Genetics and Molecular Research

Antisense expression of *Gossypium hirsutum* UDP-glucuronate decarboxylase in *Arabidopsis* leads to changes in cell wall components

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ABSTRACT. UDP-glucuronate decarboxylase (UDP-xylose synthase; UXS, EC 4.1.1.35) is an essential enzyme of the non-cellulosic polysaccharide biosynthetic pathway. In the present study, using transient expression of fluorescently labeled *Gossypium hirsutum* UXS (GhUXS3) protein in onion epidermal cells, we observed that this protein was distributed in the cytoplasm. The *GhUXS3* cDNA of cotton was expressed in an antisense orientation in *Arabidopsis thaliana* by *Agrobacterium tumefaciens*-mediated transformation. Homozygous plants showing down-regulation of UXS were analyzed with northern blots. Compared to the untransformed control, transgenic plant showed shorter roots, earlier blossom formation, and delayed senescence. Biochemical analysis indicated that levels of rhamnose, mannose, galactose, glucose, xylose, and cellulose

were reduced in some of the down-regulated antisense plants. These results suggest that *GhUXS3* regulates the conversion of non-cellulosic polysaccharides and modulates their composition in plant cell walls. We also discuss a possible cellular function for *GhUXS* in determining the quality of cotton fibers.

Key words: *Gossypium hirsutum*; UDP-glucuronate decarboxylase; Transgenic *Arabidopsis*, Down-regulation; Cell wall changes

INTRODUCTION

The spinning quality of cotton (*Gossypium hirsutum*) depends much upon its fiber properties. These fibers are unicellular seed trichomes, developed from individual epidermal cells of the ovules. They are developed during four distinct, yet overlapping, phases, namely initiation, elongation (formation of primary cell walls), secondary cell wall biosynthesis, and maturation (Basra and Malik, 1984). Building a fiber matrix during the two middle phases requires the synthesis and cross-linking of abundant complex polysaccharides, including cellulose, hemicelluloses, and pectins. Because fiber strength is determined by this cellulosic and non-cellulosic network (Hsieh, 1999; Wilkins and Jernstedt, 1999), it is critical that researchers understand this process of formation and the related system for enzyme functioning. Although, numerous studies have been conducted on cotton fiber cellulose, little is known about the non-cellulose components, e.g., xylose, arabinose, galactose, rhamnose, xyloglucan, and pectin. These components are mainly involved in the metabolism of UDP-xylose, a key nucleotide sugar (Reiter and Vanzin, 2001). In cotton fibers, xylose and arabinose contents gradually increase until secondary cell wall synthesis begins (Meinert and Delmer, 1977).

UDP-xylose is derived from UDP-glucose in two steps: 1) UDP-glucose dehydrogenase (UGD, EC 1.1.1.22) catalyzes the formation of UDP-glucuronic acid (UDP-GlcA) from UDP-glucose, and 2) UDP-GlcA is irreversibly converted to UDP-xylose by UDP-glucuronic acid decarboxylase (UDP-xylose synthase; UXS, EC 4.1.1.35; Zhang et al., 2005). The UXS family belongs to the short-chain dehydrogenase/reductase superfamily (Moummou et al., 2012). In plants, different UXS isoforms occur in the cytosol and membrane-bound compartments (Harper and Bar-Peled, 2002).

UXS has a central role in sugar nucleotide inter-conversion. The first UXS was cloned from a fungus, *Cryptococcus neoformans* (Bar-Peled et al., 2001). Its activity has now been reported in other microorganisms, vertebrate species, and plants. From various species of bacteria, 826 predicted UXS enzymes have been identified, suggesting that xylose is more prevalent in bacterial glycans (Coyne et al., 2011). In zebrafish, UXS1 activity is essential for functional deposition of proteoglycans in the extracellular matrix (Eames et al., 2010). The crystal structure, molecular dynamics, and response pathways of UXS have also been analyzed in humans (Eixelsbergeret al., 2012). At least five UXS protein isoforms have been isolated from *Arabidopsis* (Harper and Bar-Peled, 2002), while *UXS* has been cloned from pea (*Pisum sativum*) seedlings (Kobayashi et al., 2002). Six *UXS* genes have been detected in rice (Suzuki et al., 2004). In tobacco, several *UXS*s have been purified and expressed in tissues associated with secondary wall formation. Down-regulation of some of these genes by antisense expression has been associated with reduced xylan contents in such tissues (Bindschedler et al., 2007).

We previously isolated three *UXS* genes, *GhUXS1* to *3*, from cotton (Pan et al., 2010). Their roles in affecting the biosynthesis of cell wall polysaccharides and fiber traits are not well

understood. Because *GhUXS3* lacks the N-terminal extension found in the other two, we have been investigating its functioning. This gene has a 1038-bp open reading frame (ORF) and encodes a protein of 345 amino acids, with a calculated molecular mass of 38.98 kDa. Sequence alignment revealed that this protein contains a highly conserved motif of the UXS family.

Arabidopsis is a powerful model system for studying cell wall biosynthesis and the functions of various cell wall polymers (Liepmanet al., 2010). Therefore, we used an *Arabidopsis* knockout mutant for *GhUXS3* to determine the role of individual GhUXS enzymes and to test the function of this gene during cell wall formation in plants.

MATERIAL AND METHODS

Subcellular localization of GhUXS3 and GFP fusion proteins expressed in onion epidermal cells

For subcellular localization of GhUXS3 protein, we transiently expressed pCamE-UXS3:GFP fusion protein in live onion epidermal cells. *GhUXS3* was fused to the pCamE:GFP expression vector under the control of a constitutive CaMV 35S promoter, upstream of the GFP sequence. The coding region of *GhUXS3* contained digestion sites for *Xba*I and *Kpn*I restriction enzymes (TaKaRa, China). After amplification with primers 5'-TCTAGAATGGCGACAGATTCATCAAATGG-3' and 5'-GGTACCCTCTTCAGAGATTCCAAGCCTC-3' (restriction sites underlined), the polymerase chain reaction (PCR) product and the pCamE:GFP plasmid were purified, digested with *Xba*I and *Kpn*I, and ligated using T_4DNA ligase (NEB, America). The correct-sized recombinant pCamE-UXS3:GFP and the control pCamE-GFP plasmids were transferred into the epidermal cells using a gene gun (PDS-1000/He Particle Delivery System; Bio-Rad, USA), according to the manufacturer instructions. The transformed cells were incubated on Murashige and Skoog (MS) agar plates under darkness at 25°C for 36 to 48 h. Thereafter, we examined the localization of the GFP fusion protein and the control pCamE-GFP under blue light with a fluorescence microscope (Olympus BX51, Japan).

Growing of *Arabidopsis thaliana* plants

Seeds of *A. thaliana* ecotype 'Columbia-0' were placed on plates in dark for 2 to 4 days at 4°C to break their dormancy. The vernalized seeds were germinated in a greenhouse under a 16-h photoperiod and 60-70% relative humidity. The resultant plants that reached the bolting stage and formed floral inflorescences were used in the transgenic experiments.

Construction of plant antisense-expression vectors and *Arabidopsis* transformation

The *GhUXS3* coding sequence was PCR-amplified from its cDNA *Sal*I and *Xba*I site was introduced at the 5'- and 3'-end of the ORF, respectively. The amplifications were performed with primers 5'-GAGCTCGATGGCGACAGATTCATC-3' and 5'-GCTCTAGACTCTTCAGAGATTCCAA GCCTC-3' (restriction sites underlined). The PCR product was cloned into pGM-T vector (TIANGEN, China). The plasmid DNA containing an accurate *GhUXS3* ORF was digested with *Sal*I and *Xba*I. It was then sub-cloned in the antisense orientation at the corresponding sites in the expression vector, pBI121, to generate a pBI121:*GhUXS3* chimeric construct under the control of the constitutive 35S promoter.

The authenticated construct was transferred into *Agrobacterium tumefaciens* strain GV3101 and cultured for 2 days at 28°C on a Luria broth selective medium containing 100 mg/L kanamycin and 50 mg/L streptomycin. The transformed bacteria were used to infect 'Columbia-0' plants by the floral-dip method (Clough and Bent, 1998). The primary transformants (T_1) were collected from the regenerated T_0 plants, and the T_1 seeds were selected on a ½-strength MS selective medium supplemented with 100 mg/L kanamycin. The PCR positive T_1 seeds were self-pollinated to obtain homozygous T_2 transformants. After sowing, selection, and self-pollination of T_2 transformants, T_3 plants were obtained. Only those lines showing kanamycin resistance were considered homozygous and were selected for further analyses.

Northern blot analysis of *GhUXS3*

The expression of *GhUXS3* mRNA in transgenic *Arabidopsis* was analyzed by northern blot analysis. Total RNA was extracted from positive T_3 transgenic lines and one 'Columbia-0' wild-type (WT) control plant, using TRIZOL reagent (TransGen, China). The extracted RNA was combined with 8 µL formaldehyde and 5 µL 10X MOPS (morpholinopropanesulfonic acid, 200 mM MOPS, 50 mM sodium acetate, 20 mM EDTA, pH 7.0) and heated at 65°C for 10 min. Thereafter, the samples were cooled on ice for 5 min, 6 µL 10X loading buffer was added to them and they were separated on agarose gels containing 10X MOPS. The electrophoresed RNA was transferred by capillary blotting onto Hybond N^+ nylon membranes (Amersham Pharmacia, USA). Probes for the RNA gel blot were PCR-amplified from the pBI121:*GhUXS3* chimeric vector with the primers 5'-CCCCACCCACGAGGAGCAT-3' and 5'-TCCATCCACAACCCGAGACATACT-3'. The digoxigenin-labeling of the probe, its denaturation, and northern blot analysis were performed with a DIG DNA Labeling and Detection Kit II (Roche, Germany) according to the manufacturer instructions. The membranes were hybridized overnight at 42°C and washed twice, for 20 min each, in 2X SSC and 0.1% SDS followed by two washes with 0.5X SSC and 0.1% SDS at 65°C; the membranes were subsequently exposed to X-ray film.

Phenotypic analysis of transgenic *Arabidopsis* lines

After surface-sterilization with antiformin, seeds of the positive T_3 transgenic lines and the WT control were germinated on plates containing non-selective ½-strength MS. Root lengths were measured from one-week-old plants of all the genotypes. After another five weeks, we evaluated the performance of the transgenics and WT by comparing the floral stem and rosette leaf characteristics of these six-week-old plants.

Cell wall preparation and analysis

Cell wall extraction

Arabidopsis cell walls were isolated according to the method of Diet et al. (2006). Six weeks after germination, 3-g samples of stems and leaves from transgenic seedlings were homogenized with a mortar and pestle under liquid nitrogen followed by rapid addition of 30 mL 70% ethanol. The homogenate was centrifuged at 2683 *g* for 5 min at 4°C, and the supernatant was discarded. The above steps were repeated twice. A 20-mL 1:1 (v:v) chloroform:methanol mixture was added and the supernatant was centrifuged at 2683 *g* for 5 min at 4°C three times. The resulting cell wall

residues were washed three times in 100% acetone and air-dried. The residues were sequentially incubated overnight with α-amylase (5 U/mg sample) and DMSO to eliminate the starch completely. The deposit was washed three times in ultrapure water and once in 100% acetone.

Analysis of non-cellulosic monosaccharides

The non-cellulosic monosaccharides in the air-dried cell wall residues were analyzed by HPLC (high performance liquid chromatography); 1-phenyl-3-methyl-5-pyrazolone (PMP) was used for pre-column-derivatization (Fu and O'Neill, 1995; Rozaklis et al., 2002). Standard samples of rhamnose (Rha), mannose (Man), galactose (Gla), glucose (Glc), and xylose (Xyl) were dissolved in water, diluted to a concentration of 3.6 g/L, and used as the internal standard. To 50 µL standard solutions, 50 µL 0.6 M NaOH and 100 µL 0.5 M PMP was added. The mixture was heated at 70°C for 100 min, cooled for 10 min, and acidified with 10 µL hydrochloric acid. It was then extracted twice with the same volume of 1:1 (v:v) chloroform:methanol mixture before being centrifuged at 10,000 rpm for 5 min. To ensure that the excess PMP had been completely removed, the extraction was repeated twice. The aqueous phase was passed through a 0.22-µm filter membrane and injected into a Promosil C18 column (Shimadzu LC-20AB, Japan) at 40°C. The mobile phase, which was a mixture of ammonium acetate (pH 5.5) and acetonitrile (70:30, v/v), was used at a flow rate of 1.0 mL/min; the sample injection volume was 20 µL, and the detection was done at 254 nm.

A mixture of cell wall residues (5 mg, described above) and 1.5 mL 2 MTFA (methyl trifluoroacetic acid) was hydrolyzed at 121°C for 2 h. Following air-drying, 500 µL 0.3 M NaOH was added and the solution was centrifuged at 10,000 rpm for 10 min. The supernatant (100 µL) was combined with 100 µL PMP, the mixture was incubated at 70°C for 100 min and cooled. The treated product was then neutralized, extracted, filtered, and analyzed by HPLC following the method described above for preparing the monosaccharide internal standard solution.

Analysis of cellulose concentration

The level of cellulose in *Arabidopsis* cell walls was determined by acolorimetric method using anthrone reagent (Updegraff, 1969). Briefly, 5 mg treated cell wall residue was boiled for 30 min with an 8:1:2 (v:v:v) mixture of acetic acid:nitric acid:H_2O. The sample was cooled and centrifuged at 4000 rpm for 5 min at 4°C. The pellet was washed with H_2O and then with acetone before being air-dried. It was then dissolved in 72% (v/v) H_2SO_4 for 1.5 h at room temperature (RT), diluted 10 times with 72% H_2SO_4, and centrifuged. A mixture of 100 µL supernatant and 900 µL ultrapure water was prepared, to which 2 mL anthrone was added rapidly. After rapid cooling, the mixture was boiled for 15 min, transferred back to ice, and kept at RT for 5 to 10 min. Absorbance of the sample was measured at 620 nm with a Beckman DU800 spectrophotometer (Beckman, Ameica). The results were compared with a standard curve of glucose (produced simultaneously) to calculate the concentration of cellulose.

RESULTS

Cytoplasmic localization of GFP-fused GhUXS3

Onion epidermal cells transformed with the pCamE:GFP vector displayed green fluorescence in the nucleolus and plasma membrane (Figure 1A). Cells transformed with pCamE-

UXS3:GFP showed fluorescence in the mesh structure around the perinuclear region (Figure 1B) and intracellular vesicular structures (Figure 1C). This pattern of distribution indicated that GhUXS3 encoded a cytoplasmic protein. Harper and Bar-Peled (2002) also reported that UDP-Xyl biosynthesis occurred in both the cytosol and membrane-bound compartments.

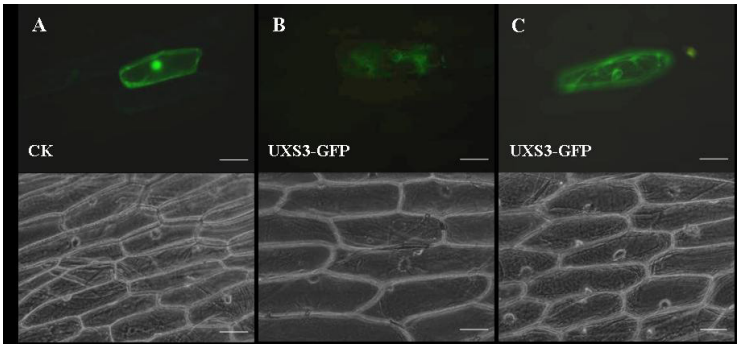

Figure 1. Subcellular localization of GhUXS3 fused with GFP in onion cells. Images of green fluorescence for pCamE:GFP vectors (**A**), and pCamE UXS3:GFP vectors (**B**, **C**). Bright-field images of cells are shown in lower panels. Scale bars = 20 μm.

Expression analysis of *GhUXS3* antisense-transgenic *Arabidopsis* plants

To gain further insights into the function of *GhUXS3* during *Arabidopsis* development, we constructed a recombinant plasmid, pBI121:*GhUXS3* (Figure 2). Using digoxigenin-labeling, we generated a 750-bp *GhUXS3* probe from the pBI121:*GhUXS3* chimeric construct (Figure 3). The transgene was detected in three transgenic lines (AU3-16-13, AU3-22-1, and AU3-26-2) using northern blot analysis; the three independent T_3 transgenic lines had a similar degree of expression, with antisense-*GhUXS3* transcript levels being higher than those detected in the WT (Figure 4).These results demonstrated that the antisense *GhUXS3* was transcribed steadily in the transgenic *Arabidopsis* plants.

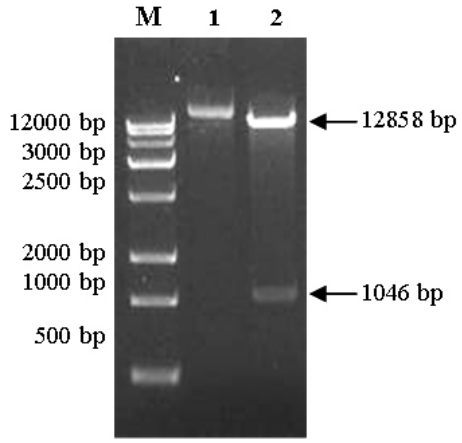

Figure 2. Characterization of antisense expression vector pBI121:*GhUXS3*. *Lane M* = wide molecular weight marker 500-12,000 bp; *lane 1* = pBI121:*GhUXS3* plasmid; *lane 2* = pBI121:*GhUXS3* plasmid digested with *Sal*I and *Xba*I.

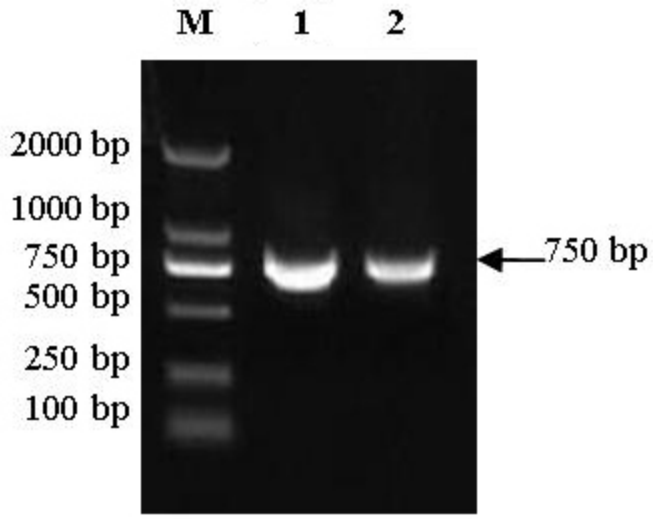

Figure 3. Antisense *GhUXS3*-amplified probe for northern blotting. *Lane M*, DL2000 molecular weight marker; *lane 1* = template DNA; *lane 2* = probe of antisense *GhUXS3*.

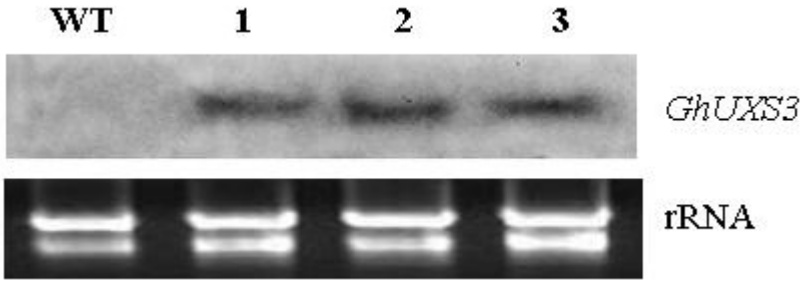

Figure 4. Northern blot analysis of T_3 transgenic *Arabidopsis* plants with pBI121:*GhUXS3*. *Lane WT* = 'Columbia-0' wild-type control; *lanes 1* to *3*, transgenic *lines* AU3-16-13, AU3-22-1, and AU3-26-2, respectively.

Phenotypic alteration of T_3 lines

To characterize the possible roles for *GhUXS3* in plants, we examined the three transgenic *Arabidopsis* lines that expressed antisense-GhUXS3 and noted their distinctive features compared to the WT. Root lengths differed significantly after 7 days of development (Figure 5), with those of transgenic lines being 7% (4.77 ± 2.26) to 30% (3.60 ± 2.30 mm) shorter than those of the WT (5.15 ± 2.31; Table 1).

Antisense-expression of *GhUXS3* continued throughout the 85- to 90-day lifespan of the transgenic *Arabidopsis*, a period that was approximately 20 days longer than the 70-day life span of the WT. The transgenic lines also bloomed earlier. For example, by day 35, the transformed plants had already flowered or bolted while the WT was still in the vegetative stage (Figure 6).

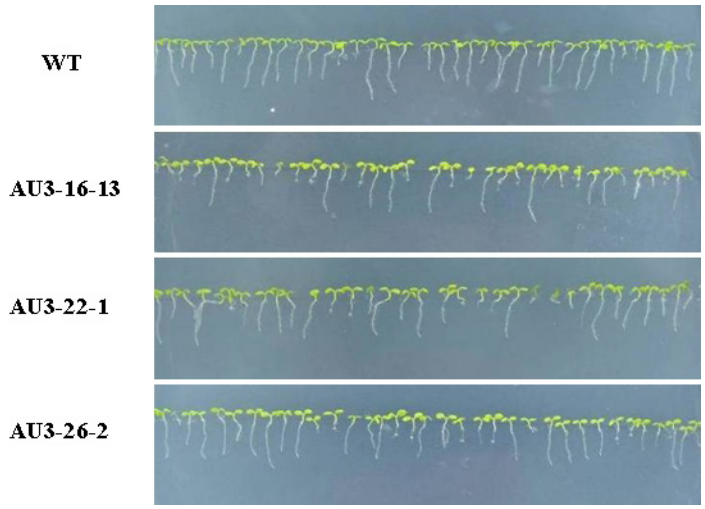

Figure 5. Roots of T_3 transgenic *Arabidopsis* plants with antisense *GhUXS3* after 7 days of growth.

Table 1. Growth characteristics of T_3 *Arabidopsis* plants with expressing antisense *GhUXS3.*

	WT	AU3-16-13	AU3-22-1	AU3-26-2
Length of floral stem (cm)	15.90 ± 3.78	27.90 ± 2.86**	23.80 ± 4.33**	16.80 ± 3.87
Diameter of floral stem (mm)	0.85 ± 0.10	1.02 ± 0.08**	0.93 ± 0.12**	0.82 ± 0.08
Length of rosette leaves (mm)	27.58 ± 3.24	31.32 ± 2.55**	30.05 ± 4.34*	22.46 ± 3.12
Toughness of floral stem (N)	14.00 ± 3.30	18.30 ± 4.70**	18.50 ± 4.30**	13.90 ± 3.30

*Differences significant at the 0.05 level, **differences significant at the 0.01 level (Two-tail *t*-test).

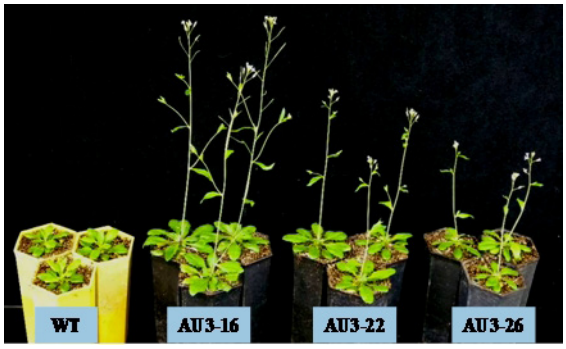

Figure 6. T_3 transgenic *Arabidopsis* plants with antisense *GhUXS3* after 5 weeks of growth.

After bolting, the stem lengths were 27.90 ± 2.86 cm (AU3-16-13) and 23.80 ± 4.33 cm (AU3-22-1), while the respective stem diameters were 20 and 9% larger (1.02 ± 0.08 and 0.93 ± 0.12 mm for AU3-16-13 and AU3-22-1 versus 0.85 ± 0.10 mm for the WT). Transgenic rosette leaves were also 2 to 4 mm longer than those of the WT. Toughness of floral stems increased more than 30% in those two transgenic lines (Table 1). In contrast, the third line, AU3-26-2, did not differ significantly from the WT in these phenotypic parameters.

Characterization of cell wall saccharides in antisense lines

Because the loss of *GhUXS3* functioning might have affected the availability of UDP sugars that are directly or indirectly derived from UDP-Xyl, we determined the compositions of sugar monomers in cell walls of the WT and transgenic plants using HPLC. The results revealed that Man, Rha, Gla, Glc, and Xyl concentrations were reduced in AU3-16-13 and AU3-22-1. In contrast, AU3-26-2 did not show significantly elevated concentrations of Man and Xyl when compared with the WT. The Glc/Xyl ratio in the cell walls from the antisense plants was 23.58 to 40.57% higher than in the controls (Table2). Using the colorimetric method, we found that the cellulose concentrations were significantly lower in the transgenics than in the control, by 16.35% for AU3-16-13, 28.30% for AU3-22-1, and 30.50% for AU3-26-2 (Figure7). This indicated that the cell wall components were modified in the antisense *Arabidopsis* plants.

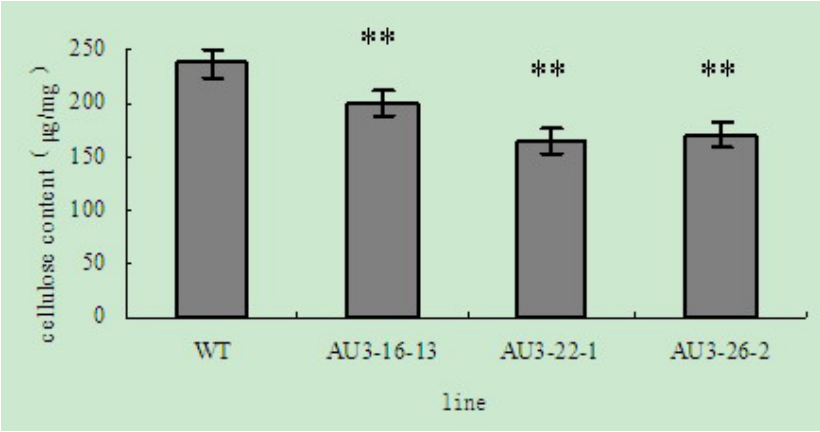

Figure 7. Cellulose concentration in the cell walls of T_3 transgenic *Arabidopsis* plants after 6 weeks of growth. **Significant differences at 0.01 level (Two-tail *t*-test with SPSS).

Table 2. Composition of cell wall saccharides (μg/mg cell wall) in *Arabidopsis* plants, as analyzed by HPLC.

	Man	Rha	Gal	Glc	Xyl	Glc/Xyl
WT	4.15 ± 0.73	7.17 ± 0.99	1.97 ± 0.45	8.58 ± 1.19	8.09 ± 1.89	1.06
AU3-16-13	3.95 ± 0.83	7.01 ± 2.79	1.87 ± 0.60	4.93 ± 0.19	6.56 ± 0.25	0.75
AU3-22-1	3.33 ± 1.42	4.26 ± 2.05	1.12 ± 0.47	3.08 ± 0.48	3.78 ± 0.21	0.81
AU3-26-2	4.20 ± 0.82	6.52 ± 1.22	1.75 ± 0.17	6.38 ± 1.10	8.20 ± 1.93	0.78

DISCUSSION

Many of the precursors for non-cellulosic polysaccharides are formed via UDP-Xyl, which is converted into pectin and hemicellulosic polysaccharides. Therefore, UXS has a crucial role for the conversion of diverse polysaccharides in the cell walls of plant, fungal, and bacterial glycans, and glycosaminoglycans in higher organisms (Reisset al., 1985; York and O'Neill, 2008; Coyne et al., 2011; Eixelsberger et al., 2012). At least three *GhUXS* genes are involved in the biosynthesis of fiber cell walls in cotton. Results from our previous reverse transcriptase-PCR analysis suggested that these genes were preferentially expressed during the different developmental stages, from

elongation to secondary cell wall synthesis, with trace levels of transcripts being detected in the roots, hypocotyls, and leaves (Pan et al., 2010). Here, we confirmed that *GhUXS3* encodes a cytoplasmic protein. We produced *GhUXS3* down-regulated transgenics of *Arabidopsis* with altered metabolism of cell wall polysaccharides as well as a distinctly modified phenotype. These findings implicate the gene in cotton fiber development.

Antisense expression of *GhUXS3* causes alterations to phenotype and polysaccharide compositions

Concentrations of Glc, Xyl, Man, Rha, and Gal were lower in AU3-16-13 and AU3-22-1 than in the WT. Antisense-transgenic tobacco also contained less Xyl when UXS was down-regulated (Bindschedler et al., 2007). Thus, the observed decline in the Xyl levels was related to lower expression of *GhUXS3*. As a result, the indirect conversion of Man, Rha, and Gal from UDP-Xyl was also decreased. UDP-Xyl feedback can inhibit the upstream enzymes (UXS, UGD, and UDP-Glc pyrophosphorylase), thereby, controlling the flux for the conversion of UDP-Glc to UDP-GlcA, UDP-Xyl, UDP-Ara, and UDP-GalA in plants (Harper and Bar-Peled, 2002).

When UXS is down-regulated, the UDP-Xyl feedback inhibition of UGD may also disappear. This can cause increased activity of UGD, a greater accumulation of UDP-GlcA, and increased conversion of UDP-GlcA to UDP-galacturonic acid. A series of changes leads to the diversification of polysaccharide composition in the cell walls. Furthermore, antisense expression of *GhUXS3* was manifested by longer floral stems and rosette leaves, tougher stems, and delayed senescence. These phenotypes contrast with those reported in the *Arabidopsis* mutant *ugd2,3*, in which a loss-of-function was associated with dwarfism and retarded development (Reboul et al., 2011). Because reduced *UXS* expression may enhance the UGD activity, it is possible that the reverse phenotype might occur with *UXS* and *UGD* mutants, thus confirming that GhUXS is active in cell wall development. Moreover, decreased expression of *UXS* was related to greater floral stem toughness and lower cellulose concentrations. Toughness is mainly reflected in the strength of the secondary cell wall; less cellulose means that mechanical strength is diminished (Hu and Han, 2008). Therefore, GhUXS can affect the quality of secondary cell walls.

In the present study, UDP-Glc and cellulose were reduced in the antisense *GhUXS3* transgenic lines. Because UDP-Glc mainly produces UDP-Xyl and cellulose in plants, down-regulation of *GhUXS3* would result in a reduction in Glc and cellulose concentrations. We observed that the levels of Man, Xyl, Rha, and Gal were higher in one of our transgenic lines than in the WT. This might have arisen because of the differences in the insertion sites or because of functional redundancy among the members of the UXS gene family (Bindschedler et al., 2007).

Speculative function of *GhUXS3* in determining cotton fiber quality

We previously proposed a model describing how GhUXS expression influenced fiber quality in cotton (Pan et al., 2010). UDP-XYL synthase 5 (UXS5) was down-regulated during the elongation stage in fuzzless-lintless ovules of a cotton *fl* mutant (Padmalatha et al., 2012). This evidence demonstrates that *GhUXS3* is closely correlated with the formation of cotton fibers.

Cell wall polysaccharides influence cell division and growth; changes in the amounts of some of these components might trigger modifications in others (Ringli, 2010; Wallace and Anderson, 2012). Lignification and microfibril orientation are thought to be mediated by alterations in the amounts and distribution of non-cellulosic polysaccharides (Donaldson and Knox, 2012).

Because fasciclin-like arabinogalactan proteins (FLAs) are related to pectin biosynthesis, *GhFLA1* RNAi lines had more pectin, arabinose, and galactose, less hemicellulose and cellulose, and a significant reduction in their proportions of glucose when compared with the WT (Huang, et al., 2013). Our results demonstrated that the antisense *GhUXS3* greatly altered the biochemical composition of cell walls, similar to the findings with the *GhFLA1* RNAi lines. These modifications imply that *GhUXS3* can affect pectin biosynthesis, stimulate changes in polysaccharide compositions, and influence cell wall development.

In conclusion, we propose that, when *GhUXS3* expression is modulated, the conversion and the concentration of non-cellulosic polysaccharides is affected. A series of alterations can enhance the levels of both cellulosic and non-cellulosic polysaccharides, and change the cross-link structure, especially during the stages of elongation and secondary cell wall biosynthesis. Further studies need to be undertaken to clarify the mechanism by which *GhUXS3* influences cotton fiber qualities.

Conflicts of interest

The authors declare no conflict of interest.

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