COMPARISON OF COTTON FIBER DEVELOPMENT BY THE USE OF ATR FT-IR SPECTROSCOPY

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Abstract

Attenuated total reflection Fourier transform infrared (ATR FT-IR) spectroscopy was used to compare the secondary cell wall (SCW) biosynthesis between two sets of cotton near isogenic lines (NILs). Previously developed simple algorithmic analyses were applied to acquire the fiber crystallinity and maturity development information on these fibers. The results revealed the difference in fiber crystallinity and maturity development between NILs (TM-1 vs. *im*), not between NILs (MD52ne vs. MD90ne).

Introduction

Cotton fiber development is considered to include four overlapping but distinctive stages: initiation, primary cell wall (PCW) formation (elongation), secondary cell wall (SCW) thickening (cellulose synthesis), and maturation (Gordon & Hsieh, 2007; Kim, 2015). Apparently, there exist significant differences in chemical, physical, and compositional attributes between cotton fibers with various developmental stages and, hence, a number of diversified and well-defined fiber testing methods have been developed to reflect these differences routinely in cotton industry (Frydrych & Thibodeaux, 2010).

Over the years, Fourier transform infrared (FT-IR) spectroscopy method has been used in cotton fiber studies (Abidi et al., 2010 & 2014; Liu et al., 2011; Nelson & O'Connor, 1964; Santiago & Hinchliffe, 2015). To acquire useful information from FT-IR spectra, different approaches have been explored, including the use of 1-band intensities directly, the estimation of 2- or 3-band intensity ratios, and the implementation of principal component analysis (PCA) (Abidi et al., 2010 & 2014; Liu et al., 2011; Santiago & Hinchliffe, 2015). In our systematic studies, we have applied simple algorithms to assess cotton fiber infrared crystallinity (CI_{IR}) and maturity (M_{IR}) for different cotton varieties (Islam et al., 2016; Kim et al., 2017; Liu & Kim, 2015 & 2017), and the results have indicated that both simple algorithms and PCA pattern can be used to describe the transition from PCW to SCW biosynthesis in cotton fibers.

The main objective of this study was to compare the CI_{IR} and M_{IR} index development during the SCW biosynthesis (20 ~ 40 days post anthesis (DPA)) between two sets of cotton near isogenic lines (NILs). One set of NILs is *Gossypium (G.) hirsutum* Texas Marker-1 (TM-1) and immature fiber (*im*) mutant fibers (Kim et al., 2017), and another set is *G. hirsutum* MD52ne and MD90ne fibers (Islam et al., 2016). Compared to its isogenic wild type TM-1 that is a standard upland cotton variety and produces normal fibers with thick walls, the *im* mutant yields thin walled fibers (Kim et al., 2017). Two NILs, MD52ne and MD90ne, show variations in bundle fiber strength (Islam et al., 2016).

Materials and Methods

TM-1, *im* Mutant, MD52ne and MD90ne Fibers

Two cotton NILs, TM-1 and *im* were grown side by side in a field of USDA-ARS in New Orleans, LA during the 2011 crop year. Cotton flowers were tagged at the day of anthesis. Two biological replicates of TM-1 and *im* cotton bolls were harvested from different cotton plants at 10, 17, 24, 28, 33, 37 and 44 DPA. In addition, TM-1 was re-planted in the same field in 2015 and the fibers were taken from two biological replicates at 12, 15, 19, 23, 27, 30, 31, 34, 37, 40 and 44 DPA. The soil type was Aquent dredged over alluvium in an elevated location to provide adequate drainage.

Separately, three biological replications at each developmental time point (10, 13, 15, 17, 20, 24, 28, 33, 37, 44 and 48 DPA) were collected from 50 plants of MD52ne and MD90ne grown in the same filed in 2013 (Islam et al., 2016).

The fibers at each DPA were collected, manually ginned, and dried in 40°C incubator. Throughout all processes from planting, tagging, harvesting, and ginning over three crop years, the plants were equivalently treated. These fibers were stored in a dark storage room with a constant temperature (23 ± 1°C) and relative humidity (50 ± 10%). ATR FT-IR spectral measurements were performed in March 2014, November 2014, and October 2015 for the respective 2011, 2013, and 2015 crop-year fibers.

ATR FT-IR Spectral Collection

ATR FT-IR spectra were collected by the use of an FTS 3000MX FTIR spectrometer (Varian Instruments, Randolph, MA) combined with a DuraSamplIR single-pass diamond-coated internal reflection accessory (Smiths Detection, Danbury, CT). 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, taking less than 10 min for each sample. 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.

Results and Discussion

ATR FT-IR Spectroscopic Characterization of Developmental TM-1 Fibers

As anticipated in Figure 1, apparent spectral intensity increases or decreases in mid-infrared (mid-IR) region of 1800-600 cm^{-1} are noticeable for these developmental TM-1 cotton fibers. Spectral intensity changes in Figure 1 are in good agreement with those reported earlier and characteristic band assignments have been summarized in these studies (Abidi et al. 2010 & 2014; Liu & Kim, 2015). In general, intensities of the bands at 1740, 1620, 1545, 1455, 1405, 1236, and 1147 cm^{-1} as well as those below 850 cm^{-1} decrease, while those at 1425, 1365, 1335, 1315, 1200, 1158, 1104, 1055 and 1028 cm^{-1} as well as those in the 1000-875 and 700-600 cm^{-1} region increase. Briefly, the vibration at 1740 cm^{-1} is assigned to C=O stretching mode of carbonyl groups due to lipids, and a broad band centered at 1620 cm^{-1} is mainly attributed to OH bending mode of adsorbed water. Bands in the region of 1500–1200 cm^{-1} represent the contributions of both CH_2 deformations and C–O–H bending vibrations, and those bands in the 1200–900 cm^{-1} region originate from the coupling modes of C–O and C–C vibrations. The bands between 800 and 700 cm^{-1} are likely due to two crystal forms (I_α and I_β) of cotton cellulose.

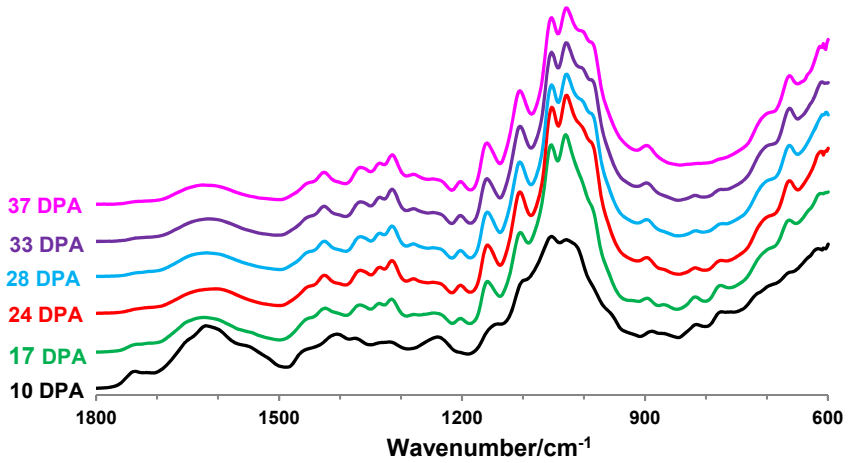

Figure 1. ATR FT-IR spectral response of TM-1 fibers to various DPA.

Algorithmic Interpretation of Developmental TM-1, *im*, MD52ne and MD90ne Fibers

As usual, algorithm analysis was applied to understand the similarity or dissimilarity of ATR FT-IR spectra between developmental TM-1 and *im* fibers. DPA-dependent infrared crystallinity (CI_{IR}) in Figure 2A indicates a steady CI_{IR} increase for 2011 TM-1 and 2011 *im* fibers from 23 to 37 DPA, as expected. In general, it suggests a slight difference in crystallinity development between the two types of fibers, in which greater CI_{IR} readings of developing 2011 *im* fibers than developmental 2011 TM-1 fibers are observed. As a comparison, the developmental TM-1 fibers grown in 2015 were included in Figure 2A. Different tendency between 2011 TM-1 and 2015 TM-1 fibers could indicate the impact of crop year or growing environment on cotton fiber crystallinity formation.

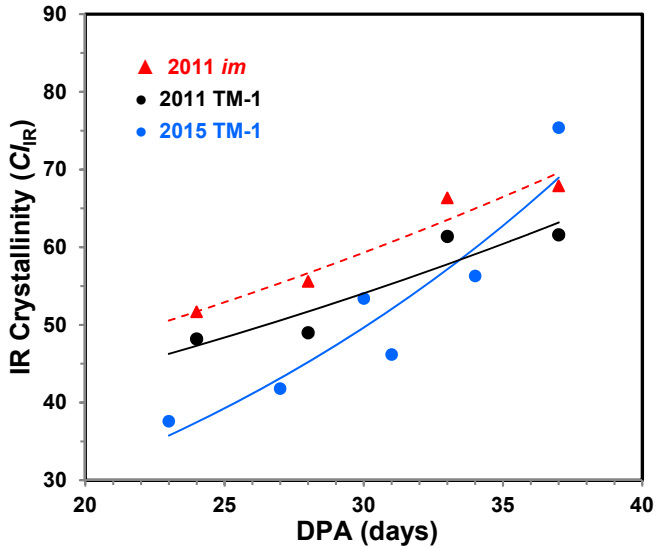

Figure 2A. IR crystallinity (CI_{IR}) from ATR FT-IR spectra of developmental 2011 *im* (▲), 2011 TM-1 (●) and 2015 TM-1 (●) fibers against DPA. Exponential regressions were applied to individual fiber sets.

As shown in Figure 2B, the M_{IR} values increase along with DPA apparently. The pattern in Figure 2B resembles that in Figure 2A, but the difference in M_{IR} values between 2011 TM-1 and 2011 *im* is insignificant.

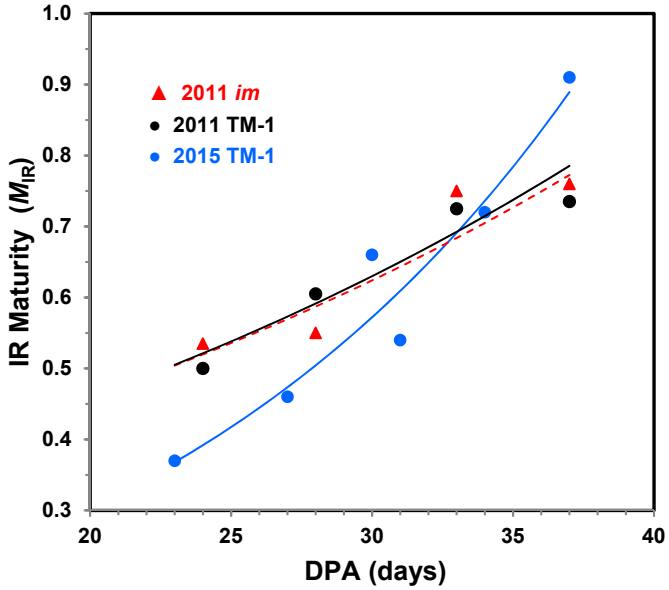

Figure 2B. IR maturity (M_{IR}) from ATR FT-IR spectra of developmental 2011 *im* (▲), 2011 TM-1 (●) and 2015 TM-1 (●) fibers against DPA. Exponential regressions were applied to individual fiber sets.

Plot of CI_{IR} against M_{IR} in Figure 2C suggests the more CI_{IR} development for *im* (○) fibers than for TM-1 (● and ●) fibers when the fibers have the similar M_{IR} values. In other words, *im* (○) fibers have more CI_{IR} development than TM-1 (● and ●) fibers. Notably, there is little difference between 2011 and 2015 TM-1 fibers.

As a comparison, the developmental NILs (MD52ne vs. MD90ne) grown in 2013 are shown in Figure 3. From 20 to 40 DPA, there was no significant difference in the CI_{IR} (Figure 3A), M_{IR} (Figure 3B), and the correlation relating CI_{IR} to M_{IR} (Figure 3C) between the two NILs.

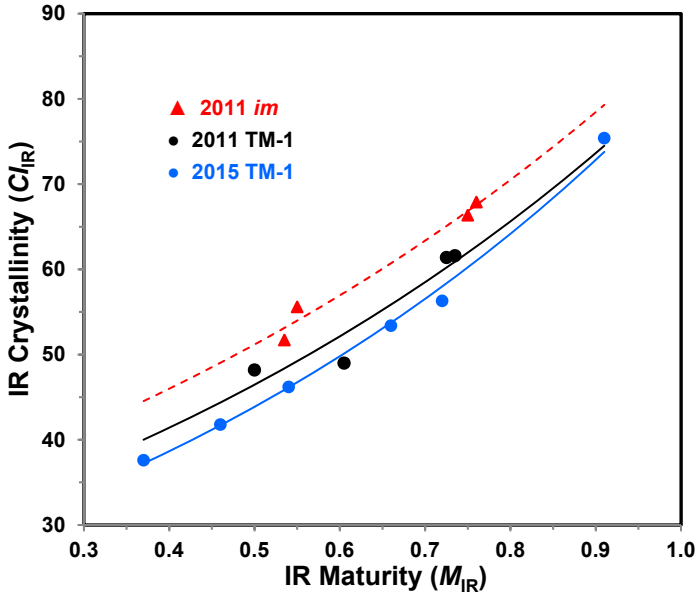

Figure 2C. IR crystallinity (CI_{IR}) against IR maturity (M_{IR}) from ATR FT-IR spectra of developmental 2011 *im* (▲), 2011 TM-1 (●) and 2015 TM-1(●) fibers. Exponential regressions were applied to individual fiber sets.

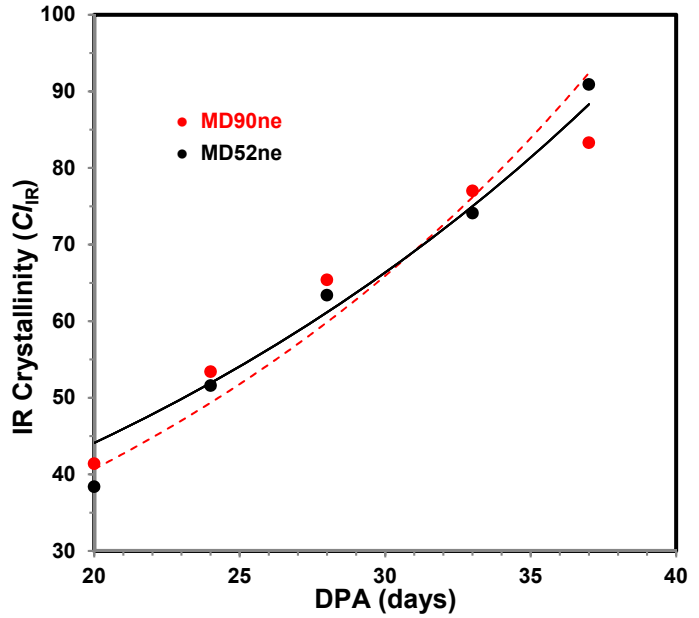

Figure 3A. IR crystallinity (CI_{IR}) from ATR FT-IR spectra of developmental 2013 MD90ne (●) and 2013 MD52ne (●) fibers against DPA. Exponential regressions were applied to individual fiber sets.

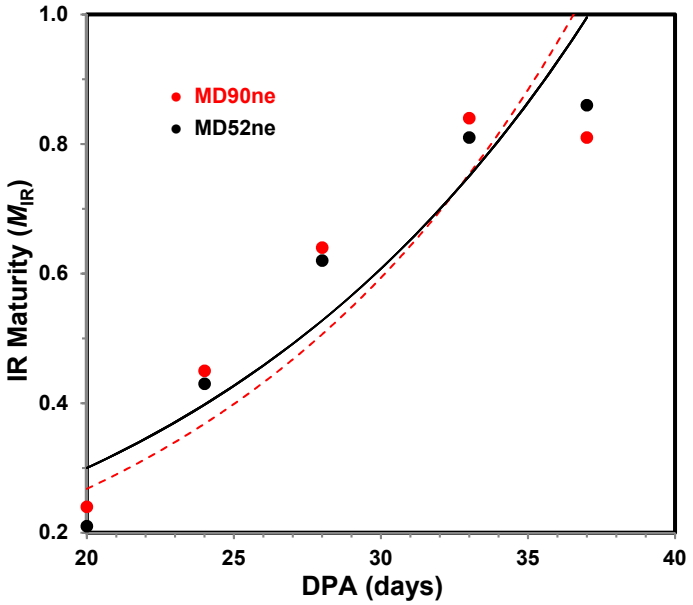

Figure 3B. IR maturity (M_{IR}) from ATR FT-IR spectra of developmental 2013 MD90ne (●) and 2013 MD52ne (●) fibers against DPA. Exponential regressions were applied to individual fiber sets.

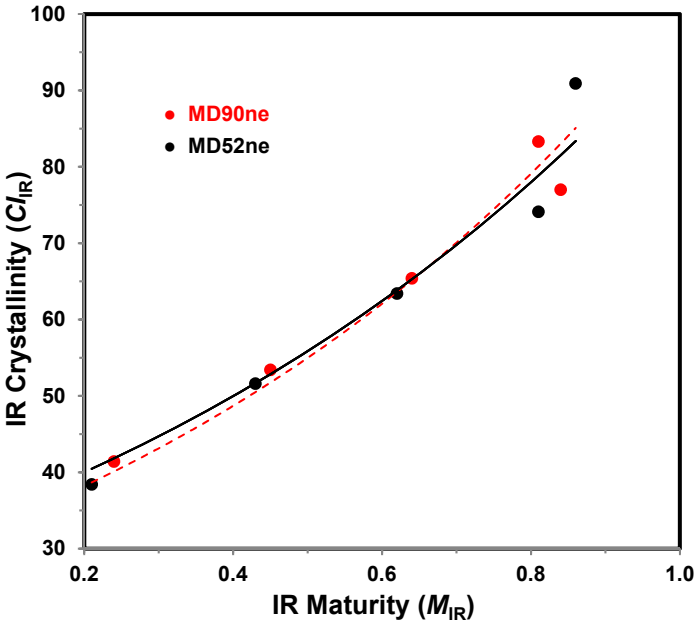

Figure 3C. IR crystallinity (CI_{IR}) against IR maturity (M_{IR}) from ATR FT-IR spectra of developmental 2013 MD90ne (●) and 2013 MD52ne (●) fibers. Exponential regressions were applied to individual fiber sets.

Summary

Significant differences in developmental cotton fibers can easily be assessed by characteristic ATR FT-IR spectral intensity decreasing or increasing. Simple algorithmic analyses enable the comparison of fiber crystallinity and maturity development during the SCW biosynthesis between cotton NILs. Preliminary results suggest that *im* fibers (having thin SCW) develop crystalline cellulose easier than its NILs, TM-1 (with thick SCW). As expected, there is insignificant difference in fiber crystallinity and maturity development between NILs (MD52ne vs. MD90ne), because they show variations in bundle fiber strength.

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