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Expression Analysis of Fiber Related Genes in Different Staple Length Genotypes in Cotton (*G. Hirsutum* L.)

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ABSTRACT

Cotton fibers, derived from the outer layer of seeds, are widely used in the textile industry for fabric production worldwide. Expression profiling techniques are valuable for identifying tissue (stem and roots) and stage-specific gene expression in cotton fibers. Expression analysis is a popular and successful technique for studying gene expression levels during tissue and stage-specific fiber development. In this study, expression profiling was conducted using real-time PCR on three different staple length genotypes of cotton (*G. hirsutum* L.): long, medium, and short staple length. The analysis was performed at five different stages of fiber elongation (0, 5, 10, 15, and 20 DPA). Three fiber development genes (*PEPc*, *XTH* and GA-*20 Oxidase*) were examined from the initiation phase (5 DPA) to the termination phase (20 DPA) of cotton fibers. Two internal control genes (ubiquitin and 18sr RNA) were used for data normalization. The primary focus of the study was to investigate the transcript levels of genes related to long staple length at different stages of cotton fiber development. All three genes showed similar expression levels during the elongation phase (15 DPA) of fiber development, but *PEPc* exhibited higher expression at 15 DPA in all three lines. The main objective of this expression profiling study was to identify and select genes associated with long staple length in cotton genotypes. The selected novel genes with high transcript levels could extend the elongation phase of cotton fiber morphogenesis, leading to improved long fiber characteristics. As a result of expression analysis studies using RT-PCR, cotton with high staple length genotypes has been commercially cultivated and utilized in the textile industry, reducing the need for imported long staple length cotton in local textile production.

Key words: Fiber elongation phase, RT- PCR, DPA, *GA*-20 *Oxidase*, *PEPc*, Long staple length.

INTRODUCTION

Cotton is unique fiber crop that supports the national economy and serves as major cash crop around the globe (Zafar *et al*., 2020). It is the primary source of fiber and also it delivers edible oil (Cotton seed oil), and other things for human use (Zafar *et al*., 2022b). Cotton fiber development is a very complicated process because there is no cell division occur during the all stages of cotton fiber development. Due to this evolutionary mechanism, cotton plant is used as an ideal plant among all fiber-related crops (Khatoon *et al*., 2018; Wang *et al*., 2019). An Industrial point of view, Due to the long fiber length; cotton is very important for both trade and industry purposes because it has been used from thousands of years around the globe (Lee and Fang 2015; Ute *et al*., 2019).

The process of fiber cells expansion is very important because it determines the subsequent increase in the final length of fiber, leading into the increase in fiber yield (Razzaq *et al*., 2021; Razzaq *et al*., 2022). In literature, there are four fiber developmental phases like i) initiation, ii) elongation, iii) secondary cell wall synthesis and iv)

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maturation (Razzaq et al., 2022). Among of them expansion (elongation) is very important for the development of a long staple length genotype because it controls and regulate the cellular growth and their progress during the long fiber development and its morphogenesis (Zafar et al., 2022a). The process of cell wall expansion starts immediately after the synthesis of primary cell wall and the elongation phase starts with the breakdown hydrogen bonds between the developing fiber cells. The breakdown of non-covalent bonds carried out after the activation of fiber elongation genes like *expansins* (Martínez-Sanz *et al*., 2017; Fry, 2018) and *XTH* (Han *et al*., 2016; McCann and Knox, 2018) resulting in the expansion of cotton cell wall (Zafar et al., 2021). During the initiation of cell wall synthesis, fiber initiation genes activate and helps in the expansion and elongation of cotton fibers tissues and cells (Yang *et al*., 2016) along with a large amount of water also entered into the fiber cells. Ultimately, other fiber expansion solutes especially potassium (k^+) also entered inside the fiber cells, results in the expansion of fiber cells due to the high turgor pressure inside the fiber cells. Fiber elongation (2^{nd} step of fiber synthesis) takes place immediately after the first stage of cell wall synthesis (Ma *et al*., 2018; Zhang *et al*., 2017; Kim *et al*., 2018). Secondary cell wall synthesis (SCW) is the third most crucial stage of fiber synthesis because it regulates the deposition of cellulose and hemicellulose fibrils along with other essential minerals inside the fiber cells. After the deposition, fiber cells turgid (enlarged) due to the increase in water potential inside the developing fibers. Critically, this cellulose deposition phase (SCW) is very essential for the regulation of final staple length (Ahmed *et al*., 2016; Nadeem *et al*., 2021).

Cell wall expansion starts immediately after the termination of initiation phase during the long fiber development in cotton (Ruan *et al*., 2004). The process of fiber cells elongation starts with breakdown of hydrogen bonds between the cellulose and hemicellulose fibrils along with the overexpression of fiber elongation enhancement genes including *expansin* (*EXP*), *sucrose synthases* (*sus*), GhCaM-7, *LTPs*, *PEPc*, *tubulins*, and *XTH*, responsible for long staple length in cotton (Liu *et al*., 2015; Cheng *et al*., 2016; Iqbal *et al*., 2016; Balasubramanian *et al*., 2016). (Iqbal, 2017) reported that the activation of these long staple length-related genes at elongation stage are thought to have necessary for the opening and closing of plasmodesmata, resulting in the intake of water and solutes. The turgor pressure inside the fiber cells increases with the entry of water and other solutes helps in the elongation of fiber tissues (Wang *et al*., 2010; Xiao *et al*., 2019).

The breakdown of hydrogen bonds between cellulose fibrils was done with the activation of these potential genes (Yuan *et al*., 2015; Fang *et al*., 2018). So, the fiber elongation genes especially *PEPc* are believed to have a positive correlation with fiber elongation. In biotechnology studies, RT-PCR analysis provided the evidences of the strong link of *PEP Carboxylase* with the elongation and expansion of fiber cells (David *et al*., 2016; Iqbal *et al*., 2016; Zhao *et al*., 2018).

The cell wall loosening genes like *expansin* and *xyloglucan endotransglucosylase* **(***XTH*) were observed during the first two stages (initiation and elongation) of fiber expansion in cotton. These genes are believed to have a starting role in fiber expansion because the breakdown of non-covalent bonds occurs with the activation of these cell wall loosening genes during the fiber morphogenesis in cotton. The cell walls loosening and the overexpression mechanism of *XTH* genes in transgenic soybean plants, helps to regulate and tolerate the flooding stress during the unfavorable environmental conditions (Naoumkina *et al*., 2015; Song *et al*., 2018). The reverse transcriptase RT-PCR analysis proved that the fiber length of *G. barbadense* is greater than *G.hirsutum*, because of the overexpression of *XTH* genes during the elongation stage of fiber morphogenesis in *G.barbadense* (Xiao *et al*., 2006, Avci *et al.*, 2013).

Real time polymerase chain reaction (RT-PCR) analysis is very important technique to explore the transcript level of tissue and time based genes at small level in a large variety of crops. In cotton, reverse transcriptase RT-PCR analysis is used to identify the transcripts of a potential gene related to long staple length. This profiling technique is very helpful to identify the novel genes and their specific regulatory regions for the long fiber (Imran and Liu, 2016; Li *et al*., 2017). After the concluded transcriptional profiling data, we can examine the genes related to long staple length along with their activation and expression stage during the fiber development. After that, the selected genes can be used to enhance the fiber propertied especially fiber length through the process of transformation into the commercially cultivated varieties (Razzaq et al., 2022).

In the present study, three lines of upland cotton (*G. hirsutum* L.) were used to see the expression of three genes *PEPc*, *XTH* and *GA-20 Oxidase* during the different fiber morphogenesis phases (0, 05, 10, 15 and 20 DPA). The simple and basic intention of this study to find out the novel genes with high expression during the different stages of cotton fiber development. After the expression profiling, transformed these genes through a specific breeding program to develop a transgenic cotton variety with long fiber qualities.

MATERIALS AND METHODS

Sowing of Cotton Genotypes with Different Fiber Length

The present study was conducted in three lines of a commercially cultivated variety *G. hirsutum*. Three cotton lines with varying fiber length (short, medium and long) were grown in the experimental field area of MNS-University of Agriculture, Multan during the cotton growing season 2020-21. These genotypes were grown in the field for fiber (samples) collection at different times of intervals (0, 5, 10, 15, and 20). These cotton lines were:

1-	Long fiber line	CIM-707
2-	Medium fiber line	CYTO-179
3-	Short fiber line	CIM-616

Flower Tagging

Fiber tissues were collected from the fully matured cotton plants in the field. The tagging of cotton bolls (samples) was done at the day of flower anthesis for accurate and defined selection of fiber tissues at 0, 5, 10, 15 and 20 DPA stages. All collected tissues were washed

carefully with Diethyl Pyro Carbonate treated water and instantly stored in liquid nitrogen for RNA extraction.

Extraction of Total RNA

Collection of Samples

The cotton bolls (fiber samples) were gathered from fully matured plants in the field at various stages of cotton boll development (0, 5, 10, 15, 20 DPA) to conduct transcript analysis (profiling) of long staple length genes. Before the assemblage of samples, cotton bolls were wash on the standing plant carefully with a spray gun. The samples were washed with DEPC (0.01% w/w) treated water to avoid any adulteration and contamination (Iqbal, 2017; Khatoon *et al*., 2018). After the collection of samples, stored in liquid nitrogen cylinder at -196℃ for a long-term storage. For the better results, accurate date and time was point out on tag before storing the samples.

Total RNA Extraction

Cotton fiber tissues were exposed by breaking the frozen boll walls by using the pestle and mortar. A plant purification RNA extraction reagent (Invitrogen, USA) was used for the total RNA extraction according to instructions given by the manufacturer.

First-strand cDNA Synthesis

A 10µg of RNA sample of all fiber developmental phases (0, 5, 10, 15 and 20 DPA) was used to transcribed first strand cDNA by using a reverse transcriptase enzyme. First strand of cDNA was reverse transcribed with the usage Revert Aid Reverse Transcriptase.

Primer Designing for PCR

A series of DNA sequences of long staple genes (*PEPc*, *XTH* and *GA-20 Oxidase*) were retrieved by commercially available online platform like NCBI and Primer 3 program (Premier Biosoft International, USA) (Wang *et al*., 2015; Iqbal, 2017). For the purpose of RT-PCR primers designing; two online available software a) Primer3 and b) AmplifX were used (Jullien, 2013). The GC contents of designed primer were ranged 40-60% and the length was almost at 18 to 20 nucleotides. At last, the sequence of primer pairs was confirmed after the checking in BLAST (http://www.ncbi.nlm.nih.gov) examination analysis. A template of 15 DPA (fiber elongation stage) of *G. hirsutum* was used for the calibration along with the optimization of primer pairs. After the calibration, the selected pairs of primers were optimized with their concentration and annealing temperature to achieve best amplification results. After that, all samples were run on 1% agarose gel and see their amplification bands under the UV light in gel electrophoresis.

Detail of Primers used for RT-PCR Studies

RT-PCR Analysis of Long Staple Length Genes

For the reverse transcriptase PCR reaction, A master mix of SYBR green included super mix dye, nucleases water and amplified primers. The setup of one sample RT-PCR reaction is as follows in Table 3.5.

For the calculation of relative gene expression level, 2^ΔΔ Ct method was used (Hung *et al*., 2015). By using this formula the temperature of a specific PCR cycle was calculated by taking the difference of temperature of interest gene to the temperature of reference gene ($Ct_{\text{interest gene}}$ -$Ct_{\text{reference gene}}$). Here "Ct" is the cycle temperature. These values were calculated after the running of all fiber samples with their respective primers in a 96 welled plate. A reference gene (*18sr RNA*) was used to compare the amplification and transcript level of all fiber genes.

RESULTS

The present study was conducted to estimate the relative expression level of three fiber developmental genes (*GA-20 Oxidase*, *PEP Carboxylase* and *XTH*) at different stages of fiber development (0, 05, 10, 15 and 20 DPA) in upland cotton. The relative gene amplification and their expression was observed at four different stages of fiber development i) initiation, ii) elongation, iii) secondary cell wall synthesis (SCW) and iv) maturation after anthesis. Expression analysis was conducted to check the transcripts of three gene families (*PEP Carboxylase*, *GA-20 Oxidase* and *XTH*) in fiber tissues through reverse transcriptase RT-PCR (Table 1). Approximately the quantity of total RNA was 0.2 µg to 0.5 µg of all fiber samples (Fig. 1). A dilution series of DEPC treated water (0.01%) was used to balance

Table 1: 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. Ubiquitin	RTUBCF	TGAATATTGTAATCAGCC	18 mer	132	CF932135
	RTUBCR	GAGCTCGGATACGATTGA	18 mer		
2. *PEP carboxylase*	RT*PEPC*F	CACCG ACCTACTACACGAGGTGTG	24 mer	227	EU032328
	RT*PEPC*R	AGAAGCCTCAAAAGGCATTCCTTG	24 mer		
3. *GA 20 Oxidase*	GA20F	CTTGCTTGGGGACTCTCTTG	20 mer	219	AY895169
	GA20R	ACGAAACTGCTTGCATACCC	20 mer		
4. *XTH*	RT*XTH*F	CCAAAATTCAGGCTGTGGAT	20 mer	231	EF546794
	RT*XTH*R1	TTGTTCCCTGTCACCCTTTC	20 mer		

Annealing temperature optimization for long staple length genes and their internal control

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

Procedure and 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

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

the concentration of all fiber samples and their amplification (Fig. 2). The first strand of complementary DNA (cDNA) was synthesized from RNA by using a reverse transcriptase enzyme and then confirmed their bands after the visualization on gel electrophoresis (Fig. 3). For PCR data normalization, an internal control (18S rRNA) is used to achieve the stable gene expression (Fig. 4). For the achievement of stable level of expression, all primer pairs were validated with a 10 times dilution series of template concentrations (Fig. 5).

A variable level of expression of *PEP Carboxylase* gene was observed in all three lines (CIM-707, CYTO-179 and CIM-616) with different stages of fiber morphogenesis i.e, 0, 05, 10, 15 and 20 DPA. Transcript analysis results showed that an elevated gene expression of *PEPc* was observed throughout the all-fiber developmental stages at different DPAs (Fig.6). A highest amplification profile was detected in elongation stage (15 DPA) of fiber development in both CIM-707 (Long fiber length) and CIM-616 (Short fiber length). A stable transcript level was also observed in (Medium fiber length) at 5 DPA. Further a stable amplification results of *PEP Carboxylase* gene were also perceived in all three lines of fiber elongation stage at 10 DPA fibers. At SCW stage of fiber development (20 DPA) nearly a small (negligible) level of expression was also observed in CIM-616. The expression level of *PEPc* was about to be doubled at 15 DPA stage in CIM-707 fibers than the others DPAs but the transcript level of *PEPc* was ultimately dropped at the same stage in both medium and short fiber lines (CYTO-179 and CIM-616).

A variable transcript pattern of oxidase gene (*GA-20*) was detected at the stages of fiber expansion and elongation. At 10 DPA (elongation) stage of fiber expansion, a stable transcript pattern of *GA-20 Oxidase* was detected during the fiber morphogenesis in CYTO-179. A stable level of transcript and continuous amplification of *GA-20 Oxidase* discovered that the transcript pattern of oxidase gene was exist at the elongation phase (15 DPA) of fiber expansion in all three fiber length genotypes (Fig. 7). A highest level of *GA-20 Oxidase* transcript was identified at 10 to 15 DPAs and then declined to 20 DPA (SCW stage). The transcripts of this gene were about to be doubled at the elongation phase (15 DPA) other than the initiation phase (0 DPA) of fiber synthesis. The transcript pattern of *GA-20 Oxidase* showed highest expression in both lines CIM-707 and CYTO-179 at 20 DPA and 10 DPA respectively. In CIM-707, a variable and unstable level of expression of *GA-20 Oxidase* was noticed at both 0 and 5 DPA stages but was much higher at 0 DPA. A large number of transcript pattern of *GA-20 Oxidase* were also detected in CYTO-179 as compared to other lines of G. *hirsutum* with the increase in days post anthesis (5 to 20 DPA) stages the level of expression of this fiber-related gene was also increased but at 15 PDA fibres the level of expression was very high. The expression profiling results showed that there is a similar transcript of *XTH* gene were also detected in all three lines of *G. hirsutum*. Expression profiling results showed that in both CIM-707 and CIM-616 cotton fiber lines, a stable and similar transcript pattern of *XTH* gene was detected at first two stages (0 DPA and 05 DPA) of fiber development. In CYTO-179, a variable transcript pattern of *XTH* gene was found at 10, 15 and 20 DPA stages of long fiber elongation stage in cotton genotypes. Transcripts of *XTH* genes were discovered in CYTO-179 at 15 DPA fibers but a low transcript level was observed at 5 DPA fibers. The RT-PCR results showed that the expression of *XTH*

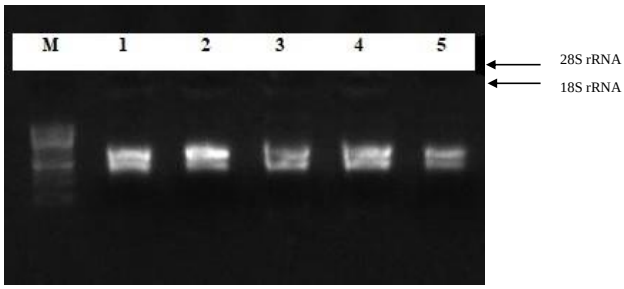

Fig. 1: Total RNA isolation from different stages of cotton fiber development. M 1 Kb ladder; Lane 1-5 total RNA extracted from five phases of three long staple length genotypes of cotton (Lane 1: 0 DPA fibers, Lane 2: 5 DPA fibers, Lane 3: 10 DPA fibers, Lane 4: 15 DPA fibers, Lane 5: 20 DPA fibers).

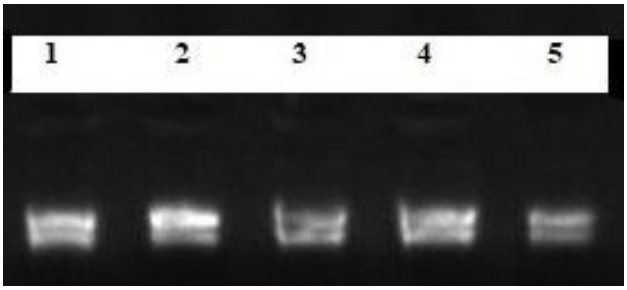

Fig. 2: Calibration of total RNA concentration in different fiber tissues. Lanes (1-5) illustrate RNA extracted from 0 DPA, 5 DPAP, 10 DPA, 15 DPA and 20 DPA fibers respectively

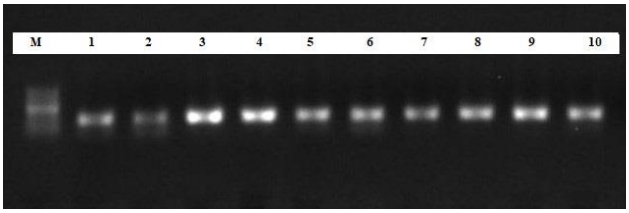

Fig. 3: Synthesis of first strand cDNA from five different phases of fiber synthesis. M- 0.5 bp ladder; Lane 1-10 cDNA synthesized from different stages of cotton fiber development. (Lane 1-5: 0, 5, 10, 15 and 20 DPA fiber of CIM-707 respectively), (Lane 6-8: 5, 10 and 15 DPA fiber of CYTO-179 respectively), (Lane 9-10: 15-20 DPA fiber of CIM-616 respectively)

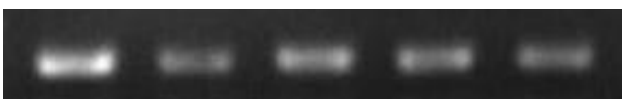

Fig. 4: Equalization and calibration of various templates concentration with *18S rRNA* primer through PCR amplification.

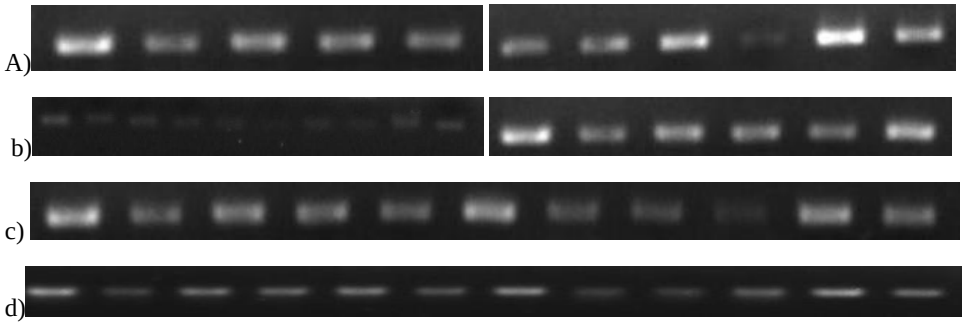

Fig. 5: Primer validation and confirmation through a series of fiber cDNA of 10 DPA stage. PCR amplification using primers of (a) *18SrRNA*, (b) GA-*20* Oxidase, (c) *PEPc* Carboxylase, d) *XTH.*

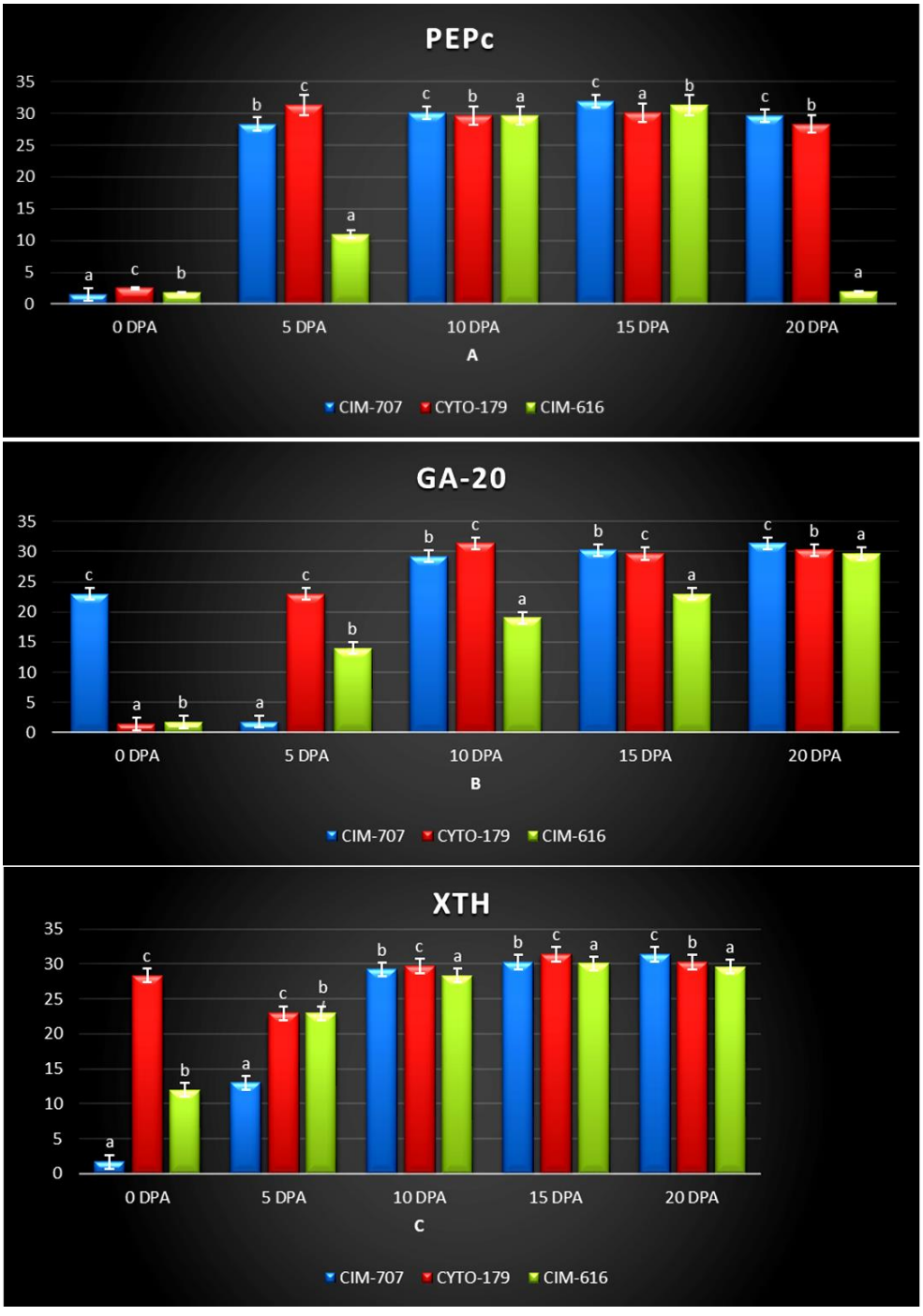

Fig. 6: RT-PCR analysis of three fiber related genes a) *PEPc*, b) *GA-20 Oxidase* and c) *XTH* of five different phases i) 0, ii) 05, iii) 10, iv) 15 and v) 20 DPA of fiber development in cotton. With the use of an internal control (18S rRNA), all transcripts' data was equalized and normalized.

gene was much similar in both CIM-707 and CIM-616 at 0 DPA and 20 DPA fibers. Real Time PCR results showed that a variable level of *XTH* transcripts were detected in all three staple length genotypes in cotton.

DISCUSSION

Fine quality and long staple length fibers are very acceptable for the textile industry. Universally, Long staple length fibers are used as raw material for fabrics production in the textile industry. A large number of long staple length related genes including *MYB* gene family are activated, results in the long fiber development around the seed trichomes (Wang *et al*., 2004). Identification and selection of long staple length related genes is very helpful for the production of new plants with high fiber length. Transcript analysis of cotton staple length related genes is very helpful to discover the transcripts of three different fiber development related genes at different phases of fiber morphogenesis. A large number of long staple length genes are highly expressed throughout the diverse phases of cotton fiber synthesis (Arpart *et al.*, 2004). Mostly of them are found to be involved during the fiber expansion and its enlargement, results in the long fiber development. In literature, most of the fiber-related genes including *EXP*, *SUS*, *LTPs*, *PEPc*, *tubulins*, *GA-20 Oxidase* and *XTH* are believed to be compulsory for the long fiber development (Li *et al.*, 2005; Huang *et al.* 2013). The successful identification and selection of long staple length related genes long with their upstream regulatory regions (promotors) was done only with expression profiling techniques (Razzaq et al., 2021).

The reverse transcriptase RT-PCR profiling is very important technique to explore the transcript level of tissue and time based genes at small level in a large variety of crops. In cotton, reverse transcriptase RT-PCR analysis is used to identify the transcripts of a potential gene related to long staple length (Yuan *et al.*, 2005). This profiling technique is very helpful to identify the novel genes and their specific regulatory regions for the long fiber (Naoumkina *et al*., 2016; Li *et al*., 2017). The transcript analysis data may also help in the investigation and confirmation of the long staple length-related genes along with their stages of activation and amplification throughout the whole process of fiber development. After that, the selected genes can be used to enhance the fiber propertied especially fiber length through the process of transformation into the commercially cultivated varieties. After the expression profiling analysis, we can change and alter the fiber properties especially fiber length through the overexpression or knockdown mechanisms of fiber-related genes into a commercially cultivated variety, results in the development of a new transgenic plant with long fiber properties (Razzaq et al., 2021).

A variable level of expression of *PEP Carboxylase* gene was observed in all three lines (CIM-707, CYTO-179 and CIM-616) with different fiber morphogenesis phases i.e., 0, 05, 10, 15 and 20 DPA. Transcript analysis results showed that an elevated gene expression of *PEPc* was observed throughout the all fiber developmental stages with different DPAs (Fig.6). A highest amplification profile was detected in elongation stage (15 DPA) of fiber development in both CIM-707 (Long fiber length) and CIM-616 (Short fiber length). A stable transcript level was also observed in (Medium fiber length) at 5 DPA. Further a stable amplification results of *PEPc* gene was also detected in all three lines with different fiber staple length (Long, medium and small) at 10 DPA fibers. At SCW (20 DPA) phase of fiber expansion, nearly a small (negligible) transcript pattern was also observed in CIM-616. The expression level of *PEPc* was about to be doubled at 15 DPA stage in CIM-707 fibers than the others DPAs but the transcript level of *PEPc* was ultimately dropped at the same stage in both medium and short fiber lines (CYTO-179 and CIM-616 (Iqbal *et al*., 2016; Nadeem *et al*., 2021).

A variable transcript pattern of Oxidase gene (GA-20) was detected at all fiber synthesis phases. A highest level of expression of *GA-20 Oxidase* of fiber expansion gene was detected at 10 DPA stage of fiber morphogenesis in CYTO-179. A stable level of transcript and continuous amplification of *GA-20 Oxidase* discovered that the transcripts of oxidase gene were noticed throughout the elongation phase (15 DPA) of fiber expansion in all three fiber length genotypes (Fig. 7). A highest level of *GA-20 Oxidase* transcript was identified at 10 to 15 DPAs and then declined to 20 DPA (SCW stage). The transcripts of this gene was about to be double at elongation phase than initiation phase. The transcript pattern of *GA-20 Oxidase* showed highest expression in both lines CIM-707 and CYTO-179 at 20 DPA and 10 DPA respectively. A variable and unstable level of expression of *GA-20 Oxidase* was detected in all three lines at first two stages including 0 and 5 DPA but was much higher at 0 DPA in CIM-707. A large number of transcript pattern of *GA-20 Oxidase* were also detected in CYTO-179 as compared to other lines of *G. hirsutum* (Iqbal *et al*., 2016; Nadeem *et al*., 2021).

With the increase in days post anthesis (5 to 20 DPA) stages, the level of expression of this fiber-related gene was also increased but at 15 PDA fibers the level of expression was very high. The expression profiling results showed that there is a similar transcript of *XTH* gene were also detected in all three lines of *G. hirsutum*. Expression profiling results showed that in both CIM-707 and CIM-616 cotton fiber lines, a stable and similar transcript level of *XTH* gene was detected in first two stages (0 DPA and 05 DPA) of fiber development. In CYTO-179, a non-constant transcript level of *XTH* gene was found at 10, 15 and 20 DPA stages of long fiber elongation stage in cotton genotypes. Transcripts of *XTH* genes were discovered in CYTO-179 at 15 DPA fibers but a low transcript level was observed at 5 DPA fibers. The RT-PCR results showed that the expression of *XTH* gene was much similar in both CIM-707 and CIM-616 at 0 DPA and 20 DPA fibers. Real Time PCR results showed that a variable level of *XTH* transcripts were detected in all three staple length genotypes in cotton.

Conclusions

From the above discussion it is determined that three fiber-related genes exhibited variable expression throughout the different levels of fiber development in cotton fiber tissues. All three fiber-related genes are highly expressed in 5, 10 and15 DPA fibers. The comparative expression studies proved that all of these fiber developing genes showed a variable and stage specific expression in fiber tissues. Highly expressed Fiber-related genes can also be used for changing the fiber characteristics through the

process of gene transformation. The comparative expression study on fiber developing genes can be used to enhance the fiber length of cotton commercial cultivars to support the national economy.

REFERENCES

Ahmed M, AA Shahid, SU Din, S Akhtar, A Ahad, AQ Rao, T Husnain, 2018. An overview of genetic and hormonal control of cotton fiber development. Pakistan Journal of Botany 50: 433-43.

Arpart AB, M Waugh, JP Sullivan, M Gonzales, D Frisch, D Main, T Wood, A Leslie, RA Wing, TA Wilkins, 2004. Functional genomics of cell elongation in developing cotton fibers. Plant Molecular Biology 54: 911-929.

Avci U, S Pattathil, B Singh, NVL Brown, MG Hahn, CH Haigler, 2013. Cotton fiber cell walls of *Gossypium hirsutum* and *Gossypium barbadense* have differences related to loosely-bound xyloglucan. Plos One 8: 56315-56328.

Balasubramanian VK, KM Rai, SW Thu, MM Hii, V Mendu, 2016. Genome-wide identification of multifunctional laccase gene family in cotton (Gossypium spp.); expression and biochemical analysis during fiber development. Science Reproduction 6: 1-16.

Cheng Y, L Lu, Z Yang, Z Wu, W Qin, D Yu, Z Yang, 2016. GhCaM7-like, a calcium sensor gene, influences cotton fiber elongation and biomass production. Plant Physiololgy Biochemistry 109: 128-136.

David A, HY Trevor, KCR Jocelyn, B David, J Reinhard, K Ljerka, K Lacey, S Lacey, 2016. Arabidopsis *LTPG* is a glycosylphosphatidyl inositol-anchored lipid transfer protein required for export of lipids to the plant surface. Plant Cell 21: 1230-1238.

Fang DD, 2018. Cotton fiber genes and stable quantitative trait loci. In Cotton Fiber: Physics, Chemistry and Biology. Springer 103:151-178.

Fry SC, 2018. Cell wall polysaccharide composition and covalent crosslinking. Annual Review Plantation 12: 1-42.

Han Y, Q Ban, Y Hou, K Meng, J Suo, J Rao, 2016. Isolation and characterization of two persimmon *xyloglucan endotransglycosylase/hydrolase* (*XTH*) genes that have divergent functions in cell wall modification and fruit postharvest softening. Frontier Plant Science 7: 624- 630.

Huang GQ, S Gong, WL Xu, W Li, P Li, CJ Zhang, XB Li, 2013. A fasciclin-like arabinogalactan protein, GhFLA1, is involved in fiber initiation and elongation of cotton (*Gossypium hirsutum*). Plant Physiology 161: 1278-1290.

Imran M, JY Liu, 2016. Genome-wide identification and expression analysis of the malate dehydrogenase gene family in Gossypium arboreum. Pakistan Journal of Botany 48: 1081-1090.

Iqbal N, 2017. Expression profiling of cotton fiber genes and evaluation of some regulatory sequences for gene expression in Dicots. Ph.D. Thesis, Department of Biotechnology University of Quaid-i-Azam, Islamabad, Pakistan.

Iqbal N, A Khatoon, M Asif, A Bashir, 2016. Expression analysis of fiber related genes in cotton (*Gossypium hirsutum* L.) through real time PCR. Pakistan Journal of Botany 48: 1099-1106.

Jullien N, 2013. AmplifX Version 1.7.0. Available on-line with updates at http://crn2m.univ-mrs.fr/pub/amplifx-dist.

Khatoon A, N Iqbal, M Asif, HMN Cheema, M Saeed, A Bashir, 2018. Comparative analysis of fiber morphogenesis genes of Calotropis procera and *Gossypium hirsutum*. International Journal of Agriculture Biology 20: 288-296.

Kim HJ, Y Liu, AD French, CM Lee, SK Kim, 2018. Comparison and validation of Fourier transform infrared spectroscopic methods for monitoring secondary cell wall cellulose from cotton fibers. Cellulose 25: 49-64.

Lee JA, DD Fang, 2015. Cotton as a world crop: origin, history, and current status. Cotton 57: 1-23.

Lee J, TH Burns, G Light, Y Sun, M Fokar, Y Kasukabe, K Fujisawa, Y Maekawa, RD Allen, 2010. Xyloglucan endotransglycosylase/ hydrolase genes in cotton and their role in fiber elongation. Planta 232: 1191-1205.

Li W, DD Li, LH Han, M Tao, QQ Hu, WY Wu, GQ Huang, 2017. Genome-wide identification and characterization of *TCP transcription factor* genes in upland cotton (*Gossypium hirsutum*). Science Reproduction 7: 1-14.

Li XB, XL Wang, L Cai, WC Yang, 2005. The cotton ACTIN1 gene is functionally expressed in fibers and participates in fiber elongation. Plant Cell 17: 859-875.

Liu X, B Zhao, HJ Zheng, Y Hu, H Lu, CQ Yang, Y Zhou, 2015. *Gossypium barbadense* genome sequence provides insight into the evolution of extra-long staple fiber and specialized metabolites. Science Reproduction 5: 14139.

Ma R, H Yuan, J An, X Hao, H Li, 2018. A Gossypium hirsutum *GDSL lipase/hydrolase gene* (GhGLIP) appears to be involved in promoting seed growth in Arabidopsis. PloS one 13: 55-56.

Martínez-Sanz M, F Pettolino, B Flanagan, MJ Gidley, EP Gilbert, 2017. Structure of cellulose microfibrils in mature cotton fibres. Carbohydration Polymerization 175: 450-463.

McCann MC, JP Knox, 2018. Plant cell wall biology: polysaccharides in architectural and developmental contexts. Annual Plant Review 12: 343-366.

Nadeem M, N Iqbal, U Waheed, Z Zia, M Ali, Z Khan, 2021. Comparative expression studies of fiber related genes in cotton spp. Journal of Bioresource Management 8: 1.

Naoumkina M, GN Thyssen, DD Fang, 2015. RNA-seq analysis of short fiber mutants Ligon-lintless-1 (Li 1) and–2 (Li 2) revealed an important role of *aquaporins* in cotton (*Gossypium hirsutum* L.) fiber elongation. BMC Plant Biology 15: 65.

Naoumkina M, GN Thyssen, DD Fang, DJ Hinchliffe, CB Florane, JN Jenkins, 2016. Small RNA sequencing and degradome analysis of developing fibers of short fiber mutants Ligon-lintles-1 (Li 1) and− 2 (Li 2) revealed a role for miRNAs and their targets in cotton fiber elongation. BMC genomics 17: 360- 361.

Razzaq, A., Zafar, M. M., Ali, A., Hafeez, A., Sharif, F., Guan, X., ... & Yuan, Y. (2022). The pivotal role of major chromosomes of sub-genomes A and D in fiber quality traits of cotton. *Frontiers in Genetics*, *12*, 642595.

Razzaq, A., Zafar, M. M., Li, P., Qun, G., Deng, X., Ali, A., ... & Yuan, Y. (2021). Transformation and overexpression of primary cell wall synthesis-related zinc finger gene Gh_A07G1537 to improve fiber length in cotton. *Frontiers in Plant Science*, *12*, 777794.

Ruan YL, SM Xu, R White, RT Furbank, 2004. Genotypic and developmental evidence for the role of plasmodesmatal regulation in cotton fiber elongation mediated by callose turnover. Plant Physiology 136:4104-4113.

Saladié M, JK Rose, DJ Cosgrove, C Catalá, 2006. Characterization of a new xyloglucan endotransglucosylase/ hydrolase (*XTH*) from ripening tomato fruit and implications for the diverse modes of enzymic action. Plant Journal 47: 282-295.

Song L, B Valliyodan, S Prince, J Wan, H Nguyen, 2018. Characterization of the *XTH* Gene Family: New Insight to the roles in soybean flooding tolerance. International Journal of Molecular Science 19: 2705.

Ute TB, P Celik, MB Uzumcu, 2019. Utilization of Cotton Spinning Mill Wastes in Yarn Production. Textile Industry and Environment. 85: 13-7

Wang J, HY Wang, PM Zhao, LB Han, GL Jiao, YY Zheng, SJ Huang, GX Xia, 2010. Overexpression of a profilin (*GhPFN2*) promotes the progression of developmental phases in cotton fibers. Plant Cell Physiology 51: 1276-1290.

Wang QQ, F Liu, SX Chen, XJ Ma, HQ Zeng, ZM Yang, 2010. Transcriptome profiling of early developing cotton fiber by deep-sequencing reveals significantly differential expression of genes in a fuzzless/lintless mutant. Genetics 96: 369-376.

Wang SJ, W Wang, N Yu, CH Li, B Luo, JY Gou, LJ Wang, XY Chen, 2004. Control of plant trichome development by a cotton fiber MYB gene. Plant Cell 16: 2323-2334.

Wang X, L Zhang, JB Evers, L Mao, S Wei, PX Pan, X Zhao, WVD Werf, Z Li, 2015. Predicting the effects of environment and management on cotton fiber growth and quality: a functional–structural plant modeling approach. Journal of Integrated Agriculture 13: 6-22.

Wang Z, Z Yang, F Li, 2019. Updates on molecular mechanisms in the development of branched trichome in Arabidopsis and nonbranched in cotton. Journal of Plant Biotechnology 17: 1706- 1722.

Xiao G, P Zhao, Y Zhang, 2019. A pivotal role of hormones in regulating cotton fiber development. Frontier Plant science 10: 87.

Xiao Y, Y Ye, Y Feng, X Li, M Luo, L Hou, Y Pei, 2006. Functional Expression of the Cotton Gibberellic Acid Oxidase Homologous Gene *GhGA20oxl* in Tobacco. Journal of Plant Physiology and Molecular Biology 32: 563.

Yang J, W Hu, W Zhao, B Chen, Y Wang, Z Zhou, Y Meng, 2016. Fruiting branch K+ level affects cotton fiber elongation through osmoregulation. Frontier Plant science 7: 13.

Yuan D, Z Tang, M Wang, W Gao, L Tu, X Jin, Y Li, 2015. The genome sequence of Sea-Island cotton (*Gossypium barbadense*) provides insights into the allopolyploidization and development of superior spinnable fibres. Science Reproduction 5: 17662.

Yuan YL, TZ Zhang, WZ Guo, JJ Pan, RJ Kohel, 2005. Diallel analysis of superior fiber quality properties in selected upland cottons. Acta Genetica Sinica 1: 79-85.

Zhang DS, M Hrmova, CH Wan, CF Wu, J Balzen, W Cai, 2004. Members of a new group of chitinase-like genes are expressed preferentially in cotton cells with secondary walls. Plant Molecular Biolology 54: 353-372.

Zhang X, DP HU, L Yuan, C Yuan, EH Abidallha, ZD Dong, L Zhang, 2017. Developmental and hormonal regulation of fiber quality in two natural-colored cotton cultivars. Journal of International Agriculture 16: 1720-1729.

Zhao Y, Y Huang, Y Wang, Y Cui, Z Liu, J Hua, 2018. RNA interference of Gh*PEPC*2 enhanced seed oil accumulation and salt tolerance in Upland cotton. Plant Science 271: 52-61.

Zafar, M. M., Jia, X., Shakeel, A., Sarfraz, Z., Manan, A., Imran, A., ... & Ren, M. (2022b). Unraveling heat tolerance in upland cotton (Gossypium hirsutum L.) using univariate and multivariate analysis. *Frontiers in plant science*, *12*, 727835.

Zafar, M. M., Manan, A., Razzaq, A., Zulfqar, M., Saeed, A., Kashif, M., ... & Ren, M. (2021). Exploiting agronomic and biochemical traits to develop heat resilient cotton cultivars under climate change scenarios. *Agronomy*, *11*(9), 1885.

Zafar, M. M., Mustafa, G., Shoukat, F., Idrees, A., Ali, A., Sharif, F., ... & Li, F. (2022a). Heterologous expression of cry3Bb1 and cry3 genes for enhanced resistance against insect pests in cotton. *Scientific Reports*, *12*(1), 10878.

Zafar, M. M., Razzaq, A., Farooq, M. A., Rehman, A., Firdous, H., Shakeel, A., ... & Ren, M. (2020). Insect resistance management in Bacillus thuringiensis cotton by MGPS (multiple genes pyramiding and silencing). *Journal of Cotton Research*, *3*(1), 1-13.