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Full Length Article

Accumulation and Structural Unit of Wall-linked Phenolics in the Cotton (*Gossypium hirsutum*) Fiber Secondary Wall

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Abstract

The development of secondary cell wall and final composition affects the quality of mature cotton fiber. The previous studies reported that the biosynthesis of phenolic compounds occur throughout the development of cotton fiber secondary wall. This study investigated the accumulation and structural unit of wall-linked phenolics in the cotton fiber secondary wall. The accumulation of phenolics in cotton fibers within a single boll increased along with the fiber development from 20 days post-anthesis (DPA) to maturity. The method, which the wall-linked phenolics was observed thoroughly by fourier transform infrared spectroscopy (FTIR) after treated by thioglycolic acid (TGA), was established. The result showed that the cell wall phenolics of cotton fiber consisted predominantly of guaiacyl units (G) with some syringyl units (S). The results of derivatization followed by reductive cleavage (DFRC) confirmed G and some S units existed in the cell wall of cotton fiber. The nuclear magnetic resonance (NMR) showed connection ways between G and S were C-C bond of β-5 and β-β, and ether linkage of β-O-4. Gel permeation chromatography (GPC) indicated the average molecular weight (Mw) distribution range of wall-linked phenolics with 1023–2169 g mol^{-1}, while number-average molecular weight (Mn) was 900–1970 g mol^{-1} and the polydispersity coefficient was 1.09–1.74. © 2019 Friends Science Publishers

Keywords: Biochemical composition; Cotton fiber; Guaiacyl unit; Phenolics; Secondary wall; Syringyl unit

Introduction

The development of secondary cell wall and final composition of cotton (*G. hirsutum* L.) fiber can affect the quality of the mature fiber. The deposition of the secondary wall begins at approximately 16 days post-anthesis (DPA), overlapping the phase of primary wall elongation. Rapid cellulose growth and progressive deposition inside the primary wall, then accompanied the secondary wall thickening (Meinert and Delmer, 1977; Kim and Triplett, 2001). It is known that mature cotton fiber predominantly contains cellulose in addition to wax-like substances, pectin, organic acids, mineral ash, and proteins (Basra and Malik, 1984; Wakelyn *et al.*, 2006), yet the extent to which, lignin or phenolic compounds contribute to the composition has remained unclear. In vascular plants, cell wall phenolics (lignin and low-molecular-weight hydroxycinnamic acids) are important components of secondary walls, which intersect cellulose micro fibrils like natural gule, providing mechanical support for plant tissues (Kärkönen and Koutaniemi, 2010; Liu, 2012). The units, come from the monolignols, which include guaiacyl (G), syringyl (S), and p-hydroxyphenyl (H) units when they were combined with the phenolic polymer (Boerjan *et al.*, 2003; Vanholme *et al.*, 2010; Shioya *et al.*, 2016). The process of cell wall growing polymer bounded to phenolics reinforces the cell wall, protects cellulose from chemical and biodegradation, affect wall mechanical strength, plant growth and morphogenesis (Iiyama *et al.*, 1994; Grabber *et al.*, 1997; Boerjan *et al.*, 2003; Fan *et al.*, 2009). Although such phenolics play an important part in plant secondary cell walls, their possible presence and role in the cotton fiber secondary walls has received little attention.

Secondary metabolism pathways in developing cotton fiber were previously considered by Shi *et al.* (2006), Gou *et al.* (2007), Chaudhary *et al.* (2008), Hovav *et al.* (2008) and Yang *et al.* (2008). For example, transcript and metabolite profiles showed seven metabolic pathways, including significant changes ($p < 0.05$) in phenolics (such as lignin) metabolism and phenylalanine metabolism during cotton fiber development (Gou *et al.*, 2007). Another study showed that genes involved in phenylpropanoid metabolism

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gradually increased expression in developmental fiber, while the genes of biosynthesis, chitinase and cellulose synthase activity in primary and secondary cell wall showed over-representation (Hovav *et al.,* 2008). Additionally, several reports have confirmed that phenolics can play an important part of determining the quality of cotton fiber. For instance, genes in the monolignol biosynthesis pathway were revealed to be expressed throughout the formation of cotton fiber secondary wall (Hovav *et al.,* 2008; Fan *et al.,* 2009; Li *et al.,* 2013; Fang *et al.,* 2014; Qin *et al.,* 2017), the expression levels of typical genes of phenylpropanoid were high connected to specific fiber characteristics, which supported the role of phenolics in determining fiber quality (Al-Ghazi *et al.,* 2009). The quality of cotton fiber was affected in essence by lignin/lignin-like phenolics (Han *et al.,* 2013). Moreover, recent research found that in developing cotton fibers, the gene of cinnamate 4-hydroxylases (*C4H*) from cotton were expressed, which catalyze the important process in the phenylpropanoid metabolic pathway (Ni *et al.,* 2014). In summary, the previous studies confirmed that monolignol biosynthesis pathway and phenolic compounds exist in cotton fiber cell wall and play an important role in determining the quality of cotton fiber. However, the phenolic compositions of monolignol units in cotton fiber secondary cell wall remain unknown. This study therefore investigated the accumulation kinetics and composition of wall-linked phenolics in cotton fiber secondary cell wall.

Materials and Methods

Plant Materials

Ten cotton cultivars (*G. hirsutum L.* including ACALA 1517; Junmian 1; TM-1; Xinluzhong 36; Xinluzhong 37; Xuzhou 142; Xinluzao 23; Zhong 35; Xinluzao 36; and Xinluzao 39) were selected for various chemical analysis. All the samples were used in the acid-insoluble phenolics analysis and FTIR spectroscopy. The cotton fiber of TM-1 was used in the experiment of DFRC for the monolignols, FTIR for functional groups related to phenylpropanoid compounds and NMR for the monolignols and their connection ways. The cotton fiber of TM-1, Zhong 35 and Xinluzao 39 were tested in the experiment of GPC for the molecular weight. The materials were planted at the Manasi experiment station in Xinjiang of China, where cotton was grown under plastic film mulching and drip irrigation. During the full flowering stage, the opening flowers were tagged. The cotton bolls were collected on 20, 30, 40, 50 DPA and at maturity based on the tagging date. The boll shells were immediately taken away and the fibers with seeds were covered with aluminum film and put at -20^{o}C. The fibers were carefully detached from seeds immersed in homogenization buffer (Müsel *et al.,* 1997). Additionally, a dried pine branch without peel (pine wood) was ground into powder with an electric grinder.

The pure fiber samples, including the mature fiber samples without any non-fibrous materials and the milled pine wood were rinsed twice with homogenization buffer, then washed twice with 80% acetone, and finally washed once by pure acetone. The residues of lignin extracted by the thioglycolate method were washed three times with distilled water. All washed samples were dried to constant weight at 45°C before further analysis.

Acid-insoluble Phenolics Analysis

The content of acid-insoluble phenolics in the developing (20, 30, 40, 50 DPA and maturity) cotton fiber was measured using the Klason method (Hatfield *et al.,* 1994; Fan *et al.,* 2009). A ϕ15 cm quantitative filter paper was wetted with 3% sulfuric acid, then washed with deionized water until the cleaning solution on the filter paper was neutral and dried to a consistent weight (W_1) in a 60^{o}C oven. A total of 2.00 g of cotton fiber (W_2) was put in a 50 mL reaction tube with 15 mL of 72% H_2SO_4 (maintained at 4°C in advance). The cotton sample was stirred until completely soaked by H_2SO_4 solution. The reaction was kept at room temperature for 2 h. The sample was churned every 10 min to assure thoroughly mixied and saturated during its hydrolysis. The contents were transferred to an Erlenmeyer flask and diluted with distilled water until the concentration of H_2SO_4 reached 3%. The sealed flask was treated with high pressure steam at 121 ± 3°C for 1 h. The hydrolysis solution was cooled at room temperature and percolated with pretreated filter paper. After filtration, the filter paper was cleaned with hot distilled water to remove the residual acid solution on the filter paper, then the paper and the filtrate were dried in an oven at 60°C to a constant weight (W_3). The dried filter papers containing the acid-insoluble phenolics were put in crucibles and heated in a muffle furnace at 575 ± 25°C until the residues (ash) reached a constant weight (W_4). The content of the phenolics was calculated as $(W_3 - W_1 - W_4) / W_2 \times 100\%$.

To obtain acid-insoluble phenolics from the mature fiber for FTIR analysis, the cotton fiber samples were acid-hydrolyzed as above. After cooling the hydrolysis solution, the acid-insoluble phenolics were collected after centrifugation (12,000 rpm, 15 min) and rinsed 3 times with deionized water. At 45°C, the resulting pellets of acid-insoluble phenolics were dried to a constant weight before further analysis.

Thioglycolic Acid (TGA) Treatment

Cotton fibers or milled pine wood (100 mg) were put in a glass vial with screwcap, added 5 mL of 2 *M* HCl and 0.75 mL of TGA (Sigma-Aldrich, T3758), then gentle shook at 98°C in an electric-heated thermostatic water bath for 4 h. After the reaction was completed, the glass vial was cooled on ice. The precipitate was obtained by centrifugation at 12,000 rpm for 15 min and washed three times with distilled

water, and then dried to constant weight at 45°C before FTIR analysis.

Cotton Fiber Residue after Thioglycolate Phenolics Extraction

The thioglycolate phenolics were obtained from cotton fiber treated by the TGA, which extracted two times with 10 mL of 0.5 *M* NaOH and then extracted once with 10 mL of 2 *M* NaOH (shook for 10–12 h in each treatment). The liquid supernatant of the extraction solutions were mixed. 1.5 mL of concentrated HCl was added to the mixture and kept at 4°C for 4 h. The wall-linked phenolics were recovered by centrifugation at 12,000 rpm for 15 min at 4°C. The fiber residue was washed with distilled water for 3 times and then dried at 45°C to constant weight before FTIR analysis.

Each 0.1 g of cotton fiber (TM-1) at different development stages was treated with TGA and 2 *M* HCl. The residue was cleaned by distilled water and dried to constant weight before FTIR test.

FTIR Spectroscopy

Infrared analysis was implemented by using a Thermo Scientific Nicolet iS10 spectrometer equipped with a rapid sampling smart accessory. The prepared powdery samples were uniformly placed on the sampling plate of the diamond crystal mounted in the stainless-steel plate of the Nicolet iS10 FTIR. The pressure tower above the specimen plate was used to exert a steady pressure to samples to ensure good contact between the sample and the ATR crystal and to minimize losses of the infrared beam.

The laboratory in which the measurements were performed was maintained at a relative humidity of 65 ± 2% and a temperature of 24 ± 1°C. FTIR spectra were obtained by scanning the spectrum from 650 cm^{-1} to 4000 cm^{-1} at a spectral resolution of 4 cm^{-1} and took the average value of 32 scans. Clean diamond crystal was scanned before scanning each sample to remove its background interference. Three sub-samples were analyzed from each sample to obtain replicate spectra. Altogether, 30 FTIR spectra were obtained for the 10 cotton varieties, and 9 FTIR spectra were obtained for 3 independent pine wood samples. The spectra were baseline amended and region standardized using the Thermo Scientific OMNIC™ Specta™ software.

Derivatization Followed by Reductive Cleavage (DFRC) Test

The DFRC test for the structural unit of wall-linked phenolics in cotton fiber was adapted from Lu and Ralph (1997). The phenolics were extracted from 500 mg of cotton fiber using the above thioglycolate extraction method. The acidification-recovered pellet was washed and dried. The pellet was put in a glass vial with screwcap and added 5 mL of AcBr stock solution (AcBr: acetic acid, volume ratio 20:80, stable for several weeks). At 50°C, the solution was gently stirred for 3 h. Finally, the solvent was fully vaporized by a rotary evaporator at less than 50°C. The precipitate was dissolved in an acid-reduction medium, which contained dioxane, acetic acid and water (volume ratio of 5:4:1, had been stable for several months). 50 mg of Zinc dust was added to the reaction solution, and stirred continuously for 30 min. After the reaction, the solution was completely transferred to a separatory funnel containing 10 mL of dichloromethane, added 10 mL of saturated NH_4Cl and 0.3 mg of tetracosane as an internal reference. The aqueous phase was regulated by adding 3% HCl till pH<3. The solution in the separatory funnel was turned upside down to blend well, and then the organic phase was separated by the funnel after standing for 5–10 min. The aqueous phase was extracted twice by 5 mL of CH_2Cl_2. The organic phase was mixed and filtered by anhydrous magnesium sulfate to dry the combined CH_2Cl_2 fractions. The filtrate was dried by reduced pressure using a rotary evaporator. The precipitate was acetylated for 40 min with a total of 1.5 mL of CH_2Cl_2 including 0.2 mL of acetic anhydride and 0.2 mL of pyridine. After acetylation, 25 mL of ethanol was added to the reaction and blended. After standing for 30 min, the solvent was evaporated under reduced pressure by a rotary evapator. The acetylated product was washed several times with ethanol until there was no pyridine odor and then the acetylated product was dried for use. At the same time, coniferyl alcohol and sinapyl alcohol were respectively acetylated as the standard substances. The precipitate was used for gas chromatography (GC) quantification. The acetylated products were dissolved in 2 mL of dichloromethane, and 1–2 μL was used for GC analysis. The phenolic monomers were analyzed by GLC (Thermo Finnigan Trace Gc2000). The column was HP-5MS with length 30 m, inner diameter of 0.25 mm and thickness of 0.5 µm. The flame photometric detector (FID) was used. The column temperature raised from 160^{o}C to 280^{o}C at a temperature rise rate of 10°C min^{-1}. Helium flowed at a speed of 1.0 mL min^{-1} as the carrier gas. The inlet temperature was 250°C and the susceptor temperature was 220°C. The combustion gas was hydrogen and the flow rate was 35 mL min^{-1}; Air flowed at a speed at 350 mL min^{-1} as the combustion gas; Nitrogen flowed at a speed of 10 mL min^{-1} as the make-up gas. The split injection was carried out at a flow rate of 10 mL min^{-1} with a separation ratio of 5:1.

Nuclear Magnetic Resonance (NMR) of Phenolics from Cotton Fiber

The method was adapted according to Crestini and Argyropoulos (1997). The cotton fiber of TM-1 was cut into pieces of 1–2 cm. Under the protection of nitrogen atmosphere, the cotton fiber were ball-milled three cycles (each cycle the ball mill ran for 30 min and stopped for 30 min) until the cotton fiber was ground into power in

stainless steel vessels by a ball mill with stainless steel ball bearings. The power of cotton fiber (50 g) was treated to fractionation in 800 mL of dioxane aqueous solution (96:4, V/V) for 24 h. After filtration, the filtrate was collected and steamed to a third of the liquid, naturally evaporated to form a powder and then washed with a dioxane/water mixed solvent, rinsed with ethanol, washed with ether and dried. About 100 mg of phenolic extracted from cotton fiber were placed in 5 mm NMR tube and dissolved in 1 mL of DMSO-d_6.

BRUKER AVANCE III 400 MHz superconducting number magnetic resonance spectrometer (NMR), manufactured by Bruker company of Switzerland, was used in the experiment. The reverse phase fitted with the spectrometer (5 mm ^{1}H and ^{13}C probe) was tested at room temperature (25°C). The solvent was DMSO-d_6 and TMS was internal standard. Standard pulse procedures were used in both NOESY (Nuclear Overhauser Effect Spectroscopy) and HMBC (Heteronuclear Multiple Bond Correlation). The column temperature was controlled at 300.2 K during the experiment. The HMBC two dimensional spectrum width is 4960.317 Hz, the sampling data dot matrix is 4096*128, accumulates 32 times, and the delay time is 1.5s. The NOESY spectrum width is 4485.646 Hz, the sampling data dot matrix is 2048*128, accumulates 8 times, and the delay time is 2 s.

Gel Permeation Chromatography (GPC) of Phenolics from Cotton Fiber

The method was slightly modified according to the methods of Koda *et al.* (2005) and Wu and Argyropoulos (2003). Weighed 6 parts of 5.0 g of cotton fiber were placed in 6 of 100 mL Erlenmeyer flask, respectively. 100 mL of 0.2 M acetic acid buffer and 325 mg cellulase was added to each flask. The cotton fiber was wetted thoroughly and mixed by the glass rod stirring. The flasks were sealed and put the thermostat oscillation tank at 50°C for 60 h. After the reaction, the pH of the reaction solution was adjusted to 2.0 by 2 *M* HCl. The solution was filtered, collected and washed them with dilute HCl (pH 2.0) 2 to 3 times, then with distilled water 2 to 3 times. The cotton fiber was mixed and freeze-dried by freeze-drying machine. The cotton fiber was put in a flask, mixed thoroughly and was refluxed for 2 h at 86°C in 300 mL of dioxane aqeuous solution (85:15, V:V) with 1.25 μL of concentrated HCl. Then the flask was cooled to room temperature. The solution was then filtered and washed 3 times with 200 mL of aqueous solution of dioxane (85:15, V:V). All filtrates were collected, neutralized with $NaHCO_3$, and then distilled to 15–20 mL under reduced pressure at 40°C. A 10-fold volume of cold water was added to the concentrated solution and stirred with a magnetic stirrer. The residues were purified (Lundquist *et al.*, 1977) and dried.

The dried phenolics were placed in a glass test tube, and then 1 mL of acetic anhydride and 1 mL of pyridine were added to the tube. After blending, the mixture was stirred continuously for 72 h at room temperature, and then 5 mL of Methanol/H_2O (1:1, V:V) was added to the acetylation soultion. The solution was mixed and kept at 4°C overnight. The precipitate was separated by centrifugation (4°C, 12,000 rpm, 5 min) and then the residues were collected. The acetylated phenolics were dried.

The molecular weights of the phenolics were performed by GPC with a Waters 1525 liquid chromatography system. The column (300 mm×7.5 mm i.d., 10 μm) used was Agilent PL-gel 5 m MIXED-C with a Waters 2414 ultraviolet UV detector. Acetylated phenolics (2 mg) were dissolved by 2 mL of THF (HPLC grade). The mobile phase was THF and calibrated with polystyrene standards. Flowed at a speed of 1 mL min^{-1} the column temperature reached at 35°C. The injection volume to the chromatographic column was 60–80 μL and the dates were processed by GPC Software.

Results

The Accumulation Kinetics of Wall-linked Phenolics Content in Developing Cotton Fiber

The acid-insoluble wall linked phenolics of cotton fiber from 20 DPA to maturity were analyzed using the Klason method. The accumulation of the acid-insoluble phenolics content during differential stages of cotton fiber was showed in Fig. 1a. The results showed that the percentage of wall-linked phenolics during earlier secondary wall development (20 DPA) was greater than in the mature cotton fibers, but it remained nearly constant from 40 DPA to maturity (Fig. 1a). The acid-insoluble phenolics content per unit boll of cotton fiber can be obtained by multiplying the weight of fiber of single cotton boll. The results showed that with the development of cotton boll, the content of acid-insoluble phenolics in the cotton fibers of single bolls increased progressively from 20 DPA to maturity (Fig. 1b).

Functional Groups Related to Wall-linked Phenolics in Cotton Fiber

FTIR analysis of mature cotton fiber: As accumulation of phenolics in the cotton secondary cell wall occurs, which compared with the FTIR spectra between the mature cotton fiber and the fiber residues after phenolics extraction with the thioglycolate method (Fig. 2). Only a small difference was observed, located approximately 1730 cm^{-1}. No any other significant differences in other aromatic ring regions was observed which showed clear differences located at the wave numbers corresponding to amides (1650 and 1550 cm^{-1}).

Exposure of the secondary wall by TGA treatment: We repeatedly cut the fibers and observed them by scanning electron microscopy (SEM). The fibers remained intact,

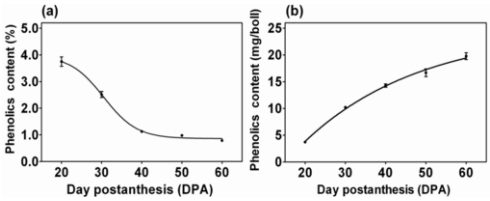

Fig. 1: Kinetic change of phenolic content in developing cotton fiber; **a:** Phenolic content as a percentage of fiber weight; **b:** Phenolic content per cotton boll. Values are means ±SE (n = 10 varieties × 3 replicates)

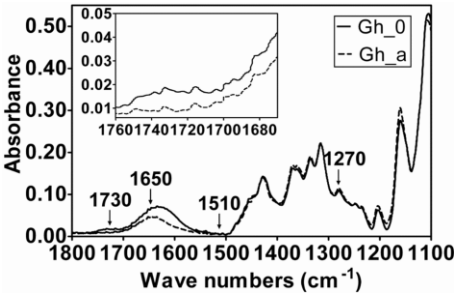

Fig. 2: FTIR spectra of cotton fibers. The solid line is the spectrum of washed and cut cotton fiber, and the dashed line is cotton fiber residues after thioglycolate extraction. Values are means of 30 spectra (n = 10 varieties × 3 replicates)

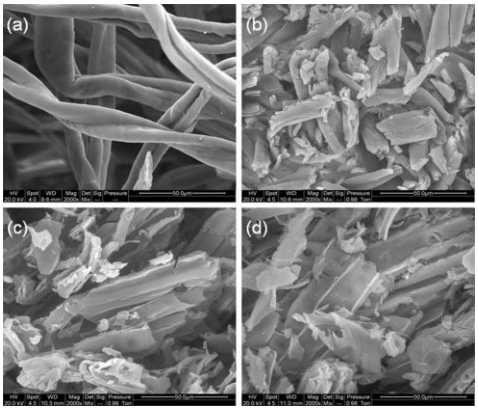

Fig. 3: Scanning electron microscopy observation of the cell wall treated by TGA. **a:** Cut mature cotton fiber; **b:** TGA-treated mature cotton fiber; **c:** Milled powder of pine wood; **d:** TGA-treated milled powder of pine wood. Bar = 50.0 μm

and the secondary walls were not exposed (Fig. 3a). It was noticed that the cotton fiber cell wall could be broken down and the secondary cell wall could be exposed by a TGA treatment. After TGA treatment, even the daily growing circles could be seen (Fig. 3b). To confirm the efficiency of the procedure, we used TGA to treat the ground powder of pine (*Pinus sylvestris*) wood (Fig. 3c), a typical high-lignin-content material. The results showed that the lignified tissue was indeed further broken down and that the secondary cell wall had become much thinner (Fig. 3d).

Compared FTIR analysis of broken cotton fiber and milled pine wood: As the TGA treatment exposed the secondary cell wall, therefore FTIR was used to compare the cotton fibers (Fig. 4a) with the pine wood (Fig. 4c). The comparison between the averaged FTIR spectra (n = 30) of the original fiber and the TGA-treated fiber as well as the pine wood powder and the TGA-treated pine wood powder showed differences in the wave number region of 800–1800 cm^{-1}, especially in the wave number of 950–1180, 1510, 1270, 1310, 1730, 1550 and 1650 cm^{-1} (Fig. 4). The effects of TGA treatment on the absorbance of phenolics and phenolic esters in the secondary walls of cotton fiber and pine wood were clearly revealed by the digital subtraction of the spectral absorbance values for the original fiber and milled pine wood from the spectral absorbance values of the equivalent regions in the TGA-treated fibers and milled pine wood respectively (Fig. 4b and 4d). After subtraction at wave number 1100–1330 cm^{-1}, the positive region in TGA-treated cotton fiber was narrower than the 1100–1530 cm^{-1} in the TGA-treated milled pine wood. The peaks at 1270 and 1310 cm^{-1} corresponded to guaiacyl (G-units) monolignol and syringyl (S-units) monolignol, respectively. Although the typical lignin peak at 1510 cm^{-1} was missing in the TGA-treated cotton fiber, the positive peak at 1730 cm^{-1} both in the cotton fiber and in the milled pine wood corresponded to the phenolic ester. These results revealed that the TGA treatment could successfully break the secondary cell walls of cotton fibers and pine wood thus allowing investigation of the secondary cell wall composition by FTIR spectroscopy.

FTIR analysis of cotton fibers at different developmental stages: TM-1 fibers at different developmental stages were treated by thioacetic acid and the infrared spectrum obtained by FTIR analysis (Fig. 5). The infrared spectral characteristic peak (Fig. 5) and the destination of absorption peak (Table 1) of some structural substances in cotton fibers were determined according to relevant literatures. It was observed that at 20 DPA most of the absorption peaks in the infrared spectrum of the same amount of cotton fiber were higher than the absorption peaks in the subsequent development process (Fig. 5). Especially at 3340, 2920, 1730, 1620, 1510, 1430, 1270 and 1230 cm^{-1}. The absorption peak was higher than other developmental stages, while the absorption peak was slightly lower during this period at 1330 cm^{-1}. The results showed that in the same weight cotton fiber, the content of hydroxyl, carbonyl, methyl and phenolics compounds in cotton fibers at 20 DPA was higher. With the development of cotton fiber, these absorption peaks gradually decreased, the absorption value at 1330 cm^{-1} increased, while the absorption peak at 1230 cm^{-1} decreased.

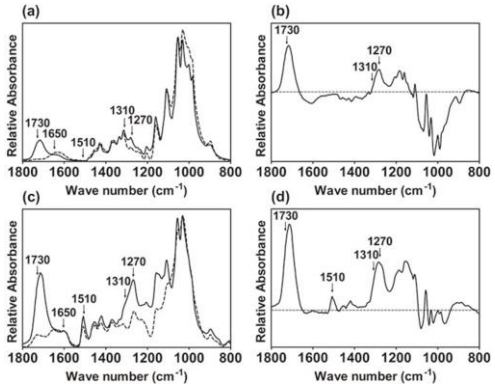

Fig. 4: FTIR spectra of broken cotton fiber and milled powder of pine wood**. a;** FTIR spectra of broken cotton fiber by TGA treatment (solid line) and original cotton fiber (dashed line); **b:** Spectrum differences between the broken cotton fiber and original cotton fiber generated by digital subtraction; **c:** FTIR spectra of broken milled powder of pine wood by TGA treatment (solid line) and milled powder of pine wood (dashed line); **d:** Spectrum differences between the broken milled powder of pine wood after TGA treatment and milled powder of untreated pine wood were generated by digital subtraction. Spectra from A and B are means of 30 spectra (n = 10 varieties × 3 replicates). Spectra from C and D are means of 9 spectra (n = 3 samples × 3 replicates)

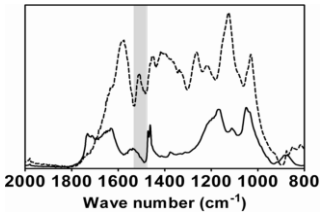

Fig. 5: FTIR spectra of acid insoluble phenolics from cotton fiber (solid line) and lignin standard (dashed line). Spectrum from the phenolics of cotton fiber is the mean of 30 spectra (n = 10 varieties × 3 replicates), and spectrum from the lignin standard is the mean of 3 replicates

FTIR analysis of acid-insoluble phenolics from cotton fibers and a lignin standard: It was shown that the typical lignin peak at 1510 cm^{-1} was missing in TGA treated cotton fibers. To investigate the absence, we compared the FTIR spectra of acid-insoluble phenolics extracted from cotton fiber with a lignin standard (Sigma-Aldrich, T3758). The acid-insoluble phenolics were extracted by using the Klason method. The acid-insoluble phenolics of cotton fiber exhibited absorption in the 900–1800 cm^{-1} wave number region. The spectrum of the acid-insoluble phenolics was similar to the lignin standard. There was a shoulder at 1510 cm^{-1} instead of the typical lignin peak (Fig. 5).

Monomers of Wall-linked Phenolics in Cotton Fiber

The wall-linked phenolics in cotton fibers were extracted with thioglycolic acid and then degraded by DFRC method. The GC analysis results of the products showed that G-lignin monomer in cotton fiber was detected at the retention time of 7.5 min, S-lignin monomer at the retention time of 8.25 min and 9.0 min. The content of G-lignin monomer was higher than of S-lignin monomer (Fig. 6).

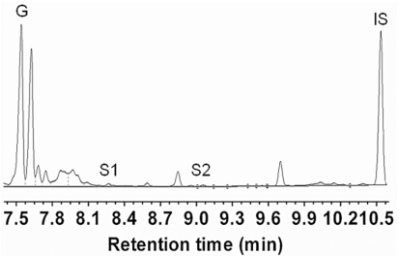

Fig. 6: GC chromatograms of DFRC monomers from cotton fiber. G is the coniferyldiacetate monomer from the guaiacyl unit. S1 and S2 are sinapyldiacetate monomers from the syringyl unit. IS is the internal standard (tetracosane)

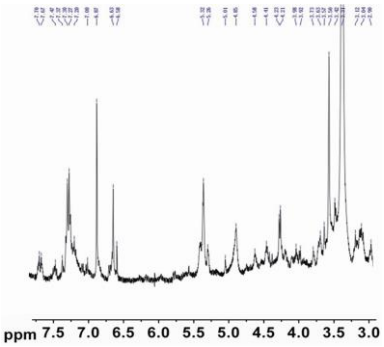

Fig. 7: ^{1}H-NMR spectrum of phenolics from cotton fiber

Monomers of Wall-linked Phenolics and their Interconnected Way in Cotton Fiber

The ^{1}H-NMR spectra of lignin in cotton fibers showed peaks at 7.2 ppm and 6.63 ppm. Therefore, it can be inferred that cotton fiber lignin belonged to guaiacyl-syringyl units (GS-type lignin). The G and S units were interconnected in the following ways: β-5 and β-β structure belonged to carbon-carbon bond, β -O-4 structures belonged to ether bond (Fig. 7 and Table 2). The spectra of NOESY and HMBC further confirmed the wall-linked phenolics in cotton fiber secondary wall were G and S units. The assignment of H on the structural units was determined (Fig. 8).

Relative Molecular Weight of Wall-Linked Phenolics in Cotton Fiber

The response curves of wall-linked phenolics chromatogram detector in different cotton varieties were slightly different (Fig. 9). The average molecular weight distribution ranged from 938 g mol^{-1} to 2169 g mol^{-1}, while the number-average molecular weight distribution ranged from 857 g mol^{-1} to 1970 g mol^{-1}, and the polydispersity coefficient ranged from 1.10 to 1.74 (Table 3).

Table 1: Corresponding relationship of absorption peak from FTIR and composition

Absorbance peak/cm^{-1}	Assignments of characteristic absorption peak	References
3340	O-H stretching	Herrera *et al.* (2018)
2920	stretching varbiation of the C-H group	Curtze *et al.* (2017); Zhao *et al.* (2014); Garg *et al*. (2007)
1730	stretching vibrations in carboxyl groups,acetyl xylan	Xie *et al*. (2000); Silverstein and Webster (1998)
1620	stretching vibration of C=C conjugated with lignin aromatic group	Garg *et al.* (2007)
1510	aromatic skeletal vibration plus C=O stretch in G-units	Antonović *et al.* (2010); Martin *et al.* (2005)
1465	band characteristic of lignin C-H vibration	Rana *et al.* (2010)
1430	stretching vibration of benzene ring skeleton in lignin polymer	Antonović *et al.* (2010); Martin *et al.* (2005)
1330~1320	syringyl	Pandey ,(1999); Jahan and Mun (2009)
1275~1270	Guaiacyl ring breathing	Pandey (1999); Faix (1991); Rana *et al.* (2010)
1230~1220	C-H stretching vibration of syringal aromatic rings	Pandey (1999)
1140	vibrations characteristic for the guaiacyl unit	Rana *et al.* (2010)

Table 2: ^{1}H-NMR absorption and main assignments of acetylated lignin obtained from cotton fiber

Chemical shift range/ppm	Maximum value/ppm	Main assignments
7.2~6.8	7.2	Aromatic ring protons on guaiacyl units
6.8~6.1	6.63	Aromatic ring protons on guaiacyl unit
5.5~5.2	5.32	$H_\alpha$of the β-5 structure
4.9~4.7	4.85	H_α of β-β structure and H of residual xylan
4.5~4.2	4.41	H_γ of β-O-4 structure
4.2~3.0	3.98	H of methoxy group and H_α of several structures

Table 3: MolecularWeight and Distribution of wall-linked phenolics of different cotton varieties

Samples	Molecular weight (g mol^{-1})	No. acreage molecular weight(g mol^{-1})	Polydispersity coefficient
TM-1	2169	1970	1.10
Xinluzao 39	1782	1018	1.74
Xinluzhong 60	938	857	1.09
Zhongmian 35	1023	900	1.13

Discussion

Transcriptome analyses have previously found that key genes coding ingredient of the phenylpropanoid pathway were expressed in the cells of developing fiber (Gou *et al.*, 2007; Hovav *et al.*, 2008; Al-Ghazi *et al.*, 2009; Padmalatha *et al.*, 2012; Hu *et al.*, 2014; Ni *et al.*, 2014). The *CAD* genes, one of important enzymes in the final monolignol biosynthesis of phenypropanoid metabolic path, which was highly expressed in the stage of the secondary cell wall synthesis (Fan *et al.*, 2009). However, it was necessary to experimentally determine the final products derived from the transcriptome and gene expression. Our results confirmed the gradual accumulation of wall-linked phenolics in the developmental cotton fibers (Fig. 1). It is well known that the cellulose deposits very fast in the secondary cell wall development (Meinert and Delmer, 1977; Haigler *et al.*, 2012). It was found that the fiber weight per boll increased with the growth of cotton fiber. The content of acid-insoluble phenolics was increasing consistent with the development trend of the secondary wall of cotton fiber cell wall.

FTIR is a simple and fast technique to analyze cell wall composition and inferring cross-link structures. It can identify macromolecules and functional components without destroying their structures and provide abundant structural component information (Chen *et al.*, 1998; Largo-Gosens *et al.*, 2014). It also can be used for evaluating cotton fiber (Abidi *et al.*, 2010). The FTIR spectra obtained in the study without TGA treatment were quite consistent with the results by Fan *et al.* (2009) and Abidi *et al.* (2010), which did not show thoroughly the absorption peaks of phenolics. No difference was found in the region of 1200–1320 cm^{-1} between the original fiber and residue following the thioglycolate method extraction for phenolic extraction. Based on SEM observations and the FTIR results, what we observed by FTIR without TGA treatment might only be the cotton fiber surface. The results showed that the accuracy of cell wall structure component detection of cotton fiber was affected by the primary wall coating, and the depth of the cell wall detected by the FTIR scan was limited. The cross-linked chemical bonds between plant cell walls could be broken by thioglycolic acid under acidic heating conditions, resulting in the breakage of plant cell walls and the exposure of secondary walls. It also overcomes the limitation that FTIR method could not be directly applied to plant fiber samples which difficult to be pulverized, thus ensuring the accuracy of FTIR detection. The absorption bands of guaiacyl nucleus and syringyl nucleus are 1270 and 1230 cm^{-1} respectively (Séné *et al.*, 1994; Fan *et al.*, 2006; Rana *et al.*, 2010; Kerr *et al.*, 2013). The ratio of A_{1270}/A_{1230} was directly related to the relative content ratio of guaiacyl and syringyl in lignin. Higher the ratio, the higher the content of guaiacyl (Chen and McClure, 2000). According to the intensity ratio of the absorption peak of mature cotton fiber at 1270 and 1230 cm^{-1}

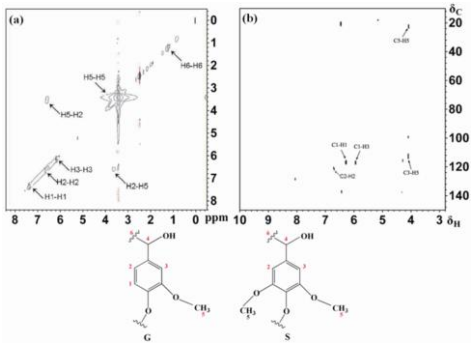

Fig. 8: 2D NMR spectrum of phenolics from cotton fiber. **a:** NOESY spectrum; **b:** HMBC spectrum. G is the coniferyldiacetate monomer from the guaiacyl unit. S is sinapyldiacetate monomers from the syringyl unit

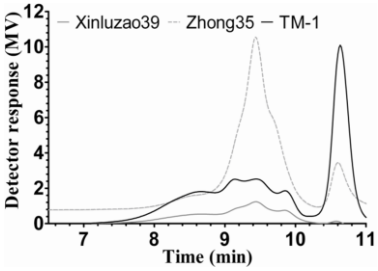

Fig. 9: GPC profiles of phenolics from TM-1, Zhong 35 and Xinluzao 39 cotton fiber. GPC spectra of phenolics from TM-1 (black solid line), Zhong 35 (gray dashed line) and Xinluzao 39 (gray solid line)

(A1270/A1230=1.69), it can be inferred that the type of lignin in cotton fiber is guaiacyl-syringyl lignin (G-S), and the relative content of guaiacyl is more than syringyl. The results of the FTIR analysis of the TM-1 fibers at different developmental stages were consistent with the previous results of the accumulation kinetics. Different studies have indicated that phenolics are synthesized and are present in cotton fibers (Fan *et al.*, 2009; Han *et al.*, 2013; Feng *et al.*, 2018). It is known that all types of recognizable structural units in lignin are formed through oxidative coupling reactions of p-hydroxycinnamyl alcohol, coniferyl alcohol, and sinapyl alcohol (Campbell and Sederoff, 1996; Grabber *et al.*, 1997; Boerjan *et al.*, 2003). The cellulose content of cotton fiber is more than 90%, while the content of wall-linked phenolic is low. The peak of structural units is masked by the interference of cellulose and other polysaccharides when wall-linked phenolic in cotton fiber was directly analyzed by DFRC method. It was necessary to exclude interference from other structural substances such as cellulose in the cotton fiber. The wall-linked phenolic are often combined with cellulose or hemicellulose in the form of chemical bonds in plants, which makes it difficult to be analyzed and extracted. The physical properties of wall-linked phenolic are related to the plant species, structure, location, growth period and other factors, as well as their separation and extraction methods. There are many methods for extracting wall-linked phenolic. Through literature search and analysis, it was found that the wall-linked phenolic, extracted by thioglycolic acid method, don't contain polyphenols and proteins and without condensation reaction. Meanwhile, wall-linked phenolic are soluble in alkaline solution and can be recovered as a precipitate after acidification with concentrated hydrochloric acid (Hatfield and Fukushima, 2005). As a robust new method for lignin analysis, the DFRC method can selectively and efficiently break the α-aryl ether bond and *β*-ether bond without any change in the methoxy group, releasing analyzable monomers. The reaction conditions are mild and the condensation reaction is very few, which provides a guarantee for further structural analysis (Lu and Ralph, 1997). Therefore, wall-linked phenolic in cotton fiber was extracted by thioglycolic acid method. The wall-linked phenolic was washed twice with 5 mL of distilled water and then dried and ground into powder. And then it was analyzed by DFRC method. The guaiacyl structural unit (G-unit) and syringyl structural unit (S-unit) were successfully detected by the method. The results were consistent with the previous FTIR results. Similarly, the typical lignin fractions of cotton stalks show a characteristic G-S lignin composition made up of predominantly 59% of G-units and significant number of 40% of S-units (Kang *et al.*, 2012). The monolignol unit is in accordance with the gene expression of monolignol biosynthesis in developing cotton fiber (Gou *et al.*, 2007; Hovav *et al.*, 2008; Al-Ghazi *et al.*, 2009; Fan *et al.*, 2009). By contrast, the bast fibers from flax (*Linum usitatissimum*), ramie (*Boehmeria nivea*), kenaf (*Hibiscus cannabinus*), and hemp (*Cannabis sativa*) generally contain a small amounts of lignin (0.5–13%) and monolignols (Neutelings, 2011).

In this study, cotton fibers were ball-milled into powder by ball mill, and then lignin was extracted by using dioxane method. The results showed that cotton fibers were guaiacyl-syringyl lignin (G-S lignin), which was consistent with the results of DFRC analysis and FTIR analysis of lignin structural monomer. The guaiacyl units and syringyl units were interconnected by carbon-carbon bond β-5 structure and β-β structure, and ether bond β-O-4 structure. The β-β and β-O-4 belonged to a non-condensed structure, while the β-5 belonged to a condensed structure. The condensed β-5 lignin structure in cotton fibers increases the complexity of its structure, causing cotton fiber to be difficult to degrade. The results of the 2D NMR again that lignin monomer structure in cotton fiber were composed of G and S unit.

The phenolics in the cotton fibers appeared to be different from typical lignin. First, the FTIR spectra of the acid-insoluble phenolics from the cotton fiber were different from the lignin standard, even though they possessed the same absorption region (Fig. 5). Second, the spectra of the phenolics in the cotton fiber secondary walls differed from those of typical lignin in pine wood treated by the TGA. In particular, the typical lignin absorbance band at 1510 cm^{-1} was missing in the spectrum of the cotton fiber (Fig. 4). We speculate on two possibilities: (1) The phenolics in the

secondary walls of the cotton fibers contain their own unique molecular structure and cross-linking with cellulose that is not yet clear. Thus, some research has shown that the carbohydrate matrix affects the lignin structure at a macromolecular level (Kärkönen and Koutaniemi, 2010). In fact, the soft, white secondary wall of cotton fiber may not be analogous to the hard, brown secondary wall of pine wood or cotton stalk. It is already known that the quality of lignin is the main reasons for the major differences in the xylem cell walls and bast fiber cells in flax (Neutelings, 2011). (2) The TGA treatment might partially degrade the structure of the phenolics from the cotton fiber because there was a shoulder at 1510 cm^{-1} in the spectra of the acid-insoluble phenolics extracted from mature cotton fibers (Fig. 5). The wave number of the shoulder ranged from approximately 1530 to 1480 cm^{-1}, which was identified to stretching vibration absorption of aromatic groups and lignin (Kerr *et al.*, 2013; Cao *et al.*, 2015). (3) The results of molecular weight distribution of wall-linked phenolics also showed that the phenolics in cotton fiber was different from that in other lignified plant cell walls. The molecular weight distribution of wall-linked phenolics in cotton fiber had its own unique characteristics: the molecular weight distribution was narrow. The molecular weight of wall-linked phenolics in cotton fiber was lower than other plants (Tolbert *et al.*, 2014). The molecular weight distribution of these phenolics in cotton fiber was narrow, which was similar to that in cotton stalk, but the polydispersity coefficient was slightly lower than that of cotton stalk (Kang *et al.*, 2012). Due to the complexity of their composition and structure, the polymeric structures of these phenolics and even of the lignin have not yet been completely revealed. Their compositions of these phenolics are different in plant group, cell type, and single cellular layers (Campbell and Sederoff, 1996).

Conclusion

In summary, this study advanced our understanding on the cell walls of cotton fibers on three aspects: 1) Accumulation of phenolics in a single boll increased along with the cotton fiber development from 20 days post-anthesis to maturity; 2) cotton fibers can be broken and exposed by thioglycolic acid treatment, and the wall-linked phenolics can be observed by fourier transform infrared spectroscopyand 3) monolignol was the structural units of the wall-linked phenolics in the cotton fiber secondary wall , but the phenolics in the cotton fibers appeared to be different from those of typical lignin. Cell wall phenolics of cotton fiber consisted predominantly of guaiacyl units (G) with some syringyl units (S). The connection ways between G and S were C-C bond of β-5 and β-β, and ether linkage of β-O-4. The average molecular weight (Mw) distribution range of wall-linked phenolics was 1023-2169 g mol^{-1}, while number-average molecular weight (Mn) was 900–1970 g mol^{-1} and the polydispersity coefficient was 1.09–1.74. Our findings might be important to agriculture, cotton processing and textile industries.

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References

Abidi, N., L. Cabrales and E. Hequet, 2010. Fourier transform infrared spectroscopic approach to the study of the secondary cell wall development in cotton fiber. *Cellulose*, 17: 309–320

Al-Ghazi, Y, S. Bourot, T. Arioli, E.S. Dennis and D.J. Llewellyn, 2009. Transcript profiling during fiber development identifies pathways in secondary metabolism and cell wall structure that may contribute to cotton fiber quality. *Plant Cell Physiol*., 50: 1364–1381

Antonović, A., V. Jambreković, J. Franjić, N. Španić, S. Pervan, J. Ištvanić and A. Bublić, 2010. Influence of sampling location on content and chemical composition of the beech native lignin (*Fagus sylvatica* L.). *Period. Biolog*., 112: 327–332

Basra, A.S. and C.P. Malik, 1984. Development of the cotton fiber. *Intl. Rev. Cytol*., 89: 65–113

Boerjan, W., J. Ralph and M. Baucher, 2003. Lignin biosynthesis. *Annu. Rev. Plant Biol*., 54: 519–546

Campbell, M.M. and R.R. Sederoff, 1996. Variation in lignin content and composition. mechanisms of control and implications for the genetic improvement of plants. *Plant Physiol.*, 110: 3–13

Cao, C., Z. Yang, L. Han, X. Jiang and G. Ji, 2015. Study on in situ analysis of cellulose, hemicelluloses and lignin distribution linked to tissue structure of crop stalk internodal transverse section based on FTIR micro spectroscopic imaging. *Cellulose*, 22: 139–149

Chaudhary, B., R. Hovav, R. Rapp, N. Verma, J.A. Udall and J.F. Wendel, 2008. Global analysis of gene expression in cotton fibers from wild and domesticated *Gossypium barbadense*. *Evol. Dev*., 10: 567–582

Chen, L., N.C. Carpita, W.D. Reiter, R.H. Wilson, C. Jeffries and M.C. McCann, 1998. A rapid method to screen for cell-wall mutants using discriminant analysis of Fourier transform infrared spectra. *Plant J*., 16: 385–392

Chen, M.H. and J.W. McClure, 2000. Altered lignin composition in phenylalanine ammonia-lyase-inhibited radish seedings: implications for seed-derived sinapoyl esters as lignin precursors. *Phytochemistry*, 53: 365–370

Crestini, C. and D.S. Argyropoulos, 1997. Structural analysis of wheat straw lignin by quantitative 31P and 2D NMR spectroscopy. The occurrence of ester bonds and α-O-4 substructures. *J. Agric. Food Chem.*, 45: 1212–1219

Curtze, J.H., F. Bär, J. Steffan, T. Dörr and H. Vogel, 2017. Hydrothermal carbonization of lignin with vanillin as a model component. *Chem. Eng. Technol.*, 40(6): 1190–1195

Faix, O., 1991. Classification of lignins from different botanical origins by FT-IR spectroscopy. *Holzforschung*, 45: 21–27

Fan, L., W.J. Shi, W.R. Hu, X.Y. Hao, D.M. Wang, H. Yuan and H.Y. Yan, 2009. Molecular and biochemical evidence for phenylpropanoid synthesis and presence of wall-linked phenolics in cotton fibers. *J. Integr. Plant Biol*., 5: 626–637

Fan, L., R. Linker, S. Gepstein, E. Tanimoto, R. Yamamoto and P.M. Neumann, 2006. Progressive inhibition by water deficit of cell wall extensibility and growth along the elongation zone of maize roots is related to increased lignin metabolism and progressive stelar accumulation of wall phenolics. *Plant Physiol.*, 140: 603–612

Fang, L., R.P. Tian, J.D. Chen, S. Wang, X.H. Li, P. Wang and T.Z. Zhang, 2014. Transcriptomic analysis of fiber strength in upland cotton chromosome introgression lines carrying different *Gossypium barbadense* chromosomal segments. *Plos One*, 4: e94642

Feng, H., X. Li, H. Chen, J. Deng, C. Zhang, J. Liu, T. Wang, X. Zhang and J. Dong, 2018. GhHUB2, a ubiquitin ligase, is involved in cotton fiber development *via* the ubiquitin-26S proteasome pathway. *J. Exp. Bot.*, 69: 5059–5075

Gou, J.Y., L.J. Wang, S.P. Chen, W.L. Hu and X.Y. Chen, 2007. Gene expression and metabolite profiles of cotton fiber during cell elongation and secondary cell wall synthesis. *Cell Res.*, 17: 422–434

Garg, U.K., M.P. Kaur, V.K. Garg and D. Sud, 2007. Removal of hexavalent chromium from aqueous solution by agricultural waste biomass. *J. Hazard. Mater*., 140: 60–68

Grabber, J.H., J. Ralph, R.D. Hatfield and S. Quideau, 1997. p-hydroxyphenyl, guaiacyl, and syringyllignins have similar inhibitory effects on wall degradability. *J. Agric. Food Chem.*, 45: 2530–2532

Haigler, C.H., L. Betancur, M.R. Stiff and J.R. Tuttle, 2012. Cotton fiber: a powerful single-cell model for cell wall and cellulose research. *Front. Plant Sci.*, 3: 1–7

Han, L.B., Y.B. Li, H.Y. Wang, X.M. Wu, C.L. Li, M. Luo, S.J. Wu, Z.S. Kong, Y. Pei, G.L. Jiao and G.X. Xia, 2013. The dual functions of WLIM1a in cell elongation and secondary wall formation in developing cotton fibers. *Plant Cell*, 25: 4421–4438

Herrera, M., K. Thitiwutthisakul, X. Yang, P. Rujitanaroj, R. Rojas, and L. Berglund, 2018. Preparation and evaluation of high-lignin content cellulose nanofibrils from eucalyptus pulp. *Cellulose*, 25: 3121-3133

Hatfield, R. and R.S. Fukushima, 2005. Can lignin be accurately measured? *Crop Sci.*, 45: 832–839

Hatfield, R.D., H.G. Jung, J. Ralph, D.R. Buxton and P.J. Weimer, 1994. A comparison of the insoluble residues produced by the Klason lignin and acid detergent lignin procedures. *J. Sci. Food Agric.*, 65: 51–58

Hovav, R., J.A. Udall, E. Hovav, R. Rapp, L. Flagel and J.F. Wendel, 2008. A majority of cotton genes are expressed in single-celled fiber. *Planta*, 227: 319–329

Hu, G., J. Koh, M.J. Yoo, D. Pathak, S. Chen and J.F. Wendel, 2014. Proteomics profiling of fiber development and domestication in upland cotton (*Gossypium hirsutum* L.). *Planta*, 240: 1237–1251

Iiyama, K., T.B.T. Lam and B.A. Stone, 1994. Covalent cross-links in the cell wall. *Plant Physiol.*, 104: 315–320

Jahan, M.S. and S.P. Mun, 2009. Isolation and characterization of lignin from tropical and temperate hardwood. *Bangl. J. Sci. Ind. Res*., 44: 271–280

Kang, S., L. Xiao, L. Meng, X. Zhang and R. Sun, 2012. Isolation and structural characterization of lignin from cotton stalk treated in an ammonia hydrothermal system. *Intl. J. Mol. Sci.*, 13: 15209–15226

Kärkönen, A. and S. Koutaniemi, 2010. Lignin biosynthesis studies in plant tissue cultures. *J. Integr. Plant Biol.*, 52: 176–185

Kerr, J.L., D.S. Baldwin, M.J. Tobin, L. Puskar, P. Kappen, G.N. Rees and E. Silvester, 2013. High spatial resolution infrared micro-spectroscopy reveals the mechanism of leaf lignin decomposition by aquatic fungi. *Plos One*, 8: e60857

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

Koda, K., A. Gaspar and D.S. Argyropoulos, 2005. Molecular weight-functional group relations in softwood residual kraft lignins. *Holzforschung*, 59: 612–619

Largo-Gosens, A., M. Hernández-Altamirano, L. García-Calvo, A. Alonso-Simón, J. Álvarez and J.L. Acebes, 2014. Fourier transform mid infrared spectroscopy applications for monitoring the structural plasticity of plant cell walls. *Front. Plant Sci.*, 5: 1–13

Li, X., D. Yuan, J. Zhang, Z. Lin and X. Zhang, 2013. Genetic mapping and characteristics of genes specifically or preferentially expressed during fiber development in cotton. *Plos One*, 8: e54444

Liu, C.J., 2012. Deciphering the enigma of lignification: precursor transport, oxidation, and the topochemistry of lignin assembly. *Mol. Plant*, 5: 304–317

Lu, F. and J. Ralph, 1997. Derivatization followed by reductive cleavage (DFRC method), a new method for lignin analysis: protocol for analysis of DFRC monomers. *J. Agric. Food Chem.*, 45: 2590–2592

Lundquist, K., B. Ohlsson and R. Simonson, 1977. Isolation of lignin by means of liquid-liquid extraction. *Sven. Pappers.*, 80: 143–144

Martin, J.A., A. Solla, S. Woodward and L. Gil, 2005. Fourier transform-infrared spectroscopy as a new method for evaluating host resistance in the Dutch elm disease complex. *Tree Physiol.*, 25: 1331–1338

Meinert, M.C. and D.P. Delmer, 1977. Changes in biochemical composition of the cell wall of the cotton fiber during development. *Plant Physiol.*, 59: 1088–1097

Müsel, G., T. Schindler, R. Bergfeld, K. Ruel, G. Jacquet, C. Lapierre, V. Speth and P. Schopfer, 1997. Structure and distribution of lignin in primary and secondary cell walls of maize coleoptiles analyzed by chemical and immunological probes. *Planta*, 201: 146–159

Neutelings, G., 2011. Lignin variability in plant cell walls: contribution of new models. *Plant Sci.*, 181: 379–386

Ni, Z.Y., B. Li, M.P. Neumann, M. Lü and L. Fan, 2014. Isolation and expression analysis of two genes encoding cinnamate 4-hydroxylase from cotton (*Gossypium hirsutum*). *J. Integr. Agric.*, 13: 2102–2112

Padmalatha, K.V., G. Dhandapani, K. Mogilicherla, S. Kumar, A. Dass, D.P. Patil, V. Rajamani, K. Kumar, R. Pathak, B. Rawat, S. Leelavathi, P.S. Reddy, N. Jain, K. Powar, V. Hiremath, I. Katageri, M. Reddy, A. Solanke, V.S. Reddy and P.A. Kumar, 2012. Genome-wide transcriptomic analysis of cotton under drought stress reveal significant down-regulation of genes and pathways involved in fiber elongation and up-regulation of defense responsive genes. *Plant Mol. Biol.*, 78: 223–246

Pandey, K.K., 1999. A study of chemical structure of soft and hardwood and wood polymers by FTIR spectroscopy. *J. Appl. Polym. Sci.*, 71: 1969–1975

Qin, Y, H. Wei, H. Sun, P. Hao, H. Wang, J. Su, and S. Yu, 2017. Proteomic analysis of differences in fiber development between wild and cultivated *Gossypium hirsutum* L. *J. Proteom. Res.*, 16: 2811–2824

Rana, R., R. Langenfeld-Heyser, R. Finkeldey and A. Polle, 2010. FTIR spectroscopy, chemical and histochemicalcharacterisation of wood and lignin of five tropical timber wood species of the family of Dipterocarpaceae. *Wood Sci. Technol.*, 44: 225–242

Séné, C.F.B., M.C. McCann, R.H. Wilson and R. Grinter, 1994. Fourier-transform raman and fourier-transform infrared spectroscopy: an investigation of five higher plant cell walls and their components. *Plant Physiol.*, 106: 1623–1631

Shi, Y.H., S.W. Zhu, X.Z. Mao, J.X. Feng, Y.M. Qin, L. Zhang, J. Cheng, L.P. Wei, Z.Y. Wang and Y.X. Zhu, 2006. Transcriptome profiling, molecular biological, and physiological studies reveal a major role for ethylene in cotton fiber cell elongation. *Plant Cell*, 18: 651–664

Shioya, T., T. Akiyama, T. Yokoyama and Y. Matsumoto, 2016. Formation rate of benzyl cation intermediate from p-Hydroxyphenyl, guaiacyl, or syringyl nucleus in acidolysis of lignin. *J. Wood Chem. Technol.*, 37: 75–86

Silverstein, R.M. and F.X. Webster, 1998. Infrared spectroscopy. *In: Spectrometry Identification of Organic Compounds*, 6th edition, pp: 71–143. John Wiley and Sons, New York, USA

Tolbert, A., H. Akinosho, R. Khunsupat, A.K. Naskar and A.J. Regauskas, 2014. Characterization and analysis of the molecular weight of lignin for biorefining studies. *Biofuels Bioprod. Biorefin.*, 8: 836 56–856

Vanholme, R., B. Demedts, K. Morreel, J. Ralph and W. Boerjan, 2010. Lignin biosynthesis and structure. *Plant Physiol.*, 153: 895–905

Wakelyn, P.J., N.R. Bertoniere, A.D. French, D.P. Thibodeaux, B.A. Triplett, M.A. Rousselle, W.R. Goynes, J.V. Edwards, L. Hunter, D.D. McAlister and G.R. Gamble, 2006. *Cotton Fiber Chemistry and Technology*, 1st edition. CRC Press, New York, USA

Wu, S. and D.S. Argyropoulos, 2003. An improved method for isolating lignin in high yield and purity. *J. Pulp Pap. Sci.*, 29: 235–240

Xie, Y.M., S. Yasuda, H. Wu and H. Liu, 2000. Analysis of the structure of lignin-carbohydrate complexes by the specific 13C tracer method. *J. Wood Sci.*, 46: 130–136

Yang, Y.W., S.M. Bian, Y. Yao and J.Y. Liu, 2008. Comparative proteomic analysis provides new insights into the fiber elongating process in cotton. *J. Proteom. Res.*, 7: 4623–4637

Zhao J., X.W. Wang, J. Hu, Q. Liu, D. Shen and R. Xiao, 2014. Thermal degradation of softwood lignin and hardwood lignin by TG-FTIR and Py-GC/MS. *Polym. Degrad .Stabil.*, 108: 133–138

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