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COMPARATIVE EXPRESSION STUDIES OF FIBER RELATED GENES IN COTTON *SPP.*

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ABSTRACT

Cotton fibers are the seed trichomes that are developed around the seed and are used to make clothes and yarn for the textile industry. Expression profiling of cotton fiber genes is very important to estimate the differential gene expression level at different fiber developmental stages. Expression analysis of fiber developing genes are very important to enhance the fiber length of cotton. The expression profiling of three gene families in five stages (0, 5, 10, 15 and 20 DPA) of cotton fiber tissues was carried out through real-time PCR. Expression analysis revealed that transcripts of GA-*20 Oxidase*, *XTH*, and *PEPc* were elevated from 5 to 20 days post-anthesis (DPA) fibers. Total RNA was extracted from various stages of cotton fiber development and was reverse transcribed to cDNA for PCR amplification. For data normalization, 18s rRNA was used as an internal control. The objective of this study was to explore the expression level of fiber developing genes at specific stages of fiber development. The results showed that most of the genes were expressed during the elongation phase in between 5 DPA to 15 DPA. Results obtained from this study may be helpful for the further identification of fiber genes and the improvement of fiber characteristics in cotton. *PEPc* and *XTH* genes that are expressed with a high rate during the fiber development may be used in breeding programmes for the improvement of fiber quality and quantity.

Keywords: Trichomes, fiber-related genes, DPA, *XTH*, *PEPc*, real time-PCR.

INTRODUCTION

Cotton is the main cash crop of a national economy; equally, it delivers clothes, edible vegetable oil, and a lot of other things for human usage and consumption. Cotton fibers are the seed trichomes that are developed around the seed and are used to make clothes and yarn that are very important for the textile industry. In the world, a cotton plant is used as a model plant to study the evolutionary and developmental mechanisms of fiber morphogenesis (Kim and Triplett, 2001; Tuttle et al., 2015; Yanagisawa et al., 2015; Khatoon et al., 2018; Qin et al., 2019; xiao et al., 2019; wang et al., 2019). Cotton fiber is called the king of all fibers because there is no cell division occurs during the different fiber developmental stages. In Industrial point of view, it is the world-leading crop because it provides very long and spinnable fiber to make textile fabrics and yarn and second in oil production (Freeland et al., 2006; Lee et al., 2015; Ute et al., 2019). Cotton fiber has unlimited financial importance for trade and industry around the globe. It is highly acceptable due to the capability to be stained, decorated, simply washed, absorbency, and porosity. upland cotton called

Gossypium hirsutum L. having 20-30 mm fiber length, is the most commonly grown cotton species worldwide (Al Ghazi et al., 2009). Though, Pakistan is one of the most important cotton-producing countries yet, it cannot fulfill the demand of cotton industries of long and spinnable fiber to make clothes and yarn. In Pakistan, to fulfill the demand of the local and fabric production textile industries, it has to import more than 50,000 million bales of very-long fiber and fine staple cotton at the cost of 157 million US dollars every year (Pakistan Economic Survey, 2019-20).

Cell wall expansion is the first step towards fiber formation in cotton. Expansion is very important for fiber development because it maintains the cellular growth in cotton. The function of cell wall loosening over and done with the breakdown of non-covalent bonds in between the hemicelluloses and cellulose fibrils through the activation of cell wall loosening genes like *expansins* (Cosgrove, 2000; Kim, 2015; Li et al., 2016; Martínez-Sanz et al., 2017; Yaqoob et al., 2018; Fry, 2018) and *xyloglucans endotransglycolyses/ hydrolase* (Fry et al., 1992; Liu et al., 2015; McCann and Knox, 2018; Han et al., 2016). Fiber expansion and elongation take place instantaneously after the cell wall synthesis. During the primary cell wall synthesis stage, the cell walls of cotton fibers are extensible (Smart et al., 1998; yang et al., 2016; Ma et al., 2018) and permit the entry of a large amount of water and other solutes (k^+) inside the fiber cell which increases turgor pressure.

The first three stages including initiation, elongation, secondary cell wall synthesis are very important because they determined the fiber quality and its staple length. Among these three-stages of fiber development, the elongation stage was very important because it determines the final staple length of fiber cells (Kim et al., 2018). This elongation phase also affects the productivity and length of the fiber. On the other hand, the second most important phase of fiber development was SCW because it determines the cellulose and other essential minerals deposition and water potential of the vacuole. This SCW phase was crucial for fiber enlargement because it controls the contents of cellulose deposition on fiber which affects the fiber length and strength (Lee et al., 2007; Ahmed et al., 2016; Zhang et al., 2017).

Fiber cell elongation mechanism was very important for the fine and long fiber development to fulfill the raw material requirements of the textile industry (Ruan et al., 2004; Liu et al., 2015). A large number of stage development specific and time-based genes such as *expansins* (*EXP*), *sucrose synthases* (*sus*), CaM-7 like genes (GhCaM-7 like), *lipid transfer proteins* (*LTPs*), *PEP Carboxylase* (*PEPc*), *tubulins,* and Xyloglucan endotransglycolyses/hydrolase (*XTH*) were reported in the literature, responsible for fiber development in cotton (Hsu et al., 1999; Arpart, 2004; Huang et al., 2013; Cheng et al., 2016; Balasubramanian et al., 2016). Out of these genes, some are fiber development-related genes that are expressed significantly/absolutely in the formation of fiber development and they are assumed to have a central role in fiber synthesis. The fiber-related genes are believed to be compulsory at the fiber initiation and cell wall elongation stage of fiber improvement (Wang et al., 2010; Yuan et al., 2015; Fang et al., 2018; Qin et al., 2019; Xia et al., 2018; Xiao et al., 2019).

The potential role of *Phosphoenolpyruvate carboxylase* (*PEPc*) and Gibberellic acid-20 oxidase (*GA-20*) genes were observed during fiber development in cotton. *PEPC* activity was higher at the elongation phase that was positively correlated with fiber elongation. RT-PCR analysis showed that *PEP carboxylase* highly expressed throughout the elongation stage of fiber development (Li et al., 2009, Xiao et al., 2010, Huang *et*

al., 2013; David et al., 2016; Zhao et al., 2018).

The three isoforms of *xyloglucan endotransglucosylase* (*XTH*) were observed during fiber development that plays a crucial role in the phase of fiber elongation in cotton. The overexpression of profilin *GhPFN2* (Wang et al., 2010) and *GhXTH1* in transgenic cotton were used to produced longer fiber as well as for fruit ripening in tomato (Saladié et al., 2006; Lee et al., 2010). In transgenic soybean plants, overexpressed *XTH* genes were used to regulate and tolerate the flooding stress (Naoumkina et al., 2015; Song et al., 2018). The expression profiling analysis verified that *XTH* expression was much greater in *G. barbadense* than *G. hirsutum* that leads to fiber elongation in *G. barbadense* (Avci et al., 2013, Xiao et al., 2006).

RT-PCR analysis provides us the opportunity to estimate and identify the expression of a potential gene during the distinct phases of fiber development in cotton. These expression profiling techniques may also help to identify the novel genes and their promoters of long fiber (Zhang et al., 2004; Imran and Liu, 2016; Naoumkina et al., 2016; Li et al., 2017). Through gene expression profiling data, we can investigate and clarify the fiber improvement process and stages and can be used to transform these novel genes of superior quality fiber (long fiber) through genetic engineering.

In the present study, three local genotypes from different cotton species were used to see the transcript level of fiber-related genes. Genes with high expression levels in fibers at different developmental stages were selected for further studies in the future. The basic aim of this study was to discover the expression level of the novel genes during the fiber developent. After the expression profiling, genes may be used in cotton breeding programmes to develop genotypes with long staple length.

MATERIALS AND METHODS

Sowing of Fiber-Related Genotypes

The seed of *G. hirsutum* (var. CYTO-179), *G. arboreum* (var. Ravi) and *G. anomalum* (wild specie) was obtained from the Central Cotton Research Institute (CCRI), Multan to conduct a field experiment. Seeds were de-linted with strong sulphuric acid (H_2SO_4) and dried under shade. Plants were cultivated in a randomized complete block (RCBD) design with three replications in the field area of MNS University of Agriculture, Multan, Pakistan, during the cotton growing season 2018-19 under normal field conditions.

Flower Tagging

Flowers of field-grown plants were tagged on the day of fertilization for precise selection of cotton bolls of particular days. A flower with the good and healthy condition was selected for tagging. More than two flowers were tagged for one sample collection. The opening of the flowers was marked as 0 DPA. Cotton bolls of different developmental stages 0, 5, 10, 15 and 20 DPA (days post anthesis) were also tagged and collected. The main purpose of tagging was a precise and timely collection of samples (bolls) for the further process in the lab.

RNA Isolation

Collection of Samples

The cotton fiber was collected from the field at different development phases (0, 5, 10, 15, 20 DPA) for the expression analysis of fiber genes. The stage of sample collection and time were the two important factors to see the exact expression of the fiber-related genes. The tagged samples were collected from the mentioned date and time for the accurate measurement and other processes related

to fiber characteristics. Before the collection of the sample (boll) from the plant washes it carefully with 0.01% of DEPC (Diethyl Pyro Carbonated) treated water to avoid any other foreign substances (RNases) contamination (Indrais et al., 2011; Iqbal et al., 2016; Iqbal. 2017; Khatoon et al., 2018). Wraped the sample in aluminum foil and stored in liquid nitrogen cylinder at -196℃ along with the date and time that was mentioned on tag.

Total RNA Extraction

Total RNA was extracted by using plant RNA extraction reagent (Invitrogen, USA) according to instructions given by the manufacturer.

Analyzing of RNA Quantity and Quality

Accurate RNA quantification was the essential step during the gene expression analysis. The total RNA concentration was determined by the nanodrop at the UV absorbance ratio of 260 nm and 280 nm. To check the reliability and concentration of total isolated RNA loaded on a gel of 2% agarose in 1X TAE buffer solution.

First-Strand cDNA Synthesis

Total RNA was reverse transcribed to cDNA using 2 µl of the dNTPs mix, 0.5 µl Ribo Lock RNase inhibitor and 0.5 µl Revert Aid Reverse Transcriptase. Reverse transcriptase was used to reverse transcribe the RNA into cDNA.

RT-PCR Primers Design

DNA sequences of fiber length related genes were retrieved from NCBI and primers were designed using Primer3 and AmplifX software (Jullien, 2013; Wang et al., 2015).

The RT-PCR primers were designed with online available program Primer 3 (Premier Biosoft International, USA). The designed primer length was almost at 18 to 20 nucleotides and GC contents were not greater than 60%. Primers were designed with no repetition of complementary structure and other secondary structures in the regions of forward and reverse primers. At last, BLAST (http://www.ncbi.nlm.nih.gov) examination analysis was done to check the specific gene sequence for each primer. The qRT-PCR assay was optimized by running a series of dilutions of a template sample and uses these results to create a standard curve. A template 15 DPA of *G. hirsutum* was used for the RT-PCR optimization with a sample of the unknown quantity (e.g., cDNA). The standard curve was generated by plotting the log of the starting quantity of template (for unknown quantities) against the C_T value obtained during the amplification of each sample dilution.

Detail of Primers used for RT-PCR Studies

Details of primers used for RT-PCRdurind this study are shown in table 1&2.

Table 1: Annealing temperature optimization of fiber genes and internal control.

Primer	Annealing Temperature (ºC)
GA-20 Oxidase	55
XTH	55
PEP carboxylase	58
18Sr RNA	46

Table 2: Description of primers used for RT-PCR studies for expression analysis of fiber-related 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	RT18SF RT18SR	AAACGGCTACCACATCCAAG CCTCCAATGGATCCTCGTTA	20 mer 20 mer	153	U42827.1
2	*PEP carboxylase*	RT*PEPC*F RT*PEPC*R	CACCGACCTACTACACGAGGTGTG AGAAGCCTCAAAAGGCATTCCTTG	24 mer 24 mer	227	EU032328.1
3	*GA20 Oxidase*	GA20F GA20R	CTTGCTTGGGGACTCTCTTG ACGAAACTGCTTGCATACCC	20 mer 20 mer	219	AY895169
4	*XTH*	RT*XTH*F RT*XTH*R	CCAAAATTCAGGCTGTGGAT TTGTTCCCTGTCACCCTTTC	20 mer 20 mer	231	EF546794

Table 3: Procedure of an RT-PCR Reaction

RT-PCR Steps	Temperature (ºC)	No. of cycles	Duration (Minutes)
First denaturation	95	1	10
Denaturation Annealing Extension	95 55 95	40	0.5 1 0.5
Final extension	72	1	10
Melt curve analysis	95	40	0.5

RT-PCR Analysis of Fiber Genes

For RT-PCR reaction, A SYBR green supermix was arranged with comprising all reagents including SYBR green supermix dye, the primers, and nucleases water. The RT-PCR reaction arrangement for every one of the samples was as follows in Table 4.

Relative gene expression was calculated by 2^ΔΔ Ct method by using this formula Ct_{sample}-$Ct_{control}$ (Hung *et al*., 2015). In this formula Ct was the temperature of a cycle. In this method Ct was calculated by Ct of interest gene – Ct of reference gene.

Table 4: Concentration of an RT-PCR Reaction

Reagents	Volume (µl)
SYBR Green Supermix	12.5
Forward primer	1
Reverse primer	1
Template (cDNA)	3
U.P water	7.5
Total volume	**25 µl**

Quantitative real time PCR based gene expression data of fiber-related genes expressing in various tissues of cotton (var. Cyto-179) 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≤0.05% was employed to determine the significance level among means of gene expression.

RESULTS

Expression profiling of three genes (*GA-20 Oxidase*, *PEP carboxylase* and *XTH*) was conducted in cotton fibers at different fiber developmental stages including initiation, elongation, secondary cell wall synthesis and maturation with different times of intervals after anthesis (0, 5, 10, 15, and 20 DPA). Expression analysis study was conducted through RT-PCR by using stage specific primers (Table 1). Transcripts of various fiber stage specific genes were examined at five different developmental stages (0, 5, 10, 15 and 20 DPA). The concentration of total RNA at all fiber developmental stages was ranged from 0.2 µg to 0.5 µg, (Figure. 1). Absorbance ratio at A260/ A280 was 1.37 to 1.75 (< 2) which revealed a good quality RNA preparation. The concentration of all samples was equalized by a dilution series of DEPC treated water (Fig. 2). Total RNA was reverse transcribed to synthesized the first strand cDNA by usin g a reverse transcriptase and visualize on gel (Figure. 3).

The concentration of all cDNA samples of cotton fibers were nearly equalize through the amplification of an internal control (*18S rRNA*) primer (Figure. 4)

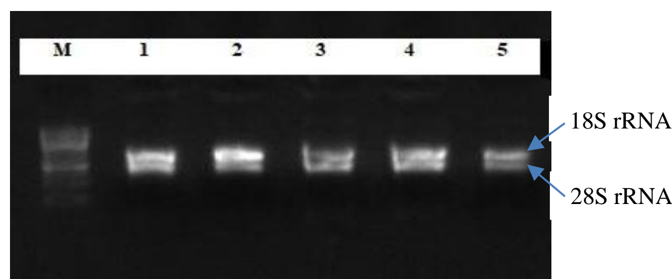

Figure 1: Total RNA isolation from cotton Fiber tissues. M 1 Kb ladder; Lane 1-5 total RNA isolated from various stages of cotton fiber (Lane 1: 0 DPA fibers, Lane 2: 5 DPA fibers, Lane 3: 10 DPA fibers, Lane 4: 15 DPA fibers, Lane 5: 20 DPA fibers).

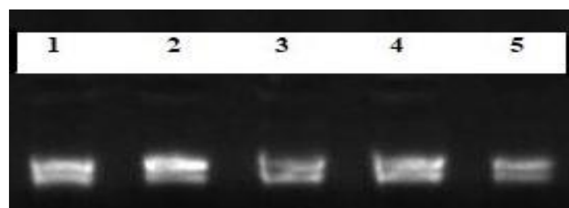

Figure 2: Equalization of RNA concentration in cotton fiber tissues. Lanes (1-5) illustrate RNA extracted from 0 DPA, 5 DAP, 10 DPA, 15 DPA and 20 DPA fibers respectively.

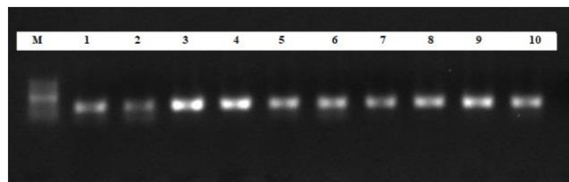

Figure 3: Synthesis and equalization of first strand cDNA from various stages of fiber development. M 0.5 bp ladder; Lane 1-10 cDNA synthesized from various stages of cotton fiber (Lane 1: 0 DPA fiber of G. hirsutum, Lane 2: 5 DPA fiber of G. hirsutum, Lane 3: 10 DPA fiber of G. hirsutum, Lane 4: 15 DPA fiber of G. hirsutum, Lane 5: 20 DPA fiber of G. hirsutum, Lane 6: : 0 DPA fiber of G. arboreum, Lane 2: 5 DPA fiber of G. arboreum, Lane 3: 10 DPA fiber of G. arboreum, Lane 4: 15 DPA fiber of G. arboreum, Lane 5: 20 DPA fiber of G. arboreum.

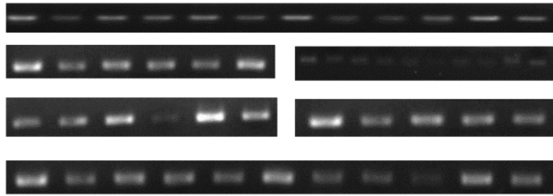

Figure 4: Primer validation through 10 folds dilution series of 10 DPA fiber cDNA. PCR amplification using primers of (a) 18SrRNA, (b) *GA-20 Oxidase*, (c) *PEPC Carboxylase*, (d) *XTH*.

An internal control was used to normalize the PCR data. All primers were confirmed by a 10 fold dilution series of template concentrations (Figure 5).

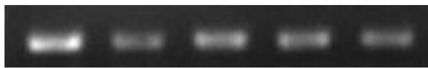

Figure 5: . Equalization of various templates concentration with 18S rRNA primer through PCR amplification

The expression analysis of *PEP Carboxylase* gene in all three genotypes showed that *PEPc* gene expression was elevated throughout the all fiber developmental stages at different times of intervals (Figure 6).

The highest gene expression was detected in 15 DPA of fiber stage in both *G. hirsutum* and *G. anomalum*. A similar gene expression was also observed in *G. arboreum* at 5 DPA. A same quantity of transcript of *PEPc* gene was observed in all three genotypes at 10 DPA fibers. At the end of elongation phase of fiber development after the 15 DPA, a small decrease in the gene expression was also observed at 20 DPA fibers.

The expression level of *PEPc* was about 1.5 fold higher in 10 and 15 DPA fibers than 0 and 20 DPA. The transcripts of *PEPc* was dropped after 15 DPA as the termination of fiber elongation phase and the deposition of cellulose started. *GA-20 Oxidase* gene showed a variable gene expression throughout the all stages of fiber development. A highest level of expression was detected in *G. hirsutum* at 10 DPA stage of fiber development in *G. arboreum*.

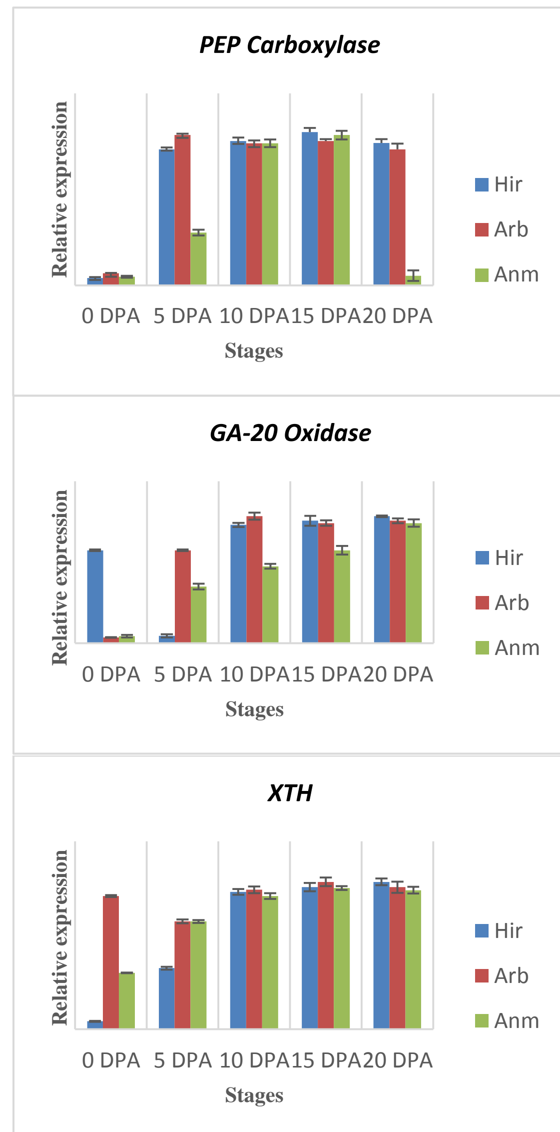

Fig. 6. Quantitative real time PCR analysis of fiber related genes in various stages of fiber development in cotton. The expression was normalized using 18S rRNA as An internal control. Genes include a) PEPc, b) GA-20 Oxidase, c) *XTH*). Error bar represents ±SE of two experimental replicates. LSD test at $p<0.05$ was used to determine the significance of expression level in various cotton fiber tissues.

The expression pattern of *GA-20 Oxidase* revealed that the most of the mRNA transcripts was present during the whole process of fiber development with different times of intervals (Fig. 7). The transcripts of *GA-20 Oxidase* was raised up from 0 DPA fiber to 10 DPA and than declined from 15 DPA to 20 DPA . The expression level of *GA-20 Oxidase* was much similer with higher concentration at

20 DPA fibers in all three genotypes. The expression level of this gene was about 1.5 fold higher in 10 DPA fiber than 0 DPA. The expression level of *GA-20 Oxidase* was remarkably high in *G. arboreum* at 10 DPA and *G. hirsutum* at 20 DPA. The transcrips of *GA-20 Oxidase* was varied in all three genotypes at 0 DPA but the level of expression in *G. hirsutum* was much higher than others. In comparison to other genotypes, the expression level of *GA-20 Oxidase* was raised up from 0 DPA to 20 DPA in *G. hirsutum*.

The transcript level of *XTH* gene was higher in 15 PDA fibers in *G. arboreum* as compared to other DPA fibers but was not much higher at 5 DPA fibers. At the termination of the fiber elongation phase, a similar level of expression of *XTH* gene was detected in many fiber samples. Expression profiling analysis showed that a very small transcript of *XTH* gene was seen in the initial stages of fiber development in cotton fiber tissues. Transcripts of *XTH* genes were discovered in large quantities in 15 DPA fibers but lacking in 0 DPA fibers. Transcripts of *XTH* gene were highest in 20 DPA fibers in *G. hirsutum* as compared to the other stages. The RT-PCR expression profiling presented that transcripts of *XTH* gene were much similar in 10 to 20 DPA fibers but lacking in the initiation stage of fiber improvement at 0 DPA. The *XTH* gene showed a high level of expression in 15 DPA of fiber samples than other developmental stages of cotton fiber development. The expression pattern of *XTH* gene was detected with low level in the first elongation phase of fiber development. The transcripts of *XTH* gene was observed in *G. hirsutum* in the all elongation stages of fiber development in cotton. The expression level of this gene was very much low in the 5 DPA stage of fiber development as compared to other

DISCUSSION

Cotton fiber is a very important commodity for industrial purposes due to its long fiber production across the globe. Cotton fibers are the seed trichomes that are developed from the epidermal layer of the seed with the activation of *MYB* gene family (Wang et al., 2004). It is used commonly as raw material for the manufacture of fabrics and other cloth stuff for the textile industry. Fiber with long staple length is very important and necessary for the fabric industry. Natural and artificial selection has been used for the selection of modern cotton cultivars with different fiber qualities. Fiber qualities like long staple length, strength and very fine fiber are highly desirable and necessary for the fabric and cloth industry.

A large number of stage development specific and time-based genes are expressed during the complex and difficult process of fiber improvement in cotton (Arpart, 2004). Out of these genes, some are fiber development related that are expressed significantly/absolutely in the formation of fiber development and they are assumed to have a central role in fiber development and improvement. These fiber-related genes are believed to be compulsory at the initiation and elongation stage of fiber development (Li et al., 2005). Expression analysis and other bioinformatics approaches are used to check, evaluate and identify the promoters of highly expressed fiber improvement genes along with their functions. Various cotton fiber developmental genes such as *expansins* (*EXP*), *sucrose synthases* (*sus*), *lipid transfer proteins*(*LTPs*), *PEP Carboxylase* (*PEPc*), *tubulins,* and *Xyloglucan endotransglycolyses/hydrolase* (*XTH*)

were reported in literature, responsible for fiber development in crop plants including cotton (Zhao et al., 2001; Li et al., 2002; Li et al., 2005; Huang et al. 2013, Zhao et al., 2018). A variety of profiling techniques are used to study the exact gene expression in any organism. At molecular and genetics level various techniques on transcript profiling analysis are being used in plant biotecnology to see the stage and tissue-specific gene expression. RT-PCR is the most efficient and reliable technique used in different types of field crops especially in cotton. RT-PCR is used to determine the quantity of the entire and absolute gene expression at any specific stage of any organism (Yuan et al., 2005). RT-PCR is mostly used to check and quantify the absolute and relative quantity of a gene in an individual. RT-PCR profiling helps to identify the expression level of highly expressed genes during definite physiological conditions in cotton fibers. RT-PCR analysis provides us the opportunity to estimate and identify the expression of a potential gene that controls fiber development during the distinct phases like fiber initiation, elongation, and secondary cell wall synthesis (SCW) in cotton. These expression profiling techniques may also help to identify and explore the novel genes and their promoters of long fiber. Through gene expression profiling data, we can investigate and clarify the fiber improvement process and stages and can be used to transform these novel genes of superior quality fiber (long fiber) through genetic engineering. For the expression profiling analysis of multiple genes in plants, real-time PCR could be used. After the PCR analysis, very expressed genes may help to alter the expression level of different types of fiber genes and the transformation of their promoters in cotton to increase its fiber length and other fiber attributes.

Expression analysis of both *PEPc* in *G. hirsutum* discovered that the transcripts records of *PEPc* genes were identified in between 5 to 20 DPA of fiber samples. The highest level of expression of *PEPc* gene was detected in 10 and 15 DPA of fiber samples. The expression level of *PEPc* was not higher in 20 DPA than 15 DPA fiber samples but a similar level of expression of *PEPc* gene was detected in the elongation stage of fiber at 10 DPA. The transcript of the *PEPc* gene was nearly twice in 10 DPA fiber samples in comparison to 5 DPA in *G. anomalum*. The transcripts of *PEPc* gene exhibited variable expression during the different cotton fiber developmental stages including initiation, elongation, secondary cell wall synthesis and maturation with different times of intervals at 0, 5, 10, 15, 15 and 20 DPA in *G. hirsutum*. The expression level of the *PEPc* gene was much higher in the elongation stage of fiber development but it began to fall over at the peak stage of fiber elongation in *G. anomalum*. In fiber developing tissues, transcripts of *PEPc* gene in all three genotypes were observed and detected in all developing fiber tissues. At 20 DPA fiber samples, transcripts of *PEPc* with low expression level were observed in 20 DPA in *G. anomalum*. The *PEPc* gene was showed a high level of expression during all-fiber developing stages. The expression level of the *PEP Carboxylase* gene was raised from 5 DPA to 15 DPA which then began to fall down till 20 DPA of *G. anomalum* fiber samples. The expression level of *PEPc* genes was to some extent higher in elongation phase during 10 and 15 DPA fiber samples as compared to the first initiation stage 0 DPA and the last maturation stage 20 DPA samples of fiber development in both *G. hirsutum* and *G. arboreum*. On the other hand, the expression level of the *PEP Carboxylase* gene presented that there was exactly the same

transcript level in between 10-20 DPA fibers in *G. hirsutum*. The expression level of the *PEP Carboxylase* gene was almost same in the elongation phase at 10 DAP fibers in comparison to 5 DPA in *G. hirsutum*.

Expression analysis of the *PEPc* gene revealed that a higher level of expression was observed and detected during the four overlapping phases of fiber development from 0 to 20 DPA fiber samples in all three cotton species *G. hirsutum*, *G.arboreum* and *G. anomalum*. On the other hand, the expression level of the *PEPc* gene was much higher in fiber elongation phase at 15 DPA fiber samples of *G. hirsutum* and *G. anomalum* as compared to *G. arboreum*. Expression profiling of *PEPc* in *G. hirsutum* confirmed that the transcripts of *PEPc* gene was expressed with higher concentration during the elongation phase at 5 DPA to 15 DPA in all-fiber developing tissues. The transcript of the *UBC* gene was much higher in 10 DPA fiber samples in comparison to 0 DPA. The expression pattern of the *PEPc* gene was highest in 5 DPA than the other fiber stages in *G. arboreum*.

The expression level of *GA-20 Oxidase* were observed in all stages of fiber development in cotton. In fiber developing tissues, a very low level of *GA-20 Oxidase* gene expression was detected in 0 and 5 DPA stages of fiber development . The transcripts of the *GA-20 Oxidase* gene also exhibited high transcript level in 10 DPA fiber samples in *G. arboreum*. The expression pattern of *GA-20 Oxidase* was not much higher in the initiation stage at 0 DPA in both *G. arboreum* and *G. anomalum*. The expression level of *GA-20 Oxidase* in *G. arboreum* was greater at elongation stage at 5 DPA fibers as compared to others. The transcripts of *GA-20 Oxidase* genes were also found to be high in the elongation phase at 10 DPA of cotton fiber development in *G. arboreum*. To the extent that, *GA-20 Oxidase* genes also showed the highest level of expression at fiber elongating phase in between 10 DPA to 15 DPA of fiber development in both *G. hirsutum* and *G. arboreum*. Transcripts of *GA-20 Oxidase* genes were showed in all four cotton fiber developmental stages with variable gene expression levels. The transcripts of *GA-20 Oxidase* genes were observed with a similar high amount in the SCW stage at 20 DPA of fiber samples in all genotypes. In some tissues of developing fiber, a low amount of transcripts of *GA-20 Oxidase* genes were detected at initial stages of fiber development in *G. anomalum*. In *G. hirsutum*, the transcripts of *GA-20 Oxidase* genes were much higher in fiber SCW stage at 20 DPA than the other stages of fiber development. The expression level of *GA-20 Oxidase* genes were almost the same in 15 and 20 DPA of fiber samples in both *G. arboreum* and *G. anomalum*. The expression level of the *GA-20 Oxidase* gene was also much higher in the elongation stage at 10 DPA fibers in *G. arboreum* as compared to the others. Therefore, the *GA-20 Oxidase* gene presented almost the same level of gene expression in between 10 DPA to 20 DPA of fiber samples. So, expression profiling revealed that the transcript of *GA-20 Oxidase* was observed in the all stages of fiber development in cotton fibers.

Expression analysis of cotton fiber development genes revealed that all fiber-related genes showed high expression levels in *G. arboreum* fibers as in *G. anomalum* and *G. hirsutum*. The transcript level of *XTH* gene was higher in 15 PDA fibers in *G. arboreum* as compared to other DPA fibers but was not much higher at 5 DPA fibers. At the termination of the fiber elongation phase, a similar level of expression of *XTH* gene was detected in many fiber samples. Expression profiling analysis

showed that a very small transcript of *XTH* gene was seen in the initial stages of fiber development in cotton fiber tissues. Transcripts of *XTH* genes were discovered in large quantities in 15 DPA fibers but lacking in 0 DPA fibers. Transcripts of *XTH* gene were highest in 20 DPA fibers in *G. hirsutum* as compared to the other stages. The RT-PCR expression profiling presented that transcripts of *XTH* gene were much similar in 10 to 20 DPA fibers but lacking in the initiation stage of fiber improvement at 0 DPA. The *XTH* gene showed a high level of expression in 15 DPA of fiber samples than other developmental stages of cotton fiber development. The expression pattern of *XTH* gene was detected with low level in the first elongation phase of fiber development. The transcripts of *XTH* gene was observed in *G. hirsutum* in the all elongation stages of fiber development in cotton. The expression level of this gene was very much low in the 5 DPA stage of fiber development as compared to others. The transcript level of *XTH* gene was nearly the same in between 10-20 DPA fibers but at extreme level in the SCW phase at 20 DPA of fiber samples in *G. hirsutum*. In cotton developing fibers, a variable transcript level of *XTH* gene was also observed in cotton.

CONCLUSION

Our results demonstrate that both *XTH* and *PEPc* genes were expressed with a variable level of expression in all stages of fiber development in all species of cotton. The genes which are constantly expressed are required by plant cells suggesting its possible involvement in the active metabolic processes essential for plant growth. The transcript level of the *GA-20 Oxidase* gene was also higher in elongating fiber. Expression of *GA-20 Oxidase* elevated gradually with fiber elongation then began to drop at the secondary cell wall synthesis phase. It demonstrates that *GA-20 Oxidase* has a pivotal role in fiber elongation. Results obtained from this study may be helpful for the further identification of fiber genes in cotton. Moreover, an expression analysis study may be useful to find out the constitutive and tissue-specific genes. Genes that were expressed specifically in fibers tissues could be used for isolation of upstream regulatory sequences. Genes that are expressed at a high rate during fiber development may be used in breeding programs for the improvement of fiber quality and quantity. Highly expressed fiber genes may be transformed in cotton for the improvement of fiber quality traits.

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