Industrial Crops and Products 100 (2017) 153–162

Contents lists available at ScienceDirect

Industrial Crops and Products

journal homepage: www.elsevier.com/locate/indcrop

Chemical variation for fiber cuticular wax levels in upland cotton (*Gossypium hirsutum* L.) evaluated under contrasting irrigation regimes

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ARTICLE INFO

Article history:
Received 28 September 2016
Received in revised form 21 February 2017
Accepted 22 February 2017

Keywords:
Cotton fiber
Fiber quality
Cuticular wax
Genomics-assisted breeding

ABSTRACT

The fiber from upland cotton (*Gossypium hirsutum* L.) makes up approximately 90% of the global cotton produced each year. Fiber quality is important to textile mills for processing and factors into bulk cotton sales. Fiber quality can be affected by many environmental factors, including water deficit, which makes identifying major fiber characteristics an important focus for breeding programs. Cotton fibers are specialized trichomes that are primarily composed of cellulose but have a cuticle composed of free waxes and cutin. Total cuticular wax of cotton fiber has been shown to act as a lubricant during textile processing, but has also been negatively correlated with important quality traits. The objectives of this study were to identify and quantify the cuticular wax compounds of cotton fiber under water-limited (WL) and well-watered (WW) irrigation treatments and assess their relationship with fiber quality from seven upland cotton lines. Through the most detailed characterization of cotton fiber cuticular wax to date, 41 quantifiable compounds were identified including free fatty acids, primary alcohols, aldehydes, alkanes, and tentatively identified alkanediols. Of these 41 compounds and their sum (total waxes), the abundance for nine were significantly different ($\alpha = 0.05$) between WL and WW conditions. Total wax and 36 compounds were highly repeatable ($r \geq 0.60$), indicating they will respond positively to selection in cotton breeding programs. Irrespective of irrigation regime, strong positive correlations (r_p 0.64–0.80) were found for fiber length and uniformity with primary alcohols, fatty acids, and aldehydes. These findings suggest that the biosynthetic pathways associated with these compounds are contributing to the phenotypic variability of these two important fiber quality traits and thus the biochemical pathways associated with cuticular fiber wax are candidates for metabolic engineering via molecular breeding approaches.
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1. Introduction

Cotton (*Gossypium* sp.) is an important natural fiber source used by the textile industry worldwide. Upland cotton (*G. hirsutum* L.) accounts for more than 90% of the 25.1 million metric tons produced globally each year (2010–2014 average, FAO, 2016). The United States is the third largest producer of cotton with approximately 70% being exported to foreign markets (Cotton Inc., 2016; National Cotton Council, 2016). Nearly 90% of the U.S. exported cotton is purchased by textile mills to produce woven fabrics and yarn for apparel and home-goods (National Cotton Council, 2016).

When purchasing bulk cotton, textile mills demand desirable fiber quality characteristics to ensure efficient production while still delivering superior products to the consumer (Bradow et al., 1997; Bradow and Davidonis, 2000). Fiber quality is quantified by a high volume instrument (HVI), which measures fiber length, elongation, uniformity, fineness, maturity, and strength (Bradow and Davidonis, 2000; Cotton Inc., 2016). Two fiber traits that are particularly important for yarn spinning are length, with increased length minimizing fiber bunching (Thibodeaux et al., 2008), and uniformity, which reduces yarn hairiness (Krifa and Ethridge, 2006). Fiber

Abbreviations: BLUE, best linear unbiased estimate; Co-A, coenzyme-A; GC–MS, gas chromatography–mass spectrometry; ITSD, internal standard; TLC, thin layer chromatography; SD, standard deviation of the BLUEs; SE, standard error of the repeatability estimates.

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http://dx.doi.org/10.1016/j.indcrop.2017.02.030
0926-6690/Published by Elsevier B.V.

quality is affected by many factors including the growing environment (Green and Culp, 1990), plant density (Bednarz et al., 2006), planting date, boll position on the plant itself (Davidonis et al., 2004), drought stress (Dağdelen et al., 2009; Dabbert et al., 2017), and genetics (Bradow and Davidonis, 2000; Paterson et al., 2003). With so many factors affecting fiber characteristics, it has been difficult to identify the primary determinants of quality, and therefore fiber characterization efforts have been renewed to meet the demands of textile mills.

Cotton fibers are specialized trichomes primarily composed of cellulose (~90%) found within the secondary cell walls of the seed coat (Gamble, 2004; Hartzell-Lawson and Hsieh, 2000; Hock et al., 1941). The cuticle is a structure that forms the outermost layer of the cotton fiber, and constitutes most of its noncellulosic components. The mature cotton cuticle itself is primarily composed of two basic classes of lipids, the free waxes and cutin (Degani et al., 2002; Yatsu et al., 1983), with minor amounts of non-cellulosic polysaccharides, proteins, pectins, ash, salts, and sugars embedded within and/or chemically linked to the cuticle (Gamble, 2003; Hartzell-Lawson and Hsieh, 2000). Of these components, wax is generally the most abundant, with known lubricant properties that have made it a focus for fiber spinning and wetting studies (Gamble, 2004; Hartzell-Lawson and Hsieh, 2000). Despite its importance in textile production, few studies have reported the composition of fiber wax and how it can vary in response to external factors including abiotic stress such as water deficit and high heat (Church and Woodhead, 2006).

Inverse relationships have been found between total fiber wax and micronaire, an indirect estimate of fiber maturity and fineness, regardless of growing environment and cotton variety (Bradow and Davidonis, 2000; Gamble, 2003). Similar negative correlations between percent fiber wax and micronaire were found by Pan et al. (2010) who also reported negative correlations of total wax with lint percentage, fiber length, strength, and uniformity in both white and colored cotton. These correlative findings led the authors to conclude that varieties with reduced total wax should be selected for use in cotton breeding programs. However, Cui et al. (2002) reported an increase in fiber breakage during carding, a textile process that aligns individual fibers, by one order of magnitude in dewaxed cotton samples, providing support that the waxes act as a lubricant to reduce friction during processing. Similar results were reported by Taylor (1997) who found that fiber wax was positively correlated with fabric strength, indicating that natural waxes are acting as lubricants during fabric production. Despite the established relationship between fiber wax, textile processing, and fiber properties, none of these studies looked at the components of the cuticular waxes themselves.

Much of the knowledge regarding the composition and synthesis of cuticular waxes comes from studies of plant leaves, stems, and fruits (Jetter et al., 2006; Parsons et al., 2013). In these organs, cuticular waxes are secreted by epidermal cells and help form a protective, hydrophobic barrier at the plant/environment interface (Jenks et al., 1994; Samuels et al., 2008). The waxes are typically composed of a complex mixture of very long-chain aliphatic molecules (C_{20}0-C_{36}) including fatty acids, primary alcohols, wax esters, aldehydes, alkanes, secondary alcohols, ketones, as well as a variety of triterpenes (Yeats and Rose, 2013). The relative proportion of each class varies greatly between plant species, as well as between organs of the same species (Lee and Suh, 2015). The biosynthetic pathway for these major components has been fairly well characterized, and some of the genes encoding relevant enzymes have been identified (Yeats and Rose, 2013; Lee and Suh, 2015). Fatty acid biosynthesis occurs in the plastids of plant cells, and C_{16} and C_{18} fatty acids are exported from plastids to the cytosol where they are conjugated with Coenzyme-A (Co-A). These fatty acyl-CoAs are then elongated two carbon units at a time by elongase enzyme complexes located in the endoplasmic reticulum, resulting in acyl-CoAs that are up to 36+ carbons in length. The acyl-CoAs are then modified by two major pathways, either the acyl-reduction pathway, which produces primary alcohols that can further be reacted with a fatty acyl-CoA to produce wax esters, or modified by an alternate pathway that produces aldehydes, alkanes, secondary alcohols, and ketones (being referred to henceforth as the decarbonylation pathway). Fatty alcohols, free fatty acids, and wax esters are expected to be identified from the cuticle of mature cotton fiber (Church and Woodhead, 2006).

Although natural waxes are known to be important for fiber processing in textile production, previous studies have shown negative correlations between total fiber wax and fiber quality traits (Bradow and Davidonis, 2000; Gamble, 2003; Pan et al., 2010). The identification of wax compounds associated with fiber quality could lead to a better understanding of how fiber quality traits, fiber wax, and textile processing are interrelated. Furthermore, a detailed description of the wax compounds coating cotton fiber would provide new insight to the biochemical pathways associated with their production, which could guide future efforts to modify fiber wax compounds and associated fiber quality traits using transgenic- and/or genomics-assisted breeding approaches. The objectives of this research were to (i) characterize the content and composition of cuticular waxes on mature upland cotton fibers (ii) determine how differential irrigation treatments affect cuticular wax compounds and fiber quality traits, and (iii) assess the relationship between these wax compounds and fiber quality traits evaluated under differential irrigation treatments.

2. Materials and methods

2.1. Plant material

The fiber from seven upland cotton lines was evaluated for cuticular wax composition: DP 393 (PI 635100), DP 491 (PI 618609), FM 958 (PVP 200100208), NM24016 (PI 612327), STV 457 (PI 633625), STV 506 (PI 529523) and, TM-1 (PI 607172) which are known to vary for fiber quality and abiotic stress tolerance. Fiber samples were taken from these lines that were included as repeated checks from a previous experiment grown at the Maricopa Agricultural Center of the University of Arizona from 2010 to 12, described by Pauli et al. (2016). Briefly, experimental plots were one-row, 8.8 m in length, and spaced 1.02 m apart with a plant density of 4.1 plants m^{-2} and arranged in an $\alpha(0,1)$ lattice design with an average of three repeated checks per replicate per treatment per year. Plots were grown under either water-limited (WL) or well-watered (WW) treatments; the WL treatment started when 50% of plots were at first flower. Prior to mechanical harvest, 25 bolls were hand collected from each experimental plot and processed with a laboratory 10-saw gin. Lint percentage was calculated by weighing the ginned fiber and dividing by the total 25 boll sample weight. Fiber sub-samples (~10 g) were sent to Cotton Inc. (Cary, NC) for fiber quality assessment on the HVI (USTER® AFIS PRO, Charlotte, NC) including fiber uniformity (%), elongation (%), micronaire (unit), strength (kN m kg^{-1}), and length (upper half mean, mm). The remaining 25 boll samples were then stored in paper bags (Kraft, 52 Lb weight) in a humidity controlled chamber (20% relative humidity) at −20 °C (Chasewood Co., Cypress, TX) before fiber wax extraction and analysis.

2.2. Extractions and analysis of cuticular waxes on cotton fibers

Fiber samples (156 in total) were removed from the −20 °C freezer and allowed to warm to room temperature (22 °C), to prevent excess fiber breakage, and organized in a completely ran-