J. Plant Biol. (2010) 53:367–373
DOI 10.1007/s12374-010-9124-9

ORIGINAL RESEARCH

Molecular Cloning of Three UDP-Glucuronate Decarboxylase Genes That Are Preferentially Expressed In *Gossypium* Fibers From Elongation to Secondary Cell Wall Synthesis

Yuxin Pan · Xingfen Wang · Hengwei Liu · Guiyin Zhang · Zhiying Ma

Received: 23 May 2010 / Revised: 4 August 2010 / Accepted: 5 August 2010 / Published online: 18 August 2010
© The Botanical Society of Korea 2010

Abstract UDP-xylose (UDP-Xyl) is a nucleotide sugar used as substrate for producing non-cellulose materials, e.g., hemicellulose and pectic polysaccharide, in the fibers of cotton (*Gossypium*). Its biosynthesis is catalyzed from UDP-glucuronic acid (UDP-GlcA) by UDP-glucuronate decarboxylase (UXS). Here, we first cloned *GhUXS1* in *Gossypium hirsutum* and *Gossypium barbadense* based on a transcript-derived fragment that originated from our cDNA–AFLP transcriptome profiling in cotton. *GhUXS2* and *GhUXS3* were also isolated via homology-based cloning. *GhUXS* nucleotide sequences were identical between the two species, and the deduced amino acid residues had the conserved motif of the UXS family, i.e., GxxGxxG, Ser residues, and YxxxK. *GhUXS1* was expressed in *Escherichia coli* and generated UXS activity that converted UDP-GlcA to UDP-Xyl. Semiquantitative RT-PCR results showed that *GhUXSs* transcripts were preferentially expressed during fiber development, from elongation through the stage of secondary cell wall synthesis. Although at the same number of days post-anthesis they were more abundant in *G. hirsutum*, expression was sustained for a longer period in *G. barbadense*. These different patterns of expression may affect quality and partially explain why the latter species has better fiber strength.

Keywords Fiber development · *Gossypium* · UDP-glucuronate decarboxylase

Y. Pan · X. Wang · H. Liu · G. Zhang · Z. Ma (✉)
North China Key Laboratory for Crop Germplasm Resources of Education Ministry, Agricultural University of Hebei, Baoding 071001, People's Republic of China
e-mail: mzhy@hebau.edu.cn

Y. Pan
Hebei Polytechnic University, Tangshan 063009, People's Republic of China

Z. Ma
College of Agronomy, Agricultural University of Hebei, No. 289 Lingyusi Street, Baoding 071001 Hebei, People's Republic of China

The quality of cotton fiber, especially its strength, is most essential to the textile industry. Therefore, its biosynthesis is an important focus within biological research. Fiber development comprises four major stages: initiation, elongation (formation of primary cell walls), deposition of secondary cell walls, and maturation (Basra and Malik 1984). Of these, secondary cell wall deposition is the key stage for determining fiber strength. Because cellulose is the main component at this stage, it has been the subject of numerous investigations (Meinert and Delmer 1977; Kurek et al. 2002; Jacob-Wilk et al. 2006). However, little is known about non-cellulose proteoglycans, which are related to fiber formation and cross-linkage polysaccharides that are synthesized during fiber elongation and secondary wall formation (Buchala 1999).

UDP-Xyl is an important glycosyl donor required for the biosynthesis of hemicellulose proteoglycans in plants (White et al. 1993; Baydoun and Brett 1997; Reiter and Vanzin 2001). It can also be epimerized to form UDP-Ara, which is used to produce pectic polysaccharides, such as arabinose, galactose, and rhamnose (Reiter and Vanzin 2001). UDP-Xyl is mainly derived from UDP-glucose (UDP-Glc) by the action of UDP-glucose dehydrogenase (UGDH, EC 1.1.1.22) and UXS (EC 4.1.1.35). First, UGDH catalyzes dehydrogenation of UDP-Glc to form UDP-GlcA. UXS then converts UDP-GlcA to UDP-Xyl.

This process probably occurs in Golgi membranes or the cytosol (Feingold et al. 1960; Hayashi et al. 1988; Harper and Bar-Peled 2002). Synthesis of UDP-Xyl can be inhibited by the product of that UXS reaction (Bar-Peled et al. 2001; Harper and Bar-Peled 2002). Because the decarboxylation reaction catalyzed by UXS is essentially irreversible, UXS represents a key enzyme for partitioning glycosyl residues between the hexosyl and pentosyl residues (Zhang et al. 2005). In addition, because of its central role in sugar nucleotide interconversion, UXS is likely ubiquitous and a target for regulatory control during cell wall biosynthesis (Seifert 2004; Zhang et al. 2005).

UXS genes have been cloned from only a few plants, including rice (Suzuki et al. 2004), *Arabidopsis thaliana* (Harper and Bar-Peled 2002), pea (Kobayashi et al. 2002), and tobacco (Bindschedler et al. 2007). Little information is available about *UXS* in cotton (*Gossypium*). We previously isolated a transcript-derived fragment (TDF), similar to *AtUXS1*, from *Gossypium hirsutum* and *Gossypium barbadense*, two species that differ in their fiber strength (Pan et al. 2007). Here, we have characterized three *UXS* gene family members (*GhUXS1*, *GhUXS2*, and *GhUXS3*) from both species. GhUXS1 was elongated and cloned based on our earlier described TDF; the other two genes were isolated by homology-based cloning. We then examined UXS activity and gene expression at different stages of fiber development to understand the role that the GhUXS enzyme might have in determining cotton fiber quality.

Materials and Methods

Plant Materials

Plants of *G. hirsutum* (cv. CRI 8) and *G. barbadense* (cv. Pima90-53) were grown in a breeding nursery. Pedicels from each cotton boll were tagged on their day of anthesis to record their ages. Some ovules were sampled at 0 DPA (days post-anthesis) and fibers were collected from the epidermis of other ovules at 3, 5, 10, 15, 20, 25, 30, and 35 DPA.

cDNA Preparation

Total RNA was extracted from ovule and fiber samples with an RNAplant Kit (TIANGEN, China) according to the manufacturer's instructions. Quality and quantity of that RNA were assessed in agarose gels (1.6%, *w*/*v*) and spectrophotometrically at 260/280 nm, respectively. cDNA was synthesized with an M-MLV Kit (TaKaRa) either for direct use in semiquantitative RT-PCR analysis or as template for amplifying *GhUXS*.

Cloning of Cotton UDP-Glucuronate Decarboxylase (*GhUXS*)

One TDF similar to *AtUXS1* was previously separated through our cDNA–AFLP transcriptome profiling of cotton fibers (Pan et al. 2007). ESTs with high similarity to this TDF were searched in the NCBI dbEST of cotton. This TDF was then elongated by an *in silico* method and *GhUXS1* was cloned. The cotton ESTs database also was searched by a BLAST program using four other *UXS* gene sequences (AT3g62830, AT5g59290, AT2g47650, and AT3g46440) from *A. thaliana*. Similar cotton EST sequences were identified and assembled with DNASTAR software. Based on this, we isolated the other two cotton *UXS* genes (*GhUXS2* and *GhUXS3*) by homology-based cloning. The derived sense and antisense primer pairs (5′ to 3′) for the three integrated ORFs were designed as follows: *GhUXS1*-sense TACGGATCCATGAAACAGTTACA-CAAGC and *GhUXS1*-antisense GACGAGCTCT-TAAGCTCCCTTCCCTT; *GhUXS2*-sense CTAGAGCTCATGGGATCGGAGCTAATAT and *GhUXS2*-antisense GCGGTCGACTTAAGATGAT-GAGTCGT; and *GhUXS3*-sense CTAGAGCTCATGGC-GACAGATTCATC and *GhUXS3*-antisense GCGGTCGACTCACTCTTTAGAGATTCC.

Our 20-DPA-fiber cDNA template was used to amplify these *GhUXS* genes. The RT-PCR reaction product was cloned into a pGM-T vector (TIANGEN), then sequenced by the Shanghai Sangon Biological Engineering Technology and Service Company, China. Based on their ORF sequences, the 5′ and 3′ sequences were also extended according to instructions for the RACE kit (Clontech).

Expression and Purification of Recombinant Proteins

Coding regions for our three *GhUXS* genes were inserted into a pET-32a(+) expression vector (Novagen), and the resulting plasmids were introduced into *Escherichia coli* host strain BL21 (DE3) pLysS (TIANGEN). Truncated versions of *GhUXS1* and *GhUXS2* that lacked the transmembrane domain also were processed as described as above. The bacterial strain, carrying either various *GhUXS* vectors or the control pET-32a(+) vector, was induced by 1 mM isopropyldithio-β-galactoside (IPTG) for approximately 3 h at 28°C. Afterward, GhUXS1 recombinant protein lacking the transmembrane domain (GhUXS1K-pET-32a(+)) was purified on an His Ni-Superflow resin and gravity column (Clontech) for enzyme assay.

Enzyme Assay

We conducted a standard 50-μL reaction assay as described by Zhang et al. (2005), using 100 mM sodium phosphate

buffer (pH 6.5), 2 mMNAD$^+$, 0.12 mM UDP-GlcA, and 10 μg of purified GhUXS1 recombinant protein (lacking the transmembrane domain). The assay was performed at 25°C for 10 min, then stopped by the addition of 50 μL of phenol/chloroform (1:1, *v*/*v*). The tube contents were mixed and centrifuged at 16,000×*g* for 5 min at room temperature. The aqueous phase was analyzed by HPLC (SHIMADZU LC-20AB) using a Shim-pack VP-ODS column (5 μm, 4.6×150.0 mm). Nucleotide sugars were eluted through an acetonitrile gradient (SHIMADZU) in 40 mM triethylamine acetate and were detected by UV A254.

Semiquantitative RT-PCR

The cDNAs from 3-, 5-, 10-, 15-, 20-, 25-, 30-, and 35-DPA fibers and 0-DPA ovules of both species were used to amplify three *GhUXS* genes by semiquantitative RT-PCR. Cycling conditions were 95°C for 2 min; then 28 cycles of 94°C for 30 s, 55°C for 45 s, and 72°C for 1.5 min; followed by a final elongation at 72°C for 10 min. Products were separated by electrophoresis on 1% agarose gels and visualized on a BIO-RAD Gel Doc 2000. The *EF-1α* gene was used as a positive control (Zhu et al. 2006; Artico et al. 2010).

Results

Molecular Cloning of GhUXS

Based on the cotton ESTs from NCBI, at least three members isolated here were revealed to have different ORFs in the cotton *UXS* gene family. Because their sequences are similar to those in *A. thaliana*, we designated them as *GhUXS1*, *GhUXS2*, and *GhUXS3* (with respective GenBank Accession Numbers EU817581, EU791897, and EU791898). Their ORFs were cloned from *G. hirsutum* and *G. barbadense* by RT-PCR, followed with analysis by 5′- and 3′-RACE. These gene sequences showed no differences between species. Full-length cDNAs of *GhUXS1*, *GhUXS2*, and *GhUXS3* were 1,922, 1,752, and 1,506 bp long, with respective ORFs of 1,311, 1,329, and 1,038 bp. The putative GhUXS protein sequences ranged from 335 to 442 amino acid residues and shared approximately 70% identity. A BLAST analysis indicated that the various *Gossypium* and *Arabidopsis* UXS isoenzymes had 66% to 91% sequence identity (Table 1).

Protein Alignment Analysis of GhUXS

The deduced amino acid sequences were compared for three UXS members in *Gossypium* and five in *A. thaliana* (Fig. 1). GhUXS protein belongs to the NAD-dependent epimerase/dehydratase superfamily, which has a conserved motif of GxxGxxG, YxxxK, and Ser (Fig. 1). The GxxGxxG motif is associated with NAD(P)-binding proteins. YxxxK and Ser complete the process that transfers hydride from the C4 position on the sugar to NAD$^+$ (Baker and Blasco 1992; Liu et al. 1997). Among the three GhUXSs, GhUXS1 and GhUXS2 are more similar to each other. The GxxGxxG motif is probably located at amino acid position 127 to 133 in GhUXS1, 124 to 130 in GhUXS2, and 39 to 45 in GhUXS3. The Ser residue is found at amino acid position 232 in GhUXS1, 230 in GhUXS2, and 146 in GhUXS3. The YxxxK motif is probably located at positions 263 to 267 for GhUXS1, 260 to 264 for GhUXS2, and 176 to 180 for GhUXS3. GhUXS1 and GhUXS2 have an N-transmembrane domain (at amino acids 1 to 48 and 1 to 42, respectively) and can be classified as a putative membrane-bound UXS. In contrast, GhUXS3 lacks the N-terminal extension and is a cytosolic protein. These structural characteristics are the same as for those of members in the *AtUXS* gene family.

Protein Expression and Assay

To verify that our isolated cDNAs encode UXS, we induced the recombinant proteins from *E. coli* under IPTG. GhUXS3 expressed its recombinant protein with a molecular mass of 60 kD. By comparison, GhUXS1 and GhUXS2 expressed truncated recombinant versions only, with molecular masses of 60 and 66.7 kD (data not shown). The 60-kD soluble protein of GhUXS1 was later purified (Fig. 2).

HPLC profiles for the enzyme reaction of GhUXS1 recombinant are shown in Fig. 3. When induced under IPTG, this protein catalyzed the UDP-GlcA conversion to UDP-Xyl. Two sharp peaks that appeared in the recombinant enzyme had an elution time (Fig. 3a) that was identical to that of the standard mixture of UDP-GlcA and UDP-Xyl (Fig. 3c). However, in the empty vector, only UDP-GlcA revealed a sharp peak (Fig. 3b). Therefore, these results demonstrate that GhUXS1 is UDP-glucuronate decarboxylase.

Transcript Levels of GhUXS at Different Developmental Stages

Expression of GhUXS-specific transcripts was determined by semiquantitative RT-PCR (Fig. 4), and patterns were similar among our three *GhUXS* genes. Transcripts for *GhUXS* remained abundant from 10 to 25 DPA, peaking at 15 DPA before dropping. At the same DPA, expression was significantly higher in *G. hirsutum* (CRI-8) than in *G. barbadense* (Pima90-53). However, expression was sustained for a longer period in the latter. Furthermore, transcripts differed in their abundance among genes, with *GhUXS1* showing a higher level of expression than the

Table 1 Comparison of sequence identities for nucleotide sugar interconversion enzymes from *Gossypium* and *Arabidopsis* based on their complete ORF amino acid sequences

Identity (%)	AtUXS1 (%)	AtUXS2 (%)	AtUXS3 (%)	AtUXS4 (%)	AtUXS5 (%)	GhUXS1 (%)	GhUXS2 (%)	GhUXS3 (%)
AtUXS1	100							
AtUXS2	68.5	100						
AtUXS3	65.4	65.7	100					
AtUXS4	68.6	89.3	65.7	100				
AtUXS5	66.8	66.8	95.3	66.8	100			
GhUXS1	84.6	68.1	66.9	68.5	66.8	100		
GhUXS2	71.0	81.8	66.3	80.8	67.1	69.5	100	
GhUXS3	66.3	67.2	89.8	67.4	90.9	68.0	67.7	100

other two. *GhUXS3* transcripts were lowest among the three and could not be quantified in samples from 25 DPA or later. Expression of all three was detected in roots and hypocotyls, and at very low levels in the leaves (data not shown). Based on these findings, we can conclude that GhUXSs are preferentially expressed in cotton fibers from the elongation stage to the synthesis of secondary cell walls. Moreover, at the same DPA, expression is greater in *G. hirsutum* than in *G. barbadense*.

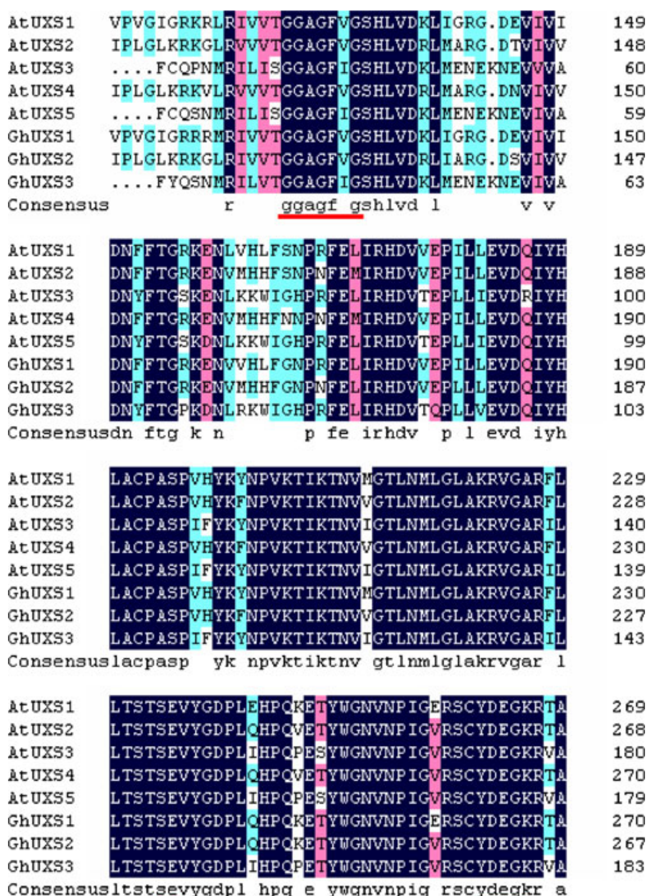

Fig. 1 Alignment of amino acid sequences for UDP-glucuronic acid decarboxylase (UXS) from *Gossypium* and *Arabidopsis*. Conserved motifs GxxGxxG (NAD^+-binding) and amino acid residues Ser and YxxxK of active site are *underlined*. Alignments were generated via DNAMAN program

Discussion

UXS proteins play essential roles in the biosynthesis of non-cellulose proteoglycans. We found at least three members of the *GhUXS* gene family, based on cotton EST databases. Here, we cloned and sequenced their corresponding cDNAs. Previous sequence analyses have demonstrated that UXS genes occur widely in plants, with five members being identified in *Arabidopsis* (Harper and

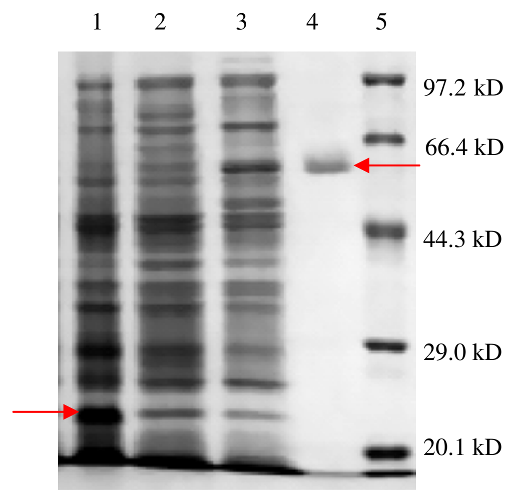

Fig. 2 12% SDS-PAGE of GhUXS1 during purification. *Lane 1*, total soluble *E. coli* protein expressing pET-32a(+) was induced under IPTG for 3 h and its His tag protein expressed (26-kD, *arrow A*). *Lane 2*, total *E. coli* protein GhUXS1 was induced under IPTG for 3 h. *Lanes 3* and *4*, total soluble *E. coli* protein expressing GhUXS1K-pET-32a(+) (*Lane 3*) was purified over Ni column (*Lane 4*, 60-kD, *arrow B*) and induced under IPTG for 3 h. *Lane 5*, marker protein with indicated molecular masses (TIANGEN)

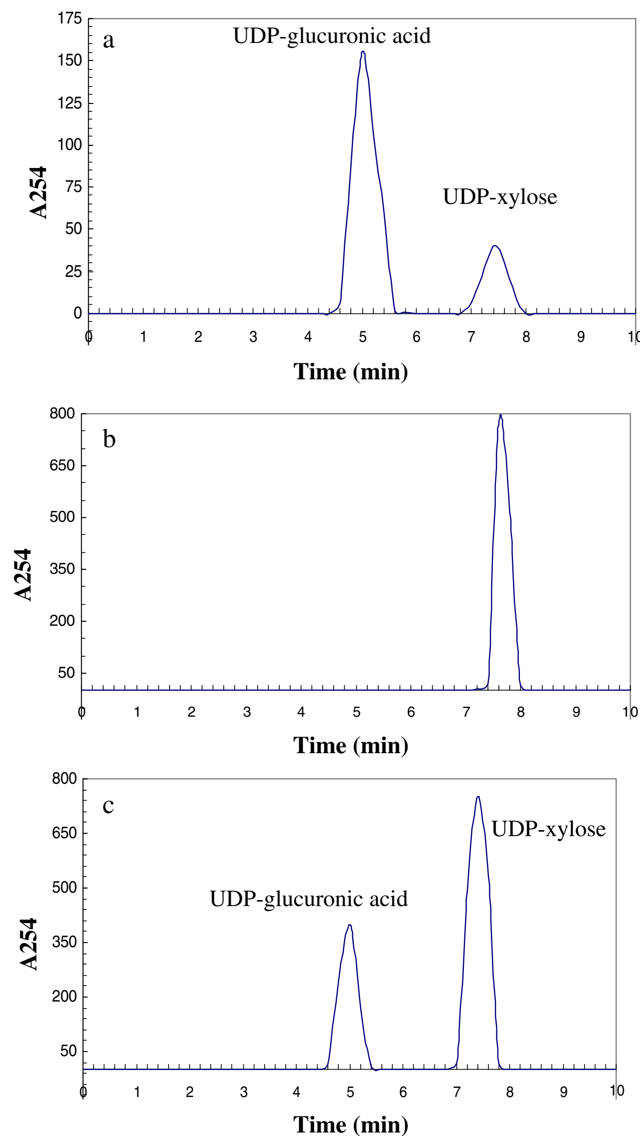

Fig. 3 Elution profiles for products of recombinant GhUXS reaction with 1 mM UDP-glucuronic acid for 10 min at 25°C. **a** Total soluble expressing combination GhUXS1 protein (10 μg) derived from *E. coli*. **b** Empty pET-32a(+) vector protein was separated on SHIMADZU HPLC. **c** Standard mixture of UDP-glucuronic acid and UDP-xylose products were separated on SHIMADZU HPLC

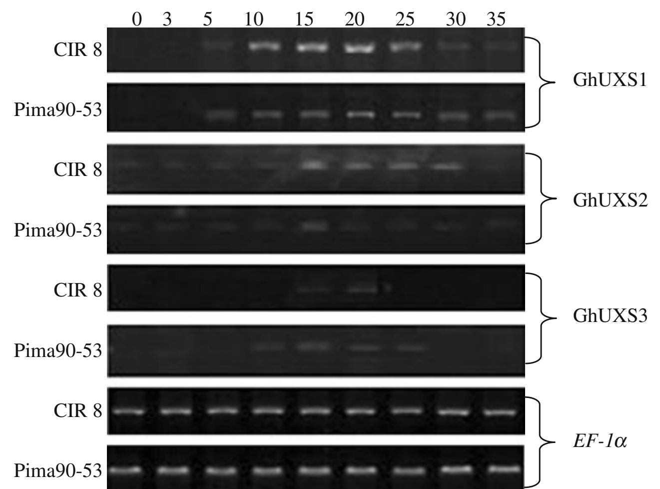

Fig. 4 RT-PCR expression of *GhUXS*1, -2, and -3 in *Gossypium*. Total RNA isolated at different developmental stages: ovule at day 0 and fiber at 3, 5, 10, 15, 20, 25, 30, or 35 days post-anthesis (DPA). Corresponding gene-specific transcript cDNAs were used for RT-PCR amplification, with *EF-1α* serving as internal control

Bar-Peled 2002), two *UXS* genes in rice (Suzuki et al. 2004), and four *HvUXS* in barley (Zhang et al. 2005). In tobacco, antisense downregulation of UDP-glucuronate decarboxylase leads to high glucose-to-xylose ratios in xylem walls due to fewer xylose-containing polymers. Such plants also have an altered vascular organization and reduced xylans in their secondary walls (Bindschedler et al. 2007).

These studies all show that *UXS* genes are expressed throughout plant development as they influence cell wall structure. Their conserved sequences, N-terminal GxxGxxG sequence, Ser residues, and YxxxK motif are believed to be involved in catalysis (Baker and Blasco 1992; Liu et al. 1997). All were present in our *GhUXSs* deduced amino acid sequences. Multiple sequence alignments suggested that GhUXS3 has diverged from the other two GhUXSs. That is, GhUXS1 and GhUXS2 have a transmembrane domain near their NH_2 termini that is absent in GhUXS3. In addition, transcript levels are lower in the latter gene at each stage of fiber development. This implies that GhUXS3 followed another branch during *GhUXS* evolution.

To confirm that our cloned genes encode UDP-glucuronate decarboxylase, we selected *GhUXS1* cDNA for expression in *E. coli* to analyze enzyme activity. GhUXS1 protein purified on an Ni-NTA column exhibited UXS activity, as measured by the conversion of UDP-GlcA to UDP-Xyl. Although activities were not analyzed for GhUXS2 and GhUXS3, we surmise that they also encode typical UDP-glucuronate decarboxylase based on their conserved domain with the GhUXS1 protein.

Using the subcellular information for homologous *UXS* genes in other species (Feingold et al. 1960; Hayashi and Matsuda 1981; Feingold 1982; Kearns et al. 1993), we speculated on the location of these *GhUXS* genes. Because *GhUXS3* lacks a putative transmembrane domain at the NH_2-terminal end, it is a soluble protein that might be located in the cytosol, whereas GhUXS1 and GhUXS2 may function in the Golgi apparatus due to their hydrophobic N-terminal extensions.

Semiquantitative RT-PCR analyses showed that expression of these *GhUXS* genes peaked during the stage of primary cell wall formation, and transcripts levels were much higher in *G. hirsutum* than in *G. barbadense*. We found similar expression patterns for polysaccharide and genes related to non-cellulose

formation. Meinert and Delmer (1977) also have reported that the contents of UDP-GlcA, xylose, and arabinose gradually increase until the beginning of secondary cell wall synthesis in cotton fibers. Pectic polysaccharide and xyloglucans also rise during the stage of primary cell wall formation (Tokumoto et al. 2002). Changes in *GhUXS*s expression leads to altered UDP-Xyl synthesis, which affects the production of hemicellulose and pectic polysaccharide. This implies that the *GhUXS* genes are closely correlated with non-cellulose polysaccharide synthesis, a process that is critical to the development of primary walls in fiber cells.

Because cellulose content is similar among fibers with dissimilar strengths (Liu et al. 1994), we can presume that the difference in strength between *G. barbadense* and *G. hirsutum* may originate during the process of fiber-structure formation. In fact, we found overlap in the stages of elongation and secondary cell wall synthesis. As described previously, the non-cellulosic and cellulose network that fixes the structure of the primary cell wall during that overlapping period and results in the first significant increase in fiber strength (Wilkins and Jernstedt 1999; Hsieh 1999). In primary cell walls, Al-Ghazi et al. (2009) have detected that the content of UDP-D–glucuronate 4-epimerase, involved in pectin synthesis, is higher in the fiber of *G. hirsutum* than in *G. barbadense*. Similar to the trend of GhUXSs expression observed in our study, Haigler et al. (2005) have found that a longer period of cellulose synthesis leads to higher-strength cotton. Therefore, we believe that GhUXS is a key enzyme in determining the quality and integrity of cotton fibers.

Acknowledgments This research was supported by the National Basic Research Program of China (Grant No. 2010CB126000), the National High Technology Research and Development Program of China (Grant No. 2006AA100105), the National Natural Science Foundation of China (Grant No. 30871561), and funding for a transgenic research project from the Ministry of Agriculture of China (Grant No. 2008ZX08009-003). The authors are grateful to the editing service of Dr. Priscilla Light.

References

Al-Ghazi Y, Bourot S, Arioli T, Dennis ES, Llewellyn DJ (2009) Transcript profiling during fiber development identifies pathways in secondary metabolism and cell wall structure that may contribute to cotton fiber quality. Plant Cell Physiol 50:1364–1381

Artico S, Nardeli SM, Brilhante O, Fátima Grossi-de-Sa M, Alves-Ferreira M (2010) Identification and evaluation of new reference genes in *Gossypium hirsutum* for accurate normalization of real-time quantitative RT-PCR data. BMC Plant Biol 10:49

Baker ME, Blasco R (1992) Expansion of the mammalian 3 beta-hydroxysteroid dehydrogenase/plant dihydroflavonol reductase super-family to include a bacterial cholesterol dehydrogenase, a bacterial UDP-galactose-4-epimerase, and open reading frames in *Vaccinia* virus and fish lymphocystis disease virus. FEBS Lett 301:89–93

Bar-Peled M, Griffith CL, Doering TL (2001) Functional cloning and characterization of a UDP-glucuronic acid decarboxylase: the pathogenic fungus *Cryptococcus neoformans* elucidates UDP-xylose synthesis. Proc Natl Acad Sci USA 98:12003–12008

Basra AS, Malik CP (1984) Development of the cotton fiber. Int Rev Cyt 89:65–113

Baydoun E, Brett CT (1997) Distribution of xylosyltransferases and glucuronosyl transferases within the Golgi apparatus in etiolated pea (*Pisum sativum* L.) epicotyls. J Exp Bot 311:1209–1214

Bindschedler LV, Tuerck J, Maunders M, Ruel K, Petit-Coni M, Danoun S, Boudet A-M, Joseleau J-P, Bolwell GP (2007) Modification of hemicellulose content by antisense down-regulation of UDP-glucuronate decarboxylase in tobacco and its consequences for cellulose extractability. Phytochemistry 68:2635–2648

Buchala AJ (1999) Noncellulosic carbohydrates in cotton fibers. In: Basra AS (ed) Cotton fibers: developmental biology, quality Improvement and Textile processing. Food Products, New York, pp 113–136

Feingold DS (1982) Aldo (and keto) hexoses and uronic acids. In: Loewus FA, Tanner W (eds) Plant carbohydrates I, intracellular carbohydrates (encyclopedia of plant physiology new series). Springer, Heidelberg, pp 3–76

Feingold DS, Neufeld EF, Hassid WZ (1960) The 4-epimerization and decarboxylation of uridine diphosphate D-glucuronic acid by extracts from *Phaseolus aureus* seedlings. J Biol Chem 235:910–913

Haigler CH, Zhang DS, Wilkerson CG (2005) Biotechnological improvement of cotton fibre maturity. Physiol Plant 124:285–294

Harper AD, Bar-Peled M (2002) Biosynthesis of UDP-xylose. Cloning and characterization of a novel *Arabidopsis* gene family, UXS, encoding soluble and putative membrane-bound UDP-glucuronic acid decarboxylase isoforms. Plant Physiol 130:2188–2198

Hayashi T, Matsuda K (1981) Biosynthesis of xyloglucan in suspension-cultured soybean cells. Occurrence and some properties of xyloglucan 4-α-d-glucosyltransferase and 6-α-d-xylosyltransferase. J Biol Chem 256:11117–11122

Hayashi T, Koyama T, Matsuda K (1988) Formation of UDP-xylose and xyloglucan in soybean Golgi membranes. Plant Physiol 87:341–345

Hsieh YL (1999) Structural development of cotton fibers and linkages to fiber quality. In: Basra AS (ed) Cotton Fibers: Developmental Biology, Quality Improvement and Textile Processing. Food Products, New York, pp 137–144

Jacob-Wilk D, Kurek I, Hogan P, Delmer DP (2006) The cotton fiber zinc-binding domain of cellulose synthase A1 from *Gossypium hirsutum* displays rapid turnover *in vitro* and *in vivo*. Proc Natl Acad Sci USA 103:12191–12196

Kearns AE, Vertel BM, Schwartz NB (1993) Topography of glycosylation and UDP-xylose production. J Biol Chem 268:11097–11104

Kobayashi M, Nakagawa H, Suda I, Miyagawa I, Matoh T (2002) Purification and cDNA-cloning of UDP-D-glucuronate carboxylyase (UDP-D-xylose synthase) from pea seedlings. Plant Cell Physiol 43:1259–1265

Kurek I, Kawagoe Y, Jacob-Wilk D, Doblin M, Delmer D (2002) Dimerization of cotton fiber cellulose synthase catalytic subunits occurs via oxidation of the zinc-binding domains. Proc Natl Acad Sci USA 99:11109–11114

Liu JH, Yin CY, Yu FY, Sun QR, Wang YM (1994) Formation mechanism and improvement approach of cotton fiber strength. Sci Agric Sin (in Chinese) 27:10–16

Liu Y, Thoden JB, Kim J, Berger E, Gulick AM, Ruzicak FJ, Holden HM, Frey PA (1997) Mechanistic roles of tyrosine 149 and serine 124 in UDP-galactose 4-epimerase from *Escherichia coli*. Biochemistry 36:10675–10684

Meinert MC, Delmer DP (1977) Changes in biochemical composition of the cell wall of the cotton fiber during development. Plant Physiol 59:1088–1097

Pan YX, Ma J, Zhang GY, Han GY, Wang XF, Ma ZY (2007) cDNA–AFLP profiling for fiber development stage of secondary cell wall synthesis and transcriptome mapping in cotton. Chin Sci Bull 52:2358–2364

Reiter WD, Vanzin GF (2001) Molecular genetics of nucleotide sugar interconversion pathways in plants. Plant Mol Biol 47:95–113

Seifert GJ (2004) Nucleotide sugar interconversions and cell wall biosynthesis: how to bring the inside to the outside. Curr Opin Plant Biol 7:277–284

Suzuki K, Watanabe K, Masumura T, Kitamura S (2004) Characterization of soluble and putative membrane-bound UDP-glucuronic acid decarboxylase (OsUXS) isoforms in rice. Arch Biochem Biophys 431:169–177

Tokumoto H, Wakabayashi K, Kamisaka S, Hoson T (2002) Changes in the sugar composition and molecular mass distribution of matrix polysaccharides during cotton fiber development. Plant Cell Physiol 43:411–418

White AR, Xin Y, Pezeshk V (1993) Xyloglucan glucosyltransferase in Golgi membranes from *Pisum sativum* (pea). Biochem J 294:231–238

Wilkins TA, Jernstedt JA (1999) Molecular genetics of developing cotton fibers. In: Basra AS (ed) Cotton fibers: developmental biology, quality improvement and textile processing. Food Products, New York, pp 231–267

Zhang Q, Shirley N, Lahnstein J, Fincher GB (2005) Characterization and expression patterns of UDP-D-glucuronate decarboxylase genes in barley. Plant Physiol 138:131–141

Zhu YC, Zhang TZ, He YJ, Guo WZ (2006) Gene expression analysis during the fiber elongation period in cotton (*Gossypium hirsutum* L.). Acta Agron Sin (in Chinese) 32:1656–1662