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Polysaccharide composition during cotton seed fibre development: temporal differences between species and in different seasons

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Abstract

Background: Cotton seed fibres are long single-celled epidermal trichomes that first appear on the surface of the ovule at anthesis and then elongate rapidly over a period of 15–25 days until a secondary cell wall (SCW) begins to develop through a rapid increase in the deposition of microfibrillar cellulose between the plasma membrane and the primary cell wall that eventually terminates elongation. Quantitative measurements of the different polysaccharide components in both wall types over time and how they influence fibre quality can direct studies involved in enhancing fibre properties for yarn quality through cell wall manipulation or molecular breeding.

Results: A detailed chemical analysis of cell wall composition by differential solvent fractionation was used to identify the range of polysaccharides present in mature cotton fibres and used to validate a simpler total cell wall monosaccharide linkage analysis protocol for wall compositional analysis. Analysis of fibres from 5 days post-anthesis through maturity for three cultivated species, *Gossypium hirsutum*, *G. barbadense*, and *G. arboreum*, showed the dynamic nature of cell wall polysaccharide composition through fibre development and that it progressed differently for each species. Plants grown in the glasshouse during either autumn to winter or spring to summer and within each species had fibre qualities and temporal aspects of cell wall development that were different for each season. Notably, the timing of the deposition of the SCW was delayed in winter grown plants and appeared to influence key fibre quality properties.

Conclusions: These results suggest that the temporal aspects of cell wall polysaccharide biogenesis during fibre development influence final fibre quality, and this timing is determined by both genetic and environmental factors. The onset of SCW synthesis appears to be a critical factor coinciding with termination of fibre elongation and specifying the duration of wall thickening that then affects fibre length and other wall-associated quality parameters that ultimately determine yarn quality.

Keywords: Fibre development, *Gossypium hirsutum*, *Gossypium barbadense*, *Gossypium arboreum*

Background

Cotton is an important commercial crop providing a natural fibre primarily for textiles. Cotton fibres are single elongated cells that grow as specialised seed epidermal trichomes composed predominantly of cellulose (>95% by weight) at maturity. The cell walls of these unusual cells have long been studied and shown to undergo compositional changes through development. The seed fibres develop through a series of distinct phases starting with fibre initiation around the time of anthesis [0 day post-anthesis (DPA)] when epidermal cells destined to become fibres begin to bulge from the seed surface (Basra and Malik 1984). Post-initiation, the fibre initial cells, which

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are surrounded by a pectin-rich primary cell wall (PCW) (Singh et al. 2009), undergo a period of anisotropic elongation to achieve a length of up to 4 cm before transitioning into the production of a thickened secondary cell wall (SCW). The transition period is marked by a reduced rate of cell elongation, increased deposition of cellulose microfibrils associated with increased expression of a set of SCW-specific cellulose synthases (CesA genes) (Kim et al. 2012; Li et al. 2013) and overlapping with a period of callose (β-1,3-glucan) deposition whose role is not yet fully understood (Maltby et al. 1979). The SCW layers of cellulose continue to be deposited between the plasma membrane and the PCW until only a small lumen remains and the cells move into a final maturation phase where they are no longer biologically active, dehydrate and collapse to form the twisted ribbon-like fibres that can be spun into yarn.

The cotton fibre is an ideal single cell system to study cell wall elongation and biogenesis (Haigler et al. 2012) and considerable effort has gone into understanding the cell wall-related genes and enzymes, such as CesAs that are differentially expressed throughout fibre development (e.g. Li et al. 2013; Kim and Triplett 2001; Haigler et al. 2009; MacMillan et al. 2017). The highly crystalline cellulose walls of the fibre have hindered the chemical analysis of fibre polysaccharide composition and structure, but there has been a growing body of literature on the other polysaccharide types present in the fibres. Their arrangements at a cellular level are starting to be unravelled using microscopy and immunohistochemistry (e.g. Vaughn and Turley 1999; Avci et al. 2013; Hernandez et al. 2015). These studies, as well as FTIR spectrophotometric profiling (Abidi and Manike 2018) indicate that during development fibres undergo dramatic changes in polysaccharide composition and structure that are thought to reflect the different physical properties needed of the cell wall during those different phases of expansion, growth, and SCW deposition. The initial PCW, for example, is rich in pectic polysaccharides and xyloglucans (Huwyler et al. 1979; Maltby et al. 1979; Singh et al. 2009), and under the influence of a high internal turgor pressure the cell is able to rapidly elongate because of the plastic behaviour of the wall conferred by these polysaccharides. As the increase of cellulose levels when the fibre transitions to SCW production, the wall becomes more rigid and pectins and xyloglucans decrease in relative abundance (Tokumoto et al. 2002). Pectin is a mixture of different polymers of galacturonic acid (GalA), the most common being homogalacturonan (HG) which is initially deposited with many of the GalA residue carboxyl groups methylesterified. The extent and distribution of methylesterification on the pectin chains can be subsequently modified *in muro* to alter the physical and chemical properties of the pectin matrix as the fibre grows. The degree of pectin methylesterification (DE) is controlled by various pectin methylesterases (PMEs) (Liu et al. 2013; Singh et al. 2009) and DE decreases over time which increases the potential for cross-linking of pectin chains by calcium bridges and also the lability of the pectin to degradative enzymes (polygalacturonases) also present in the wall. The shift in pectin DE and total pectin content, and the corresponding increase in crystalline cellulose content beginning at the transition phase, all contribute to rigidifying the wall and hindering further cell elongation.

Not surprisingly, a number of studies (Abidi et al. 2010; Avci et al. 2013; Li et al. 2013) have suggested that differences in cell wall development between different cotton species may influence their final contrasting fibre qualities [commercially defined in terms of either average fibre length, fibre strength or tenacity and various measures of fineness (linear density) and maturity (related to wall thickness)] as these properties must all be determined by the changing chemical and physical nature of the PCW and SCW as the fibres grow. Subtle differences in microfibril structure (Martinez-Sans et al. 2017) and the timing of cellulose deposition in *Gossypium barbadense* (Gb) fibre walls relative to that of *Gossypium hirsutum* (Gh), for example, have been implicated in the vastly superior quality of Gb fibre (Avci et al. 2013; Li et al. 2013; Rajasundaram et al. 2014) which is longer, finer and stronger than that of Gh, among other differences. Despite this, Gh is the more common species grown commercially (90% of global cotton production) as it is more adaptable across wider environmental conditions than Gb, and as a result, there is considerable interest in transferring the superior properties of Gb fibre to Gh by conventional plant breeding (Lubber and Chee 2009). Understanding the biological mechanisms involved in regulating fibre cell wall development and fibre quality is therefore critical for rationally enhancing the fibre properties of the major cultivated cotton species.

We focus here on the relationship between the timing of cell wall polysaccharide deposition and fibre quality. We undertook a quantitative study of temporal cell wall polysaccharide compositional changes in the fibres of three species of cotton, the two tetraploid species Gb (cultivar SiPima 280) and Gh (cultivar Coker 315-11) and the diploid species *Gossypium arboreum* (Ga) (cultivar BM13H), each with very contrasting fibre quality properties. SiPima 280 has superior fibre, followed by Coker 315 and then BM13H. Coker 315 is an older commercial Gh variety now only used for genetic modification research because it is one of the few varieties that can be regenerated in tissue culture and hence are transformable. BM13H is a Chinese Ga variety that produces relatively

coarse and short fibres that are only suitable for low-end uses such as in rugs, and, like other Ga varieties, is now only grown on a very small scale globally. In addition to examining the differences of cell wall development and composition among the species, we also grew each of the varieties under glasshouse conditions during either spring to summer or autumn to winter months to determine whether they grew similarly in both environments (differing mainly in light exposure) and to establish if fibre quality was affected. Studying the fibre development across different seasons will help to understand the impact on the commercial cotton fibre product when faced with shifting environmental conditions, such as climate change or expanding production into new growing regions, and potentially provide guidance to manage the crop for better outcomes. As monosaccharide linkage analysis had not previously been used on cotton fibre to estimate overall polysaccharide composition of intact cell walls, and a concurrent compositional analysis by more conventional solvent fractionation of SiPima 280 mature fibre was completed to establish the methodology for cotton fibres.

Results

Cell wall polysaccharide composition of mature cotton fibre determined by monosaccharide linkage analysis

To establish and validate a monosaccharide linkage analysis protocol for whole cell walls of cotton fibres, mature fibre cell walls [as an alcohol insoluble residue (AIR)] from SiPima 280 were first extracted into five sequential sub-fractions, CDTA (3.4 mg out of the total AIR 152 mg preparation), Na_2CO_3 (3.0 mg), 1 mol·L^{-1} KOH (1.5 mg), 4 mol·L^{-1} KOH (1.3 mg) and the insoluble residue (138.0 mg) for comparison with the unfractionated fibre wall preparations. Monosaccharide composition by linkage type of each fraction differed considerably from one fraction to another (Table 1) reflective of the different types of polysaccharides extracted by the different solvents. The most abundant fraction was the final residue fraction (90.8% of recovered material) which is predominantly composed of 1,4-Glc(p) derived from cellulose. The CDTA and Na_2CO_3 fractions were next in abundance (2.2% and 2.0%, respectively), followed by the 1 mol·L^{-1} KOH (1.0%) and 4 mol·L^{-1} KOH (0.9%) fractions. The monosaccharide linkage composition of these soluble fractions was considerably more complex than that of the insoluble residue (Table 1). To simplify the interpretations of these data, monosaccharides of each linkage type were combined and reclassified according to the most likely polysaccharide components from which they might be derived to generate an overall estimate of the polysaccharide composition (Table 2) of the different wall fractions and the unfractionated total walls. Pectic components of the wall included homogalacturonan (HG), rhamnogalacturonan I (RGI), arabinan, type I arabino-3,4-galactan (Type I AG) and type II arabino-3,6-galactan (Type II AG). These were the most soluble of the polysaccharides with 45% or more extracted with CDTA and the majority of the remainder extracted with Na_2CO_3. The type II AG component also represents the AG present on arabinogalactan-proteins (AGPs) since the AGP reactive β-glucosyl Yariv reagent precipitated a water-soluble component of the mature fibre cell wall accounting for approximately 0.1% (w/w). Callose was distributed almost evenly between the Na_2CO_3 and 1 mol·L^{-1} KOH fractions suggesting that it existed as at least two populations within the mature fibre wall with one being more soluble than the other. Xyloglucan (XG) was extracted predominantly with 4 mol·L^{-1} KOH and almost 80% of the heteromannan (HM) remained in the insoluble residue fraction with the cellulose component. Heteroxylan (HX), identified as a glucuronoxylan (GX) from the linkage analysis data and what is known of dicot xylans, was distributed amongst the 1 mol·L^{-1} KOH (50%), 4 mol·L^{-1} KOH (25%) and insoluble residue (20%) fractions. Importantly, the polysaccharide compositions predicted from the monosaccharide linkage analysis of the total cell walls was very close to that calculated from the data for the fractionated AIR wall preparation (Table 2), confirming that the methodology is appropriate to use for the time-course experiments on the different species.

Cell wall compositional changes throughout fibre development in three cotton species

Cell wall polysaccharide composition deduced from monosaccharide linkage analysis of AIR without any prior wall fractionation was determined for each of the fibre samples from SiPima 280, Coker 315, and BM13H grown in either the "summer" or "winter" regimes and over the full period of fibre development from 5 DPA to maturity, with more frequent sampling around the transition period (Figs. 1 and 2, Additional file 1: Tables S1 and Additional file 2: Table S2). Over this time course, fibre length increased steadily until maximum length was reached, generally coinciding with the time when the crystalline cellulose deposition rate was the greatest and this occurred earliest for BM13H, followed by Coker 315 and then SiPima280. All three species elongated at similar rates from 5 DPA to 15 DPA, but slowed after that, again in the order of BM13H, Coker 315, and SiPima280 elongating for the longest time. Crystalline cellulose content began to increase earliest for BM13H, then Coker 315 and finally SiPima280. Early in development (5 DPA), fibre walls consisted of 30∼40 mol% cellulose, 35%∼50% pectic polysaccharides, 8%∼15% XG, 2%∼5% HM,

Table 1 Monosaccharide composition (mol%) by linkage type of SiPima 280 mature cotton fibre cell wall fractions

Monosaccharide	Deduced linkage*	Total unfractionated walls (AIR)	CDTA (2.2%)	Na_2CO_3 (2.0%)	1 mol·L^{-1} KOH (1.0%)	4 mol·L^{-1} KOH (0.9%)	Insoluble residue (90.8%)
Fuc(p)	Terminal	0.1	0.1	0.1	–	1.0	–
Rha(p)	Terminal	tr	1.6	1.7	0.2	–	–
	1,2-	0.1	0.8	0.5	0.3	0.3	–
	1,2,4-	0.1	0.6	0.5	0.4	0.3	–
Ara(f)	Terminal	0.5	18.2	6.9	14.2	6.7	–
	1,2-	0.1	1.5	0.5	0.2	–	–
	1,3-	tr	0.4	0.3	0.2	tr	–
	1,5-	0.3	8.7	3.6	1.0	0.6	–
	1,2,5-	–	0.2	0.2	–	0.2	–
Ara(p)	Terminal	–	0.3	0.1	0.1	0.3	–
	1,2-	–	0.1	0.1	–	–	–
Xyl(p)	Terminal	0.3	0.3	0.8	0.6	10.7	–
	1,2-	0.1	–	–	–	4.2	–
	1,4-	0.6	0.2	1.6	9.3	8.6	0.2
	1,2,4-	0.3	–	–	18.1	8.3	–
	1,2,3,4-	0.1	–	–	0.2	–	–
Man(p)	1,4-	1.2	0.5	2.8	0.5	0.9	0.4
	1,4,6-	0.3	0.6	0.5	0.1	0.5	–
Gal(p)	Terminal	0.4	4.6	1.9	0.1	2.4	–
	1,2-	–	–	–	–	1.8	–
	1,3-	0.1	2.5	1.1	0.2	0.1	–
	1,4-	0.1	0.6	0.4	0.6	0.5	–
	1,6-	0.1	4.2	1.8	0.2	0.1	–
	1,2,4-	–	0.1	0.2	–	–	–
	1,3,4-	–	0.1	–	–	tr	–
	1,3,6-	0.4	11.4	4.3	0.5	0.2	–
	1,4,6-	–	0.2	0.4	–	–	–
	1,3,4,6-	–	0.1	–	–	–	–
Glc(p)	Terminal	0.3	0.9	2.2	1.9	–	1.2
	1,2-	–	0.3	0.7	0.2	0.2	–
	1,3-	0.5	1.2	7.3	15.6	0.3	–
	1,4-	85.9	2.1	35.5	15.1	26.1	94.8
	1,2,3-	–	–	–	0.1	–	–
	1,2,4-	1.2	–	–	0.1	0.1	1.0
	1,3,4-	1.3	–	0.5	0.1	0.2	0.8
	1,3,6-	–	tr	0.3	0.5	–	–
	1,4,6-	2.4	0.1	0.9	0.3	11.2	1.5
	1,2,4,6-	0.3	–	–	–	–	–
	1,3,4,6-	0.5	–	–	0.1	–	0.1
	1,2,3,4,6-	0.9	–	–	–	–	–
GalA(p)	Terminal	tr	0.8	1.0	0.7	–	–
	1,4-	1.2	31.2	18.8	1.7	4.1	–
	1,2,4-	–	0.2	0.2	–	–	–
	1,3,4-	–	0.3	0.2	–	–	–
GlcA(p)	Terminal	0.3	2.6	2.2	16.9	9.9	–
	1,4-	–	2.6	–	–	–	–

Percentage of fraction recovery (w/w) relative to starting material is indicated in brackets

– not detected, *tr* trace < 0.05 mol%

*Terminal Fuc(p) is deduced from 1,5-di-O-acetyl-6-deoxy-2,3,4-tri-O-methyl hexitol, etc.

Table 2 Calculated polysaccharide composition (mol%) of mature cotton fibre cell wall fractions based on monosaccharide linkage analysis (Table 1)

	Relative proportion of polysaccharide in each fraction						Percentage of each polysaccharide across fractions				
Polysaccharide	**Total**	**CDTA**	**Na_2CO_3**	**1 mol·L^{-1} KOH**	**4 mol·L^{-1} KOH**	**Residue**	**CDTA**	**Na_2CO_3**	**1 mol·L^{-1} KOH**	**4 mol·L^{-1} KOH**	**Residue**
Homogalacturonan	1.0 (1.1)	30.9	19.0	1.7	3.4	0.0	62.1	33.7	1.5	2.6	0.0
Rhamnogalacturonan I	0.3 (0.1)	2.7	1.9	1.3	1.2	0.0	50.0	31.3	10.1	8.6	0.0
Arabinan	0.3 (0.3)	9.1	4.0	1.0	1.0	0.0	67.5	26.5	3.2	2.8	0.0
Type I Arabinogalactan	0.1 (0.1)	1.4	1.4	0.6	0.5	0.0	45.4	40.4	7.8	6.5	0.0
Type II Arabinogalactan	1.0 (1.0)	31.5	12.2	1.8	0.7	0.0	72.7	24.8	1.8	0.6	0.0
Xyloglucan	1.9 (0.4)	0.3	2.8	0.8	41.9	0.0	1.7	12.8	1.9	83.6	0.0
Callose	0.5 (0.4)	1.3	7.6	16.1	0.3	0.0	8.3	44.3	46.7	0.7	0.0
Heteromannan	2.8 (1.0)	2.2	6.6	1.2	2.9	0.9	4.8	13.0	1.2	2.5	78.6
Glucuronoxylan	1.2 (0.9)	0.2	1.6	43.1	25.2	0.2	0.6	3.7	50.1	25.4	20.2
Cellulose	90.4 (93.8)	2.7	34.8	16.1	14.6	98.9	0.1	0.8	0.2	0.1	98.9
Other	0.6 (0.8)	17.6	7.9	16.3	8.2	0.0	50.5	19.9	20.6	9.0	0.0

Values in brackets represent estimates based on the sum of polysaccharides in each fraction as a proportion of their recovery

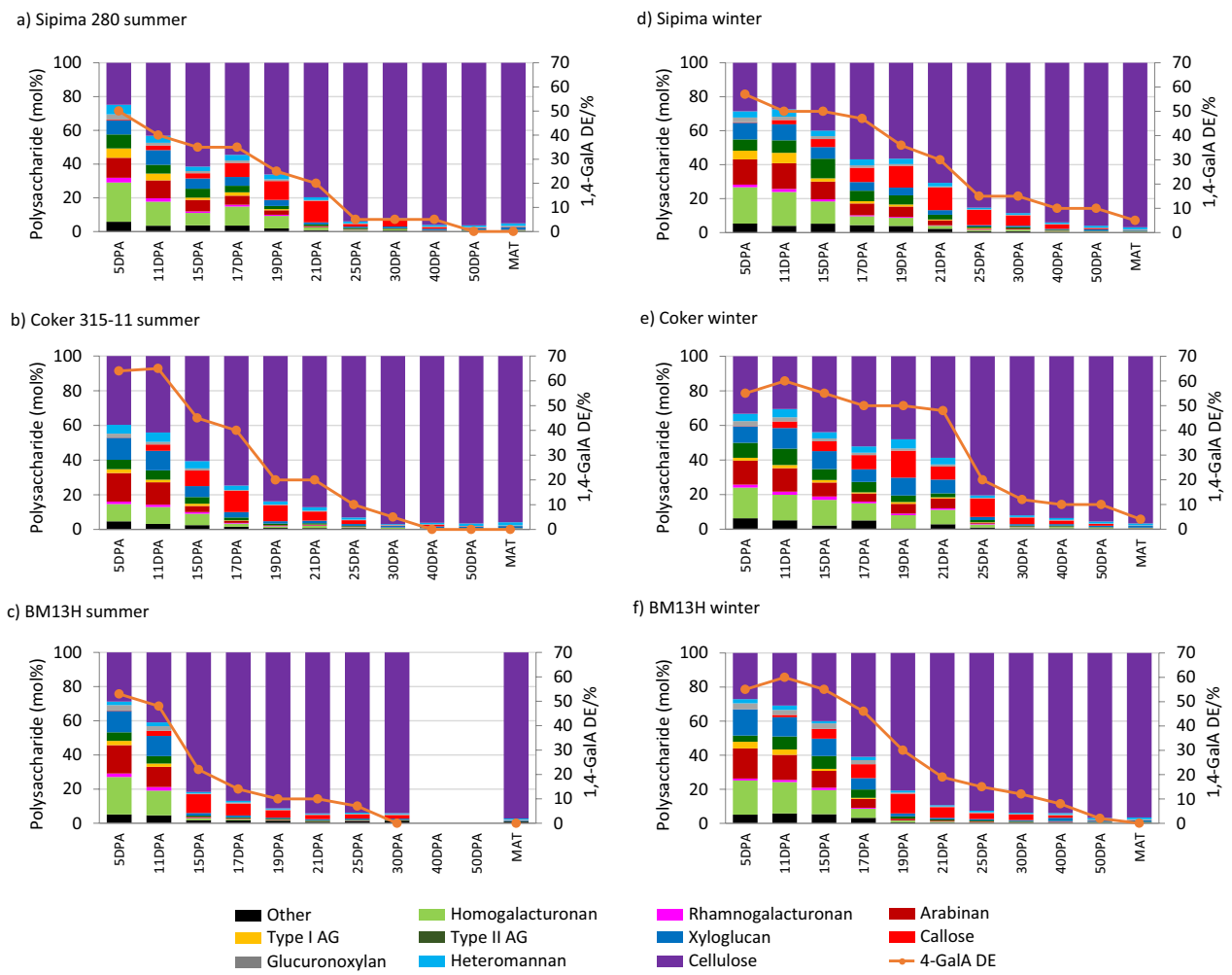

Fig. 1 Changes in polysaccharide composition (mol%) and pectin methylesterification (1,4-GalA DE) for **a** SiPima 280 (*G. barbadense*), **b** Coker 315-11 (*G. hirsutum*) and **c** BM13H (*G. arboreum*) cotton varieties through summer development and **d** SiPima 280 (*G. barbadense*), **e** Coker 315-11 (*G. hirsutum*) and **f** BM13H (*G. arboreum*) cotton varieties through winter development. Deduced from primary data in Additional file 1: Tables S1 and Additional file 2: Table S2

2%∼4% GX and less than 0.5% callose. As the fibre developed, the composition of the wall underwent a number of changes that were similar in all three species (e.g., compare Figs. 1 and 2, Additional file 2: Table S2). Cellulose increased massively, while XG, GX, HM and pectin and its neutral side chains (arabinan and AG) all decreased in relative terms. Callose also increased, but only until the transition into secondary cell wall development. The structure of the pectin also changed through development, with the degree of esterification (DE) decreasing from 50%∼65% at 5 DPA to 0∼5% at maturity (Fig. 1).

Despite showing the same general pattern of changes in the synthesis of specific polysaccharides throughout development, each of the three species progressed through fibre development and fibre cell wall biogenesis at different rates within any one growing season, as indicated by the temporal differences in the increase in fibre length, crystalline cellulose content and detailed changes in individual polysaccharide compositions (Figs. 1, 2 and Additional file 2: Table S2). Fibre from BM13H (Figs. 1c and 2c) progressed most rapidly through development, followed by Coker 315 (Figs. 1b and 2b) and then SiPima 280 (Figs. 1a, 2a), in line with their final fibre lengths. The differences in physical development could all be accounted for by differences in the biogenesis of different cell wall polysaccharides. BM13H, for example, had the most rapid proportional decline in content of non-cellulosic polysaccharides (including the pectic polysaccharides, as well as XG, GX and HM), and the earliest rapid deposition of cellulose that was also accompanied

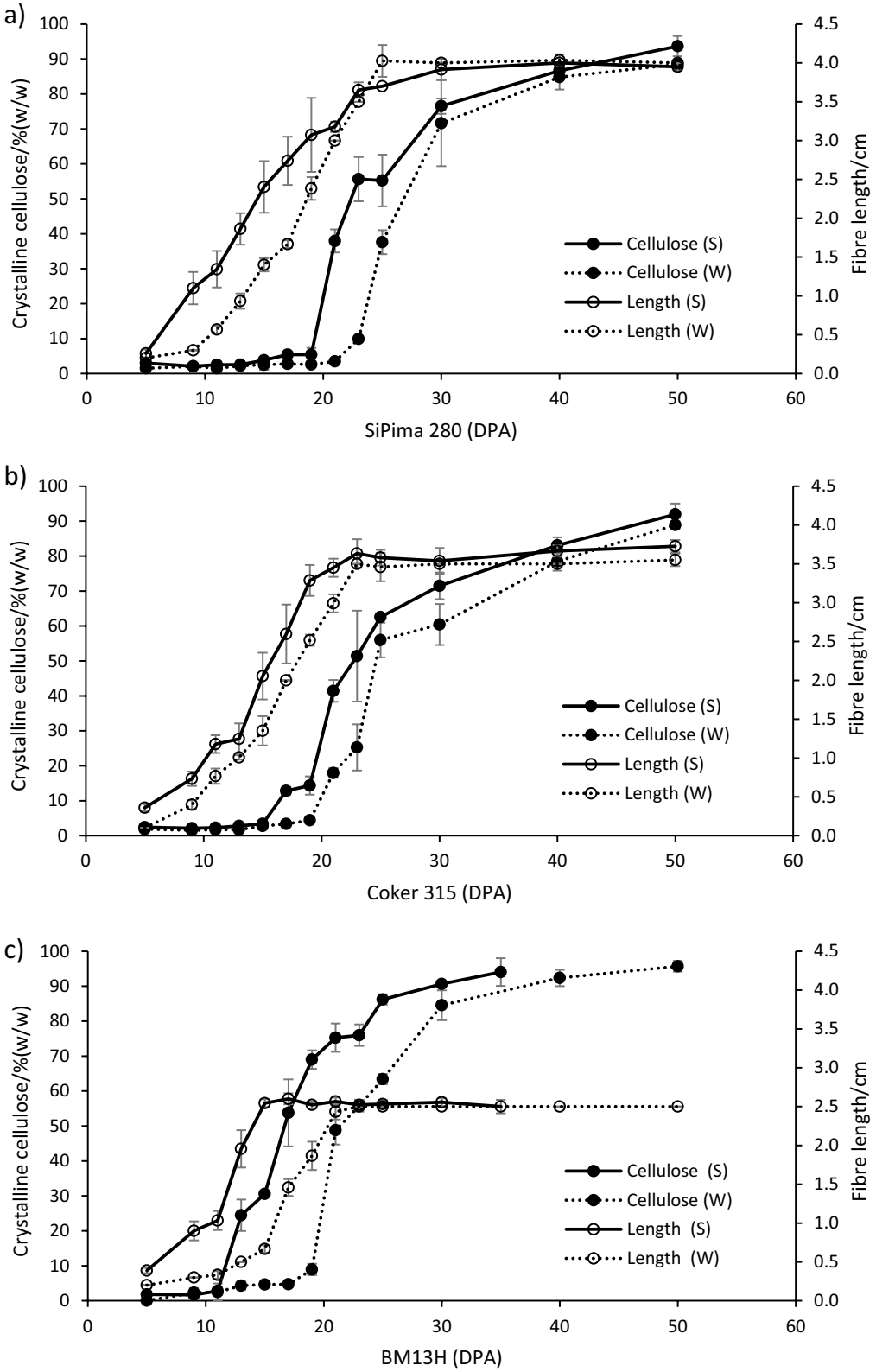

Fig. 2 Changes in fibre length (cm) and crystalline cellulose [% (w/w)], through development for **a** SiPima 280 (*G. barbadense*), **b** Coker 315-11 (*G. hirsutum*) and **c** BM13H (*G. arboreum*) cotton varieties over different seasons (W, winter; S, summer)

by an earlier peak in callose deposition in BM13H relative to Coker 315 and SiPima280 (Fig. 1, Additional file 2: Table S2).

Fibre cell wall polysaccharide compositional differences within a species grown in two different environments

In addition to the fibre cell wall compositional differences seen through development among the three species grown in any one environment, there were also obvious differences in the rate of fibre development and cell wall biogenesis between the two different "seasons" for each of those species. In the winter season, all three species took longer to reach their maximum fibre length and were later in depositing the crystalline cellulose of the SCW than the same species grown through the summer (Fig. 2). This coincided with a delay in the proportional decline in all the other polysaccharides (Fig. 1).

Fibre quality differences between species and environments

The quality of mature fibre (fibre length, strength, elongation, fineness and maturity) from each species grown over the two seasons was measured to investigate whether the different measured rates of fibre development and wall compositional changes impacted on the final fibre properties that are normally used to determine the commercial value of cotton. Despite all plants being grown in the glasshouse under similar conditions, there were some small variations in temperature due to the large outside seasonal variation in temperature at the site where the material was grown. Light in the glasshouse was not controlled, resulting in large differences in the quantity and quality of the solar radiation exposure of the plants between the "winter" and "summer" periods. Table 3 shows fibre quality data alongside recorded glasshouse temperatures, day length, solar radiation exposure and the calculated Day Degrees Post Anthesis (DDPA) by which crystalline cellulose levels in the SCW had at least doubled for the first time (a quantitative estimate of the onset of SCW deposition). There were clearly differences in fibre quality within the same species when grown at different times of the year (Table 3). Although the differences look to be quantitatively small, they were significant with respect to the differences considered in both valuing cotton commercially and in breeding. As expected, in both seasons, SiPima 280 fibre was much longer, finer and stronger than Coker 315 fibre, with BM13H having the shortest, coarsest and weakest fibre by these standard measures. The winter grown fibre was generally slightly longer, but was considerably finer and with greater elongation than its summer counterparts. Strength, however, was greater for the summer than winter grown fibres, at least for SiPima 280 and Coker 315. Linear regression analysis between the timing of crystalline cellulose deposition and glasshouse temperature, day length, solar radiation and an indicator of fibre development was performed and the R^2 value and corresponding significance value (P) were recorded in Table 3. Temperature (max and average) and solar radiation exposure showed a negative correlation with rates of fibre development, however solar radiation was the stronger correlation and was statistically significant. Fibre grown through the winter experienced around half or less of the solar radiation of the summer grown material.

Discussion

Cell wall composition of mature cotton fibre is complex and unique among plants

Summation of polysaccharides from each fraction based on weight recovery indicated that the total wall composition by monosaccharide linkage analysis was indicative of the polysaccharide composition determined by fractionation (Table 2). Polysaccharide estimation from a total cell wall fraction by-passed the need to fractionate the wall prior to compositional analysis, thereby simplifying the analytical process without loss of information. This provided confidence in the use of the method of polysaccharide estimation for analysis of the wall composition throughout development. Despite the mature cotton fibre being composed predominantly of cellulose, its polysaccharide composition is relatively complex, mostly due to the remnants of the PCW, but also from other polysaccharides embedded within the SCW. All of the polysaccharide components detected in this study have been identified in cotton fibre previously (Bowling et al. 2011; Singh et al. 2009; Buchala and Meier 1981; Maltby et al. 1979; Hernadez-Gomez et al. 2015) but their relative proportions are rarely reported due to the technical challenges of the methodology and analytical methods of detection have improved considerably over time making it opportune to re-visit. Fractionation of the mature cotton fibre by different solvents allowed for the easier identification of cell wall components and provided glimpses into the variety of structures present and, from their accessibility, how they might be arranged within the cell wall. Mature cotton fibre pectin is not purely HG, but a combination of HG, RG, and the associated neutral galactans and arabinans. Arabinans and galactans have been detected in young fibres (Singh et al. 2009), but it is apparent that a small proportion is retained in the wall through to maturity. Hayashi and Delmer (1988) described the structure of XG isolated from 19 DPA cotton fibre composed of Glc, Xyl, Gal, and Fuc in a ratio of 50:29:12:7. This differs from that seen in this study for XG from mature fibre which shows lower levels of Gal and Fuc relative to Glc. Monosaccharide linkage analysis of

Table 3 Fibre quality, crystalline cellulose deposition doubling timepoint (DDPA) and environmental conditions under which different cotton species were grown

Fibre sample	Length/inch	Length uniformity	Short Fibre index	Strength/ $cN \cdot tex^{-1}$)	Elongation/%	Mic	Mat	Fineness/ mTex	Solar radiation/ $(MJ \cdot m^{-2})$	Day length ave/h	GH temp min–max/°C	GH temp. mean/°C	Time to flower/d	Cellulose increase/ DDPA^
SiPima 280 (Spring/Summer)	1.38	85.0	5.4	55.6	5.7	4.2	0.87	155.9	24.4	13.7	13–37	27	59.8	247
SiPima 280 (Autumn/Winter)	1.41	85.9	5.3	52.6	5.9	4.1	0.87	145.8	11.1	10.7	19–35	26	54.9	315
Coker 315-11 (Spring/Summer)	1.23	85.0	8.1	33.5	7.5	4.8	0.87	190.8	24.4	13.7	13–37	27	57.1	195
Coker 315-11 (Autumn/Winter)	1.29	85.3	7.0	32.1	8.0	4.3	0.85	177.8	11.1	10.7	19–35	26	54.9	285
BM13H (Spring/Summer)	0.85	81.7	18.7	30.0	8.1	7.0	0.91	307.4	27.8	14.3	22–36	30	50.7	187
BM13H (Autumn/Winter)	0.89	81.6	15.7	30.7	8.5	6.5	0.90	300.2	11.1	10.7	19–35	26	52.4	255
Correlation with DDPA (R^2)	0.32	0.26	0.32	0.24	0.16	0.36	0.28	0.29	0.73	0.73	0.02–0.46	0.58	0.020	–
P	0.238	0.297	0.240	0.328	0.439	0.210	0.281	0.268	0.030	0.031	0.781–0.141	0.079	0.789	–

DDPA: Day degrees post anthesis, calculated as [(Max temp. − 12) + (Min temp. − 12)]/2

the XG-enriched 4 mol·L^{-1} KOH fraction also suggests that cotton XG is arabinosylated, as is seen in oleander and *Nicotiana plumbaginifolia* XGs (Hoffman et al. 2005; Sims and Bacic 1995). The callose extracted from cotton fibre walls with Na_2CO_3 is more branched (6% of the Glc exists as 1,3,6-Glc) than that extracted with 1 mol·L^{-1} KOH (3% branched Glc) which likely account for the difference in solubility of these two forms. Solubility differences and degree of backbone substitution is also evident for the GX present in cotton fibre, with the degree of GlcA backbone substitution decreasing with sequential extractions of increasing strength (Table 1; compare 1,4-Xyl, 1,2,4-Xyl, and t-GlcA) which is consistent with the tendency of this type of polysaccharide to form strong non-covalent interactions at regions of low substitution (Kabel et al. 2007; Simmons et al. 2016). Cellulose is, as expected, the major polysaccharide component of the mature cotton fibre and distinguishes it from other thickened specialised cells in dicots. Interestingly, 1,4-Glc(p) not already assigned to XG and HM is a major component of the Na_2CO_3 fraction. Starch was not detected in the mature fibre cell wall preparations with iodine staining (data not shown), consistent with observations by Maltby et al. (1979), however it is possible that small amounts are present and only obvious upon extraction and hydrolysis. Alternatively, the 1,4-Glc(p) could represent a soluble, non-crystalline form of cellulose, but further work is required to confirm this.

Fibre cell wall composition dynamically reflects function through development

The changes in cell wall composition of developing cotton seed fibre reflects the functional properties needed of the wall in moving from a readily extendable structure undergoing rapid rates of cellular elongation to a more solid, but still flexible structure of high tensile strength that is one of the longest plant cells in nature. The pectic material of the wall, initially highly abundant as the flexible matrix for cellulose microfibrils during fibre elongation, undergoes both abundance and structural changes through development, including a reduction in neutral arabinans and galactans and 1,4-GalA methylesterification. A small number of fibre-specific pectin methylesterase (PMEs) out of the almost one hundred PME genes found in cotton (Liu et al. 2013), have been identified that are responsible for this shift in the DE during fibre development. Each fibre PME is expressed at different stages of development suggesting that their roles are stage specific.

Even during the period of the most rapid increase in fibre length, the relative proportions of each of the wall polysaccharides changed reasonably consistently for each of the species. This suggested that the mechanisms responsible for cell wall changes with respect to polysaccharide synthesis, deposition and modification are the same for each species, but that they are regulated differently, resulting in the observed variations in the rate of fibre development between different species. A key biochemical event that appears to define the developmental differences between species is the timing of the onset of SCW cellulose deposition at the transition point. This increased cellulose deposition is coincident with a period of callose deposition spanning 5∼10 days that may be responsible for the so-called winding layer (Kerr 1946) that occurs between the PCW and SCW proper. The causal connections between callose deposition and increased cellulose deposition are still unclear but this should be an area for further investigation at the molecular level now that a number of cotton genome sequences have become available (Paterson et al. 2012; Li et al. 2014).

Environment has a significant impact on fibre cell wall development and affects final fibre quality

When grown under the same conditions the fibre of the three species tested here had the same general cell wall composition at maturity but developed at different rates (with the most dramatic difference observed for the diploid cultivar BM13H), so their genetics must determine the common biochemical events controlling the rate of fibre development in all three species. As these biochemical differences affect the quality of the end product fibre, the considerable effort put into breeding for improved fibre quality in cotton is justifiable. However, environmental conditions also have a significant role to play as seen in our analysis of the same varieties grown in different seasons. Environmental effects are known to account for much of the field variation in fibre quality observed for individual cotton varieties and poor seasonal conditions particularly during fruit boll development can have a major impact on the saleability of whole regions and even the national outputs of cotton.

Reduced solar radiation, or shading, has been reported to have an impact on fibre quality in field grown cotton, including increasing fibre length and decreasing micronaire through reductions in carbon assimilation via decreased photosynthesis (Eaton and Ergle 1954; Pettigrew 2001). This would explain the differences in rate of cell wall development observed in our summer and winter grown cotton as both light quantity and light quality were the main variables different between those seasons when grown in a temperature-controlled glasshouse. There was some variation in actual glasshouse temperature due to external temperature fluctuations between seasons. This was accounted for by using the day degree calculation (Constable and Shaw 1988) to determine the timepoint shift from PCW to SCW stages. The fibre

produced in the lower solar exposure environment had decreased micronaire and strength but was longer, finer, and had increased elongation (extension before breakage) compared with fibre from the same species grown under high solar radiation. Much of these changes could result from a later onset of SCW deposition that would allow the fibre to reach a longer length before becoming too rigid to elongate any further. The reduced carbon available for cellulose synthesis would result in a thinner and weaker SCW, but it is not clear how light quality is impacting on the timing of the onset of SCW cellulose deposition and that requires further research. These responses of SCW deposition to environment mean that there may be opportunities to manipulate fibre quality through management practices that shift the conditions in which the cotton fibre is developing on the plant.

Conclusions

Different cotton species with fibres having very different physical properties, such as length, strength and fineness, have very similar final contents of most polysaccharides, but differ in their rates of development and deposition of those polysaccharides. A key milestone in fibre development appears to be the onset of SCW deposition when large amounts of crystalline cellulose are laid down under the PCW to fill much of the internal contents of the fibre cell. The timing and rate of SCW formation can determine when the fibre cell stops elongating and so can influence the final physical properties of the fibre. Plants grown in the winter when there is a lower quality and quantity of light are delayed in the deposition of the SCW and this results in fibres that are longer and finer, but slightly weaker. A better understanding of the genes involved in specifying the timing of SCW formation may provide opportunities for improving fibre quality through breeding or biotechnology.

Methods

Plant materials

All plants were grown under glasshouse conditions in Canberra, Australia (35°18′27″ S 149°07′27.9″ E, 580 m) using natural light and with a temperature minimum set to 20 °C from 10:00 pm to 6:00 am, and maximum set to 32 °C from 6:00 am to 10:00 pm, although periodically departed from these set values because of the extremes in outside temperature. Environmental conditions were recorded directly (glasshouse temperature) or deduced from data provided by the Australian Bureau of Meteorology (www.bom.gov.au) for the Canberra region over the growing period. Plants were watered daily via an automatic watering system and fertilised with slow-release commercial fertiliser (Osmocote) just before flowering and then fertilised as required. To limit boll position influences the first and second flowers to emerge were tagged for harvesting of a minimum of 5 biological replicates at each time point. Flowers were tagged on the day of anthesis and harvested at 5, 9, 11, 15, 17, 19, 21, 25, 30, 35, 40, 50 DPA and maturity when bolls had fully opened. First flowers were used preferentially and only for 5 DPA when fibre mass was limiting was the second flower used. The samples of SiPima 280 and Coker 315 tagged from October to December in 2011 and BM13H tagged from December 2012 to January 2013 are referred to as "summer" samples and the samples of SiPima 280, Coker 315 and BM13H tagged from April to May 2012 are referred to as "winter" samples. At the time of harvest, the boll coat was removed and locules were immersed in liquid nitrogen. Fibre was removed from seeds under liquid nitrogen and then ground with a mortar and pestle under liquid nitrogen. The fibre released from older fibres (25 DPA—mature) was freeze-dried before grinding in stainless steel jars with a Tissue-Lyser (Qiagen).

Cell wall preparation as alcohol insoluble residue (AIR)

Ground fibre material was extracted consecutively with 70% ethanol (v/v) (three times), chloroform:methanol (1:1), 100% methanol and the AIR either stored in 100% ethanol or washed with 100% acetone before air drying. Cell wall preparations (AIR) were not de-starched as they were expected to contain little if any starch (Maltby 1979).

Mature fibre cell wall fractionation

The AIR of SiPima 280 mature fibre (152 mg) was fractionated by sequential extraction with 50 mmol·L^{-1} CDTA (cyclohexanediaminetetraacetic acid) in 50 mmol·L^{-1} sodium acetate (pH 6.5), 50 mmol·L^{-1} sodium carbonate (Na_2CO_3) with 0.3% (w/v) $NaBH_4$ (sodium borohydride), 1 mol·L^{-1} potassium hydroxide (KOH) with 0.3% (w/v) $NaBH_4$ and 4 mol·L^{-1} KOH/0.3% (w/v) $NaBH_4$. Each extraction was performed by stirring in extractant at RT for 6 h followed by a second extraction O/N. Between extractions, the residue was separated from the extract by centrifugation at 3 000 *g* for 10 min before the next treatment. The first and second extracts were pooled and neutralised with acetic acid, dialysed against water, or for the CDTA extract, against 100 mmol·L^{-1} ammonium acetate (pH 5.2) and then water, before freeze-drying. The final 4 mol·L^{-1} KOH residue was neutralised, washed twice with 70% ethanol and once with acetone before drying in a vacuum oven at 40 °C.

Monosaccharide linkage analysis and polysaccharide estimation

AIR preparations and mature fibre fractions were carboxyl reduced to include uronic acid and methyl-esterified uronic acid residues in the monosaccharide linkage analysis according to Pettolino et al. (2012). Due to the large number of replicate samples collected for the developmental series, 1 mg from each of five biological replicates was pooled for carboxyl reduction and subsequent methylation. Monosaccharide linkage analysis was performed by methylation (twice for AIR preparations and the KOH residue), hydrolysis, reduction and acetylation to generate partially methylated alditol acetates that were separated and identified by GC–MS. Polysaccharide levels were estimated according to Pettolino et al. (2012) by addition of appropriate deduced monosaccharide linkages for each polysaccharide based on knowledge from the wider literature and what was detected in cell wall fractions of mature fibre. AGP content was measured using the radial gel diffusion assay with β-glucosyl Yariv reagent as described by van Holst and Clarke (1985).

Fibre measurements

Fibre mass per seed was determined by weighing freeze-dried fibre removed from 3 seeds per sample. Fibre length through development was determined by boiling seeds with fibres intact in lightly acidified water (one drop of HCl in 25 mL of water) for 30 min then spreading the fibre out onto plastic film and measuring the distance from the seed centre to the fibre ends at 4 locations on the seed and for 3 replicates each. Dry fibre from the fibre mass determination was used to determine cellulose content by a modified Updegraff method (1969) using acetic acid: nitric acid as described in Burton et al. (2010) except that intact fibre was used directly. Mature fibre was tested for fibre quality on a High Volume Instrument (HVI) (Uster; Knoxville, TN) and fineness and maturity ratio were measured using Cottonscope (Rodgers et al. 2013).

Abbreviations

SCW: Secondary cell wall; PCW: Primary cell wall; DPA: Days post-anthesis; Gb: *Gossypium barbadense*; Gh: *Gossypium hirsutum*; Ga: *Gossypium arboretum*; AIR: Alcohol insoluble residue; DE: Degree of esterification; PME: Pectin methylesterase; HG: Homogalacturonan; RG: Rhamnogalacturonan; Type I AG: Type I arabino-3,4-galactan; Type II AG: Type II arabino-3,6-galactan; AGP: Arabinogalactan-protein; XG: Xyloglucan; HM: Heteromannans; HX: Heteroxylan; GX: Glucuronoxylan; Fuc(p): Fucopyranose; Rha(p): Rhamnopyranose; Ara(f): Arabinofuranose; Ara(p): Arabinopyranose; Xyl(p): Xylopyranose; Man(p): Mannopyranose; Gal(p): Galactopyranose; Glc(p): Glucopyranose; GalA(p): Galacturonopyranose; GlcA(p): Glucuronopyranose.

Supplementary Information

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Additional file 1: Table S1. Monosaccharide linkage analysis (mol%) for Coker 315-11, Sipima 280 and BM13H through development in different seasons.

Additional file 2: Table S2. Polysaccharide composition based on linkage analysis for cotton.

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Author contributions

Pettolino FA conceived of the study while Llewellyn DJ and Bacic A participated in the analysis of the data. Pettolino FA and Yulia D carried out the fibre growth measurements and chemical analyses. Pettolino FA drafted the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

All data generated or analysed during this study are included in this published article [and its Additional files 1 and 2].

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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