Rate and Amount of Cellulose Synthesis in Developing Fibers of *Gossypium arboreum* and *Gossypium hirsutum* Cotton

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ABSTRACT: Cotton fiber cellulose synthesis and cellulose content at various post anthesis maturity stages of boll' development were studied in sixteen cotton varieties of *Gossypium arboreum* and *Gossypium hirsutum* species, grown in the 2001 crop year in New Delhi, under identical agro-climatic conditions. Cellulose content was estimated using Updegraff's method. Field matured seed cotton was harvested at the end of the crop season and ginned fibers were subjected to physical testing in an AFIS-HVI facility. X-ray orientation parameters were computed from the normalized azimuthal diffracted X-ray intensity scans of (002) reflections. Simple correlations were worked out among various parameters measured. Cotton varieties were indeed observed to vary in their synthesized cellulose content and such differences are genetically inherent. Varieties of *Gossypium arboreum* synthesized less cellulose compared to the *Gossypium hirsutum* cultivars, although the rate of cellulose synthesis within each variety, regardless of species, was observed to be practically uniform at any five-day interval. The range of variation in cellulose content was observed to be more than 260%. Uniformity ratio, HVI tenacity and average leaf area were positively correlated with cellulose content. X-ray angles of 40% and 50% correlated significantly with a negative cellulose content, (thereby) indicating an increased orientation of cellulose crystallites to the fiber axis, with a greater amount of cellulose synthesized. © 2003 Wiley Periodicals, Inc. J Appl Polym Sci 90: 1453–1462, 2003

Key words: X-ray; orientation; strength

INTRODUCTION

Cotton consists of about 90–96% pure cellulose, which is almost wholly crystalline in nature.[1,2] In light of its purity, cotton cellulose has been the subject of extensive investigations, employing diverse physical and chemical techniques for biosynthetic pathways, length of polymer chain, degree of polymerization, density, crystallite size, crystal lattice type, unit-cell size and structure, orientation of crystallites within the microfibrillar polymer and within the matrix of diurnal secondary growth layers of developing cotton fibers.[1–7] All of these parameters have found practical applications in determining structure-property correlations and in assessing the technological performance of fibers with considerable success,[2,6,8–14] and yet, several aspects of the structure of native cotton fibers are still open to debate. Some such areas are the rate and amount of cellulose synthesis[6,7,15,16] in cotton varieties and species, and the orientation and disposition of crystalline cellulose to the fiber axis within individual diurnal secondary layers of developing cotton fiber, which are complicated by the generation of reversal extinction bands[17,18] and convolution twists.[18,19] There has been debate over the constancy or variation of this orientation among varieties and species of cotton.[20–22]

Among all of the physical properties of cotton fiber, micronaire (including maturity and fineness) and tenacity depend mainly on the property of the fiber's secondary wall and the disposition of cellulose within the diurnal secondary layers.[2,19,23,24] The exact mechanism of the deposition of cellulose within the matrix of developing cotton fiber and its influence on the strength of fibers is still not completely understood[25–28] although X-ray cellulose crystallite orientation parameters have been shown to correlate best with the tensile strength of cotton fibers.[2,6,10–14] However, recent investigations[14] have indicated that X-ray orientation parameters for individual cotton varieties were practically invariant when the varieties were grown at different agro-climatic locations and in different crop years. The only parameter, that was observed to vary within individual varieties with location of growth of cotton was the rate and amount of cellulose synthesized and deposited into the fibers.[7]

Several studies on cellulose biosynthesis in cultured and plant grown cotton fibers have been reported,[19,29,30] and these studies have led to the recognition of differing sensitivity to low temperatures shown by different varieties of cotton.[31,32] Factors, other than orientation of cellulose crystallites have also been shown to affect the tensile strength of fibers.[33–35] The rate of cellulose synthesis is directly related to night temper-

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TABLE I (A)
Data on Fiber physical Properties and X-ray Orientation Parameters of *Gossypium arboreum* Cotton Varieties

Serial No.	Variety	2.5% Span Length (mm)	MIC Micronaire	UR Uniformity ratio (%)	Elongation (%)	Maturity Ratio	Average Leaf Area (cm^2)	Bundle Tenacity g/tex (3mm gauge)		HVI Tenacity 3.2 mm (g/tex)	X-RAY ANGLES (*)		
											50% (002 Peak)	Normalized X-Ray Angle (002 Peak)	
								Pressley	Stelometer			40%	50%
1	HD-107	19.75	7.60	49.40	6.20	0.935	11.49	23.30	18.10	15.64	23.6	33.0	29.0
2	NCA-5	22.58	5.57	42.75	6.25	0.925	8.77	25.50	19.50	18.36	23.6	25.0	21.5
3	NCA-7	25.24	5.03	49.40	5.30	0.915	9.08	23.35	16.95	19.32	21.4	24.0	20.0
4	NCA-1	25.11	5.12	48.00	5.90	0.925	10.97	25.10	22.20	19.59	23.6	25.0	22.0
5	LD-327	21.13	7.00	51.05	6.25	0.940	20.35	23.70	16.80	16.72	22.9	25.0	21.0
6	HD-123	18.01	7.69	50.65	6.40	0.925	11.80	15.40	12.40	13.69	23.3	26.0	22.0
7	AAH-1	20.29	6.78	50.00	6.35	0.910	—	18.40	15.90	17.05	24.0	25.0	21.5

TABLE I (B)
Data on Fiber Physical Properties and X-ray Orientation Parameters of *Gossypium hirsutum* Cotton Varieties

Serial No.	Variety	2.5% Span Length (mm)	MIC Micronaire	UR Uniformity Ratio (%)	Elongation (%)	Maturity Ratio	Average Leaf Area (cm^2)	Bundle tenacity g/tex (3mm gauge)		HVI Tenacity 3.2 mm (g/tex)	X-RAY ANGLES (*)		
											50% (002 Peak)	Normalized X-Ray Angle (002 Peak)	
								Pressley	Stelometer			40%	50%
1	BB	17.16	3.78	46.00	4.75	0.86	24.73	20.55	17.95	20.43	22.15	25.0	22.0
2	RS-875	24.59	4.72	48.65	5.50	0.885	26.14	26.15	20.15	19.40	21.40	27.0	24.0
3	RS-2013	25.78	4.63	51.85	5.65	0.935	22.80	29.80	24.40	22.66	21.20	24.0	21.0
4	LH-1556	26.81	4.76	48.45	5.20	0.94	28.15	27.25	21.65	20.93	20.80	23.0	19.0
5	HS-6	25.13	5.26	52.20	5.50	0.935	31.24	28.05	24.60	22.31	20.65	21.5	18.0
6	PUSA 343	27.37	4.32	53.20	5.85	0.925	—	31.10	27.60	25.20	22.50	—	—
7	H-1098	24.76	4.64	49.20	4.66	0.885	24.09	23.55	18.50	18.95	19.50	22.0	19.0
8	PBKH-4	25.86	4.40	50.15	5.75	0.895	31.10	25.05	19.30	20.75	24.00	24.5	21.5
9	F-846	26.38	4.67	47.75	5.25	0.985	36.15	24.30	20.75	19.02	19.15	23.0	20.0

TABLE II
Cellulose Content of Cotton Fibers in Varieties of Cotton

DPA	HD-107	NCA-5	NCA-7	BB	NCA-1	RS-875	LD-327	RS-2013	AAH-1	LH-1556	HS-6	HD-123	Pusa-343	H-1098	PBKH-4	F-846
5	0.0200	0.0141	0.0133	0.0334	0.0124	0.0183	0.0183	0.0158	0.0158	0.0225	0.0208	0.0150	0.0212	0.0285	0.0396	0.0721
10	0.0083	0.0125	0.0141	0.0173	0.0208	0.0208	0.0235	0.0258	0.0266	0.0268	0.0390	0.0443	0.0475	0.0514	0.0542	0.0679
15	0.1101	0.030	0.0583	0.0663	0.0269	0.0946	0.0612	0.0546	0.0416	0.0981	0.0941	—	0.0779	0.0799	0.0677	0.0921
20	0.2100	0.0762	0.1322	0.2579	0.0961	0.3833	0.2011	0.2995	0.1594	0.1878	0.0880	0.1896	0.1794	0.1650	0.1250	0.1560
25	0.2783	0.2292	0.2435	0.5237	0.2790	0.4980	0.2834	0.5178	0.3321	0.3979	0.6959	—	0.5033	0.4733	0.2223	0.1436
30	0.2617	0.3078	0.2782	1.0521	0.2869	0.7679	0.5106	0.8121	0.4922	0.7443	1.0339	0.7888	1.5155	1.1152	0.5160	0.9158
35	—	0.3963	0.5301	1.0305	0.6312	0.8448	1.0389	1.1042	1.4196	—	—	1.0831	1.3753	—	1.3858	1.1521
40	—	—	—	1.5231	—	1.1763	—	1.2570	—	—	1.4176	—	—	1.5287	—	—

DPA - Days post anthesis
Content measured in grams cellulose/grams seed in cotton boll.

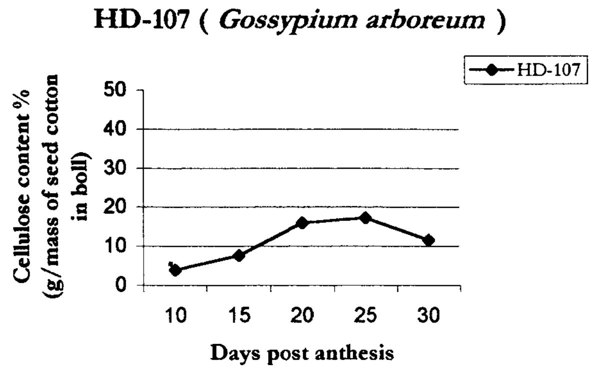

Figure 1 Progressive variation of cellulose content in seed fibers against days post anthesis.

ature, which is one of the greatest deterents to optimum fiber development. A decrease in temperature from the optimum causes numerous modifications in fiber structure and ultimately in the physical and chemical properties of the cotton fibers.[36–39] Furthermore, the sub-optimum night temperatures in the boll development process cannot be compensated by high day temperatures. The rate of cellulose synthesis, therefore, appears to be the key to cotton fiber structure[40–50] and a useful factor in determining the location specificity of cotton varieties, although the weight-molecular distribution of cellulose around the fiber axis has also been proposed to affect the tensile strength of cotton fibers.[50] With the advent of open-end spinning technology, the emphasis on breeding cotton varieties for increased staple length has reversed in favor of breeding types for increased tensile strength of fibers. One promising way to address this problem is to identify cotton genotypes for parental stock for hybridization, based on a higher rate of cellulose synthesis or amount of cellulose deposition in their fibers.

The objective of this investigation was to determine whether there are any inherent variations in the rate and amount of cellulose synthesis among cultivars of different species and if there is any variation, the

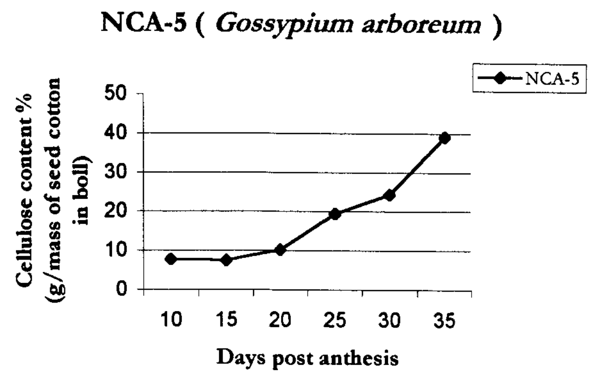

Figure 2 Progressive variation of cellulose content in seed fibers against days post anthesis.

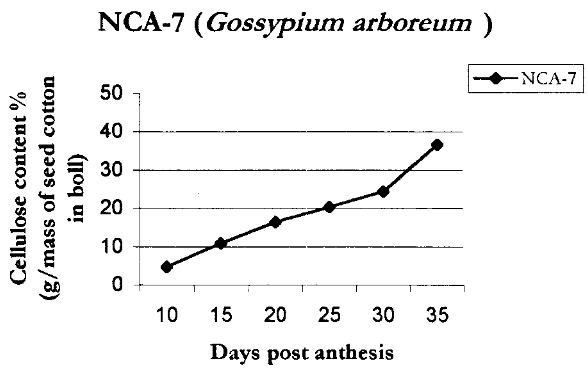

Figure 3 Progressive variation of cellulose content in seed fibers against days post anthesis.

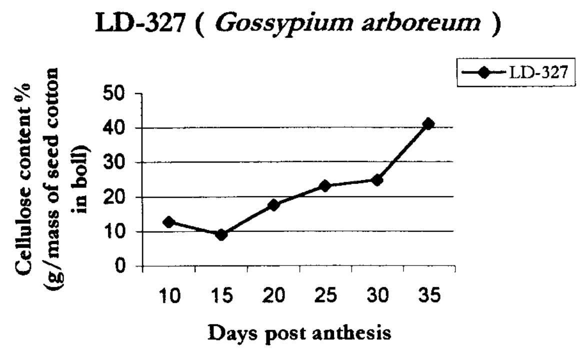

Figure 5 Progressive variation of cellulose content in seed fibers against days post anthesis.

extent of such variation within and among species of cotton and its relationship to the metabolic and physiological response of the cotton to uniform agro-climatic growth conditions. The results of examining seven varieties of *Gossypium arboreum* and nine varieties of *Gossypium hirsutum,* grown on the same farm under uniform agro-climatic conditions at New Delhi, in the 2001 crop year season, are reported below.

EXPERIMENTAL

Cotton varieties

Seven cotton varieties, of *Gossypium arboreum* called HD-107, NCA-5, NCA-7, NCA-1, LD-327, AAH-1 and HD-123, and nine varieties of *Gossypium hirsutum*, called LH-1556, PBKH-4, F-846, Bray Brown, RS-875, HS-6, H-1098, RS-2013 and Pusa-343, were grown on the Indian Agricultural Research Institute farm in New Delhi in 2001. Uniform agro-climatic conditions were provided for all varieties.

Flower tagging

Flowers were tagged every day in large numbers during the peak flowering period for all sixteen varieties.

Collection of green cotton bolls

Green developing cotton bolls at 5, 10, 15, 20, 25, 30, 35 and 40 days post anthesis, as measured from the date of flowering and tagging, were collected from all sixteen varieties and dehydrated at 65°C in an oven. The dry bolls were stored in a cool place.

Seed cotton

Field-matured seed cotton from all sixteen varieties was harvested at the end of the crop season and ginned on a CTRL-model laboratory gin. Lint fiber was collected for physical testing and fine structure study.

Physical testing of fibers

Lint cotton was analyzed for physical fiber properties and color with an AFIS-HVI facility. Independent evaluations of the same fibers, under different code numbers, were conducted from two test houses: the Central Institute for Research on Cotton Technology (ICAR) and the Bombay Textile Research Association, based in Mumbai. The data are given in Table I.

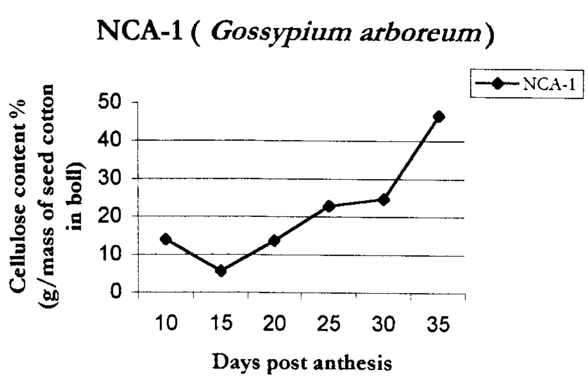

Figure 4 Progressive variation of cellulose content in seed fibers against days post anthesis.

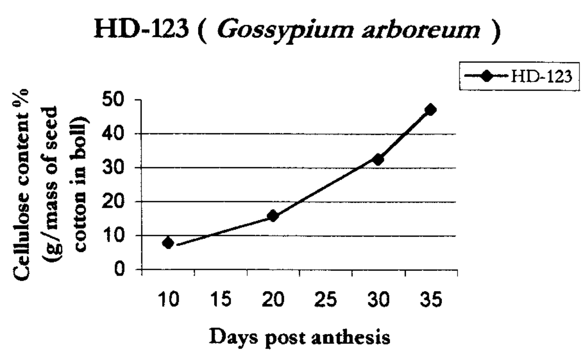

Figure 6 Progressive variation of cellulose content in seed fibers against days post anthesis.

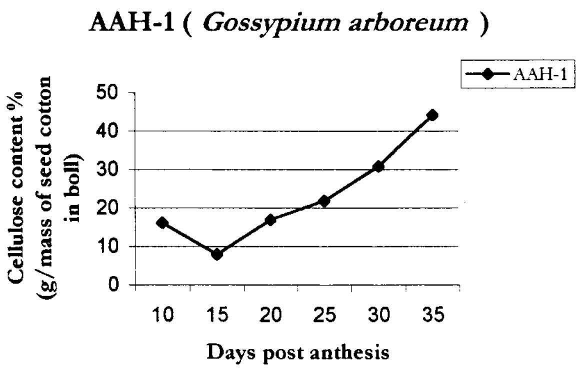

Figure 7 Progressive variation of cellulose content in seed fibers against days post anthesis.

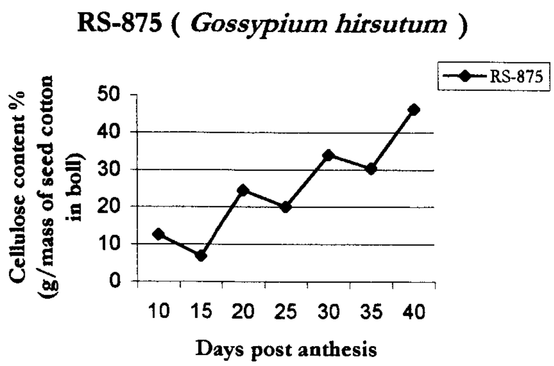

Figure 9 Progressive variation of cellulose content in seed fibers against days post anthesis.

Purification of cotton fibers

About 50 g of lint cotton from each of the sixteen varieties studied were purified for removal of waxes, pectic materials and protoplasmic residues by soaking for 6 h in carbon tetrachloride and methanol and then boiling in 2% sodium hydroxide for 3 hs. The boiled fibers were neutralized in 0.1*N* HCl for 1 h and then washed with distilled water.

X-ray diffraction

Azimuthal X-ray diffractograms were recorded from bundles of well-parallelized cotton fibers rotated through 360° in a plane perpendicular to the direction of X-ray radiations, keeping the glancing angles fixed at 2 θ by scanning through the range from 8° to 40° on a Philips model PW-1720 X-ray diffractometer using a 35 kV acceleration voltage, a 20 mA current and a Copper K_α (0.15418 nm) wavelength. The 50% X-ray orientation angle was calculated from both the half width of the diffracted X-ray intensity distribution curves corresponding to the intense (002) reflection, and also 40% and 50% X-ray orientation angles from the normalized intensity curves. The data are given in Table I.

Estimation of cellulose content

Cotton bolls harvested at various maturity stages and dehydrated in an oven at 65°C from all sixteen varieties were digested in 67% sulphuric acid, and the total cellulose content per gram mass of total seed cotton was estimated using Updegraff's spectro-photometric method[51] and the standard calibration curve of pure cellulose. Three replicates were taken for each stage of boll maturity for each variety, and the average values were recorded. The data are presented in Table II.

RESULTS AND DISCUSSION

It may be observed from Table that 2.5% span length varies from the lowest 17.2 mm for the Bray Brown variety to 27.4 mm for the Pusa-343 cultivar. Fineness was observed to vary from 3.8 for the Bray Brown to 7.7 for the HD-123 variety. Percentage of elongation varied from 4.6% for H-1098 to 6.4% for the HD-123 variety. The range of variation for uniformity was only 10%, between 42.7% for NCA-5 to 53.2% for Pusa-343. Likewise, the range of variation for maturity values was found to vary by only 10%. A 50% X-ray angle was observed to range from 18.0° to 29.0° for the most intense (002) reflection. Also, the 50% X-ray angles

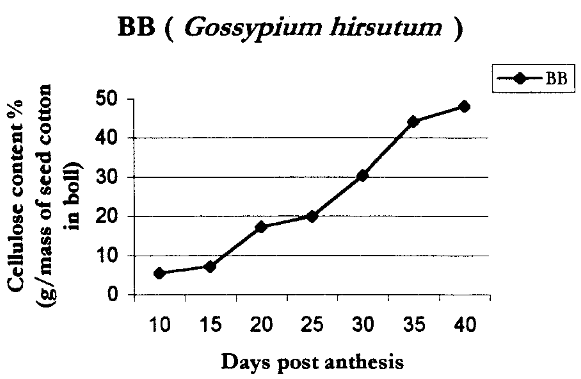

Figure 8 Progressive variation of cellulose content in seed fibers against days post anthesis.

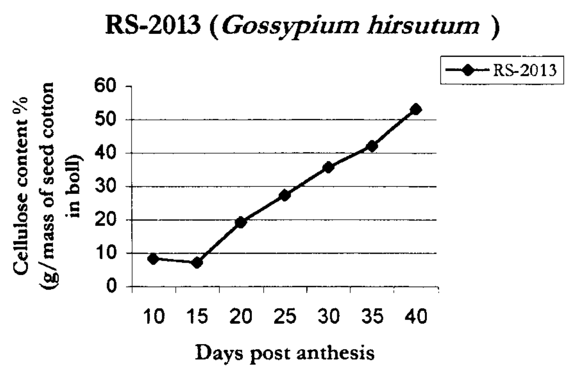

Figure 10 Progressive variation of cellulose content in seed fibers against days post anthesis.

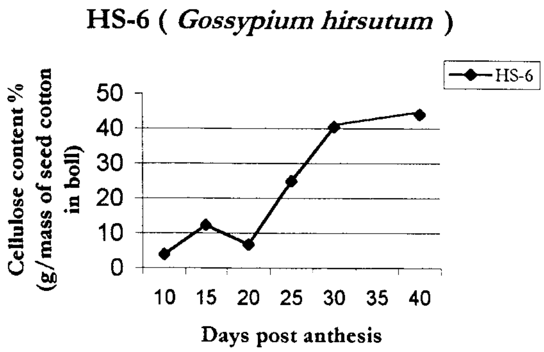

Figure 11 Progressive variation of cellulose content in seed fibers against days post anthesis.

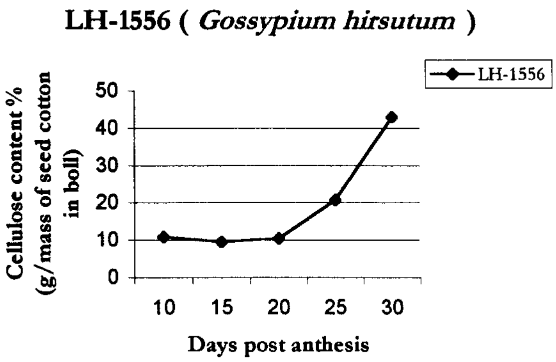

Figure 12 Progressive variation of cellulose content in seed fibers against days post anthesis.

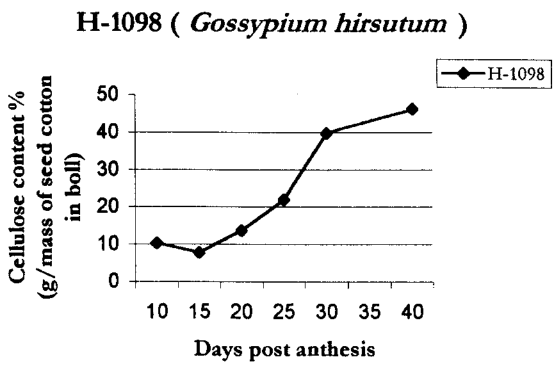

Figure 13 Progressive variation of cellulose content in seed fibers against days post anthesis.

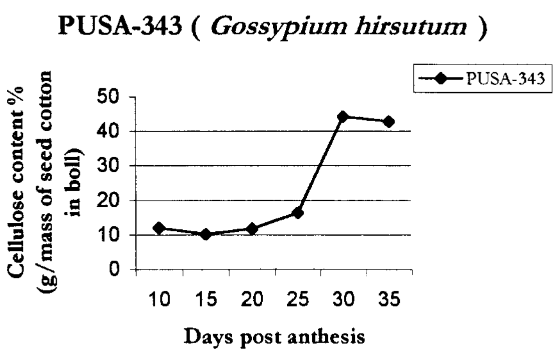

Figure 14 Progressive variation of cellulose content in seed fibers against days post anthesis.

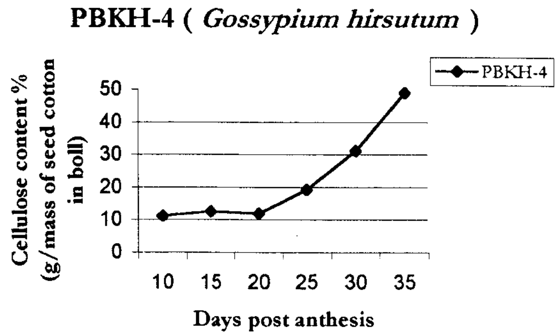

Figure 15 Progressive variation of cellulose content in seed fibers against days post anthesis.

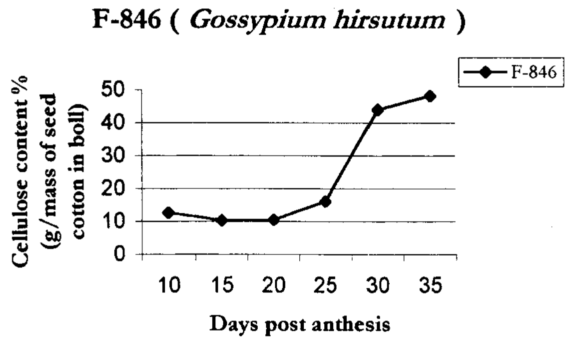

Figure 16 Progressive variation of cellulose content in seed fibers against days post anthesis.

TABLE III
Cotton Varieties Arranged in Order of Increasing Value for Average Cellulose Content at Any Five Day Interval

Serial No.	Variety	Average Cellulose Content at Any Five Day Interval (g/mass of seed cotton)
Gossypium arboreum		
1	HD-107	0.1480
2	NCA-5	0.1523
3	NCA-7	0.1813
4	NCA-1	0.1933
5	LD-327	0.3052
6	AAH-1	0.3553
7	HD-123	0.4241
Gossypium hirsutum		
1	LH-1556	0.2462
2	PBKH-4	0.3443
3	F-846	0.3713
4	Bray Brown	0.3851
5	RS-875	0.4755
6	HS-6	0.4841
7	H-1098	0.4917
8	RS-2013	0.5108
9	Pusa-343	0.5314

computed from the normalized X-ray intensity curves for the (002) peak for each variety *were* observed to be almost identical to the 50% X-ray angles computed from the half width of the (002) diffraction peaks, as shown in Table I. The values of the short fiber content

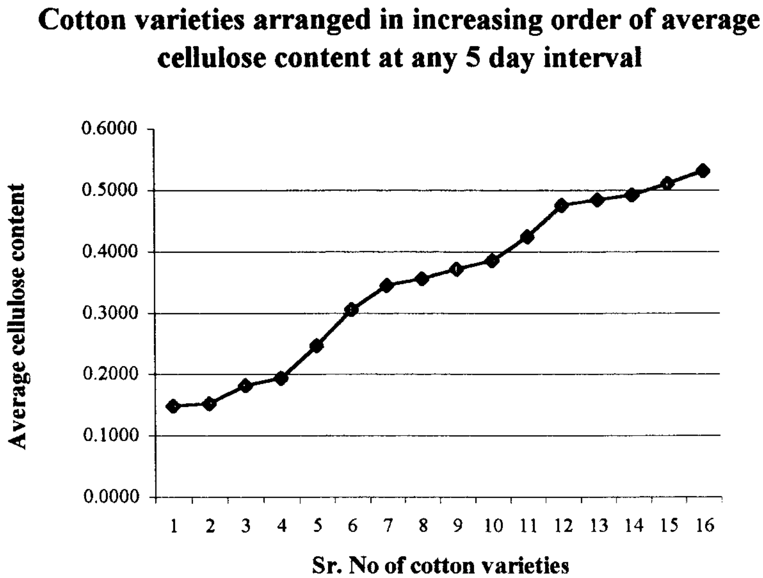

Figure 17 Cotton varieties arranged in increasing order of average cellulose content in fibers over any five day interval.

(SFC) was found to vary among varieties and species, but was also found to vary for fibers of the same variety from the same crop year and location of growth, as evaluated by two independent test houses. It is not certain if this wide variation is due to instrumental limitations or inherent instrumental inaccuracy of measurement of this parameter by HVI instruments.

It may be observed from Table II that the total cellulose content of cotton fibers varied both within and among species of cotton when grown at the same location, in the same crop season and under identical agronomic production conditions. Differences in the rate of cellulose synthesis are genetically inherent, as is evident from the very wide variation of about 260% between the lowest and highest values in the *Gossypium arboreum* and *Gossypium hirsutum* varieties. It may be observed from Table II and particularly from Figures 1–16, that in varieties of *Gossypium arboreum,* cellulose synthesis picks up rapidly between 15 and 35 days post anthesis, whereas in varieties of *Gossypium hirsutum,* cellulose synthesis picks up from 20–40 days post anthesis. Arranging the varieties of both species in order of increasing average cellulose content at any five day interval (Table III), it may be observed that the smallest value of 0.1480 g seed cotton corresponds to variety HD-107 and the highest value of 0.5314 g seed cotton corresponds to the Pusa-343 variety. A cursory look at the values would indicate that the varieties of *Gossypium hirsutum* synthesized more cellulose than the varieties of *Gossypium arboreum,* and the range of this variation among varieties of individual species was 216% and 286%, respectively. Similar conclusions were reported by Jihua Liu[19] and Hsieh-Youlo et al.[23] Figure 17 strikingly displays the inherent relative variation of the average cellulose content of fibers at any five day interval, arising as a metabolic and physiological response to

TABLE IV
Correlations of Days Post Anthesis (DPA) with Average Cellulose Content at Any Five Day Interval, Within Individual Varieties

Gossypium arboreum			*Gossypium hirsutum*		
Serial No.	Variety	Correlation	Serial No.	Variety	Correlation
1	HD-107	r = 0.7846	1	Bray Brown	r = 0.9178
2	NCA-5	r = 0.8439	2	RS-875	r = 0.9019
3	NCA-7	r = 0.8902	3	RS-2013	r = 0.9112
4	NCA-1	r = 0.9027	4	LH-1556	r = 0.8467
5	LD-327	r = 0.9013	5	HS-6	r = 0.8784
6	HD-123	r = 0.8969	6	Pusa-343	r = 0.8891
7	AAH-1	r = 0.8646	7	H-1098	r = 0.8999
			8	PBKH-4	r = 0.8987
			9	F-846	r = 0.8898

Note: All correlations are significant at $P > 0.01$

TABLE V
Linear Correlations of Various Physical and X-ray Orientation Parameters in Varieties of Gossypium arboreum and Gossypium hirsutum

	Pressley Tenacity	Stelometer Tenacity	HVI Tenacity	2.5% Span Length	MIC	UR %	Elongation %	MR	Av. Leaf Area	50% X-Ray Angle	Normalized X-Ray Angle 40%	Normalized X-Ray Angle 50%	Av. Cellulose Content
Pressley Tenacity	1	r = 0.927 P > 0.01	r = 0.832 P > 0.01	r = 0.794 P > 0.01	r = −0.562 P > 0.05	r = 0.230 NS	r = −0.21 NS	r = 0.154 NS	r = 0.363 NS	r = −0.318 P > 0.05	r = −0.259 NS	r = −0.218 NS	r = 0.207 NS
Stelometer Tenacity		1	r = 0.894 P > 0.01	r = 0.728 P > 0.01	r = −0.597 P > 0.5	r = 0.275 NS	r = −0.221 NS	r = 0.134 NS	r = 0.446 NS	r = −0.307 NS	r = −0.342 NS	r = −0.275 NS	r = 0.194 NS
HVI Tenacity			1	r = 0.700 P > 0.01	r = −0.811 P > 0.01	r = 0.269 NS	r = −0.432 NS	r = −0.152 NS	r = 0.540 NS	r = −0.334 NS	r = −0.567 NS	r = −0.494 NS	r = 0.427 NS
2.5% Span Length				1	r = −0.584 P > 0.05	r = 0.248 NS	r = −0.305 NS	r = 0.275 NS	r = 0.418 NS	r = −0.489 NS	r = −0.512 P > 0.05	r = −0.478 NS	r = 0.179 NS
MIC					1	r = 0.139 NS	r = 0.752 P > 0.01	r = 0.335 NS	r = −0.538 P > 0.05	r = 0.5 NS	r = 0.582 P > 0.05	r = 0.459 NS	r = −0.329 NS
UR %						1	r = 0.138 NS	r = 0.161 NS	r = 0.258 NS	r = −0.074 NS	r = −0.088 NS	r = −0.123 NS	r = 0.563 P > 0.05
Elongation %							1	r = 0.318 NS	r = −0.520 NS	r = 0.770 P > 0.01	r = 0.492 NS	r = 0.432 NS	r = 0.028 NS
MR								1	r = 0.078 NS	r = −0.217 NS	r = −0.054 NS	r = −0.114 NS	r = −0.013 NS
Av Leaf Area									1	r = −0.590 NS	r = −0.479 NS	r = −0.414 NS	r = 0.608 P > 0.05
50% X-Ray Angle										1	r = 0.554 P > 0.05	r = 0.532 NS	r = −0.418 NS
X-Ray Angle (002) 40%											1	r = 0.986 P > 0.01	r = −0.383 NS
X-Ray Angle (002) 50%												1	r = −0.354 NS
Av. Cellulose Content													1

uniform agro-climatic conditions of growth. The days post anthesis (DPA) shows a significant positive correlation of more than 0.80 to the average cellulose content at any five-day interval, within all individual varieties irrespective of species, as shown in Table IV. This indicates that the rate of cellulose synthesis, regardless of species and variety under identical agro-climatic conditions of growth, appears to be similar, although the actual amount of cellulose varies among varieties and species (Table II).

Table V gives the values of various simple linear correlation coefficients and probability values among the various parameters measured. It may be observed that a 2.5% span length correlates significantly with the Pressley, Stelometer and HVI tenacity values. Micronaire fineness showed significant negative correlation with all the three tenacity measures, indicating that all of the longer fibers are finer cottons and vice-versa. The uniformity ratio indicated no significant correlation with tenacity measures, span length or fineness. The elongation percent, however, showed significant positive correlation with micronaire fineness. The maturity ratio did not show any significant correlation with any of the parameters studied and listed in Table V. The average leaf area showed significant negative correlation with micronaire fineness. However, a direct consequence of this correlation is not clear. The average cellulose content also shows a negative, though not significant, correlation with 40 and 50% X-ray angles ($r = -0.383$ and $r = -0.354$, respectively), indicating that, with increased cellulose synthesized and deposited during cotton fiber development, the orientation of crystalline cellulose to the fiber axis also increases. This result seems to be logical in light of current knowledge and understanding of the structure of native cotton fiber. The 40 and 50% X-ray angles show negative correlations with all three tenacity measures. Although these correlations are not significant, their trend has been reported in several earlier publications.[6,10,11,21,22]

CONCLUSIONS

Cotton varieties do indeed vary in their amount of cellulose synthesized and deposited within the matrix of developing cotton fibers. Differences in the amount of cellulose synthesized and deposited into fibers are genetically inherent. Varieties of *Gossypium arboreum* synthesized less cellulose than varieties of *Gossypium hirsutum.* The cultivar Pusa-343 showed not only the highest cellulose content and the highest tenacity, but also the lowest value for 40 and 50% X-ray angles. This relationship in native cotton fibers has been derived from several publications referred to in the discussion above. The authors feel that it can be exploited by progressive cotton breeders in evolving new strains with increased fiber tenacity by incorporating cellulose synthesis as a direct parameter for screening parent genotypes for hybridization.

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