

**Effect of chromosome on important  
quantitative traits of agronomic and  
fiber traits using *Gossypium  
barbadense* chromosome-specific  
recombinant lines of *Gossypium  
hirsutum***

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## ABSTRACT

The exceptional fiber length, strength and fineness of Pima and Sea Island cotton (*Gossypium barbadense* L.) give it a 30% to 50% price advantage over the more widely grown Upland cotton (*G. hirsutum*) because of its superior spinning and manufacturing performance. Attempts to incorporate genes from *G. barbadense* into Upland have generally not achieved stable introgression of the fiber properties due to incompatibility between the genomes. We developed a set of backcrossed chromosome substitution lines for different chromosomes of *G. barbadense* (3-79) in *G. hirsutum* (TM-1) covering about 80% of the genome. Selfed seeds from cytologically identified euploid (normal 26 II chromosomes) backcrossed chromosome substitution lines were used for crossing with TM-1 to develop chromosome specific hybrids ( $F_1$ ). Hybrid plants were selfed to develop chromosome specific  $F_2$  hybrid populations. Fourteen chromosome specific  $F_2$  hybrid populations, TM-1 and 3-79 were planted in three replicated plots at three locations (Mississippi, New Mexico, and Arizona) in 2001 for evaluation of agronomic and fiber properties based on bulk sample analysis of individual lines. Considering that each substituted chromosome or chromosomal segment carried a significant fraction of the genetic factors from 3-79 and that all of the lines were in a similar Upland (TM-1) genetic background, the comparative results reflected the effects of traits associated with particular chromosomes. Results indicated that several chromosome specific  $F_2$  hybrid populations had superior fiber and agronomic properties compared to their parental lines. Chromosome 18 and 22 of 3-79 significantly improved lint percent in TM-1 at all locations. Chromosome 25 of 3-79 had significantly reduced micronaire at all locations and increased fiber strength at Mississippi and Arizona in TM-1. Several chromosome specific  $F_2$  hybrid populations had significantly lower fiber yield and decreased boll size compared to TM-1, suggesting that genes controlling fiber yield and boll size were located on many chromosomes. The chromosome specific  $F_2$  hybrid populations used in identifying chromosomal locations of important agronomic and fiber traits and results demonstrated that some of the backcrossed chromosome substitution lines might be useful for improving germplasm of Upland cotton.

## Introduction

Competition from synthetic fibers and the need to improve fiber quality are two major economic forces driving the current global cotton market. Technological changes in the textile industries demand higher quality fiber; however, the primary focus in cotton production is usually higher yield rather than fiber quality. The high value per hectare of cotton and the recent demands for high quality of fibers, clearly justify the importance of new and innovative approaches toward understanding genetic mechanisms of fiber quality.

The exceptional fiber length, strength, and fineness of Pima and Sea Island cotton (*Gossypium barbadense* L.) give 30% to 50% price advantage over the more widely grown Upland cotton (*G. hirsutum* L.) because of its superior spinning and manufacturing performance. However, Upland cotton occupies over 90% of the cotton growing area. Pima and Sea Island Cottons require longer growing seasons to produce yields of high quality fiber and are thus primarily suited to production in Arizona, California, and New Mexico. However, most US cotton farmers prefer to grow early-maturing varieties because of their higher yielding capacity and less insect and agronomic problems.

The complexity of improving fiber qualities through conventional breeding methods has been well documented in cotton (Meredith, 1984; 1994). The breeder frequently faces many challenges in the process of introgressing *G. barbadense* genes into Upland cotton: 1) breeders work with chromosome pieces of different sizes at the whole genome level, leading to a large amount of unwanted DNA around the target gene resulting in conflicting linkage associations; for example, lint yield is positively correlated with micronaire but negatively correlated with fiber length and fiber strength (Meredith, 1984); 2) many of the fiber quality traits are quantitatively inherited with low heritability and are subject to large environmental effects (Meredith, 1984); and 3) infertility, cytological abnormalities and distorted segregation may cause problems in interspecific crosses. Thus, *G. barbadense*-level fiber strength in an Upland genetic background is not currently available. The utilization of backcrossed chromosome or chromosome arm substitution lines as the donor parent will help to overcome the problems of interspecific incompatibility at the whole genome level while targeting fiber traits for Upland cotton improvement. Examples of transfer of genetic material from alien chromosomes have been well documented in wheat and other agronomic crops (Knott, 1987).

Recently, we have developed backcrossed chromosome substitution lines in cotton. By hypo-aneuploid-based backcross chromosome substitution, we have replaced individual *G. hirsutum* (TM-1) chromosome pairs with the respective *G. barbadense* ('3-79') chromosome pairs. Collectively, the set of substitutions cov-

ers about 80% of the genome. Currently, we are in the process of releasing these backcrossed chromosome substitution lines as improved germplasm. In this research, such 14 different euploid ( $2n = 52$ ) backcrossed chromosome substitution lines were crossed with TM-1 (*G. hirsutum*) to develop chromosome-specific hybrids ( $F_1$ ) and subsequently by selfing to produce chromosome-specific recombinant (CRB) lines. The CRB lines resulting from this manipulation are genetically identical except that each differs by the recombination of a specific homologous pair of chromosomes from Pima 3-79 and TM-1. Observation and measurement of different quantitative traits in such a uniform genetic background will detect the effect of the group of genes that a specific chromosome carries. The overall goal of this research is to identify the chromosomal association of some important agronomic and fiber traits including yield, micronaire, fiber strength, and boll size based on comparative analysis of the CRB lines.

### Experimental procedure

$BC_5S_1$  seeds from euploid ( $2n=52$ ) backcrossed, chromosome substitution lines specific to 14 different chromosomes were used in crossing with TM-1 (*G. hirsutum*) to develop chromosome-specific hybrids ( $F_1$ ). The  $F_1$  seeds were sent to the winter cotton nursery in Tacoman, Colima, Mexico to produce chromosome-specific  $F_2$  hybrid seeds by selfing. The chromosome-specific  $F_2$  hybrid populations, parental lines (TM-1 and 3-79) and a commercial cultivar DeltaPine 90 (DP90) were planted in a randomized complete block design with four replications at three locations including Mississippi (MS), New Mexico (NM) and Arizona (AZ) in summer 2001 for evaluation of agronomic and fiber properties. Considering that each substituted chromosome or chromosomal segment from a chromosome specific  $F_2$  hybrid population carried a significant fraction of the genetic factors from 3-79 and that all of the lines are in a similar Upland (TM-1) genetic background, a comparative analysis on the field performance will provide the interaction effect between the substituted 3-79 chromosome or chromosome arm and the TM-1 genetic background. Analysis of variance approach was used to data analysis and the least significance differences (LSD) for each trait at each of three locations were calculated. A significant positive or negative deviation of LSD value was considered to show the significant chromosomal effect of 3-79 compared to TM-1.

### Results and Discussion

A major limitation to the genetic improvement of cotton is the lack of information about genes that control quantitative traits such as fiber yield or fiber quality in Upland cotton. The chromosome specific  $F_2$  hybrid populations provided a means to identify association of specific chromosomes with important fiber and agronomic traits in Upland cotton. Among all three loca-

tions, chromosome specific  $F_2$  hybrid populations performed best in MS and poorest in NM in overall agronomic and fiber traits (Tables 1 and 2). We observed that 3-79 chromosomal effects for fiber and agronomic traits could be broadly grouped under two categories: 1) presence of a chromosomal effect at all locations, indicating the chromosomal effect was stable across environment and 2) absence of a specific chromosomal effect in one or two locations showing chromosomal effect was dependent on environmental conditions.

The statistical method used in this study did not partition the chromosomal effect into different components. We are in the process of analyzing two years field data following similar experiments including parental euploid backcrossed chromosome substitution lines using a more powerful mixed model of statistical analyses (Zhu, 1994).

### Yield (LYHA)

The average lint yields for TM-1, 3-79 and the conventional interspecific  $F_2$  family over the three locations (2650 kg/ha, 1101 kg/ha and 698 kg/ha, respectively) demonstrated lower yield of 3-79 and negative overall interaction resulting from genome wide segregation. Several lines had yields significantly lower than the recurrent TM-1 parent, suggesting genes with significant effect on yield were located on many chromosomes (Tables 1 and 2). We observed that the short arm of chromosome 5 of 3-79 significantly reduced fiber yield at all locations, suggesting that environment had little interaction with genes associated with this chromosome arm. Results revealed several chromosomes of 3-79 had significant negative effect on yield in one or two locations. None of the chromosome specific  $F_2$  hybrid populations had a higher yield than TM-1, so further breeding will be needed to separate loci with alien beneficial and deleterious alleles in coupling.

### Lint percent (LP)

The average lint percent for TM-1, 3-79, and the conventional interspecific  $F_2$  population over three locations were 35.25, 33.85 and 31.65. The chromosome 18 specific  $F_2$  hybrid population and chromosome 22sh (short arm for chromosome 22) specific  $F_2$  hybrid population had significantly higher lint percent than the recurrent TM-1 parent at all locations, suggesting that genes on chromosome 18 and 22sh of 3-79 affecting lint percent were little influenced by environment (Table 1). Several other chromosome specific  $F_2$  hybrid populations had higher lint percent at one or two locations compared to TM-1, suggesting that the genes located on these chromosomes of 3-79 contributed to higher lint percent, lint percent in 3-79 and in the combined interspecific  $F_2$  family was lower than TM-1, suggesting that the positive influence imparted by individual 3-79 chromosomes were due to epistatic affects. The chromosome 5sh specific  $F_2$  hybrid population showed significant negative effect on lint percent at AZ, suggesting a possible effect involving the 3-79 chromo-

some (Table 1). Our results showed that many of the backcrossed chromosome substitution lines would be useful in crossing program to improve lint percent in Upland cotton.

### **Boll size (BS)**

The average boll size over three locations of TM-1, 3-79 and the conventional  $F_2$  population was 5.92 g, 3.53 g and 3.18 g, respectively, suggesting that 3-79 chromosomes had significant negative impact on boll size in the hybrid. Many of the 3-79 chromosomes showed an effect of significantly decreased boll size in the chromosome specific  $F_2$  hybrid populations, demonstrating that they carry alleles that lead to reduced boll size in a TM-1 genetic background (Table 1). The chromosome specific  $F_2$  hybrid populations for the substituted chromosomes of 18, 5sh, 14sh and 22Lo respectively had lower boll size compared to TM-1 at all locations, indicating that effects of the respective genes on these chromosomes were largely independent of specific environment (Table 1).

### **Micronaire (MIC)**

The average micronaire values for TM-1, 3-79 and the conventional  $F_2$  population were 4.83, 3.77, and 3.53 respectively, showing the potential of 3-79 chromosomes in reducing micronaire in Upland cotton. The chromosome 25 specific  $F_2$  hybrid population had significantly lower micronaire compared to TM-1 at all locations, indicating that gene(s) controlling micronaire are located on the substituted chromosome 25 (Table 2). The results suggested that backcrossed chromosome substitution line for the chromosome 25 would be very useful in a crossing program with high yielding cultivars in reducing micronaire in Upland cotton.

### **2.5% Span length (SL)**

Our results showed that none of the 3-79 chromosomes had any effect on 2.5% span length except the short arm of chromosome 14, which showed a significant positive effect only at the NM location.

### **Elongation (EI)**

The average elongation of TM-1, 3-79, and the conventional  $F_2$  population were 7.61%, 7.21% and 7.98%, respectively, over the three locations. However, we did not find any specific 3-79 chromosomes showing significant effect on elongation at all locations. The short arm of chromosome 22 from 3-79 had a significant positive effect on elongation in TM-1 background only at AZ location. However, genes located on chromosome 6 and the long arm of chromosome 22 had a significant lowering effect on elongation at MS in TM-1.

### **Fiber strength (T1)**

The average fiber strength (g/tex) of TM-1, 3-79 and the regular  $F_2$  line over three locations was 20.47, 29.61 and 25.66, respectively, showing the

potential of 3-79 chromosomes in improving fiber strength in Upland cotton. Chromosome 25 of 3-79 had a significant positive effect in fiber strength at the AZ and MS.

### **Genetic potential of CRB lines in improving fiber traits**

Attempts to incorporate genes from *G. barbadense* into Upland cotton generally have not achieved stable introgression of the *G. barbadense* fiber properties (Stephens, 1949; McKenzie, 1970). Associated with these attempts at introgression have been poor agronomic qualities of the progeny, distorted segregation, sterility, mnote formation, and limited recombination due to incompatibility between the genomes. Inadequate results from previous efforts of introgression of *G. barbadense* germplasm suggest different approaches should be used. Successful introgression program by conventional backcrossing methods requires a long-term commitment. Such approaches will lead to fiber quality improvement but require the long term effort, which public agencies only can deliver.

An alternative approach to introgress *G. barbadense* fiber quality genes into an Upland background would be to rely on chromosome substitution lines rather than trying to achieve stable recombination between the partially incompatible, complete genomes. The primary thrust of this research is to utilize this novel approach for germplasm enhancement of agronomic and fiber qualities that will benefit the cotton producers and industries.

Our results (Table 1 and 2) revealed that several chromosome specific  $F_2$  hybrid populations had an overall negative effect on fiber yield, but high genetic potential of improving fiber traits in Upland cotton. The overall effects on yield do not mean that we will not find individual 3-79 alleles that enhance yields. But the beneficial effects of 3-79 chromosomes clearly points to the importance of backcrossed chromosome substitution lines in improving Upland cotton germplasm. Several of the chromosome specific  $F_2$  hybrid populations have the potential to increase lint percent in Upland cotton. Our results also showed that the chromosome 25 specific  $F_2$  hybrid population has the potential to reduce micronaire and increase fiber strength in Upland cotton. Low micronaire with high fiber strength are essential requisites for a higher price of Upland cotton. Results showed that several of the backcrossed chromosome substitution lines will be useful in crossing program with high yielding Upland cultivars. Our results clearly demonstrated that these chromosome specific  $F_2$  hybrid populations are not only useful to identify chromosomal locations of important quantitative traits, but the backcrossed chromosome substitution lines may be useful in crossing program to improve germplasm of Upland cotton.

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**Table 1.** Means for agronomic traits of CRB lines grown at three environments.

Entry**	Arizona			New Mexico			Mississippi		
	Lint yield (kg ha <sup>-1</sup> )	Lint %	Boll size (g)	Lint yield (kg ha <sup>-1</sup> )	Lint (%)	Boll size (g)	Lint yield (kg ha <sup>-1</sup> )	Lint (%)	Boll size (g)
CRB2 F <sub>2</sub>	755	33.60*	5.00*	598	35.63	5.83*	984	34.31	5.58
CRB4 F <sub>2</sub>	697*	33.02	5.03*	540	35.30	6.04	1051	33.68	5.63
CRB6 F <sub>2</sub>	883	32.88	5.83	515	34.23	5.98*	992	32.80	5.55
CRB7 F <sub>2</sub>	659*	32.52	5.28	516	35.45	6.00*	1073	32.66	5.69
CRB16 F <sub>2</sub>	584*	31.73*	5.40	514	35.08	6.42	971*	33.34	6.03
CRB17 F <sub>2</sub>	792	32.27	5.70	543	34.28	6.44	1025	32.99	5.91
CRB18 F <sub>2</sub>	625*	33.90*	5.05*	561	36.60*	5.91*	866*	34.57*	5.28*
CRB25 F <sub>2</sub>	830	32.59	4.83*	522	34.80	5.88*	984	33.09	5.48
CRB5sh F <sub>2</sub>	664*	31.61*	5.05*	472*	34.85	5.73*	970*	32.30	5.26*
CRB14sh F <sub>2</sub>	584*	32.83	4.40*	482*	35.03	5.35*	998	34.03	5.15*
CRB15sh F <sub>2</sub>	791	32.69	5.53	570	35.43	6.34	1037	33.26	5.34
CRB22sh F <sub>2</sub>	650*	33.42*	4.70*	658*	37.60*	5.72*	1040	35.15*	5.70
CRB22Lo F <sub>2</sub>	787	34.95*	4.60*	534	36.43*	5.53*	1043	35.18*	5.06*
CRB26Lo F <sub>2</sub>	612*	31.79	4.80*	561	35.25	6.32	958*	33.29	5.59
F <sub>2</sub> †	100	28.09	2.20	221	31.65	3.85	308	29.57	3.50
TM1	956	32.59	5.65	549	35.25	6.26	1154	33.41	5.84
3-79	233	28.96	2.93	333	33.85	3.91	519	34.26	3.74
DP90	1460	36.60	4.63	885	41.68	5.19	1228	40.23	5.21
LSD (p=0.05)	242	0.81	0.42	61	1.08	0.25	172	1.11	0.46

† Regular F<sub>2</sub> family from the cross of TM-1 X 3-79

\* Significantly different at the 0.05 level from TM-1

\*\* CRB2 F<sub>2</sub> represents chromosome specific F<sub>2</sub> hybrid or recombinant population developed from the cross of backcrossed chromosome substitution lines for the chromosome 2 of 3-79 (*G. barbadense*) with TM-1 (sh = short arm, Lo = long arm)

**Table 2.** Means for fiber traits of CRB lines grown at three environments.

Entry**	Arizona				New Mexico				Mississippi			
	MIC†	2.5 S.L. (inch)	E1 (%)	T1 (g/tex)	MIC	2.5 S.L. (inch)	E1 (%)	T1 (g/tex)	MIC	2.5 S.L. (inch)	E1 (%)	T1 (g/tex)
CRB2 F <sub>2</sub>	4.73	1.17	7.08	19.90	5.08	1.22	6.99	21.04	4.78	1.14	7.56	19.83
CRB4 F <sub>2</sub>	4.73	1.14	7.53	19.40	5.00	1.21	7.63	19.47	4.48	1.19	8.38	19.35
CRB6 F <sub>2</sub>	4.70	1.15	6.58	19.58	4.95	1.21	7.00	20.17	4.55	1.15	7.31*	19.51
CRB7 F <sub>2</sub>	5.05	1.15	6.95	18.60	5.20	1.18	7.12	20.13	4.68	1.14	7.69	19.64
CRB16 F <sub>2</sub>	4.65	1.15	6.83	19.93	5.00	1.16	7.32	19.94	4.45	1.14	7.94	19.91
CRB17 F <sub>2</sub>	4.63	1.15	7.70	19.08	4.93	1.16	7.50	19.85	4.45	1.13	8.44	19.99
CRB18 F <sub>2</sub>	4.65	1.16	7.28	19.30	5.08	1.21	7.44	19.32	4.53	1.15	8.44	19.24
CRB25 F <sub>2</sub>	4.40*	1.16	6.75	21.50*	4.55*	1.22	7.01	21.01	4.20*	1.19	7.94	21.23*
CRB5sh F <sub>2</sub>	4.83	1.15	7.20	19.25	4.93	1.18	7.85	19.75	4.58	1.15	8.69	19.53
CRB14sh F <sub>2</sub>	4.75	1.16	7.18	19.00	5.10	1.24*	6.94	19.82	4.58	1.18	7.63	19.63
CRB15sh F <sub>2</sub>	4.78	1.17	7.53	19.38	5.13	1.22	7.86	19.61	4.38	1.15	8.25	19.26
CRB22sh F <sub>2</sub>	4.92	1.12	8.13*	19.83	5.08	1.16	7.72	18.88	4.70	1.13	8.56	19.53
CRB22Lo F <sub>2</sub>	4.95	1.15	6.90	19.83	5.15	1.15	6.95	18.93	4.75	1.14	7.06*	20.01
CRB26Lo F <sub>2</sub>	4.68	1.18	7.13	19.45	5.00	1.22	7.21	19.88	4.53	1.18	8.31	19.34
F <sub>2</sub> †	3.30	1.22	7.88	25.25	3.85	1.35	7.70	25.66	3.45	1.25	8.38	23.24
TM1	4.80	1.15	7.10	19.45	5.13	1.17	7.54	20.47	4.58	1.16	8.19	19.38
3-79	3.98	1.37	7.03	27.70	3.88	1.44	7.04	29.61	3.45	1.40	7.56	26.24
DP90	4.78	1.18	6.33	21.83	4.45	1.22	6.91	22.52	4.45	1.14	7.06	20.39
LSD(0.05)	0.32	0.04	0.66	1.62	0.31	0.06	NS	1.22	0.30	0.03	0.72	0.91

† MIC= Micronaire, 2.5 S. L. = 2.5 % span length, E1= elongation, T1= fiber strength.

‡ Regular F<sub>2</sub> family from the cross of TM-1 X 3-79.

\* Significantly different at the 0.05 level from TM-1.

\*\* CRB2 F<sub>2</sub> represents chromosome specific F<sub>2</sub> hybrid or recombinant population developed from the cross of backcrossed chromosome substitution lines for the chromosome 2 of 3-79 (*G. barbadense*) with TM-1 (sh = short arm, Lo = long arm).