DIRECT, NON-DESTRUCTIVE, AND RAPID EVALUATION OF DEVELOPMENTAL COTTON FIBERS BY ATR FT-IR SPECTROSCOPY

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Abstract

Chemical, compositional, and structural differences within the fibers at different growth stages have been investigated considerably through a number of methodologies. Due to its direct, non-destructive, and rapid attribute, this study reports the utilization of attenuated total reflection Fourier transform Infrared (ATR FT-IR) spectroscopy technique to acquire compositional and structural information of fibers grown in planta and in culture. Principal component analysis (PCA) approach suggested approximately 10-day slower in the transition from primary to secondary cell wall biosyntheses for the fibers grown in culture than for the fibers grown in planta. Relatively, the IR crystallinity index is of interest, as it can detect the slight difference in fibers with day of post anthesis (DPA) larger than 25 days. Furthermore, spectral intensities of two bands at 708 and 730 cm^{-1} utilized in the ratio algorithm development indicated differing patterns between two types of cotton fibers.

Introduction

Cotton fiber is formed from the protodermal cells on the outer integument layer of a fertilized cotton seed. Its growth requires approximately 1.5 ~ 2 month period to produce mature fibers in the field. In general, this development is considered to include at least four overlapping but distinctive phases: initiation, primary cell wall formation (elongation), secondary cell wall thickening (cellulose biosynthesis), and maturation (Gordon & Hsieh, 2007; Kim, 2015). Such a growth phase occurs along with a number of significant changes in fiber chemical compositions and structures.

Chemical compositions of cotton fiber cell walls during the development have been extensively analyzed by a set of extraction, separation, and isolation steps, followed by chemical and instrumental determination of targeted components (Abidi et al., 2010a; Huwyler et al., 1979; Kim et al., 2013; Meinert & Delmer, 1977). Undoubtedly, analysis of cellulose and non-cellulose components in cotton fiber through extraction and separation steps is classical. However, it has some degree of concerns because cotton cellulose is not easily dissolved in solvents and also the dissolving process could modify the original molecular structures of complicated components in cotton fibers. In addition, the extraction and separation process encounters significant limitations that include the tedious procedures of optimal solvent and temperature selection as well as extracted specimen identification.

Cellulose and non-cellulose components in cottons have been simultaneously identified by a number of available analytical methods, including attenuated total reflection (ATR) sampling device based Fourier transform infrared (ATR FT-IR) spectroscopy, differential scanning calorimeter (DSC), thermogravimetric analysis (TGA), and pyrolysis-gas chromatography/mass spectroscopy (GC/MS) methods (Abidi et al., 2010b; Abidi et al., 2014; Gordon & Hsieh, 2007; Hartzell-Lawson & Hsieh, 2000). These measurements provided clear evidence of specific non-cellulosic components in developing cotton fibers through the onset of secondary cell wall synthesis. Among the techniques, ATR FT-IR spectroscopy has some merits as it requires minimal sample preparation, permits routine analysis on any stages of fiber growth and is easy to operate.

To extract useful information from ATR FT-IR spectra, approaches to include the calculation of three-band ratios and the adoption of principal component analysis (PCA) have been explored (Abidi et al., 2010b; Abidi et al., 2014; Liu & Kim, 2015; Liu et al., 2011 & 2012; Santiago & Hinchliffe, 2015). The results indicated that both simple algorithms and PCA pattern can be used to describe the transition from primary to secondary cell wall biosyntheses.

The main objective of this study was to compare chemical, compositional, and structural differences between the fibers grown in planta (field) and in culture, from the PCA characterization, IR cellulose crystallinity index (CI_{IR}) assessment, and direct spectral intensity examination on respective ATR FT-IR spectra. The cotton ovule culture method was taken as an important option for cotton researches in the laboratories (Kim & Triplett, 2001).

Materials and Methods

Planta and Culture Cotton Fibers

In 2011, cotton plants (*Gossypium hirsutum* L. TM-1) were grown in the field of USDA-ARS-SRRC (New Orleans, LA). Cotton flowers were tagged at day of post anthesis (DPA). Cotton bolls were harvested at 10, 17, 24, 28, 33, and 37 DPA. These planta fibers at each DPA were collected by manually removing the seeds, prior to dry in 40°C incubator. The soil type was Aquent dredged over alluvium in an elevated location to provide adequate drainage.

At flowering day (0 DPA), unfertilized cotton ovules from TM-1 variety harvested from the cotton field were cultured on Beasley and Ting (BT) medium containing 0.5 µM GA and 5.0 µM NAA (Beasley & Ting, 1974). The cultures were kept in a dark environment at 30°C in a 5% CO_2 atmosphere. The cultured ovules at different developmental stages (16, 20, 24, 28, and 38 DPA) were harvested, manually ginned, and dried for further analyses. Both planta and culture fibers were well conditioned at a constant relative humidity of 65 ± 2% and temperature of 21 ± 1°C for at least 48 hours prior to spectral measurement.

ATR FT-IR Spectral Collection and Interpretation

An FTS 3000MX FTIR spectrometer (Varian Instruments, Randolph, MA) attached with a DuraSamplIR single-pass diamond-coated internal reflection accessory (Smiths Detection, Danbury, CT) was used to collect the ATR FT-IR spectra. At least five different locations for individual sample were scanned over the range of 4000-600 cm^{-1} at 4 cm^{-1} and 16 co-added scans. All spectra were given in absorbance units and no ATR correction was applied.

After exporting to GRAMS IQ application in Grams/AI (Version 9.1, Thermo Fisher Scientific, Waltham, MA), mean spectrum was taken for each sample and then was smoothed with a Savitzky–Golay function (polynomial = 2 and points = 11). The spectral set was exported into Microsoft Excel 2000 to execute simple algorithm analysis. The spectra were also normalized by dividing the average intensities in the 1800-600 cm^{-1} region, prior to PCA characterization in the 1800–600 cm^{-1} IR region with mean centering (MC), Savitzky–Golay first-derivative (2 degrees and 13 points), and with leave-one-out cross-validation method.

Results and Discussion

Visual Comparison of Fibers Grown in Planta and in Culture

As shown in Figure 1, fiber growth in culture was slow compared to that in planta. Previous result indicated that culture fibers differ from planta fibers in some characteristics, for example, fiber length, cellulose content, the degree of branching of carbohydrate polymers, and protein profiles (Kim & Triplett, 2001).

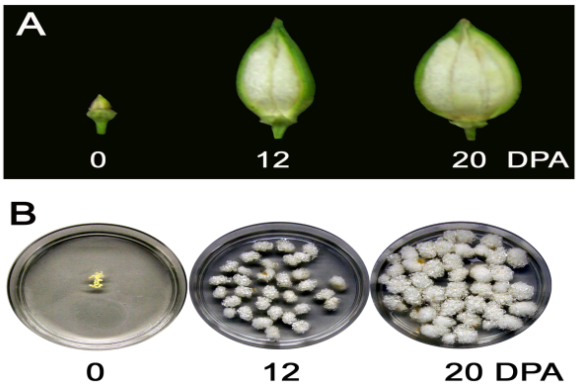

Figure 1. Schematic of fiber growth at 0, 12, and 20 DPA in planta (A) and in culture (B).

ATR FT-IR Spectral Feature of Developing Fibers

ATR-FTIR spectra of developing cotton fibers of different varieties have been studied in detail previously (Abidi et al., 2010b; Abidi et al., 2014; Liu & Kim, 2015; Liu et al., 2011 & 2012; Santiago & Hinchliffe, 2015). Apparent spectral intensity increasing or decreasing of unique IR bands have been related to chemical, compositional, and structural changes during cotton fiber cellulose development, demonstrating the effectiveness of this technique in monitoring the increasing dominance of secondary cell wall cellulose.

With the continuous deposition of cellulose in fibers, spectral intensity differences of the developing fibers between in planta and in culture with similar DPA are anticipated (Figure 2). Comparison of young fibers at the early growth stages that included a 10 DPA fibers in planta and a 16 DPA fibers in culture, three bands at 1565, 1525, and 1405 cm^{-1} are much distinctive due to the difference in fiber growing environments (in culture vs. in planta), with two intense bands (1565 and 1405 cm^{-1}) in the 10 DPA fibers in planta and one intense band (1525 cm^{-1}) in the 16 DPA fibers in culture. While old fibers at the later developmental stage including the 33 DPA fibers grown in planta and the 38 DPA fibers grown in culture, the spectral intensity changes are not apparent between two types of fibers. Like those grown in planta, fiber growth in culture medium results in the dominant production of the major common chemical component in cotton fibers, cellulose.

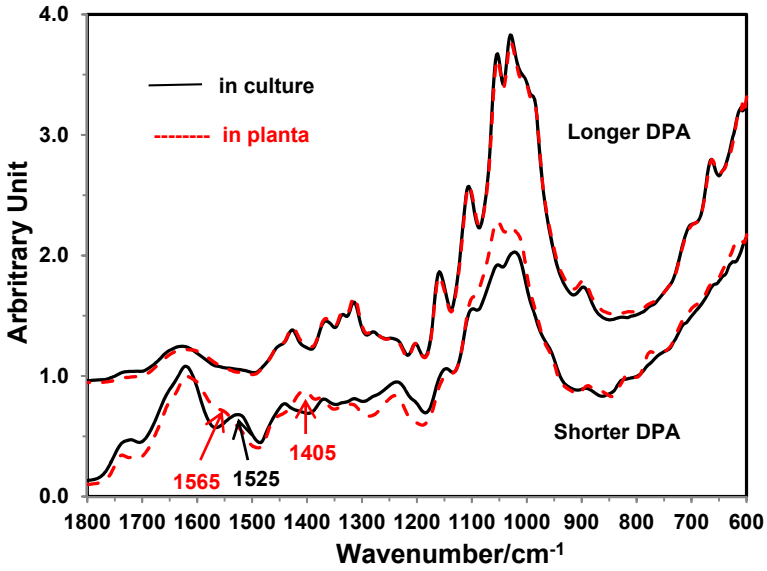

Figure 2. Representative of normalized ATR FT-IR spectra of shorter DPA (bottom) and longer DPA (top) fibers; the former included the 10 DPA fibers in planta and 16 DPA fibers in culture while the latter included the 33 DPA fibers in planta and 38 DPA fibers in culture. Spectra of longer DPA cotton fibers were shifted up vertically for direct comparison.

On the basis of relative ATR FT-IR spectral intensities and band positions, cotton fibers from the plant developed faster than those from the cultures at earlier stages, which is consistent with visual observation of fiber growth (Figure 1). However, subjective interpretation of spectra in Figure 2 cannot be applied for comparing or assessing the degree of fiber secondary wall biosynthesis in a semi-quantitative way.

PCA Classification of ATR FT-IR Spectra

In order to understand the similarity or dissimilarity of spectra that are indicative of fiber growth in planta and in culture, all spectra were submitted for PCA recognization. The first two PCs accounted for 95.2% of the total variation, with the PC1 explaining 90.3% of the variation. Figure 3, a plot of PC1 score vs. DPA, provides a good visualization of sample distribution between two sets of fibers. For fibers grown in planta, PC1 increases rapidly between 10 and 24 DPA before reaching the nearly constant PC1 score. It implies the occurrence of phase transition from primary to secondary cell wall synthesis between 10 to 24 DPA, and also is consistent with the earlier results showing that elongating fibers at 10 DPA contain no secondary walls whereas thickening fibers at 24 DPA are composed of relatively more content of secondary wall cellulose. Regard to fibers grown in culture, PC1 raises

significantly 20 through 28 DPA. Therefore, secondary cell wall synthesis of developing fibers grown in planta started between 10 and 17 DPA, and those grown in culture started between 20 and 28 DPA, showing a 10-day delay in growth. Notably, PC1 scores for both fiber sets are nearly independent of fiber DPA when DPA is older than 25 days, probably the spectral intensity changes at this period of fiber maturation are insignificant.

Algorithm for Analyzing ATR FT-IR Spectra

In above PCA approach, a number of 624 datapoints or variables (from the 1800 to 600 cm^{-1} IR region with 1.949 cm^{-1} interval in this study) were decomposed to a set of spectra with significantly reduced datapoints known as principal components (PCs). As a different strategy, three-band based ratios or algorithms were attempted to extract useful information in describing the deposition of secondary wall cellulose from ATR FT-IR spectra. One of recent developments was to estimate CI_{IR} by utilizing the respective IR bands at 708 and 730 cm^{-1} (Liu & Kim, 2015; Liu et al., 2011 & 2012). Plot of CI_{IR} against DPA in Figure 4 indicates a steady CI_{IR} increase for each fiber set. Due to obvious fiber growth environmental conditions, there is a reasonable lag in crystallinity development between two types of fibers, in which greater CI_{IR} of the developing fibers grown in planta than those in culture is anticipated. Relative to PCA result in Figure 3, Figure 4 apparently suggests a difference in CI_{IR} among the fibers with 25 DPA and older, likely implying the ability of this algorithm in detecting the subtle difference within these fibers.

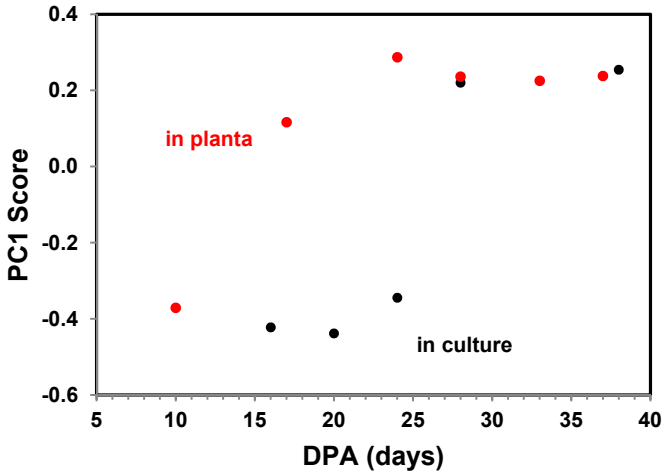

Figure 3. Relationship between PC1 scores and DPA from normalized ATR FT-IR spectra of fibers collected in planta (●) and in culture (●).

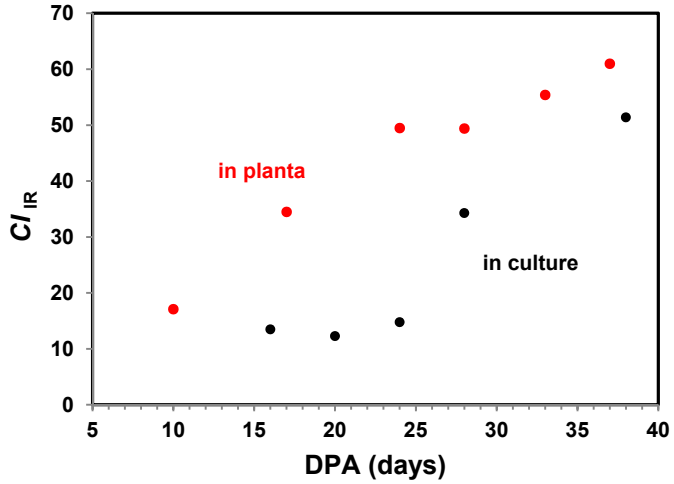

Figure 4. Plot of CI_{IR} against DPAs from ATR FT-IR spectra of fibers collected in planta (●) and in culture (●).

Based on original concept of assessing CI_{IR} from ATR FT-IR measurement (Liu & Kim, 2015; Liu et al., 2011 & 2012), intensities of two bands at 708 and 730 cm^{-1} (assignable to I_β cellulose in crystalline part and to I_α cellulose in amorphous part, respectively) were used to compute the algorithmic ratios, which were then converted cotton CI_{IR}. Figure 5 compares the spectral intensity changes of two bands at 708 cm^{-1} and 735 cm^{-1} with DPAs between the fibers collected in planta and in culture. For either 708 cm^{-1} or 735 cm^{-1} band, there are obvious differing trends between two types of cotton fibers, which is reasonable mostly due to their growing environments. Fibers in planta show nearly consistent 708 cm^{-1} intensity from 10 to 38 DPA, but those in culture reveal an increase up to 24 DPA before becoming unchanged. Interestingly, the 735 cm^{-1} band reduces its intensity sharply for planta fibers from 10 to 24 DPA, whereas that occurs for culture fibers from 24 to 28 DPA. Differing patterns in Figure 5 imply the usefulness of two bands at 708 cm^{-1} and 735 cm^{-1} in monitoring the changes of crystalline and amorphous cellulose concentrations within fibers grown in planta and in culture.

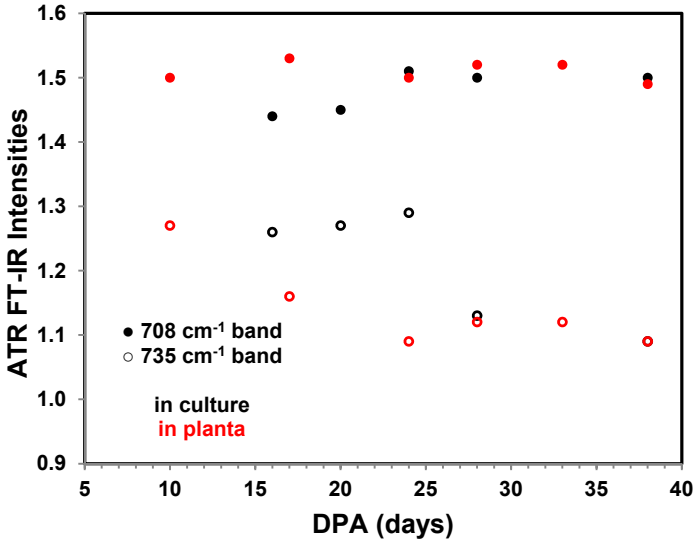

Figure 5. Plots of ATR FT-IR intensities at 708 cm^{-1} (●, ●) and 735 cm^{-1} (○,○) against DPAs from ATR-FTIR spectra of fibers collected in planta (●,○) and in culture (●,○).

Summary

A number of significant changes in chemical, physical, and structural aspect occur among the fibers grown in planta and in culture. Although these changes can simply be monitored by unique ATR FT-IR spectral features, it is not easy to acquire semi-quantitatively information on these fibers from the spectra directly. From PCA approach, the transition from primary to secondary cell wall biosyntheses of the fibers in culture happened approximately 10 days later than that of the fibers in planta. Notably, the IR crystallinity index algorithm is of interest, as it can detect the slight difference in fibers with DPA larger than 25 days. Examination of spectral intensities of two bands at 708 and 730 cm^{-1} that were used in the ratio algorithm indicates obviously differing patterns between two types of cotton fibers, which is reasonable due to their growing environments.

Acknowledgements

Authors thank Tracy Condon of USDA-ARS-SRRC for technical assistance in collecting the experimental samples.

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