

BREEDING AND GENETICS

Comparative Phenotypic Analysis of *Gossypium raimondii* with Upland Cotton

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ABSTRACT

Gossypium raimondii Ulbrich, a wild diploid species of cotton, was sequenced due to its small genome size and similarity with the cultivated allotetraploid Upland cotton. The D-genome of *G. raimondii* has become the reference sequence used extensively in cotton genomic and genetic studies. However, phenotypic information is limited because photoperiodicity prevents flowering outside its native environment and its fiber quality cannot be measured by conventional methods. Fiber and seed properties of *G. raimondii* were measured and compared with those of Upland cotton cultivars. Fiber length, fineness, cellulose content, and seed lint percentage were all significantly reduced in *G. raimondii* compared to Upland cotton, whereas fiber maturities were comparable. Spectrophotometric properties of *G. raimondii* fibers were similar to green Upland cotton fibers but differed from white and brown Upland fibers. Seed kernels of *G. raimondii* were smaller but their chemical compositions were similar to those of Upland cotton. Quantitative traits of *G. raimondii* will aid in interpreting its genome and accelerating comparative genomics approaches for identifying potential genes regulating fiber and kernel properties among *Gossypium* species.

Cotton is an important source of textile fiber, animal feed, and food-grade vegetable oil (Wakelyn et al., 2010). Greater than 90% of the world's cotton production is derived from *Gossypium*

hirsutum L, known as Upland cotton, due to its high yield and generally good fiber properties (Zhang et al., 2008). The remaining cotton supply is produced from the closely related *G. barbadense* L., *G. arboreum* L., and *G. herbaceum* L. species.

The *Gossypium* genus includes approximately 35 species, including more than 30 species with diploid ($n = 13$) genetics designated by classes A, B, C, D, E, F, G, and K (based on chromosome pairing relationships) and five species with polyploidy genetics ($n = 26$) designated as AD₁ to AD₅. Diploid *Gossypium* spp. with A and D genomes and the polyploid Upland cotton with the AD₁ genome have been used to study plant genome size evolution and plant polyploidization (Adams, 2003; Chaudhary et al., 2009; Hovav et al., 2008; Senchina, 2003). Among the diploid species, *G. raimondii* Ulbrich (D₅ genome) is a wild Peruvian species that is cytologically, morphologically, and phenogenetically similar to Upland cotton plants (Endrizzi et al., 1985; Hutchinson et al., 1947; Wendel et al., 1989). *G. raimondii* is believed to have served as the pollen parent for the allotetraploid Upland cotton (Fig. 1). *G. herbaceum* (A₁) and *G. arboreum* (A₂) are the closest relatives to the maternal parent of Upland cotton (Applequist et al., 2001; Wendel and Cronn, 2003).

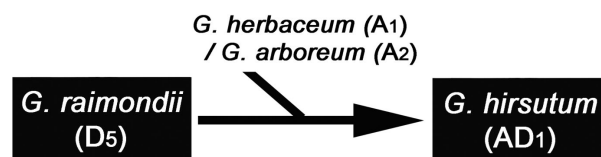


Figure 1. Polyploidization of *G. hirsutum* or Upland cotton (AD₁) from diploid cotton species, with *G. raimondii* (D₅) as the pollen parent, and *G. herbaceum* (A₁) or *G. arboreum* (A₂) as the maternal parent.

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Because of the relatively large size of the *G. arboreum* (1745 Mbp) and Upland cotton (2400 Mbp) genomes, the *G. raimondii* genome (885 Mb) was the first cotton species to be sequenced. Monsanto and Illumina deposited raw sequences of the species into Genbank (www.ncbi.nlm.nih.gov/sra/SRP003645) in 2010. The D-genome draft sequence (Wang et al., 2012) and the D-genome reference sequence (Paterson et al., 2012) were later sequenced from *G. raimondii*

and assembled. Several other draft genomes have been reported recently from cultivated cotton species: *G. arboreum* (Li et al., 2014), Upland cotton (Li et al., 2015; Zhang et al., 2015), and *G. barbadense* (Liu et al., 2015). However, the *G. raimondii* reference sequence, which represents approximately 99% of the D-genome (Paterson et al., 2012), has been used widely to identify candidate genes for Upland cotton genetic improvement (Fang et al., 2013; Gore et al., 2014; Islam et al., 2014), to determine transcriptome profiles of Upland cotton varieties (Gilbert et al., 2014; Kim et al., 2013; Naoumkina et al., 2014), and to gain insight into the genomes of cultivated species (Jin et al., 2013; Li et al., 2014; Olsen and Wendel, 2013; Page et al., 2013; Yoo and Wendel, 2014).

Phenotypic data have been crucial for deciphering genomic information and identifying candidate genes responsible for fiber quality (Islam et al., 2016; Kim et al., 2013). Conventional methods using High Volume Instrument (HVI) and Advanced Fiber Information System (AFIS) instruments generally have been used to determine indirectly cotton fiber properties for classifying commercial value of cotton, evaluating fiber quality, and performing genetic and genomic research. Advanced and innovative techniques also are available to determine directly quantitative traits of cotton fibers that are botanically unicellular trichomes elongating from epidermal cells of cotton ovules (Kim, 2015; Kim and Triplett, 2001). However, *G. ramondii* fibers described as tightly bound or non-spinnable (Fryxell, 2000; Paterson et al., 2012; Saunders, 1961) have not been characterized by conventional methods or advanced techniques. Among major fiber properties of *G. ramondii* such as length, maturity, fineness, and cellulose content, the short fiber lengths (~10 mm) were reported frequently (Applequist et al., 2001; Boza and Madoo, 1941; Hutchinson et al., 1947; Li et al., 2014). Other properties have not been reported because they are below the detection limit of the conventional methods. Hutchinson et al. (1947) reported *G. raimondii* from northern Peru had seeds of a greenish color that were covered with hairs. In naturally colored Upland cotton, green and brown colors are caused by suberin and proanthocyanidin, respectively (Ryser et al., 1983; Xiao et al., 2014). In green lint of an Upland cotton variety, suberin is associated with wax that is localized in the secondary cell wall and generates florescence (Ryser et al., 1983). The natural green color is not stable and fades to brown when exposed to light (Dickerson et al., 1999). The color pigments in the naturally colored Upland cotton can be characterized by near-infrared

(NIR) spectroscopy (Conard and Neely, 1943; Dickerson et al., 1999; Elesini et al., 2002; Pan et al., 2010; Xiao et al., 2014), whereas those in *G. raimondii* fibers have yet to be identified. Among seed kernel properties, gossypol containing insecticidal and antimicrobial activities was measured previously from *G. raimondii* seeds (Benbouza et al., 2002; Stipanovic et al., 2005), but other major properties including sugar, protein, and fatty acid profiles have not been characterized.

In this paper, we determined quantitative traits of *G. raimondii* fibers and seeds and compared their phenotypes with Upland cotton varieties. We grew *G. ramondii* and Upland cotton at three locations that differed in geographical and environmental conditions. Fiber properties from non-spinnable *G. raimondii* and spinnable Upland cotton Texas Marker-1 (TM-1) were compared by advanced instrumental techniques (Kim et al., 2014; Liu and Kim, 2015; Liu et al., 1998) as well as other methods, including image analysis microscopy, cellulose chemical analysis, and gravimetric methods (Kim, 2015). Seed kernel properties were measured by chemical analyses, including nitrogen analyzer, gas chromatography (GC), and high-performance liquid chromatography (HPLC). The results showed that *G. raimondii* was a naturally green-colored cotton containing suberin. *G. raimondii* fibers have a lower cellulose content and were significantly shorter and finer than Upland cotton fibers composed of higher cellulose content. Fiber maturity and seed kernel chemical profiles of *G. raimondii* were similar to those of the Upland cotton TM-1, despite the size differences.

MATERIALS AND METHODS

Plant Materials and Growth Conditions. Three *G. raimondii* accessions, D₅-3 (PI530900), D₅-6 (PI530903), and D₅-8 (PI5309050), were grown in a 44 gallon pot (one plant per pot, two replications) at greenhouses located at the National Cotton Germplasm Center (College Station, TX) during the winters of 2008 to 2010 that were naturally short-day conditions. Each plant produced on average two bolls during a winter season. The bolls became mature and opened approximately 45 d after floral anthesis. These plants were used for leaf and seedling characterization and for fiber trait tests. To obtain sufficient *G. raimondii* seeds for the tests, three *G. raimondii* plants (D₅-31, PI 530928) were grown perennially in the ground at the Cotton Winter Nursery (Kothari et al., 2011) at Tecoman, Colima, Mexico during the 2011 to 2013 seasons. The plants grew 8 ft tall

and 8 ft wide and produced 400 bolls (340 g) in the second winter season that were naturally short-day conditions. Fiber samples were manually collected from *G. raimondii* seeds that were harvested at the two locations. Upland cotton varieties TM-1 (Texas Marker-1, PI 607172), SA-1 (PI 528418), and SA-481 (PI 528787) as well as the three *G. raimondii* accessions (D₅-3, D₅-6, and D₅-8) were grown side by side in 5-gal pots (one plant per pot, three replications) with Metro-Mix 350 soil at the greenhouses located at the Southern Regional Research Center (New Orleans, LA) during the winters of 2008 to 2009 that were naturally neutral-day conditions. TM-1 has been a standard variety for cotton genetics analyses (Kohel et al., 1970). The SA-1 and SA-481 varieties were included as representative examples of brown and green cotton fiber types, respectively.

Fiber Length. Fiber lengths of both *G. raimondii* and Upland cotton were measured as described by Schubert et al. (1973). After dispersing the seed in warm acidic water, the relaxed fibers at the chalazal end of the seed were measured to the nearest 0.01 mm with a digital caliper. Thirty seeds were measured to determine an average value.

Single-Seed Weight, Fiber Weight, and Lint Percentage. Fibers from the Upland cotton TM-1 variety were ginned with a laboratory roller gin; hence, linters were not included with the lint. *G. raimondii* fibers were removed by hand with tweezers. Single seed and fiber weights were calculated based on 30 seed samples, with each seed type analyzed in triplicate. Lint percentage was calculated from the triple measurements by dividing the lint weight by the initial seed cotton weight.

Fiber Maturity Determination by Image Analysis Microscopy. Approximately 300 mg of fibers were manually ginned from 200 seeds from *G. raimondii* and 10 seeds from TM-1 that were randomly selected. The fiber samples were mixed, combed, and used to make multiple fiber bundles composed of several thousand individual fibers. They were embedded, thin-section cut, and photographed using a light microscope. Average wall area excluding lumen (A) and perimeter (P) of the fiber cross sections were measured from 300 cross sections using image analysis software (Xu and Huang, 2004). Circularity representing the degree of fiber cell wall thickness was obtained from the equation, $\theta = 4\pi A/P^2$, and the maturity ratio (MR) was converted from circularity by the equation, $MR = \theta/0.577$ (Thibodeaux and Rajasekarn, 1999).

Cottonscope Measurements. Fiber fineness, MR, and ribbon width were measured with the Cottonscope software version 1.54 (Cottonscope Pty Ltd, Perth, Australia) according to the procedure described by Rodgers et al. (2013). Five hundred *G. raimondii* seeds and 20 TM-1 seeds were used to collect 500 mg of fibers from each cotton species. The ginned fibers were first equilibrated at 65 ± 2 % relative humidity and 21 ± 1 °C for a minimum of 24 h. *G. raimondii* and Upland cotton fiber samples were chopped into segments (i.e., snippets) with a knife-blade cutter. The snippets (50 mg mass, approximately 0.7 mm length) were then placed in a water bowl where they were dispersed by agitation. The snippet population was digitally measured with a polarized color camera. Birefringence was used to estimate maturity, whereas length and width of the snippet population were used to measure fineness and ribbon width. Approximately 20,000 snippets were counted in each loading. Each replicate instrument loading was measured twice and the results were averaged to give a value for the sample. Two to three instrument loadings were evaluated for each sample.

Gravimetric Fineness. Fiber fineness was measured as mass-per-unit length and reported as millitex (mtex = milligrams per kilometer of fiber length) (ASTM, 2012a). Upland cotton fibers were combed into a parallel tuft and then trimmed from the top and bottom to leave 15 mm of length. *G. raimondii* fiber samples were prepared using a comb-sorter (ASTM, 2012b) to isolate fibers of a known average length of 14.3 mm. Three hundred fibers were counted and weighed with a microbalance. Each sample was replicated three times.

Cellulose Content. Fiber cellulose content was measured as described by Updegraff (1969) with minor modifications. Dried fiber samples were cut into small pieces. Ten milligrams of each sample were added to a 5 ml heavy-walled glass vial. Noncellulosic material was hydrolyzed with a solution of acetic and nitric acids. The cellulose was then hydrolyzed with sulfuric acid and measured by colorimetric assay with anthrone. Avicel PH-101 (FMC, Rockland, ME) was used as a cellulose standard. Each sample was measured three times. Average cellulose content was calculated from two replications.

NIR and Fourier Transform Infrared (FTIR) Spectral Measurements. A Foss XDS rapid content analyzer (Foss NIR Systems Inc., Laurel, MD) was used to acquire visible and NIR reflectance spectra of the fibers. Each sample was pressed into a sample round in a cylindrical cell (3.8-cm diameter x 1-cm

thick). Log (1/Reflectance) readings were obtained over a 400 to 2500 nm range at 0.5-nm intervals. Thirty-two scans were recorded for each spectrum. Three spectra were obtained for each sample by re-packing the samples and these spectra were averaged.

Attenuated total reflection Fourier transform infrared (ATR-FTIR) spectra were collected in absorbance units with an FTS model 3000MX spectrometer (Varian Instruments, Randolph, MA) equipped with a ceramic source, a KBr beam splitter, and a deuterated triglycine sulfate detector. The ATR sampling device utilized a DuraSAMPLIR single-pass diamond-coated internal reflection accessory (Smiths Detection, Danbury, CT), and consistent contact pressure was applied by a stainless steel rod and an electronic load display. Ten spectra were collected for each sample over the range of 4000 to 600 cm^{-1} at a 4- cm^{-1} resolution with 16 co-added scans. Their average was taken as the spectra for the sample. Spectra were imported to Grams/AI software (Version 9.1, Thermo Fisher Scientific, Waltham, MA) and were smoothed with a Savitzky–Golay function (polynomial = 2; points = 11). The dataset was then loaded into Microsoft Excel to calculate the MR from a previously reported algorithm (Liu et al., 2011, 2012).

Microscope Imaging. Images of glands on leaves and seeds were taken with an Olympus SZX stereomicroscope and model DP11 digital camera. Image composites were constructed with Adobe Photoshop CS6 software.

Seed Kernel Analyses. Seeds of *G. raimondii* and Upland cotton were dehulled by a brief milling in a Waring blender and the hull and kernels pieces were separated using a series of screens followed by hand removal of hull fragments. The kernel pieces were then ground with a food processor to pass through a #20 sieve and freeze dried. Kernel protein was determined by combustion and measurement of the resulting nitrogen with a Leco FP-528 nitrogen analyzer (St. Joseph, MI). Oil was extracted with petroleum ether in a Foss HT-6 Soxtec apparatus (Prairie Eden, MN) and was determined gravimetrically. Soluble carbohydrates were measured by derivatizing 50-mg samples of ground kernel tissue with hexamethyldisilazane followed by gas chromatographic separation of the silyl sugars. Isomeric and total gossypol was measured by forming a diastereometric complex with *R*-(–)-2-amino-1-propanol and the individual enantiomers were separated by reverse-phase HPLC. Oil fatty acids

were transesterified with Methanolic base (Supelco, Bellefonte, PA), and the resulting methyl esters were separated and detected by gas chromatography. All analyses were conducted in duplicate. Full details of these methods can be found in a prior report (Pet-tigrew and Dowd, 2011).

Statistical Analyses. Statistical analyses and construction of graphs were performed using t-test, two-way ANOVA, and Prism version 5 software (Graph-Pad Software, Inc., San Diego, CA). Paired comparisons were conducted by the Tukey method with $p < 0.05$. For the cellulose analyses, the differences between individual plants of the same accession were minor; hence, the individual plant and replicate samples were combined and the analysis was conducted with the pooled dataset.

RESULTS AND DISCUSSION

Plant Characteristics. *G. raimondii* consists of creamy flowers similar to those commonly found in Upland cotton (Fig. 2). Unlike most Upland cotton genotypes, the bottom parts of petals from *G. raimondii* have a large deep-red petal spot. *G. raimondii* leaves on the sympodial branch are cordate (heart shaped) unlike the corresponding Upland cotton leaves, which are palmate (hand-like) with three to five lobes. Both *G. raimondii* and Upland cotton seed capsules (i.e., bolls) contain three to five locules, each containing up to several seeds. A comparison between pre-ginned and ginned seeds showed short fibers extending from the surface of the *G. raimondii* seeds (Fig. 2). On average, *G. raimondii* seed weighed approximately one-third of Upland cotton seed (Table 1), and the fiber yield per seed from the D₅-31 accession seed was 3 mg compared to 71.5 mg for Upland cotton seed (Table 1). This gives a fiber-to-seed ratio of 1:12.0 for the D₅-31 seed compared to a ratio of 1:1.8 for the TM-1 seed. Typical cotton fiber-to-seed ratios are approximately 1:1.7 (Tharp, 1948), although this number has varied in recent years and ratios as low as 1:1.5 are common.



Figure 2. Phenotype of *G. raimondii*. The photos were taken from a flower (day of anthesis, *G. raimondii*, D₅-3), a leaf, a cotton boll, pre-ginned single seed, and ginned single seed (*G. raimondii*, D₅-8).

Table 1. Fiber properties of *G. raimondii* and Upland cotton accessions

	<i>G. raimondii</i>			Upland cotton		
	D ₅ -6	D ₅ -8	D ₅ -31	TM-1	SA-481 ^Z	SA-1 ^Y
Single seed wt., mg			35.9 ± 1.1 b	129.0 ± 6.6 a		
Single seed lint wt., mg			3.0 ± 0.1 b	71.5 ± 10.3 a		
Lint %			7.8 ± 0.2 b	35.6 ± 2.1 a		
Length, mm	11.7 ± 1.0 b	11.3 ± 1.5 b	10.1 ± 0.9 c	31.1 ± 1.3 a		
Gravimetric fineness, mtex	29 ± 1 e	51 ± 2 d	28 ± 1 e	170 ± 6 b	110 ± 1 c	254 ± 3 a
Fineness, mtex, by Cottonscope			73 ± 2 d	165 ± 5 b	129 ± 2 c	289 ± 7 a
MR, by image analysis			1.04 ± 0.26 a	0.92 ± 0.26 b	0.54 ± 0.33 d	0.84 ± 0.37 c
MR, by FTIR spectroscopy	0.52 ± 0.06c	0.59 ± 0.06 c	0.55 ± 0.07 c	0.70 ± 0.17 b	0.57 ± 0.07 c	0.89 ± 0.07 a
MR, by Cottonscope			1.27 ± 0.03 a	0.88 ± 0.01 b	0.60 ± 0.01 d	0.79 ± 0.01 c
Ribbon width, μm			10.5 ± 0.1 d	15.6 ± 0.01 c	16.4 ± 0.1 b	17.3 ± 0.2 a
Cellulose content, %	75 ± 2 b	78 ± 2 b	74 ± 2 b	96 ± 3 a		
Water insoluble, %			96.1 ± 0.9 a	93.7 ± 0.6 a		
Acetic/nitric acid insoluble, %			74 ± 2 a	96 ± 3 b		

^Zgreen cotton.

^Ybrown cotton.

^Xmeans within a row followed by a different letter are significantly different by Tukey's mean comparison test at the 5% level.

Fiber Length. *G. raimondii* fiber length of the D₅-6, D₅-8, and D₅-31 accessions averaged between 10.1 and 11.7 mm (Table 1). These lengths were approximately one-third the length of the fibers from the TM-1 cotton variety (Fig. 3). Cotton fiber properties, including fiber length, are measured with HVI, defined as a standard method by the International Cotton Advisory Committee. HVI cannot be used on the small quantities of short, non-spinnable *G. raimondii* fibers available. Thus, fiber lengths of both *G. raimondii* and Upland cotton were manually measured by the method described by Schubert et al. (1973).

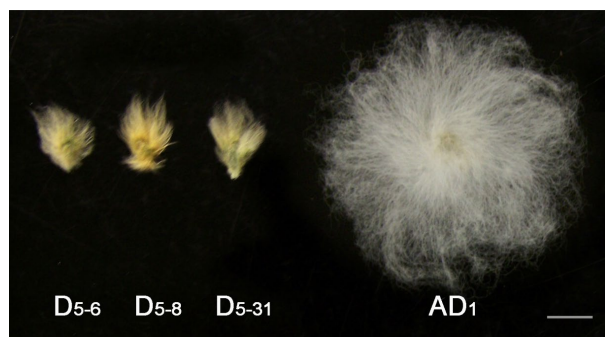


Figure 3. Comparison of fiber length between *G. raimondii* (D₅-6, D₅-8, and D₅-31) and Upland cotton TM-1 fibers (AD₁). The bar represents 1 cm of length.

Although *G. raimondii* has been classified in literature as a lintless species (Hutchinson et al.,

1947), the short fibers are lint fibers that are initiated on the day of anthesis (Applequist et al., 2001) and are different from the linter fibers that are initiated a few days post anthesis on Upland cotton seed (Lang, 1938). The *G. raimondii* fiber lengths grown at two different locations (College Station, TX and Tecoman, Mexico) were in good agreement with the lengths reported by others (Applequist et al., 2001; Fryxell, 2000; Hutchinson et al., 1947; Li et al., 2014) for the species.

Fiber Color. *G. raimondii* is naturally colored cotton (Fig. 4). IR and NIR spectral differences are known for naturally colored Upland cottons fibers, with green and brown fibers tending to show increased reflectance band intensities in the 400- to 800-nm range compared with white cotton fibers (Elesini et al., 2002; Liu et al., 1998). The reflectance band position and intensities of the D₅-31 *G. raimondii* spectrum were almost identical to the spectrum for the green fibers of Upland cotton variety SA-481 (Fig. 5A). Notably, these fibers show greater log (1/R) intensities at 632 and 675 nm within the visible region (400-750 nm), a small additional band near 2312 nm, and unusual band shapes between 1700 to 1800 nm and 2340 to 2380 nm when compared to the spectra of the TM-1 (white) and SA-1 (brown) cotton fibers.

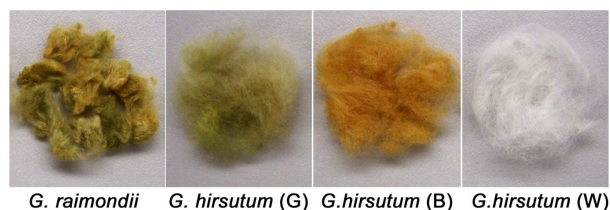


Figure 4. Comparisons of fiber colors between *G. raimondii* and three Upland cotton colored cottons. Fiber color of *G. raimondii* D₅₋₃₁ (left) compared with SA-481 green (G), SA-1 brown (B), and TM-1 white (W) fibers from Upland cottons.

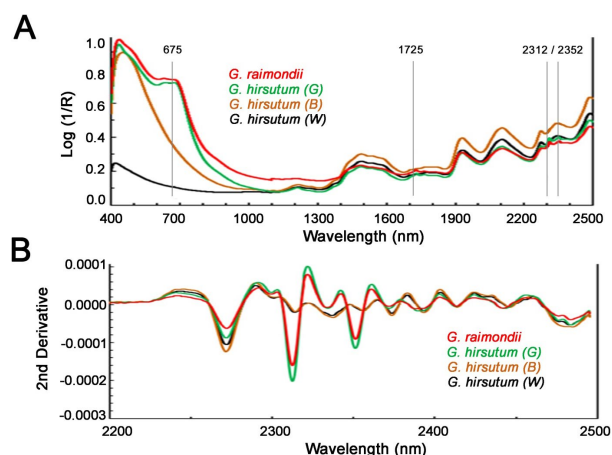


Figure 5. NIR spectroscopic analyses of cotton fibers. A. Visible/NIR log (1/R) spectra of *G. raimondii* D₅₋₃₁ fiber, Upland cotton SA-481 green (G), SA-1 brown (B), and TM-1 white (W) fibers. B. The second derivative of visible/NIR spectra of color and white cottons.

Differences in the spectral band shapes and intensities can be enhanced by viewing the second-derivative spectral response. Between 2200 to 2500 nm (Fig. 5B), the second-derivative spectra for the SA-481 and D₅₋₃₁ fibers were different from the spectra of the white and brown Upland cotton fibers. A new second-derivative band at 2352 nm was present, and the band at 2312 nm was more intense, whereas the band at 2271 nm decreased in intensity. These three bands are assigned to the second overtone of the C-H deformation vibration (2352 nm), a combination of C-H stretching and C-H deformation modes (2312 nm), and the combination of the O-H stretching and C-C/C-O stretching modes (2271 nm), respectively. Their intensity differences suggest that the green SA-481 fiber and *G. raimondii* fiber have components with more C-H and fewer O-H and C-C/C-O moieties than other cotton fibers.

NIR light reflectance showed that *G. raimondii* fibers appear spectrally similar to Upland cotton green fibers. The color trait in cottons is genetically inherited; however, fiber color has been observed to vary over seasons and geographic locations due

to climate and soil differences (Dickerson et al., 1999). Additionally, colored cotton fibers tend to be unstable; some darkening when exposed to light and others fading. Green fibers tend to be light sensitive (Dickerson et al., 1999; Pan et al., 2010).

The ultrastructure of Upland cotton green fibers is characterized by a high content (14-17%) of wax material that is referred to as suberin (Conard and Neely, 1943; Pan et al., 2010). This material is deposited in concentric, osmiophilic layers that alternate with layers of cellulose in the secondary cell wall of the cotton fibers (Ryser et al., 1983). In contrast, brown cotton fiber pigments are derived from proanthocyanidins (Xiao et al., 2014). The suberin of the green color fibers appears to be a complex material that has not been well characterized. It is composed of both polyaliphatic and polyaromatic domains, possibly cross-linked, and could function to regulate intercellular transport processes (Ranathunge et al., 2011). The aliphatic components are believed to be ω -hydroxyacids, α,ω -diacids, fatty acids, and primary alcohols, whereas hydroxycinnamic acids, especially ferulic acid, make up the polyaromatic domain. The similar spectral patterns indicate similar chemical and physical components between *G. raimondii* fibers and Upland cotton green fibers.

Fiber Fineness. Average fineness values were manually measured by gravimetric fineness methods because the short *G. raimondii* fibers could not be measured by AFIS, which generally is used to determine fineness values from Upland cotton fibers. Average gravimetric fineness of the fibers from *G. raimondii* accessions D₅₋₆, D₅₋₈, and D₅₋₃₁ was 29.0, 51.1, and 28.1 mtex, respectively. Hence, these samples exhibited some variation, but all of the accessions evaluated were significantly finer than the fibers from the three studied *G. hirsutum* varieties (Table 1). In addition to gravimetric fineness, the Cottonscope method also confirmed that *G. raimondii* fibers (73 mtex) were finer than TM-1 fibers (165 mtex). Previous evaluations have shown that, even though these methods yield different values, the gravimetric and Cottonscope fineness results tend to track each other (Rodgers et al., 2013), which was observed here. Fiber ribbon width, also determined by Cottonscope, was smaller for D₅₋₃₁ fibers (10.5 μ m) than it was for TM-1 fibers (15.6 μ m). The green Upland cotton fibers are known to have lower micronaire (2.0-3.0) than brown or white cottons (4.0-5.5) (Pan et al., 2010). The lower fineness of green fibers was consistent with low micronaire value found for these fibers in this study.

Fiber Cellulose Content. The cellulose contents of the D₅-6, D₅-8, and D₅-31 accessions were 75.3, 78.1, and 74.5%, respectively (Table 1). The cellulose levels of *G. raimondii* accessions were approximately 20% lower than for the TM-1 variety of Upland cotton. To further characterize the non-cellulosic material, *G. raimondii* D₅-31 fibers were extracted with water and then subsequently with an acetic acid/nitric acid reagent. The noncellulosic components from *G. raimondii* fibers were insoluble in water, but soluble in the acid solution, unlike the noncellulosic components of the TM-1 fibers that were soluble in water. A recent analysis of cellulosic and noncellulosic components from various cotton lines and hybrids has shown that a high content of noncellulosic components also is found in naturally green fibers (Pan et al., 2010), but not in white or brown fibers.

Fiber Maturity. Fiber maturity describes the degree of fiber thickening relative to the fiber perimeter. Among various methods and measurements used to estimate MR values, cross-section image analysis microscopy has been considered as the reference method because it directly measures the degree of fiber thickness, despite being a time consuming and difficult assay to perform (Kim et al., 2014; Thibodeaux and Rajasekaran, 1999). The direct image analysis showed that the D₅-31 *G. raimondii* accession exhibited a high MR value of 1.04 ± 0.26 , which was 1.1-fold greater than the TM-1 fibers (0.92 ± 0.26) (Table 1).

An indirect method, Cottonscope, was used as a supplement to the direct image analysis. Cottonscope measures the degree of birefringence intensity of the fiber's crystalline cellulose. The birefringence intensity is converted to MR values using calibrations versus standard MR values measured from Upland cotton and normalized with the fiber ribbon width as a proxy for fineness (Brims and Hwang, 2010; Kim et al., 2014; Rodgers et al., 2013). Only the D₅-31 *G. raimondii* accession was tested because insufficient fiber was available from the other accessions. The average MR of *G. raimondii* was 1.266, which was 1.4-fold greater than that of the TM-1 fibers (0.882). Cottonscope MR values suggest that *G. raimondii* fibers are substantially more mature than Upland TM-1 fibers.

Another indirect FTIR method also was used to compare MR values between *G. raimondii* and TM-1 fibers (Table 1). The FTIR spectra of the three available *G. raimondii* accessions gave FTIR MR values

of 0.52 (D₅-6), 0.59 (D₅-8), and 0.55 (D₅-31). In comparison, TM-1, SA-1, and SA-481 fibers produced values of 0.79, 0.91, and 0.55, respectively (Table 1). The FTIR approach is based on the reflected light that results from the functional chemical groups present on the near-surface of the fiber cellulose (Abidi et al., 2014; Liu, 2013; Liu et al., 2011). If MR is associated with the presence of more cellulose in the fibers, then it might be possible to exhibit distinctive IR bands whose intensities change with cellulose amount. The measured FTIR MR value of the green SA-481 fiber was lower compared to the other *G. hirsutum* fibers, but was similar to the *G. raimondii* fibers, suggesting that the green trait could affect cellulose deposition in both plant species. Smaller relative standard deviations for the *G. raimondii* values indicate a narrower maturity distribution than what was observed for the TM-1 fibers.

Direct image analysis determines MR values from cross-sectional areas of the fiber cell wall, and the result of this technique showed that the *G. raimondii* fibers were slightly more mature than Upland cotton TM-1. The two indirect methods also suggested that *G. raimondii* fibers were relatively mature, but there were some variations in the maturity values between the indirect methods and the direct image analysis (Table 1). Cottonscope data suggested the *G. raimondii* fibers to be substantially more mature than the Upland cotton TM-1 fibers, whereas the FTIR spectra showed the *G. raimondii* fibers to be somewhat less mature than the Upland cotton TM-1 fibers.

Those variations of MR values between direct and indirect methods were likely caused by differences of calibration methods among the three measurement methods. Unlike the image analysis microscopy that directly measured MR from the cross-sectioned fiber cell wall, the indirect measurement methods detect birefringence (Cottonscope) and cellulose (FTIR) from cotton fibers and convert them to MR values using the algorithms developed from the calibrated fibers ranging various MR. Due to a lack of calibrated *G. raimondii* fibers, the Upland cotton fibers whose birefringence and cellulose levels are different from *G. raimondii* fibers were used as calibrations for determining MR values from *G. raimondii*. The differences of birefringence and cellulose levels between the calibrated fibers and sample fibers potentially cause the variations of the MR values between direct and indirect methods.

The different MR values between the two indirect methods were likely caused by distinct principles between Cottonscope and FTIR. With the Cottonscope, polarized light is used to measure the birefringence intensity that would be generated from crystalline cellulose. The polarized microscope can detect not only birefringence from the cellulose but also fluorescence generated from the suberin that is autofluorescent in *G. raimondii* green fibers (Hewitt, 1938; Ryser et al., 1983). Thus, Cottonscope tended to inflate MR values of the *G. raimondii* green fibers containing the fluorescent suberin. In contrast, the FTIR spectroscopy method measured the IR band intensities generated from cellulose and converted to MR using algorithms (Liu et al., 2011). Standard Upland cotton fibers composed of nearly pure cellulose (> 90%) are used as calibrator to determine *G. raimondii* fibers consisted of substantially lower cellulose content (74%). The lower cellulose content of the *G. raimondii* fibers reduced the IR band intensities, and the FTIR tended to exclude fiber maturity that was contributed by non-cellulosic materials (26%) in the *G. raimondii* green fibers. As results of both direct and indirect methods, we concluded that the maturity of *G. raimondii* green fibers is similar to that of Upland cotton white fibers, although noncellulosic materials contributed more to the maturity of *G. raimondii* green fibers than the Upland cotton white fibers.

Seed Kernel Properties. In most respects, *G. raimondii* D₅-31 kernel composition was similar to the kernel composition of the TM-1 variety. The dehulled kernels of D₅-31 and TM-1 contained comparable levels of oil and protein (Table 2). These levels are within the range typically reported for cottonseed varieties (Cherry and Leffler, 1984). Similarly, both seed types contained approximately 7 to 8% soluble sugar with raffinose accounting for 70 to 75% of this sugar.

The distribution of major fatty acids from *G. raimondii* seed oil differed from those of the TM-1 seed oil in that greater levels of linoleic acid and lower levels of palmitic and oleic acids were found in the wild species (Table 2). This difference, however, was mostly due to the oil of the TM-1 seed being modestly different than the oil of typical commercial cottonseed varieties. The fatty acid profile of *G. raimondii* seed oil was essentially in the middle of the range of the Codex trading standard for commercial cottonseed oil (FAO/WHO, 1999) and is therefore essentially the same as most cotton varieties. The *G. raimondii* oil also contained the same secondary

fatty acids as the TM-1 oil, including small amounts (< 1%) of the unusual cyclopropyl fatty acids that are characteristic of many Malvaceae family plants (Badami and Patil, 1980).

Table 2. Seed kernel composition of *G. raimondii* (D₅-31) and Upland cotton (TM-1)^Z

Component	<i>G. raimondii</i> D ₅ -31	Upland cotton TM-1
Protein (N x 6.0), %	41.1 ± 0.4 a	40.6 ± 0.1 a
Crude oil, %	29.6 ± 0.0 b	30.4 ± 0.2 a
Gossypol, %	2.52 ± 0.07 a	1.11 ± 0.01 b
% (+)-isomer	58.8 ± 0.1 a	52.8 ± 0.1 b
Soluble sugars		
sucrose, %	0.58 ± 0.01 b	1.44 ± 0.07 a
raffinose, %	5.05 ± 0.04 b	5.95 ± 0.21 a
stachyose, %	1.09 ± 0.01 a	0.91 ± 0.06 b
total sugars, %	6.71 ± 0.04 b	8.30 ± 0.33 a
Oil fatty acid profile		
myristic acid	0.63 ± 0.00 b	1.14 ± 0.01 a
palmitic acid	23.2 ± 0.0 b	27.6 ± 0.10 a
palmitoleic acid	0.76 ± 0.00 a	0.68 ± 0.00 b
heptadecanoic acid	0.10 ± 0.00 a	0.10 ± 0.00 a
stearic acid	3.68 ± 0.01 a	2.83 ± 0.01 b
malvalic acid	0.60 ± 0.03 a	0.26 ± 0.01 b
oleic acid	16.3 ± 0.00 b	23.8 ± 0.20 a
cis-vaccenic acid	0.53 ± 0.09 b	0.74 ± 0.00 a
sterculic acid	0.16 ± 0.00 b	0.18 ± 0.01 a
linoleic acid	53.1 ± 0.10 a	41.9 ± 0.10 b
arachidic acid	0.45 ± 0.00 a	0.38 ± 0.00 b
linolenic acid	0.08 ± 0.00 b	0.09 ± 0.00 a
benhic acid	0.20 ± 0.00 a	0.17 ± 0.00 b
lignoceric acid	0.10 ± 0.00 b	0.12 ± 0.00 a

^Zall values except for the fatty acid profile are expressed in terms of % kernel weight on a dry weight basis. Fatty acids are expressed as the percentage of the total fatty acid profile.

^Ymeans within a row followed by a different letter are significantly different by Tukey's mean comparison test at the 5% level.

Gossypol levels differed between the two seed types, with the *G. raimondii* seed containing more than double the total gossypol level found in the Upland TM-1 seed. The 2.5% level of total gossypol found in the *G. raimondii* seed is also higher than the 0.6 to 1.3% range of total gossypol reported for 22 *G. raimondii* accessions from the National Cot-

ton Germplasm Collection (Stipanovic et al., 2005). Kernel and half-kernel images showed distinct pigments glands that are typical of *Gossypium* spp. seed (Fig. 6) and are the storage sites for gossypol. Gland density was not measured formally but appeared to be greater in the *G. raimondii* kernels than in the Upland cotton kernels, which is likely a direct manifestation of the greater gossypol levels in these seed. This difference is likely related to the conditions used to grow the plants, because seed gossypol levels are sensitive to environmental conditions, with increased seed gossypol levels resulting from lower field temperatures and increased rainfall (Pons et al., 1953). Although this factor has not been studied for wild *Gossypium* species, gossypol level differences of greater than 50% are known for seeds of plants grown in dryland and irrigated fields for the same variety (Pettigrew and Dowd, 2011).

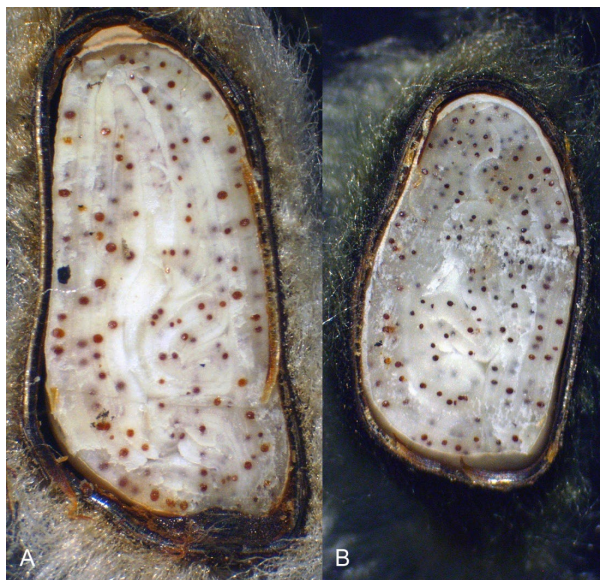


Figure 6. Half sections of individual *G. hirsutum* TM-1 (A) and *G. raimondii* D₅-31 (B) seed showing the glanding pattern. Seeds are shown at comparable scale.

Environmental Effects. There were insignificant differences among fiber properties obtained from *G. raimondii* grown in two separate locations that differed in geographical and environmental conditions. *G. raimondii* plants were grown in pots at College Station, TX, whereas they were grown in the ground at Tecoman, Mexico. Regardless of the conditions, average fiber lengths (10.1-11.7 mm) of *G. raimondii* were similar to each other (Fig. 3). The fineness values of the two *G. raimondii* accessions grown at College Station (29-51 mtex) were similar to those grown at Tecoman (28 mtex) (Table 1).

Similar MR values (0.52-0.59), cellulose contents (74.5-78.1%), and color also were observed from *G. raimondii* fibers grown in two different environments (Table 1; Fig. 4). We also observed similar fiber and seed properties from the Upland TM-1 grown at New Orleans, LA and College Station, TX (Kim et al., 2013) and concluded that the different environmental conditions in the US and Mexico did not impose serious implications to the properties of the *G. raimondii* and TM-1.

Photoperiod Sensitivity. Photoperiodic conditions were crucial for producing flowers in *G. raimondii*. *G. raimondii* produced flowers in short-day conditions during winter seasons at College Station, TX and Tecoman, Mexico. In contrast, they were unable to produce flowers in neutral-day conditions during winter seasons at New Orleans, LA. The photoperiod sensitivity appears to be a barrier for utilizing *G. raimondii* in Upland cotton breeding programs. Several genes were suggested to involve in the short-day photoperiod response in an Upland cotton variety (Lewis and Richmond, 1957) and a *G. barbadense* line (Lewis and Richmond, 1960).

Due to the differences in photoperiod sensitivity between *G. raimondii* and Upland cotton, we grew *G. raimondii* in short-day conditions and Upland cotton varieties in neutral-day conditions, and compared quantitative traits of the two species.

CONCLUSION

Fiber properties and kernel tissue composition were compared between *G. raimondii* and Upland cotton varieties. *G. raimondii* generally had inferior properties for lint percentage, length, fineness, cellulose content, and seed size, compared with Upland cotton. In contrast, similar fiber maturity and seed kernel composition were found between *G. raimondii* and Upland cotton. The results also showed that *G. raimondii* fibers were spectrometrically similar to green Upland cotton fibers but differed from white and brown Upland cotton cottons.

The comparative and quantitative phenotypic results of *G. raimondii* with Upland cotton varieties will be useful for cotton researchers to better interpret the D₅ and AD₁ genome data and apply the findings for improving Upland cotton. The results also suggest that the *G. raimondii* genome data can be useful for mining information pertaining to photoperiod and naturally green color biosynthesis in addition to the fiber and seed development.

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DISCLAIMER

Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the USDA that is an equal opportunity employer.

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