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Analysis of the physical properties of developing cotton fibres

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ABSTRACT

Cotton fibres develop over four stages: initiation, elongation, secondary-wall thickening, and maturation. They develop a significant crystalline structure during the secondary wall thickening stage of development. Cotton fibres were harvested from 17 days to 60 days after flowering (dpa). Transmission Electron Microscopy (TEM), Interferometry, Attenuated Total Reflectance Fourier–transform Infrared (ATR–FTIR) spectroscopy, immunofluorescence labelling, and fluorescence spectroscopy were used to characterise the cotton fibres in different stages. It was found that, secondary wall thickening and micronaire remain fairly constant from 17 to 24 dpa, after that time significant change occurs until maturity. Maturity ratio increases as the fibres develop. Birefringence increases rapidly from 17 dpa to 26 dpa, then levels off up to 60 dpa. It is evident by comparing the lateral order index (LOI) and results from the binding of a crystalline-cellulose binding probe (CBM3a) that there is a significant increase in the degree of cellulose crystallinity from 17 dpa to 26 dpa. Hydrogen Bond Intensity (HBI) increased to 24 dpa and decreased from 24 to 40 dpa indicating significant changes in inter-molecular hydrogen bonds. From 40 to 60 dpa an increase of HBI was observed. It is concluded that during the maturation stage of cotton fibre development, water loss from lumen allows the cellulose chains to come closer together and to form intermolecular hydrogen-bonds. TEM, Interferometry, ATR–FTIR spectroscopy, and immunofluorescence labelling combined with fluorescence spectroscopy, were demonstrated to be useful techniques in quantifying physical changes in cotton fibres during development, offering advantages over traditional analytical techniques.

1. Introduction

Cotton is the purest form of cellulose found in nature and cotton fibres have considerable economic significance. Therefore a fundamental understanding of cotton fibre structure and properties is important. Studies on the development of cotton fibre have concentrated on biochemical and cell structures from genetic and environmental perspectives [1].

Cotton fibres develop over four stages: (i) initiation; (ii) elongation; (iii) secondary-wall thickening; and (iv) maturation. Fibre initiation begins during flowering and fibres arise from the epidermal cells on the ovule surface [2,3]; the days after flowering are referred to as days post anthesis (dpa). Fibre elongation begins on the day of flowering by spherical expansion above the ovular surface and continues with primary cell wall deposition for 20–25 days until reaching final fibre lengths of 22–35 mm. Secondary cell wall synthesis begins around 15–22 dpa and continues for 30–40 days. Fibre maturation is evident by a twisted ribbon-like structure beginning 45–60 dpa [2,4]. While cells are growing, cellulose

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is synthesised by the condensation of glucose molecules at enzyme complexes, each of which generates 36 cellulose molecules; these lie in the same direction and crystallise into long microfibrils [5]. In studies of the crystalline structures of developing cotton fibres, Paralikar [6] showed that the crystalline structure at 5 dpa belonged to cellulose I; Tuichiev et al. [7] suggested that immature cotton fibres up to 10 days after flowering showed crystalline structure cellulose III, while the cellulose I was shown only for mature fibres; Chanzy et al. [8] noted that cellulose of the primary wall of cotton at 15 dpa possesses the crystalline structure of cellulose IV; Hsieh et al. [4,9] and Hu and Hsieh [1,10] demonstrated that crystallinity increases with fibre development; Hsieh et al. [4] showed that the most significant increase in crystallinity occurs between 20 and 35 dpa, equivalent to the first two weeks of secondary cell wall synthesis or the fourth and fifth weeks of entire fibre development. It is reported that no change in crystallinity is contributed by development beyond 35 dpa [4].

There is a loss of water and the fibres dehydrate when the cotton boll starts to open and fibres become flattened and twisted. After dehydration, the cross-section of cotton fibres is kidney-shaped, however the shape is near circular in fully developed, thick-walled, mature fibres and curled in thin-walled, immature fibres [11,12]. Fibre maturity is probably the most misunderstood and least well-defined term in textile industry [13,14]; the term, fibre maturity, is used to describe the degree of development of the fibre wall [14,15]. The degree of cell wall thickening (θ) is calculated from the measured parameters of the cross-sectional area and the perimeter of the cell wall. Although determining θ is theoretically the most accurate approach of measuring fibre maturity, measurements are affected by significant experimental error because of fibre preparation and the limited number of fibres that can be practically measured [15]. Some studies on measuring the development of cotton cell-wall thickening have been reported using different techniques: cross-section image analysis [14,16,17]; scanning probe microscopy (SPM) [11]; scanning electron microscopy (SEM) [18]; X-ray fluorescence spectroscopy (XRF) [19]; Fourier-transform infrared spectroscopy (FT-IR) [20]; and Goldthwait's method using a combination of two dyes, C.I. Direct Red 81 and C.I. Direct Green 26 [21].

Long et al. [15] measured the maturity of developing Pima (*Gossypium barbadense*) and Upland (*Gossypium hirsutum*) cotton fibres using an automated polarized light microscopy technique (SiroMat instrument). They pointed out that the fibre maturity increased as fibres developed – Upland fibres have higher average maturity, but Pima fibres matured quicker than Upland fibres. Wartelle et al. [19] studied cotton fibre maturity by X-ray fluorescence spectroscopy and Advanced Fibre Information System (AFIS) – they pointed out that these two techniques used together provide a more direct, quantitative measure of cotton maturity than other methods in use.

Abidi et al. [22] studied structural changes of developing cotton fibres using FT-IR; differences in structural evolution was reported for two cotton cultivars (*G. hirsutum* L. cv. TX19 and TX55) where transition between primary and secondary cell wall occurred between 17 and 18 dpa for TX19 cultivar fibres, and between 21 and 24 dpa for fibres from TX55 cultivar. The same authors supported these findings by thermogravimetric analysis [23], and changes in sugar composition and cellulose content using HPLC [24]. Seagull et al. [25] showed significant increases in fibre diameter during the first 30 days of fibre development for 4 genotypes from two species (*G. hirsutum* and *G. barbadense*); all genotypes started secondary wall synthesis by 20 dpa, as indicated by significant increases in wall birefringence.

Ceylan et al. [26] studied moisture sorption in developing cotton fibres (*G. hirsutum*) by dynamic vapour sorption. Two distinct stages of developing cotton fibres were reported: At the first stage from 21 to 25 dpa elongation of the fibres occurs and at the second stage above 25 dpa the secondary cell wall becomes dominant over the primary cell wall; during the first stage moisture sorption is very high in comparison to the second stage. Young fibres showed preference for polylayer water adsorption, probably due to the hygroscopic nature of the fibre components, and due to the fairly large total surface area of immature fibres. During the second stage cellulose content increases with the dpa and the maximum absorbance capacity is lower in comparison to the first stage.

Herein, the aim of this work is to analyse whether the application of novel analytical techniques such as interference polarized-light microscopy and immunofluorescence analysis using carbohydrate-binding modules (CBM), enables a better understanding of the structural properties of and crystallinity changes in developing cotton fibres. It has been previously reported that CBMs can be used to study the cell wall composition of cellulose [27,28], and in our previous research we have demonstrated that CBMs can be successfully employed to monitor changes in crystallinity in cotton treated with different concentrations of sodium hydroxide [29]. In this work, CBMs were used to study crystallinity changes of developing cotton fibres and results were compared to both structural changes monitored using interferometric methods and also crystallinity changes measured by Attenuated Total Reflectance Fourier–transform Infrared (ATR–FTIR) spectroscopy.

2. Experimental section

2.1. Materials

Plants from a conventional FiberMax cotton (*G. hirsutum*) variety were grown in 5 L pots containing a Perlite soil mixture, in a greenhouse with 16 h of artificial sunlight per day and maintained between 25 °C and 30 °C. Flowers were tagged as soon as they appeared, and harvested at the relevant dpa or days after flowering. Fibres from bolls at different developmental stages including green bolls that were 17, 20, 22, 24, 26, 35, 40, 45, 50, and 55 dpa and one boll that was matured and opened on the plant at 60 dpa were included. Bolls from 12 different plants were studied for all assays. Green and mature bolls were opened and the fibres were separated from the seeds by hand within 1 h of harvesting. Fibres in young fruit were packed