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Production of New Cotton Interspecific Hybrids with Enhanced Fiber Fineness

Nacoulima Lalaissa Nafissatou[1], Diouf Fatimata Hassedine[1], Konan N'guessan Olivier[2] & Mergeai Guy[1]

[1] Laboratory of Tropical Agroecology and Horticulture, Gembloux Agro-Biotech, University of Liège, Gembloux, Belgium

[2] Université Jean Lorougnon Guédé, Daloa, Côte d'Ivoire

Correspondence: Nacoulima Lalaissa Nafissatou, Laboratory of Tropical Agroecology and Horticulture, Gembloux Agro-Biotech, University of Liège, Passage des Déportés, 2-B-5030 Gembloux, Belgium. E-mail: nacoulima@gmail.com

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Abstract

To improve cotton fiber fineness, the (*Gossypium hirsutum* L. × *Gossypium longicalyx* Hutch. & Lee)² allohexaploid and the [(*Gossypium hirsutum* L. × *Gossypium thurberi* Tod.)² × *G. longicalyx*] allotetraploid were backcrossed to *G. hirsutum* to produce introgressed genetic stocks. The ribbon width (RW) of 600 swelled fibers produced by the hybrids, their parents, and their backcross progeny were analyzed for each compared genotype using an optical microscope. The RWs varied between 6.41±2.15 µm for *G. longicalyx* to 17.45±2.98 µm for the *G. hirsutum* parent cultivar C2. Fibers produced by the trispecific hybrids and their progeny were finer than the bispecific hybrid material. For the introgressed stocks, the lowest RWs were observed for the trispecific hybrid (10.79±2.14 µm) and certain backcross progenies (between 11.98±1.27 µm to 12.71±1.61 µm). The allohexaploid RW was 13.58±1.41 µm. One of its tetraploid progeny produced approximately the same value (13.94±2.48 µm). These results show that *G. longicalyx* is a potential genetic stock for cotton fiber fineness improvement. The genetic stocks produced are valuable materials for improve the fineness of cotton fiber.

Keyswords: *Gossypium*, interspecific hybridization, fiber fineness, RW

1. Introduction

Cotton fiber is an important raw material for the textile industry. The world production of cotton fiber was estimated at 119.7 million bales in 2012/2013 (USDA, 2013). The cotton fiber price is determined based on the fiber quality. The quality is defined by parameters, such as length, strength, fineness, maturity, and color. These parameters are established during fiber development. Among the fiber physical properties, fineness is considered as a major trait that determines the cotton fiber quality. This character influences the spinning limit, yarn strength, yarn uniformity and productivity. The fiber fineness determines the number of fibers required to spin a yarn at a particular thickness. The finer a fiber is, the more significant the quantity of fibers in the yarn cross-section. Spinning finer fibers produces stronger and uniform yarns (May, 1999). Long et al. (2010) found that the strongest yarns were produced from genotypes with the longest and finest fibers.

Cotton fiber fineness is expressed as the perimeter, diameter or RW, cross sectional area, and standard fiber weight (Gordon & Hsieh, 2006). Most cotton breeders and geneticists estimate cotton fiber fineness in terms of linear density (tex) values, which are conventionally determined using a gravimetric fineness method or AFIS, and micronaire values are measured using HVI. More recent methods have been verified for determining fiber fineness. The methods are based on an image analysis system (Huang et al., 2002), electromagnetic scattering using a laser system (Thomasson et al., 2009), light-scattering experiments (Aslan et al., 2003; Adedoyin et al., 2011) and vibroscopic techniques (Delhom et al., 2010). An instrument was recently developed using polarized light microscopy and image analysis, wherein a cottonscope measures the fiber RW, maturity and linear density (Rodgers et al., 2010, 2012).

The cotton genus *Gossypium* (Malvaceae) contains 45 diploid species (2n = 2x = 26) and five tetraploid species (2n = 4x = 52) (Fryxell et al., 1992). The diploid species are differentiated into eight genome groups designated by the capital letters A, B, C, D, E, F, G and K (Stewart, 1995). The sole F genome species, *G.*

longicalyx, is separated from the remaining genome groups based on its distinctive geography, morphology and ecology (Fryxell et al., 1992). All diploid *Gossypium* genomes contain an exclusive wild species, except for the A genome. The tetraploid species contain two distinct subgenomes related to the A and D diploid genomes (Wendel & Cronn, 2003). The tetraploid species *G. hirsutum* L., which is cytogenetically known as (A_hD_h) or $(AD)_1$, is the main cotton cultivated throughout the world; it accounts for 95% of the world lint production. The level of genetic diversity among agriculturally elite types of *G. hirsutum* is low (Gingle et al., 2006a, 2006b). Diploid species are an important reservoir for alleles of interest that can be exploited to improve cotton fiber qualities (Mergeai, 2006). The results generated thus far in numerous interspecifc breeding projects in different parts of the world show cryptic, beneficial alleles in wild germplasms and that multigenic traits can be introgressed through interspecific breeding using the main cultivated cotton species, irrespective of the donor species performance. According to Demol et al. (1978), cotton fiber fineness can be improved by using the African wild diploid species *G. longicalyx* Hutch & Lee. To verify this hypothesis, the (*Gossypium hirsutum* L. x *G. longicalyx*)² allohexaploid and [(*G. hirsutum* × *G. thurberi* Tod.)² × *G. longicalyx*] allotetraploid were created at GemblouxAgro Bio-Tech (University of Liège). The objective of this study was to evaluate the possibility of enhanced fineness through introgression in the backcross progenies of these two interspecific hybrids.

2. Material and Methods

2.1 Plants Material

The basic plant material includes the following:

i). The parents of the (*Gossypium hirsutum* L. × *Gossypium longicalyx*)² and [(*G. hirsutum*x *G. thurberi* Tod.)² × *G. longicalyx*] interspecific hybrids are the *G. hirsutum* cultivar C2 (2n = 4x = 52, $A_h A_hD_h D_h$) (G 107), RNR, and the two diploid species *G. longicalyx* (2n = 2x = 26, F_lF_l) (G 17) and *G. thurberi* (2n = 2x = 26, D_1D_1) (G 27). The G numbers correspond to the classification of the accessions and hybrids in the Gembloux Agro Bio-Tech cotton gene bank (Maréchal, 1983).

ii). Selfed progenies of MAALS (Monosomic Alien Addition Lines) obtained by Koto (1983) from the bi-specific hexaploid (*G. hirsutum*cv. C2 × *G. longicalyx*)² (G 368) were created in Gembloux using the scheme illustrated in Figure 1. 10 out of 13 possible MAALs of *G. longicalyx* on *G.hirsutum* numbered from I to XIII provided by the CIRAD (France) gene bank were used in this study.

iii). The BC1 and BC2 progenies of the tri-specific hybrid HTL {(*G. hirsutum* × *G. thurberi*)² × *G. longicalyx*} were created and backcrossed to the *G. hirsutum* var RNR variety following the pseudophyletic method (Mergeai, 2006) illustrated in Figure 2.

All plant materials were grown at the same time in Gembloux Agro Bio-tech greenhouses. The seed-cotton was randomly harvested without regard to the boll position.

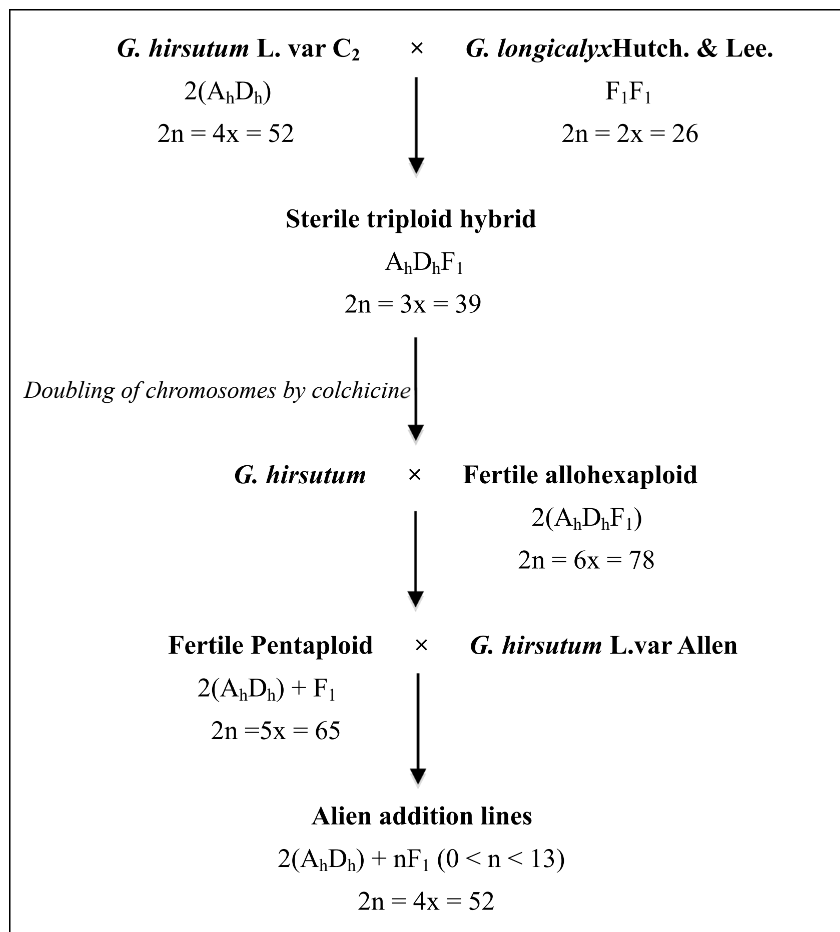

Figure 1. Scheme of the aphyletic method

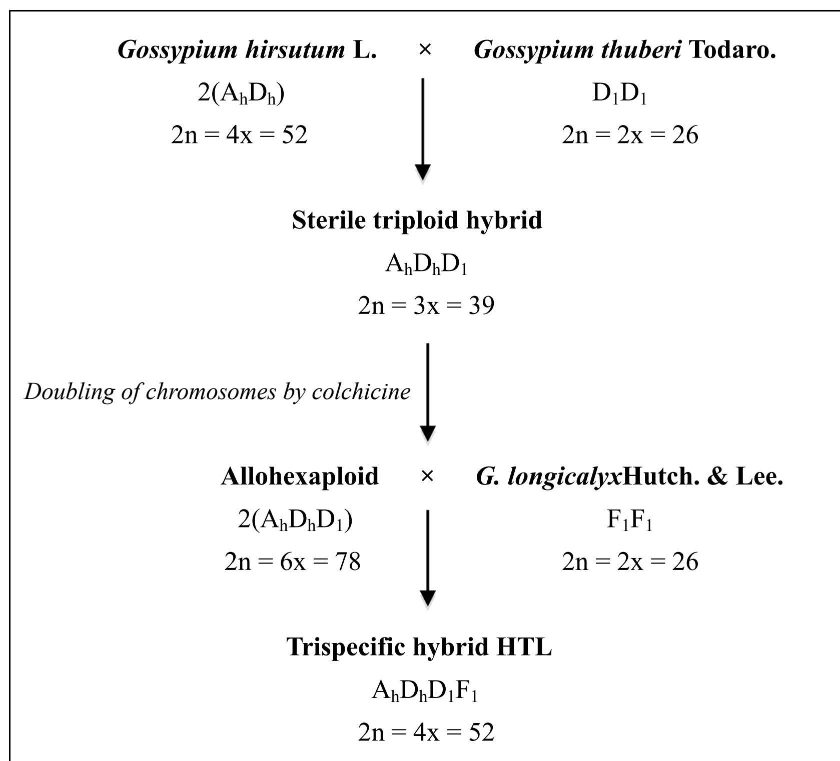

Figure 2. Scheme of the pseudophyletic method

2.2 Mitotic Observation

The karyotype of MAALS progenies likely to carry a supernumerary chromosome was determined based on their phenotype using mitotic cells and the method described by D'Hont et al. (1995). Roots were collected from growing seeds germinated in Petri dishes on filter paper at 30 °C for 48 hours. First, the roots were pre-treated in 0.04% Hydroxyquinoline at room temperature for 4 hours in the dark and were fixed at metaphase in Carnoy's I solution (glacial acetic acid 1: ethanol 3) for 48 hours. Thereafter, the roots were stored in 70% ethanol at 4 °C, the roots tips were digested in an enzyme solution (5% cellulose Onozuka R-10 and 1% pectolyase Y-23 in citrate buffer) at 37 °C for 1 hour, and they were compressed onto slides in a fresh fixative (3:1 ethanol: acetic acid). Chromosome preparations were visualized and counted under a fluorescent light microscope after staining with 4',6-diamidino-2-phenylindole (DAPI)/Vecta-shield.

2.3 Ribbon Width Measurement

RW measurements were performed on fibers produced by three seeds randomly harvested without regard to the boll position and opening date. Certain HTL progenies were quasi sterile and did not produced sufficient seeds; thus, the measurements were performed on 2 seeds. For each seed, the diameters of 200 swollen fibers were measured using the software NIS-Elements BR 2.30 and a Nikon Eclipse E800 microscope equipped with a digital Nikon camera. To perform the measurements, the fibers were straightened and paralleled through combing. The median region of the fibers was used for the observations. Each parallel fiber sample was drawn along a glass slide. The fibers were moistened and swollen in an 18% NaOH solution. The measurements were performed on one side of the slide, and the slide was moved across the field of vision to avoid repeating the measurements on any fiber based on Roehrich's (1947) recommendations. The fiber RWs were determined by dividing the mean of the measured diameters by the 1.3 Summers coefficient (Roehrich, 1947).

2.4 Statistical Analysis

The mean and standard deviation were calculated. The kurtosis and skewness of the fiber fineness frequency distributions (Pearson, 1895, 1905) were calculated using Minitab 17 Statistical Software 2010. ANOVA was performed to compare the RW means between and within genotypes. The multiple comparisons were examined for significance at $P < 0.05$ using Tukey's test, and the output was condensed into letter grouping.

3. Results and Discussion

Monosomic alien addition lines (MAALs) with only one chromosome from *G. longicalyx* in the genetic background of *G. hirsutum* and HTL trispecific hybrids containing the two wild diploid species genomes *G. longicalyx* and *G. thurberi* in a *G. hirsutum* genetic background were used in this study.

Seed setting and seedlings were obtained through backcrossing and selfing the MAALs progenies as well as the HTL trispecific hybrid to *G. hirsutum* (Table 1). The material morphological traits are also reported in Table 1.

The HTL plants presented no phenotypic segregation, and the BC1 plants exhibited segregation based on morphological characters. The leaf shape varied, and the number of lobes per leaf ranged from 1 to 5. The BC1 plant leaf size varied, but all were larger than the *G. thurberi* and *G. longicalyx* leaves and similar to *G. hirsutum*.

Most of the MAALS progeny plants presented *G. hirsutum* morphological traits. The remaining plants (38.5%) differed morphologically from the *G. hirsutum* plants. The MAALS progeny karyotypes were determined. RW measurements were performed on plants with 2n = 53 and plants with 2n = 52, presenting a sign of introgression.

Table 1. Seed germination and number of seedlings of plants

Line	Total number of seeds	Germinated seed(%)	Seedlings that survived(%)	Morphological traits
MAAL-I	14	71	40	Small plant, well-branched, small and dark-green leaves with 3-5 lobes, globular boll
MAAL-II	35	71	76	Glabrous plant, many small leaves with 3-5 pointed lobes, rounded three- or four-celled boll
MAAL-III	32	81	42.3	Slender stem, little branching, large leaves with 4-5 lobes, abundant anthers and pollen, few bolls produced, large amount of cottonseed per boll, ovoid capsule
MAAL-IV	22	50	63.7	Globular plant, few fruiting branches, small leaves, capsule globular and pointed
MAAL-V	29	79	82.6	Small plant, small leaves, slow growing, round boll
MAAL-VII	10	75	50	Light green leaves with 3-5 lobes, few fruiting branches, few bolls produced
MAAL-VIII	19	58	18.2	Small bushy plant, many vegetative branches, light green leaves with 3 lobes, small round boll with 3-4 lobes
MAAL-IX	30	70	38	Slow-growing plant, large leaves with 5-7 lobes, low pollen production, short fruiting branches
MAAL-XI	25	56	14.3	Dark green leaves with 3-5 lobes, many vegetative branches, few small bolls, large number of cottonseeds
MAAL-XII	13	77	70	Slender stem, small leaves with 3 lobes, few fruiting branches, large number of small globular bolls
HTL	10	90	100	Small plant, well-branched, small and dark-green leaves with 3-5 lobes, globular boll
BC1	27	48	92	Glabrous plant, many small leaves with 3-5 pointed lobes, rounded three- or four-celled boll

We examined the normalities of the fiber RW distributions for all genotypes, and skewness and kurtosis were used to characterize the symmetry and peakedness of the fiber RW distributions. The skewness coefficient measures the degree of symmetry in the variable distribution. A positive or negative skewness, respectively, indicates a long asymmetric right tail and a long asymmetric left tail (Doane & Seward, 2011; Hall, 2014). A skewness value of zero indicates normal distribution, whereas a skewness value greater than 1 indicates that the distribution differs significantly from a normal distribution.

Genotypes that present a negative skewness exhibit a distribution with more coarse fibers, while genotypes with a positive skewness exhibit more fine fibers.

Kurtosis measures the degree to which a distribution is more or less peaked compared with a normal distribution. A high kurtosis value indicates a relatively peaked distribution with a long tail, and a low kurtosis signifies a relatively flat distribution with thinner tails (Zenga, 2014). A high kurtosis indicates a narrow fineness range for the fibers.

The parental species *G. hirsutum* var. C2 and *G. longicalyx* exhibited positive skewness and opposite kurtosis. (Table 2). The *G. hirsutu*m var C2 presented a negative skewness with larger fibers than the wild parental species *G. longicalyx*.

MAAL-V # 1, MAAL-V # 1, MAAL-V # 1 and MAAL-XII # 4 exhibit skewness values near 0 (-0.08 and -0.06), corresponds to a normal distribution. Among the genotypes presenting a reduced RW, MAAL-F_l-II-8 and MAAL-III # 2 showed positively skewed distributions, which indicates more fine fibers than coarser fibers, while MAAL-XII # 6 and MAAL-F_l-IV-3 exhibited negatively skewed distributions with many coarse fibers. The Skewness and the kurtosis valus are both negative for MAAL-XI # 1. This MAAL may shown a wide range of coarse fibers.

Table 2. Skewness and Kurtosis values of MAALs and their parental species

Genotype	Skewness	Kurtosis
G. longicalyx	0.67	0.58
(*G. hirsutum* × *G. longicalyx*)²	0.10	-0.35
MAAL-I # 6	-0.13	-0.1
MAAL-I # 10	0.23	-0.19
MAAL-II # 8	0.20	0.09
MAAL-III # 2	0.13	0.01
MAAL-IV # 3	-0.41	-0.25
MAAL-V # 1	-0.08	-0.25
MAAL-VII # 3	0.65	0.74
MAAL-VIII # 10	0.40	0.83
MAAL-IX # 2	-0.44	0.21
MAAL-XI # 1	-0.14	-0.64
MAAL-XII # 4	-0.06	0.16
MAAL-XII # 6	-0.39	0.47

The genotypes MAAL-XII-6, MAAL-III # 2 and MAAL-IX # 2 exhibit approximately the same mean (13.97±1.65 µm, 13.94±2.48 µm and 13.85±2.79 µm) but different skewness (0.13, -0.39, and -0.44) and kurtosis values (0.01, 0.47, and 0.21).

The RW distributions for these cotton samples are shown in Figure 3 wherein the x-axis represents the fiber RW, and the y-axis represents the fiber frequencies. Despite exhibiting the same mean, the three genotypes presented different distributions. We presume that MAAL III # 2 contains more fine fibers than the remaining genotypes, which indicates that the mean cannot be used to differentiate the cotton fineness levels.

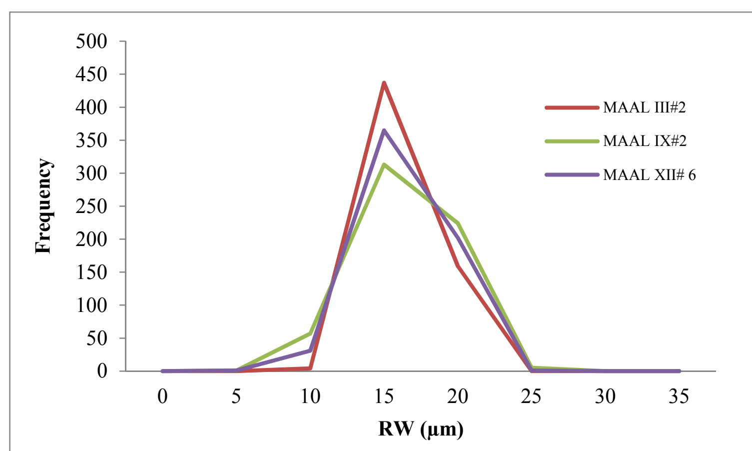

Figure 3. RW distribution of some MAALs

Statistical parameters such as skewness and kurtosis, are necessary to further characterize the RW of different cottons presenting the same mean. A fiber RW distribution that is symmetrical (skewness = 0) around the mean and as peaked (kurtosis > 0) as possible is necessary.

G. hirsutum var. RNR, G. *thurberi* and BC2-7 presented a leptokurtic distribution are positively skewed (< 1) and leptokurtic, which indicates a narrow range of distribution of fiber RWs less than the mean (Table 3). The hexaploid (*G. thurberi* × *G. longicalyx*) had a negative kurtosis (-0.16) a wide fineness range of fibers.

The HTL and the genotypes BC2-5 and BC1-6 exhibit a negative skewness and positive kurtosis value, which

indicates a narrow range of fiber RWs with more coarse fibers than the mean.

Table 3. Skewness and Kurosis value of HTL plants and their parental species

Genotype	Skewness	Kurtosis
G. hirsutum var RNR	0.01	0.13
G. longicalyx	0.67	0.58
G. thurberi	0.61	0.93
(*G. hirsutum* × *G. thurberi*)^2	-0.15	-0.16
HTL	-0.52	0.40
HTL F1 BC2-16	0.26	0.93
HTL F1 BC2-7	0.07	0.14
HTL F1 BC2-5	-0.13	0.26
HTL F1 BC1-6	-0.38	0.78

The mean RWs of the fibers were similar for the three seeds of *G. hirsutum* var C2 and HTL F1 BC2-16 plants (Tables 2 and 3).

Bradow et al. (1997) observed significant variations in Pee Dee cotton (*G. hirsutum* L.) fiber diameters, cross-sections, and circularities at the boll and locule levels measured on 2500 fibers with the AFIS lengths and diameter modules (Zellweger-Uster, Knoxville, TN). In this study, the overall average Pee Dee 3 fiber diameter was 12.7±0.9 µm. Studying the influence of seed location on fiber quality, Davidonis and Hinojosa (1994) found that fiber perimeters of apical seeds were thinner in the micropylar region than the chalazal regions, and, conversely, the fiber perimeter of basal seeds were larger in the micropylar region than the chalazal regions. These authors hypothesized that each fiber cell receives a certain allotment of assimilates for development.

G. hirsutum var. C2 presented an average fiber RW of 17.45±2.98 µm, and *G. longicalyx* exhibited the finest fiber with an average RW of 6.41±1.39 µm (Table 4). The hexaploid (*G. hirsutum* L. × *G. longicalyx*)^2 exhibited a RW of 13.58±1.41 µm. The MAAL-III # 2 (2n = 53 chromosomes) and the following euploids (2n = 52 chromosomes) MAAL-II # 8, MAAL-II # 8, MAAL-IX # 2 and MAAL-XII # 6 exhibited means similar to the hexaploid with, respectively 14.07±2.53 µm, 13.85±2.79 µm and 13.94±2.48 µm. The MAAL-XI # 1 exhibited the largest RW (18.09±3.56 µm).

According to Tukey's test, MAAL-III # 2 (2n = 53) and three introgressed stocks (2n = 52) produced through the bi-specific introgression method did not exhibit significantly different fiber RWs compared to the hexaploid (*G. hirsutum* L. × *G. longicalyx*)^2.

Table 4. Average RW (μm) of the fibers of the MAALs and their parental species

Genotype	Chr Nb	Genomic formula	RW±standard deviation			F value	RW±SD for 600 fibers	Tukey Grouping
			200 fibers Seed 1	200 fibers Seed 2	200 fibers Seed 3			
G. hirsutum var C2	52	$2(AD)_1$	17.59a±3.45	17.59a±2.41	17.17a±2.98	1.40	17.45±2.98	B
G. longicalyx	26	$2F_1$	6.00a±1.29	6.37b±2.20	6.84b±1.46	20.03*	6.41±1.39	H
(*G. hirsutum* × *G. longicalyx*)²	78	$2(A_hD_hF_1)$	13.41a±1.27	13.29b±1.15	14.03b±1.66	15.22*	13.58±1.41	G †
MAAL-I # 6	52	$2(AD)_1$+1	15.74a±1.40	15.39ab±1.51	15.92b±1.63	6.07*	15.69±1.53	D
MAAL-I # 10	53	$2(AD)_1$+1	15.31a±2.26	14.4ab±1.79	14.85b±2.07	8.64*	14.86±2.08	F
MAAL-II # 8	52	$2(AD)_1$	14.69a±1.74	13.80b±2.90	13.73b±2.69	10.79*	14.07±2.53	G†
MAAL III # 2	53	$2(AD)_1$+1	14.07a±1.65	14.52b±1.49	13.31c±1.59	30.32*	13.97±1.65	G†
MAAL-IV # 3	53	$2(AD)_1$+1	15.91a±3.02	17.79b±2.50	16.93c±2.90	22.49*	16.89±2.91	C
MAAL-V # 1	53	$2(AD)_1$+1	15.93a±2.75	16.01a±3.38	14.91b±3.39	7.83*	15.62±3.22	DE
MAAL-VII # 3	53	$2(AD)_1$+1	14.83a±1.51	15.31a±2.78	16.49a±2.68	1.86*	15.54±2.38	EF
MAAL-VIII # 10	53	$2(AD)_1$+1	15.67a±1.81	15.78b±2.99	16.86b±2.41	14.62*	16.10±2.51	D
MAAL-IX # 2	52	2(AD)1	13.46a±2.86	14.13ab±3.06	13.97b±2.39	2.94*	13.85±2.79	G†
MAAL-XI # 1	53	$2(AD)_1$+1	17.56a±3.88	18.14ab±3.44	18.31b±3.32	3.89*	18.09±3.56	A
MAAL-XII # 4	53	$2(AD)_1$+1	14.84a±3.46	15.11a±3.42	15.56a±3.08	2.74*	15.17±3.33	EF
MAAL-XII # 6	52	2(AD)1	13.96a±2.21	13.53ab±2.54	14.35b±2.62	4.94*	13.94±2.48	G†

Note. * Significant at the 0.05 probability level; † Means followed by the same letter are not significantly different from each other; Chr Nb: Chromosome number; SD: Standard deviation.

The tri-specific hybrid parents *G. hirsutum* (cv. 11240-RNR) and *G. thurberi* exhibited the RW 19.75±3.55 μm and 9.09±1.82 μm (Table 3), respectively.

The *G. hirsutum* variety RWs ranged from 15.1 to 17.5 μm, consistent with other studies, which indicate that Upland cotton fiber RWs vary between 17 to 20 μm (Roehrich et al., 1947) and 12 to 25 μm (Mishra, 2000).

The (*G. hirsutum* L. × *G. thurberi* Tod.)² allohexaploid presented a RW of 14.78±1.67 μm, which is consistent with Demol et al. (1978), who estimated that the fiber RW of the hexaploid (*G. hirsutum* cv. C2 × *G. thurberi*)² is 15.20 μm. These authors also reported RWs for allohexaploids involving *G. hirsutum* cv. C2 and certain wild diploid species. The mean RWs of hexaploids involving *G. anomalum* (B1), *G. sturtianum* (C1), *G. harknessi* (D4), *G. raimondii* (D3), and *G. areysianum* (E) genomes were 20.30 μm, 22.30 μm, 20.90 μm, 19.00 μm and 22.80 μm, respectively.

The HTL and HTL F1 BC2-5 RWs were, respectively, 10.79±2.14 and 12.71±1.61 μm. These values were lower than the average hexaploid RW; these genotypes carry several *G. longicalyx* and *G. thurberi* chromosomes in a *G. hirsutum* background. Tukey's test showed a significant difference between the progenies of [(*G. hirsutum* × *G. thurberi*.)² × *G. longicalyx*] hybrids and their parental species.

Table 5. Average RW (μm) of the fibers of the HTL progenies and parental species

Genotype	Chr Nb	Genomic formula	RW±standard deviation			F value	RW±SD for 600 fibers	Tukey Grouping
			200 fibers Seed 1	200 fibers Seed 2	200 fibers Seed 3			
G. hirsutum var C2	52	2(AD)1	17.59a±3.45	17.59a±2.41	17.17a±2.98	1.40	17.45±2.98	B
G. hirsutum var RNR	52	2(AD)1	17.95a±9.43	20.41a±3.62	20.88b±2.79	72.77*	19.55±3.55	A
G. longicalyx	26	2F1	6.00a±1.29	6.37b±2.20	6.84b±1.46	20.03*	6.41±1.39	H
G. thurberi	26	2(AD)1	8.81a ± 1.83	9.34ab±1.90	9.13b ± 1.71	4.38*	9.09±1.82	G
(*G. hirsutum* × *G. thurberi*)^2	78	$2(A_hD_hD_1)$	14.94a±1.70	14.86ab±1.68	14.55b±1. 66	3.96*	14.78±1.67	C
HTL	52	$A_hD_hD_1F_1$	12.52a±1.22	9.06b±1.31		651.51*kjm	10.79±2.14	F
HTL F1 BC2 - 16	52	$A_hD_hD_1F_1$	17.67a±1.64	17.28ab±2.16	17.03b±2.12	5.02*	17.32±1.94	B
HTL F1 BC2 - 7	52	$A_hD_hD_1F_1$	11.84a±1.42	12.12b±1.12	-	5.39*	11.98±1.27	E†
HTL F1 BC2 - 5	52	$A_hD_hD_1F_1$	12.68a±1.32	12.75a±1.86	-	0.026	12.71±1.61	D†
HTL F1 BC1 - 6	52	2(AD)1	12.83a±1.24	11.83b±1.09	12.33c±1.19	35.20*	12.33±1.24	DE†

Note. *Significant at the 0.05 probability level; † Means with the same letter are not significantly different from each other; Chr Nb: Chromosome number; SD: Standard deviation.

The coefficients of variation ranged from 9.26 to 21.44%; this value ranged between 21.04 and 21.44% for the wild species *G. longicalyx* and was lower at 12% for the hexaploid MAAL-F_l-I-6, MAAL-III # 2, and HTL F1-BC1. The high variation observed in this study is first due to the large variation within the cotton seed. A source of error with a lower effect may the microscope measurement, but the reliability of the fiber RW measurements greatly increased through mercerization of the fibers before the measurements.

Huang et al. (2002) performed longitudinal measurements of 25 cotton fiber samples from one bale; they analyzed 1.000 to 4.000 fibers for each sample using an imaging analysis method, and the coefficient of variation for the mean fiber width from one cotton bale was 2.91%.

Adedoyin et al. (2011) reported that the coefficient of variation from laser diffraction system measurements on 20 fibers from one bale of cotton was 9.35%.

Few studies have focused on cotton fiber quality measurements at the seed level because modern instruments require a large sample size, and non-instrumental measurement methods are tedious and time consuming. As a result, the full differences in fiber quality within a single plant and seed have not been estimated. The cotton scope was recently developed and only requires 50 mg of lint per replication, which would be useful for fiber fineness characterization at the seed level.

Baezinger et al. (2007) reported that to enhance the probability of genetic improvement for a specific trait of interest, the trait must be satisfactorily measured and heritable, and sufficient genetic variability must exist to ultimately realize long-term genetic improvement for the trait through plant breeding. Cotton fineness measured as perimeter, linear density or micronaire is moderately to highly heritable (May, 1999). Enhanced fineness is particularly important because selecting a smaller perimeter could facilitate selection of a low-micronaire, longer, and stronger fiber (Kloth, 1998).

According to Long and Bange (2012), RW provides the best average indication of fiber fineness or coarseness regardless of how the external fiber architecture is influenced by other components, such as convolutions, level of fullness affected by fiber maturity, and fiber perimeter. RW is an attribute that relates to how fibers will best pack together in yarn. RW is more related to yarn strength than either micronaire or gravimetrically determined linear density; that is, fibers with smaller RWs spin stronger yarns (Bange et al., 2010). These authors found that less mature cotton from a cooler growing season produced stronger yarns and fabric partly due to the fiber's smaller RW, which had a greater effect on fiber packing density and inter-fiber friction. RW measurements through microscope analysis remains a reliable and accurate means for highlighting the broad differences observed in the analyzed material.

Although a genetic basis for the fiber fineness is unknown, many studies have localized the QTL associated with this trait to 1 to 8 different chromosomes (Nacoulima & Mergeai, 2014), which indicates that the cotton fiber fineness is a trait controlled by several genes. The tri-specific introgression pathway is more suitable for introgression of genes located on several chromosomes (Mergeai et al., 1997). Under this approach, all of the chromosomes for the wild diploid species encountered the *G. hirsutum* chromosomes. The recombination frequency for homoeologous chromosomes between the donor species and the sub-genomes A_h and D_h is typically higher for trispecific hybrids than bispecific hybrids (Mergeai, 2006).

However, exploiting trispecific hybrids is difficult because this genetic material is highly unbalanced with low fertility. Linkages among desirable and undesirable traits by the donor and bridge species also complicate successful use of this genetic material. Several backcrossing and selection schemes are necessary to produce fertile offspring and eliminate uninteresting traits as well as fix the selected agronomic traits.

Within the (*G. hirsutum* × *G. longicalyx*)^2 allohexaploid progeny, three introgressed stocks (2n = 52) present enhanced fineness, which suggests that multiple introgressions likely occurred in these plants; thus, they are valuable material for fineness introgression into Upland cotton.

The introgressed stocks with enhanced fiber fineness are from three different MAALs. Presumably; one or several QTLs from the *G. longicalyx* chromosomes that influence fineness were integrated into the *G. hirsutum* background. Another hypothesis is that the different MAALs carry the same alien chromosome, which was preferentially transmitted to the pentaploid progenies. This phenomenon was observed in various studies (Ahoton et al., 2004; Benbouza et al., 2008; Sarr et al., 2012).

Recombination may also have occurred at the hexaploid level. *G. longicalyx* loci from different linkage groups were detected on different MAALs (data not shown), which highlights the importance of using molecular markers to monitor chromosome fragment introgression.

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