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Characterization of the early fiber development gene, Ligon-lintless 1 (*Li1*), using microarray

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ABSTRACT

Cotton fiber length is a key factor in determining fiber quality in the textile industry throughout the world. Understanding the molecular basis of fiber elongation would allow for improvement of fiber length. Ligon-lintless 1 (*Li1*) is a monogenic dominant mutation that results in short fibers. This mutant provides an excellent model system to study the molecular mechanisms of cotton fiber elongation. Microarray technology and quantitative real time PCR (qRT-PCR) were used to evaluate differentially expressed genes (DEGs) in the Ligon-lintless 1 (*Li1*) mutant compared to the wild-type. Although the results showed only a few differentially expressed genes at −1, 3 and 7 days post anthesis (DPA); at 5 DPA, there were 1915 DEGs, including 984 up-regulated genes and 931 down-regulated genes. The critical stage for early termination of *Li1* fiber elongation was 5 DPA, as there were the most differentially expressed genes in this sample. The transcription factors and other proteins identified might contribute to understanding the molecular basis of early fiber elongation. Gene ontology analysis identified some key GO terms that impact the regulation of fiber development during early elongation. These results provide some fundamental information about the TFs that might provide new insight into understanding the molecular mechanisms governing cotton fiber development.

1. Introduction

Cotton fiber is not only the largest renewable resource of the textile industry throughout the world but also an excellent model for testing gene expression associated with fiber elongation. Cotton fiber is a highly elongated single cell derived from the ovule epidermis on or around the day of anthesis (Basra and Malik, 1984; Lee et al., 2007). Only approximately 25% of ovule epidermal cells differentiate into spinnable fiber cells during the initiation stage of cotton fiber (Tiwari and Wilkins, 1995). Cotton fiber development can be grouped into four overlapping stages: initiation (−3 ~ 0 DPA), elongation (1–25 DPA), secondary cell wall formation (16–40 DPA) and maturation (40–50 DPA) (Tiwari and Wilkins, 1995). Fiber initiation and elongation have a great effect on the number, length and fineness of fibers, which are major factors in determining lint quality and yield. In recent years, by comparing the transcriptomes of different tissues, transcription factors such as MYB and WRKY and genes encoding proteins involved in auxin, gibberellins, ethylene, brassinosteroid acid, abscisic acid and other metabolic pathways were identified to play a main role in fiber cell development (Samuel Yang et al., 2006). Cotton fiber development is regulated by a network of genes rather than a single gene; however, the lack of information at the molecular level concerning the genes and regulatory components that regulate fiber development is one of the main impediments in understanding the genetics to improve cotton fiber. Cotton fiber mutants are useful tools for understanding the genetics and physiology of cotton fiber development (Bolton et al., 2009; Ding et al., 2014; Ji et al., 2003; Kwak et al., 2009; Wang et al., 2010). Several cotton fiber mutants have been discovered, one of which is the dominant mutation in Ligon-lintless1 (*Li1*) (Rong et al., 2005). This mutant exhibits abnormal morphological characteristics including distorted leaves, stems and extremely short lint fiber length on the mature seed, which only reach 4–6 mm in length (Gilbert et al., 2013b). To understand the abnormal growth of the *Lil* mutant, it is necessary to gain insight into the genes and elements that are required to control fiber length, strength and cell wall characteristics (Gilbert et al., 2013b). A previous study indicated that the *Li1* gene is located on chromosome 22 (Karaca et al., 1999). Compared to the wild-type ovules, there were only a few genes in the Ligon-lintless1 mutant ovules that showed differential expression during the fiber initiation stage (Liu et al., 2012). Co-expression of some secondary cell wall development-related genes, such as expansin,

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tubulin and sucrose synthase, that were found to be expressed during the early fiber development of *Li1*; however, they were expressed during the fiber elongation stage in wild-type cotton, possibly elucidating the mechanism underlying the phenotype of the Ligon-lintless1 mutant (Bolton et al., 2009; Gilbert et al., 2013b). Prior studies using microarray and RNA-seq analysis have attempted to identify the genes that impact the fiber that is produced by the *Li1* mutant during the elongation stages (Bolton et al., 2009; Gilbert et al., 2014; Liu et al., 2012; Naoumkina et al., 2015; Rong et al., 2005). In this study, we used microarray technology to identify the genes and their functions during early fiber development (−1, 3, 5 and 7 DPA). Genes that were differentially expressed between the *Li1* mutant and the wild-type were analyzed in reference to the AD cotton genome. This research provides new information on the development of cotton fiber that may impact further studies on the molecular mechanisms that regulate cotton fiber development.

2. Materials and methods

2.1. Plant materials

Upland cotton (*Gossypium hirsutum* L.) Ligon-lintless 1 (*Li1*) mutants and wild-types (li1) were planted in the experimental field at the Institute of Cotton Research, the Chinese Academy of Agricultural Sciences (ICR, CAAS) under conventional field management conditions. Flowers on one day before anthesis were tagged for self-pollination. Developing bolls were collected at the following time points: −1, 3, 5 and 7 days post anthesis (DPA). Harvested bolls were frozen in liquid nitrogen and stored at −08 °C until the RNA was extracted.

2.2. RNA extraction and microarray hybridization

Total RNA was extracted from ovules and fibers from Ligon-lintless 1 and wild-type using the CTAB-sour phenol extraction method. RNA samples were treated using DNase 1 to remove genomic DNA. RNA extracted from −1, 3, 5 and 7 DPA cotton fibers was used to make cDNA probes. The mutant cDNA was labeled using Cy-5 (red) and the wild-type cDNA was labeled using Cy-3 (green) (CapitalBio). There was one stable control, five hybridization controls and eight exogenous system controls for each microarray; 50% DMSO was used as the negative control. The wild-type and mutant cDNAs were hybridized to the 12 K Cotton cDNA Array (CapitalBio Corp) (Shi et al., 2006); three biological replicates were performed.

2.3. Data acquisition, statistical analysis and clustering

All microarray slides were scanned using a LuxScan 10KA two-stand laser scanner (CapitalBio Corp) and the raw data were extracted using LuxScan 3.0 software (CapitalBio Corp). For double passage microarrays, the scanning set for the Cy-3 and Cy-5 passages were normalized by the optical examination of an external spot set control on the gene chip. The LOWESS (Locally Weighted Scatterplot Smoothing) method was used to normalize the double passage data by using the signals from the Cy3-labeled sample. The signal intensity ratios for the experimental and control samples were used to perform cluster analysis; duplicate spots on each slide were used as two single values for analysis. SAM software was used to screen for differentially expressed genes. The standard false discovery rate (FDR) below 0.05 was used to control for multiple testing errors (Hochberg and Tamhane, 1987). Differentially expressed genes were identified based on a ratio of mutant/wild-type expression; ratios ≥1.5 were considered up-regulated and those ≤0.67 were considered down-regulated. Bio-informatic analysis was performed using MAS (microarray analysis system). Hierarchical clustering, using the average linkage method, was used on those genes that showed significant fiber-specific expression during different stages of development; cluster data were visualized using Genesis_v1.7.6.30.09.10-DIGERATI software. GO annotations were analyzed using WEGO (Web Gene Ontology Annotation Plot).

2.4. Semi-quantitative RT-PCR and quantitative reverse transcription PCR (qRT-PCR) validation

Total RNA used for qRT-PCR was extracted from four cotton ovules and four fibers from the developmental time points specified before; three biological replicates were performed. Following genomic DNA removal using DNase 1, approximately 3 μg of RNA was used for reverse transcription using a cDNA synthesis kit (Shanghai Sangon). The specific primers were designed using Oligo 6 software (Supplementary Table 1), and the primer templates used for microarray probes were synthesized by TAKARA Biotechnology. Cotton UBQ 7 (accession number AY189972) and 18S RNA were used as internal controls. The qRT-PCR was carried out using SYBR® Green Premix kit (TaKaRa Biotechnology, Dalian, China). The composition of the PCR mix was as follows: 0.4 μl of each primer (10 μM), 10 μl of 2 × SYBR Premix ExTaq™, 1 μl of cDNA and 8.2 μl RNase-free water. The qRT-PCR was performed on a Lightcycle-480 (Roche). The qRT-PCR began with 10 min at 95 °C, followed by 45 cycles at 95 °C for 15 s and 60 °C for 20 s and 30 s of extension at 72 °C. Three independent experiments were performed for each biological replicate, and the amplification data were analyzed using Roche 480 software.

2.5. Bioinformatics analysis

Nucleotide sequence similarity was analyzed using EMBOSS (http://www.ebi.ac.uk/Tools/emboss/align/), and the ORF was identified using ORFfinder (Wheeler et al., 2003) (http://www.ncbi.nlm.nih.gov/projects/gorf/). The DNA regulatory sequences were analyzed using PlantCARE (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/) and gene structure analysis was performed using InterPro (http://www.ebi.ac.uk/Tools/InterPro Scan/). Expasy ProtParam (http://cn.expasy.org/tools/protparam.html) was used to predict the isoelectric point (PI) and molecular weight of each protein. Genes were annotated by using the BGI v1.0 assembly & the annotated genome sequences of *Gossypium hirsutum L.* (AD1) (https://www.cottongen.org/data/download/genome_BGI_AD1).

3. Results

3.1. Nature of Li1 mutant fiber during initiation and early elongation

Mutation in the Ligon-lintless1 (*Li1*) gene produces pleiotropic effects both on vegetative growth and fiber development. The Ligon-lintless1 mutant exhibits a stunted growth during the preliminary seedling stage and produces an extremely short fiber (Fig. 1). For early fiber development, MAS analyses showed no discernable differences in the appearance of ovules from Ligon-lintless1 and the wild-type on −1 and 3 DPA. The mutant fiber began normal initiation as visualized at −1 DPA with no apparent differences in the distribution of initial fibers on the ovule surfaces. Similarly, *Li1* mutant and wild-type fiber morphology appeared the same during the early elongation stage of fiber development until 3 DPA. Significant differences were observed at 5 DPA. The 5 DPA stage was critical for the early termination of *Li1* fiber elongation, as there were the most differentially expressed genes in the 5 DPA samples; transcription factors and other proteins identified might contribute to understanding the molecular basis of early fiber elongation.

3.2. Identification of differentially expressed genes during the early elongation stage between the Li1 mutant and wild-type using microarrays

To elucidate the molecular mechanisms regulating early fiber development, four samples of ovules and fibers were harvested from the *Li1*

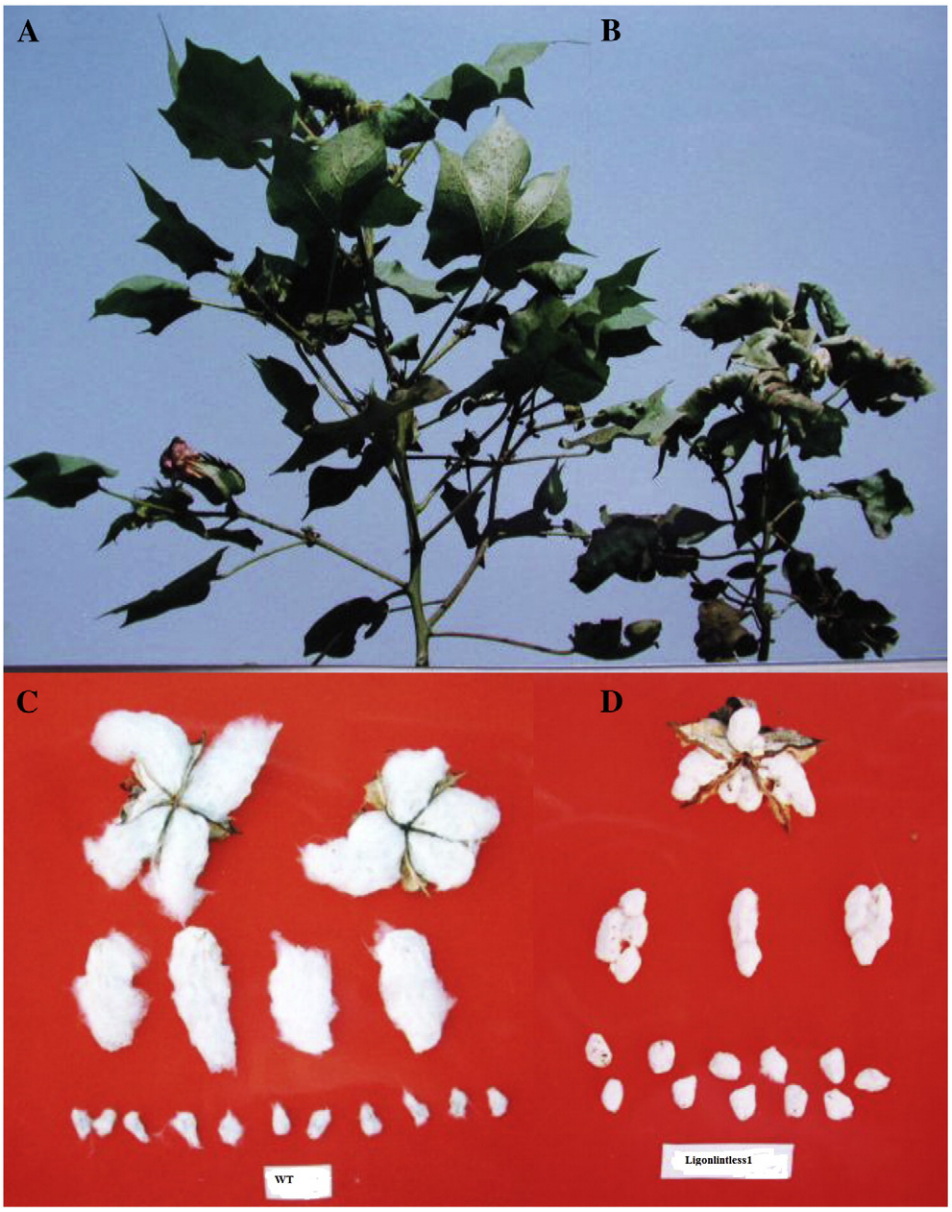


Fig. 1. The Ligon-lintless1 and its wild-type plant, and the opening boll and seed fibers of Ligon-lintless1 and its wild-type. Wild-type plant (A), Ligon-lintless1 plant (B), wild-type seed fibers (C) and Ligon-lintless1 seed fibers (D).

mutant and the wild-type. cDNA microarrays were used to measure gene expression levels in the ovules and fibers during the initiation and early elongation stages. We identified 2364 DEGs, including 12 DEGs at −1 DPA, 128 DEGs at 3 DPA, 1915 DEGs at 5 DPA and 309 DEGs at 7 DPA. When comparing the differentially expressed genes across 3, 5 and 7 DPA, only three genes were up-regulated, and they were annotated as CotAD_20959 (CCCH-type Zinc finger), CotAD_73235 (Calcium-binding EF-hand) and CotAD_66768 (1-aminocyclopropane-1-carboxylate synthase). There were 34 genes specific to 3 DPA, 70 genes specific to 7 DPA and 894 genes that were specific to 5 DPA (Fig. 2A). For down-regulated genes, there were only two common genes found at 3, 5 and 7 DPA; there were 72 genes specific to 3 DPA, 124 genes were specific to 7 DPA and 911 genes were specific to 5 DPA (Fig. 2B). Interestingly, this result reveals that a majority of DEGs were found at 5 DPA between the *Li1* mutant and the wild-type. Thus, 5 DPA is most likely the turning point for early fiber development in *Li1* mutant. For further analysis of DEGs, we focused only on genes that are regulated during 3, 5 and 7 DPA.

3.3. Gene ontology (GO) categories associated with differentially expressed genes

To determine the possible biological functions that were altered during early fiber development; we compared DEGs at 3, 5 and 7 DPA using WEGO gene ontology annotation. Our results indicate that the number of genes represented by each GO term was different at 3, 5 and 7 DPA in the categories of biological process and molecular function. In contrast, there were few differentially expressed genes at 3, 5 and 7 DPA that were found in the cellular component category (Fig. 3). For the biological process category, genes annotated with the following GO terms, multi-organism process, multicellular organismal process, reproduction and reproductive process, were expressed only at 5 DPA. The genes annotated with the terms anatomical structural formation and cellular component biogenesis were expressed at 5 and 7 DPA. For molecular function, genes with the terms electron carrier and enzyme regulator were expressed only at 5 and 7 DPA; genes with the term molecular transducer were expressed only at 5 DPA, while the genes annotated

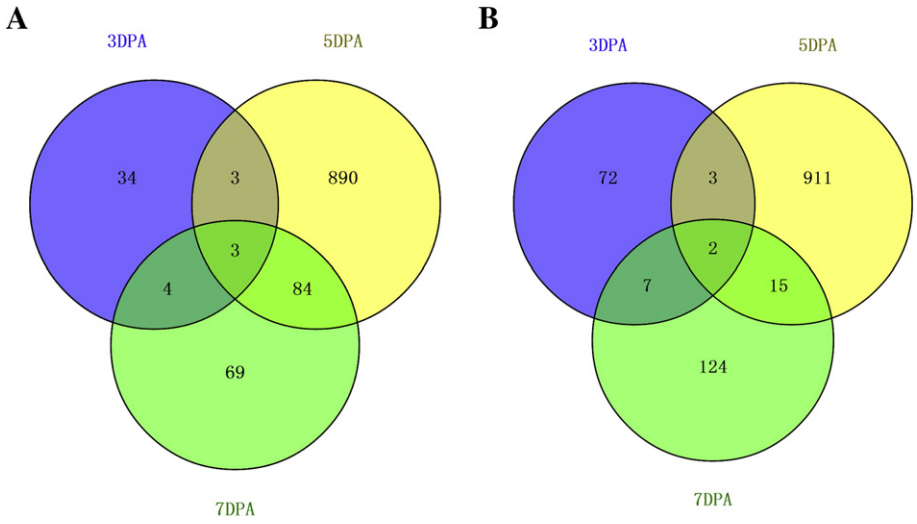


Fig. 2. Venn diagram showing of the gene expressed in three stages of ovules and fiber development. Up-regulation genes (A) and Down-regulation genes (B) showed DEGs during the fiber elongation at 3, 5 and 7 DPA in *Li1*/wild-type (WT).

with the GO term nutrient reservoirs were expressed only at 3 DPA (Supplementary Table 2).

3.4. Cluster analysis of differentially expressed genes during early fiber development

Genes with parallel expression patterns are often functionally correlated. We performed a cluster analysis of the gene expression patterns to identify clusters with functional enrichment; the cluster analysis identified at least four groups. The majority of these genes have been shown to be up-regulated at 5 DPA and 7 DPA and down-regulated at 3 DPA (Fig. 4). The first group included four genes, CotAD_05343, CotAD_14056, CotAD_25811 and CotAD44037, that were all down-regulated at 5 DPA; three were up-regulated at 7 DPA, but only one gene, CotAD_05343 (glycoside hydrolase, family 28), was up-regulated at 3 DPA. The second group included 17 genes, including four members of the Hsp20 heat shock protein family (CotAD_09470, CotAD_26951, CotAD_29552, CotAD_40001), two zinc finger proteins (CotAD_37072, CotAD_04644), one helix–loop-helix DNA-binding protein (CotAD_27190) and one protein kinase (CotAD_35157), all of which were highly down-regulated at 3, 5 and 7 DPA. The third group included 66 genes most of which were up-regulated at 5 and 7 DPA and down-regulated at 3 DPA. Only two signal transduction response regulator genes were up-regulated at 3 DPA and down-regulated at 5 DPA. However, the genes encoding 1-aminocyclopropane-1-carboxylate(CotAD_06129), ornithine/DAP/Arg decarboxylase (CotAD_19166) and C2 calcium-dependent membrane targeting (CotAD_57989), SANT/Myb domain (CotAD_16726) were all highly expressed at 5 and 7 DPA in the *Li1* mutant compared with the wild-type. The fourth group comprised 41 genes and a majority of these genes were highly up-regulated at 5 DPA. This group can be divided into three sub-groups: Sub-group 4–1 includes five genes, four of which were up-regulated at 5 and 7 DPA; one gene (Glutathione S-transferase, N-terminal: CotAD_57509) was down-

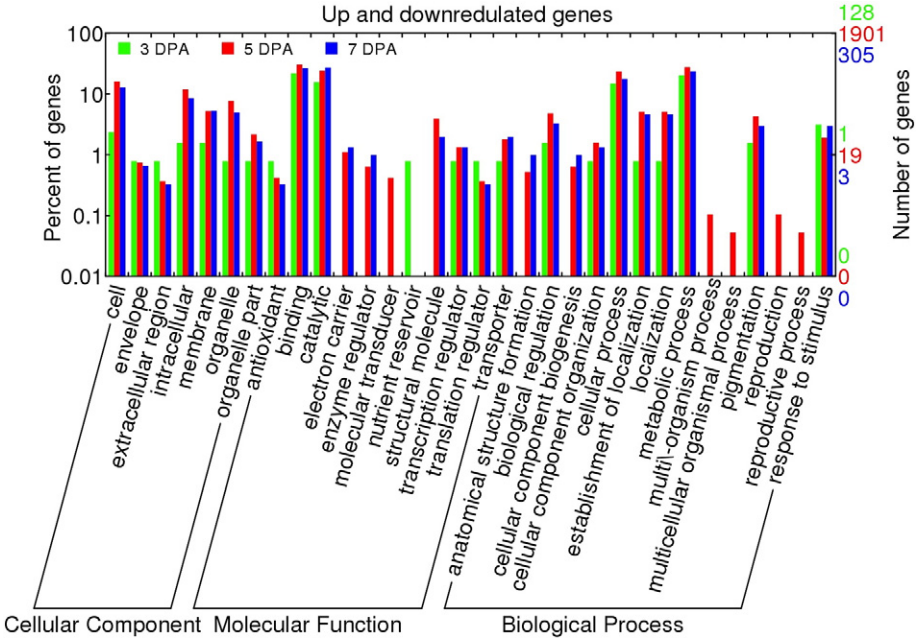
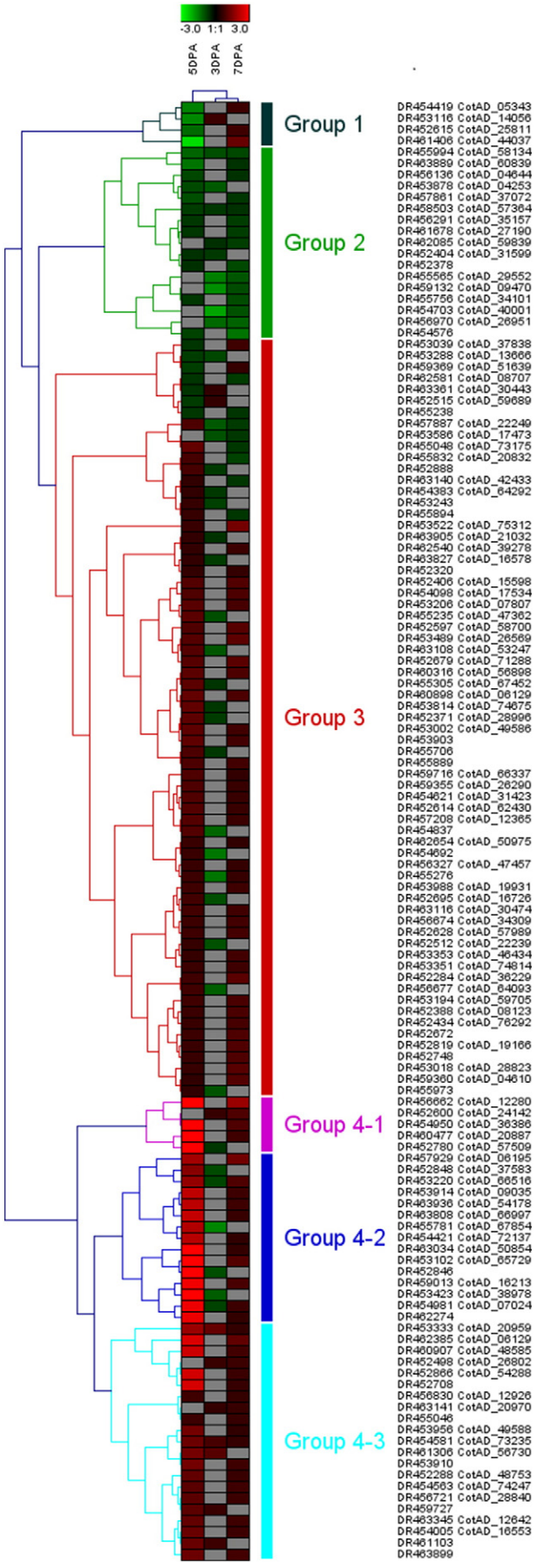


Fig. 3. Functional categorization of differentially expressed genes during early cotton fiber development in Li1/wild-type analyzed by WEGO Gene Ontology Annotation. Gene Ontology categories that were significantly enriched were analyzed in pairwise comparisons (3, 5 and 7 DPA). Percentages are based on the proportion of the number of genes in each set.



regulated at 3 DPA, and another gene (RNA recognition motif domain: CotAD_24142) was up-regulated at 3 DPA. Sub-group 4–2 includes 15 genes. All of these genes were up-regulated at 5 and 7 DPA and include: xyloglucan endotransglucosylase (CotAD_06195), fumarate reductase/succinate dehydrogenase and ribonuclease T2 (CotAD_65729). The translation initiation factor SUI1 (CotAD_07024) and NAD-dependent epimerase/dehydratase (CotAD_66516) were both down-regulated at 3 DPA. Sub-group 4–3 included 21 genes, all of which exhibited up-regulation at 3, 5 and 7 DPA. As examples, 1-aminocyclopropane-1-carboxylate (CotAD_06129), calcium-binding EF-hand (CotAD_73235), zinc finger (CotAD_56730), plant peroxidase (CotAD_54288), pyridine nucleotide-disulfide oxidoreductase (CotAD_12642) and protein kinase (CotAD_49588) were up-regulated in the mutant (Supplementary Table 3).

3.5. Validation of microarray data by RT-PCR and qRT-PCR

We used qRT-PCR to validate the expression patterns identified by the microarray results, with three biological replicates. We selected six genes to confirm the microarray results: ornithine decarboxylase (ODC, CotAD_19166), 1-aminocyclopropane-1-carboxylate (ACO, Cot AD_06129), xyloglucan endotransglycosylases/hydrolases (XET or XEH, CotAD_06195), protein kinase (CIPK, CotAD_35157), C2 calcium-dependent membrane targeting (JLL, CotAD_57989) and auxin-binding protein (ABP, CotAD_00361). Expression patterns for the six genes were analyzed using RT-PCR (Fig. 5). The results indicate a good correlation between the transcript abundance assayed using the microarrays and the expression profile obtained using qRT-PCR (Fig. 6A and B).

In summary, the validation tests indicate that there is a high degree of concordance between the qRT-PCR results compared to the microarray data. Thus, the genes encoded by ODC, ACO, ABP, JLL, XET and CIPK are the best candidates for genes responsible for early cotton fiber development in the *Li1* mutant.

4. Discussion

Cotton fiber development is a complex process that is regulated by multiple factors. The *Li1* cotton mutant can initiate lint fiber formation normally from an ovule at the day of anthesis and continuously develop normal fiber cells until 3 DPA. Abnormal primary elongation, cellulose synthesis and deposition that happen subsequently can produce a significantly shorter fiber. Recently, the gene expression profile of the *Li1* mutant fiber at 15 DPA was shown to be similar to that of wild-type using microarray methods (Bolton et al., 2009). Differentially expressed genes were detected in both 6 DPA (Liu et al., 2012) and 8 DPA fibers (Gilbert et al., 2014). When and why *Li1* mutant fiber elongation is stopped remains largely unknown. The main goal here was to investigate gene expression profiles in the *Li1* mutant during early fiber development. At first, we found that 5 DPA was a critical stage for early fiber elongation. Although our microarray analysis indicated that only a few genes were significantly different at −1 DPA, 3 DPA and 7 DPA between the *Li1* mutant and the wild-type, there were many genes that showed significantly different expression levels at 5 DPA in the *Li1* mutant when compared to the wild-type (Fig. 2). The GO results suggest that early fiber development of the *Li1* mutant was mostly related to the functional categories biological process and molecular function (Fig. 3). The differences in the biological process and molecular function genes between the *Li1* mutant and the wild-type across 3, 5 and 7 DPA may have caused gene expression changes during early fiber development in *Li1* mutant (Supplementary Table 2). Cotton fiber elongation is a

Fig. 4. Complete linkage hierarchical cluster analysis of 129 differentially expressed genes in Li1 mutant and its wild-type. Log 2 Ratio was indicated on a color scale from red (high) to green (low). Gene's names are on the right side of the figure, and the three stages of fiber development.

very complicated process that involves many signaling pathways (Gilbert et al., 2014). Therefore, we concluded that the *Li1* mutant is a critical gene that can both directly and indirectly affect many other genes that are involved in cotton energy metabolism, plant growth, development and morphogenesis. Cluster analysis of genes that were differentially expressed between the *Li1* mutant and the wild-type, at three different stages of fiber elongation, showed that a majority of DEGs were up-regulated at 5 and 7 DPA and down-regulated at 3 DPA, suggesting that they may contribute to short fiber production in the *Li1* mutant. From our results, we identified two families of zinc finger proteins; the first is the zinc finger RING-type family that had two genes that were both down-regulated at 3 and 5 DPA in the *Li1* mutant. This result is consistent with results obtained by Padmalatha, who suggests that zinc finger family transcription factors are down-regulated at the fiber initiation and elongation stages (Padmalatha et al., 2012). The second is the zinc finger CCCH-type family that has only one gene and was up-regulated at 5 and 7 DPA in *Li1* mutant compared to the wild-type. This result agrees with the result obtained by Thyssen et al. (2014). It has also been reported that zinc fingers regulate trichome cell development through gibberellin (GA) and cytokinin signaling in *Arabidopsis* (Zhou et al., 2013). Recent reports suggest that zinc fingers are candidate genes affecting fiber development in the *Li2* mutant (Thyssen et al., 2014). We propose that the two families of zinger finger genes could be involved in fiber development. SANT/Myb transcription factor was up-regulated at 5 DPA and down-regulated at 3 DPA, suggesting that this transcription factor may play a critical role in controlling fiber development in the *Li1* mutant. Moreover, MYB was significantly differentially expressed between *Li2* and wild-type during the later stage of fiber development (Thyssen et al., 2014). However, our study demonstrates that myb TFs could be playing a role in early fiber development. The last transcription factor identified in this work, the leucine zipper domain, was up-regulated at 5 and 7 DPA in the mutant. Transcription factor of the basic-leucine zipper (bZIP) family controls important processes including, morphogenesis, seed formation and abiotic and biotic stress responses in plants (Alves et al., 2013). In addition, bZIP factors play a central role in regulating cotton fiber development through GA (Shan et al., 2014). Our results suggest that three families of transcription factors (zinc finger, MYB and leucine zipper) are the most likely candidates for genes that are responsible for early fiber development in cotton.

For the transcripts that encode protein kinases and the catalytic domains, we identified three protein kinase genes that were down-regulated at 5 and 7 DPA in *Li1* mutant. Our results strongly suggest that protein kinases might play major roles in the modulation of early fiber elongation. Previous studies have shown that high levels of protein kinases are required for normal fiber development (Liu et al., 2012); consistent with this, CDPK (Huang et al., 2008) and MAPK (Wang et al., 2005) are expressed to higher levels in the wild-type compared to the *Li1* mutant, which had lower levels of kinase expression. Prior studies found that protein kinases were present at high levels of expression during fiber development (Liu et al., 2012). The expression level of CIPK was significantly reduced in the *Li1* mutant compared to the wild-type during the elongation stage. Transcripts encoding protein phosphatases were down-regulated at 5 and 7 DPA in the *Li1* mutant compared to the wild-type. A recent study showed that protein phosphatases, *i.e.*, serine/threonine phosphatases, play diverse roles in transcription, translation, differentiation, cell cycle and signal transduction in many organisms (Du et al., 2013).

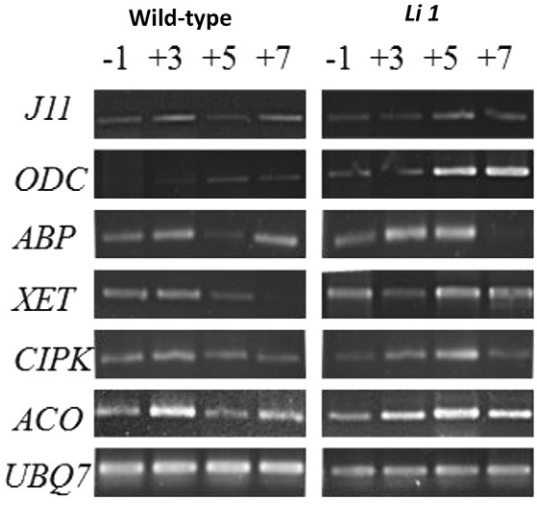


Fig. 5. Expression patterns of the candidate genes (6 genes) in the *Li1* mutant and its wild-type were analyzed by semi-quantitative PCR of ovules, and fiber at different developmental stages of between Ligon-lintless1 mutant (*Li1*) and its wild-type (WT) at −1, 3, 5 and 7 DPA (days post anthesis).

Genes that encode heat shock proteins included six members of the heat shock protein Hsp20 family, chaperonin Cpn10 and heat shock protein DnaJ; all were down-regulated at 3 and 7 DPA, while heat shock protein DnaJ was expressed to high levels at 5 DPA in the *Li1* mutant compared to the wild-type. However, until now there have been no studies to indicate that HSP proteins can regulate fiber development (Ding et al., 2014). These results suggest that heat shock proteins could be involved in the disruption of important biological processes and abnormal fiber development in the *Li1* mutant. ACO (1-aminocyclopropane-1-carboxylate) was significantly up-regulated at 5 and 7 DPA in the *Li1* mutant compared to wild-type during the same period. It has been reported that ACO genes responsible for ethylene production are expressed to significantly higher levels during fiber development (Shi et al., 2006). In this work, we found higher levels of ethylene produced during fiber development at 5 and 7 DPA in the *Li1* mutant, possibly leading to short fiber development. This result is consistent with previous studies that reveal that high levels of ethylene during the fiber elongation stage might be responsible for early senescence during fiber development (Li et al., 2014; Shi et al., 2006). Auxin-binding protein (ABP) was up-regulated at 5 DPA in the *Li1* mutant. We suggest that high levels of ABP could stop early fiber development in *Li1* mutant. This result is consistent with the report by Gilbert, who suggests that ABP accumulation is high in mutant fibers (Gilbert et al., 2013a). Previous studies have suggested that high levels of ABP can inhibit cotton fiber cell elongation in the *in vitro* cotton ovule culture system (Beasley and Ting, 1974). Moreover, ABP mediates auxin-induced cell elongation and, directly or indirectly, cell division (Ji et al., 2003). In our study, transcript encoding the signal transduction response regulator was up-regulated at 3 DPA and down-regulated at 5 DPA; however, cotton fiber elongation is regulated by a complex system of hormones and signal transduction proteins (Gilbert et al., 2014). Transcripts encoding plant peroxidase were up-regulated at 5 and 7 DPA in *Li1* mutants contrary to the results of Ding, which suggest that H_2O_2 was down-regulated in *Li1* mutant leaves. However, previous studies suggest that H_2O_2 was accumulated and predominantly expressed in rapidly elongating cotton fibers (Huang et al., 2008; Mei et al., 2009; Qin et al., 2008). In addition, transcripts encoding calcium-binding EF-hand family proteins were up-regulated at 3, 5 and 7 DPA in the *Li1* mutant compared to the wild-type. This result was different from the previous report, which indicated that the calcium-binding EF-hand was highly down-regulated during fiber initiation and development in mutants (Padmalatha et al., 2012). Transcripts encoding suppressor of K^+ transport growth defect 1, an AAA-type ATPase family protein, were up-regulated at 5 and 7 DPA in the mutant. Our results were in agreement with the recent reports that suggested that the concentrations of K^+ and other detected ions were significantly higher in *Li1* mutants than in wild-type (Naoumkina et al., 2015). Our results show that high accumulation of ions in the *Li1* mutant could lead to the production of short fibers. The transporting ATPase, F1 complex, delta was up-regulated at 5 and 7 DPA in the mutant. This gene is required for mitochondrial ATP synthesis and to keep a higher ATP:ADP ratio, which facilitates cotton

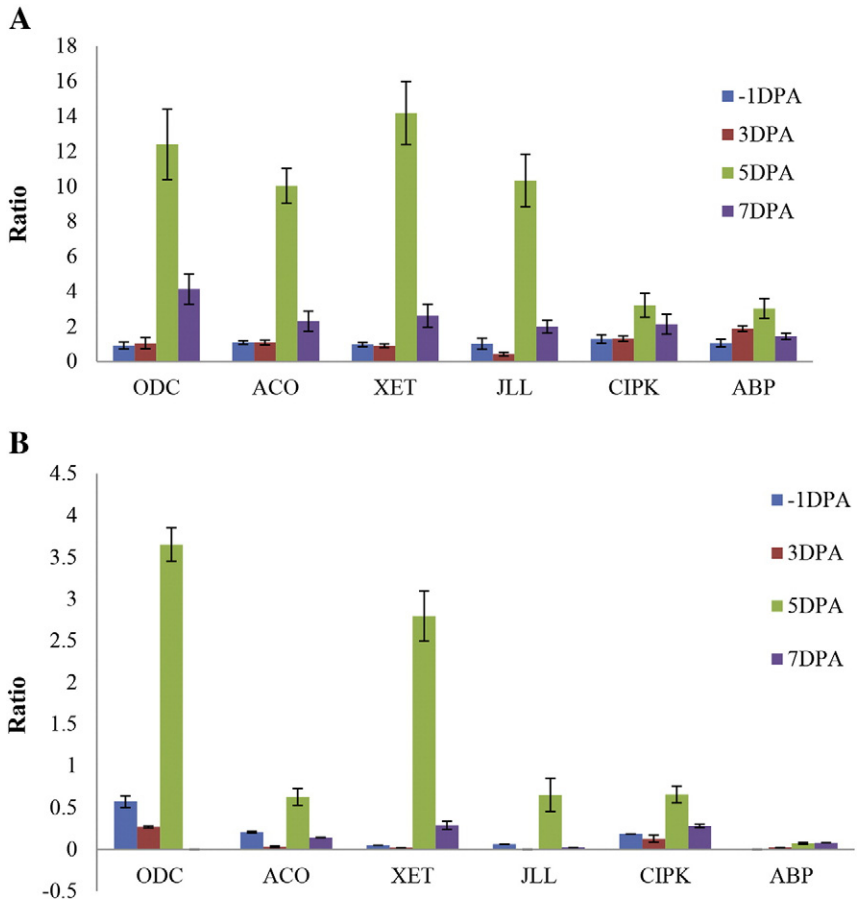


Fig. 6. The trend line of six DEG by microarray method (A) and the trend line of six DEG by qRT-PCR (B).

fiber elongation (Pang et al., 2010). However, transcript encoding the oligopeptide transporter was up-regulated in the mutant at 5 and 7 DPA. This oligopeptide transporter in *Arabidopsis* is believed to be important for the transport of small peptides across cellular membranes (Koh et al., 2002).

Ornithine decarboxylase (ODC) was up-regulated at 5 and 7 DPA in the *Li1* mutant. Our results suggest that lower expression of this gene is required for the normal fiber development. This suggests that ODC is associated with rapid cell growth and cell division in eukaryotes (Theiss et al., 2002). It was found to be active during growth and somatic embryogenesis and could contribute to a polyamine biosynthesis (Loukanina and Thorpe, 2008). Xyloglucan endotransglucosylase (XET) was up-regulated at 5 and 7 DPA in *Li1* mutant compared to wild-type. Our results suggest that the high expression during early fiber development in *Li1* mutant may cease or slow the early fiber development in cotton. However, this result is not in agreement with results obtained by Shao, who suggests that higher XET activity can cleave the xyloglucan-cellulose chains thereby loosening the fiber cell wall and promoting fiber cell elongation in wild-type compared to the mutant (Shao et al., 2011). Previous reports indicate that xyloglucan endotransglycosylases/hydrolases (XTH) can cleave and reattach xyloglucan polymers that make up the hemicellulose matrix of wild-type cell walls (Ding et al., 2014). XTH influences plant cell wall polysaccharide structure (Perrin, 2008) and provides clues about the cellular makeup of the cotton fiber (Kumar et al., 2013). C2 calcium-dependent membrane targeting (JLL) was up-regulated at 5 and 7 DPA in *Li1* mutant compared to wild-type. This result did not agree with earlier reports that suggest that C2 calcium-dependent membrane targeting protein was highly down-regulated at initiation and elongation fiber in the *fl* mutant (Padmalatha et al., 2012). Our study provides important information for understanding the molecular mechanisms regulating cotton fiber development.

5. Conclusion

This is the first report that has explored the effects of the *Li1* mutant on the AD cotton genome. We used microarray methods and the AD cotton genome to compare the transcripts of *Li1* mutants to the wild-type during fiber initiation and early elongation. The most significant DEGs occur at 3, 5 and 7 DPA; GO analysis and cluster analysis of these DEGs provide important insights into the underlying molecular mechanisms that control cotton fiber elongation. The most critical stage for the early termination of *Li1* fiber elongation was 5 DPA, as there were the most differentially expressed genes in these samples. We identified a large number of transcription factors, such as zinc finger, MYB and basic-leucine zipper, hormones (ACO and ABP) and other proteins that might contribute to the understanding of the molecular basis of early fiber elongation. These results provide some fundamental information about the transcription factors and other proteins that might provide new insight into understanding the molecular mechanisms governing cotton fiber development. Further hypothesis-driven molecular analyses will allow us to fully elucidate the molecular mechanisms regulating early fiber development.

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Conflict of interest

The authors declared that they have no conflict of interest.

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