

Selection for Increased Fiber Length in Cotton Progenies from Acala and Non-Acala Types

Luiz Paulo de Carvalho,* Francisco José Correia Farias, and Josiane Isabela da Silva Rodrigues

ABSTRACT

Cotton (*Gossypium hirsutum* L. r. *latifolium* Hutch.) is one of the crops of greatest economic importance in Brazil. The changes in weaving technology, competition with synthetic fibers, and the globalization of cotton and textile production have increased the demand for better quality fibers. One of the characteristics to be improved is fiber length. Brazil traditionally produced and exported longer cotton fibers through the production of *G. hirsutum* L. r. *marie galante*, but this race has not been produced during the last 10 yr because of its perennial growth habit, which hinders control of the cotton boll weevil, *Anthonomus grandis*. This work studied some genetic aspects of fiber length in segregating generations of crosses between two upland cultivars, Guazuncho 2 (PI 606819) and Acala SJ4 (PI 529538). Single-seed descent protocol and pedigree breeding procedures were used to advance generations. The fibers of the recombinant inbred lines (RILs) and those originated by pedigree procedure were evaluated by high-volume instrument for length, lint percentage, and strength. The results suggest that it is possible to select materials with improved upper-half mean length (UHML) that have values approaching a commercial extra-long staple upland cultivar. A high percentage of the RILs also exhibited a UHML greater than 32 mm. Genetic gain for fiber length from the F_4 to the F_5 generation was 6.8%, indicating significant variability for UHML. The result indicates that simple methods of intrapopulational breeding may lead to genetic gains in UHML.

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Abbreviations: ELS, extra-long staple; HVI, high-volume instrument; RIL, recombinant inbred lines; UHML, upper-half mean length.

COTTON (*Gossypium hirsutum* L. r. *latifolium* Hutch.) is one of the crops of greatest economic importance in Brazil. One of the main goals of a cotton breeding program is selection of genotypes with the fiber quality required by the textile industries while maintaining yield potential (Hoogerheide et al., 2007). The changes in weaving technology, competition with synthetic fibers, and the globalization of cotton and textile production have increased the demand for better quality fibers (Smith et al., 2008). One of the characteristics to be improved is fiber length. Brazil produced 1,489,000 ton of lint cotton and exported 500,000 ton in 2013 through 2014 that averaged 28-mm upper-half mean length (UHML). Brazil traditionally produced and exported longer cotton fibers through the production of *G. hirsutum* L. r. *marie galante*, but this race has not been produced during the last 10 yr because of its perennial growth habit, which hinders control of the cotton boll weevil, *Anthonomus grandis*. To remain competitive in the global market and to supply the demand for upland cotton with longer UHML, breeding programs seek to develop cultivars with greater UHML. Along with the increase in the international standard for UHML, industries require greater resistance to breakage during processing, greater fiber bundle strength, decreased short-fiber content, greater uniformity of length, and mature fibers. All these traits are desirable for increased machine

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processing speeds (Smith et al., 2008). High-volume instrument (HVI) technology allows breeders to rapidly determine UHML and strength and thereby select among thousands of individual plants promptly and efficiently.

Studies seeking to evaluate genetic gains in relation to fiber length have been performed in different time periods. Miller and Rawlings (1967), Bridge et al. (1971), Bridge and Meredith (1983), Wells and Meredith (1984), and Culp and Green (1992) observed little or no increase in genetic gain for fiber length. Other authors, however, have observed genetic gains for this trait (Turner et al., 1976; Bayles et al., 2005). Turner et al. (1976) estimated genetic advances in fiber quality in some US breeding programs and found, based on regional variety test data, that most of them showed substantial gains for length. Bayles et al. (2005) evaluated twelve upland cultivars in multiple environments to estimate selection progress and observed increases in fiber length of 0.04 to 0.06 mm yr⁻¹ and of 0.02 mm yr⁻¹ for 2.5 and 50% span length, respectively.

According to Cotton Incorporated (www.cottoninc.com/fiber/quality/US-Fiber-Chart/Ratings-Of-Fiber-Properties/), extra-long staple fiber (ELS) of upland cotton in the United States exhibit a UHML value ≥ 32 mm, while pima cottons (*G. barbadense* L.) traditionally are referred to as ELS cotton and the shortest nondiscount UHML is 34.9 mm. Practically 100% of Brazilian upland cotton production is classified as medium UHML. Average UHML of cotton produced in Brazil in 2013 was 28 mm with little long or extra-long fiber cotton production during the last 10 yr. For that reason, it is important that breeding programs focus on the selection of these types of cotton to supply the expected future demand.

The objective of this study was to evaluate a breeding population grown for improvement of UHML and to analyze the genetic gains from selection of UHML.

MATERIALS AND METHODS

Two cultivars were chosen for this study: Guazuncho 2 (PI 606819), for its yield capacity; and Acala SJ4 (PI 529538), for its long UHML. These two parents were crossed in 2008 and advanced to the F₂ generation at Campina Grande, PB, Brazil. Soil type was a regosol and standard production practices were followed.

Single-seed descent protocol was used to produce 67 F₅ recombinant inbred lines (RILs), with all generations grown under greenhouse culture at Campina Grande, PB, Brazil from 2009 to 2013. These 67 F₅ plants, referred to hereafter as RILs, were harvested individually and seed-cotton ginned on a roller gin. The fiber of each plant was divided in two lots and HVI fiber properties determined at Campina Grande. The data originating from this HVI determination were analyzed as a completely randomized design with two replications.

Pedigree breeding procedures were used to advance additional F₂ seeds of Guazuncho 2/Acala SJ4 to the F₄ generation through self-pollination under field cultural practices. The F₃ generation was field grown at Campina Grande without irrigation. Soil type was a regosol. Fifty-six F₃ plants were harvested

and their F₄ progenies were planted in 5-m rows, without replication, at Itatuba, PB, Brazil in 2011. Cultural practices were standard for this area of Brazil and included insect control and irrigation. Soil type at Itatuba was an orthic chromic luvisol. Approximately five plants were selected randomly from each of the 56 progeny rows for a total of 271 F₄ plants. Seed-cotton was ginned on a roller gin at Campina Grande and HVI fiber properties determined at EMBRAPA (Brazilian Agricultural Research Corporation). Fifty-one F₄ plants were selected based on HVI UHML ≥ 31 mm and their F_{4:5} progenies planted in the greenhouse in 3-m length rows at Campina Grande, PB, Brazil, in 2013. Two samples of 12 bolls each were harvested from each of the F_{4:5} progeny rows at maturity, with seed-cotton ginned and HVI fiber properties determined as described above. Adopting the criterion of selecting lines with UHML ≥ 32 mm, 34 F_{5:6} lines were selected for this trial. These lines were evaluated in the field in Ipanguaçu, RN, Brazil, in a randomized complete block trial with two replications grown with irrigation. Cultural practices were those used in the region and the soil in Ipanguaçu was an oxisol red yellow-eutrophic. At maturity, hand-harvested samples consisting of 20 bolls each were taken from each plot, with seed-cotton ginned and HVI fiber properties determined as described above.

Using the F_{4:5} and F_{5:6} progenies tests described above, a variance analysis was conducted for each generation and heritability (H^2) in the broad sense estimated based on the mean value of the progenies ($H^2 = \sigma_g^2 / \sigma_f^2$) as well as based on parent-offspring regression in the F₄ generation on the F₅ generation and calculated according to Smith and Kinman (1965):

$$H_{\text{reg}}^2 = \frac{b}{2r_{F_i, F_{i+1}}} = \frac{b}{1 + F_t} = \frac{b}{1 + F_4}$$

where

$r_{F_i, F_{i+1}}$ = relationship of parent-offspring,

$b = \frac{\text{cov}(F_4, F_5)}{V(F_4)}$ = regression coefficient,

$F_t = 1 - \left(\frac{1}{2}\right)^{t-2}$ = inbreeding coefficient of the t generation,

$\text{cov}(F_4, F_5)$ = covariance between the values of the individual F₄ plants and the mean values of the F₅ progenies, and

$V(F_4)$ = variance of the F₄ population.

The genetic gains obtained from the selection were calculated by the expression

$$G_s = H_{(i)}^2 DS_i$$

in which

G_{s_i} = gain from direct selection made in the i generation,

$H^2_{(i)}$ = heritability in the i generation, and

DS_i = selection differential based on the genotypes of best performance.

In the expression of gain, the genetic variance expressed in the F_4 generation, $\sigma_g^2(F_4)$, was calculated indirectly from the genetic variance in the F_5 generation obtained in analysis of variance:

$$\sigma_g^2(F_4) = 2F\sigma_a^2 + F(1-F)\sigma_d^2$$

where

σ_a^2 = additive variance,

σ_d^2 = variance attributed to the dominance deviations, and

F = inbreeding coefficient considering that it is equal to 3/4 and 7/8 in the F_4 and F_5 generations, respectively.

Disregarding the nonadditive part or making $\sigma_d^2 = 0$, we have

$$\sigma_g^2(F_5) = \frac{7}{4}\sigma_a^2,$$

$$\sigma_a^2 = \frac{4}{7}\sigma_g^2(F_5),$$

$$\sigma_g^2(F_4) = \frac{3}{2}\sigma_a^2 = \frac{6}{7}\sigma_g^2(F_5), \text{ and}$$

$$\sigma_g^2(F_5) = \text{MSG} - \text{MSR} / 2,$$

where MSG is the mean square of genotypes and MSR is the mean square of the residue.

To estimate heritability in the F_4 generation, it was used as phenotypic variance (V_{F_4}), an estimate made from the 51 F_4 plants selected in Itatuba and lately evaluated in a greenhouse. The variance analysis of data and the other estimates were made using the program GENES (Cruz, 2001) from Federal University of Viçosa, Brazil.

RESULTS AND DISCUSSION

As noted above, 51 F_5 lines were selected from the F_4 population derived by pedigree breeding. From these 51 $F_{4:5}$ lines, 34 were selected to advance to $F_{5:6}$ lines. Genetic and total variance components from the analysis of variance of

Table 1. Phenotypic, genetic, and environmental parameters obtained from the F_5 generation for genotypes evaluated for upper-half mean length.

Parameter†	Estimate
σ_f^2	1.09
σ_g^2	0.60
σ_e^2	0.48
H^2	55.40
Interclass correlation	38.30
CV_e/CV_g	0.78

† σ_f^2 , phenotypic variance; σ_g^2 , genotypic variance; σ_e^2 , environmental variance; H^2 , heritability in the broad sense; CV_e , coefficient of environment variation; CV_g , coefficient of genetic variation.

Table 2. Phenotypic, genetic, and environmental parameters obtained from the F_6 generation of genotypes evaluated for upper-half mean length.

Parameter†	Estimate
σ_f^2	1.94
σ_g^2	1.17
σ_e^2	0.77
H^2	0.60
Interclass correlation	43.18
CV_e/CV_g	0.87

† σ_f^2 , phenotypic variance; σ_g^2 , genotypic variance; σ_e^2 , environmental variance; H^2 , heritability in the broad sense; CV_e , coefficient of environment variation; CV_g , coefficient of genetic variation.

Table 3. Direct gain from selection in F_4 and F_5 generations for upper-half mean length in cotton.

Gain	X_s^\dagger	X_o^\ddagger	$H^2\%$	G_s^\P	G_s
F_4 direct	31.92	29.26	0.75	1.99	6.80
F_5 direct	32.60	32.44	0.55	0.29	0.90

† X_s , mean value of the lines selected.

‡ X_o , mean value of the lines evaluated.

§ H^2 , heritability, in the broad sense.

¶ G_s , gains from selection.

the F_5 and F_6 generations indicated broad-sense heritabilities (H^2) for UHML of 55 and 60%, respectively (Table 1, 2), for fiber length, showing the possibility of genetic gains from intrapopulational selection. The heritability value in the F_4 generation (Table 3) of 75% was the greatest among the others estimated, which may be attributed, among other causes, to the large variability found for fiber length in the F_4 , the generation in which selection began. The genetic gain derived from the 51 lines, as explained in the materials and methods section, was 6.8% (Table 3).

Differences were observed among the 51 $F_{4:5}$ lines ($P < 0.05$), allowing one to infer that there is still genetic variation among these lines in regard to UHML and that additional genetic gain from selection for UHML in this population is possible. The results of the analysis of variance shown in Table 4 allowed estimation of the phenotypic, genotypic, and environmental parameters, as well as the ratio between CV_e and CV_g (Table 1). Although this ratio

Table 4. Summary of analysis of variance for upper-half mean length in the F_5 generation.

Source of variance	df [†]	MS [‡]
Genotypes	50	2.18**
Residue	51	0.97
Mean	32.44	
CV _e [§] (%)	3.04	
CV _g [§] (%)	2.32	

** Significant at the 0.01 probability level by the F test.

[†] df, degree of freedom.

[‡] MS, mean square.

[§] CV, coefficient of variation.

Table 5. Mean values and phenotypic variances of selected lines and heritability through parent–offspring regression method for upper-half mean length.

Description [†]	Estimate
Number of genotypes	51
$F_{4(P)}$ means (51 plants selected in the field, Itatuba)	31.92
$F_{5(F)}$ means (51 progenies evaluated at the greenhouse)	32.44
σ^2_p	0.68
σ^2_f	1.09
COV(P, F)	0.38
Correlation	0.44
$H^2_{(SK)} (%)$	0.32

[†] σ^2_p , parent phenotypic variance; σ^2_f , progeny phenotypic variance; COV(P, F), parent–progeny covariance; $H^2_{(SK)}$, heritability estimated according to Smith and Kinman (1965).

was less than one, genetic variance is still considerable, indicating possibilities of gains in UHML from selection. A breeding program depends primarily on the quantity of genetic variation and particularly on its value in relation to the total phenotypic value (Berti et al., 2011). A large part of the phenotypic value was explained by genetic variation in the F_5 generation (Table 1). Adopting the criteria cited, an average gain of 0.9% in UHML was obtained from the selection of 34 $F_{5:6}$ lines from among the 51 $F_{4:5}$ lines (Table 3). It should be noted that the heritability in the F_5 was 0.55 (Table 4), less than that of F_4 . According to Falconer (1987), the main function of heritability is to make predictions on expressing the reliability of the phenotypic value as an estimator of the genotypic value, such that the greater the heritability, the greater the potential genetic gain from selection. Tables 1, 2, and 5 illustrate the probability of G_{s_i} involving different generations and also indicate the progression toward pure inbred lines in a pedigree program. This conclusion is logical since there was good experimental precision in all the trials and the lower H^2 values in the $F_{4:5}$ and $F_{5:6}$ lines must be attributed to a reduction in variability among lines by the selection of only those with high values for UHML. The lower estimate of H^2 in Table 5 was based on parent–offspring regression, which also may be explained by the fact of the $F_{4:5}$ generation was derived from individual F_4 plants with high UHML, thus reducing genetic variation.

Table 6. Summary of analysis of variance for upper-half mean length in the F_6 generation.

Source of variance	df [†]	MS [‡]
Treatments (T)	34	3.95**
Genotypes (G)	33	3.88**
G vs. T	1	6.22**
Residue	34	1.54
Overall mean	31.51	
Mean of genotypes	31.46	
Mean of check cultivar	33.25	
CV _e [§] (%)	3.93	
CV _g [¶] (%)	3.43	

** Significant at 0.01 probability level by the F test.

[†] df, degree of freedom.

[‡] MS, mean square.

[§] CV_e, coefficient of environment variation.

[¶] CV_g, coefficient of genetic variation.

The field evaluation of the $F_{5:6}$ lines showed differences ($P < 0.05$) among lines (Table 6) and allowed the comparison of these lines among themselves and with the a check cultivar, SM3, which exhibits longer UHML than the average produced in Brazil during the past decade (Table 7). Although several were numerically longer, none of the $F_{5:6}$ lines exceeded ($P < 0.05$) the mean value of the check cultivar; although, three of the lines with UHML from 34.1 to 33.4 mm significantly exceeded germplasm line CNPA 12/106, which exhibited the shortest UHML at 28 mm. Sixteen of the lines averaged 32 mm or more, which is the lower limit for upland ELS according to Cotton Incorporated (www.cottoninc.com/fiber/quality/US-Fiber-Chart/Ratings-Of-Fiber-Properties/). Smith et al. (1999) reports that breeders have long sought introgression of alleles of *G. barbadense* L. genotypes in *G. hirsutum* L. genotypes as a way of improving the fiber quality while maintaining the yield potential of upland. The progenitor of Acala SJ4 used in this study has introgression of *G. barbadense* L (Smith et al., 1999). It is also possible that the Guazuncho 2 has additional alleles that interact for an increase in UHML when crossed with Acala SJ4. Beyer et al. (2013), in a test cross study, found that one of the materials evaluated had the capacity of increasing fiber length when crossed with the long fiber tester. Hague et al. (2011) used an upland ELS genotype developed by Smith et al. (2008) in a cross with the cultivar DP 50 (PVP 8400154), a medium UHML cultivar, and evaluated the F_2 generation in two locations, observing significance for genotype and location effects. They concluded that the use of an upland ELS in crosses with materials that produce medium fibers could result in the development of cultivars with better fiber quality. Upland supposedly has a narrow genetic base due to the constriction associated with polyploidy, with domestication, and with modern breeding practices (Brubaker et al., 1999). Many investigators suggest that the limited genetic diversity in cotton could have

Table 7. Overall means of high-volume instrument fiber traits in relation to long-staple upland cotton F_6 lines and a commercial control evaluated under irrigated condition in Ipan-guaçu, RN, Brazil.

Genotype	UHML	Lint	Strength
	mm	%	kN m kg ⁻¹
30-CNPA 2012/143	34.1	31.1	326.6
19-CNPA 2012/122	33.4	35.8	332.5
15-CNPA 2012/117	33.4	28.6	326.6
31-CNPA 2012/144	32.9	33.2	293.2
14-CNPA 2012/116	32.8	28.1	344.2
11-CNPA 2012/113	32.6	26.8	349.1
25-CNPA 2012/132	32.5	30.5	346.2
26-CNPA 2012/138	32.4	32.3	312.8
01-CNPA 2012/97	32.4	30.5	332.5
07-CNPA 2012/107	32.2	29.7	305.0
12-CNPA 2012/114	32.2	28.3	342.3
17-CNPA 2012/120	32.1	34.8	325.6
10-CNPA 2012/112	32.1	26.3	329.5
22-CNPA 2012/128	32.1	30.7	305.0
27-CNPA 2012/140	32.0	36.3	323.6
03-CNPA 2012/102	32.0	28.4	346.2
32-CNPA 2012/145	31.9	34.2	315.8
13-CNPA 2012/115	31.8	33.7	329.5
33-CNPA 2012/146	31.5	33.7	332.5
23-CNPA 2012/129	31.3	35.9	293.2
02-CNPA 2012/98	31.2	28.6	372.7
04-CNPA 2012/103	31.2	28.6	354.0
28-CNPA 2012/141	31.1	37.8	318.7
18-CNPA 2012/121	31.0	36.2	332.5
05-CNPA 2012/105	30.9	31.6	341.3
20-CNPA 2012/123	30.3	38.7	326.6
34-CNPA 2012/148	30.1	37.8	320.7
16-CNPA 2012/118	29.7	36.9	331.5
08-CNPA 2012/108	29.7	39.3	276.6
29-CNPA 2012/142	29.6	39.2	300.1
09-CNPA 2012/111	29.6	35.6	302.1
21-CNPA 2012/127	29.4	37.0	283.4
24-CNPA 2012/130	29.3	37.1	267.7
06-CNPA 2012/106	28.0	38.7	327.6
SM3 [†]	33.2	30.8	385.4
LSD _{0.05} [‡]	5.1	7.6	6.1
CV [§] (%)	3.9	5.5	5.5

[†] Check, cultivar from the Finobrasa group.

[‡] LSD_{0.05}, least significant difference.

[§] CV, coefficient of variation.

an impact on breeding for yield and fiber qualities (Lewis, 2001; May et al., 1995). However, according to Kuruparthi and Bowman (2013), there has been recent improvement of upland cotton fiber quality in the United States.

One aspect of this study was to consider lint percentage, the ratio of lint weight to lint + seed weight, which, in most of the lines reported herein, was low (Table 7). Generally, the lines with longer UHML showed a lower lint percentage, a fact also reflected in the high negative correlation between UHML and lint percentage in Table 8.

Table 8. Genotypic correlations between upper-half mean length (UHMS) and other traits in the F_5 and F_6 generations.

Trait	Genera-tion	Lint per-centage	Boll size	Unifor-mity	Strength	Micro-naire
Length	F_5	-0.81**	0.02ns [†]	0.30*	0.43**	0.14ns
UHML	F_6	-0.80**	-0.31ns	0.64**	0.59**	-0.13ns

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

[†] ns, not significant.

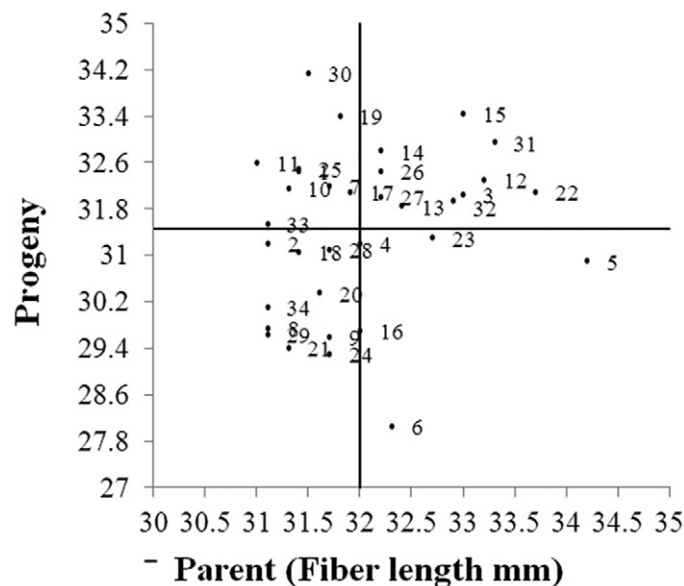


Figure 1. Scatter plot of fiber upper-half mean length (UHML) (mm) of the 35 cotton lines selected and evaluated in the F_6 generation (progenies) in Ipan-guaçu/RN in 2013 and their corresponding F_4 (parent) UHML.

Culp and Harrel (1977) observed a negative correlation between fiber production and fiber length in interspecific crosses. In relation to fiber strength, most of the lines in this study, including those with length greater than 32 mm, retained high values, greater than 304 kN m kg⁻¹, which also is reflected in the positive but nonsignificant correlation of UHML and strength.

In Fig. 1 are the mean values of the $F_{5,6}$ lines and the respective values of the $F_{3,4}$ plants that gave rise to the lines. The occurrence of values in the upper left quadrant and in the lower right quadrant indicates that in spite of the positive correlation between mean values of generations for some lines, selection in early generations does not always result in G_{s_i} . Positive correlation does not ensure that there will be simultaneous gains in both generations when selections are made, for example, of the best lines in lower right or upper left quadrant. The selection of individuals in the upper right quadrant reveals effectiveness of selection in the initial generations since, in this quadrant, individuals are found that show gains in the F_4 generation that was reflected in the F_6 generation. Lines CNPA 2012/117 (15), CNPA 2012/144 (31), CNPA 2012/128 (22), and CNPA 2012/114 (12) are the most promising in

Table 9. Analysis of variance for recombinant inbred lines (RILs) in relation to upper-half mean length.

Source of variance [†]	df [‡]	MS [§]
RIL	66	3.58**
Residue	67	0.32
Mean	31.08	
σ^2_a	1.62	
CV _e (%)	1.83	
CV _g (%)	2.23	
σ^2_f	1.79	
$H^2(n)$	90.90	

** Significant at the 0.01 probability level by the F test.

[†] σ^2_a , additive variance; CV_e, coefficient of environment variation; CV_g, coefficient of genetic variation; σ^2_f , phenotypic variance; $H^2(n)$, narrow-sense heritability.

[‡] df, degree of freedom.

[§] MS, mean square.

regard to fiber length (Table 7, Fig. 1), since they exhibited the greatest UHML in the F₆ generation and were above the mean in the F₄ generation.

The analysis of variance indicated differences ($P < 0.01$) among the RILs developed and evaluated in this study (Table 9). Figure 2 shows that the UHML range for the 67 RILs was 27 to 34 mm, essentially the same numerically as accomplished by the pedigree method. Almost all of the phenotypic variance (1.79) is explained by the genotypic variance (1.60) (Table 9), which in this case was considered as additive variance since RILs should show no dominance effects. The H^2 was 0.90, suggesting the importance of additive gene effects in the expression of UHML. Al-Rawi and Kohel (1969) showed that the technological characteristics of fiber, including fiber length, were polygenically inherited. The yield of seed-cotton and fiber quality are controlled by polygenes and affected by the environment (Magadum et al., 2012). Many studies show the importance of additive values of genes for length, which favors the programs of intrapopulational selection for increasing UHML. Al-Rawi and Kohel (1970) observed that heritability defined as the ratio of the additive variance or variance due to additive \times additive epistasis, or both, and the total phenotypic variance, of almost all the characteristics analyzed, including length, was relatively high, which suggests that most of the total phenotypic variance was additive in their study. Figure 1 shows a normal distribution for the frequency of UHML categories in this study, further supporting that UHML is controlled predominately by additive gene action.

Data reported herein suggest that it is possible to select materials with improved UHML, with values approaching a commercial ELS upland cultivar, for production in Brazil. A high percentage of the RILs also exhibited a UHML greater than 32 mm. Genetic gain for UHML from the F₄ to the F₅ generation was 6.8%, indicating significant variability for UHML and indicating that simple methods of intrapopulational breeding may lead to genetic gains in UHML.

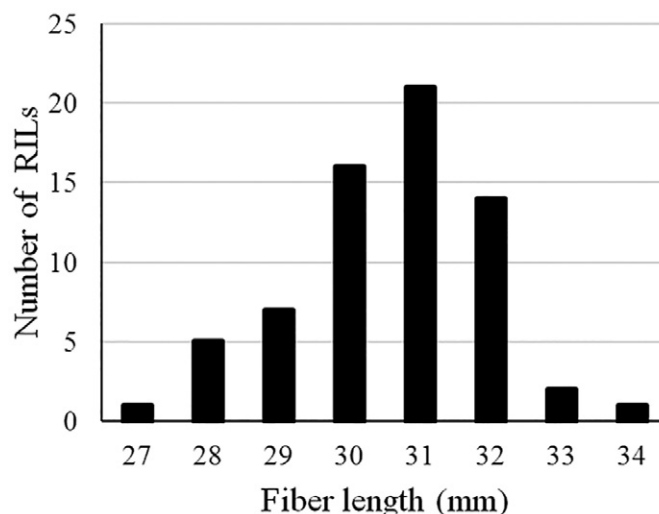


Figure 2. Distribution in relation to upper-half mean length (mm) of the recombinant inbred lines (RILs) from the cross between Guazuncho 2 and Acala SJ4 cultivated in Campina Grande/PB in 2013.

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