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MOLECULAR BIOLOGY AND PHYSIOLOGY

Isolation and Characterization of Genes Differentially Expressed in Fiber of *Gossypium barbadense* L.

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ABSTRACT

Cotton breeders face many problems, such as negative association between lint yield and fiber strength, in their efforts to improve fiber quality through conventional breeding methods. The primary goal of this project was to identify and characterize genes at the molecular level related to cotton fiber development. Messenger RNAs were isolated from fibers at 20 day-post-anthesis (dpa) of a high yielding cotton accession TM-1 (*Gossypium hirsutum* L.) and of a high fiber quality cotton accession 3-79 (*G. barbadense* L). A cDNA library was constructed from mRNA obtained from cotton fiber (20 dpa) of 3-79 and screened sequentially with cDNA probes from TM-1 and 3-79. Thirteen cDNAs were identified. Five of the thirteen cDNAs (*Gb*Fb$_1$, *Gb*Fb$_2$, *Gb*Fb$_3$, *Gb*MAPK and *Gb*LTP) were highly expressed in the 20-dpa fiber tissue of 3-79 cotton. Sequence analysis indicated a cotton lipid transfer protein (*Gb*LTP), a mitogen-activated protein kinase (*Gb*MAPK), and a novel gene with no homologous sequence (*Gb*Fb$_1$) in the GenBank database. The remaining two cDNAs with unknown functions, *Gb*Fb$_2$ and *Gb*Fb$_3$, showed high identity to fiber ESTs from *G. hirsutum* at 6 d and *G. arboreum* L. at 7 to 10 dpa, respectively. These five genes are developmentally regulated, and they showed much higher expression levels in the 20-dpa fiber than in the other tissues tested, except the gene for *Gb*Fb$_1$, which was abundantly expressed in the flower. These results are among the few reports of putative gene expression associated with fiber in *G. barbadense*.

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Cotton is the leading natural fiber crop. Because of competition in the global economy, yarn and textile manufacturers have adopted more efficient production machinery capable of generating more products per unit of time. The widespread use of high speed spinning technology has increased the demand for raw cotton fiber with higher strength. Cotton fiber quality must be improved to remain competitive with synthetic fiber and meet the needs of new spinning and weaving methods.

Cotton (*Gossypium* spp.) contains about 50 diploid and disomic tetraploid species indigenous to Africa, Central and South America, Asia, Australia, the Galapagos, and Hawaii (Fryxell, 1992). The four domesticated species of cotton are *G. arboreum* L., *G. herbaceum* L., *G. barbadense* L., and *G. hirsutum* L. *Gossypium barbadense* provides about 10% of the current world production of fiber, and it is used mostly for the production of high quality fabrics and sewing thread. *Gossypium hirsutum* contributes most of the current world production because of its high productivity and wide adaptability. Because of its superior spinning and manufacturing performance, the unique high quality characteristics of *G. barbadense* lint provides a higher price than *G. hirsutum*. *Gossypium barbadense* has poor productivity and limited adaptation to most cotton-growing areas, so cotton farmers prefer to grow early maturing *G. hirsutum* cultivars because of their higher yielding capacity and fewer insect and agronomic problems. Therefore, improving the fiber quality of *G. hirsutum*, while maintaining its high fiber yield, could have a significant economic impact.

Classical cotton breeding methods improve fiber quality through the tedious process of selection based on measuring the fiber traits in advanced generations of the selected lines (Green and Gulp, 1990), but genetic improvement has been hampered by the association of poor fiber properties with high yields in cotton (Kloth, 1998; Kohel, 1999). Stable introgression of fiber quality is difficult to achieve by incorporating genes of *G. barbadense* into *G. hirsutum*

(McKenzie, 1970). Another barrier to fiber quality improvement through conventional breeding in cotton is the length of time required. The development of an alternative approach to introduce fiber quality genes of *G. barbadense* into an Upland background is necessary to avoid these difficulties. Genetic engineering is a highly desirable approach for meeting these objectives. Genetic transformation enables the transfer of individual genes from *G. barbadense* into *G. hirsutum*. A logical prelude to cotton improvement strategies through a molecular approach is the establishment of programs that identify and characterize fiber-associated genes in cotton.

The development of cotton fiber, like any specialized cell, requires the expression of specific genes. If these genes can be identified, they may provide useful tools for understanding the mechanisms of fiber development. They also can be used for improving cotton fiber quantity and quality through biotechnology. Many "cotton fiber genes" have been cloned and characterized. These reports are split between those genes only expressed in fiber (fiber-specific) and those expressed in both fiber cells and other tissues (fiber-associated), although the function is known only for some of these genes (Kim and Triplett, 2001; Wilkins and Jernstedt, 1999). The primary goal of this study was to identify and characterize the genes involved in cotton fiber development. To accomplish this goal, *G. barbadense* accession 3-79 and *G. hirsutum* accession TM-1 were chosen as the experimental materials, because they are the genetic and cytogenetic standards for their respective species (Kohel et al., 2001). These two cultivars have been widely used as parents for interspecific crosses for improvement of fiber properties due to the unique superior fiber quality of 3-79 and the high productivity and wide adaptation of TM-1 (Kohel et al., 2001; Yu and Kohel, 2001). In this research, the fiber tissues of the high yielding accession TM-1 and the high fiber quality accession 3-79 were used for mRNA isolation. Then a cDNA library of 20-dpa fibers from 3-79 was constructed and screened with the cDNA probes from mRNAs of TM-1 and 3-79. The genes that were more highly expressed in the high fiber-quality accession 3-79 than in TM-1 at the same developmental stage were identified.

MATERIALS AND METHODS

Plant materials. Plants of *G. hirsutum* accession TM-1 and *G. barbadense* accession 3-79 were grown in pots in the greenhouse at 30±2 °C/16 h light and 20±2 °C/8 h darkness on the Alabama A&M University campus. Each plant was watered once every 2 d as needed. Standard fertilizer, pest, and disease control practices were used. Flowers were dated and tagged the day they opened as zero day post-anthesis (0 dpa) as a reference point for the age of the fruit. The cotton bolls were collected on 10, 15, 20, and 25 dpa. Ovules were removed and frozen immediately in liquid nitrogen. The fiber layer was then scraped from the developing ovule with a scalpel and a forceps under liquid nitrogen and stored at -70 °C until it was used for RNA preparation. To determine tissue specificity for fiber-related gene expression, root tips, flowers (0 dpa), and young leaf tissues were harvested from mature plants of accession 3-79 at flowering and stored at -70 ºC until their use for RNA isolation.

RNA isolation. Total RNA was extracted according to the method of Wan and Wilkins (1994). Poly $(A)^+$ mRNA was isolated from total RNA of 3-79 and TM-1 fiber with an mRNA Purification Kit (Amersham Pharmacia Biotech; Piscataway, NJ) according to the vendor's instructions.

Construction and screening of cDNA library of 20-dpa fiber from *G. barbadense* accession 3-79. The fiber cDNA library of 3-79 was prepared from 20-dpa fiber mRNA from *G. barbadense* accession 3-79 using a SMART cDNA Library Construction Kit (Clontech Laboratories, Inc.; Palo Alto, CA) following the manufacturer's instructions. The cDNA library of 20-dpa fiber from 3-79 was screened with fiber cDNA probes from TM-1 and 3-79. The cDNA probes were synthesized from the 20-dpa fiber mRNAs of TM-1 and 3-79 cotton using a Strip-EZ RT Stripable cDNA Probe Synthesis and Removal Kit (Ambion; Austin, TX). The cDNA library was plated and transferred to duplicate sets of 132-mm colony/plaque screen membranes (DuPont-NEN; Boston, MA). The membranes were denatured with 0.5 M NaOH, neutralized with 1.0 M Tris-HCl/pH 7.5, and cross-linked with an XL-1500 UV Cross-linker (Spectronics Corporation; Westbury, NY). Overall, duplicate membranes were hybridized with the cDNA probes of TM-1, scored, stripped, and hybridized with the cDNA probes of 3-79. The membranes were first prehybridized at 65 °C for 2-3 h in a hybridization tube containing prehybridization buffer (2X SSC, 5X Denhart's solution, 1.0% SDS, 100 µg/ml sheared carrier DNA, and 10% dextran sulfate). The probes (10^6 cpm/mL hybridization buffer) were denatured at 95 °C for 5 min, chilled on ice

for 15 min, and added to the prehybridization buffer in the tube. The hybridization was conducted at 65 °C overnight. After hybridization, the membranes were washed twice with 2X SSC for 15 min at room temperature, then twice with 2X SSC/0.1% SDS at 60 °C for 15-30 min, and once with 0.1X SSC at room temperature for 30 min. The hybridized membranes were exposed to X-OMAT AR X-ray film (Kodak; Rochester, NY) at -70 °C for about 70 h. To reduce the false positive clones and minimize redundancy, the screening was conducted three times. The clones differentially hybridizing to the cDNA probe of 3-79 on the duplicate membranes were located, picked, re-plated, transferred to 82-mm colony/plaque screen membranes (DuPont-NEN; Boston, MA), and hybridized sequentially with both probes using the same procedure as the first round of screening.

Sequencing cDNA and sequence analysis. Plasmid DNA was prepared from each selected clone using a Plasmid Midi Kit (Qiagen; Valencia, CA) following the manufacturer's instructions. The DNA sequencing was carried out with automated sequencers (ABI Prism 3100, Applied Biosystems; Foster City, CA). The cDNA sequences were analyzed using BLAST (available at http://www.ncbi.nlm.nih.gov/blast), primary and secondary structure of the deduced translation product were analyzed using ExPASy (available at au.expasy.org) and Predictprotein (available at http://cubic.bioc.columbia.edu/predictprotein), and nucleotide sequences of the selected cDNA clones were aligned and compared with other related gene sequences available from GenBank (at http://www.ncbi.nlm.nih.gov/genbank/index.html). Sequence similarity inferences were deduced from the BLAST results. The predicted amino acid sequences were translated for the longest open reading frame (ORF) from the first in-frame ATG after the first in-frame stop codon in each cDNA of the unknown function clones and terminated at the first stop codon encountered in all of the selected cDNAs.

RNA gel blot analysis. An equal amount (30 µg) of total RNAs from 20-dpa fibers of TM-1 and 3-79 was denatured at 65 °C with formaldehyde, fractionated on the formaldehyde-denatured 1.2% agarose gel, and transferred to BrightStar-Plus membranes (Ambion). The RNA gel blot analysis was carried out using the NorthernMax Kit (Ambion) following the vendor's instructions. Radiolabeled (^{32}P-α-dATP, DuPont-NEN) probe was synthesized from the plasmid DNA of each selected clone using the StripAble PCR Probe Synthesis and Removal Kit (Ambion). After development of the RNA blot film, transcript expression was estimated semi-quantitatively by capturing and analyzing the image of the film with an AlphaImager 2000 Documentation and Analysis System (Alpha Innotech Corporation; San Leandro, CA). Similar/identical areas were selected for integration to compare expression levels between the two genotypes. Films were scanned under visible light and the pixel intensity of bands was quantified using the spot density tool of the image analysis software. The data were normalized using 18S rRNA pixel values (band intensities) for the samples in question.

DNA gel blot analysis. DNA gel blot analysis was used to determine the copy number of the cDNAs in the genome. The copy number of cDNAs was scored according to Walden et al. (1999). Genomic DNA was isolated from young leaves of 3-79 and TM-1 using the DNeasy Plant Maxi Kit (Qiagen). DNA gel blot was carried out according to standard methods (Sambrook et al., 1989).

RESULTS

The cDNA library of 20-dpa fiber from 3-79 contained 2 X 10^6 independent clones, 88% of which contained inserts. Approximately 10^5 clones in the cDNA library of 3-79 fiber were screened by sequentially hybridizing duplicate membranes with each of the two total cDNA probes prepared from the 20-dpa fiber mRNAs of TM-1 and 3-79. After screening, 22 clones that differentially hybridized were selected for sequencing. Sequence comparison between these clones showed that some clones shared high sequence identity (97% to 99%) with each other, suggesting that they represented duplicate clones from the same genes. Therefore, only thirteen clones were analyzed by RNA gel blot analyses. Five of the thirteen clones (*Gb*Fb$_1$, *Gb*Fb$_2$, *Gb*Fb$_3$, *Gb*MAPK, and *Gb*LTP) showed high differential expression in the 20-dpa fiber tissue of 3-79 (Fig. 1). Semi-quantitative analysis of gene expression as measured by the AlphaImager 2000 Documentation and Analysis System showed that the expression level of *Gb*Fb$_1$ and *Gb*Fb$_3$ was about 2 to 3 times greater in fiber of 3-79 than in TM-1. Transcript levels of the three remaining genes, *Gb*Fb$_2$, *Gb*MAPK, and *Gb*LTP, expressed differentially in 20-dpa fiber of 3-79, were doubled compared with the levels found in TM-1 (Fig. 2).

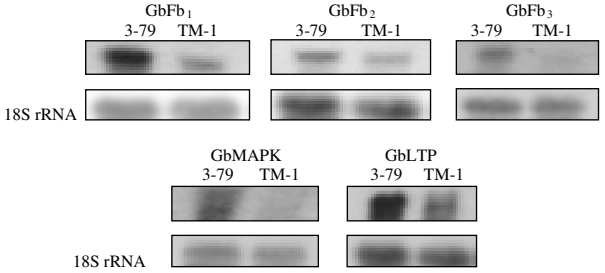

Fig. 1. Differential expression of *Gb*Fb$_1$, *Gb*Fb$_2$, *Gb*Fb$_3$, *Gb*MAPK, and *Gb*LTP in 20-dpa cotton fiber from *G. barbadense* accession 3-79. RNA blots with 30 µg of total RNA in each lane on all membranes were hybridized with *Gb*Fb$_1$, *Gb*Fb$_2$, *Gb*Fb$_3$, *Gb*MAPK, or *Gb*LTP probes. Each blot was stripped to remove the probe, and re-hybridized with an 18S rRNA probe from *Arabidopsis* to evaluate the loading differences in each lane.

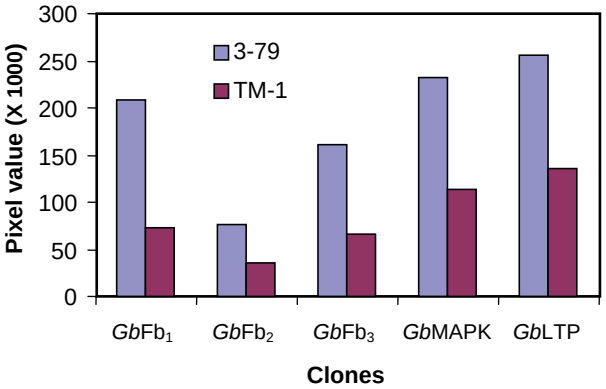

Fig. 2. Relative transcript abundance of five cDNAs in 20-dpa fibers of *G. barbadense* accession 3-79 and *G. hirsutum* accession TM-1 as determined semi-quantitatively by the spot density tool of AlphaImager 2000 Documentation and Analysis System software. The data were normalized using 18S rRNA pixel values (band intensities) for the samples in question.

To further investigate the expression characteristics of the cDNA clones, RNA gel blot analyses with the five cDNAs as probes using total RNA extracted from roots, leaves, and flowers of 3-79 and fibers at different developmental stages (10, 15, 20, 25 dpa) of 3-79 and TM-1 were conducted. RNA gel blot analysis showed that the expression of the five cDNA clones was developmentally regulated (Fig. 3). The genes for all the five cDNAs were expressed at much higher levels in 20-dpa fiber than in the other tissues tested with the exception of the gene for *Gb*Fb$_1$ that was more abundantly expressed in the flower. The expression of these genes increased abruptly at about 15 dpa and then decreased sharply after 20 dpa. Interestingly, the expression pattern of the gene for *Gb*MAPK differed between fibers of 3-79 and TM-1. The expression level of this gene in 3-79 fibers steadily increased during primary wall synthesis (about 10-15 dpa), reached a maximum at about 20 dpa, and declined after 25 dpa. Expression of the gene in TM-1 fibers was very low at about 10 dpa, but peaked at about 15 dpa and decreased sharply after 20 dpa.

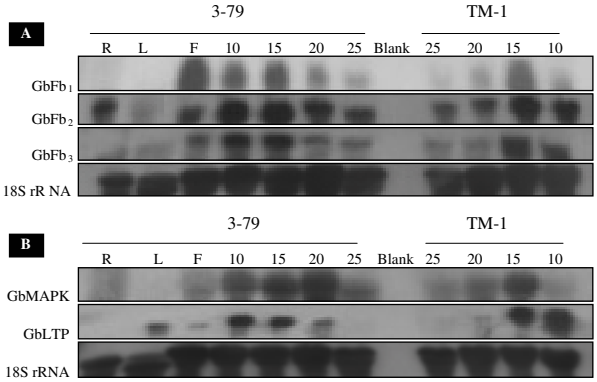

Fig. 3. Expression pattern of the five cDNA clones in different tissues [root (R), leaf (L), and flower (F)] of 3-79 and developing fibers (10, 15, 20, and 25 dpa) of plants of *G. barbadense* accession 3-79 and *G. hirsutum* accession TM-1. Two RNA blots (A and B), with 30 µg of total RNA in each lane, were sequentially hybridized with the cDNA probes indicated (blot A for probes of *Gb*Fb$_1$, *Gb*Fb$_2$, and *Gb*F$_3$; blot B for probes of *Gb*MAPK and *Gb*LTP). After hybridization, each blot was stripped to remove the probe and then re-hybridized to another probe. The loading differences in each lane were assessed by hybridization with an 18S rRNA probe from *Arabidopsis*.

DNA gel blot analysis of genomic DNA from 3-79 and TM-1 revealed that cDNAs *Gb*Fb$_2$, *Gb*LTP, and *Gb*MAPK represented genes of intermediate (average 3-7 bands per digestion) copy number (Fig. 4), and cDNAs *Gb*Fb$_1$ and *Gb*Fb$_3$ represented low (average 1-2 bands per digestion) copy number (data not shown). The copy numbers of the five genes in the genomes of 3- 79 and TM-1 was not different.

Sequence analysis revealed that *Gb*Fb$_1$ with 655 nucleotides (GenBank accession No. AF531362) had no significant homology with any nucleotide or protein sequences in the GenBank database (Jan. 2005). The assumed ORF of *Gb*Fb$_1$ was 330 nucleotides long. The deduced polypeptide of *Gb*Fb$_1$ contained 109 amino acid residues with a calculated molecular mass (MW) of 12.3 KD and an isoelectric point (pI) of 5.04. The amino acid sequence of *Gb*Fb$_1$ had no signal peptide and was characteristic of a soluble protein. Also, an EF-hand calcium-binding domain (a calcium-binding site formed by helices E and F of the calcium-binding protein that are positioned like a forefinger and thumb) was present in the *Gb*Fb$_1$ polypeptide.

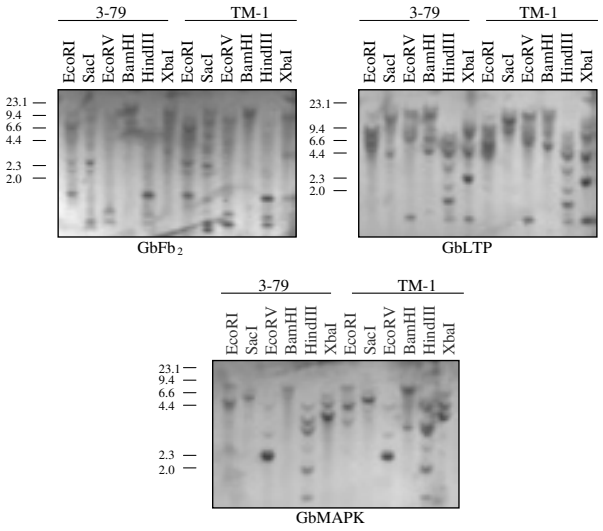

Fig. 4. DNA lot analysis of *G. barbadense* accession 3-79 and *G. hirsutum* accession TM-13 genomic DNA. Genomic DNA (10 µg per lane) was digested with the indicated restriction enzyme and probed with the indicated cDNA probe. *HindIII* contains recognition sites within the cDNA sequences of *Gb*LTP and *Gb*MAPK; *EcoRV* sites are within the cDNA sequences of *Gb*Fb$_2$ and *Gb*MAPK; and *EcoRI and Sac I* sites are within the *Gb*Fb$_2$ sequence. Molecular mass markers (kb) are shown on the left margin of each blot.

Clone *Gb*Fb$_2$ with 1145 nucleotides (GenBank accession No. AF531363) had 96% identity to a cDNA of 6-d cotton fiber from *G. hirsutum* (GenBank GI: 5047798) with unknown function. Clone *Gb*Fb$_2$ had a long ORF of 663 nucleotides. The deduced polypeptide of clone *Gb*Fb$_2$ had 220 amino acid residues. The theoretical pI and MW of the *Gb*Fb$_2$ polypeptide were 5.20 and 24.6KD, respectively. The amino acid sequence of *Gb*Fb$_2$ had no signal peptide and was also characteristic of a soluble protein. Structure analysis showed that some motifs for putative post-translational phosphorylation by kinases were found in the amino acid sequences of *Gb*Fb$_1$ and *Gb*Fb$_2$. The *Gb*Fb$_2$ amino acid sequence contained two possible N-glycosylation sites. Interestingly, *Gb*Fb$_2$ was also abundantly expressed in 3-79 root tissue.

Clone *Gb*Fb$_3$ with 428 nucleotides (GenBank accession No. AF531364) was 99% identical in nucleotide sequence to another cDNA of unknown function from 7-10 dpa cotton fiber of *G. arboreum* (GenBank GI: 13352805). Clone *Gb*Fb$_3$ might be a partial cDNA.

One clone with 1242 nucleotides (GenBank accession No. AF531365) was named *Gb*MAPK because of its sequence similarity to plant mitogen-activated protein (MAP) kinases (MAPKs). Comparison of the *Gb*MAPK nucleotide sequence to other plant MAPK orthologs showed that *Gb*MAPK was 83% identical to alfalfa (GenBank GI: 4456681), 81% identical to *Arabidopsis* (GenBank GI: 6579252), 80% identical to tobacco (GenBank GI: 12718823), 80% identical to maize (GenBank GI: 4239888), 79% identical to leafy spurge (GenBank GI: 7649152), and 73% identical to pea (GenBank GI: 20207).

Comparison of the deduced *Gb*MAPK amino acid sequence to MAPK orthologs from the plants above indicated that *Gb*MAPK was 87% identical to alfalfa (GenBank GI: 4456682), 82% identical to *Arabidopsis* (GenBank GI: 8439881), 82% identical to tobacco (GenBank GI: 1278824), 70% identical to maize (GenBank GI: 4239889), 70% identical to pea (GenBank GI: 20208), and 69% identical to leafy spurge MAPKs (GenBank GI: 7649153). The ORF of *Gb*MAPK was 715 nucleotides long and encoded a polypeptide of 237 amino acid residues. Sequence alignments (data not shown) indicated that the open reading frame of *Gb*MAPK was missing 130 to 165 amino acid residues at the N terminus, and five, sub-domain I to sub-domain V, of the eleven catalytic sub-domains were missing.

The last clone with 621 nucleotides (GenBank accession No. AF531366) had highly significant sequence similarity to cotton lipid transfer proteins (LTPs), so it was named *Gb*LTP. The alignment of the *Gb*LTP nucleotide sequence with several cotton LTPs of *G. hirsutum* in GenBank indicated that the *Gb*LTP shared high identity with LTPs of *G. hirsutum* by 99% (GenBank GI: 2829203), 96% (GenBank GI: 7012718), 95% (GenBank GI: 11095209), 94% (GenBank GI: 7012723), 94% (GenBank GI: 999314), and 94% (GenBank GI: 995906). Also, the predicted amino acid sequence of *Gb*LTP shared identity with the LTPs of *G. hirsutum* above by 98% (GenBank GI: 2829204), 90% (GenBank GI: 7012719), 90% (GenBank GI: 11095210), 89% (GenBank GI: 7012724), 88% (GenBank GI: 999315), and 87% (GenBank GI: 995907). The ORF of *Gb*LTP contained 363 nucleotides. The deduced polypeptide of *Gb*LTP had 120 amino acid residues with a calculated MW of 11.6 KD and an pI of 8.88. Sequence analysis showed that the predicted polypeptide of the *Gb*LTP contained a highly hydrophobic signal peptide in the N-terminal region.

DISCUSSION

Five cDNAs expressed differentially in 20-dpa fiber tissue of *G. barbadense* accession 3-79 were identified by screening a cDNA library of cotton fiber (20 dpa) from 3-79. One of the differentially expressed genes, *Gb*Fb$_1$, had no significant sequence identity with any gene in GenBank. Two of the differentially expressed genes, *Gb*Fb$_2$ and *Gb*Fb$_3$, were similar to ESTs of unknown function previously identified from *G. hirsutum* and *G. arboreum*. The two remaining genes, *Gb*MAPK and *Gb*LTP had sufficiently high levels of sequence similarity to other plant genes in public databases to make tentative functional assignments.

*Gb*MAPK is the first member of the MAPK family to be identified in *G. barbadense* cotton fiber. *Gb*MAPK is a member of the Group B MAPKs (MPKs), having significant nucleotide sequence similarity to *At*MPK11 and *At*MPK13 from *Arabidopsis* (Ichimura et al., 2002). MAPKs are serine/threonine kinases, becoming activated when both threonine and tyrosine residues in a TXY motif are phosphorylated (Anderson et al., 1990). The TXY motif, located in the kinase catalytic subdomain VIII, is conserved in all MAPKs, so all MAPKs share a very similar activation mechanism (Nishihama et al., 1995). MAPKs are part of the signal transduction modules known as MAPK cascades. These phosphorylation cascades represent core units for signal transduction from the cell surface to the nucleus (Kültz 1998), and some MAPKs directly stimulate the transcription of specific genes in the nucleus.

In yeast, *Saccharomyces cerevisae*, one of the MAPK signal pathways (protein kinase C-MAPK or PCK-MAPK) has been shown to function in the integrity of the yeast cell wall and thus, to control the yeast cell growth (Atienza et al., 2000; Lee et al., 1993; Levin et al., 1994; Rajavel et al., 1999). *Saccharomyces cerevisae* also has another MAPK signal pathway, an osmosensing transduction pathway known as the high osmolarity glycerol response MAPK pathway or HOG-MAPK (Brewster et al., 1993), which is implicated in yeast cell wall biosynthesis (Cid et al., 1995; Reynolds et al., 1998; Roemer et al., 1993). Reynolds et al. (1998) proposed that both the HOG-MAPK pathway and the PKC-MAPK pathway control the plasticity of the yeast cell wall, and coordinate the organization and synthesis of the cell wall composition during the cell growth. Our finding that *Gb*MAPK is differentially expressed in fiber of 3-79 from 10 to 20 dpa and was highest at 20 dpa (during primary wall elongation and secondary wall formation) suggests that *Gb*MAPK functions, as MAPK does in yeast, in the organization and biosynthesis of the fiber cell wall. The production and extension of the primary wall and the building of the secondary wall apparently occur simultaneously during fiber development (DeLanghe, 1986; Seagull et al., 2000); therefore, during this time the cell wall must be plastic enough to allow for increases in both fiber length and diameter. Cell turgor pressure obviously plays an important role in fiber elongation (Ruan and Chourey, 1998; Smart et al., 1998). By comparison with the role of MAPK in regulating cell osmotic pressure in yeasts, we propose that *Gb*MAPK might be involved in mediating the cell turgor and/or wall plasticity of cotton fibers, especially at the late transition period.

A second identified gene that was more abundantly expressed in fiber of 3-79 at 20 dpa was *Gb*LTP, a cotton lipid transfer protein (LTP). The predicted polypeptide of *Gb*LTP was similar to other plant LTPs with such characterisitcs as low MW, high (basic) pI, a high content of proline, glycine, alanine, and serine, a low number of aromatic acids (Phe, Trp and Tyr), seven to eight Cys at conserved positions, and a signal peptide that could be processed during trans-membrane passage (Ma et al., 1995; Orford and Timmons, 1997). *Gb*LTP shared high sequence identity (94-99% in nucleotide sequence and 87-98% in amino acid sequence) with other cotton LTPs of *G. hirsutum*, suggesting that this gene is homologous to one or more of these LTPs or is, at least, a member of the same gene family. Plant LTPs are small, basic proteins that constitute up to 4% of the total soluble protein in higher plants (Kader, 1996). Originally plant LTPs were thought to participate in membrane biosynthesis and regulation of the intercellular fatty acid pools (Kader et al., 1984); however, a highly hydrophobic signal peptide present in all cDNA clones and gene sequences of plant LTPs (Ma et al., 1995) suggested that plant LTPs could enter the secretory pathway (Kader, 1996). Also, LTPs were shown to be secreted to and located in cell walls and outer cellular layers (Pyee, et al 1994; Pyee and Kolattukudy, 1995; Sterk et al., 1991; Thoma et al., 1994), indicating that plant LTPs were extracellular proteins (Ma et al., 1997). These findings are not consistent with either a role in membrane biosynthesis or a role in fatty acid synthesis as proposed earlier. Therefore, several novel roles for plant LTPs, such as participation in the

synthesis of cutin and cuticular wax, embryogenesis, defense reactions against phytopathogens, symbiosis, and responses to environmental stresses, have been suggested (Kader, 1996; Blein et al., 2002). During cotton fiber development, LTPs may play a role in cutin synthesis (Ma et al., 1995). *Gb*LTP was highly expressed at 10 to 15 dpa during the cell elongation stage of development when the fiber has only a primary wall. Expression of *Gb*LTP decreased sharply after 20 dpa during secondary wall synthesis.

Cotton fiber growth traditionally occurs in four phases: initiation, elongation, secondary cell wall thickening, and maturation (Basra and Malik, 1984), and each of the developmental phases overlaps the succeeding phase. The duration of fiber developmental phases in *G. hirsutum* have been identified (Ryser, 1985); however, much less information is available on the fiber development of the other cultivated species. Recently, Applequist et al. (2001) found that there was considerable interspecific variability for the cultivated and wild diploid and tetraploid species in the duration of fiber elongation phase, during the period of the maximum rate of elongation, and in the absolute growth rate during different stages of development. In most wild diploids tested in the B-, C-, and D-genome groups, fiber growth reached the final length by about 14 dpa, but fibers in the A-genome species (*G. herbaceum* and *G. arboretum*) continued to elongate for an additional week. The accession TM-1 of *G. hirsutum* showed an increased rate of elongation between 10 and 15 dpa, whereas in the putatively wild accession of *G. hirsutum* (TX2094), the comparative period of maximum growth was delayed until between 15 and 20 dpa (Applequist et al., 2001). Therefore, it is possible that differences in the duration of fiber developmental phases exist between *G. barbadense* and *G. hirsutum*, so the expression profile of fiber genes would be different between these two species at a given time period. Further experiments are planned to investigate the exact relationship between the five genes reported here and fiber development.

To determine the functions of *Gb*Fb$_1$, *Gb*Fb$_2$, and *Gb*Fb$_3$, the RNA interference (RNAi) technique will be used in future studies. Using RNAi to suppress sucrose synthase (Sus) gene expression in cotton fiber, Ruan et al. (2003) found that the number of fiber initials and fiber length were reduced dramatically, suggesting that Sus plays a crucial role in cotton fiber initiation and elongation. Also, RNAi of *G. hirsutum* ACTIN1 (*Gh*ACTN1) gene caused significant reduction in cotton fiber elongation, indicating that the *Gh*ACTIN1 gene functions in cotton fiber elongation (Li et al., 2005). To further characterize the functions of the five selected genes in cotton fiber development, it is important to know whether the expression differences of these genes between the two species exist only at the mRNA (transcription) level or also at the protein (translation) level. Also, identification and characterization of the corresponding gene promoters will be valuable for determining how developmentally regulated gene expression is accomplished and the function(s) of the genes in fiber development.

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REFERENCES

Anderson, N.G., J.L. Maller, N.K. Tonks, and T.W. Sturgill. 1990. Requirement for integration of signals from two distinct phosphorylation pathways for activation of MAP kinase. Nature 343:651-653.

Applequist, W.L., R. Cronn and J.F. Wendel. 2001. Comparative development of fiber in wild and cultivated cotton. Evol. Dev. 3: 3-17.

Atienza, J.M., M. Suh, I. Xennarios, R. Landgraf, and J. Colicelli. 2000. Human ERK1 induces filamentous growth and cell wall remodeling pathways in *Saccharomyces cerevisiae*. J. Biol. Chem. 275:20638-20646.

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

Blein, J.-P., P. Coutos-Thevenot, D. Marion, and M. Ponchet. 2002. From elicitins to lipid-transfer proteins: a new insight in cell signaling involved in plant defence mechanisms. Trends Plant Sci. 7:293-296.

Brewster, J.L., T. de Valoir, N.D. Dwyer, E. Winter, and M.C. Gustin. 1993. An osmosensing signal transduction pathway in yeast. Science 259:1760-1763.

Cid, V.J., A. Duran, F. del Rey, M.P. Snyder, C. Nombela, and M. Sanchez. 1995. Molecular basis of cell integrity and morphogenesis in *Saccharomyces cerevisiae*. Microbiol. Rev. 59:345-386.

DeLanghe, E.A.L. 1986. Lint Development. p. 325-349. *In* J.R. Mauney and J.McD. Stewart (ed.) Cotton Physiology. The Cotton Foundation, Memphis, TN.

Fryxell, P.A. 1992. A revised taxonomic interpretation of *Gossypium* L. (Malvaceae). Rhoedea 2:108-165.

Green, C.C., and T.W. Gulp. 1990. Simultaneous improvement of yield, fiber quality, and strength in Upland cotton. Crop Sci. 30:66-69.

Ichimura, K., K. Shinozaki, G. Tena, J. Sheen, Y. Henry, A. Champion, M. Kreis, S. Zhang, H. Hirt, C. Wilson, E. Heberle-Bors, B.E. Ellis, P.C .Morris, R.W. Innes, J.R. Ecker, D. Scheel, D.F. Klessig, Y. Machida, J. Mundy, Y. Ohashi, and J.C. Walker. 2002. Mitogen-activated protein kinase cascades in plants: a new nomenclature. Trends Plant Sci. 7:301-308.

Kader, J.-C. 1996. Lipid-transfer protein in plants. Annu. Rev. Plant Physiol. Plant Mol. Biol. 47:627-654.

Kader, J.-C., M. Julienne, and C. Vergnolle. 1984. Purification and characterization of a spinach-leaf protein capable of transferring phospholipids from liposomes to mitochondria or chloroplasts. Eur. J. Biochem. 139:411-416.

Kim, H.J., and B.A. Triplett. 2001. Cotton fiber growth in planta and in vitro. Models for plant cell elongation and cell wall biogenesis. Plant Physiol. 127:1361-1366.

Kloth, R.H. 1998. Analysis of commonality for traits of cotton fiber. J. Cotton Sci. 2:17-22 [Online]. Available at http://www.cotton.org/journal/1998-02/1/17.cfm.

Kohel, R.J. 1999. Cotton germplasm resources and the potential for improved fiber production and quality. p. 167-182. *In* A.S. Basra (ed.) Cotton Fibers. The Haworth Press, Inc., Binghamton, NY.

Kohel, R.J., J. Yu, Y.-H. Park, and G.R. Lazo. 2001. Molecular mapping and characterization of traits controlling fiber quality in cotton. Euphytica 121:163-172.

Kültz, D. 1998. Phylogenetic and functional classification of mitogen- and stress-activated protein kinases. J. Mol. Evol. 46:571-588.

Lee, K.S., K. Irie, Y. Gotoh, Y. Watanabe, H. Araki, E. Nishida, K. Matsumoto, and D.E. Levin. 1993. A yeast mitogen-activated protein kinase homolog (*Mpk1p*) mediates signaling by protein kinase C. Mol. Cell. Biol. 13:3067-3075.

Levin, D.E., B. Bowers, C.-Y. Chen, Y. Kamada, and M. Watanabe. 1994. Dissecting the protein kinase C/MAP kinase signaling pathway of *Saccharomyces cerevisiae*. Cell. Mol. Biol. Res. 40:229-239.

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

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

Ma, D.-P., H. Tan, Y. Si, R.G. Creech, and J.N. Jenkins. 1995. Differential expression of a lipid transfer protein gene in cotton fiber. Biochim. Biophys. Acta 1257:81-84.

McKenzie, W.H. 1970. Fertility relationships among interspecific hybrid progenies of *Gossypium*. Crop Sci. 10:571-574.

Nishihama, R., H. Banno, W. Shibata, K. Hirano, M. Nakashima, S. Usami, and Y. Machida. 1995. Plant homologues of components of MAPK (mitogen-activated protein kinase) signal pathways in yeast and animal cells. Plant Cell Physiol. 36:749-757.

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

Pyee, J., and P.E. Kolattukudy. 1995. The gene for the major cuticular wax-associated protein and three homologous genes from broccoli (*Brassica oleracea*) and their expression pattern. Plant J. 7:49-59.

Pyee, J., H. Yu, and P.E. Kolattukudy. 1994. Identification of a lipid transfer protein as the major protein in the surface wax of broccoli (*Brassica oleracea*) leaves. Arch. Biochem. Biophys. 311:460-468.

Rajavel, M., B. Philip, B.M. Buehrer, B. Errede, and D.E. Levin. 1999. *Mid2* is a putative sensor for cell integrity signaling in *Saccharomyces cerevisiae*. Mol. Cell. Biol. 19:3969-3976.

Reynolds, T.B., B.D. Hopkins, M.R. Lyons, and T.R. Graham. 1998. The high osmolarity glycerol response (HOG) MAP kinase pathway controls localization of a yeast Golgi glycosyltransferase. J. Cell Biol. 143:935-946.

Roemer, T., S. Delaney, and H. Bussey. 1993. *SKN1* and *KRE6* define a pair of functional homologs encoding putative membrane proteins involved in beta-glucan synthesis. Mol. Cell. Biol. 13:4039-4048.

Ruan, Y.-L., and P.S. Chourey. 1998. A fiberless seed mutation in cotton is associated with lack of fiber cell initiation in ovule epidermis and alterations in sucrose synthase expression and carbon partitioning in developing seeds. Plant Physiol. 118:199-406.

Ruan, Y.-L., D.J. Llewellyn and R.T. Furbank. 2003. Suppression of sucrose synthase gene expression represses cotton fiber initiation, elongation, and seed development. Plant Cell 15: 952-964.

Ryser, U. 1985. Cell wall biosynthesis in differentiating cotton fibers. Eur. J. Cell Biol. 39: 236-256.

Sambrook, J., E.F. Fritsch, and T. Maniatis. 1989. Molecular Cloning: A Laboratory Manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Seagull, R.W., V. Oliver, K. Murphy, A. Binder, and S. Kothari. 2000. Cotton fiber growth and development. 2. Changes in cell diameter and cell wall birefringence. J. Cotton Sci. 4:97-104 [Online]. Available at http://www.cotton.org/journal/2000-04/2/97.cfm.

Smart, L.B., F. Vojdani, M. Maeshima, and T.A. Wilkins. 1998. Genes involved in osmoregulation during turgor-driven cell expansion of developing cotton fibers are differentially regulated. Plant Physiol. 116:1539-1549.

Sterk, P., H. Booij, G.A. Schellekens, A.V. Kammen, and S.C. De Vries. 1991. Cell-specific expression of the carrot EP2 lipid transfer protein gene. Plant Cell 3:907-921.

Thoma, S., U. Hecht, A. Kippers, J. Botella, and S.C. De Vries. 1994. Tissue-specific expression of a gene encoding a cell wall-localized lipid transfer protein from *Arabidopsis*. Plant Physiol. 105:35-45.

Walden, A.R., C. Walter and R.C. Gardner. 1999. Genes expressed in *Pinus radiate* male cones include homologs to anther-specific and pathogenesis response genes. Plant Physiol. 121:1103-1116.

Wan, C.Y., and T.A. Wilkins. 1994. A modified hot borate method significantly enhances the yield of high-quality RNA from cotton (*Gossypium hirsutum* L.). Anal. Biochem. 223:7-12.

Wilkins, T.A., and J.A. Jernstedt. 1999. Molecular genetics of developing cotton fibers. p. 231-269. *In* A.S. Basra (ed.) Cotton Fibers. The Haworth Press, Inc. Binghamton, NY.

Yu, Z.H., and R.J. Kohel. 2001. Cotton genome research in the United States. p. 103-121. *In* J.N. Jenkins and S. Saha (ed.) Genetic Improvement of Cotton. Science Publishers, Inc., Enfield, NH.