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Plant Special Cell – Cotton Fiber¹

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1. Introduction

Cotton fibers are single-celled outgrowths from individual epidermal cells on the outer integument of the ovules in the developing cotton fruit. Fibers of upland cotton (*G. hirsutum* L.) generally grow up to 30 to 40 mm in length and ~15 μm in thickness at full maturity. Their development consists of four overlapping stages: fiber initiation, cell elongation, secondary wall deposition, and maturation. The thickened secondary walls of mature cotton fibers have long been considered unique in that they were thought to consist of nearly pure cellulose and to be devoid of hemicellulose and phenolics. However, other plant derived fibers such as flax (*Linum usitatissimum* L) and ramie (*Boehmeria nivea* L) fibers have been shown to contain phenolics except cellulose (Angelini et al., 2000; Day et al., 2005).

Plant cell wall phenolics consist of two groups of compounds: (1) lignin, the polymer of monolignol units, linked by oxidative coupling; and (2) low molecular weight hydroxycinnamic acids, that are bound to various cell wall components and are involved in cross-linkages (Iiyama et al., 1994; Wallace & Fry 1994). From a functional point of view, plant cell wall phenolics protect cellulose fibers in plant cell wall from chemical and biological degradation (Grabber et al., 2004) and can influence wall mechanical strength, growth, morphogenesis and responses to biotic and abiotic stresses (Wallace & Fry, 1994; Boerjan et al., 2003).

The emergence of lignin during evolution is believed to be a crucial adaptation for terrestrial plants from aquatic ancestors (Kendrick & Crane, 1997; Peter & Neale, 2004). It is mainly present in secondary thickened cell walls within xylem tissues, where it provides rigidity and impermeability to the cell walls. Later on, "lignin-like" compounds have been identified in primitive green algae (Delwiche et al., 1989), and lignins have been found exist within a red alga's calcified cells that lack hydraulic vasculature and have little need for additional support (Martone et al., 2009). Lignin is also an integral constituent of the primary cell walls of the dark-grown maize (*Zea mays* L.) coleoptile (Müsel et al., 1997), elongation zone of maize primary root (Fan et al., 2006), the juvenile organs those are still in the developmental state of rapid cell extension. Molecular and biochemical evidences have also showed phenylpropanoid synthesis and presence of wall-linked phenolics in white, soft cotton

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fibers. The aim of this chapter is to present our new results about cotton fiber growth and development. Molecular, spectroscopic and chemical techniques were used to prove the possible occurrence of previously overlooked accumulation of phenolics during secondary cell wall formation in cotton fibers.

2. Cotton fiber growth

2.1 Cotton fiber elongation

North Xinjiang in China is an upland cotton (*G. hirsutum* L.) growing area. Local effective accumulated temperature ($\geq 10^{\circ}\text{C}$) is less than 3500°C . Frost-free period is less than 150 d. Under this growing condition, we observed the fiber cell elongation of the cultivar Xinluzao36 (*G. hirsutum* L.) and cultivar Xinhai 22 (*G. barbadense* L.). The fiber length was determined kinetically by the method of Gokani and Thaker (2000). The final values were taken as means ($n=6$ balls \times 20 seed with fibers). The fiber elongation of both varieties went through slow-fast-slow-stop process and showed typical sigmoid growth curve (Fig. 1). During 0-10 DPA, fibers were initiating and elongating very slowly in 0-5 DPA, and fibers were accelerately elongating in 5-10 DPA. During 10-15 DPA, the fiber elongation rate was actively accelerated. Both varieties showed similar fiber elongation rate in 0-15 DPA. However, the fiber elongation rate of Xinhai 22 was continuously actively accelerated until 19 DPA. The fiber elongation rate was decelerated during 15-21 DPA for Xinluzao36 and during 19-23 DPA for Xinhai 22 respectively. The results suggested that longer-fiber-length variety Xinhai 22 not only had longer fiber elongation period, but also had longer actively accelerated fiber elongation period.

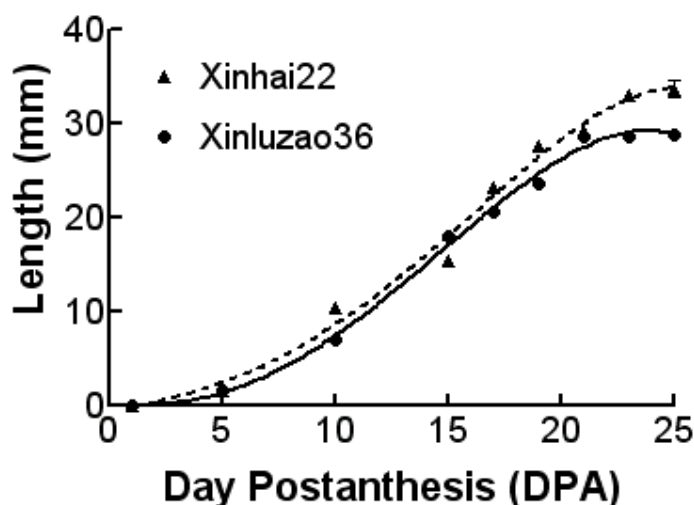


Fig. 1. Kinetics of cotton fiber elongation postanthesis

2.2 Cotton fiber secondary wall deposition

The Ultra-structural developmental process of fiber cell from early maturing cotton cultivar Xinluzao 36 was observed by using transmission electron microscopy. At 10 DPA, when the primary cell wall of cotton fiber was thin and even thickness, and a big vacuole located at the central of fiber cell, there were rich organelles, such as mitochondria, ribosomes and Golgi bodies in the cytoplasm (Fig. 2 A-D). At 20 DPA, a thin layer of the secondary cell wall formed inside the primary cell wall clearly and a part of the organelles disappeared (Fig. 2 E-F). Subsequently, the secondary cell wall thickened rapidly (Fig. 3). The average thickness

increased around $0.14 \mu\text{m}$ per day from 30 DPA to 40 DPA and around $0.47 \mu\text{m}$ per day from 40 DPA to 50 DPA (Fig. 4). The secondary cell wall gradually thickened and formed daily growth rings (Fig 3. E). Then the vacuum inside fiber cell became a narrow gap and the thickness of cell wall became thinner as the fiber maturing and water loss (Fig 3. F). Results showed that the fiber development process of early-maturing cotton was quite similar to other varieties reported (Xu, 1988), despite flowering and maturing earlier.

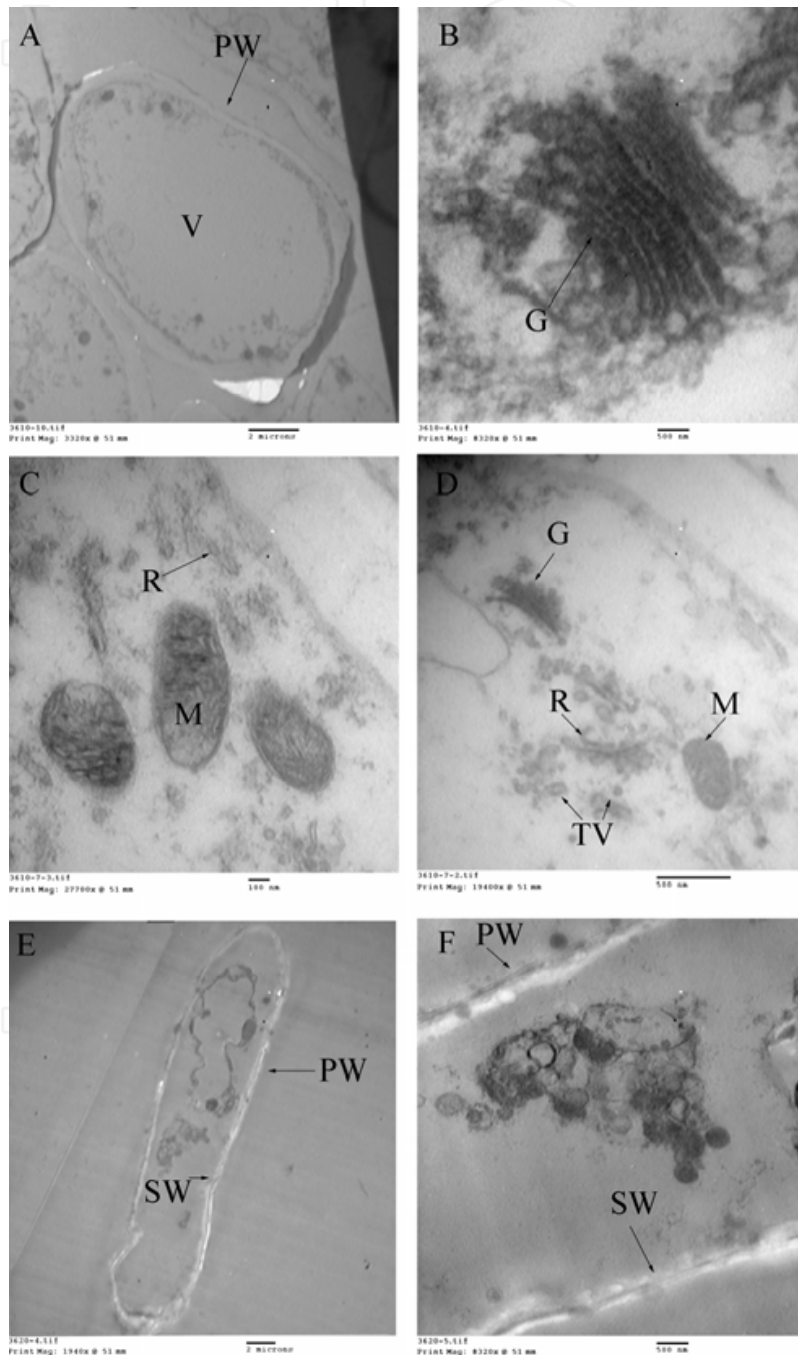


Fig. 2. Transverse section characters of 10 DPA and 20 DPA fiber cell
 10DPA:A($\times 6000$),B($\times 15000$),C($\times 60000$); 20DPA:E($\times 3500$),F($\times 15000$); DPA(Day post anthesis);
 PW(Primary wall); G(Golgi apparatus); M(Mitochondrion); R(Rough endoplasmic
 reticulum); SW(Secondary wall); TV(Transport vesicles); V(Vacuole)

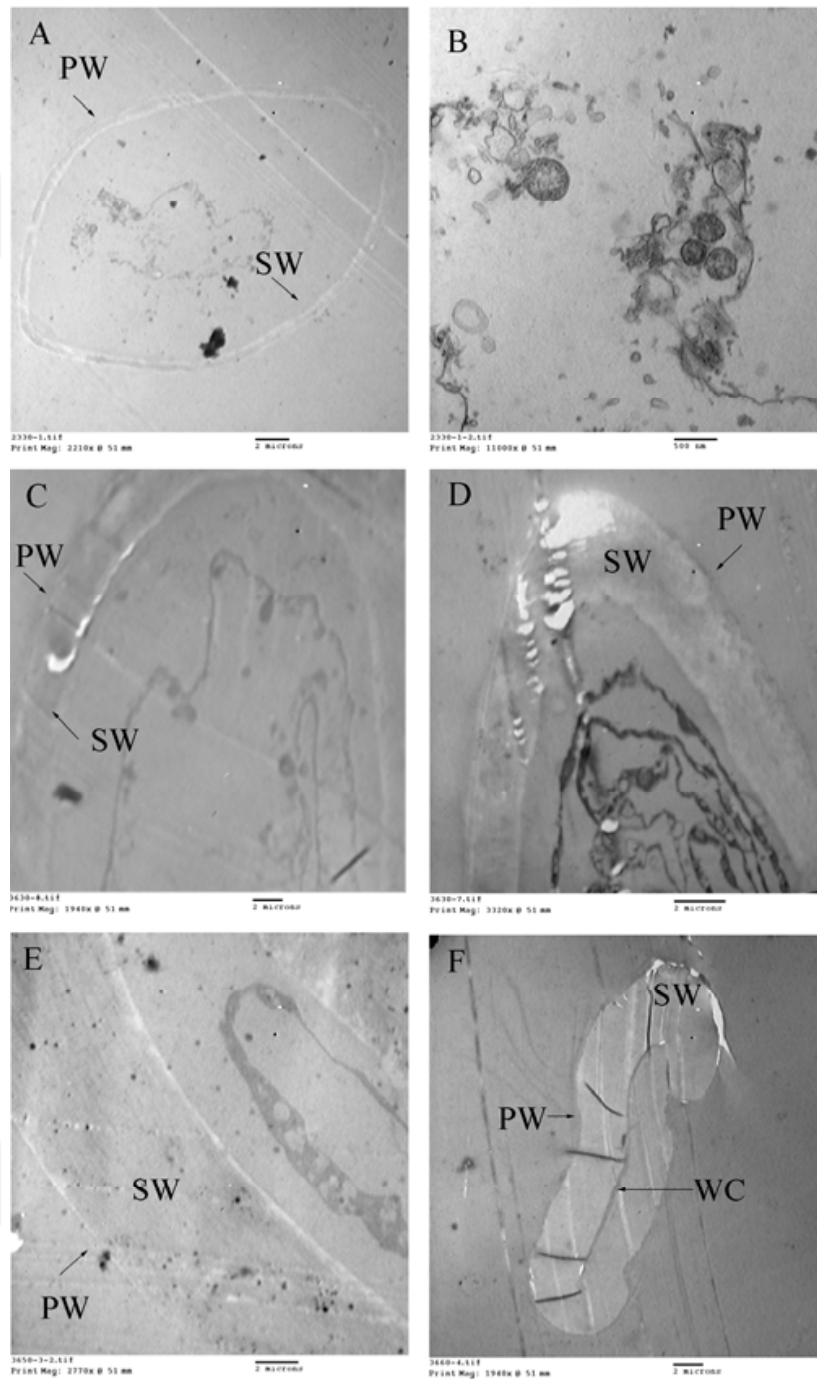


Fig. 3. Transverse section characters of 30 DPA ,40 DPA, 50 DPA and mature fiber cell 30DPA:A($\times 4000$),B($\times 20000$); 40DPA:C($\times 3500$); 50DPA:E($\times 5000$); Mature: F($\times 15000$);DPA(Day post anthesis); PW(Primary wall); SW(Secondary wall); MC(Mesocoele).

2.3 Ovule culture fed by middle product in phenylpropanoid pathway

Based on the system of ovule cultured *in vitro*, ferulic acid (FA), which is middle product in phenylpropanoid pathway, was fed in the media for either fertilized or unfertilized ovules. With the assistance of image digitization technology (Fan et al. 2011), the growth condition of the fibers at different period was analyzed. The results revealed that higher concentration of exogenous FA (100 $\mu\text{mol/L}$) inhibited the normal growth of the fibers(not show), while low level of FA (50 $\mu\text{mol/L}$) had not showed such inhibited phenomenon, in the opposite, fiber elongation was accelerated (Fig. 5). The results showed that FA had the effects on the cotton fiber growth by adjusting the FA concentration in the media. The turning point for accelerating and decelerating growth of cotton fiber was possibly at the concentration ranged between 50 $\mu\text{mol/L}$ and 100 $\mu\text{mol/L}$. TEM observation on fiber transverse section indicated that the cell wall thickness was significantly increased by exogenous FA($P<0.01$). In addition, acetyl bromide chemical analysis revealed that the content of phenylpropanoid compound in cotton fiber was increased 5.2% for the fibers fed with 50 $\mu\text{mol/L}$ FA. The investigation of cotton fiber growth, cell wall thickness and chemical analysis revealed that exogenous FA had significant effects on cotton fiber development in ovule culture system. This work improved our understanding of cotton fiber development mechanism and the effect of phenylpropanoid pathway and its products on cotton fiber development.

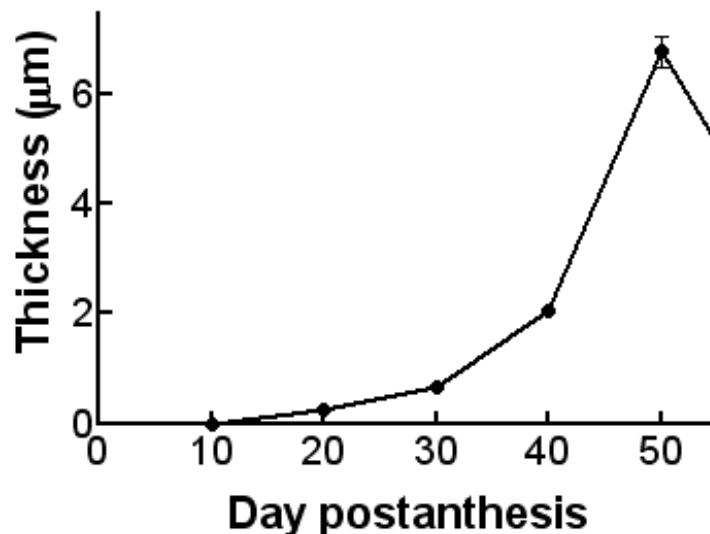


Fig. 4. Increasing thickness of secondary wall in fiber development

15 images of cell wall from each period were randomly selected, the perpendicular thickness of the secondary wall of 15 evenly distributed points at each image were measured. Values =Means \pm SE, n = 225 measurements.

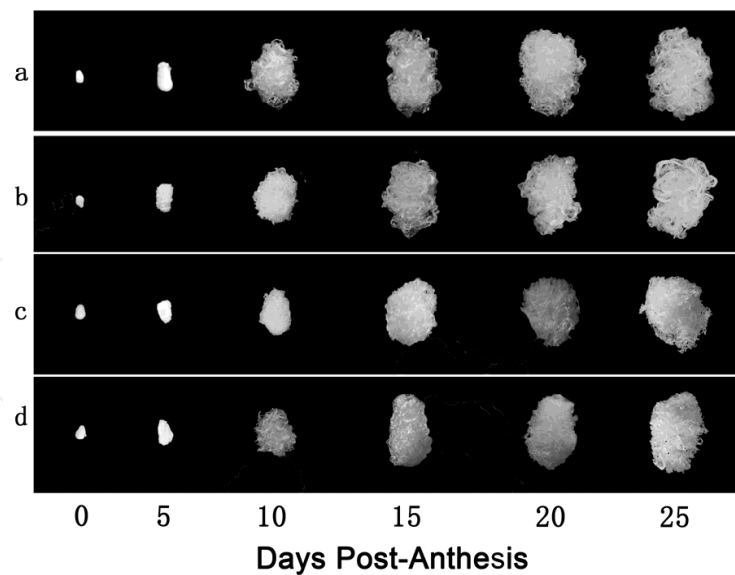


Fig. 5. The representative ovule expansion images of FA treatment under time course (a) represents fertilized ovules; FA final concentrations of 50 μ M; (b) represents fertilized ovules control (no FA adding); (c) represents unfertilized ovules; FA final concentrations of 50 μ M; (d) represents unfertilized ovules control.

3. Cotton fiber secondary wall development

3.1 Genes in phenylpropanoid pathway were expressed in developing cotton fibers

To explore the expression regularity of genes in phenylpropanoid pathway in developing cotton fibers and to investigate the relationships between cotton fiber quality and genes expression at transcriptional level, the relative quantitative expression of genes in phenylpropanoid pathway, such as *GhPAL*, *Gh4CL*, *GhC4H*, *GhCOMT*, *GhCCoAOMT*, *GhCCR* and *GhCAD*, were studied by semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR) in different organs and different time of developmental cotton fibers respectively (Fig. 6). The expression of *GhC4H*, *GhCOMT* and *GhCCoAOMT* had higher levels in both root and stem. Along with the initiation of fiber cell wall thickening, most of the genes displayed high expression levels. The expression of *GhCAD1*, *GhCCR2* and *GhCCR3* were gradually reduced in the development of cotton fiber, but they were not have significant differences in different quality of cotton fiber in all developmental stages.

Since CAD has been considered a key enzyme in the phenylpropanoid biosynthesis pathway (Steeves et al. 2001, Boerjan et al. 2003), *GhCADs* expressions in cotton fiber were analyzed. Relative quantitative RT-PCR analysis was carried out at the time of secondary cell wall development (25 DPA) in the cotton fiber cell using the gene-specific primers. The analysis showed that *GhCAD6* and *GhCAD1* were predominantly expressed among seven gene homologs. The relative expression of *GhCAD6* was increased during the period of secondary wall development in cotton fibers, and reached the higher level at 20 DPA.

The relationship between phenylpropanoid compounds and cotton fiber development was explored in our laboratory. The higher expression time of key enzyme in phenylpropanoid pathway CAD genes was coincident with the secondary wall development of both the fiber cells and the organs with vascular elements. In addition, the time of *GhCAD6* higher

expression coincided with the time of secondary wall formation of cotton fiber (Fig. 7). This is a kind of indicator for the biosynthesis of the phenylpropanoid unit and cell wall phenolics. Those results explained that the pathway was active not only in vascular tissues but also in fiber cells with secondary wall thickening. Guo et al. (2007) found that the regulation of seven metabolic pathways including pathways of phenol metabolism, showed significant changes during the maturation of cotton fiber. Recent research by Hovav et al. (2008) found that genes of the phenylpropanoid metabolism in cluster 6 gradually increase in expression during fiber development. Their studies provide indirect support for our findings.

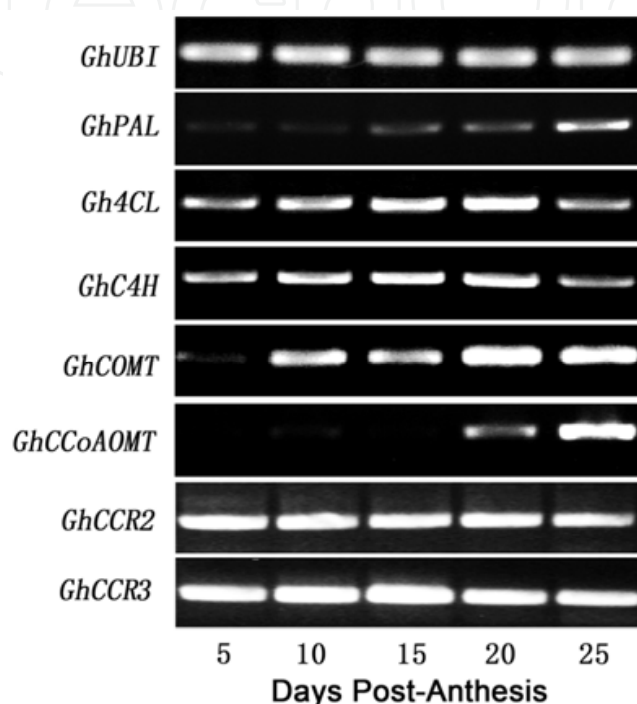


Fig. 6. Relative quantitative RT-PCR analysis of phenylpropanoid pathway genes in developing cotton fiber

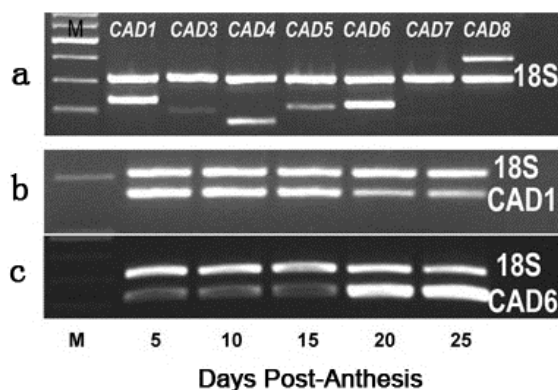


Fig. 7. Relative quantitative RT-PCR analyses of *GhCADs* in developing cotton fiber. (a) Relative quantitative RT-PCR analyses of seven *GhCAD* gene homologs at 25 DPA of developing cotton fiber; (b) Relative quantitative RT-PCR analyses of *GhCAD6* as time course; (c) Relative quantitative RT-PCR analyses of *GhCAD1* as time course. Lane M shows nucleotide markers; 18S indicates a 315 bp fragment of ribosomal RNA used as the internal standard.

3.2 Cloning and characterization of genes in phenylpropanoid pathway in developing cotton fibers

Sixteen gene complete cDNAs in this pathway had been cloned from developing cotton fibers. Their amino acid sequences had high homology with those corresponding genes from other plants (Table 1).

Genes	NCBI No.	Genes	NCBI No.
<i>GhCAD1</i>	EU281304	<i>Gh4CL1</i>	FJ479707
<i>GhCAD6</i>	EU281305	<i>GhCOMT1</i>	FJ479708
<i>GhCAD3</i>	FJ376601	<i>GhCOMT2</i>	FJ479709
<i>GhCAD7</i>	FJ376602	<i>GhC4H1</i>	FJ848866
<i>GhCCR1</i>	FJ376603	<i>GhC4H2</i>	FJ848867
<i>GhCCR4</i>	FJ376604	<i>GhCAD5</i>	FJ848868
<i>GhCCoAOMT1</i>	FJ376605	<i>GhCOMT3</i>	FJ848869
<i>GhCCoAOMT2</i>	FJ376606	<i>Gh4CL2</i>	FJ848870

Table 1. Genes cloned from developing cotton fiber and their NCBI numbers

3.2.1 Gene of initiation (*GhPAL*)

PAL is the first enzyme of the general phenylpropanoid pathway and catalyzes the nonoxidative deamination of phenylalanine to trans-cinnamic acid and NH₃. Subsequent enzymatic steps involving the actions of cinnamate 4-hydroxylase (C4H), 4-coumarate:CoA ligase (4CL), hydroxycinnamoyl-CoA transferase (HCT), p-coumarate 3-hydroxylase (C3H), caffeoyl-CoA O-methyltransferase (CCoAOMT), cinnamoyl-CoA reductase (CCR), ferulate 5-hydroxylase (F5H), caffeic acid O-methyltransferase (COMT), and cinnamyl alcohol dehydrogenase (CAD) catalyze the biosynthesis of monolignols (Andersen et al.2008). Semi-quantitative RT-PCR analysis revealed that *GhPAL* gene was differentially expressed in different developmental stages, Along with the initiation of fiber cell wall thickening, *GhPAL* gene displayed high expression levels (Fig. 8).

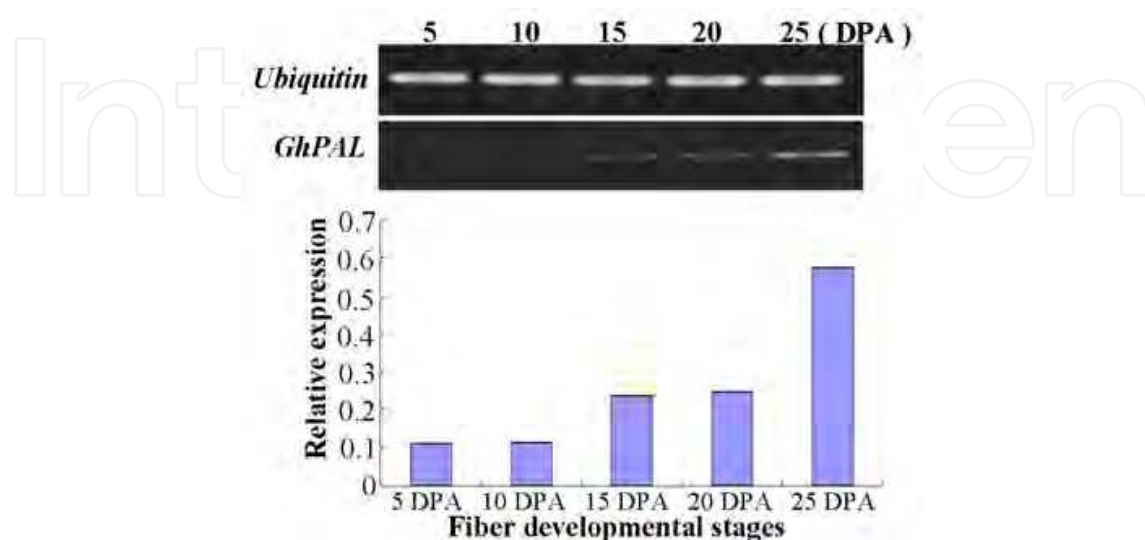


Fig. 8. Expression patterns of *GhPAL* in developing cotton fibers

3.2.2 Genes of methylation reaction (*GhCOMT* and *GhCCoAOMT*)

Caffeic acid O-methyltransferase (COMT) and caffeoyl-CoA-3-O-methyltransferase (CCoAOMT) genes encode two methyltransferases at different substrate levels in lignin biosynthesis (Yoshihara et al.2008). COMT is essential for syringyl lignin (S unit) biosynthesis and has long been considered as the only methylating enzyme involved in lignification; CCoAOMT is involved in the biosynthesis of guaiacyl (G) and syringyl (S) lignins.

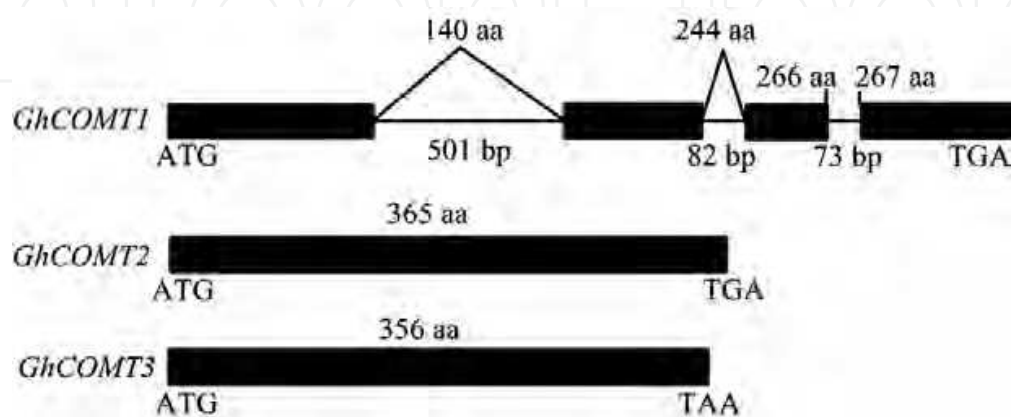


Fig. 9. The structures of *GhCOMT1*, *GhCOMT2* and *GhCOMT3* genes in cotton

Exons are denoted by black boxes. Introns are denoted by lines. The lengths of the introns in base pairs are indicated. The number at the boundaries of each exon indicates the codon at which the intron is located. The translation initiation and termination codons are shown. aa, amino acids.

Full-length cDNA of a key enzyme genes *GhCOMT1*, *GhCOMT2* and *GhCOMT3* related to lignin metabolism in cotton (*G. hirsutum* L.) were isolated, and their cDNA and the amino acid sequences were analyzed by bioinformatics methods (Fig. 9). Semi-quantitative RT-PCR analysis revealed that *GhCOMT1*, *GhCOMT2* and *GhCOMT3* were differentially expressed in different tissues, and *GhCOMT1* and *GhCOMT2* mRNA accumulated most abundantly in root, *GhCOMT3* was highly expressed in stem. For the fiber developing, from 5 to 25 DPA, *GhCOMT1* and *GhCOMT3* were consistently expressed, while *GhCOMT2* was increasingly expressed.

Two *GhCCoAOMT* genes were cloned in developing cotton fiber. Semi-quantitative RT-PCR analysis revealed that *GhCCoAOMT1* and *GhCCoAOMT2* can be expressed in different kinds of cotton tissues (Fig. 10), and their mRNA accumulated most abundantly in stem *GhCCoAOMT1* expression in cotton tissues was stem > root > petal > hypocotyl > 10 DPA fiber > stamen > ovule > leaf. Meanwhile, the expression peak of *GhCCoAOMT1* appeared at 25 DPA, while *GhCCoAOMT2* was at 10 DPA and 15 DPA (Fig. 11). The results confirmed that all of the genes of the two methyltransferase gene families had higher expression quantity, which was coincident with vascular tissues, such as root and stem, while tissue specific and development period specific expression patterns were detected in other organs and growing fibers.

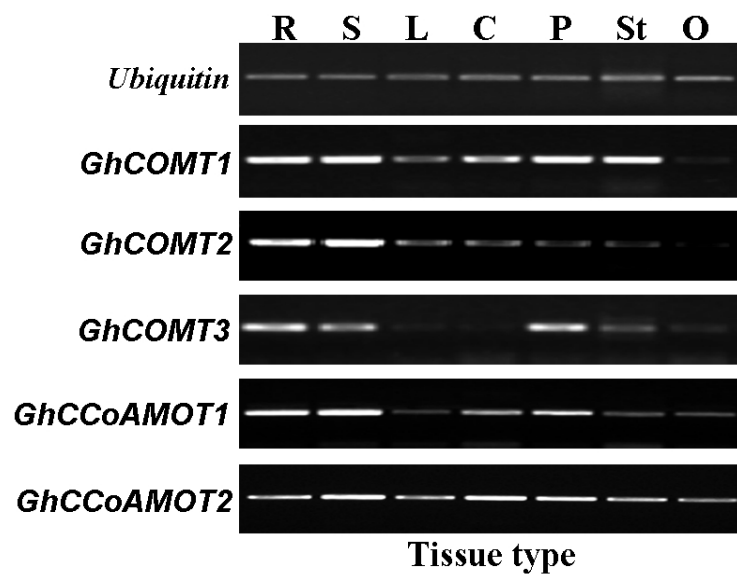


Fig. 10. *GhCOMT1*, *GhCOMT2*, *GhCOMT3* and ubiquitin gene RT-PCR products from different cotton tissues. R: Root; S: Stem; L: Leaf; H: Hypocotyl; P: Petal; St: Stamen; O: Ovule.

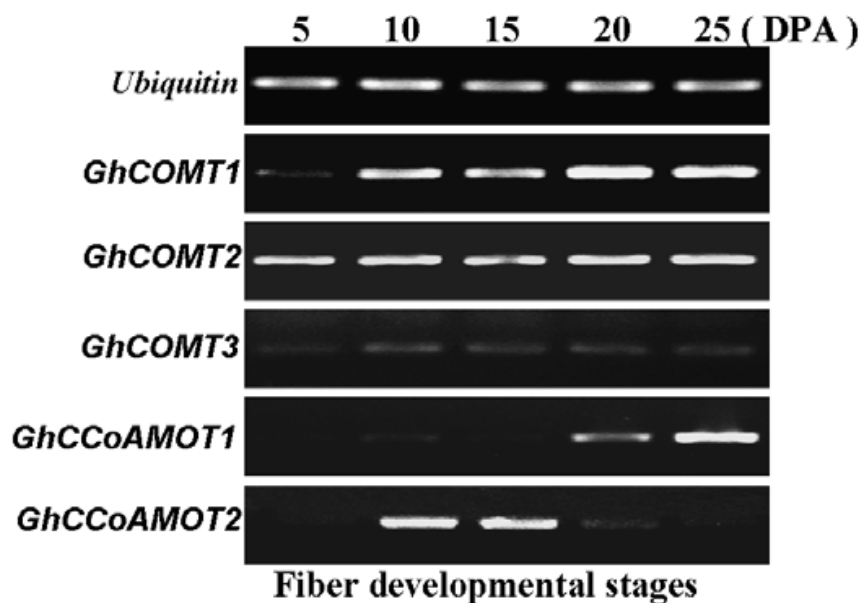


Fig. 11. *GhCOMT1*, *GhCOMT2*, *GhCOMT3* and ubiquitin gene RT-PCR products from different developmental stages

3.2.3 Genes of reduction reaction(*GhCCR* and *GhCAD*)

Cinnamoyl-CoA reductase (CCR), one of the key enzymes in the first step of the phenylpropanoid pathway, catalyzes the conversion of cinnamoyl-CoA esters to their respective cinnamaldehydes and is the first enzyme of the monolignol-specific part of the lignin biosynthetic pathway; Cinnamyl alcohol dehydrogenase (CAD) is the last enzyme on the pathway to the monolignols coniferyl and sinapyl alcohols, from which lignins are normally derived (Goujon et al., 2003).

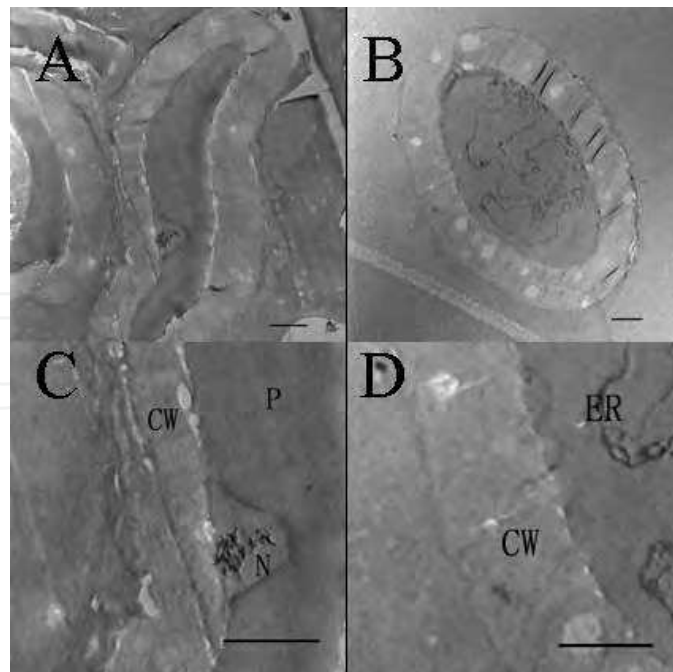


Fig. 12. Effects of the *GhCCR4* on secondary wall thickness of fibers under a transmission electron microscope. A: Check; B: Transgenic; C: Magnif of check; D: Magnif of transgenic; N: Nucleus; P: Pusule; CW: Cell Wall; ER: Endocytosplasmic Reticulum; Bar=2 μ m.

In order to analyze the function of these genes, five *GhCAD* and two *GhCCR* genes were isolated from cotton fiber, and transient expression vector of cotton *GhCCR4* gene was constructed. The transient expression vector pGUS-*GhCCR4* are driven by 35s promoter with *GUS* reporter gene and the target gene, *GUS* reporter gene and target gene simultaneously express in single cell. They were transformed into cotton ovule using PDS-1000/He biolistic particle delivery system. The results indicated that the transient expression vector pGUS-*GhCCR4* could be high efficiency expressed in the epidermal cells of cotton fiber. Transmission electron microscopy demonstrated that the wall thickness of transgenic fiber was increased to 17% of that of the wild type (Fig. 12). These findings suggest that *GhCCR4* could play a critical role in the processes of secondary cell wall formation during fiber development.

4. Cotton fiber chemical component

The cotton fiber wall phenolics were observed by UV induced auto-fluorescence (Leica DMI6000B microscope) with co-observation by scanning electron microscopy (SEM). The washed cotton fiber, especially the cut ends, clearly showed the green-blue auto-fluorescence indicative of wall phenolics (Fig. 13 A). The cotton fiber residues after extraction of lignin-like phenolics by the thioglycolate method showed less green-blue auto-fluorescence (Fig. 13 B). Apparently, the thioglycolate method removed some but not all of the phenolic compounds from the cell walls of the cotton fibers. Notably, the autofluorescence of the remaining residues became lower after saponified phenolics were extracted (Fig. 13 C). Moreover, the secondary layers of cell wall separated and the residual fibers lost their tubular shape (Fig. 13 F).

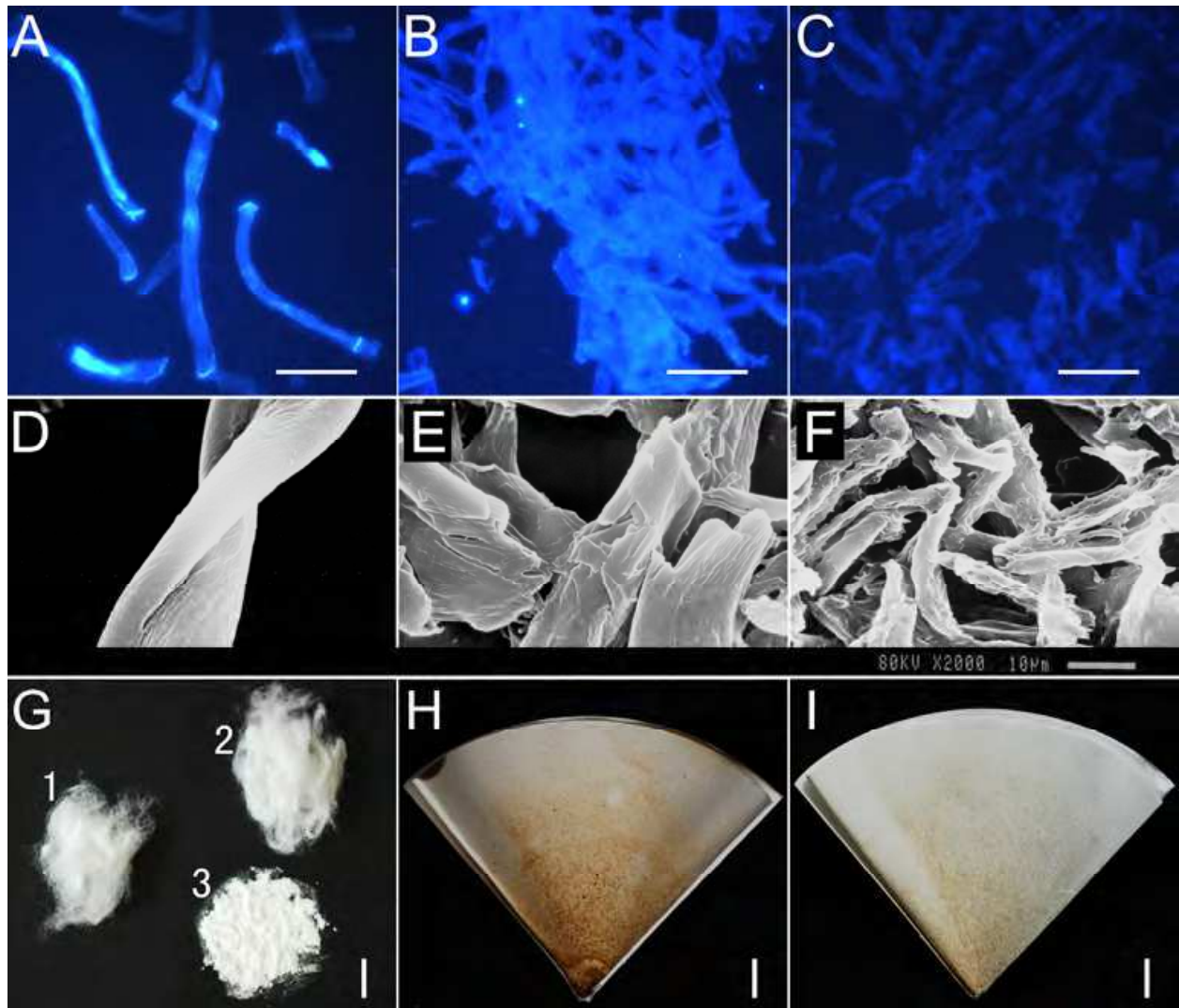


Fig. 13. Microscopic observation of cotton fibers and their residues.

(A) Auto-fluorescence of cut cotton fiber; (B) Auto-fluorescence of the residues left after thioglycollate extraction; (C) Auto-fluorescence of the fiber residues after further extraction of saponifiable phenolics; (D) Scanning electron microscopy (SEM) observation of cotton fiber; (E) SEM observation of the residues left after thioglycollate extraction; (F) SEM observation of the fiber residues after further extraction of saponifiable phenolics; (G) Cotton fiber before washing (1), after washing (2), and residues after thioglycollate extraction (3); (H) The brown insoluble residues left on the filter paper by Klason extracts of cotton fiber; (I) Decreased amount of brown insoluble residue after thioglycollate extraction; Horizontal bars in color figures represent 100 μm , and in black and white figures represent 10 μm . Vertical bars represent 1 cm.

The classical methods of analyzing lignin content including Klason, thioglycolate and acetyl bromide were optimized in order to suit for analyzing cotton fiber. We analyzed the contents of lignin-like phenolics in mature cotton fiber samples using the optimized Klason, thioglycolate, and acetyl bromide methods (Table 2). Although the contents varied between samples and test methods, all of the analyzed samples (14 cultivars and two lines) of cotton fiber contained lignin-like phenolics (Table 2). With the Klason method, 72% H₂SO₄ dissolved away the polysaccharides and acid soluble small molecular phenolics, leaving lignin-like phenolics as an insoluble residue. The brown insoluble residue from cotton fiber clearly showed on the filter paper (Fig. 13 H). The content of Klason phenolics in cotton fiber was relative lower (0.37–1.08%). The thioglycolate method only extracted partial phenolics from cotton fiber (Fig. 13 B,I). There was still some brown residue left after thioglycolate extraction (Fig. 13 I). Therefore, the content of thioglycolate phenolics in cotton fiber was quite low (0.13–0.35%). The acetyl bromide method might reveal both lignin and hydroxycinnamic acids in cotton fibers, since the cotton fibers were completely digested. The content of acetyl bromide phenolics was 2.23–2.63%. The content of lignin-like phenolics in cotton fiber in single boll was kinetically increased with the fiber developing during 20DPA to mature, which had the same trend as the secondary wall thickening by ultra-structure observation (Fig. 14). Compared with lignin accumulation and secondary wall thickness observed by ultra-structure, we found the same increasing trend. The results suggested that monolignol biosynthesis and wall-linked lignin-like phenolics involved in the secondary wall thickening of cotton fibers (Fan et al., 2009).

No.	Sample	Origin	Planting place	Lignin-like phenolics in cotton fiber(%)		
				Thioglycolate	Acetyl bromide	Klason
1	Xinluzao6	China	Manasi,China	0.330±0.006	2.558±0.018	0.902±0.044
2	CJ01	China	Manasi,China	0.252±0.004	2.353±0.004	0.683±0.068
3	Liao3206	China	Manasi,China	0.249±0.009	2.420±0.037	0.468±0.025
4	Tashkent3	Uzekistan	Manasi,China	0.217±0.016	2.578±0.033	0.713±0.013
5	Zhong35	China	Kuche,China	0.172±0.007	2.455±0.032	0.663±0.003
6	Zhong49	China	Kuche,China	0.127±0.010	2.566±0.040	0.675±0.005
7	KK1543	USSR	Kuche,China	0.230±0.008	2.472±0.015	0.783±0.045
8	MC50	USA	Kuche,China	0.347±0.017	2.602±0.017	0.660±0.010
9	CB1135	USA	Kuche,China	0.184±0.008	2.234±0.043	0.703±0.015
10	DP2156	USA	Kuche,China	0.281±0.023	2.633±0.021	0.723±0.038
11	NC33B	USA	Anyang,China	0.340±0.034	2.465±0.025	0.578±0.018
12	NC32B	USA	Anyang,China	0.207±0.012	2.545±0.030	0.738±0.013
13	NC20B	USA	Anyang,China	0.146±0.024	2.578±0.020	0.502±0.019
14	DP99B	USA	Anyang,China	0.190±0.017	2.449±0.030	0.860±0.030
15	DPH37B	Hybrid	Anyang,China	0.197±0.023	2.336±0.020	0.370±0.007
16	Xuzhou142	China	PKU,China	0.169±0.021	2.601±0.058	1.078±0.033

Table 2. The content of lignin-like phenolics in cotton fiber tested by Thioglycolate, Acetyl bromide and Klason methods

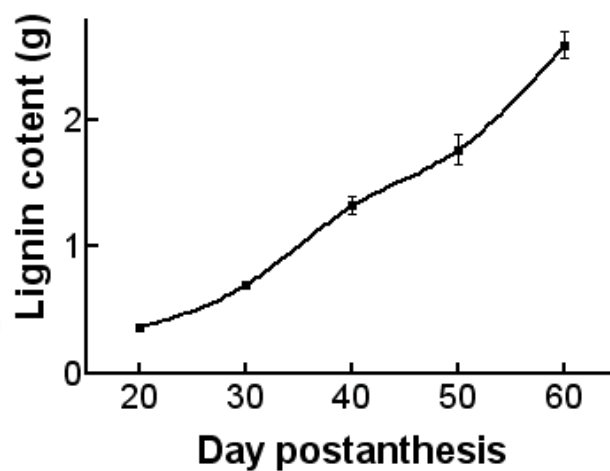


Fig. 14. Kinetics of lignin content of cotton fiber in single boll.

The samples were taken from different fiber developmental stages. The fiber were carefully detached from the seeds. The fiber samples were washed twice with homogenization buffer (50 mmol/L Tris-HCl, 10 g/L Triton X-100, 1 mol/L NaCl; pH8.3), twice with 80% (v/v) acetone and once with acetone as described Niklas(2000). The lignin content of 2.00g of cotton fiber was analysed by Klason method. Values =Means \pm SE, n = 3 measurements.

5. Conclusion

The mature cotton (*G. hirsutum* L.) fiber have long been considered unique in that their thickened secondary cell were thought to consist of nearly pure cellulose. However, we found that secondary wall deposition in cotton fiber and in water-conducting xylem cells shares common elements. RT-PCR analysis showed that genes in the monolignol biosynthesis pathway were expressed in the secondary wall formation of cotton fiber. Sixteen gene complete cDNAs in this pathway had been cloned from developing cotton fibers. Their amino acid sequences had high homology with those corresponding genes from other plants. Some of these genes were predominantly expressed during secondary wall formation in cotton fibers. Chemical analysis confirmed the presence of lignin-like phenolics in mature cotton fiber from germplasm resources in different areas of China, USA, Australia, Russia and Mexico. We concluded that monolignol biosynthesis and wall-linked lignin-like phenolics involved in the secondary wall thickening of cotton fibers.

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This book is devoted to botany and covers topical issues in this diverse area of study. The contributions are designed for researchers, graduate students and professionals. The book also presents reviews of current issues in plant-environment interactions making it useful to environmental scientists as well. The book is organized in three sections. The first section includes contributions on responses to flood stress, tolerance to drought and desiccation, phytotoxicity to Chromium and Lead; the second has aspects of economic botany including a review of Smut disease in sugarcane and properties of plant extract used Tassaboount date juice; the last covers topical issues on morphogenesis and genetics on cotton fiber special cell, secretory glands *Asphodelus aestivus* flower, pollen tube growth in *Leucojum aestivum*, morphological studies of *Ardisia crenata* complex, and hybrid lethality in the Genus *Nicotiana*.

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