sequence. The small letters were untranslated sequence and the capital letters were coding sequence. The ATG and TGA in the grey box indicated initiation and termination codons, respectively. The modified polyadenylation signals showed in italics with underline. The potential signal peptide domain and the possible CAM combining region was marked with double underline and boldfaced underline, respectively. And the potential transferase glycosyltransferase glycosyl expressed a Golgi family-like coiled coil region showed in broken lines. The potential N-glycosylation sites were pointed in boxes.

To obtain more information on the putative *GbGAUT1* gene, signal peptide, catalytic domains, CAM potential binding sites and other predicted motifs were analyzed. Using neural network (NN) and hidden Markov models (HMM) trained on eukaryotes, a max cleavage site probability signal peptide was between pos. 22 and 23 or 28 to 29 (data not shown). The sequence was predicted to encode glycosyltransferases with type II membrane topology, C-terminal catalytic domains (278-572 AA) with consensus sequences clustered in pfam glycosyltransferase family 8. Analysis of CAM potential binding sites indicated that possible CAM combining region was at the C-terminus near the No. 500 AA. The transferase glycosyltransferase glycosyl F25I16.8 expressed a Golgi family-like coiled coil (PD191560) region was found between 203 and 429 AA. Further analysis indicated that the deduced amino acid sequence included 5 potential N-glycosylation sites (Asn112, Asn382, Asn463, Asn496 and Asn528) (Figure 1).

Homology of deduced amino acid sequence of the *GbGAUT1* gene

Searches of current nucleotide and protein databases with the BLAST network service revealed that the sequence was unique. Using BLASTP programs, GbGAUT1 putative protein shared 54, 51 and 48% identity with AtGAUT6, OsI-035509 and AtGAUT1, respectively. The predicted catalytic domains contained DxD motifs, a conserved motif present in many well characterized glycosyltransferase families, which directly interacted with the ribose of the UDP molecular as well as the coordination of divalent cations. Furthermore, it had a D-[DHS]-Dxxxx-D motif, which was a distinction between GAUT1-related family and other family-8 proteins. The conserved GAUT1-related superfamily motif, which was unique to the entire GAUT1-related superfamily, was also found in the deduced acid amino sequence (Figure 2). The sequence conservation also suggested that the newly cloned gene product could be a novel homogalacturonan galacturonosyltransferase of GAUT1-related superfamily.

Signal peptide, GT8 family conserved domain and the golgi anchor region were found in GbGAUT1, AtGAUT1, AtGAUT3, AtGAUT4 and AtGAUT5. To sum up, the deduced protein backbones of GbGAUT1 and the 4 AtGAUTs contained an N-terminal signal sequence, a Golgi anchor region, a GT8 family conserved domain and a GAUT1-related superfimily motif (Figures 3 and 4).

To get insight about the structure/function relationships in plant GT8 domains, 3D structure of GT8 domains was investigated using swiss model algorthm. The 3D model of GT8 domains was constructed using 3D structure 1ga8 chain “A” (Figure 4A) (Persson et al., 2001) as template. 3D Structure of GbGAUT1 shared 17.95% identity with the template 1ga8A (Figure 4B). The main-chain conformation and the folding partterns were similar to structural feature characteristic of galactosyl transferase lgtc. The result further showed that the newly cloned cDNA was a cotton homogalacturonan galacturonosyltransferase gene.

Phylogenetic analysis

The phylogenetic analysis of the 18 GAUT1-related superfamily members including GbGAUT1 showed that they were grouped into 4 distinct clades by using ClustalW (Slow/accurate, Gonnet) method (Figure 5). The GAUT proteins were clusterred into 3 clades, including GAUT-A, GAUT-B and GAUT-C. The members of the GATL (GAUT-like) family were clusterred tightly into a distinct clade. This result was similar to the current previous research (Sterling et al., 2006). GbGAUT1 was just in the GAUT-A group and more closely related to AtGAUT5 and AtGAUT6 than AtGATL1. The data also suggested that the GbGAUT1 and other members of the GAUT1-related superfamily were highly conserved.

Expression profiles of *GbGAUT1* in different fiber developmental stage and multi-tissues

To determine the expression pattern of *GbGAUT1*, semiquantitative RT-PCR analysis was performed. The results showed that *GbGAUT1* expressed differently during the period of fiber development (Figure 6A). Remarkably, higher level was detected in the fibers of 35 and 40 DPA, which were just about late stage of the fiber secondary cell wall deposition. And there was slightly expression in the fibers of 10 DPA, which was in the primary cell wall development stage. Expression of *GbGAUT1* was detected in hypocotyls and leaves, but no expression in roots (Figure 6B). This revealed that *GbGAUT1* was a tissue specific and developmentally controlled expression gene in cotton.

DISCUSSION

In this study, *GbGAUT1* (GenBank accession No. EF552-432) was cloned by bioinformatics methods, RT-PCR and RACE. As far as we know, this should be the first molecular evidence that multiple pectin polysaccharide biosynthetic homogalacturonan galacturonosyltransferase genes exist in cotton. The ORF was 1797 bp long and putatively encoded a polypeptide of 598 amino acids, with a predicted molecular mass of 69 kDa. The *GbGAUT1* gene had the conserved amino acids and features shared with the GT8 gene family and GAUT-related superfamily. *GbGAUT1* could be considered as a HG transferase protein since the conserved GAUT1-related superfamily motif had been found. Semiquantitative RT-PCR showed that the expression levels of

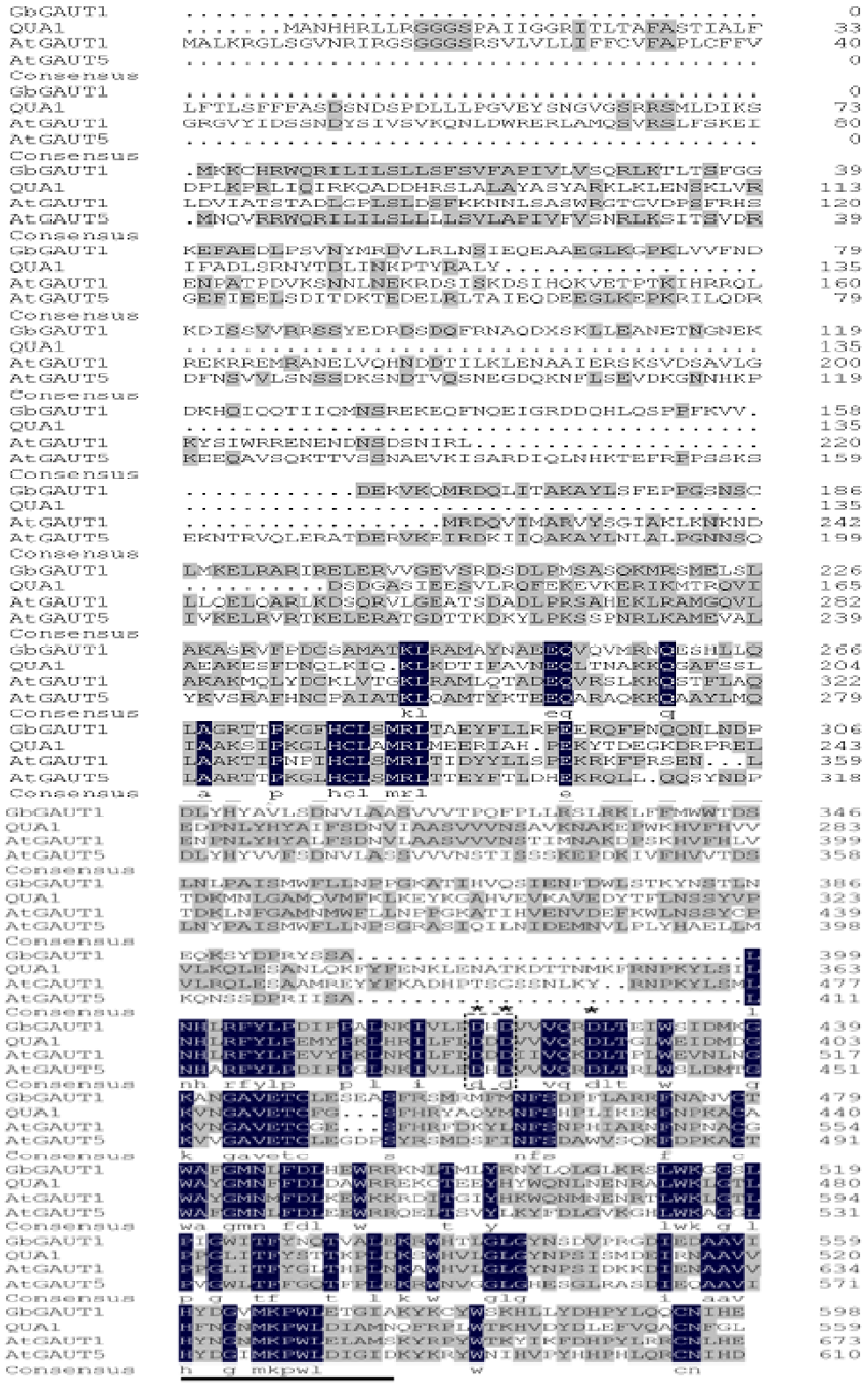

Figure 2. Alignment of the GbGAUT1 protein sequence with related GAUT proteins. Amino acid sequences were aligned for GbGAUT1 (EF552432), AtGAUT1 (NP-191672), AtGAUT5 (NP-850150) and QUA1 (AtGAUT8, NP-189150), Residues common to all four proteins were shown by white letters on black ground, whereas those shared by 3 or 2 were shadowed. The conserved motif DxD and D[DHS]-DxxxxxD appeared in the broken line box and stars, respectively. The black line showed the conserved GAUT1-related superfamily motif [H-[FWY]-[DNS]-G-x(2)K-P- W-x(2)-[ILM]-[ADGS].

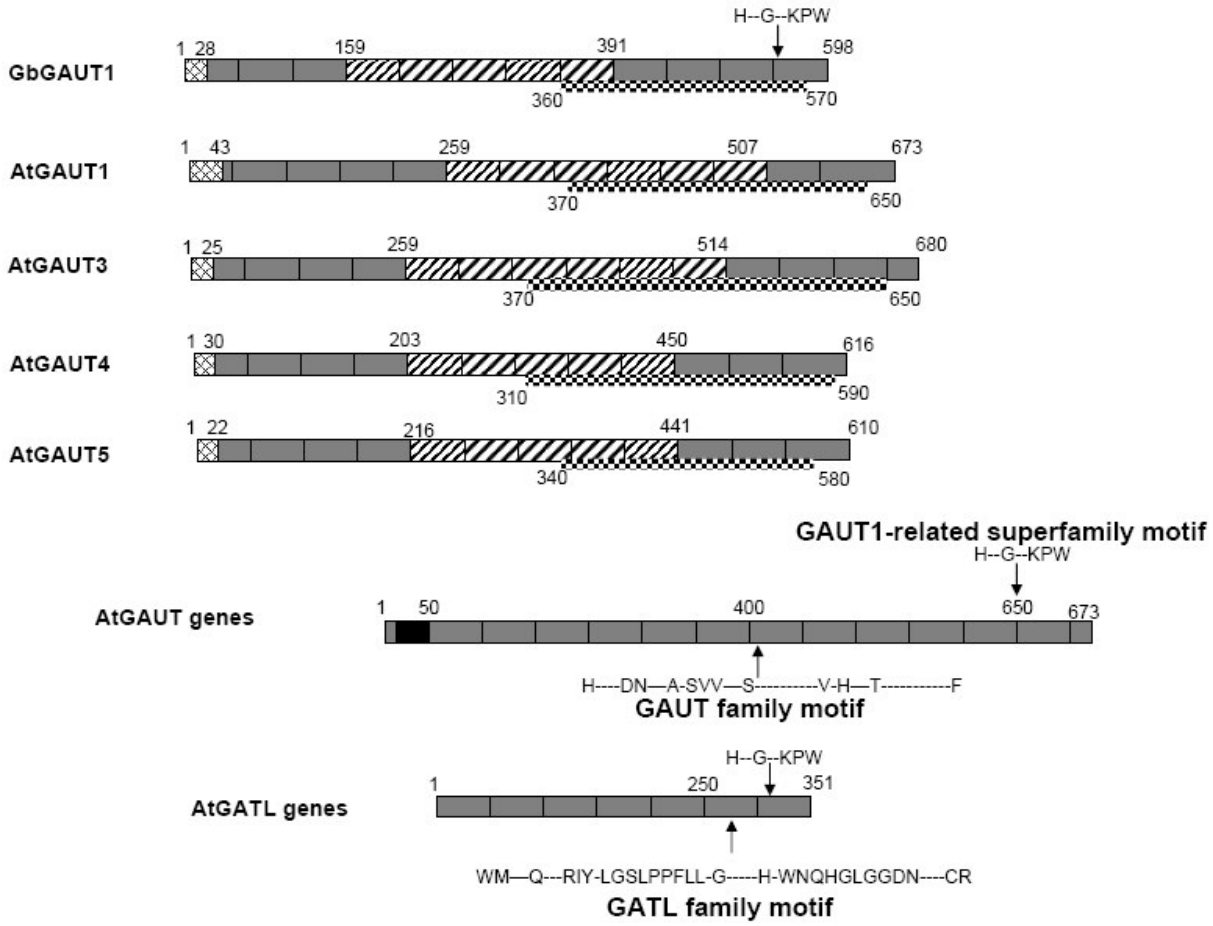

Figure 3. Scheme of GbGAUT1 structure. Schematic representation of domain structure and conserved amino acid motif were found in GbGAUT1, AtGAUT1 (NP-182171), AtGAUT3 (NP-195540), AtGAUT4 (NP-568688) and AtGAUT5 (NP-850150). Residues 22-30 represent a prediced signal peptide. The position of conserved residues were numbered. The conserved amino acid residues within the motifs diagnostic for GAUT1-related superfamily and the GAUT subfamily were shown.

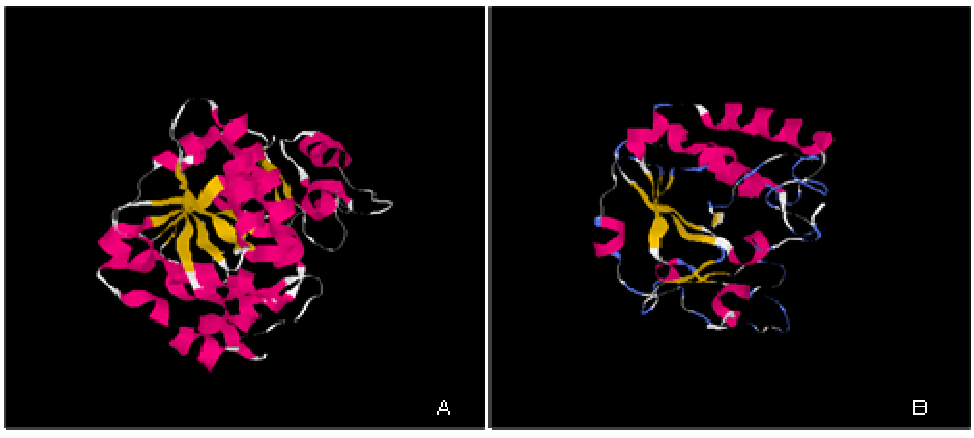

Figure 4. Homology modeling of 3D structure of GbGAUT1 (B) and the template model 1ga8 (A).

GbGAUT1 in the 35 and 40 DPA fibers were markedly higher than in the other fibers and tissues. These results suggested that the novel cotton GbGAUT1 might play an important role in fiber development. GbGAUT1 showed high sequence similarity with *Arabidopsis* homogalacturonan galacturonosyltransferases, although its catalytic

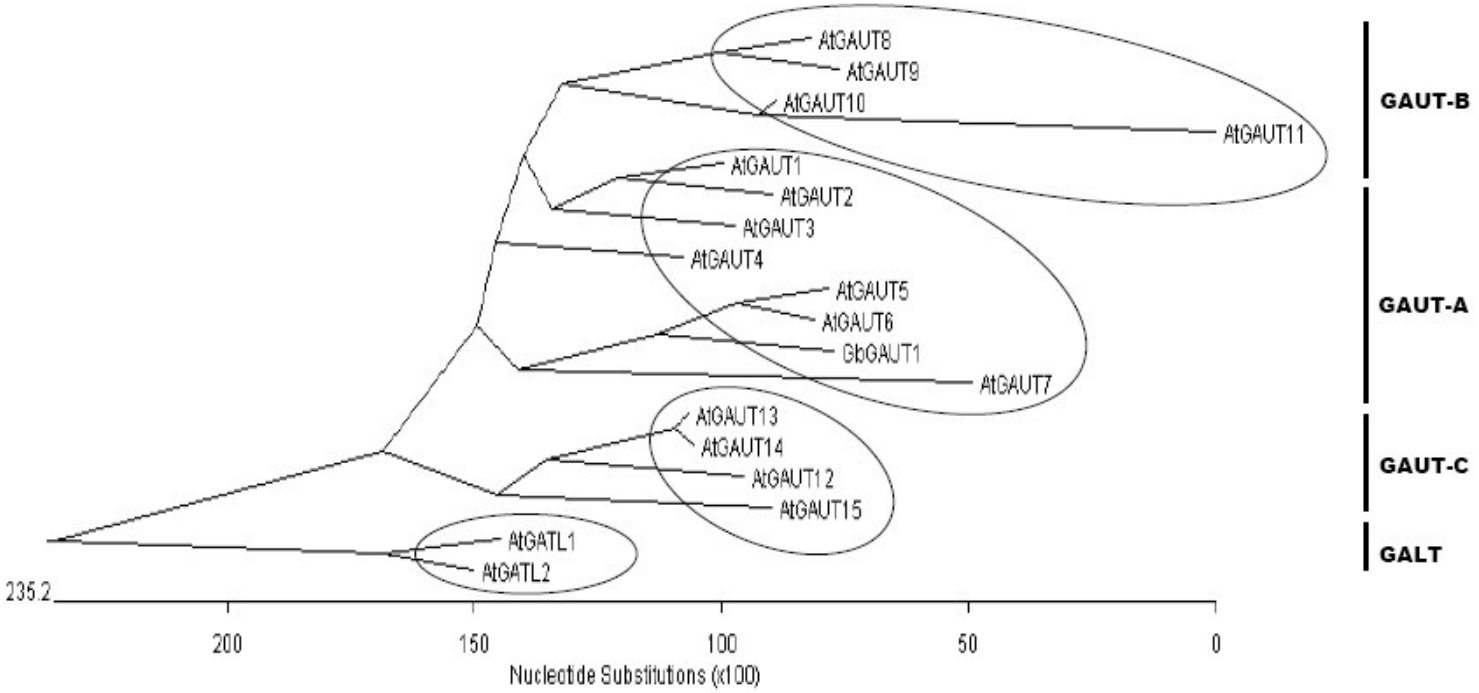

Figure 5. GAUT phylogeny Protein sequences phylogeny showed all 15 AtGAUTs and representative AtGATLs. Some accession numbers were the same as Figures 2 and 3 and other accession numbers were as follows: AtGAUT2 (NP-182171), AtGAUT6 (NP-563771), AtGAUT7 (NP-565893), AtGAUT9 (NP-566170), AtGAUT-10 (NP-565485), AtGAUT11 (NP-564057), AtGAUT12 (NP-200280), AtGAUT13 (NP-186753), AtGAUT14 (NP-197051), AtGAUT15 (NP-191438), AtGATL1 (NP-564077), AtGATL2 (NP-190645). The Arab numbers meant the nucleotide substitutions. Subclasses were indicated by ellipsoid and vertical bars on the right.

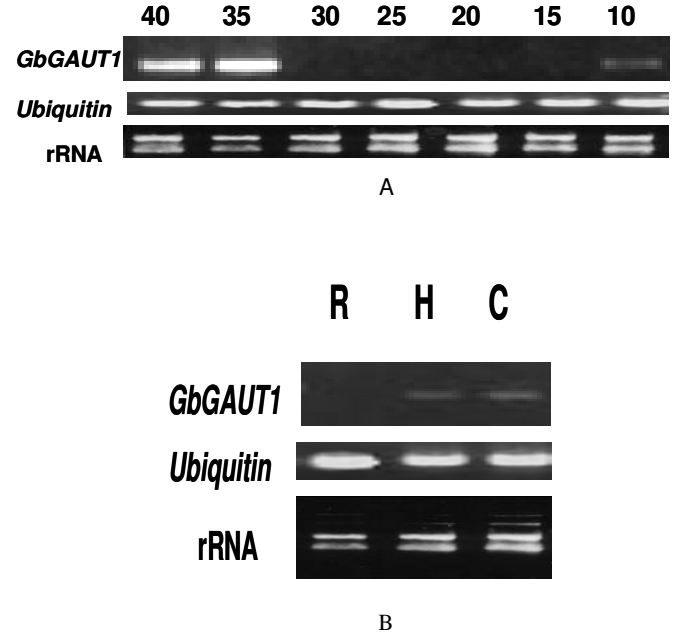

Figure 6. Semi-quantitative RT-PCR analysis of *GbGAUT1* expressed in different fiber developing stages (A) and different tissues (B). 40: 40DPA, 35: 35DPA, 30: 30DPA, 25: 25DPA, 20: 20DPA, 15: 15DPA, 10: 10DPA, R: root, H: hypocotyl, C: cotyledon. Ubiquitin: the internal control for RT-PCR.

activity had not been demonstrated. Furthermore, it is the first cloned putative homogalacturonan galacturonosyltransferase in cotton. Sequence analysis of *GbGAUT1* revealed that the protein encoded by this gene consisted of catalytic domains. GbGAUT1 encoded conserved domains not only including the pfam GT8 motif, but also 2 conserved motif, D[DHS]-DxxxxxD and[H-[FWY]-[DNS]-G-x(2)K-P-W-x(2)-[ILM]-[ADGS] (Sterling et al., 2006), which indicated that GbGAUT1 belonged to the GAUT1-related family. Phylogenetic analysis further demonstrated that GbGAUT1 belonged to the GAUT1-related family and GAUT-A group.

GbGAUT1 contained a signal peptide in the N-terminal region. It was reported that AtGAUT3, AtGAUT4 and AtGAUT5 had an N-terminal signal peptide rather than a transmembrane domain (Sterling et al., 2006). AtGAUT2 was the only AtGAUT family member that had no N-terminal transmenbrane domain and no signal peptide. As we know, most of the *GAUT* genes are likely to encode type II membrane proteins (Sterling et al., 2006) that possess a putative transmembrane domain (TMD) in their hypervariable N-terminal region (Breton et al., 2001). So, some differences might exist in the hypervariable N-terminal region in *GAUT* gene family.

In pea, GAUT1 had been confirmed to have HG:GalAT activity and localize to Golgi lumen (Sterling et al., 2001).

In *Arabidopsis*, GAUT1 protein had also been localized to the Golgi (Dunkley et al., 2004). *GbGAUT1* had a Golgi family-like coiled coil region (PD191560), so it was deduced that the GbGAUT1 might be localized to the Golgi.

As is well known, cotton fiber development is a complicated process, which was divided into 4 overlapping stages, initation, elongation, secondary wall thickening and maturation. The extractable matrix (pectin and hemicellulose) polysaccharides account for 30-50% of the total sugar content in the cotton fiber elongation stage, but less than 3% in the fiber cell wall thickening stage (Tokumoto et al., 2002). But more and more researches indicated that cross-linkage polysaccharides were synthesized not only in fiber elongation but also secondary wall formation (Brett et al., 1997; Wu and Liu, 2005). Pectin mainly consists of HG, RG-I and RG-II. HG was a polymer of α-1, 4-linked GalA that accounted for about 65% of pectin. RG-I and RG-II were complex branched polysaccharides which had a backbone of α-1,4-linked GalA (Nakamura et al., 2002). In the fiber developmental stage, the semi RT-PCR indicated that *GbGAUT1* expressed in the fibers of 10 DPA, the primary cell wall stage and then highly expressed in the fibers of 35 and 40 DPA, the stage of secondary cell wall deposition. The expression analysis meant that *GbGAUT1* expressed in both cell wall synthesis and preferentially in the fiber secondary cell wall thickening stage. There might be a similar structure with primary cell wall in the secondary cell wall that the matrix polysaccharides including pectin and hemicellulose were served as cross-links between cellulose microfibris. The strength of the wall might be increased by the quantity and cross-linked density of matrix polysaccharides. The expression pattern of *GbGAUT1* coordinated with requirement of the matrix polysaccharides in fiber development, suggesting that *GbGAUT1* must play an important role in the synthesis of pectin polysaccharides during plant cell wall morphogenesis.

The function of *GbGAUT1* in cotton fiber development would require more detailed research. The protein purification and functional analysis of the *GbGAUT1* gene is currently being investigated in our laboratory.

ACKNOWLEDGEMENTS

We are grateful to Prof. Yuxian Zhu (the National Laboratory of Protein Engineering and Plant Genetic Engineering, Beijing University, P. R. China) and his laboratory staff for technical assistance. This research was supported by the National 973 Project (No. 2004CB117302), the National 863 Project (No. 2006011001044), the National Transgenic Breeding Project (2008ZX08009-003), the Natural Science Foundation of China (No. 30671322, 30871561) and the Natural Science Foundation of Hebei Province (No. C2006001034).

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