TITLE: **Cellulose Synthase Catalytic Subunit (*Cesa*) Genes Associated With Primary or Secondary Wall Biosynthesis in Developing Cotton Fibers (*Gossypium hirsutum*)**

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AUTHORS:

Hee Jin Kim
Department of Biological Sciences
University of New Orleans
2000 Lakeshore Drive
New Orleans, LA 70148

Barbara A. Triplett (Corresponding author)
USDA-ARS
Southern Regional Research Center
1100 Robert E. Lee Blvd.
New Orleans, LA 70124
Phone: 504-286-4275
Fax: 504-286-4419
Email: btriplet@srrc.ars.usda.gov

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ABBREVIATIONS: *CesA*s, cellulose synthase catalytic subunits; DOA, day of anthesis; DPA, days post-anthesis.

Cellulose Synthase Catalytic Subunit (*Cesa*) Genes Associated With Primary or Secondary Wall Biosynthesis in Developing Cotton Fibers (*Gossypium hirsutum*)

ABSTRACT

Cotton fibers are unicellular seed trichomes and consist of almost pure cellulose. During the transition from elongation growth to secondary wall thickening, the rate of cellulose biosynthesis in fibers rises nearly 100-fold. Although the first two cellulose synthase catalytic subunits (*CesA*s) were isolated from developing cotton fibers, it is not clear how many *CesA*s are involved in either primary or secondary wall synthesis in fibers, or how these genes are regulated during cell wall biogenesis. Cotton (*Gossypium hirsutum*) gene sequences from public databases that contained *CesA* motifs were classified into fourteen distinct *CesA* genes. The expression pattern of these genes was measured in field grown fibers for two growing seasons using quantitative RT-PCR. The first group of six *GhCesA* genes is preferentially expressed in elongating fibers, hypocotyls, and roots. The second group of six *CesA* genes is expressed specifically during the secondary wall thickening stage of fiber development. A third group comprised of two *CesAs* is expressed throughout fiber development. The results show that most *GhCesA* genes are developmentally regulated during fiber development, and that distinct multiple *CesA* genes are involved in either primary or secondary wall cellulose biosynthesis in cotton fibers. Recent progress on understanding the molecular basis for regulation of secondary wall *CesA* genes will also be discussed.

KEY WORDS:

Cellulose synthase catalytic subunit; cotton fiber; gene expression; *Gossypium hirsutum*; primary cell wall; quantitative RT-PCR; secondary cell wall.

A mature cotton fiber consists of almost pure cellulose and is a unicellular seed trichome. Cotton fibers (*Gossypium hirsutum*) elongate from the ovule epidermis during the elongation stage from the day of anthesis (DOA) to approximately 21 to 26 days post anthesis (DPA) (Basra and Malik, 1984). When synthesis of the fiber's secondary wall begins at approximately 15 to 16 DPA, fiber elongation declines, the rate of cellulose synthesis in cotton fibers is estimated to increase nearly 100-fold *in vivo*, and synthesis of other cell wall polymers ceases (Meinert & Delmer, 1977; Tokumoto et al., 2002). There are multiple developmental programs controlling gene expression throughout cotton fiber development (Delmer et al., 1995; Pear et al., 1996; Smart et al., 1998; Shimizu et al., 1997; Whittaker and Triplett, 1999).

Cellulose, a polymer of β-(1,4) glucose, is synthesized by a plasma membrane associated, multisubunit enzyme called cellulose synthase that is localized in six-fold symmetric complexes referred to as rosettes in the plasma membrane (Muller and Brown, 1980; Kimura et al., 1999). Cellulose synthase is a large multisubunit enzyme utilizing uridine diphosphate-glucose (UDP-Glc) as a substrate (Delmer, 1999; Doblin et al., 2002). The first two catalytic subunits (*CesA*s) of cellulose synthase were isolated from developing cotton fibers by comparing the nucleotide sequences of cotton fiber genes expressed during secondary wall formation with a bacterial cellulose synthase sequence (Pear et al., 1996). In *Arabidopsis,* at least 10 *CesA* isoforms exist (Richmond and Somerville, 2000). All *CesA* isoforms contain the conserved D, D, D, QXXRW motif characteristic of β-glycosyltransferases (Saxena et al., 1995) and zinc-binding domains (Kurek et al., 2002). Analyses of *Arabidopsis* cellulose-deficient mutants showed that each *CesA* plays a distinct role in the cellulose synthetic process. *AtCesA1* (Arioli et al., 1998), *AtCesA3* (Scheible et al., 2001), and *AtCesA6* (Fagard et al., 2000; Scheible et al., 2001) are involved in cellulose synthesis in primary walls of root and hypocotyl. *AtCesA4, AtCesA7*, and *AtCesA8*

(Taylor et al., 1999, 2000, 2003) are involved in secondary wall cellulose synthesis in xylem cells. Thus, it appears that three different *AtCesA*s are essential components of cellulose synthase, and the presence of all three *CesA* proteins is required for correct assembly and function (Taylor et al., 2003).

Although cotton fibers are a good experimental model for studying cellulose synthesis (Kim and Triplett, 2001), the progress of *CesA* research in *G. hirsutum* has been relatively slower than for other plants. The genomic sequences of only three *G. hirsutum CesA* genes (*GhCesA1, GhCesA3,* and *GhCesA4*) are in public databases (Pear et al., 1996; Laosinchai et al., 2000; Kim & Triplett, 2001; Grover et al., 2004). *GhCesA1, 2*, and *4* are up-regulated immediately prior to the secondary wall cellulose biosynthesis stage of fiber development (Pear et al., 1996; Triplett & Kim, 2006). *GhCesA3* and *5* were shown to be expressed throughout fiber development by non-quantitative analyses (Laosinchai et al., 2000; Li et al., 2002).

To determine if *CesA* gene*s* are specifically involved in primary or secondary wall cellulose biosynthesis, we analyzed *CesA* sequences in public databases and determined gene expression patterns of fourteen different *CesAs* in developing fibers and other tissues from *G. hirsutum* by quantitative RT-PCR. Our results show that most *GhCesA* genes are developmentally regulated, and distinct sets of *CesA* genes are involved in cellulose biosynthesis in either primary or secondary walls of cotton fibers.

MATERIALS AND METHODS

Plant materials. Cotton plants (*Gossypium hirsutum* L., DPL90) were grown in the field in New Orleans during the summer of 2002 and 2003. Immature seeds with fiber were harvested by 9AM and frozen in liquid nitrogen. Developing bolls were collected at 4-d intervals from 8 through 20 DPA. Fibers were carefully scraped from the frozen ovules using a scalpel. Fully

grown leaves (15 cm in diameter), expanding young leaves (5 cm in diameter), hypocotyls and roots were harvested from 1- or 6-wk old plants grown in a greenhouse at 25^{o}C to 32^{o}C. All tissues were frozen in liquid nitrogen, and stored at -80^{o}C.

RNA extractions and quantitative RT-PCR. Total RNA was extracted from cotton fibers and other tissues at different developmental stages by phenol extraction and LiCl precipitation (Schultz et al., 1994). Isolated total RNAs were treated with DNase I (Ambion, Austin, TX) and purified with an RNeasy kit (Qiagen, Valencia, CA). First-strand complementary DNA was synthesized from total RNA by priming with hexamers using TaqMan Reverse Transcription Reagents (Applied Biosystems, Foster City, CA). Gene-specific primers (Table I) were designed using Primer Express software Version 2.0 (Applied Biosystems, Foster City, CA). Quantitative RT-PCR was performed according to the method of Kim and Triplett (2004). Statistical analyses were performed using Graphpad Prism version 4.00 software.

RESULTS AND DISCUSSION

Fourteen putative *CesA* genes from *Gossypium hirsutum*. *Gossypium hirsutum CesA* genes (*GhCesAs*) belong to a multigene family. Other than the published nucleotide sequences of *GhCesA1* (Pear et al., 1996; Grover et al., 2004), *GhCesA2* (Pear et al., 1996), *GhCesA3* (Laosinchai et al., 2000), *GhCesA4* (Kim and Triplett, 2001), and *GhCesA5* (Li et al., 2004), only fourteen ESTs containing the *CesA* motif were in the National Center for Biotechnology Information (NCBI) database when this project was launched. Thus, nineteen putative *CesA* genes from diverse cultivars of *G. hirsutum* (Table II) were analyzed by homology searches (Altschul et al., 1997), Clustal W (Higgins et al., 1994) and T-Coffee (Notredame et al., 2000). Subsequently they were classified as 14 different *GhCesA*s consisting of three full-length (*GhCesA1, 3, and 4*) and 11 truncated sequences (*GhCesA2* and 10 ESTs). Three of them

(*GhCesA1, 2,* and *4*) were reported to be involved in secondary wall cellulose biosynthesis (Pear et al., 1996; Triplett and Kim, 2006). Sequence analysis of two BAC sequences (AY632359 and AY632360) (Grover et al., 2004) and expression analyses (Triplett and Kim, 2006) revealed that *GhCesA1* and *GhCesA4* are homeologous genes of the D and A subgenomes. *GhCesA2* is a 5'-truncated partial cDNA from *G. hirsutum* L., Acala SJ-2, and shares 65 % nucleotide similarity with *GhCesA1* (Pear et al., 1996). In an attempt to obtain the promoter of *GhCesA2* (AF254895), a primer designed from the 5'-terminal sequence of 5'-truncated *GhCesA2* cDNA was used to amplify genomic DNA from *G. hirsutum* L., Coker 130 using PCR-based walking. The *GhCesA2* genomic sequence consists of 1.7 kb of 5'-flanking region and 6 exons of 5'-terminal region of *GhCesA2* cDNA; however, there are no overlapping sequences between the *GhCesA2* cDNA and *GhCesA2* gene. Intergenic chimeras can form during PCR amplification from allotetraploid cotton, *G. hirsutum* (Cronn et al., 2002). Thus, it is not clear if the putative *GhCesA2* genomic sequence is identical to or merely similar to *GhCesA2* cDNA. A full-length *GhCesA3* was reported to be constitutively expressed throughout fiber development (Laosinchai et al., 2000). Nine ESTs (*GhEST1~ 9*) show differing levels of sequence similarity with full-length *GhCesA1, 3,* and *4*. *GhEST2* consists of three overlapping ESTs, and one of them, named *GhCesA5* (BM356396), was expressed throughout fiber development (Li et al., 2002). *GhEST8* and *GhEST9* were identified from unpublished EST sequences obtained from a limited survey of ESTs from 15 DPA cotton fiber and the NCBI database. Since the deduced amino acid sequences of *GhEST8* and *GhEST9* are similar to an Arabidopsis *CesA* gene predominately expressed in cells producing secondary walls (*AtCesA7*; Taylor et al., 1999), they were subsequently named *GhCesA6* and *GhCesA7*.

Recently, collaborating efforts within the cotton research community have substantially increased the numbers of cotton ESTs in the DFCI Cotton Gene Index (CGI, Release 8.0 at http://compbio.dfci.harvard.edu/tgi/cgi-bin/tgi/gimain.pl?gudb=cotton). According to CGI Release 8.0, there are 31 different *CesA* genes from *G. hirsutum* composed of 22 tentative consensus sequences (TC) and 9 singletons. As a result, our expression analyses reported here cover approximately half of the *CesA* genes from *G. hirsutum*. Interestingly, CGI classifies two homeologous genes (*GhCesA1* and *GhCesA4*) as an identical gene (TC6623). Also, CGI erroneously classifies *GhEST2* (*GhCesA5,* AI729981, and AI725882) and *GhEST4* (AI726753) as the same *CesA* (TC66614). We have verified that *GhEST2* (*GhCesA5*) is different from *GhEST4* by careful sequence analyses, although we cannot conclude if they are homeologous genes at this time. We suspect the CGI programs for analyzing TC sequences may be designed to overlook a few sequencing errors that are typically found many times in ESTs. As a result, very similar sequences in a multigene family or homeologous genes containing a very low rate (ks = 0.041) of substitutions were mistakenly analyzed as identical genes.

Phylogenetic comparison of plant *CesA* genes. Phylogenetic relationships of *CesA* genes from *G. hirsutum* (*GhCesAs*) were compared with those from Arabidopsis (*AtCesA*), maize (*ZmCesA*), aspen (*PtrCesA*) and rice (*OsCesA*) (Fig. 1). Three Arabidopsis *CesA* genes (*AtCesA4*, *AtCesA7*, and *AtCesA8*) and three rice *CesA* genes (*OsCesA4, OsCesA7*, and *OsCesA9*) are known to be coordinately involved in secondary wall synthesis in diploid plants (Taylor et al., 1999, 2000, 2003; Tanaka et al., 2003). Another set of three Arabidopsis *CesA*s (*AtCesA1, AtCesA3*, and *AtCesA6*) are expressed primarily in cells having primary cell walls, principally in roots and hypocotyls (Arioli et al., 1998; Scheible et al., 2001; Fagard et al., 2000). Thus, we predict that there will be at least six *GhCesA* genes for secondary wall cellulose

biosynthesis in tetraploid cotton. Phylogenetic analyses of *GhCesA1* and *GhCesA4* (*AtCesA8* orthologs), *GhCesA2* and *GhCesA2* gene (*AtCesA4* orthologs), and *GhCesA6* and *GhCesA7* (*AtCesA7* orthologs) clustered with secondary wall *CesA* genes from Arabidopsis, rice, maize, and aspen (Fig. 1). *GhCesA5*, *GhEST1*, and *GhEST5* (*AtCesA1* orthologs), *GhCes3*, *GhEST4*, *GhEST6*, and *GhEST7* (*AtCesA3* orthologs), and *GhEST3* (*AtCesA6* ortholog) clustered with primary wall *CesA* genes from other plants (Fig. 1).

Secondary wall *CesA* genes from *G. hirsutum*. Transcripts levels of *GhCesA*s were measured by quantitative RT-PCR. DNA-free total RNAs were extracted from field-grown cotton fiber at 4 d intervals from 8 to 20 DPA in two separate years. Primer sets were designed to recognize unique *GhCesA* genes using Primer Express software (Applied Biosystems, Foster City, CA) (Table 1). Sequence specificities of primers sets were confirmed by checking the dissociation of the final PCR products and finding a single peak (data not shown). The transcript levels of *GhCesAs* in developing cotton fibers were normalized with respect to those of *α-tubulin4*, expressed constitutively throughout fiber development (Whittaker and Triplett, 1999). For specific detection of *α-tubulin4*, primers sets were designed from the 3' UTR sequence of *α-tubulin4* (Kim and Triplett, 2004). Figure 2A shows that all six *GhCesA* genes (*GhCesA1, GhCesA2, GhCesA2 gene, GhCesA4, GhCesA6,* and *GhCesA7*) were similar to Arabidopsis secondary wall *CesA* genes that are up-regulated prior to secondary wall biosynthesis in developing cotton fibers. During the rapid fiber elongation stage (8-12 DPA), very low levels of this set of six *GhCesA* genes were detected (Fig. 2A). When secondary wall synthesis began at 14 - 16 DPA, the levels of all six *GhCesA*s increased, continuing to rise at 20 DPA (Fig. 2A). The homeologous genes of *GhCesA1* and *GhCesA4* are developmentally regulated very similarly during secondary wall cellulose biosynthesis. The developmental regulation of *GhCesA2* gene

was almost identical to that of *GhCesA2* cDNA. The results of Fig. 2B show that the *GhCesA2* gene with few overlapping sequences of *GhCesA2* cDNA can be either identical or homeologous to authentic *GhCesA2*. The expression patterns of *GhCesA6* and *GhCesA7* (*AtCes7* orthologs) strongly suggest that they are involved in secondary wall biosynthesis in developing fiber with *GhCesA1, 2,* and *4*.

To compare the tissue-specific expression patterns of *GhCesA*s, quantitative RT-PCR was performed using RNA isolated from young expanding (YL) and fully expanded leaves (EL), 1-wk old hypocotyls (S-1) , 6-wk old stems (S-6), and 1- and 6-wk-old roots (R1 and R6) in addition to 20 DPA fibers (F-20). Since *α-tubulin4* is preferentially expressed in cotton fibers and not expressed in other tissues, *18S rRNA* was used as a normalizer. Although low levels of all six secondary wall *GhCesA* genes were detected in tissues other than fiber, there are some differences in their tissue specificities. Figure 2B shows more abundant expression of *GhCesA1, GhCesA2, GhCesA2* gene, and *GhCesA4* in actively elongating 1-wk old hypocotyls and roots than 6-wk old hypocotyls and roots. In contrast, *GhCesA6* and *GhCesA7* show higher levels of expression in expanded leaves and 6-wk-old stems and roots than young and actively growing 1-wk tissues. Overall, Figure 2 shows that *GhCesA1, GhCesA2, GhCesA2 gene, GhCesA4, GhCesA6,* and *GhCesA7* are developmentally regulated and preferentially expressed in cotton fibers at the secondary wall synthesis stage of fiber development.

Primary wall *CesA* genes from *G. hirsutum*. Another group of six *GhCesA* genes (*GhCesA3, GhCesA5, GhEST1, GhEST3, GhEST4,* and *GhEST5*) also exhibit developmentally regulated patterns of expression during cotton fiber development; however, their expression patterns are quite distinct from those of secondary wall *GhCesA* genes. Previously, *GhCesA3* and *GhCesA5* (*GhEST2*) were detected throughout cotton fiber development (Laosinchai et al.,

2000; Li et al., 2002), however Figure 3A shows that transcript levels of *GhCesA3, GhCesA5,* and other *CesA* genes *(GhEST1, GhEST3, GhEST4,* and *GhEST5)* peaked during the elongation stage (8–12 DPA), and declined coincident with the initiation of secondary wall synthesis (16 DPA). Their expression patterns are very similar to those of other genes expressed primarily during the cell elongation stage of fiber development (Smart et al., 1998; Whittaker and Triplett, 1999). Figure 3B shows that all six *GhCesAs* are highly expressed in elongating tissues such as 1-wk old hypocotyls and 1-wk old roots in addition to elongating fibers. Preferential expression in young tissues suggests that this set of *CesA* genes is involved in synthesizing cellulose for primary cell walls.

Constitutive expression of *CesA* genes. The expression patterns of *GhEST6* and *GhEST7* in fiber were unique (Fig. 4A). These genes were expressed at similar levels throughout fiber development (Fig. 4A). The expression patterns of *GhEST6* and *GhEST7* in other tissues were also distinct. *GhEST6* and *GhEST7* expression was most abundant in fully expanded leaves (Fig. 4B). The sequence analysis of CGI, Release 8.0 has classified *GhEST6* (AI727450) and *GhEST7* (TC77125) as two different genes, and the expression patterns of *GhEST6* and *GhEST7* showed recognizable differences in tissue specific expression (Fig. 4). Sequence analysis and expression patterns of Arabidopsis *CesA*s suggests that two different groups of *CesA*s are coordinately regulated in either elongating or thickening tissues (Hamann et al., 2004); however, some *CesA* genes are expressed in both conditions. The expression of barley (*HvCesA3*) and maize (*ZmCesA1, ZmCesA7* and *ZmCesA8*) genes significantly overlapped in both elongating and thickening tissues (Burton et al., 2004; Appenzeller et al., 2004). Thus, they were classified as a third type of *CesA* gene that cannot be categorized as either associated with primary or secondary wall cellulose biosynthesis (Burton et al., 2004).

In this study, we compared the expression levels of fourteen different *GhCesA* genes from developing fibers and other tissues using quantitative RT-PCR. The expression patterns obtained from field-grown fibers during two years consistently show that most *CesA*s are developmentally regulated during fiber development, and distinct multiple *CesA*s are required to synthesize cellulose in the primary and secondary wall of cotton fibers.

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Table 1. Genes and oligonucleotides used in quantitative RT-PCR.

Name	Primer Sequences	Accession No.
GhCesA1	5'-TGGACTACCCGGTGGATAAGGT-3' 5'-CTTTCTTGCAAAGTCGGCTGTT-3'	U58283
GhCesA2	5'-CACTCGTGATCATCCTGGAATG-3' 5'-AAGTCGAGGCAGCTCTTTGC-3'	U58284
GhCesA2 gene	5'-ACCGTTGACCCTCTCAAGGAA-3' 5'-GGTAATCGACGGCCAAGATC-3'	AF254895
GhCesA3	5'-CATTGTGACGAGGTTCTCGTAA-3' 5'-GCCCCTTCAACACCCTCTTCTA-3'	AF150630 AI728899
GhCesA4 gene	5'-CCTTGCCTTGGACTACCCTGTA-3' 5'-CTTTCTTGCAAAGTCGGCTGTT-3'	AF413210
GhCesA5 (GhEST2)	5'-TGGCATCCAAGGTCCTGTCT-3' 5'-CATACCCATATAGAGCTTGCCTGTT-3'	AI729981 BM356396
GhCesA6	5'-ATACTGCATGCCAAAGTTGCC-3' 5'-AGATTGATGGGAGCTGAACCC-3'	CO496136
GhCesA7	5'-AAAACAGGACGCCCACCAT-3' 5'-TTGAAGCCAGCAGAACTGACC-3'	DT048689
GhEST1	5'-CCATCACAGCTATCCCCTGTAG-3' 5'-CCACTGCAAGGATTGACAAGA-3'	AI729285
GhEST3	5'-TCAGATCGTCTGCACCAGGTT-3' 5'-TGCCTGCTCAAGAAAATTTCAA-3'	AI728789
GhEST4	5'-GCTGTTTTCCAAGGGCTTCTC-3' 5'-GAACATGTAAAGATCAGGGAAATCC-3'	AI726753
GhEST5	5'-AAACGAACAGTTCTGGGTCATTG-3' 5'-ACTTTCAGAAGACCCTGGAAAACA-3'	AI729626 AW587492
GhEST6	5'-TGAAAACTCACATCTTCTGTCACAGA-3' 5'-TCCATAAGTGTTGAAGCAACAAAAA-3'	AI727450
GhEST7	5'-CCTTGCTGGCATTGATACCA-3' 5'-TTTGCGAAATCCCCATCTTC-3'	AI731696
GhTua4 (3'UTR)	5'-GATCTCGCTGCCCTGGAA-3' 5'-ACCAGACTCAGCGCCAACTT-3'	AF106570
Gh18S rRNA	5'-CGTCCCTGCCCTTTGTACA-3' 5'-AACACTTCACCGGACCATTCA-3'	U42827

Table 2. Cellulose synthase catalytic site (*CesA*) genes of *Gossypium hirsutum*.

Stage of Predominant Expression	Name	NCBI		Cultivars	Reference	Cotton Gene Index 8.0	
		Accession number	Sequence			*CesA*	Sequence
Elongation (1° wall)	GhCesA3	AF150630	3,723 nt	TM1	Laosinchai et al. (2000)	TC67362	3,765 nt
		AI728899		Acala Maxxa	Blewitt et al. (unpublished)		
	GhEST1	AI729285	689 nt	Acala Maxxa	Blewitt et al. (unpublished)	TC66615	915 nt
	GhCesA5 (GhEST2)	BM356396	641 nt	Xu 142	Li et al. (2002)	TC66614	2,436 nt
		AI729981	577 nt	Acala Maxxa	Blewitt et al. (unpublished)		
		AI725882	706 nt	Acala Maxxa	Blewitt et al. (unpublished)		
	GhEST4	AI726753	678 nt	Acala Maxxa	Blewitt et al. (unpublished)		
	GhEST3	AI728789	590 nt	Acala Maxxa	Blewitt et al. (unpublished)	TC60482	2,702 nt
	GhEST5	AI729626	625 nt	Acala Maxxa	Blewitt et al. (unpublished)	TC66613	1,455 nt
Cellulose Synthesis (2° wall)	GhCesA1 (GhCesA1-Dt)	U58283	3,186 nt	Acala SJ-2	Pear et al. (1996)	TC66213	3,186 nt
		AY632360	BAC	Acala Maxxa	Grover et al (2004)		
	GhCesA4 gene (GhCesA1-At)	AF413210	6,886 nt	DPL90	Kim & Triplett (2001)		
		AY632359	BAC	Acala Maxxa	Grover et al (2004)		
	GhCesA2	U58284	2,229 nt	Acala SJ-2	Pear et al. (1996)	TC66201	2,273 nt
	GhCesA2 gene	AF254895	4,003 nt	Coker 130	Hogan & Delmer (unpublished)	TC78496	1,111 nt
	GhCesA6 (GhEST8)	CO496136	693 nt	DPL90	Haigler et al (2005)	TC68655	1,011 nt
	GhCesA7 (GhEST9)	DT048689	530 nt	Xu142	Gou et al (unpublished)	DT048689	530 nt
Both stages	GhEST6	AI727450	600 nt	Acala Maxxa	Blewitt et al. (unpublished)	AI727450	600 nt
	GhEST7	AI731696	671 nt	Acala Maxxa	Blewitt et al. (unpublished)	TC77125	1,386 nt

FIGURE CAPTIONS

Figure 1. Phylogenetic relationships of cotton fiber (*Gossypium hirsutum*) cellulose synthase (*CesA*) genes and those from Arabidopsis (*AtCesA*), maize (*ZmCesA*), aspen (*PtrCesA*) and rice (*OsCesA*). The phylogenetic tree was generated with the ClustalW program. The *CesA* genes associated with either primary or secondary wall synthesis are differentially highlighted.

Figure 2. Expression profiles of six cotton *CesA*s involved in secondary wall cellulose synthesis. (A). Quantitative RT-PCR was performed using the SYBR Green PCR Master Mix in the ABI Prism 7900HT Sequence Detection System (Applied Biosystems) with gene specific primers. The transcript levels in developing cotton fibers were normalized with respect to the transcript level of *α-tubulin4*. (B). Preferential expression of in other cotton tissues. DNA-free total RNAs were extracted from young leaves (YL), fully expanded leaves (EL), 1-wk old hypocotyls (S-1), 6-wk old stems (S-6), 1-wk old roots (R-1), 6-wk old roots (R-6), 8 DPA fibers (F-8), and 16 DPA fibers (F-16). The transcript levels were normalized with respect to 18S ribosomal RNA.

Figure 3. Expression profiles of six cotton *CesA*s involved in primary wall cellulose synthesis in developing fibers (A) and other tissues (B) by quantitative RT-PCR, carried out as described in Fig. 2.

Figure 4. Expression profiles of two cotton *CesA*s expressed throughout fiber development (A) and other tissues (B) by quantitative RT-PCR, carried out as described in Fig. 2.

Figure 1.

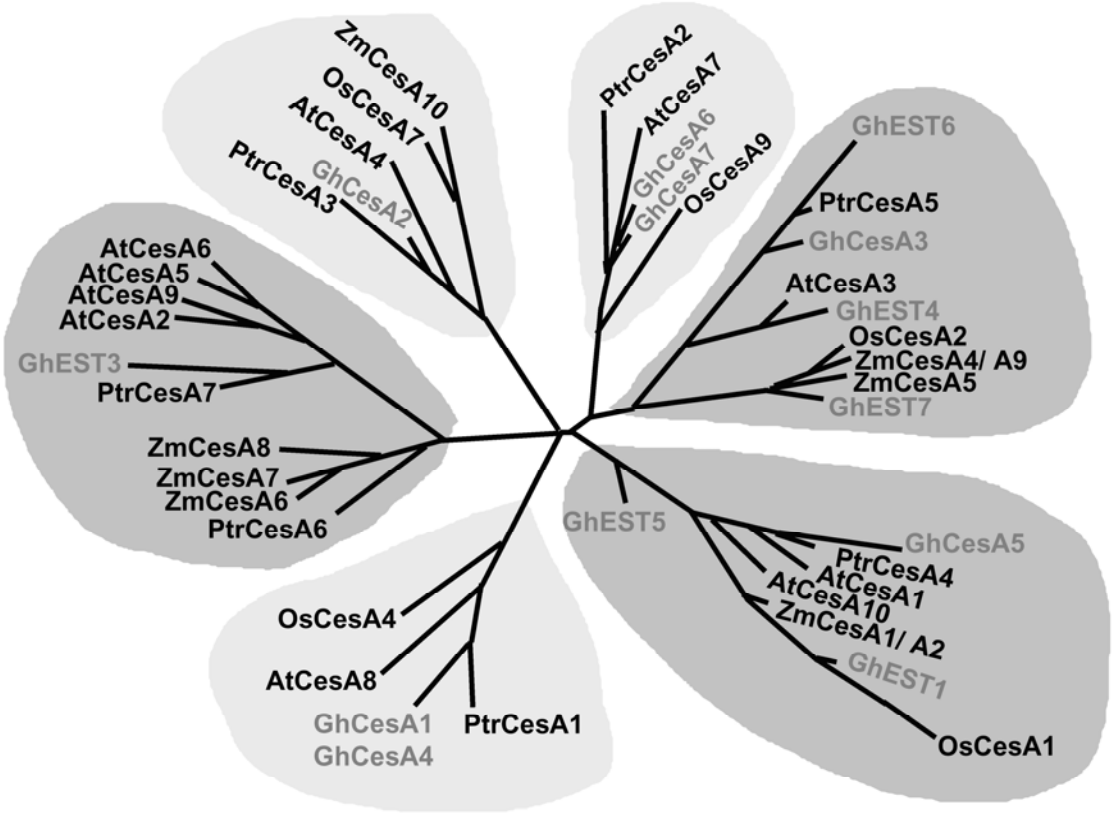

ZmCesA10
OsCesA7
AtCesA4
PtrCesA3
GhCesA2
PtrCesA2
AtCesA7
GhCesA6
GhCesA7
OsCesA9
GhEST6
PtrCesA5
GhCesA3
AtCesA3
GhEST4
OsCesA2
ZmCesA4/ A9
ZmCesA5
GhEST7
AtCesA6
AtCesA5
AtCesA9
AtCesA2
GhEST3
PtrCesA7
ZmCesA8
ZmCesA7
ZmCesA6
PtrCesA6
GhEST5
GhCesA5
PtrCesA4
AtCesA1
AtCesA10
ZmCesA1/ A2
GhEST1
OsCesA1
OsCesA4
AtCesA8
GhCesA1
GhCesA4
PtrCesA1

Figure 2.

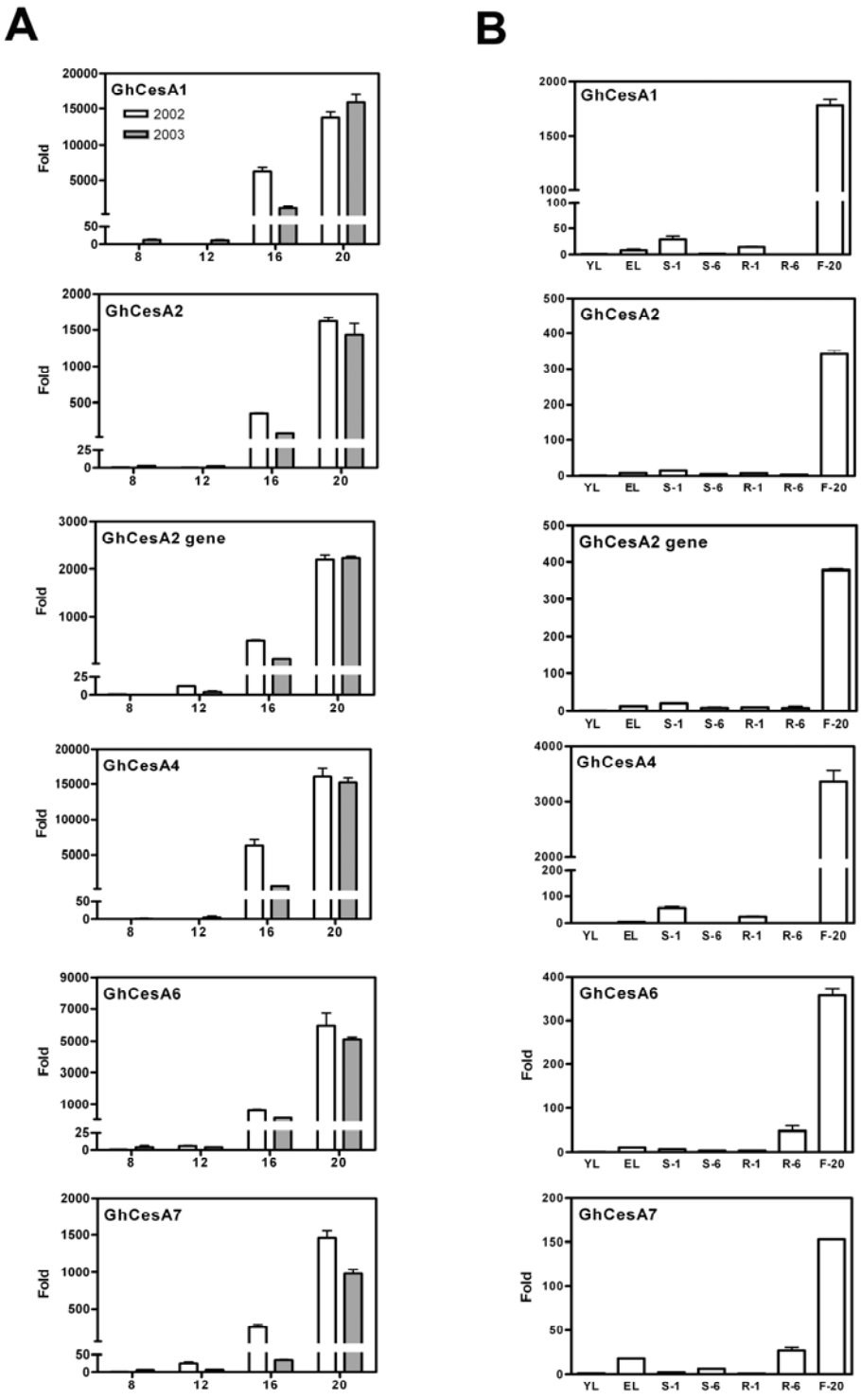

A
B
GhCesA1
2002
2003
GhCesA2
GhCesA2 gene
GhCesA4
GhCesA6
GhCesA7
Fold
8
12
16
20
YL
EL
S-1
S-6
R-1
R-6
F-20

Figure 3.

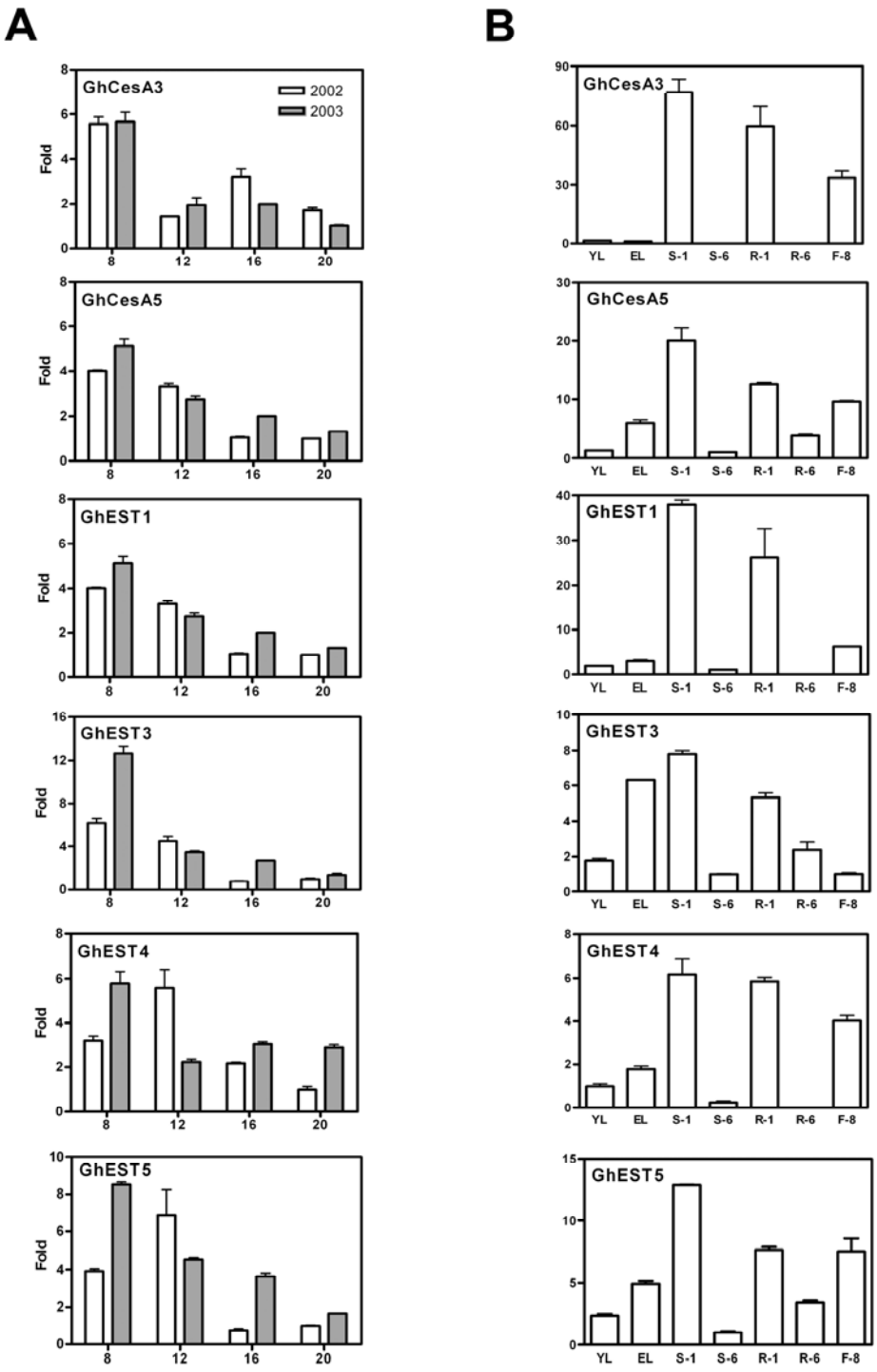
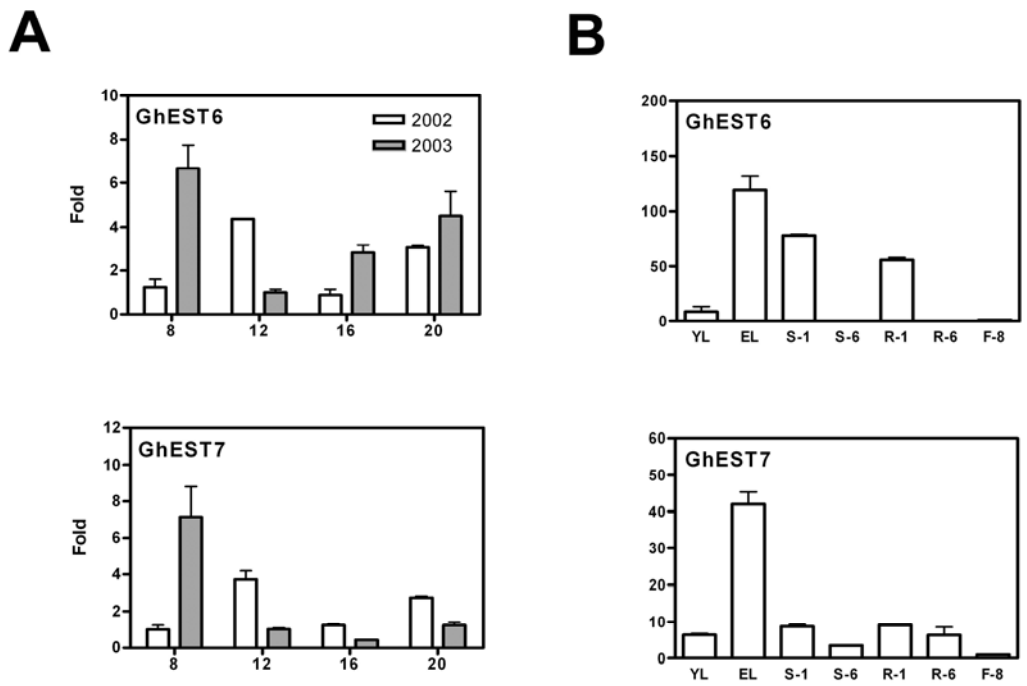

A
B
GhCesA3
2002
2003
Fold
8
12
16
20
GhCesA5
GhEST1
GhEST3
GhEST4
GhEST5
YL
EL
S-1
S-6
R-1
R-6
F-8

Figure 4.


A
B
GhEST6
2002
2003
Fold
8
12
16
20
GhEST7
GhEST6
GhEST7
YL
EL
S-1
S-6
R-1
R-6
F-8