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BREEDING & GENETICS

Genetic Linkage Map and QTL Analysis of Agronomic and Fiber Quality Traits in an Intraspecific Population

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INTERPRETIVE SUMMARY

Molecular techniques have been developed that can enhance breeding efficiency for quantitatively inherited traits, many of which are economically important. Examples include yield, yield components, and fiber properties. Such traits do not consistently fall into discrete classes because environmental conditions greatly modify their performance.

Molecular markers such as restriction fragment length polymorphisms (RFLPs) can aid in selecting for quantitative traits because they identify the segments of chromosomes that contain useful quantitative trait loci (QTL) or gene locations. Thus, once identified, breeders can select for desirable QTLs without interference from environmental effects.

An RFLP genetic linkage map of cotton was developed from 119 $F_{2:3}$ families derived from a cross of MD5678ne × 'Prema' and used for QTL analysis of yield, yield components, and fiber traits.

Twenty-six QTLs were identified that were associated with agronomic and fiber quality traits. The identification of QTLs may indicate the presence of genes that influence expression of specific traits. Most QTLs contributed by the Prema parent conditioned low yield of fibers that were long, strong, and fine, while those from MD5678ne imparted high yield of short, weak, and coarse fibers.

Analysis of these QTLs will identify specific chromosome regions that affect economic traits, and can allow breeders to search germplasm for useful genes and ultimately accelerate the breeding progress.

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ABSTRACT

Molecular markers may enhance breeding for quantitatively inherited traits. We developed a restriction fragment length polymorphism (RFLP) genetic linkage map of cotton (*Gossypium hirsutum* L.) from 119 $F_{2:3}$ progeny from the cross MD5678ne × Prema, and used it for quantitative trait loci (QTL) analysis for agronomic and fiber quality traits. The linkage map comprises 81 loci mapped to 17 linkage groups with an average distance between markers of 8.7 centiMorgans (cM), covering 700.7 cM, or approximately 15% of the recombinational length of the cotton genome. Lint percentage and fiber strength were negatively correlated (r = -0.41), as were fiber strength and fiber perimeter (r = -0.38). In addition, fiber strength was positively correlated with 50% fiber span length (r = 0.36) and 2.5% span length (r = 0.31). Twenty-six QTLs were detected on nine linkage groups, and explained from 3.4 to 44.6% of the trait variation. Two QTLs were detected for lint yield and three for lint percentage, explaining from 5 to 20% of the variation in each trait. Three QTLs for fiber strength and two QTLs for fiber 2.5% span length were detected. A QTL near locus A42B1b explained 4.8% of the phenotypic variation in lint percentage, 24.6% of the variation in fiber strength, 11.5% of the variation in 2.5% span length, and 11.3% of the variation in perimeter. As expected, the Prema parent contributed QTLs for low yield of fibers that were long, strong, and fine while QTLs from MD5678ne imparted high yield of short, coarse, and weak fibers. The QTL positions on the linkage groups suggest that genes conferring fiber quality may cluster on the same cotton chromosome(s).

Cotton is the world's most-utilized natural textile fiber. This genus comprises about 50 diploid

Abbreviations: AFLP, amplified fragment length polymorphism; LOD, log-odds ratio; QTL, quantitative trait loci; RAPD, random amplified polymorphic DNA fragment; RFLP, restriction fragment length polymorphism; SSR, simple sequence repeat.

and tetraploid species (Fryxell, 1984). Two tetraploid species, *G. hirsutum* L. and *G. barbadense* L., account for 90 and 5%, respectively, of the world's cotton production (Wendel et al., 1992).

Recent developments in molecular genetics offer plant breeders a rapid and precise alternative approach to conventional selection schemes for improving cultivars for yield, adaptability, pest resistance, etc. Molecular markers are important tools for generating genetic linkage maps and have provided a significant increase in genetic knowledge of many cultivated plant species (Tanksley and Hewitt, 1988).

The majority of these genetic maps have been developed through interspecific hybridization, which currently have little use in a conventional breeding program (Reinisch et al., 1994; Yu et al., 1998). The most commonly used DNA markers are restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), simple sequence repeat (SSR), and random amplified polymorphic DNA (RAPD).

The association between markers and traits of interest can be revealed from studies based on measurements of the trait in mapped populations. Traditionally, morphological characteristics of an individual parent have been used to predict the characteristics of its progeny. Molecular markers are a promising genetic tool with the potential to enhance selection efficiency in cotton (Meredith, 1995).

In an attempt to map QTLs for yield components and fiber quality traits, a major effort to map the cotton genome is ongoing by several research groups (Reinisch et al., 1994; Shappley et al., 1998b; Ulloa and Cantrell, 1998; Yu et al., 1998).

As a part of this ongoing study, our objectives were to map the cotton genome through populations obtained from intraspecific crosses and to develop a core of markers with more practical application for cotton breeders. Herein we report a partial linkage map developed from *G. hirsutum* × *G. hirsutum* population. It was used to reveal QTLs that influence cotton yield, yield component characteristics, and fiber quality traits.

MATERIALS AND METHODS

Mapping Population

The mapping population was developed from crossing MD5678ne × Prema. MD5678ne is a nectariless isoline of 'DES 56,' and is derived from the bulk of a single, fourth-backcross,fifth-generation(BC_4F_5) plant selection. DES 56 is a Delta type cultivar that has high yield, low fiber strength, and coarse fiber. DES 56 is in the pedigree of at least 25 cultivars grown in the Mid-South (Calhoun et al., 1997). Prema is a high fiber quality Acala cultivar grown in California, but is not ubiquitous in eastern USA cultivar pedigrees. One hundred-nineteen bulk-sampled plots from the $F_{2.3}$ MD5678ne × Prema population were sampled for DNA to expose RFLPs.

Agronomic and Fiber Quality Data

The 119 $F_{2.3}$ families were grown in one-row plots at two sites near Stoneville, MS in 1991. Plot size was 1 m wide and 5 m long. One site, planted on 15 May, was a Beulah fine sandy loam soil type (coarse-loamy, mixed, active, thermic typic dystrudepts), and the other site, planted on 7 May, was a Dubbs silt loam (fine-silty, mixed, active, thermic typic hapludalfs) with three entries of MD5678ne and Prema each used as parental checks. Nitrogen rates were 112 kg ha^{-1} applied about 30 d prior to planting. Two replications of plots at each location were used to determine yield, yield components, and fiber properties. Plant density was about 113 000 plants ha^{-1}. Weed control, irrigation, and insect control were standard practices for production of cotton in the Mississippi Delta.

Boll weight was determined from 50 hand-harvested bolls, just prior to first harvest from each plot. Lint percentage was determined from the 50-boll sample by ginning on a small 10-saw experimental gin. Seed weight was determined by the weight of 100 seeds from each plant. For each plot, total lint yield was determined by multiplying lint percentage by total seed cotton weight. Seed cotton yield was determined by hand harvest. Total seed cotton weight was determined as the sum of the first and second harvest.

No QTLs were detected for first harvest (data not shown), so only the total yield results are reported. A commercial fiber testing company, Star Laboratories, Knoxville, TN, determined fiber properties. The following agronomic traits were evaluated: lint yield, lint percentage, boll weight, and seed weight. The following fiber quality traits were evaluated: 2.5 and 50% fiber span length, fiber bundle strength, fiber elongation, micronaire reading, fiber maturity, and fiber perimeter.

Fiber 2.5 and 50% span lengths were measured with a digital Fibrograph[1] instrument. Span length is the distance spanned by the indicated percentage of fibers in the fiber sample scanned by the Fibrograph. Fiber span length at 2.5% estimates the length of the longest 2.5% of fibers expressed in millimeters (mm). Fiber strength is the strength (kN m kg^{-1}) of a bundle of fibers measured by the stelometer. Elongation (%) is an estimate of the elasticity of the bundle sample. Micronaire is a measure of fiber fineness and maturity. Arealometer is an instrument that measures fiber fineness and shape based on the resistance a given sample offers to the flow of air at two pressures. Fineness and shape provide estimates of maturity and perimeter. The maturity measure is based on the simple linear regression prediction of the caustic soda percent maturity method by Hertel and Craven (1951). Perimeter is defined as the distance around the outside wall of the fiber cross section, and is determined in micrometers.

Probe Construction and RFLP Analysis

A cDNA library was developed from leaf tissue of six different cotton cultivars, generating a set of probes using the pGem-11zt(-) vector (Promega) at Biogenetic Service, Inc., Brookings, SD. The radiolabeling of the probes was done by random priming. In 1991 a bulk sample of DNA from 50 $F_{2.3}$ individuals from the two planted locations (25 from each location) was assayed with 106 probe × enzyme (*EcoRI* and *EcoRV*) combinations for purposes of finding RFLPs.

[1] Mention of name proprietary product, or specific equipment does not constitute a guarantee or warranty by the USDA and does not imply its approval to the exclusion of other products that may be suitable.

Genetic Linkage Analysis and Map Construction

Informative fragments were scored as present (+) or absent (-) for a dominant marker (3:1 segregation ratio), and if both alleles from the parents were identified, then the marker was scored as codominant (1:2:1). The JOINMAP (Stam and Van Ooijen, 1995) computer program was used to test for Chi-square goodness-of-fit to determine the fit of observed genetic segregation ratios of alleles compared with expected segregation ratios, and to develop the final map. The MAPMAKER/Exp 3.0 (Lander et al., 1987) also was used to develop this genetic map. Log-odds ratio (LOD) scores of 3 to 6 were examined, using the Kosambi map function to determine distances, and using a maximum distance of 40 cM to determine linkage between two markers to develop the map. A log-odds ratio score of 4.0 was set to develop the linkage map.

Statistical and QTL Analyses

Data Analysis

Statistical analyses were performed on the agronomic and fiber quality data at each location. Location by family (G × E) interactions were not significant ($P > 0.05$) for agronomic and fiber quality traits, therefore effects of location by family were small for most traits. Means averaged across the two locations were used in further analyses. The general linear model [PROC GLM of SAS (SAS Inc., Cary, NC)] and a set of contrasts performed the analyses of variance and mean separation. For the association of lint yield, lint percentage, boll weight, seed weight, 50 and 2.5% fiber span length, fiber strength, fiber elongation, micronaire, fiber maturity, fiber perimeter, stepwise regression and correlations (PROC REG/stepwise and PROC CORR, respectively, SAS Inc., Cary, NC) were performed.

Quantitative Trait Loci (QTL) Analysis Programs

Three different computer programs, MAPMAKER/QTL (Lander et al., 1987), MapQTL (Van Ooijen and Maliepaard, 1996), and QTL Cartographer (Basten et al., 1994) performed QTL

Table 1. RFLP loci exhibiting deviations from Mendelian segregation ratios derived from 119 bulk-sampled $F_{2:3}$ plots of a MD5678ne × Prema cotton population evaluated at two sites near Stoneville, MS. Expected Mendelian segregation ratio for a dominant marker is 3:1, where C = 3 (Prema fragment present) and A = 1 (fragment absent) and D = 3 (MD5678ne fragment present) and B = 1 (fragment absent). Co-dominant marker segregation ratios should be 1:2:1, where A = 1 (Prema), H = 2 (fragment present from both parents), and B = 1 (MD5678ne). Given 119 families, a normal segregation ratio should be A = 21 families, H = 77 families, and B = 21 families. The minus sign represents missing families. Chi-square values are listed, all with one degree of freedom. ***

Locus	RFLP allelic segregation						
	A†	H‡	B§	C¶	D#	-	X^2
A17F2	0	0	55	0	64	0	28.57
A20D6	0	0	53	0	66	0	24.23
A29A2	0	0	53	0	66	0	24.23
A45B6	0	0	52	0	67	0	22.19
A47B1	0	0	49	0	70	0	16.61
A48B4	0	0	55	0	64	0	28.57
A50D1	0	0	58	0	61	0	35.77
A58A1	0	0	56	0	63	0	30.88
A66C3	0	0	47	0	72	0	13.34
A87D6	0	0	49	0	70	0	16.61
A89C3	0	0	50	0	69	0	18.38
A41D6a	46	66	7	0	0	0	26.98
A22C3b	48	0	0	71	0	0	14.93
A44B4	55	0	0	64	0	0	28.57
A48F2	56	0	0	63	0	0	30.88
A40E1	59	0	0	60	0	0	38.34

***** Significant at $P < 0.001$.**

† A = absent (-) informative fragment if marker scored as dominant if it is not = D.

‡ H= heterozygote.

§ B = absent (-) informative fragment if marker scored as dominant - if it is not = C.

¶ C = alleles from Prema parent.

 # D= informative fragment present (+) from MD5678ne parent.

analysis. MAPMAKER/QTL uses interval mapping to detect and locate a QTL. MapQTL conducts QTL analysis by interval mapping and composite interval mapping. It also calculates the estimated mean of the distribution of the quantitative trait associated with each parental source as well as the heterozygote. The computer program QTL Cartographer version 1.12 uses simple linear regression, stepwise regression, interval mapping, and composite interval mapping that extends the regression equation to include more markers as cofactors in order to remove the effects of multiple QTLs.

Linkage groups were scanned to determine whether the likelihood ratio (LR) test statistic is increasing or decreasing. Assume L_1 is the likelihood that the QTL is located in the interval flanked by the markers and L_0 is the likelihood there is no QTL in the interval (i.e., the null hypothesis or H_0), the log-odds ratio (LOD) is defined as:

$$LOD = -log(L_0 / L_1)$$

The likelihood ratio test statistic (LR) calculated by QTL cartographer according to Basten et al. (1997) is:

$$LR = -2l\,n\,(L_0 / L_1) = -2l\,n\,10^{-LOD}$$
$$= 2\,(ln\,10)\,LOD = 4.605\,LOD$$

And thus,

$$LOD = -log\,exp\,\{-(LR / 2)\}$$
$$= (1/2)(log\,e)LR = 0.217\,LR.$$

In this study, the threshold value of 9.21 for LR was set for declaring a QTL, corresponding to a LOD score of 2.0. In addition, the reported QTLs were detected and located by at least two of the three QTL analysis programs used in this study. R^2 reports the genotypic contribution to trait variation as a percentage of the total variation. The detection and estimation of association of trait data with marker data were performed by using the original data from each fiber trait and the overall pooled mean for each trait from the two locations.

RESULTS

Marker Segregation

A total of 106 cDNA probes were examined with *EcoRI* and *EcoRV* restriction enzymes, revealing 113 RFLP loci. The informative fragments hybridized to 119 DNA samples obtained from bulk-sampled progenies from F_2-derived F_3 (MD5678ne × Prema) population. Segregation distortion was observed in 36 RFLP loci as deviation from the expected 3:1 ratio for a dominant locus and 1:2:1 for a codominant locus. Nineteen of the 36 markers exhibiting non-Mendelian segregation were from the Prema parent. In addition, 11 of the 16 loci exhibiting the greatest distortion ($X^2 > 25.0$) have higher than expected allelic frequency from the

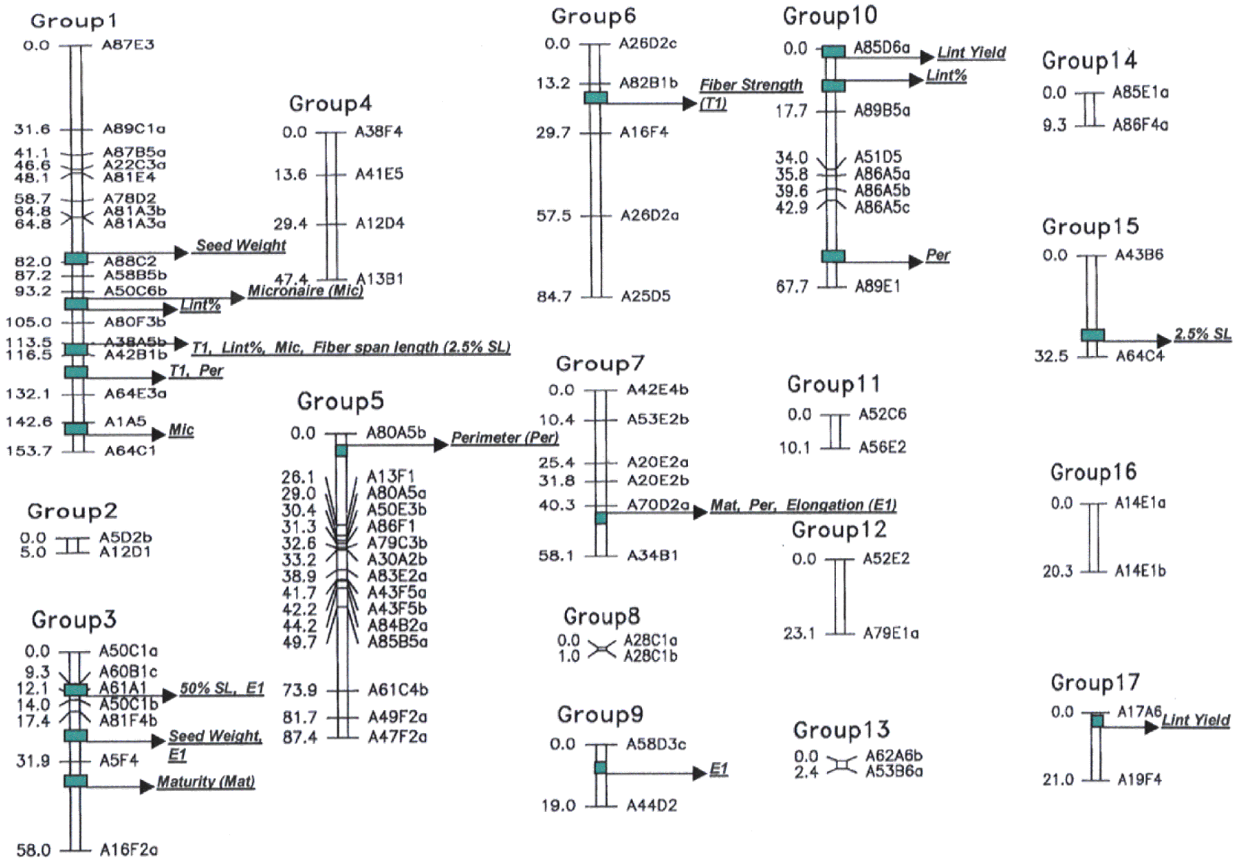

Fig. 1. Cotton RFLP linkage map from 119 bulk-sampled plots of $F_{2.3}$ (MD5678 × 'Prema') population with 81 loci, 17 linkage groups, covering 700.7 cM of the genome. Map distances between adjacent markers are in centiMorgans. The map was constructed by using the JOINMAP (Stam and Van Ooijen, 1995) computer program, with Kosambi function and log odds ratio of 4.0. The location of QTLs for agronomic and fiber quality traits was detected with log odds ratio >2.0 by at least two different QTL programs (MAPMAKER/QTL, QTL Cartographer, and MapQTL). E1=fiber elongation. T1=fiber strength.

Prema parent, indicating an allelic preference for this parent in this population (Table 1).

Cotton Genetic Linkage Map

The majority of the RFLP markers used to construct the linkage map showed normal Mendelian segregation. Ninety cDNA probes yielded 97 loci, of which 36 segregated as codominant and 61 as dominant. From linkage analysis performed on the 97 RFLP markers, 81 were found to be linked and 16 remained unlinked.

Our cotton linkage map comprises 17 linkage groups with at least two markers per group, and with an average distance between two markers around 8.7 cM (Fig. 1). The map includes 81 loci covering 700.7 cM based on the JOINMAP computer program (Stam and Van Ooijen, 1995). Estimated map distance of the cotton genome is around 4660 cM (Reinisch, et al., 1994), thus the 81 loci in our map covered about 15% of the total recombinational length of the cotton genome.

Because the RFLP markers exhibiting the most segregation distortion were discarded, the map shows a slight excess of MD5678ne alleles for certain loci. However, these loci showed at least 70% confidence for normal Mendelian segregation. Reinisch et al. (1994) observed disomic chromosome pairing in a population derived from an interspecific population. The alternate allele is probably masked by monomorphic, co-migrating, duplicated DNA fragments. The segregation distortion and the high proportion of dominant RFLP markers in this intraspecific population presumably result from polyploidy.

Agronomic and Fiber Quality Trait Correlations

The frequency distribution of agronomic and fiber quality traits data shows genetic variation consistant with multigenic inheritance. Correlations among agronomic and fiber traits based upon $F_{2.3}$ family means are given in Table 2, and agree with previous studies (Meredith et al., 1991; Kloth, 1998; Shappley et al., 1998b; Ulloa et al., 2000). Lint yield was correlated with all agronomic traits, and with 50% fiber span length, micronaire, and fiber maturity (Table 2). Lint percentage was correlated with most agronomic and fiber traits. A negative correlation between lint percentage and fiber strength (r = -0.41) was observed. Three of the components of fiber strength, 50% fiber span length, 2.5% fiber span length, and fiber perimeter (Meredith, 1992), were correlated (r = 0.36, 0.31, and -0.38, respectively). Seed weight was correlated with all traits (Table 2).

Quantitative Trait Loci for Agronomic and Fiber Traits

Based on the program recommendation for the accepted log odds ratio threshold of QTL detection (e.g., LOD > 2.0), MAPMAKER/QTL yielded 26 QTLs, MapQTL yielded 45 QTLs, and QTL Cartographer yielded 34 QTLs. The 26 QTLs identified by two of the three detection programs were assigned to nine linkage groups of the cotton genetic map. These QTLs explain from 3.4 to 44.6% of the variation of the traits (Table 3). The map distance from the nearest molecular marker for each QTL was excluded from Table 3 due to the different values from each QTL program. However, the discrepancy was an average of only 3 cM.

Two QTLs were detected for lint yield. The one in linkage group 10 near locus A85D6a explained 9.2% of the variation, and the one in linkage group 17 near locus A17A6 explained 15.7% of the trait variation.

For lint percentage, three QTLs were detected. Two QTLs were detected in linkage group 1, one QTL near locus A80F3b and the other near locus A42B1b. The third QTL was detected on group 10 near locus A85D6a. The agronomic traits, lint yield and lint percentage, had a QTL near locus A85D6a that explained 9.2% of the variation of lint yield and 12.6% of the variation of lint percentage.

For fiber quality traits, three QTLs were identified for fiber strength. Two QTLs were detected on linkage group 1, one near locus A38A5b and the other near locus A42B1b. The QTLs near locus A38A5b explained 24.6% of the variation while the QTLs near locus A42B1b explained 10.6% of the variation. The third QTL was detected on linkage group 6 near locus A82B1b and explained around 11.3% of fiber strength variation.

For 2.5% fiber span length, two QTLs were detected. One QTL was detected on linkage group 1 near locus A38A5b and explained 11.5% of the variation. The second QTL was detected on group 15 near locus A64C4 and explained 44.6% of the variation.

The fiber quality traits of fiber strength, 2.5% fiber span length, and micronaire had a QTL near locus A38A5b. The QTL for fiber strength explained 24.6%. The QTL for 2.5% span length explained 11.5%, and the QTL for micronaire explained 21.7%.

Table 2. Correlations among cotton lint yield, yield components, and fiber quality traits from 119 $F_{2\cdot3}$ bulk-sampled plots of a MD5678ne × Prema population evaluated at two sites near Stoneville, MS.

	Agronomic traits					Fiber quality traits				
Cotton traits	**Lint yield**	**Lint percentage**	**Boll weight**	**Seed weight**	**50% Fiber span length**	**2.5% Fiber span length**	**Fiber strength**	**Fiber elongation**	**Micronaire**	**Maturity**
Lint percentage	0.16‡									
Boll weight	0.35‡	-0.09*								
Seed weight	0.27‡	-0.42‡	0.52‡							
50% Fiber span length	0.10*	-0.43‡	0.24‡	0.50‡						
2.5% Fiber span length	0.04	-0.58‡	0.26‡	0.52‡	0.71‡					
Fiber strength	-0.02	-0.41‡	0.12†	0.32‡	0.36‡	0.31‡				
Fiber elongation	0.01	0.15†	-0.08	-0.27‡	-0.17‡	-0.17‡	-0.49‡			
Micronaire	0.41‡	0.27‡	0.23‡	0.17‡	-0.06	-0.34‡	-0.08	-0.07		
Fiber maturity	0.36‡	0.06	0.25‡	0.25‡	0.12†	-0.12†	0.18‡	-0.19‡	0.72‡	
Fiber perimeter	-0.01	0.29‡	-0.08	-0.21‡	-0.26‡	-0.25‡	-0.38‡	0.17‡	0.05	-0.59‡

***, †, ‡ significant at P < 0.05, 0.005, 0.0005, respectively.**

Table 3. Twenty-six QTLs [detected with log odds ratio (LOD) score > 2.0] affecting agronomic and fiber quality traits assayed in a $F_{2:3}$ MD5678ne × Prema cotton population evaluated at near Stoneville, MS. The 26 QTLs were assigned to nine linkage groups of the cotton genetic map.

					Trait means				
Trait	**Program†**	**Locus**	**Group**	**LOD‡**	**MD5678ne§**	**Heterozygote§**	**Prema§**	**SD¶ ±**	**%ExpVar#**
Lint yield (kg ha^{-1})	**M, Q, J**	**A85D6a**	**10**	**2.2**	**1262.5**	**1256.3**	**1168.2**	**96.6**	**9.2**
	Q, J	**A17A6**	**17**	**2.2**	**1313.6**	**1239.9**	**1207.3**	**88.7**	**15.7**
Lint percentage (%)	**M, Q, J**	**A80F3b**	**1**	**2.8**	**38.3**	**38.4**	**37.0**	**1.32**	**19.6**
	Q, J	**A42B1b**	**1**	**2.8**	**38.0**	**38.2**	**36.6**	**1.30**	**4.8**
	M, Q, J	**A85D6a**	**10**	**3.1**	**37.5**	**38.0**	**36.7**	**1.33**	**12.6**
Seed weight (g seed^{-1})	**Q, J**	**A88C2**	**1**	**2.4**	**0.1039**	**0.1081**	**0.1119**	**0.0075**	**3.8**
	M, Q, J	**A81F4b**	**3**	**2.3**	**0.1009**	**0.1050**	**0.1098**	**0.0078**	**11.2**
Fiber strength (kN m kg^{-1})	**M, J**	**A38A5b**	**1**	**3.1**	**217.5**	**223.1**	**231.4**	**11.9**	**24.6**
	M, Q, J	**A42B1b**	**1**	**2.5**	**217.6**	**223.4**	**232.2**	**11.8**	**10.6**
	M, Q, J	**A82B1b**	**6**	**2.3**	**218.4**	**227.6**	**217.4**	**9.8**	**11.3**
Fiber elongation (%)	**Q, J**	**A50C1b**	**3**	**2.8**	**8.3**	**7.85**	**7.55**	**0.52**	**3.4**
	M, Q, J	**A81F4b**	**3**	**3.8**	**8.4**	**7.71**	**7.52**	**0.48**	**11.9**
	M, Q, J	**A58D3c**	**9**	**6.4**	**8.0**	**7.78**	**7.39**	**0.47**	**31.6**
50% Fiber span length (mm)	**M, Q, J**	**A50C1b**	**3**	**2.5**	**0.56**	**0.57**	**0.57**	**0.01**	**10.4**
2.5% Fiber span length (mm)	**M, Q, J**	**A38A5b**	**1**	**2.5**	**28.25**	**29.01**	**29.87**	**1.15**	**11.5**
	Q, J	**A64C4**	**15**	**2.2**	**29.37**	**27.73**	**29.75**	**0.65**	**44.6**
Micronaire (units)	**Q, J**	**A50C6b**	**1**	**3.2**	**4.6**	**4.4**	**4.3**	**0.26**	**9.1**
	M, Q, J	**A38A5b**	**1**	**3.2**	**4.7**	**4.4**	**4.3**	**0.23**	**21.7**
	M, Q, J	**A42B1b**	**1**	**4.2**	**4.7**	**4.4**	**4.2**	**0.24**	**19.3**
	Q, J	**A1A5**	**1**	**3.3**	**4.5**	**4.4**	**4.2**	**0.27**	**6.2**
Fiber maturity (%)	**M, Q, J**	**A5F4**	**3**	**2.3**	**83.3**	**86.2**	**86.8**	**3.4**	**15.2**
	Q, J	**A70D2a**	**7**	**2.5**	**83.5**	**85.4**	**86.1**	**1.8**	**33.6**
Fiber perimeter (µm)	**M, Q, J**	**A42B1b**	**1**	**2.9**	**49.8**	**49.4**	**47.8**	**1.9**	**11.3**
	Q, J	**A80A5b**	**5**	**2.3**	**49.8**	**48.6**	**48.9**	**1.8**	**5.8**
	M, Q, J	**A70D2a**	**7**	**3.0**	**49.7**	**48.3**	**48.3**	**1.74**	**9.3**
	Q, J	**A89E1**	**10**	**2.3**	**50.7**	**49.0**	**51.3**	**1.47**	**28.8**

† Programs detected the same QTL near the same locus, M = MAPMAKER/QTL, Q = QTL Cartographer, and J = MapQTL.

‡ LOD = The LOD scores for each QTL were derived from the likelihood ratio test statistic from QTL Cartographer.

§ Means for each parent and the heterozygote from informative fragments calculated by MapQTL program.

¶ SD = Standard deviation calculated by MapQTL.

 # %Exp = The trait percentage variation explained by the QTL, calculated by MapQTL.

Four QTLs for micronaire were detected and explained from 9.1 to 21.7% of the trait variation.

Four QTLs for fiber perimeter were identified on different linkage groups. One QTL was on linkage group 1 close to locus A42B1b and explained 11.3% of the variation. The second was placed on linkage group 5 near locus A80A5b and explained 5.8% of the variation. The third QTL was detected on linkage group 7 near locus A70D2a and explained 9.3% of the variation. The fourth QTL for fiber perimeter was detected on Linkage Group 10 near locus A89E1 and explained 28.8% of the variation (Table 3).

Each of the following fiber traits - lint percentage, fiber strength, 2.5% fiber span length, and fiber perimeter - had a QTL within the vicinity of locus A42B1b. The QTL for lint percentage explained 4.8% of the variation. The QTL for fiber strength explained 24.6% of the variation. The QTL for 2.5% fiber span length explained 11.5% of the variation, and the QTL for fiber perimeter explained 11.3% of the variation. (Fig. 1 and Table 3).

Two linkage groups carried most QTLs: linkage group 1 with 10, and linkage group 3 with five.

Several QTLs affecting fiber quality traits were detected within the vicinity, e.g., linkage groups 1 and 3. The clustering of QTLs within linkage groups may indicate that genes for fiber quality traits are linked on the same chromosome(s). These results agree with similar studies in other crops (Alber et al., 1991; Paterson et al., 1991; Veldboom et al., 1994; Xiao et al., 1996) as well as in cotton (Shappley et al., 1998b), where correlated traits often have QTL mapping to the same chromosomal locations. The same trend was observed for the fiber quality traits in this study.

DISCUSSION

As a step toward further understanding the structural genomics of upland cotton, we generated a genetic linkage map from 119 bulk-sampled plots of an $F_{2.3}$ MD5678ne × Prema population. JOINMAP and MAPMAKER/Exp 3.0 linked the same number of loci in linkage groups, except for two RFLP loci. However, some discrepancy was observed in map distances within linkage groups. Following JOINMAP marker order, MAPMAKER/Exp 3.0 covers 1033 cM or about 23% of the map distance of the cotton genome. The discrepancy between the two programs is probably due to markers exhibiting distorted segregation due to the rationale used by these programs to assign markers to linkage groups and their ordering to construct the map. JOINMAP uses the test of independence, which is not affected by segregation distortion rather than the normal log odds ratio score employed by MAPMAKER/Exp 3.0.

This is the second map developed from an intraspecific population and uses the same set of cDNA probes developed from the same cDNA library. The present map shares 16 RFLP loci with that of Shappley et al. (1998a), plus seven that were discarded because of their high segregation distortion in this population. Linkage group 3 shares four RFLP loci with the map of Shappley et al. (1998b) on linkage group 10. In both studies, these linkage groups contain QTLs for fiber quality traits. The remainder of RFLPs common to the two studies were scattered throughout the linkage groups in this study. In addition, both maps report possible allelic RFLPs (e.g., A86A5a, A86A5b, and A86A5c in group 10). After carefully examining RFLP X-ray films, we noticed that occasionally more than one allele was observed to behave independently from the remainder of the alleles for a particular locus. It is possible that a few RFLP alleles were scored as dominant markers in both *EcoRI* and *EcoRV* digestions. Reinisch et al. (1994) observed in a population derived from an interspecific cross that the alternate allele is probably masked by monomorphic co-migrating duplicated DNA fragments. The segregation distortion and the high proportion of dominant RFLP markers in this intraspecific population may also result from polyploidy.

The analysis of the parental origin of each RFLP allele revealed slight preference for the Prema alleles in this mapping population. Markers exhibiting segregation distortion also were observed with the same tendency. Segregation distortion was observed in 36 RFLP loci that deviated significantly from Mendelian segregation. Segregation distortion due to higher recombination rates, resulting in preferential transmission of DNA segments to its offspring from one of the parents, has been found in populations from other crops (Rhoades, 1941; Robertson, 1984; Paterson et al., 1991). Occasionally, such preferential segregation can be used as a basis for genotypic selection among individuals, accelerating introgression of a desired chromosomal segment into a new genetic background (Young and Tanksley, 1989).

Breeding for high cotton lint yield is still the primary goal of any breeding program, but improving fiber quality has become increasingly important (Meredith et al., 1991). Many agronomic and fiber quality traits were correlated based on the 119 $F_{2.3}$ family means. These correlations may be useful in developing selection criteria to simultaneously improve yield and fiber quality traits.

Qualitative trait loci explaining from small to moderately high proportions of phenotypic variance (3.4 to 44.6% of trait variation) were common in our study and support a model for quantitative inheritance for most agronomic and fiber quality traits (Landen and Thompson, 1990; Paterson et al., 1991). The majority of the RFLP alleles associated with long fiber span length, fineness, and strong fiber were contributed from Acala Prema, while most of the alleles associated with high yield and high lint percentage were contributed from the MD5678ne parent.

These results suggest that gene introgression at the DNA level within *Gossypium hirsutum* was successfully accomplished for the agronomic and fiber quality traits in the $F_{2.3}$ families. However, these small additive and dominant effects do not preclude other types of gene action for the expression of these agronomic and fiber quality traits. Linkage groups 1 and 3 locate and share strongly the above RFLP allelic information, suggesting that genes for fiber traits may recombine as DNA blocks during meiosis (Fig. 1 and Table 3). Even though a QTL for fiber strength was located near marker A42B1b, this marker also affects lint percentage, micronaire, 2.5% fiber span length, and fiber perimeter (Fig. 1 and Table 3).

Multiple traits can be correlated due to linkage, pleiotropy, or the correlated traits may be components of a more complex variable. Two components of bundle fiber strength are fiber length and perimeter (Meredith, 1992). Fine fiber (e.g., small perimeter) results in more fibers per bundle, which can confer greater fiber strength. Additionally, longer fibers promote fiber-to-fiber contact, tending to increase fiber strength. Micronaire and fiber length, which both influence lint percentage, are another example of components of a more complex trait.

Linkage group 1 contains nine QTLs, all of which are components of one or more other traits. The wide map distance (62.6 cM) between seed weight and micronaire suggests more than one gene may be located within this region. Linkage group 1 has genes for multiple fiber functions with several linked QTLs for fiber quality.

Unexpected sources of variation may also be attributed to epistasis. Favorable (or unfavorable) alleles may be present in a parental line but not expressed in its genetic background. When crossed to another individual, however, epistatic interaction may affect the expression of the alleles (Veldboom et al., 1994). Further research is needed on recombination rates to monitor these events.

The partial linkage map developed by the *G. hirsutum* × *G. hirsutum* population promises to provide a better understanding of the cotton crop by possibly providing a core of markers with more practical application than those developed in interspecific populations.

In this ongoing study, research is in progress toward developing a consensus linkage map through intraspecific populations. The combination of correlations, QTLs, and molecular marker information will help breeders to understand and dissect agronomically important traits and to develop new methods of multi-directional selection.

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