



## Inheritance and variation of lint yield and fiber traits in a partially introgressed (*Gossypiumbarbadense* L. × *G. hirsutum* L.) population of cotton

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### Abstract

The objective of this study was to evaluate a partially introgressed (*Gossypiumbarbadense* L. × *G. hirsutum* L.) population of cotton for two years. The traits that studied were lint yield per plant, lint %, fiber length, fiber strength and micronaire. A total of 31 cytogenetically stable lines (2n=4x=52) were produced from a cross between a *G. barbadense* line, Carnak and a *G. hirsutum* line, 4S, through an alien pollination production system and three generations of self-pollination. Normal distribution was observed for all traits that studied apart from lint yield per plant. Coefficients of variation were the highest for lint yield per plant and lint %. Heritability for lint yield ranged from 0.77 in 2004 to 0.71 in 2005. Correlation coefficients were the highest between fiber length and fiber strength which may indicate the possibility of concurrent selection of lines within the population for these two traits. In addition, micronaire and lint yield per plant were favorably associated. In the partially introgressed population (PI), Carnak × 4S, several lines that combined better yield, fiber quality performance or both than the introgressed parental lines were identified.

**Keywords:** Alien pollinations; Fiber quality; Heritability.

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### Introduction

In cotton genus (*Gossypium* sp.), two diploid (*G. arboreum* and *G. herbaceum*) and two tetraploid (*G. hirsutum* and *G. barbadense*) species have been cultivated for fiber (Brubaker et al., 1999). Tetraploids lead commercial cotton production. More specifically, *G. hirsutum* L. provides over 90% of the world production, because of its high yield potential and broader environmental adaptability while *G. barbadense* L. production has been limited due to its lower yield potential, heat sensitivity and longer growing period (Percy et al., 2006). Nevertheless, *G. barbadense* possesses superior fiber quality that includes fiber length, strength, uniformity and fineness, attributes that are unavailable in *G. hirsutum* (McCreight, 1992).

Currently, although breeding programs continue to make progress, it becomes more and more difficult to develop new varieties using the existing germplasm because of the very limited genetic diversity (Linos et al., 2002). Germplasm narrowness of the primary gene pool has been reported as a major factor for improvement plateaus and increased genetic vulnerability (May and Jividen, 1995). This reality has resumed interest of cotton researchers in interspecific introgression as a mean of increasing genetic variation.

*G. barbadense* and *G. hirsutum* are both interspecific disomic ( $2n=52$ ) tetraploids (Cronn and Wendel, 2004). Interspecific crosses between these two species can produce hybrids with economically valuable breeding characters (Basbag and Gencer, 2007). *G. hirsutum* ( $AD_1$ ) and *G. barbadense* ( $AD_2$ ) present cytogenetic similarity (Reinisch et al., 1994), despite their morphological distance (Wise et al., 2000). Yet, genetic improvement through interspecific hybridization has been hampered by genetic breakdown in advanced populations. In addition, infertility, cytological abnormalities, and distorted segregation have been reported (Kohel et al., 1977; Paterson and Smith, 1999; Galanopoulou-Sendouca and Roupakias, 1999; Saha et al., 2004).

New breeding techniques and a more thorough knowledge of the genetic basis for yield and fiber quality are required for developing cotton lines that combine high yield potential with fiber quality. Cytogenetical approaches could be used for the creation of genotypes that possess specific chromosomes from both species. These genotypes are known as partial interspecific hybrids or measured hybrids (White et al., 1967). Mavromatis and Roupakias (1994) described 'measured hybrid' as a plant that carries in a homozygous condition, part of the chromosomes or chromosome segments from one species with the remainder from the other one, evolving in this way the concept of partial interspecific hybrids. Additionally, Mavromatis et al. (2005) reported the development of partial interspecific cotton plants via alien pollination of  $F_1$  interspecific hybrids between *G. barbadense* and *G. hirsutum* with pollen from *Hibiscus cannabinus* L. Some of these plants combined characteristics from both cotton species, they were fertile and their chromosome number was increased in three generations back to 52, the normal chromosome number of the tetraploid cottons.

The objective of this study was to evaluate the effectiveness of a partially introgressed (PI) population developed via pollen from *H. cannabinus* L. for improving lint yield and fiber quality properties.

## Materials and Methods

### Plant Materials

As described in detail by Mavromatis et al. (2005), field grown  $F_1$  plants from *G. barbadense*, variety Carnak and *G. hirsutum*, cultivar 4S provided the original test material. Flower buds were emasculated one day prior to anthesis and isolated with paper bags (Kearney and Potter, 1926). The following morning, the stigmas were pollinated with fresh pollen of an early flowering *H. cannabinus* genotype (kindly provided by C.G. Cook, USDA) and rebagged. In each  $F_1$  hybrid plant, 28-45 flowers pollinated with alien pollen were left on the plant to grow naturally until maturity. In total, thirty-eight partial interspecific plants (Carnak  $\times$  4S) produced. These hypoaneuploid plants were self-pollinated for 3 generations in a row. The cytogenetic analysis of all the  $PI_3$  plants-168 in

total-with chromosome counts in mitosis and flow cytometric analysis, showed that 31 of those were euploid with 52 chromosomes (Table 1). Seed obtained from the 31 euploid PI<sub>3</sub> plants was utilized in the 2-year field experiment.

Table 1. Cytogenetic analysis of the parental cotton plants, F<sub>1</sub> and F<sub>2</sub> *G. barbadense* × *G. hirsutum* hybrids PI<sub>3</sub> plants and reference standard pea plant (*Pisum sativum*) with chromosome counts in mitosis and flow cytometric analysis (Mean G<sub>0</sub>/G<sub>1</sub> and DNA index).

Genotype	No. of plants	Mean chr. no ± StDev	Mean G <sub>0</sub> /G <sub>1</sub> ± StDev	DNA index
<i>G. hirsutum</i> (4S)	20	51.77±0.13	106.28±2.55	0.70
<i>G. barbadense</i> (Carnak)	20	51.62±0.24	99.49±1.19	0.65
F <sub>1</sub> - <i>G. barbadense</i> × <i>G. hirsutum</i>	20	51.67±0.24	105.42±2.22	0.69
F <sub>2</sub>	20	51.91±0.11	100.43±0.87	0.66
PI <sub>3</sub> (hypoaneuploids)	137	42.01±3.92	87.56±3.13	0.53
PI <sub>3</sub> (euploids)	31	51.33±0.70	99.89±0.96	0.66
Control ( <i>Pisum sativum</i> )	20	14.00±0.00	152.50±5.23	1

### Field Experiments

Thirty-one PI<sub>4</sub> lines were evaluated during the seasons 2004 and 2005 for lint yield and fiber properties in the experimental field on the farm of the Aristotle University of Thessaloniki, Greece. Two parental lines, Carnak and 4S were used as checks in these studies. Each plot consisted of a single row, 1m apart and 10m long with two replications. Plots were seeded on April 22<sup>nd</sup> in 2004 and April 29<sup>th</sup> in 2005. All plots were grown under sprinkler irrigation conditions with standard agronomic practices for the specific location. Plots were sprayed for controlling aphids, lygus, and bollworm with Danitol, Thiodane, Methomyl and Steward. At maturity, all plots were hand-harvested and ginned to obtain estimates of lint % and lint yield. Approximately 30 g of lint from each line was analyzed for fiber quality properties using high volume instrument (HIV) (Riley, 1996).

### Statistical Analyses

Genotype means, ranges, coefficients of variation (CV) and standard errors of the lint %, lint yield per plant and quality properties (length, strength and micronaire) were calculated for both years for the PI lines and the checks from raw data. Skewness and kurtosis were calculated and also normality of distribution was tested by Shapiro-Wilk W test for all studied traits. Also, deviations of the PI population trait means from their parental values were tested by independent sample test. Analyses were performed on JMP 8.02 (SAS Institute Inc., Cary, NC, USA). Separate analyses of variance were performed for years 2004 and 2005 and for the combined experiment under a fixed model in the R statistical environment (R Development Core Team, 2009). For variance component and heritability estimation, years and genotypes were considered random, and calculations were done through restricted maximum likelihood (REML) with the use of the R package lme4 (Bates and Maelcher, 2010). Additionally, Pearson's coefficients of correlation were estimated for all pairs of variables under two schemes. For the first one, genotype scores were averaged for each year; this scheme captures also the correlation due to variation in year effects. The second one was done in an entry mean basis across years. Significance of

correlations was evaluated by the two-sided *t* test. Scatterplots were drawn to visualize association between pairs of variables. This analysis was performed in the R environment as well (R Development Core Team, 2009).

## Results

### PIs compared to parental lines

HVI analyses of PI<sub>4</sub> plants showed a broad range of variation for fiber properties that evaluated. There was broad variation for fiber length (range of 27.2 to 37.6), fiber strength (range of 27.3 to 40.3) and micronaire (range of 2.1 to 5.1). The ranges for lint yield per plant (42.57-129.10) and lint % (22.10-43.90) were also broad. The means and standard deviations for the traits measured for PIs were compared to the parental means over the years (Table 2). The *G. hirsutum* parent (4S) had a significantly higher average ( $P < 0.001$ ) only for lint yield per plant compared to the *G. barbadense* parent (Carnak). For all the other traits (lint %, fiber length, strength and micronaire), Carnak exceeded significantly ( $P < 0.05$ ) 4S. The PI population means for lint yield per plant did not differ from the best parent. Lint % and the three fiber properties showed to be either the same or significantly improved compared to *G. hirsutum* but not as good as those of *G. barbadense* (Table 2).

Table 2. Means, ranges of PI lines, parental and check means, and *t* tests for deviation of PI lines from parental lines for lint%, lint yield per plant and fiber traits (length, strength, and micronaire).

Traits	PI lines		Means ( $\pm$ St.Dev.)		<i>t</i>	
	Mean ( $\pm$ St. Dev.)	Ranges	4S ( <i>G. hirsutum</i> )	Carnak ( <i>G. barbadense</i> )	H <sub>0</sub> : Mean=4S	H <sub>0</sub> : Mean=Carnak
Lint yield/plant (g)	72.39 ( $\pm$ 15.95)	42.57-129.10	72.90 ( $\pm$ 0.28)	56.15 ( $\pm$ 0.78)	-0.2532 <sup>ns</sup>	8.0148 <sup>***</sup>
Lint %	34.28 ( $\pm$ 5.15)	22.10-43.90	34.65 ( $\pm$ 0.27)	35.91 ( $\pm$ 0.49)	-0.5625 <sup>ns</sup>	-2.482 <sup>***</sup>
Length (mm)	32.02 ( $\pm$ 2.32)	27.20-37.60	28.75 ( $\pm$ 0.21)	37.14 ( $\pm$ 0.09)	11.091 <sup>***</sup>	-17.341 <sup>***</sup>
Strength (gtex <sup>-1</sup> )	34.48 ( $\pm$ 3.43)	27.30-40.30	33.97 ( $\pm$ 0.06)	44.45 ( $\pm$ 0.39)	1.169 <sup>ns</sup>	-22.891 <sup>***</sup>
Micronaire	3.39 ( $\pm$ 0.73)	2.10-5.10	3.87 ( $\pm$ 0.09)	3.13 ( $\pm$ 0.06)	-5.146 <sup>***</sup>	2.891 <sup>ns</sup>

<sup>ns</sup> non-significant.

<sup>\*</sup> Significant at the 0.05 probability level.

<sup>\*\*</sup> Significant at the 0.01 probability level.

<sup>\*\*\*</sup> Significant at the 0.001 probability level.

### Variability and heritability

Coefficients of variation were calculated for all traits in the PI population. The CVs were the largest for agronomic traits lint yield per plant and lint % (22.04 and 15.02 respectively). Among fiber traits, the largest CV was obtained for fiber strength (9.94). The lowest levels of genotypic variation within the PI population were obtained for micronaire (3.39) (Table 3).

Table 3. Coefficients of variation for yield and fiber properties of the PI population.

Trait	Coefficient of variation	P values <sup>†</sup>	Skewness	Kurtosis
Lint yield/plant (g)	22.04	0.0130	0.997	1.918
Lint %	15.02	0.1393	-0.455	-0.285
Length (mm)	7.25	0.6286	0.206	0.003
Strength (g tex <sup>-1</sup> )	9.94	0.1742	-0.141	-0.810
Micronaire	3.39	0.2668	-0.095	-0.383

<sup>†</sup> Shapiro-Wilk W goodness-of-fit test for normal distributions (H<sub>0</sub>=the data is from Normal distribution. P-values lower than 0.05 reject H<sub>0</sub>).

Analysis of variance for years 2004 and 2005 separately gave highly significant results for differences among genotypes in lint yield (Table 4). For the combined analysis across years, the effects of genotypes and interaction genotype  $\times$  year were significant, albeit average difference between years was not significant (Table 4). The fact that there was no significance between years may obey to the high mean square of replications per year. In fact, although for year 2004 the replication means were quite similar (70.91 vs. 66.19) this was not the scenario for 2005, in which replications were highly different (81.84 vs. 70.61). It is necessary to point out that year effects were considered fixed for this analysis, thus the significant difference between genotypes was considered historical. On the other hand, if conclusions are to be general, years should be considered as random, and in this case differences among genotypes were not significant. Restricted maximum likelihood was used to calculate heritability of lint yield. The values ranged from 0.77 in 2004 to 0.71 in 2005.

Table 4. Analysis of variance for lint yield for year 2004, 2005 and across years.

Year 2004					
Source of variability	df	Sum Sq	Mean Sq	F value	Pr (>F)
Genotype	30	10453.8	348.5	5.6	4.5 <sup>e-06</sup> ***
Replications	1	345.5	345.5	5.6	0.02498*
Residuals	30	1860.9	62.0		
Year 2005					
Source of variability	df	Sum Sq	Mean Sq	F value	Pr (>F)
Genotype	30	18763.5	625.5	3.4	0.0005***
Replications	1	1954.0	1954.0	10.8	0.0026**
Residuals	30	5437.6	181.3		
Across years					
Source of variability	df	Sum Sq	Mean Sq	F value	Pr (>F)
Year	1	1826.7	1826.7	1.6	0.33 <sup>ns</sup>
Reps/year	2	2299.4	1149.7	9.5	0.0003***
Genotype	30	9291.5	309.7	2.5	0.001***
Genotype $\times$ year	30	19925.9	664.2	5.5	1.2 <sup>e-08</sup> ***
Residuals	60	7298.5	121.6		

#### Frequency distribution of the PIs

Figure 1 (a-e) presents the frequency distribution of the overall means of PIs for lint yield (a), lint % (b) and fiber quality traits (c-e). Shapiro-Wilk W goodness-of-fit test showed that all the traits were normally distributed apart from lint yield per plant (Table 3). The distribution of lint yield per plant means deviated significantly from normality and a 0.997 skewness value was obtained within PI population. Distribution for this trait seemed to be shifted toward the high yielding parent, *G. hirsutum*. Approximately 39% of the PI lines exceeded the high lint yield per plant of 4S (Figure 1a) and nine PI lines showed to have better lint % than Carnak (Figure 1b). As for the fiber quality properties, 32.25% of the PI lines showed to have improved micronaire values that were lower than that of the best parent, Carnak (Figure 1e). Eighteen lines showed not to have significant differences ( $P < 0.05$ ) in fiber length from *G. barbadense* and also four lines had fibers as strong as ( $P < 0.05$ ) Carnak (Figure 1c-d). None of the fiber quality traits departed from normality with the highest skewness not to exceed 0.206 (Table 3).

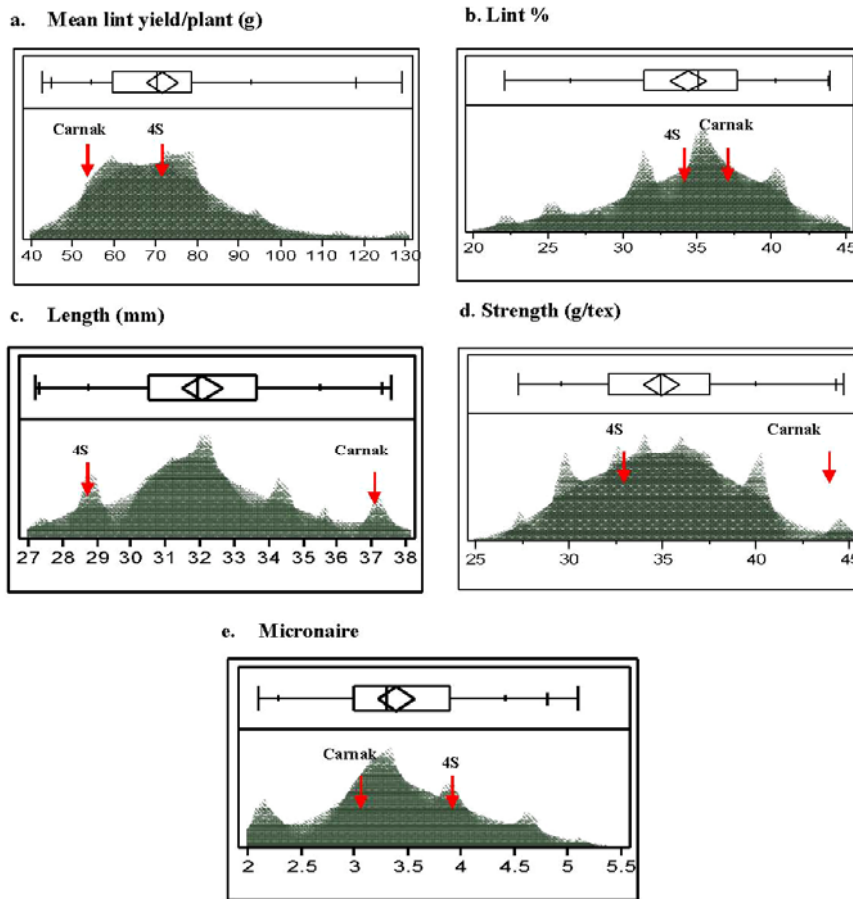


Figure 1. Distribution of PI population and parental lines (4S and Carnak) for lint yield per plant (a), lint percentage (b) and fiber properties (c-e) (length, strength and micronaire) over the years.

#### Phenotypic correlations

Correlation coefficients were calculated for each pair-wise combination of studied traits from PI means (Table 5). Significant phenotypic correlations were observed among the traits in an entry-mean per year basis. Lint yield per plant was observed to be positively associated ( $r=0.7170^{***}$ ) with lint% and moderately associated with micronaire ( $r=0.2602^*$ ). Also, lint yield was negatively correlated with both fiber length ( $r=-0.7749^{***}$ ) and strength ( $r=-0.7195^{***}$ ). Lint %, in addition to being correlated with lint yield per plant, was negatively associated with fiber length ( $r=-0.4297^{**}$ ) and strength ( $r=-0.4949^{**}$ ). No association observed between lint % and micronaire. Among fiber quality properties, the strongest correlation was estimated between length and strength ( $r=0.8662^{***}$ ). Even though it was statistically significant, micronaire had a low negative association with fiber length

( $r=-0.2673^*$ ). Correlation coefficient for micronaire-fiber strength was not significant. Scatterplot in Figure 2 depicts the above mentioned associations. The signs and statistical significances of these correlations were maintained when they were estimated from the entry means of the traits across years, an indication of their robustness.

Table 5. Correlations among lint %, lint yield per plant and fiber traits (length, strength, and micronaire) of PI lines.

Traits	Lint %	Length (mm)	Strength (gtex <sup>-1</sup> )	Micronaire
Lint yield/plant (g)	0.7170 <sup>***</sup>	-0.7749 <sup>***</sup>	-0.7195 <sup>***</sup>	0.2602 <sup>*</sup>
Lint %		-0.4949 <sup>***</sup>	-0.6143 <sup>***</sup>	0.1566 <sup>ns</sup>
Length (mm)			0.8663 <sup>***</sup>	-0.2674 <sup>*</sup>
Strength (gtex <sup>-1</sup> )				-0.0830 <sup>ns</sup>

<sup>ns</sup> non-significant.

<sup>\*</sup> Significant at the 0.05 probability level.

<sup>\*\*</sup> Significant at the 0.01 probability level.

<sup>\*\*\*</sup> Significant at the 0.001 probability level.

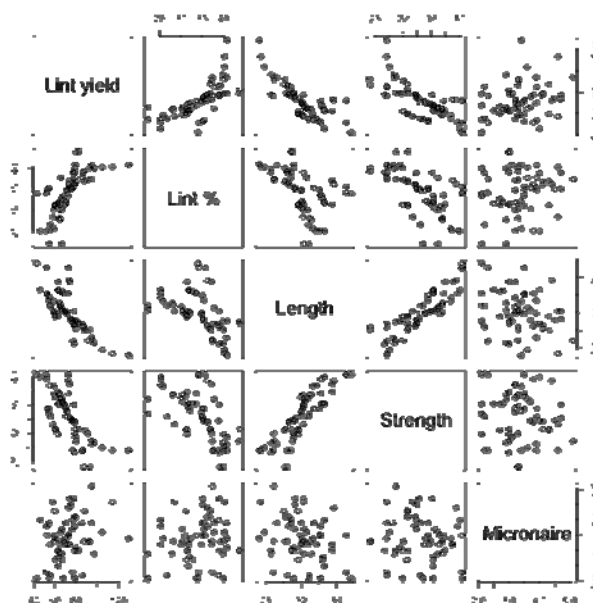


Figure 2. Scatterplot of associations among lint%, lint yield per plant and fiber traits (length, strength, and micronaire) of PI lines.

## Discussion

This study evaluated yield and fiber quality properties of a partially introgressed population between *G. barbadense* and *G. hirsutum*, produced through an alien pollination approach from the cross Carnak × 4S. The majority of traits (lint yield per plant, lint % and fiber strength) were closer to the values of 4S than of Carnak, suggesting partial dominance of the *G. hirsutum* parent for these traits. This observation is in agreement with a previous report on cotton interspecific hybrids between *hirsutum* and *barbadense* (Jiang et al., 2000).

Significant variations as those described by CVs occurred in the PI population for both lint yield per plant and lint %. Among fiber properties higher genotypic variation observed for micronaire. Fiber length produced the lowest CV of 7.25 while fiber strength did not exceed of 9.94. The observed variation within PI population can have practical application for both yield and fiber improvement efforts (Percy et al., 2006).

The PI population included 16 lines that have better mean yield which exceeded 4S by an average of 17.06%. Although 58% of the lines showed lower lint % than *G. barbadense* parent, 22.5% of the lines had equivalent or better values. Carnak parent possessed a desirable micronaire of 3.08 and 12.9% of the PI lines showed micronaire values lower than *G. barbadense* check. It is known that high micronaire is an undesirable fiber property in mature cotton because it results in price discounts (Calhoun and Bowman, 1999).

Genotypes were significantly different for lint yield within each year. Also, there is evidence of genetic differences from the combined experiment across the two years. However, this cannot be generalized, because if their effects are random, genetic significances disappear.

Heritability for lint yield in a single year basis showed consistent values across years: 0.77 and 0.71 for 2004 and 2005 respectively. Looking these results as a whole, one can say that the interaction of genotype  $\times$  year may have been so high that it concealed a general genetic variance.

Positive phenotypic correlations among fiber traits that studied in the PI population may suggest that individual lines can be selected for the simultaneous improvement of quality properties. The previously reported association of fiber length with fiber strength in hybrid (Basal and Smith, 1997) or recombinant inbred populations (Percy et al., 2006) was observed in the current investigation. This positive correlation indicated that it should be possible to concurrently identify lines with both desirable fiber length and strength. As mentioned previously, lower micronaire is highly desirable in cotton breeding. The negative phenotypic correlation between micronaire and fiber length is useful in selecting lines for desired genes. More specifically, 8 PI lines identified to possess fiber lengths not significantly different from the ones of the *G. barbadense* parent and micronaire lower than Carnak. Overall, a closer examination of the population allowed us to identify one line that had a combination of fiber length, strength and micronaire better than *G. barbadense*.

It was expected, based on previous reports (Calhoun and Bowman, 1999; Percy et al., 2006), lint % to be positively and strongly associated to lint yield per plant. In addition, it was not surprising the negative correlation between these two yield components with fiber strength and length. In the PI population 4 lines were selected that had lint yield per plant higher than 4S and micronaire values lower than Carnak.

A limited molecular analysis with 56 genome-wide microsatellites of PI population, parental lines and F<sub>1</sub> hybrid (Carnak  $\times$  4S) was conducted to determine the existence of unique loci. Two markers, BNL2805 (Chr 3) and BNL1047 (Tel 2sh) revealed a different profile of some PI lines with unique alleles not presented in 4S and Carnak. This preliminary molecular study may suggest that PI population is genetically atypical from a conventional segregating *G. barbadense*  $\times$  *G. hirsutum* interspecific population, but more extensive research needs to be done.



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