Pak. J. Bot., 48(3): 1099-1106, 2016.

EXPRESSION ANALYSIS OF FIBER RELATED GENES IN COTTON (*GOSSYPIUM HIRSUTUM* L.) THROUGH REAL TIME PCR

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Abstract

Cotton fibers are unicellular seed trichomes and the largest known plant cells. Fiber morphogenesis in cotton is a complex process involving a large number of genes expressed throughout fiber development process. The expression profiling of five gene families in various cotton tissues was carried out through real time PCR. Expression analysis revealed that transcripts of expansin, tubulin and E6 were elevated from 5 to 20 days post anthesis (DPA) fibers. Three Lipid transfer proteins (LTPs) including LTP1, LTP3, LTP7 exhibited highest expression in 10 - 20 DPA fibers. Transcripts of LTP3 were detected in fibers and non fiber tissues that of LTP7 were almost negligible in non fiber tissues. Sucrose phosphate synthase gene showed highest expression in 10 DPA fibers while sucrose synthse (susy) expressed at higher rate in 5-20 DPA fibers as well as roots. The results reveal that most of fiber related genes showed high expression in 5-20 DPA fibers. Comprehensive expression study may help to determine tissue and stage specificity of genes under study. The study may also help to explore complex process of fiber development and understand the role of these genes in fiber development process. Highly expressed genes in fibers may be transformed in cotton for improvement of fiber quality traits. Genes that were expressed specifically in fibers or other tissues could be used for isolation of upstream regulatory sequences.

Key words: Cotton, Fiber related genes, Real time PCR, Expression profiles, DPA.

Introduction

Cotton fibers are unicellular seed trichomes used as raw material for manufacture of fabrics in textile industry. Cotton fiber is one of the largest single cell in the higher plants that passes through rapid elongation without mitosis during seed development. Fiber development in cotton takes place through four over lapping phases including initiation, elongation, secondary cell wall synthesis and maturation (Kim & Triplett, 2001). All stages are crucial for determining the quality of fiber but first three stages are more important as they determine yield and length of fiber. Fiber development initiates before anthesis and then it keeps on elongating rapidly for 16 DPA before the start of secondary cell wall formation (Tiwari & Wilkins, 1995). High turgor pressure inside fiber cell is mainly responsible for elongation (Smart *et al.,* 1998). When turgor pressure inside cell exceeds the threshold level, it causes loosening of cell wall matrix assisted by various proteins which cause cell to expand. In fiber cells high turgidity is attained through intake of water mediated by high concentration of solutes along with proton pump (Cosgrove, 1997). Fiber development is a complex process and diverse sets of genes are expressed during each developmental stage. Out of these genes, some are upregulated or down regulated while few of these genes are expressed exclusively in fiber during distinct phase of development. In allotetraploid cotton, there are about 36, 00 genes half of these are active during particular fiber developmental phase (Havov *et al.,* 2008). A large number of genes have been isolated which are developmentally regulated and assumed to play important role in determining fiber quality. Numerous genes involved in various processes like cell elongation, cellulose synthesis, cell division regulation and lipid metabolism have been discovered in cotton (Qin *et al.,* 2007; Kim *et al.,* 2012). Many full length fiber specific genes have been isolated and cloned but there is very brief knowledge about their exact role and expression pattern (Li *et al*., 2005).

For improvement of cotton fiber quality through transgenic technology, the preliminary step is the identification of genes crucial for cotton fiber development. A large number of genes which are highly expressed in fibers have been cloned and characterized (Wang *et al.,* 2010). Many novel fiber related genes have been explored through screening of cotton fiber cDNA libraries (Ji *et al.,* 2003). These genes encode for various structural proteins and enzymes including expansins, lipid transfer proteins, sucrose synthases, Phosphoenol pyruvate carboxylase (PEPc), a tubulins, xyloglucan endotransglycolase (XTH), transcription factors, phyto hormones and metabolic pathways genes These genes are widely studied in cotton and other plant species (Lee *et al.,* 2010; Huang *et al.,* 2013)

Expansin proteins are present in all plants, take part in cell enlargement under low pH conditions (Cosgrove, 2000). These proteins act non-enzymatically and break the hydrogen bonds between cellulose microfibrils. Tubulin is another high conserved gene family expressed in fiber tissues. Tubulins are mainly components of microtubules in plant cells that play important role in many cellular processes like cell division, cell motility, intracellular transport as well as cell morphology. In cotton, nine α-tubulins and seven ß-tubulins isotypes have been identified (Dixon *et al*., 1994).

Lipid transfer protein genes encode for LTPs which are small basic proteins. These proteins enhance cell wall extension by non hydrolytic disruption of cell wall polymers (Nieuwland *et al.,* 2005). Several members of LTP gene family are expressed distinctly in developing cotton fiber (Orfard & Timmis, 2000).

E6 a structural protein, is also found to be expressed in developing cotton fibers.that plays role in metabolism cell wall polysaccharides (Bacic *et al*., 1988). E6 has also role in cellulose deposition in cotton fibers (Preston, 1986).

Sucrose phosphate synthase (SPS) is an important enzyme that mediates sucrose synthesis and increases rate of cellulose synthesis. It provides excess UDP glucose which is used as substrate for cellulose synthesis (Haigler *et al.,* 2001). This enzyme is supported by the (susy) sucrose synthase which degrades sucrose into UDP glucose and fuctose. Sucrose synthase is also involved in osmoregulation and conversion of sucrose into useable hexoses crucial during fiber development (Amor *et al.,* 1995).

Previously, several studies have been conducted to determine qualitative and quantitative expression of fiber genes (Li *et al*., 2005; Yang *et al.,* 2014) but the knowledge about exact timing and extent of expression of most fiber genes is still very brief. For improvement and productivity of cotton fiber through transgenic technology, there is need to explore more fiber genes and determine their role during distinct phase of development. The objective of the study was to determine expression level of five genes families in cotton tissues through RT qPCR in local cotton genotype of Pakistan. Real time or qPCR is the most suitable technique (Bustin, 2000) for gene expression analysis because it allows specific detection of each member of gene family. The results obtained through present study show that all genes exhibited high expression in 5-20 DPA fibers. So, these genes may have crucial role in fiber elongation and strength. The systematic gene expression study may help to explore complex metabolic pathways governing during fiber cell development. Genes that would found to be highly expressed in fiber or any other organs can be selected for isolation of regulatory sequences. These fiber specific promoters may used to express transgene for cotton fiber improvement without IPR issues.

Materials and Methods

Plant material used: Seeds of *Gossypium hirsutum* L. (var. CIM707) were provided by Central Cotton Research Institute (Multan, Pakistan). Cotton seeds were delinted with 10% H_2SO_4 and dried for 48-72 hours under shade. Plants were cultivated at NIBGE, Faisalabad under normal field conditions during cotton growing season. The bolls were tagged on the day of fertilization in open fields. Day of flower opening was taken as 0 DPA (days post anthesis). Cotton bolls of different developmental ages (0 DPA, 5 DPA, 10 DPA, 15 DPA and 20 DPA) were also marked and collected. Other plant tissues including leaves, roots, shoots and stem were also collected from field grown 10-15 days old plants. All cotton tissues were washed with DEPC treated water, dried and frozen in liquid nitrogen immediately after sampling.

RNA isolation and first strand cDNA synthesis: Fibers of different developmental ages were detached carefully from frozen bolls with fine forceps in liquid nitrogen and ground in liquid nitrogen using pestle and mortar. Other tissues were also ground to fine powder for RNA isolation. Total RNA was extracted based on guanidinium thiocyanate based method using the "Plant RNA purification reagent" (Invitrogen, USA) as described by the manufacturer. RNA integrity was checked on 1% agarose gel containing 0.5µg/ml ethidium bromide. RNA samples were quantified on ultrospec 3100 Amersham Biosciences spectrophotometer and equalized through dilution with DEPC water before cDNA synthesis. Total RNA was treated with DNase I (Promega, USA) according to manufacturer's instruction.

High quality total RNA from each cotton tissue was reverse transcribed to cDNA using oligo dT (20 mer) primers and the "RevertAid H¯ cDNA Synthesis Kit" (Fermentas, USA). The cDNA was used as template in PCR to amplify fiber genes, as detailed in Table 1. Different cDNA samples were equalized both spectrophotometrically and by PCR amplification of 18S rRNA and Ubiquitin gene primers to compensate variation in concentration of starting material.

Table 1. Description of primers used for real time PCR studies for expression analysis of fiber genes in cotton.

No.	Name of gene	Primer pair	Primer sequence (5'-3')	Primer length	Amplicon size (bp)	Gene bank accession No.
1.	18S rRNA	RT18SF2	AAACGGCTACCACATCCAAG	20 mer	153	U42827.1
		RT18SR2	CCTCCAATGGATCCTCGTTA	20 mer		
2.	β-Tubulin	RT β-TUB F2	TGATGTGTGCAGCTGATCCT	20 mer	180	AFA84959
		RT β-TUB R2	TTGGAGGGATGTCACAAACA	20 mer		
3.	Expansin	RTEXP2F	GCATTCTTGCAGATCGCGGA	20 mer	143	DQ023525
		RTEXP2R	GCATTCTTGCAGATCGCGGA	20 mer		
4.	Lipid Transfer Protein 1	RTLTP1F1	CCTCCCACGTTGGTTATCAA	20 mer	194	AF195863
		RTLTP1R1	ATGGTATTGCAAGCGGACTC	20 mer		
5.	Lipid Transfer Protein 3	RTLTP3 F2	AGCTGGTGAACTTTCCATGC	20 mer	167	AF228333
		RTLTP3 R2	GCCGTAACCTGTGGTCAAGT	20 mer		
6.	E6	RTE6 F2	GCCGTAACCTGTGGTCAAGT	20 mer	142	U30506.1
		RTE6 R2	CTTTTGATGCATCTGCAAGC	20 mer		
7.	Lipid Transfer Protein 7	LTP7 F2	CTCAACTCCGCCGCCCAA	18 mer	162	KC342638.1
		LTP7 R2	AGCCCTAGCACTGACTGC	18 mer		
8.	Sucrose synthase	RT SS2 F1	CACTGGTGGTCAGGTTGT	18 mer	160	HQ702185
		RTSS2 R1	CAGCCGCTGATTGCAACT	18 mer		
9.	Sucrose phosphate synthase	SPSF1	CAGTTACCTGGAAGCGAT	18 mer	148	JQ043231
		SPSR1	GAGACCGATCTCCACCGT	18 mer		
10.	Ubiquitin	RTUBC	TGAATATTGTAATCAGCC	18 mer	132	CF932135
		RTUBC	GAGCTCGGATACGATTGA	18 mer		

qPCR primer design: Primers for real time PCR were designed using Beacon Designer 7 (premier Biosoft international, USA) for each gene with amplicon length of 100 to 250 bp. Primer length was ranged from 18 to 20 nucleotides and guanine-cytosine contents were between 40 to 60%. Primers were designed from template region having no secondary structure for efficient primer template binding. BLAST short (http://www.ncbi.nlm.nih.gov) was used to ensure that each sequence was unique for specific gene.

Efficiency of each primer was determined by a dilution series of 10 DPA fiber cDNA. The specificity of real time PCR was confirmed by a single peak in the melting temperature curve analysis and single band on agarose gel electrophoresis.

Real time PCR analysis: Real time PCR was conducted in 25µl reaction volume using 12.5µl SYBR Green Super Mix (Bio-Rad, USA) and 25ng/µl of each gene specific primer. For normalizing transcript level of each gene under investigation 18S rRNA was used as reference gene. The PCR reactions were performed in 96 well reaction plates sealed with optically clear film in iQ5 cycler (BioRad). All samples a were preheated at 94ºC for 4 min as initial denaturation step, followed by 40 cycles of denaturation at 94ºC for 1 min, annealing at 48ºC for 1 min and extension at 72ºC for 30Sec. A melt curve was generated for each sample (55 to 95ºC) to monitor any non specific amplification. The reaction was run in triplicate for each gene and their average Ct values were used for relative quantification. Parallel reactions were run using cotton 18S rRNA primers to normalize the amount of template added. Cotton ovules (0 DPA) of medium fiber length genotype MNH-786 were taken as control to calculate relative expression of particular gene. Relative fold expression was calculated by $2^{-\Delta\Delta Ct}$ method using relation $\Delta Ct_{sample}-\Delta Ct_{control}$ (Huang *et al.,* 2015). In this relation ΔΔCt was (Ct of target gene – Ct 18 S RNA) of CIM 707 cotton tissues - (Ct of target gene – Ct 18 S RNA) of MNH-786 ovules (0 DPA). Final data obtained was expressed as $\log_2$ scale (Caldana *et al.,* 2007).

Quantitative real time PCR based gene expression data of fiber related genes expressing in various tissues of cotton (var. CIM707) was statistically analyzed by taking mean values of three replicates for each gene. Standard error of each relative gene expression values was also calculated. Mean gene expression data was compared and least significance difference (LSD) at $p \leq 0.05\%$ was employed to determine the significance level among means of gene expression.

Results

Expression analysis of five gene families was carried out to investigate their expression level in developing fibers and other cotton tissues. Expression study was conducted through reverse transcriptase real time PCR using gene specific primers (Table 1).Transcripts abundance of selected genes was examined in fiber at five developmental periods (0-20 DPA) along with root, shoot, leaves and stem tissues. Total RNA concentration in various tissues ranged from 0.2µg to 0.5µg, (Fig. 1). Absorbance at A260/A280 was 1.5 to 1.8 (less than 2) which reflected good quality RNA preparation. Concentrations of all samples were nearly equalized by dilution with DEPC treated water (Fig. 2). All primers were validated through wide range of dilution series of template (Fig. 3). Primers that gave no dimers and efficiencies more than 85% were selected for qPCR assay. Concentrations of all cDNA samples were nearly equalized by amplification of 18S rRNA and ubiquitin genes primers (Fig. 4a & b). For normalization of real time PCR data, 18S rRNA was used as internal control.

Expression analysis of both expansin and tubulin genes revealed that both genes showed elevated expression in 5 to 20DPA fibers (Fig. 5a & b). The highest expression level of both genes was detected in 10DPA fibers. Very low transcripts of both genes were detected in non fiber tissues. The expression of tubulin was about 1.5 folds higher in 10 DPA fibers as compared to cotton ovules at 0DPA. The transcripts of both exapnsin and tubulin began to drop after 10 DPA as fiber elongation is terminated and cellulose deposition started.

Lipid transfer proteins showed variable expression in different cotton tissues. Expression pattern of LTP3 gene showed its mRNA transcripts in most of tissues aincluding fibers during all fiber development stages (Fig. 5c). The transcripts of LTP3 gene were elevated from 5 DPA to 15 DPA which then began to decline. In tissues other than fiber, expression of this gene was slightly high in leaves and shoot as compared to root and stem but not at that extent as in elongating fiber. Expression of LTP1 gene was also remarkably high in rapid elongating fiber during 5 to 15 DPA but it began to fall at the end of peak elongation period (Fig. 5d). In non fiber tissues low expression was monitored in all tissues except leaves. On the other hand, expression pattern of LTP7 revealed that there was almost steady expression level from 10 to 20 DPA fibers. Transcripts of LTP7 gene were more than double in 10 DAP fiber as compared to 5 DPA. In other tissues, the expression of this gene was almost negligible (Fig. 5e).

Expression profilies of E6 gene showed that its transcripts were detected in rapidly elongating fibers along with cotton roots, leaves and stem (Fig. 5f). Expression of this gene was detected in all fiber stages but reached at maximum level in 15 DPA fibers. On the other hand, expression pattern of SPS and susy demonstrated that both genes expressed at higher level during 5-20 DPA Fig. 5g & h). Further transcript level of susy was almost stable from 10 to 20 DPA fiber whereas transcripts of SPS were maximum in 10 DAP fibers (about 3.5 folds higher than 0 DPA ovules) then began to drop gradually with the start of secondary cell wall deposition phase. In non fiber tissues, both genes showed nearly much low expression but susy mRNA was detected in roots to some extent. On the other hand, Very low transcripts were detected in non fiber tissues.

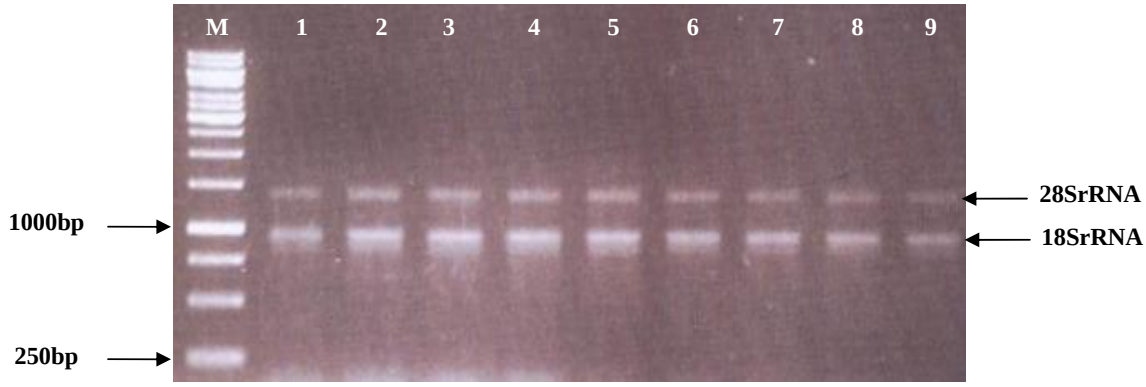

Fig. 1. Total RNA isolation from cotton tissues. M 1 Kb ladder; Lane 1-9 total RNA isolated from various tissues of cotton plant (Lane 1: Root, Lane 2: Shoot, Lane 3: Stem, Lane 4: Leaves, Lane 5: 0 DPA fibers, Lane 6: 5 DPA fibers, Lane 7: 10 DPA fibers, Lane 8: 15 DPA fibers, Lane 9: 20 DPA fibers).

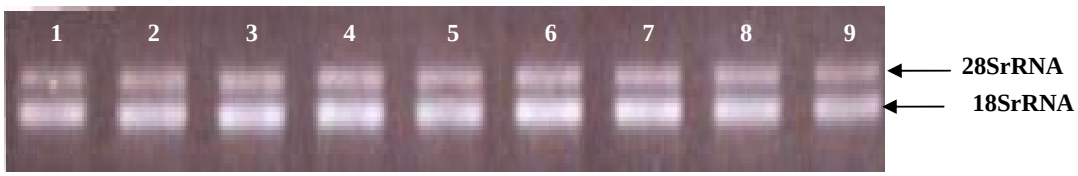

Fig. 2. Equalization of RNA concentration in cotton tissues. Lanes (1-9) illustrate RNA extracted from Root, shoot, stem, leaves, 0 DPA, 5 DAP, 10 DPA, 15 DPA and 20 DPA fibers respectively.

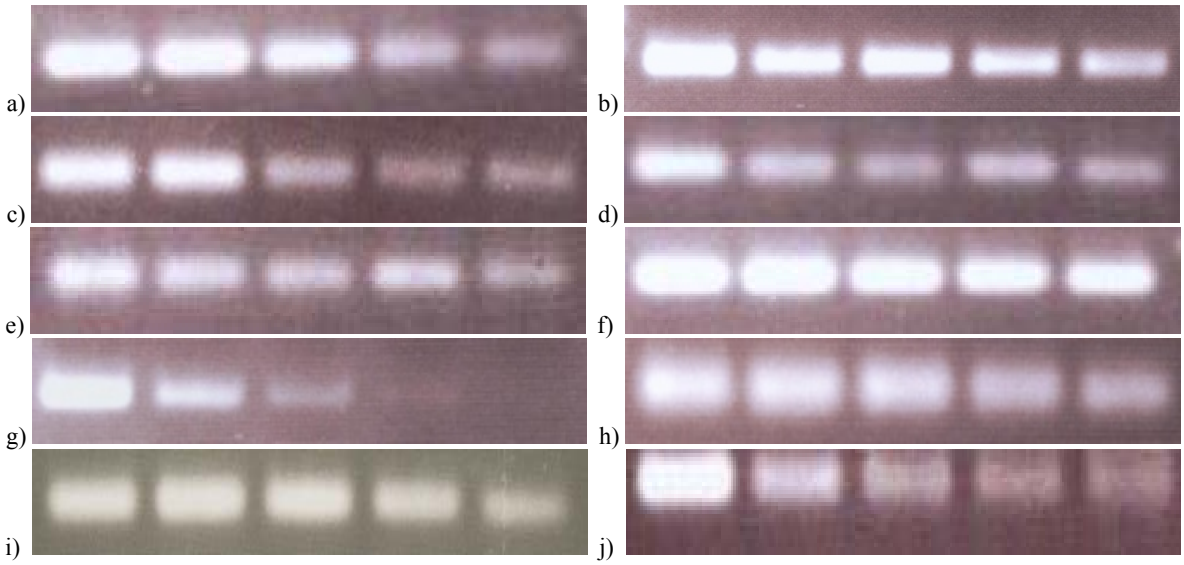

Fig. 3. Primer validation through 10 folds dilution series of 10 DPA fiber cDNA as template. PCR amplification using primers of (a) 18SrRNA, (b) Expansin, (c) Tubulin, (d) LTP1, (e) LTP3, (f) LTP7, (g) Ubiquitin, (h) E6, (i) SPS, (j) Susy.

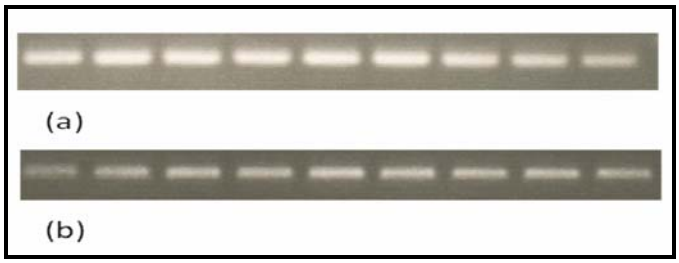

Fig. 4. Equalization of various templates concentration through PCR amplification. a)18S rRNA primer, b) Ubiquitin primer.

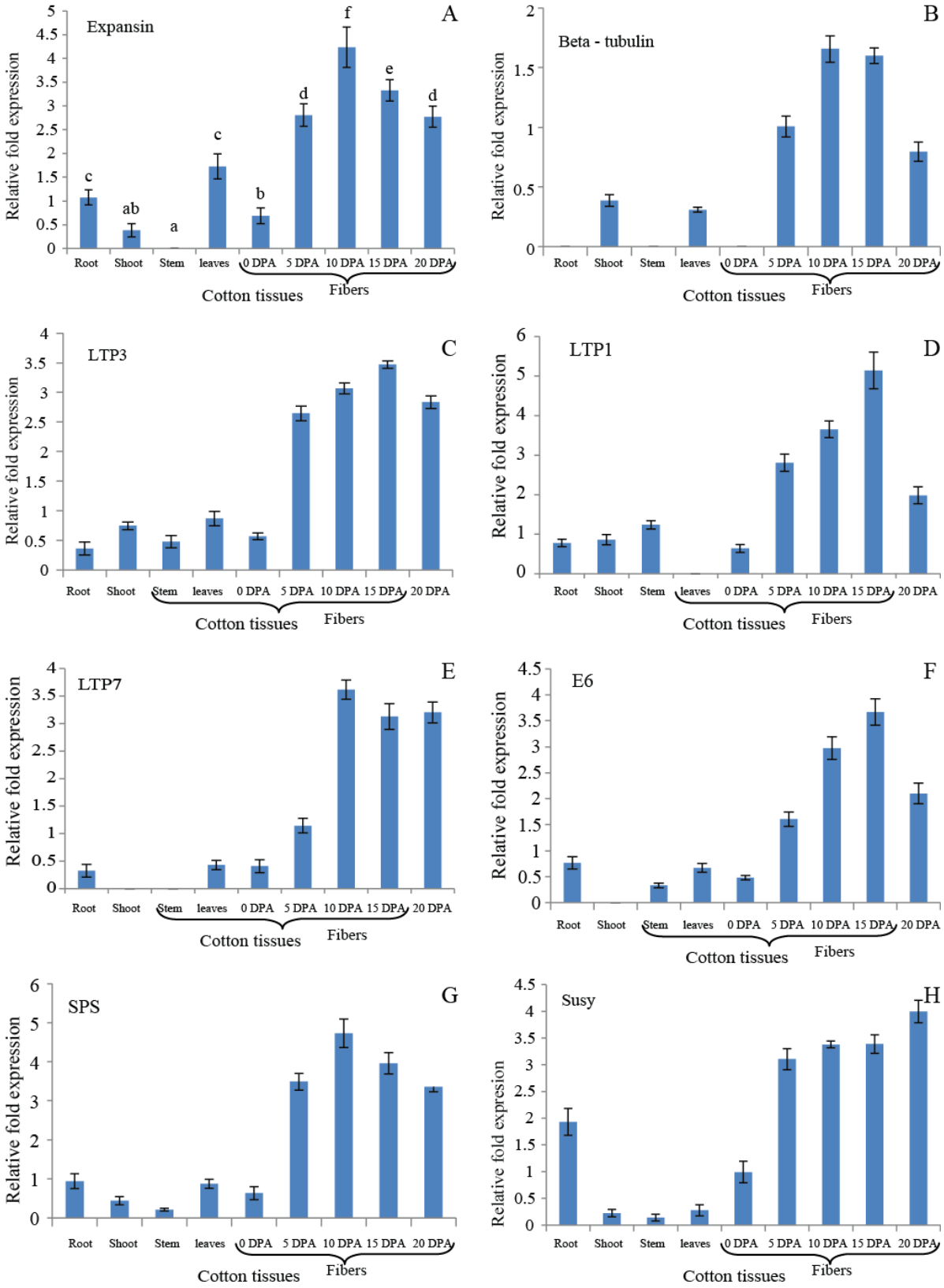

Fig. 5. Quantitative reverse transcriptase analysis of fiber related genes in various tissues of cotton (var. CIM707). The expression was normalized using 18S rRNA as a normalizer. Genes include a) Expansin, b) Tubulin, c) LTP1, d) LTP3, e) LTP7, f) E6, g) SPS, h) Susy. Error bar represents ±SE of three experimental replicates. LSD test at $p<0.05$ was used to determine the significance of expression level in various cotton tissues.

Discussion

A large number of genes are involved in complex process of fiber development (Arpat, 2004). Several genes including LTPS, actins, arabinogalactans, endoglucanases, sucrose synthases, annexins, ascorbate peroxidases, expansins and some others have been found to express at high rate in developing fiber (Mei *et al.,* 2012). Many fiber genes are expressed exclusively in fibers and are thought to have pivotal role in fiber morphogenesis. Some fiber related genes are expressed distinctly during particular developmental phase which reflect their stage specificity. In the present study reverse transcriptase real time PCR technique was used to monitor the relative transcript abundance of some cotton genes using SYBR green fluorescent dye. For each gene more than two primer pairs were tested from which best primer pair was selected for each gene.

Expression analysis of fiber genes revealed that expansin showed maximum level of expression infiber during elongating phase especially during peak elongation period (10 DPA). In rapidly elongating fibers elevated expression of expansin depicts its role in fiber cells expansion. Expansin proteins under low pH conditions along with various hydrolases cause cell expansion by loosening bond between cellulose and matrix polysaccharides (Cosgrove, 2000). Similar results were obtained in previous study which describes high expression of expansin in fast extending fibers (Ruan *et al.,* 2001). High expression of expansin in fibers depicts its role in fiber elongation. So, expansions gene may be transformed in cotton for improvement of fiber length as describer previously (Bajwa *et al*., 2013). In non fiber tissues the expression of expansin was slightly higher in roots. This may be associated with active cell division in root tissues.

Level of β-tubulin gene showed similar expression pattern in fibers as expansins and was highest during rapid fiber elongation stage (Fig. 5b). After reaching at maximum level at 10 DPA, its transcript level began to drop as secondary cell wall deposition initiated. Similar expression pattern of five tubulin genes in cotton was observed as described previously (Feng *et al*., 2004). Earlier study of expression analysis of tubulin gene by northern analysis showed similar expression as by real time PCR analysis (Indrias *et al.,* 2011). So, real time PCR assay can also be used to validate northern blot results. Tubulins are major components of microtubules that constitute cytoskeleton in plants. The organization of microtubules have crucial role in alignment of cellulose microfibrils during fiber development (Fisher & Cyr, 1998). Like expansins gene family tubulins are also multigene family having diverse functions (Li *et al.,* 2002). The results of our study revealed that transcript abundance of cell wall loosening enzyme expansin along with tubulins have direct relationship with elongation of fiber.

We have also investigated the transcription abundance of three lipid transfer protein (LTP1, LTP3 and LTP7) genes in cotton fibers to explore their role in fiber development. The expression pattern of three LTPs revealed that their expression was much higher in elongating fibers. In non fiber tissues, the expression of LTP3 was found to be relatively higher in leaves whereas LTP1 showed slightly higher expression in shoot and stem (Fig. 5c & d). In contrast, LTP7 gene showed almost negligible expression in non fiber tissues (Fig. 5e). This gene showed much higher expression in fast extending fibers that continued even at the onset of secondary cell wall synthesis. High expression throughout fiber development may be associated with fiber specificity of this gene and suggests that LTP7 may not be involved in the processes than fiber morphogenesis. This also suggests that LTP7 gene may be transformed in cotton to improve fiber qualities as well as for isolation of upstream regulatory sequences.

There are various isoforms of LTPs, some of which are constitutive while others are tissue specific. Lipid transfer proteins bind to large range of lipid molecules to their hydrophobic cavity and cause cell wall loosening. Both LTPs and expansins have cell wall loosening activity but they have some difference in their mode of action. Expansins are activated by lowering pH while LTP work at neutral pH. Both of these are associated with cell wall cellulose/xyloglucan network, expansin by carbohydrate binding domain while LTPs with their hydrobhobic cavity which is essential for their function. Several plant LTPs have been previously identified in cotton fibers express from 1-20 DPA (Orford and Timmis, 2000; Feng *et al*., 2004). Our results demonstrate that both LTP1 and LTP3 genes are expressed in almost all tissues and all stages of fiber development with variable expression levels. The genes which are constantly expressed are required by plant cells suggesting its possible involvement in the active metabolic processes essential for plant growth.

Transcript level of E6 gene was also much higher in elongating fiber than in non fiber tissues. Expression of E6 elevated gradually with fiber elongation then began to drop at secondary cell wall synthesis phase. This demonstrates that E6 protein has pivotal role in fiber elongation not in secondary cell wall deposition. E6 protein has many active sites which are activated by Casein kinase II. Many proteins of various metabolic pathways that have to perform crucial functions in individual fiber cells are activated by E6 protein (Pinna, 1990). The reason behind raising of E6 level in fibers might be that many metabolic events including biosynthesis of polysaccharides take place at fast rate in extending fibers.

Sucrose phosphate synthase (SPS) is also another abundantly expressed gene in developing cotton fibers especially in 10 DPA fibers which then began to drop with progression of fiber development. High expression of SPS in fast elongating fiber might be due to synthesis of sucrose, which along with other hexoses in fiber cell increases cell turgidity, allows fiber cell to expand. Previous study reported that SPS level rises parallely with the start of secondary cell wall deposition (Babb & Haigler, 2001). However, in the present study highest transcript level of SPS was detected in fast elongation stage (10 DPA). As fiber elongation period is metabolically active phase of cell and a large number of cells are expressed during this phase. There fore expression level of SPS might be high during active transcription period in the cell (Haigler *et al.,* 2001).

Compared to Susy which have almost constitutive expression during 5-20 DPA of fiber development, SPS expression seems to stage specific. Due to highest expression during fast elongation stage, this gene may be over expressed in cotton to gain premium quality fiber. Previous reports showed improved quality fiber in cotton when transformed with transgenic cotton with spinach sucrose phosphate synthase gene (Haigler *et al.,* 2007). Therefore, expression studies provide useful information about genes those can be used to engineer cotton with improved qualities.

As far as sucrose synthase (susy) is concerned, there is almost steady expression during fiber development with slight upregulate during secondary cell wall deposition stage (Fig. 5h). This may be due to its involvement in cellulose synthesis during secondary cell wall synthesis (Brill *et al.,* 2011). Sucrose synthase is an important enzyme found to have major role in cotton fiber development (Ruan *et al.,* 2003). Expression of susy gene was much higher in roots as compared to other non fiber tissues. Various isoforms of sucrose synthase exhibit diverse functions in plant tissues like stem root and leaves that is associated with starch/cellulose storage in plants (Barratt *et al.,* 2001).

Present study reveals that through expression analysis, we can expound the role of fiber related genes in fiber development. Real time PCR can be best technique utilized, to monitor the changes in the gene expression level in various cotton tissues and fiber during particular developmental phases. Although many studies have under taken but there is very brief knowledge about developmental regulation of cotton fibers. There are also differences among genotypes and species of cotton for expression of fiber genes. So, there is need to fully determine the variation in gene expression pattern among different genotype and species. The above study conducted in a long fiber cotton variety of Pakistan may be helpful to determine accurate timing and level of expression of some fiber related genes and may help to improve cotton fiber through biotechnological approaches.

Conclusion

From the above study it is concluded that five gene families exhibited variable expression pattern in fiber and non fiber tissues. Most of the genes are expressed at higher rate in 5-15 DPA fibers. The comprehensive expression profiles demonstrated tissue and stage specific expression of these genes. Selected highly expressed genes can be used for transformation in cotton to alter fiber properties. Upstream regions of these genes could also be analyzed as novel promoters and used to express foreign genes in transgenic plants.

Acknowledgement

This study was supported by the Ministry of Food and Agriculture (Min FA), Government of Pakistan and HEC, Government of Pakistan.

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(Received for publication 24 February 2015)