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Full Length Article

Temporal Expression Analysis and Cloning of Cotton (*Gossypium hirsutum*) Fiber Genes

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ABSTRACT

Cotton fiber, a seed trichome and the longest plant cell, is an important model system to dissect the molecular genetic basis of fiber cell development processes. The expression patterns of 19 fiber specific genes in cotton (*Gossypium hirustum* L.) were determined by RT-PCR and Northern blots. Total RNA, extracted from cotton fibers at 0, 2, 5, 10, 15 and 20 days post anthesis (DPA), was reverse transcribed to cDNA for PCR amplification and cloning. Transcripts of expansin and tubulin were observed from 5 to 15 DPA. Lipid transfer protein (*LTP*) and *E6* gene transcription was evident throughout fiber development. Out of the ten partially characterized genes, five showed expression patterns similar to *LTP* and *E6*, while the remainder showed distinct profiles. This information is valuable in selecting promoters for the fiber specific expression of transgenes in cotton species.

Key Words: Expression profiling; RT-PCR; Fiber specific genes; *Gossypium hirsutum*

INTRODUCTION

The cotton (*Gossypium hirsutum* L.) fibers represent the longest cells known in plants. The fiber cells originate from specific cells of the epidermal layer of cotton ovules and attain a length of 2.5-3.0 cm in 16-21 days post anthesis (DPA) (Basra & Malik, 1984). During development, the cotton fiber passes through various stages that include initiation, elongation, secondary wall thickening and maturation (Gokani & Thaker, 2000). Multiple sets of genes are expressed at various phases of fiber cell development. The identification of fiber development specific genes would help to identify the metabolic pathways involved in fiber cell development.

Work on gene discovery from the developing cotton fibers has helped to identify dozens of genes that take part in cotton fiber development (Zhao *et al*., 2001; Ji *et al*., 2003; Wilkins & Arpat, 2005). Several genes have been characterized for their specific role in fiber development (Orford & Timmis, 1995; Yu *et al*., 2000; Wang *et al*., 2001; Zhao *et al*., 2001). Expansins (*EXPA*), tubulins (*TUB*), lipid transfer proteins (*LTP*s), arabino-galactan proteins (*AGP*s), proline rich proteins (*PRP*s), actins, *MYB* transcription factors and E6 are by far the most extensively studied gene families involved in fiber elongation and development (Cedroni *et al*., 2003; Orford & Timmis, 1997 & 1998; Li *et al*., 2005; Ji *et al*., 2002; Li *et al*., 2002b; Yuanli *et al*., 2002). Expansins, tubulins and actins are believed to be key gene families responsible for fiber cell elongation and structural organization.

Expansins are family of proteins encoded by a highly conserved multigene family that are differentially expressed in cotton fibers (Martin *et al*., 2001) in a developmentally regulated manner (Rose *et al*., 1997; Orford & Timmis, 1998; Ruan *et al*., 2001; Feng *et al*., 2004). In elongating cotton fibers the complex of cellulose microfibrils and matrix glucan is believed to be loosened by expansins (Cosgrove, 1997 & 2000; Rose *et al.,* 1997; Harmer *et al.,* 2002). Microtubules consist mainly of α and β-tubulins, both of which are encoded by multigene families in plants (Cleveland & Sullivan, 1985; Silflow *et al*., 1987; Li *et al*., 2002b). Nine α and seven ß-tubulin isotypes have been identified in cotton (Dixon *et al*., 1994; Whittaker & Triplett, 1999). During fiber development, microtubules exhibit specific developmental changes in orientation, organization, number, length, and proximity to the plasmalemma (Seagull, 1992). These changes are most apparent during the transition from rapid elongation to secondary cell wall synthesis (Kloth, 1989).

About half of the cotton genome is active during fiber development process (Wilkins *et al*., 2005). Although a number of gene families have been characterized for their specific role in the fiber elongation and maturation process yet dozens of other genes need yet to be characterized for their exact role in cotton fiber development (Wilkins & Arpart, 2005). The identification of genes involved in fiber

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development has paved the way for improvement of fiber characteristics by modifying their expression. Fiber characteristics may be modified by modulating their expression in the fiber cells, alternatively the regulatory sequences may be selected to regulate these genes as desired. The genetic modification of fiber traits requires that the relative timings of expression for the key genes involved in fiber development may be investigated. The present study was undertaken to determine the expression profiles of 19 genes during fiber development using RT-PCR and Northern blot analysis.

MATERIALS AND METHODS

Plant growth: Cotton (*Gossypium hirsutum* L. var. CIM707) seed were obtained from Central Cotton Research Institute (Multan, Pakistan). The seeds were delinted with 10% H_2SO_4 and dried for 48-72 h at room temperature. Plants were grown in open field. Each flower was tagged at the onset of flowering. Opening of the flower was taken as 0 DPA. Various stages of fiber development were selected by studying the boll development from 0-20 DPA. Five bolls were collected from various plants at different location of the field for total RNA isolation from their fibers.

RNA isolation and RT-PCR: Cotton bolls were frozen in liquid nitrogen immediately upon collection. Total RNA was extracted from developing cotton fibers using the plant RNA isolation kit (Invitrogen, USA). Total RNA from different developmental stages of cotton fibers (0, 2, 5, 10, 15 & 20 DPA) was reverse transcribed to cDNA using oligo dT primers and the H^- first strand cDNA synthesis kit (Fermentas, USA). The cDNA was used as template in PCR (RT-PCR) to amplify 19 genes, as detailed in Table I.

Primer designing: The nucleotide sequences of the nineteen fiber specific genes were retrieved from GenBank and primers were designed using Primer3 program available at www.justbio.com. The accession numbers of the reported nucleotide sequences, which were used for primer designing, are given in Table I.

Expression profiling cloning and sequencing: The amplification products for each gene at six fiber development stages were separated by electrophoresis. The gel fragments were cut and the eluted amplified products were ligated into the plasmid vector pCR2.1 (Invitrogen, USA). The resulting clones were sequenced on an ABI Prism 310 Genetic Analyzer.

Sequence deposition to the GenBank: The sequences of the genes isolated from *G. hirsutum* cultivar CIM-707 by RT-PCR were deposited to the Genbank at http://www.ncbi.nlm.nih.gov/WebSub/?tool=genbank and their accession numbers were obtained.

RNA blot analysis: The temporal expression pattern of selected genes was analyzed by RNA blot analysis. Total RNA isolated at the various developmental stages was spotted onto a nylon membrane (Hybond-N^+, Amersham, USA) and UV cross-linked (Stratalinker, Stratagene, USA). Specific probes were prepared using the Hexalabel DNA labeling kit (Fermentas, USA) with α ^{32}P dATP (Amersham, USA). Blots were hybridized overnight with specific probes at 65^{o}C and were washed at high stringency. The auto-radiograms were obtained by overnight exposure of the blots to X-ray film (Fuji Super XR).

RESULTS

The members of some gene families (*EXPA*s, *LTP*s, *TUB*s & *E6*) along with a few uncharacterized genes were studied to determine their timings of expression. The selected stages to study the temporal expression of the genes represent the initiation (0-2 DPA), elongation (5 DPA), rapid elongation (10 DPA), an overlapping elongation and secondary cell wall synthesis phase (15 DPA) and an overlapping secondary cell wall synthesis and the start of maturation phase (20 DPA) (Fig. 1).

Cloning and sequence analysis: Using primers derived from published sequences, 19 fiber development specific genes were amplified by RT-PCR and cloned. The homology based sequence analysis indicated 95-100% identity to the sequence used to design primers (Table I). These sequences are available in the Genbank, EMBL and DDJB databases under the accession numbers detailed in Table II.

Expression profiles of genes in developing fibers: The expression of selected genes was examined at six time periods during fiber development by semi-quantitative RT-PCR. Expansin and tubulin gene amplifications indicated that their transcripts were present at 5 DPA and continued until 15 DPA (Fig 2). The mRNA for lipid transfer proteins (*LTP1* & Fs*LTP3*) were present in cotton fibers at 0 DPA and the expression continued until 20 DPA. Fiber specific *E6*-1 and *E6*-2 transcripts were detected from 0-15 DPA (Fig 2). *E6*-3 expression started at 0 DPA and continued until 20 DPA. *E6*-4 and *E6*-h transcripts were detected from 2 to 20 DPA. The expression pattern observed for ten uncharacterized fiber genes, indicated that five of them (*Fb*14, *Fb*22, *Fb*29, *Fb*35 & *Fb*37) were expressed throughout the fiber development similar to *LTP* and *E6* genes (Table II, Fig. 3). *Fb*27 had an expression pattern similar to that of expansin and tubulin. This gene was expressed from 5-15 DPA. The results for *Fb*2, *Fb*22, *Fb*28 and *Fb*31 indicated that their expression was only transient or confined to the late stages of development (Table II).

RNA blot analysis: Northern blots of total RNA obtained from six time points during the development of fibers probed for the presence of the transcripts of six genes (*EXPA1, Fb22, FsLTP3, E6-1 & β-TUB*) is shown in Fig. 4. The expansin and tubulin gene transcripts were detected from 5-15 DPA. *Fb*22 expressed from 0-2DPA. Fs*LTP*3 and *E6*-1 expressed throughout the fiber development, except that the transcript level for *E6*-1 was very low at 20 DPA.

Table I: Genes, primer sequences, expected amplicon sizes and accession numbers of 19 fiber development specific genes used to design gene specific primers

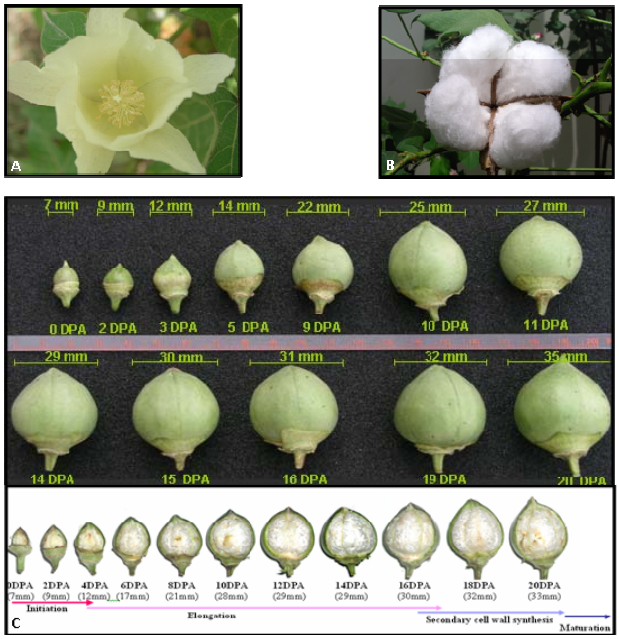
Gene	Primer pairs	Primer sequence	Size of amplified product	Accession numbers of reported genes
E6-1	E6F	5' GCAACCATAGCCATGGCTTCC 3'	741 bp	GBU30505
	E6R	5' ATCAGGGTTCGAACTGTTCCTCG 3'		
E6-2	CFF5	5' GCAACCATAGCCATGGCTTCC 3'	726 bp	GHU30507
	CFR5	5' GTGATCAGGGTTCGAACTCTTCC 3'		
E6-3	CFF6	5' GCAACCATAGCCATGGCTTC 3'	741 bp	GBU30508
	CFR6	5' ATCAGGGTTCGAACTCTTCCT 3'		
E6-4	CFF6	5' GCAACCATAGCCATGGCTTC 3'	735 bp	GHU30506
	CF78R	5' TCAGGGTTCGAACTCTTCCTC 3'		
E6-h	CFF7	5' GCCCTCTTCTCCATGCAAAT 3'	666 bp	AF218378
	CFR7	5' ATCAGGGTTCGAACTCTTCC 3'		
EXPA1	EXF	5' GCTCTTACTCAAATGGCAACC 3'	777 bp	AY189969
	EXR	5' TCTTAAAACTGGCCTCCTTCAAAA 3'		
β-TUB	BTF	5' TTTCCCGAGAAAATGAGAGAA 3'	1338 bp	AF487511
	BTR	5' TTTAAGCCTCTGCCTCGTA 3'		
LTP1	CFF16	5'TGATTAATCGATATGGCTAGCTCAATGTC3	351 bp	GHU15153
	CFR16	5'CTCATACGAACCTGTTGCAGTCAGT 3'		
FsLTP3	CFF17	5' ATGGCTAGCTCAATGTCCCTT 3'	363 bp	AF228333
	CFR17	5' TTCACTTGACGCTGTTGCAGT 3'		
Fb2	CFF34	5 'GGGTGATTTAGAAATGGATGC 3'	663 bp	AF531363
	CFR34	5' TTTATAAGATATCATCAACAATTGTAGA3'		
Fb14	CFF32	5' GAAAAATAGTGTGATGGCATC 3'	252 bp	AY271664
	CFR32	5'CTATGCATGATGATGAACAAC 3'		
Fb17	CFF25	5' CCGGACTCCAAATGCAAGA 3'	647 bp	AY375330
	CFR25	5' TCCTCAGTAACTTCCTCAAG 3'		
Fb22	CFF28	5' GGTCCGGGAAAAGTCTATAGC 3'	261 bp	AY271672
	CFR28	5'TGTCATAGAAGGCTTGGTCTCC 3'		
Fb27	CFF20	5'CAACCTTGTGAAAATGGCGAC 3'	585 bp	AY375337
	CFR20	5' CTCAATTAGTCTTGGGATCAGG 3'		
Fb28	CFF13	5' TTAATCCATATGGCTAGCTCA 3'	216 bp	AY375340
	CFR13A	5' CCTATGTATCACCACAGAG 3'		
Fb29	CFF12	5'GAAAATTGCATGGATCTTTCTTTTAAACA3'	183 bp	AY375341
	CFR12	5' TCAAGCCGGGGCAACACT 3'		
Fb31	CFF11	5' CTCTTCTCTTTCATTCTCATC 3'	531 bp	AY375342
	CFR11	5' ACTACGGGGTGAACTTCA 3'		
Fb35	CFF4	5'GGCCACGAGTCAGGCTCAG 3'	489 bp	AY429439
	CFR4	5'ATCAGGGTTCGAACTCTTCCTCGA 3'		
Fb37	CFF2	5' GGAGATTGAAAAAATATGGCTGAAG 3'	414 bp	AY429441
	CFR2	5' ATCAAAAGCACAAGCATGTTGTTC 3'		

Table II: Expression profiling and percent identity of the cloned genes to the best aligned sequences

Gene name	Accession No.	Identity of cloned genes to the best aligned sequences (%)	Expression profile (DPA) 0 2 5 10 15 20
E6-1	DQ023518	99% to GBU30505	
E6-2	DQ023519	96% to GBU30507	
E6-3	DQ023520	99% to GBU30508	
E6-4	DQ023521	97% to GBU30506	
E6-h	DQ023522	99% to AF218378	
EXPA1	DQ023525	98% to AY189969	
β-TUB	DQ023526	98% to AF487511	
LTP1	DQ023527	100% to GHU15153	
LTP3	DQ023529	100% to AF228333	
Fb2	DQ023530	99% to AF531363	
Fb14	DQ023531	100% to AY271664	
Fb17	DQ023536	99% to AY375330	
Fb22	DQ023537	95% to AY271672	
Fb27	DQ023523	98% to AY375337	
Fb28	DQ023532	99% to AY375340	
Fb29	DQ023533	97% to AY375341	
Fb31	DQ023534	98% to AY375342	
Fb35	DQ023535	99% to AY429439	
Fb37	DQ023524	100% to AY429441	

Fig. 1: Cotton boll and fiber developmental stages

(A) Fully bloomed unfertilized creamy flower at 0 DPA; **B)** An opened cotton boll with fully mature white cotton fibers; **C)** Cotton boll at various stages of development ranging from 0 DPA to 20 DPA presented by measuring the diameter of the boll with and without the integument. The increase in boll size is directly related to an increase in fiber length



The Northern blot results are in agreement with those obtained by RT-PCR. However, the expression level for each gene can be better interpreted from the Northern blots than from the RT-PCR profiles. These blots indicated that Fs*LTP*3 and *E6*-1 had the highest transcript level from 2-5 DPA and 2-10 DPA respectively and the hybridization signal of these genes at the early stages of development was several fold higher than other genes studied by northern blot.

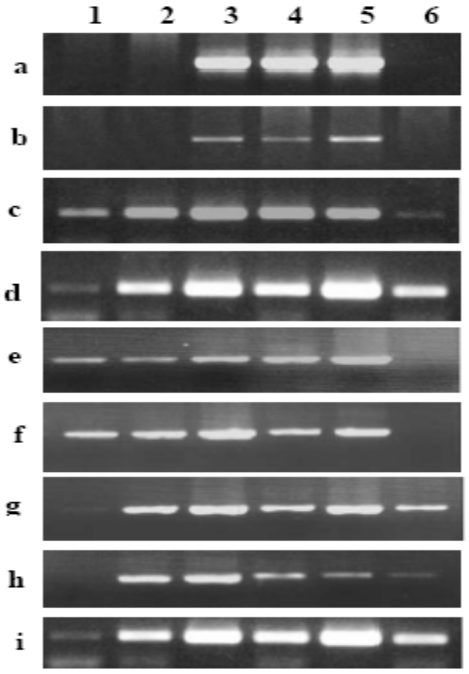
DISCUSSION

Fiber morphogenesis is a complex process and only a few of the gene involved have been characterized (Wilkins *et al*., 2005). Some of the characterized genes belong to *EXP*s, LTPs, *E*6, *TUB*s and actins (Orford & Timmis, 1995; Yu *et al*., 2000; Wang *et al*., 2001; Zhao *et al*., 2001). Fiber specific *EXPA*s, LTPs, *TUB*s and *E*6 are reported to express abundantly in developing cotton fibers (Orford & Timmis, 1997; Li *et al*., 2002b; Yuanli *et al*., 2002; Li *et al*., 2005). The efforts have so far been concentrated on identifying the genes involved in fiber development and determining the potential role of these genes. However, limited information is available on the timings, duration and levels of gene expression during fiber development.

The RT-PCR and RNA blotting of *EXPA*1 gene (DQ023525) at six development stages showed that this gene is detected between 5-15 DPA, which is the rapid elongation phase of fiber development. The RT-PCR (Fig. 2) and RNA blot (Fig. 4) did not show the presence of

Fig. 2: Expression profiles of characterized genes.

a: *EXPA1(777bp), b: β-TUB(1338bp),* c: *LTP*1 (363 bp), d: Fs*LTP*3 (351 bp), e: *E6*-1 (741 bp), f: *E6*-2 (726 bp), g: *E6*-3 (741 bp), h: *E6*-4 (735 bp) and i: *E6*-h (666 bp). Lanes 1-6 represent RT-PCR results obtained at six different cotton fiber development stages (Lane 1: 0DPA, Lane 2: 2DPA, Lane 3: 5DPA, Lane 4: 10DPA, Lane 5: 15DPA and Lane 6: 20DPA)

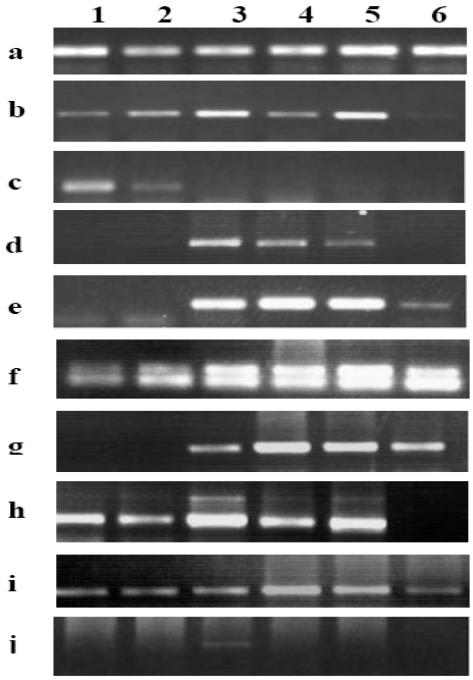


*EXPA*1 (DQ023525) at any stage of fiber development. It has been reported that expression of several *EXPANSIN* genes parallels fiber elongation (Orford & Timmis, 1998; Ruan *et al.,* 2001; Harmer *et al.,* 2002; Ji *et al.,* 2003). The abundance of *EXPA*s transcripts during 5-15 DPA indicates its key role in fiber elongation by loosening the elongating cellulose fibers according to its reported mode of action (Cosgrove, 2005). This suggests the potential role of expansin genes in affecting economically significant cotton fiber properties such as length and elongation.

One of the β-*TUB* (DQ023526) was detected from 5-15 DPA that represents the rapid stage of cell elongation. The detected expression is in accordance with the expression patterns of five out of six tubulins reported by (Feng *et al*., 2004). Microtubules are composed of α and β-tubulins (*TUB*). These structures are very important in carrying out various functions in the cell including cell division, transport and morphogenesis (Heald & Nogales, 2002). The Northern blot for the β-*TUB* (Fig. 4) indicated similar hybridization signal intensity as that of *EXPA1*. The size of β-*TUB* gene is almost double the size of expansin. The hybridization signal for tubulin may be expected to be higher than expansin at an equal transcript level and probe activity. A comparable hybridization signal for both the genes might indicate that tubulin transcripts are lower than that of expansin at the rapid phase of fiber elongation.

Fig. 3: Expression profiles of uncharacterized fiber specific genes

a: *Fb*14 (252 bp), b: *Fb*17 (647 bp), c: *Fb*22 (261 bp), d: *Fb*27 (585 bp), e: *Fb*28 (216 bp), f: *Fb*29 (183 bp), g: *Fb*31 (531 bp), h: *Fb*35 (489 bp), i: *Fb*37 (414 bp) and j: *Fb*2 (663 bp). Lanes 1-6 represent RT-PCR results obtained from the fiber specific transcripts at six different cotton fiber development stages (Lane 1: 0DPA, Lane 2: 2DPA, Lane 3: 5DPA, Lane 4: 10DPA, Lane 5: 15DPA and Lane 6: 20DPA)

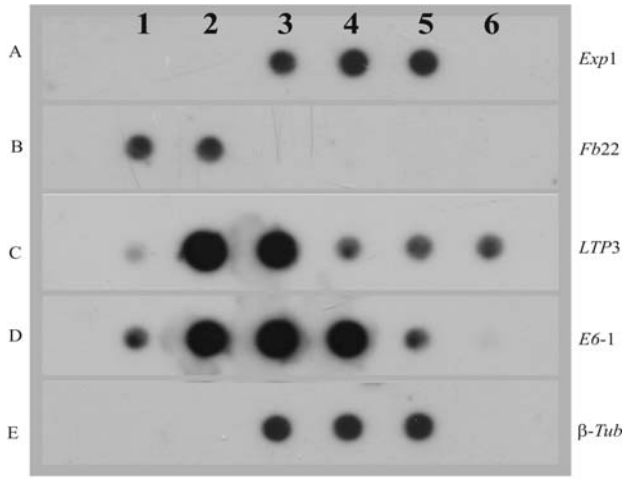


The transcription levels of two lipid transfer proteins (*LTP*1 & Fs*LTP*3) were investigated in cotton fibers. Both the RT-PCR (Fig. 2) and Northern blot results (Fig. 4) indicated that *LTP*1 and Fs*LTP*3 genes are expressed throughout the six developmental stages (0 -20 DPA). The Northern blot signal for *LTP*1 was comparable to *EXPA*1, while Fs*LTP*3 signal was several fold higher from 2-5 DPA and was comparable to *LTP*1 and *EXPA*1 at other fiber development stages. The result is in accordance with the presence of LTPs in cotton fibers during fiber development (Ma *et al*., 1997; Liu *et al*., 2000; Orford & Timmis, 2000; Feng *et al*., 2004). The transcript abundance of *LTPs* during elongation stage of fiber development indicated the potential of utilizing the promoter of this gene family in a gene modification strategy to express the transgene during this important phase of fiber development.

Five *E*6 genes (*E6*-1, *E6*-2, *E6*-3, *E6*-4 & *E6*-h) were cloned. The transcripts of *E6*-1 and *E6*-2 were not detectable after 15 DPA (Fig. 2), indicating that these genes may not be involved in the later stage of fiber elongation and secondary cell wall deposition. *E6*-3 was detectable from 0-20 DPA, while *E6*-4 and *E6*-h were detected from 2-20DPA. The Northern blot analysis for *E6*-1 indicated that its hybridization signal from 2-10 DPA was several fold higher than the other genes studied through Northern blot (Fig. 4).

Fig. 4: Expression profiles of some fiber specific genes by Northern blot analysis

Lanes 1-6 represent total RNA blotted from 0, 2, 5, 10, 15, and 20DPA cotton fibers. Strip A hybridized with expansin (*EXPA*1) probe. Strip B hybridized with *Fb*22 probe. Strip C hybridized with Fs*LTP*3 probe. Strip D hybridized with *E6*-1 probe. Strip E hybridized with β-tubulin probe



This observation predicted the involvement of this gene family during rapid elongation phase of cotton fiber but the exact role of these genes need to be determined for their utilization in the fiber trait modification program. It was proposed that *E*6 protein is a substrate for casein kinase II (Pinna, 1990). The known substrates for casein kinase II are involved in transcription and translation of regulatory proteins. It has also been suggested that E6 might be involved in the deposition of cellulose; which occurs during the secondary cell wall deposition stage of fiber development (Preston, 1986). Other possible functions that could be assigned to E6 are synthesis and degradation of polysaccharides or performing structural role in the cotton fibers (Bacic *et al*., 1988).

The transcript analysis of 10 partially characterized fiber specific genes indicated that the timings of expression for these genes were variable as compared to the characterized genes reported in this study. The transcript analysis of characterized genes and one member of the uncharacterized gene were carried out both by the RT-PCR and the classical RNA blotting. The results indicated that both the techniques were comparable, although RNA blotting was better for quantitative expression analysis as the signal is proportional to the transcript copy number and thus may help to identify the genes associated with high transcription rate. This study highlights the timings of expression for some of the genes reported in cotton fiber development and relative expression for a few of them.

In conclusion, transcript analysis of 10 partially characterized fiber specific genes indicated that their timings of expression are variable. Comparison of RT-PCR and the classical RNA blotting techniques indicated that both were comparable, although the latter was better for quantitative

expression analysis of fiber development specific genes. These results may be utilized to select regulatory sequences for controlled expression of genes in developing cotton fibers. Resources for several promoters/regulatory sequences that express at restricted stages of fiber development have been identified. Moreover, some of uncharacterized fiber specific genes were found to have similar expression patterns as *LTP*s and provide alternate sources for promoter selection to meet controlled expression of genes in developing cotton fibers.

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REFERENCES

Bacic, A., P.J. Harris and B.A. Stone, 1988. Biochemistry of plants; *In*: Stumpf, P.K. and E.E. Conn (eds.), *Structure and Function of Plant Cell Walls*, pp: 297–371. Academic Press, New York

Basra, A. and C.P. Malik, 1984. Development of the cotton fiber. *Int. Rev. Cytol.,* 89: 65–113

Cedroni, M.L., R.C. Cronn, K.L. Adams, T.A. Wilkins and J.F. Wendel, 2003. Evolution and expression of *MYB* genes in diploid and polyploid cotton. *Plant Mol. Biol.,* 51: 313–325

Cleveland, D.W. and K.F. Sullivan, 1985. Molecular biology and genetics of tubulin. *Annu. Rev. Biochem.,* 54: 331–365

Cosgrove, D.J., 1997. Relaxation in a high-stress environment: the molecular bases of extensible cell walls and cell enlargement. *Plant Cell,* 9: 1031–1041

Cosgrove, D.J., 2000. Loosening of plant cell walls by expansins. *Nature,* 407: 321–326

Cosgrove, D.J., 2005. Growth of the plant cell wall. *Nat. Rev. Mol. Cell Biol*., 6: 850–861

Dixon, D.C., R.W. Seagull and B.A. Triplett, 1994. Changes in the accumulation of α- and β-tubulin isotypes during cotton fiber development. *Plant Physiol.,* 105: 1347–1353

Feng, J.X., S.J. Ji, Y.H. Shi, Y. Xu, G. Wei and Y.X. Zhu, 2004. Analysis of five differentially expressed gene families in fast elongating cotton fiber. *Acta Biochim. Biophys. Sin.,* 36: 51–56

Gokani, S.J. and V.S. Thaker, 2000. Physiological and biochemical changes associated with cotton fiber development. VIII. Wall components. *Acta Physiol. Plant.*, 22: 403–408

Harmer, S.E., S.J. Orford and J.N. Timmis, 2002. Characterization of six alpha-expansin genes in *Gossypium hirsutum* (upland cotton). *Mol. Genet. Genomics,* 268: 1–9

Heald, I.R. and E. Nogales, 2002. Microtubule dynamics. *J. Cell Sci.,* 115: 3–4

Ji, S.J., Y.C. Lu, J. Li, G. Wei, X.J. Liang and Y.X. Zhu, 2002. A beta-tubulin-like cDNA expressed specifically in elongating cotton fibers induces longitudinal growth of fission yeast. *Biochem. Biophys. Res. Commun*., 296: 1245–1250

Ji, S.J., Y.C. Lu, J.X. Feng, G. Wei, J. Li, Y.H. Shi, Q. Fu, D. Liu, J.C. Luo and Y.X. Zhu, 2003. Isolation and analyses of genes preferentially expressed during early cotton fiber development by subtractive PCR and cDNA array. *Nucleic Acids Res.,* 31: 2534–2543

Kloth, R.H., 1989 Changes in the level of tubulin subunits during development of cotton (*Gossypium hisutum*) fiber. *Plant Physiol*., 76: 37–41

Li, C.H., Y.Q. Zhu, Y.L. Meng, J.W. Wang, K.X. Xu, T.Z. Zhang and X.Y. Chen, 2002a. Isolation of genes preferentially expressed in cotton fibers by cDNA filter arrays and RT-PCR. *Plant Sci.,* 163: 1113–1120

Li, X.B., L. Cai, N.H. Cheng and J.W. Liu, 2002b. Molecular characterization of the cotton *GhTUB1* gene that is preferentially expressed in fiber. *Plant Physiol*., 130: 666–674

Li, X.B., X.P. Fan, X.L. Wang, L. Cai and W.C. Yang, 2005. The cotton *ACTIN1* gene is functionally expressed in fibers and participates in fiber elongation. *Plant Cell,* 17: 859–875

Liu, H.C., R.G. Creech, J.N. Jenkins and D.P. Ma, 2000. Cloning and promoter analysis of the cotton lipid transfer protein gene *LTP3*. *Biochim. Biophys. Acta,* 1487: 106–111

Ma, D.P., H.C. Liu, H. Tan, R.G. Creech, J.N. Jenkins and Y.F. Chang, 1997. Cloning and characterization of a cotton lipid transfer protein gene specifically expressed in fiber cells. *Biochim. Biophys. Acta,* 1344: 111–114

Martin, C., K. Bhatt and K. Baumann, 2001. Shaping in plant cells. *Curr. Opin. Plant Biol.,* 4: 540–549

Orford, S.J. and J.N. Timmis, 1995. Specific genes expressed during cotton fiber development. *J. Cellular Biochem.,* 21: 447–447

Orford S.J. and J.N. Timmis, 1997. Abundant mRNAs specific to the developing cotton fibre. *Theor. Appl. Genet.,* 94: 909–918

Orford, S.J. and J.N. Timmis, 1998. Specific expression of an expansin gene during elongation of cotton fibers. *Biochem. Biophys. Acta*, 1398: 342–346

Orford, S.J. and J.N. Timmis, 2000. Expression of a lipid transfer protein gene family during cotton fiber development. *Biochem. Biophys. Acta,* 1483: 275–284

Pinna, L.A., 1990. Casein kinase 2: an 'eminence grise' in cellular regulation? *Biochem. Biophys. Acta,* 1054: 267–284

Preston, R.D., 1986. Natural cellulose, *In*: Young, R.A. and R.M. Rowell (eds.). *Cellulose: Structure, Modification and Hydrolysis*, pp: 3–27. John Wiley and Sons, New York

Rose, J.K.C., H.H. Lee and A.B. Bennett, 1997. Expression of a divergent expansin gene is fruit-specific and ripening-regulated. *Proc. Natl. Acad. Sci.,* 94: 5955–5960

Ruan, Y.L., D.J. Llewellyn and R.T. Furbank, 2001. The control of single-celled cotton fiber elongation by developmentally reversible gating of plasmodesmata and coordinated expression of sucrose and K+transporters and expansin. *Plant Cell,* 13: 47–63

Seagull, R.W., 1992. A quantitative electron microscopic study of changes in microtubule arrays and wall microfibril orientation during in vitro cotton fiber development. *J. Cell Sci*., 101: 561–577

Silflow, C.D., D.G. Oppenheimer, S.D. Kopczak, S.E. Ploense, S.R. Ludwig, N.A. Haas and D.P. Snustad, 1987. Plant tubulin genes: structure and differential expression during development. *Dev. Genet.,* 8: 435–460

Wang, X.D., Y.X. Zhu, D.F. Ji, S.L. Jiang and Y.Y. Li, 2001. Cloning of fiber-specific cDNAs and their structural variations in 4 fiber mutants. *Chinese Sci. Bull.,* 46: 234–237

Whittaker, D.J. and B.A. Triplett, 1999. Gene-specific changes in α-tubulin transcript accumulation in developing cotton fibers. *Plant Physiol*., 121: 181–188

Wilkins, T.A. and A.B. Arpat, 2005. The cotton fiber transcriptome. *Physiol. Plant,* 124: 295–300

Wilkins, T.A., A. Apart and B. Sickler, 2005. Cotton Fiber Genomics: Developmental mechanisms. *Pflanzenschutz-Nachrichten Bayer*, 58: 119–139

Yuanli, L., S. Jie, L. Chunhong, Z. Yongqing and X. Guixian, 2002. Specific expression of β- tubulin gene(*GhTub1*) in developing cotton fiber. *Sci. China,* 46: 236–242

Yu, X.H., Y.Q. Zhu, S. Lu, T.Z. Zhang, X.Y. Chen and Z.H. Xu, 2000. A comparative analysis of a fuzzless-lintless mutant of *Gossypium hirsutum* L. cv. Xu-142. *Sci China, Series C; Life Sci.,* 43: 623–630

Zhao, G.R., J.Y. Liu and X.M. Du, 2001. Molecular cloning and characterization of cotton cDNAs expressed in developing fiber cells. *Biosci. Biotechnol. Biochem*., 65: 2789–2793

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