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Quantitative analysis and QTL mapping for agronomic and fiber traits in an RI population of upland cotton

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Abstract Genetic mapping is an essential tool for cotton (*Gossypium hirsutum* L.) molecular breeding and application of DNA markers for cotton improvement. In this present study, we evaluated an RI population including 188 RI lines developed from 94 F_2-derived families and their two parental lines, 'HS 46' and 'MARCABUCAG8US-1-88', at Mississippi State, MS, for two years. Fourteen agronomic and fiber traits were measured. One hundred forty one (141) polymorphic SSR markers were screened for this population and 125 markers were used to construct a linkage map. Twenty six linkage groups were constructed, covering 125 SSR loci and 965 cM of overall map distance. Twenty four linkage groups (115 SSR loci) were assigned to specific chromosomes. Quantitative genetic analysis showed that the genotypic effects accounted for more than 20% of the phenotypic variation for all traits except fiber perimeter (18%). Fifty six QTLs (LOD $>$ 3.0) associated with 14 agronomic and fiber traits were located on 17 chromosomes. One QTL associated with fiber elongation was located on linkage group LGU01. Nine chromosomes in sub-A genome harbored 27 QTLs with 10 associated with agronomic traits and 17 with fiber traits. Eight chromosomes in D sub-genome harbored 29 QTLs with 13 associated with agronomic traits and 16 with fiber traits. Chromosomes 3, 5, 12, 13, 14, 16, 20, and 26 harbor important QTLs for both yield and fiber quality compared to other chromosomes. Since this RI population was developed from an intraspecific cross within upland cotton, these QTLs should be useful for marker assisted selection for improving breeding efficiency in cotton line development.

Keywords Linkage mapping · QTL · Recombinant inbred lines · Upland cotton · Agronomic traits · Fiber traits

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Introduction

Upland cotton (*Gossypium hirsutum* L. 2n = 52), one of four cultivated *Gossypium* species, is the world's

leading fiber crop and renewable resource, providing natural fiber for the manufacture of textiles. Demands for enhancement of fiber quality traits such as fiber length and fiber strength have been increasing because of changes in spinning technology in the textile industry; however, most commercial cultivars, although high in yields, are lacking in desirable fiber quality. The primary breeding goal for the worldwide cotton researcher is how to genetically improve both yield and fiber quality. Many previous studies showed that agronomic and fiber traits of interest were negatively associated and controlled by multiple environmental sensitive quantitative genes. These genetic and non-genetic factors greatly confound the conventional cotton breeding schemes that many cotton breeders employed.

Molecular linkage map construction has been recognized as an essential tool for plant molecular breeding using DNA markers because they have the properties of neutrality, lack epistasis, and are simply inherited Mendelian characters (Tanksley and McCough 1997). Therefore, the use of DNA markers, highly associated with traits of importance will be an important approach to reaching this breeding goal through marker assisted selection (MAS). To date, much effort in detection of various types of DNA markers for linkage map construction in cotton has been investigated. For example, restriction fragment length polymorphism (RFLP) markers have been widely used in both interspecific populations of *G. hirsutum* × *G. barbadense* L. (i.e. Reinisch et al. 1994; Jiang et al. 1998; Kohel et al. 2001; Lacape et al. 2003, 2005; Rong et al. 2004) and intraspecific populations within *G. hirsutum* (Shappley et al. 1998a; Ulloa et al. 2002, 2005). On the other hand, the PCR based DNA markers such as amplified fragment length polymorphism (AFLP), random amplified polymorphic DNAs (RAPD), simple sequence repeats (SSR), sequenced-target sites (STS), and expressed sequence tags-SSR (EST-SSR) have also been widely used in cotton linkage mapping (i.e. Lacape et al. 2003; Zhang et al. 2003; Rong et al. 2004; Nguyen et al. 2004; Han et al. 2004, 2006). In addition, several new types of DNA markers have been discovered (Tomkins et al. 2001; Qureshi et al. 2004; Park et al. 2005; Zhang et al. 2005; Frelichowski et al. 2006). As expected, it is not surprising that more DNA markers were detected between *G. hirsutum* and *G. barbadense* than within *G. hirsutum* (Brubaker and Wendel 2001; Gutierrez et al. 2002; Lacape et al. 2003, 2005; Rong et al. 2004). Two interspecific genetic maps with wide genome coverage (>80%) have been recently reported derived from an F_2 population (Rong et al. 2004) and BC_1 progenies (Lacape et al. 2003).

With DNA markers and linkage maps available, precisely identifying QTLs contributing to agronomic and fiber traits of interest has become possible. Most studies have been focused on fiber traits (Jiang et al. 1998; Shappley et al. 1998b; Ulloa and Meredith 2000; Kohel et al. 2001; Ulloa et al. 2002, 2005; Zhang et al. 2003, 2005; Lin et al. 2005; Park et al. 2005; Shen et al. 2005, 2006; Frelichowski et al. 2006). However, very few papers regarding QTL for agronomic traits, especially for yield have been reported (Shen et al. 2006). One major reason is that most mapping populations were F_2 or backcross, which have difficulties measuring agronomic traits over repeated plots and /or environments especially when the heritability for these traits are low.

Three types of mapping panels, F_2, backcross, and recombinant inbred (RI) populations have been popularly used for plant genetic mapping. Among these in cotton, F_2 or backcross inter- and intra-specific populations predominate. Unlike F_2 or backcross populations, an RI population consists of a number of RI lines, which are in high homozygousity under multiple cycles of self pollination, can be repeatedly evaluated in different environments, by different researchers, and at different times (Burr et al. 1988; Burr and Burr 1991). Thus, better QTL assessments would result especially for low-heritability traits (Wu et al. 2003a). However, developing a large RI population is not easy, especially in *G. hirsutum* × *G. barbadense* due to possible distorted segregation. To date, three RI populations in tetraploid cotton have been reported (Wu et al. 2004; Park et al. 2005; Shen et al. 2006; Frelichowski et al. 2006). Two of these RI populations represent intraspecific crosses within *G. hirsutum* (Wu et al. 2004; Shen et al. 2006). QTLs contributing to yield and other agronomic traits in an RI population were only reported by Shen et al. (2005). The RI population we previously reported included 188 RI lines, which were developed from 94 F_2-derived families using bulk-based procedure (Wu 2003; Wu et al. 2004). The feasibility of the use of such a bulk-based RI population for linkage and QTL mapping was evaluated by Monte Carlo simulation (Wu 2001; Wu et al. 2003a).

Many individual markers or linkage groups have been assigned to the specific chromosomes using chromosome substitution lines (i.e. Reinisch et al. 1994; Liu et al. 2000a, b; Kohel et al. 2001; Rong et al. 2004; Ulloa et al. 2005). The assignment of these DNA markers have greatly facilitated the detection of QTLs contributing to traits of importance, not only linked to DNA markers, but also on specific chromosomes or chromosome arms. More importantly, such work provides an opportunity for different reports being comparable regardless of the parental lines and types of DNA markers in different mapping populations. Many QTLs associated with quantitative traits were also assigned to specific chromosomes (i.e. Jiang et al. 1998; Kohel et al. 2001; Lacape et al. 2003; Park et al. 2005; Shen et al. 2005, 2006; Zhang et al. 2005; Frelichowski et al. 2006).

In this present study, we evaluated an RI population including 188 RI lines developed from 94 F_2-derived families using bulk-based procedure (Wu et al. 2004) and its two parental lines at Mississippi State, MS for two years. Fourteen agronomic and fiber traits were measured. One hundred forty one (141) polymorphic SSR markers were screened for this population and 125 markers were used to construct a linkage map. The objective of this study was to identify QTLs associated with agronomic and fiber traits in an RI population of upland cotton. The results will provide molecular mapping information on marker assisted selection for the improvement of multiple traits of interest.

Materials and methods

Materials and field experiments

One hundred eighty-eight RI lines (F_8) were developed by a modified single-hill (bulked progeny row) procedure (Fehr 1987) from the *G. hirsutum* intraspecific cross HS46 (P_1) × MARCABUCAG8US-1-88 (P_2) (Shappley et al. 1998a, b). A cross between P_1 and P_2 was made at Mississippi State, MS in 1991 and the F_1 generation was grown in 1992. One-hundred F_2 seeds from one F_1 individual were planted in the greenhouse and selfed in 1992. The F_3 seeds were planted in 12-m single row plot (named as single-hill) at Mississippi State in the spring of 1994 and plants were self-pollinated and bulked. In the winter of 1994, F_4 selfed seeds were sent to a nursery in Mexico for generation increase by selfing and bulked to obtain F_5 seeds. In the spring of 1995, two-row F_5 plots from each F_2-derived family were planted and 25 individual plants were selfed to obtain F_6 seeds. In the winter of 1996, one seed from each of 25 selfed plants from each F2-derived family was sent to Mexico. Up to 8 plants from each family were selfed to produce F_7 seeds. In the winter of 1998, up to 8 individual plant progenies from each of 94 F_2-derived families were planted and hand harvested separately (F_8 seeds). Two lines were then randomly chosen from each F_2-derived family.

These 188 RI lines and two parental lines were grown at the Plant Science Research Center, Mississippi State, MS in 1999 and 2000. The seeds used for the 2000 test were boll samples collected in the 1999 test. The experimental design was a randomized complete block with four replicates for each of the two years. Plot size was two rows, 12 × 0.97 m, with an unplanted row between plots. Plots were thinned to single plants spaced approximately 10 cm. The planting date was May 12 each year. Soil type was a Leeper silty clay loam (Fine, smectitic, nonacid, thermic Vertic Epiaquepts). Standard cultural practices were followed throughout the growing season. A 50-boll sample was collected from each plot before machine harvest. Each sample was weighed to determine boll weight and then ginned on a laboratory 10-saw gin to determine lint percentage and seed index (SI, weight in gram of 100 gin-run seeds) and to provide lint samples for fiber analyses. Lint samples were sent to STARLAB, Inc., Knoxville, TN, for determination of conventional single instrument fiber quality: micronaire reading (MIC), elongation (EL), fiber strength (T1), 2.5% span length (SL2.5), and 50% span length (SL50). Additional fiber measurements were made using the arealometer instrument for maturity (M), perimeter (P), weight fitness (WF), and wall thickness (WT). The plots were harvested with a machine picker, with lint yield ha^{-1} determined by multiplying seed cotton yield by lint percentage.

Genomic DNA extraction

Young leaves for each RI line were collected in the summer of 1999 in the field plot and freeze-dried

following described protocols (Saha et al. 1997). DNA was isolated from 20 mg (dry weight) of cotton leaf tissue previously ground with a Qiagen Mixer Mill MM 300 and using the DNeasy Plant mini kit (Qiagen, Santa Clarita, CA) following the manufacture's protocol with the following modification. Sodium metabisulfite was added to the lysis buffer at concentration of 10 μM (Horne et al. 2004).

SSR amplification and analysis

SSR primers were obtained from four different sources: BNL, CM, JESPR, and CIR primers whose sequences are available at http://www.mainlab.clemson.edu/cmd/projects. PCR was performed using 12.5 ng of DNA as template, 0.15 μM each fluorescently labeled forward (5′) and non-labeled reverse (3′) SSR specific primer pairs (Sigma Genosys, The Woodlands, TX; Applied Biosystems, Foster City, CA, USA), 0.2 mM each dNTP, 1X GeneAmp PCR Gold Buffer, 3.0 mM $MgCl_2$, 1% (w/v) PVP (10,000 mw, Sigma, St. Louis, MO) and 0.5 units Ampli*Taq* Gold DNA polymerase (Applied Biosystems, Foster City, CA, USA) in a 10 μl reaction solution following the described methods (Gutiérrez et al. 2002).

Capillary electrophoresis was performed using the automated ABI PRISM 3100 Genetic Analyzer™ (Applied Biosystems, Foster City, CA, USA). Computer-assisted analysis of the data was performed with GeneMapper™ 4.0 software (Applied Biosystems, Foster City, CA, USA) using the default values for the local Southern method.

Linkage analysis

We realized that this RI population was developed from 94 F_2-derived families, each containing two inbred lines, so this is not a traditional SSD RI population (Wu et al. 2004). However, this RI population can be considered two sub SSD RI populations. We conducted the linkage analysis in two ways. First we ran the linkage map analysis for each of two sub-data sets (94 lines) and then joinmaped two linkage maps. Also we ran the linkage map analysis using all RI lines. All linkage analyses were conducted by JoinMap 3.0 (Van Ooijen and Voorrips 2001). The Kosambi mapping function (Kosambi 1944) was used to convert recombination units into genetic distances. We observed that the linkage maps constructed by both ways were almost identical except slight map distance changes and few marker order reversions for tightly linked markers. We determined that the linkage maps using both methods were acceptable. In this study, we reported the results obtained from all RI lines rather than two separate data sets.

Phenotypic data analysis

A genotype with genotype × environment interaction model was subjected to the phenotypic data analysis. Variance components were estimated by the minimum norm quadratic unbiased estimation (MINQUE) approach suggested by Rao (1971) with all prior values being set to 1.0 (Zhu 1989). The genotypic effects were predicted by the adjusted unbiased prediction (AUP) approach by which the predicted effects had variance close to the true variance (Zhu 1993). The group based Jackknife procedure was applied to calculate the standard error (SE) for each parameter by successive removal of one block within each environment (Miller 1974). An approximate t-test was used to test the significance of each parameter (degrees of freedom = 7). Skewness and kurtosis among predicted genotypic effects were calculated by SAS 8.0 (SAS Institute 1999). Variance components and genotypic effects were calculated using a program written in C++ (Wu et al. 2003b).

QTL mapping

An additive genetic model was applied to our QTL analysis for all agronomic and fiber traits based on the predicted genotypic values from 188 RI lines (over two years). A mixed model based approach (Wang et al. 1999) and its corresponding software package QTLMaper 1.6 were used to detect the QTLs contributing to these fourteen traits. The QTLs were selected based on LOD value greater than 3.0. The contribution (heritability) of a single QTL is calculated by dividing a squared QTL effect by the phenotypic variance. For unlinked QTLs, the total contribution (heritability) for a quantitative trait is the summation of each QTL contribution. For linked QTLs, the linkage distances among QTLs need to be considered in calculating the total contribution.

Results

Linkage mapping construction

We used 945 SSR primer pairs to screen for polymorphisms between HS46 and MARCABU-CAG8US-1-88. One hundred forty one polymorphic loci were identified. On average, about 5.65% (ranging from 0.00 to 14.97%) out of 188 RIL were heterozygous for each single marker, suggesting slight existence of heterozygosity among this RIL population. These 141 SSR markers were used to construct linkage maps in two ways as described in Materials and Methods.

The linkage maps constructed by all RI lines and by two separate RI lines showed very similar results except for slight distance changes and few linkage order changes. The results indicated that loosely linked markers will not be grouped together when two sub-data sets are used (each of which has a small population size), as showed in a simulation study (Wu 2001). Thus, the linkage group obtained by using the whole population has a longer distance than that by using two separate data sets. Thus, we determine that both linkage maps are acceptable. In this paper we reported the linkage mapping results obtained by using the whole data rather set than two sub-data sets and we used such a linkage map for our QTL analysis.

Twenty six linkage groups loci covering 125 SSR markers were established with the use of JoinMap 3.0 (Van Ooijen and Voorrips 2001) and twenty four of them map to specific chromosomes (Fig. 1). Even though a criterion ranging from 50 to 60 centiMorgan (CM) provides a good power for grouping (Wu et al. 2003c), with the knowledge of some loci on specific chromosomes or chromosome arms, an increased criterion for join-mapping several groups was applied.

The loci associated with a particular chromosome ranging from 2 to 13 (Fig. 1). Map distances between flanking loci varied from less than 1 to 71 cM. The average distance between loci was 9.2 cM, which was similar to the value (9.6 cM) in the F_2 population from the same cross (Shappley et al. 1998a).

The total distance covered by individual linkage groups ranged from 2 cM (Ch08) to 162 cM (Ch09) (Fig. 1). The overall map distance covered by all 26 linkage groups was 965 cM, covering 20.7% of minimum map distance which has been estimated to be approximately 4660 cM for the cotton genome (Reinisch et al. 1994). Out of 125 loci, 115 markers were mapped on 24 chromosomes, with an average of 4.8 loci on each chromosome. Chromosomes 3, 5, 9, 10, 12, 14, and 16 had more loci than the other linkage groups, ranging from 6 to 13. A total distance of 425 cM was covered by these six linkage groups, contributing 44% of overall distance among these twenty four map chromosomes.

Seventy loci were associated with A sub-genome, and 51 loci were located on D sub-genome (Fig. 1). A recent study conducted by Ulloa et al. (2005) also showed that more RFLP markers and QTLs were associated with A sub-genome more so than with D sub-genome. Thus, our results showed a similar pattern to the previous studies (Shappley et al. 1998a; Ulloa et al. 2005) even though different types of DNA markers were used.

Quantitative analysis for agronomic and fiber traits

Estimated proportions of variance components relative to the phenotypic variances for agronomic and fiber traits are summarized in Table 1. Approximately 18 to 69% of the phenotypic variation was attributable to genotypic effects, 4 to 19% was contributable to G × E interaction effects, and 26 to 76% to residual. More than 60% of the phenotypic variation for all arealometer measurements, 50% span length, and elongation was attributed to residual effects, indicating that these fiber traits are controlled by large residuals. The ratio V_G/V_{GE} ranged from 1.6 (seed cotton yield) to 14.6 (lint percentage), suggesting that genotypic effects were more important than G × E interaction effects for these traits under these two environments (years). Genetic performance for lint percentage, boll weight, seed index, and most fiber traits were more stable over these two years than those for seed and lint yield. The ratio, V_G/V_P which can be considered an estimated narrow sense heritability, was greater than 20% for all traits except fiber perimeter (18%). The small values of V_{GE}/V_P for most traits (Table 1) will make the detection of QTL positions with significant QTL × E effects quite difficult using this population with this two-year data set.

The genotypic value for parent 1 (P_1) was greater ($P \leq 0.05$) than that for parent 2 (P_2) with respect to seed cotton yield, lint cotton yield, lint percentage,

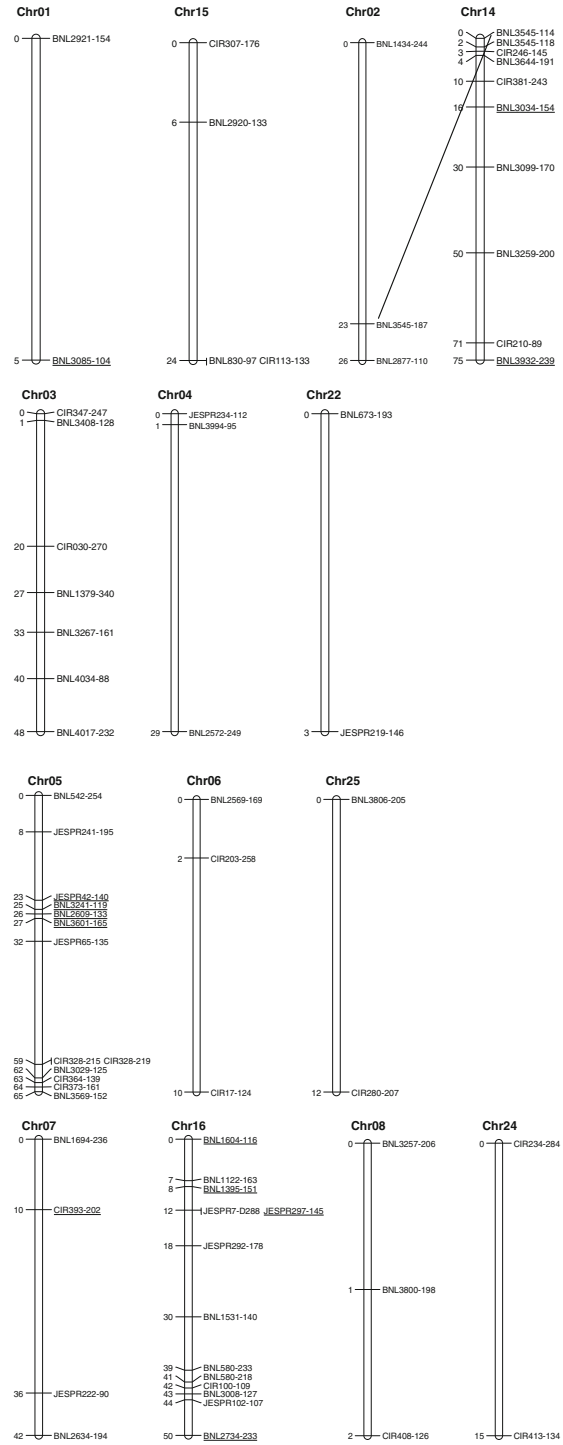

Fig. 1 Genetic linkage map constructed using a 188 RIL► population derived from the intraspecific cross: *G. hirsutum L.* cv. HS46 and MARCABUCAG8US-1-88. Chromosomes are organized by 12 homeologous pairs. Unknown linkage groups are designated as LGU01 and LGU02. SSR loci designations are composed by the SSR primer name and the fragment size. Genetic distances between loci are expressed in centiMorgans (Kosambi 1944). Distorted loci are presented as underlined and homeologous loci are connected by a straight line

maturity, weight fitness, wall thickness, micronaire, and fiber strength (Table 2). P_1 values were lower than P_2 for boll weight, seed index. No significant difference between the two parents was detected for perimeter, 50% span length, 2.5% span length, and fiber elongation. Even though the two parents were phenotypically similar regarding these four traits, due to genetic dissimilarities between the two parents significant differences in this RI population existed. The values of the skewness and kurtosis suggested the predicted genotypic values within this RI population were suitable for QTL analysis.

QTL positions and effects

The positions and effects of QTLs contributing to fourteen agronomic and fiber traits are summarized in Table 3 (LOD value $\geq$3.0). In this study, a negative QTL effect is designated that P_2 has a positive effect while a positive QTL effect is designated that P_1 has a positive effect. The numbers of QTLs associated with different chromosomes (linkage groups) ranged from zero to eight QTLs per chromosome or linkage group. Chromosomes 2, 6, 8, 11, 21, 22, 25 and linkage group LGU02 had no associations with any QTLs for these traits. This could be due to narrow marker coverage or no detectable QTLs on these chromosomes or linkage groups.

Agronomic traits

Four QTLs contributed 42.3% to the phenotypic variation for seed cotton yield and were located on chromosomes 12, 16, 20, and 26. Five QTLs accounted for 36.4% of the phenotypic variance for lint yield and were located on chromosomes 1, 5, 13, 16, and 26. Five QTLs responsible for 38.1% of the phenotypic variance for lint percentage and were located on chromosomes 3, 4, 9, 12, and 26. Three QTLs accounted for 30.2% of the phenotypic variance and were located on chromosomes 15, 16, and 26. Six QTLs contributed 50.5% of the phenotypic variance for seed index and were located on chromosomes 3, 7, 14, 23, 24, and 26.

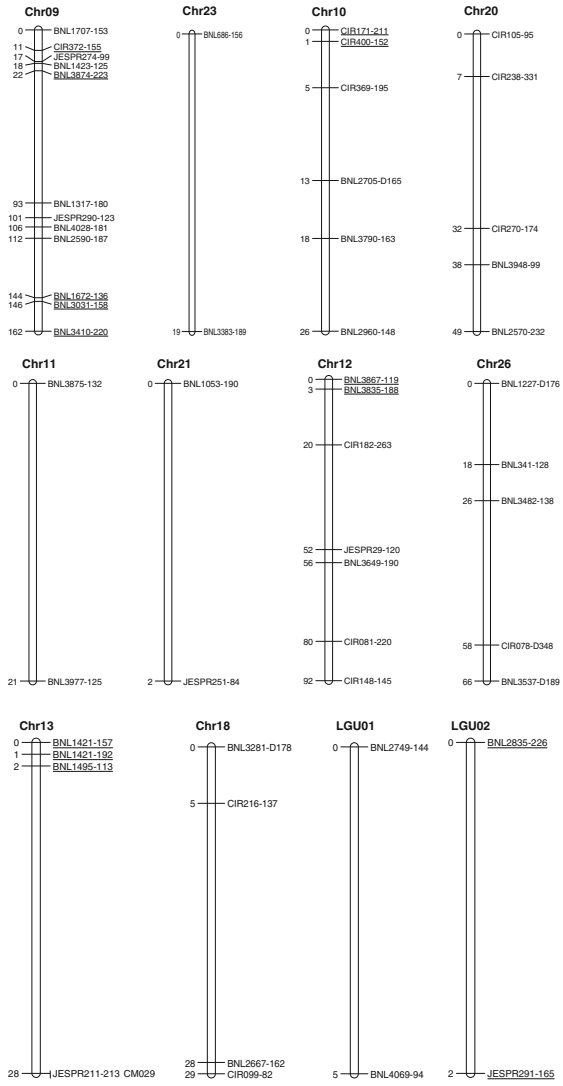

Fig. 1 continued

Fiber traits

Five QTLs contributed 35.6% to the phenotypic variance for fiber maturity and were located on chromosomes 3, 5, 9, 16, and 18. Two QTLs contributed 13.4% of the phenotypic variance for fiber perimeter and were located on chromosomes 10 and 14. Three QTLs accounted for 19.8% of the total variance for fiber weight fineness and were located on chromosomes 10, 14, and 20. Four QTLs contributed 24% to the phenotypic variance for fiber wall thickness and were located on chromosomes 3, 5, 9, and 18. Three QTLs contributed 26.1% to the phenotypic variance for fiber micronaire and were located chromosomes 3, 5, and 13. Three QTLs contributed 20.1% to the phenotypic variance for 50% fiber span length and were located on chromosomes 12, 24, and 26. Four QTLs contributed 38.6% of the phenotypic variance for 2.5% fiber span length and were located on chromosomes 12, 13, 14, and 20. Four QTLs contributed 28.3% to the phenotypic variance for fiber elongation and were located on chromosomes 14, 20, 26, and linkage group LGU1. Six QTLs accounted for 56.9% to the phenotypic variance for fiber strength and were located on chromosomes 5, 9, 12, 16, 20, and 26.

In summary, twenty seven QTLs were distributed within A sub-genome, covering nine of 13 chromosomes, whereas 29 QTLs were distributed within D sub-genome, covering eight chromosomes (Table 4). Among 23 QTLs contributing to five agronomic traits, ten were associated with A sub-genome and 13 with D sub-genome. Among 33 QTLs for fiber traits, 17 were associated with A sub-genome and 16 with D sub-genome.

Table 1 Estimates of variance components for agronomic and fiber traits expressed as a proportion of phenotypic variance

	YLD	LY	LP	BW	SI
V_G/V_P	0.30**	0.31**	0.69**	0.46**	0.58**
V_{GE}/V_P	0.19**	0.18**	0.05**	0.07**	0.08**
V_e/V_P	0.52**	0.51**	0.26**	0.47**	0.34**
	MIC	SL50	SL2.5	EL	T1
V_G/V_P	0.52**	0.26**	0.52**	0.31**	0.49**
V_{GE}/V_P	0.07**	0.05**	0.04*	0.08**	0.05**
V_e/V_P	0.42**	0.70**	0.45**	0.62**	0.47**
	MAT	PER	WF	WT	
V_G/V_P	0.23**	0.18**	0.23**	0.28**	
V_{GE}/V_P	0.05*	0.06*	0.11**	0.10**	
V_e/V_P	0.72**	0.76**	0.66**	0.62**	

*, ** significantly different from zero at probability levels of 0.05 and 0.01, respectively

YLD = seed cotton yield; LY = lint cotton yield; LP = lint percentage; BW = boll weight; SI = seed index; MIC = micronaire reading; SL50 = 50% span length; 2.5%SL = 2.5% span length; EL = elongation; T1 = fiber strength; MAT = maturity; PER = perimeter; WF = weight fitness; and WT = wall thickness

Table 2 Descriptive statistics of values for agronomic and fiber traits

	Statistics					
	RI Min	RI Max	P_1	P_2	Skewness	Kurtosis
YLD (kg/ha)	2693d*	4746a	4467a	3538c	0.08	0.46
LY (kg/ha)	973d	1748a	1699a	1254c	0.30	0.48
LP (%)	32.70e	39.20a	38.32b	35.39d	0.35	0.20
BW (g)	4.62d	5.76a	4.97c	5.31b	−0.03	−0.64
SI (g)	9.63d	12.12a	10.15c	11.06b	0.04	−0.49
MAT	81.43d	93.13a	88.04b	86.18c	−0.15	0.10
PER	42.33d	49.10a	47.22ab	46.22b	0.27	0.34
WF	3.29d	4.42a	4.23a	3.94c	−0.26	0.38
WT	2.40f	3.03a	2.86b	2.70e	0.01	−0.23
MIC	3.72e	4.68a	4.52a	4.09d	−0.06	−0.49
SL50 (mm)	13.50c	14.83a	14.31b	14.14b	−0.13	0.11
SL2.5 (mm)	27.83c	30.34a	29.29b	29.13b	0.17	−0.41
EL (%)	4.75c	9.75a	6.44b	6.66b	0.22	0.37
T1 (kNm/kg)	189.5e	241.1a	225.7b	203.8d	−0.09	−0.58

* Values followed by the same letter within a row in the first three columns are not significantly different based on 95% confidence interval test

YLD = seed cotton yield; LY = lint cotton yield; LP = lint percentage; BS = boll weight; SI = seed index; MAT = maturity; PER = perimeter; WF = weight fitness; and WT = wall thickness; MIC = micronaire reading; SL50 = 50% span length; SL2.5 = 2.5% span length; EL = elongation; T1 = fiber strength

P_1 = Parent 1, P_2 = Parent 2

Discussion

The advantages of an RI population for QTL mapping over an F_2 or BC population were well documented and discussed by Burr et al. (1988), and Burr and Burr (1991). With the single-hill procedure (Fehr 1987), we developed this RI population containing 94 founding families with two lines per family from the cross HS46 × MARCABUCAG8US-1-88, the same cross used by Shappley et al. (1998a, b). Therefore, the population in this study is slightly different from a traditional SSD RI population; however, based on the simulations in our previous study (Wu 2001; Wu et al. 2003a), such an RI population is adequate for conducting QTL mapping. To further consider the possibility of the influence of genetic similarity of lines from the same families on both linkage mapping and QTL mapping, we conducted additional data analyses in multiple ways. First, the genotypic variance for each trait was partitioned into the between-family and within-family variance components. Results (data not shown) indicated that the importance of within-family variance and between-family variance was trait-dependent, suggesting that both between-family and within-family variations are contributors to the total genotypic variance. Second, we divided the complete data set into two parts each of which can be considered as a traditional SSD RI population and we conducted linkage analyses of the two sub-data sets and the complete data set. Results suggested that the linkage maps from two separate data sets and the complete data were very similar with few exceptions. Furthermore, we compared the QTL mapping results from two sub-data sets and the complete data set. The results indicated that QTL effects and positions were similar with large genetic effects, while detectability of QTL with small effects varied between two sub-data sets. With the complete data set, the detectability of QTLs with small genetic effects increased, indicating the QTL mapping power increased with the combined data which had a larger population size as expected (Wu et al. 2003a). Based on the above additional analyses, we decided to use the complete data set for this study.

In this study, we detected 57 QTLs contributing to 14 agronomic and fiber traits. It appeared that on the

Table 3 QTL chromosome, QTL flanking markers and position, and QTL additive effects by parent for agronomic and fiber traits

Trait	Chrom/LG	FM1*				FM2*				QTL				
		Name	Position**	P1 Allele	P2 Allele	Name	Position***	P1 Allele	P2 Allele	Position**	LOD	P1 Effect	P2 Effect	H^2(Ai)***
YLD	12	CIR182	20	263	261	JESPR29	52	120	126	50.5	4.18		91.6	0.086
	16	BNL580	41	218	205	CIR100	42	109	107	40.9	7.25	118.3		0.144
	20	CIR238	7	327	331	CIR270	32	175	174	11.3	3.78		94.9	0.093
	26	BNL1227	0	A	176	BNL341	18	128	153	19.5	5.1	98.7		0.100
Total heritability														0.423
LY	1	BNL2921	0	154	156	BNL3085	5	104	102	4	4.05	26.96		0.053
	5	CIR373	64	167	161	BNL3569	65	158	152	64.1	6.83	35.08		0.089
	13	BNL1421	1	235	192	BNL1495	2	163	113	0.6	5.66	32.46		0.076
	16	BNL580	39	233	211	BNL580	41	218	205	39.1	9.09	38.61		0.108
	26	BNL1227	0	A	176	BNL341	18	128	153	6	2.55	22.97		0.038
Total heritability														0.364
LP	3	BNL3408	1	126	128	CIR030	20	268	270	11.3	9.29	0.45		0.121
	4	JESPR234	0	110	112	BNL3994	1	93	95	0	6.16	0.32		0.062
	9	BNL3874	22	223	225	BNL1317	93	180	178	23.6	4.94	0.3		0.054
	12	CIR182	20	263	261	JESPR29	52	120	126	20.5	7.59		0.36	0.078
	26	BNL3482	26	138	136	CIR078	58	348	A	30.3	5.8		0.33	0.066
Total heritability														0.381
BW	15	CIR307	0	170	176	BNL2920	6	135	133	2	4.14	0.07		0.073
	16	BNL1531	30	140	136	BNL580	39	233	211	30.3	7.83	0.09		0.117
	26	BNL341	18	128	153	BNL3482	26	138	136	17.5	7.56	0.09		0.112
Total heritability														
SI	3	CIR347	0	245	247	BNL3408	1	126	128	0	2.44	0.09		0.030
	7	BNL1694	0	236	246	CIR393	10	202	199	0	8.65		0.17	0.112
	14	BNL3034	16	154	152	BNL3099	30	170	172	23.7	2.98		0.1	0.039
	23	BNL686	0	159	156	BNL3383	19	189	190	0	5.7		0.13	0.065
	24	CIR234	0	290	284	CIR413	15	134	138	0	4.36	0.11		0.052
	26	BNL341	18	128	153	BNL3482	26	138	136	23.5	15.32	0.23		0.207
Total heritability														0.505

Table 3 continued

Trait	Chrom/LG	FM1*				FM2*				QTL				
		Name	Position**	P1 Allele	P2 Allele	Name	Position***	P1 Allele	P2 Allele	Position**	LOD	P1 Effect	P2 Effect	H^2(Ai)***
MAT	3	BNL3267	33	165	161	BNL4034	40	86	88	39.1	3.4		0.63	0.066
	5	BNL3601	27	165	146	JESPR65	32	135	163	29	5.17	0.84		0.116
	9	BNL1707	0	163	153	CIR372	11	155	153	2	2.7		0.59	0.058
	16	JESPR297	12	145	172	JESPR292	18	178	168	12	3.31	0.65		0.070
	18	BNL3281	0	178	A	CIR216	5	141	137	0	2.71		0.53	0.046
Total heritability														0.356
PER	10	CIR369	5	197	195	BNL2705	13	165	A	6.8	2.77		0.27	0.043
	14	BNL3644	4	191	195	CIR381	10	243	246	6.4	5.71	0.39		0.091
Total heritability														0.134
WF	10	CIR369	5	197	195	BNL2705	13	165	A	4.8	3.7		0.04	0.052
	14	CIR381	10	243	246	BNL3034	16	154	152	10.2	6	0.06		0.094
	20	CIR105	0	95	89	CIR238	7	327	331	0	3.28		0.04	0.052
												0.198		
WT	3	BNL4034	40	86	88	BNL4017	48	232	223	45.5	2.63		0.03	0.060
	5	BNL3601	27	165	146	JESPR65	32	135	163	27	4.38	0.04		0.092
	9	JESPR274	17	99	101	BNL1423	18	125	127	17	2.29		0.02	0.040
	18	BNL3281	0	178	A	CIR216	5	141	137	0	2.65		0.03	0.048
Total heritability														0.240
MIC	3	BNL4034	40	86	88	BNL4017	48	232	223	45.5	2.82		0.04	0.048
	5	BNL3241	25	119	115	BNL2609	26	133	135	25.3	8.68	0.07		0.133
	13	BNL1421	0	233	157	BNL1421	1	235	192	0	5.01		0.06	0.080
Total heritability														0.261
SL50	12	CIR081	80	220	221	CIR148	92	142	145	90.3	6.52		0.08	0.102
	24	CIR234	0	290	284	CIR413	15	134	138	2	2.28	0.05		0.036
	26	BNL341	18	128	153	BNL3482	26	138	136	21.5	4.01	0.06		0.063
Total heritability														0.201
SL2.5	12	CIR081	80	220	221	CIR148	92	142	145	88.3	3.54		0.12	0.048
	13	JESPR211	27.6	213	215	CM029	27.6	166	167	27.6	6.11	0.14		0.060
	14	CIR381	10	243	246	BNL3034	16	154	152	10.2	16.29		0.24	0.189
	20	CIR105	0	95	89	CIR238	7	327	331	0	7.86	0.17		0.089
Total heritability														0.386

Table 3 continued

Trait	Chrom/LG	FM1*				FM2*				QTL				
		Name	Position**	P1 Allele	P2 Allele	Name	Position***	P1 Allele	P2 Allele	Position**	LOD	P1 Effect	P2 Effect	H^2(Ai)***
EL	14	BNL3545	0	128	114	BNL3545	2	182	118	2	3.26	0.1		0.056
	20	BNL3948	38	101	99	BNL2570	49	232	228	47.6	2.4		0.09	0.047
	26	BNL1227	0	NS	176	BNL341	18	128	153	16	5.38		0.13	0.097
	LGU1	BNL2749	0	144	145	BNL4069	5	94	96	4	4.66		0.12	0.083
Total heritability														0.283
T1	5	CIR328	59	215	200	BNL3029	62	133	125	61.4	5.96	3.88		0.139
	9	BNL3031	146	158	161	BNL3410	162	222	220	158.3	3.99		2.9	0.078
	12	CIR081	80	220	221	CIR148	92	142	145	84.3	6.7		3.63	0.122
	16	JESPR7	11.6	288	A	JESPR297	12	145	172	11.6	3.63		2.33	0.050
	20	BNL3948	38	101	99	BNL2570	49	232	228	47.6	2.73	2.15		0.043
	26	BNL3482	26	138	136	CIR078	58	348	A	26.3	10.05	3.85		0.137
Total heritability														0.569

YLD = seed cotton yield; LY = lint cotton yield; LP = lint percentage; BW = boll weight; SI = seed index; MAT = maturity; PER = perimeter; WF = weight fitness; and WT = wall thickness; MIC = micronaire reading; SL50 = 50% span length; SL2.5 = 2.5% span length; EL = elongation; T1 = fiber strength

* Flanking marker name is designated by the primer name. FM1 and FM2 are flanking markers 1 and 2, respectively. P1 and P2 allele sizes in this RI population are provided for each flanking marker (A indicates the absence of an allele i.e. a dominant marker)

** Flanking marker or QTL position from the starting point in unit of cM

*** Heritability of single QTL effect, equivalent to contribution from a single QTL

Table 4 Chromosomes or linkage groups that harbored QTLs contributing significantly to agronomic and fiber traits

Chromosome/ Linkage group	YLD	LY	LP	BW	SI	MAT	PER	WF	WT	MIC	SL50	SL2.5	EL	T1
1		P1*												
3			P1		P1	P2*			P2	P2				
4			P1											
5		P1				P1			P1	P1				P1
7					P2									
9			P1			P2			P2					P2
10							P2	P2						
12	P2		P2								P2	P2		P2
13		P1								P2		P1		
14					P2		P1	P1				P2	P1	
15				P1										
16	P1	P1		P1		P1								P2
18						P2			P2					
20	P2							P2				P1	P2	P1
23					P2									
24					P1						P1			
26	P1	P1	P2	P1	P1						P1		P2	P1
LGU1													P2	

YLD = seed cotton yield; LY = lint cotton yield; LP = lint percentage; BW = boll weight; SI = seed index; MAT = maturity; PER = perimeter; WF = weight fitness; and WT = wall thickness; MIC = micronaire reading; SL50 = 50% span length; SL2.5 = 2.5% span length; EL = elongation; T1 = fiber strength

* P1 means parent one has an increased QTL effect and P2 means parent two has an increased QTL effect

average the QTL number for each of five agronomic traits (23/5 = 4.6 QTLs) was slightly higher than that of nine fiber traits (34/9 = 3.7 QTLs). A total of 15 QTLs with large genetic effects (≥10%) were detected for agronomic and fiber traits and they were mainly located on chromosomes 3, 5, 12, 14, 16, 20, and 26, which harbored 14 QTLs with at least 10% of contribution effects for yield or fiber quality of importance (Table 3). For example, chromosome 5 had four QTLs with large genetic effects for lint yield (8.9%); fiber maturity (11.6%); fiber wall thickness (9.2%); fiber micronaire (13.3%); and fiber strength (13.9%). In addition, the QTLs for lint yield and fiber strength were closely linked (positions 64.1 cM and 61.4 cM, respectively) and QTLs for the fiber maturity, fiber wall thickness, and fiber micronaire were closely linked (positions 29, 27, and 25.3 cM, respectively).

In our previous studies of chromosome substitution lines, we reported more chromosome associations for agronomic traits than for fiber traits (Saha et al. 2004, 2006; Jenkins et al. 2006, 2007). The results suggested that the number of genetic factors for agronomic traits seemed greater than for fiber traits, indicating improvement for agronomic traits might be more difficult than that for fiber traits. Several previous studies showed that D sub-genome harbors more genetic factors than A sub-genome in tetraploid cotton species (Saha et al. 2004, 2006; Ulloa et al. 2005; Jenkins et al. 2006). Our study showed similar results, with 27 QTLs associated with nine chromosomes in A sub-genome whereas 29 QTLs associated with eight chromosomes in D sub-genome. However, this difference may not be significant.

Since the parental lines, types of mapping populations, and DNA markers varied among different experiments reported in the literature, detailed comparisons among different reports are very difficult. With the assignments of DNA markers or QTLs to specific chromosomes, however, such comparisons can be more readily made. For example, chromosomes 16 and 18 were associated with cotton yield and boll weight using cotton chromosome substitution lines (Saha et al. 2004, 2006; Jenkins et al.

2006). Our current results are consistent with those previously reported in chromosome substitution lines. These previous reports with chromosome substitution lines show that chromosome 25 was associated with several fiber traits (i.e. fiber length, fiber micronaire, and fiber strength), whereas in this study no QTLs were detected for these fiber traits. This is not surprising because (1) we only had a limited number of markers segregating for this chromosome and (2) the mapping population used in this study is from a *G. hirsutum* intraspecific cross.

Despite the differences in parents, type of mapping populations, and DNA markers, our results are comparable with many other reports. For example, results from Lacape et al. (2005) showed that fiber length was associated with chromosomes 12, 14, 20, and 26, fiber micronaire with chromosomes 3, 5, and linkage group A01 (chromosome 13 in this study), and fiber strength with chromosome 5. Zhang et al. (2005) found that fiber length was associated with chromosome 20 and fiber strength and micronaire were associated with chromosome 5. Jiang et al. (1998) reported that fiber strength was associated with chromosome 20. Shen et al. (2005) found that fiber strength was associated with chromosome 16. Shen et al. (2006) also reported that fiber strength, fiber length, and lint yield were associated with chromosome 16, lint percentage with chromosome 12, and boll weight and seed index with chromosome 26. Our results showed consistence with the above reports even though different genetic backgrounds and markers were used.

On the other hand, we also compared our results with those reported by Shappley et al. (1998b) in the F_2 population from the same cross though different types of population (F_2 vs. RI) and different types of DNA markers (RFLP vs. SSR) were used. Results reported by Shappley et al. (1998b) showed that chromosome 3 was associated with QTLs for lint percentage, seed index, fiber micronaire, fiber maturity, and wall thickness. Our results showed that these traits were associated with the chromosome 3. Chromosome 5 in Shappley et al.'s study (1998b) showed the associations with wall thickness, fiber strength, and maturity. Our results showed that chromosome 5 was also associated with these three traits. Chromosome 14 was associated with elongation, micronaire, wall thickness, maturity. Our results showed that this chromosome 14 was also associated with these four traits. Chromosome 26 was associated with elongation and lint percentage (Shappley et al. 1998b). Chromosome 26 in this study was also associated with these two traits.

Marker coverage is an important factor for genetic mapping; however, marker coverage for most cotton chromosomes or linkage groups is still very limited, especially when using crosses within *G. hirsutum*. Thus, for detecting more QTLs or QTLs with large effects, a larger number of polymorphic DNA markers within *G. hirsutum* are needed. Due to the different parents and/or markers being used, common markers that are polymorphic in a wide range of parental lines are needed to construct a more dense linkage map. For example, the marker types used in this RI population and the F_2 population from the same two parents differed (Shappley et al. 1998a). Although we found consistent results between two populations, it should be valuable to run the RFLP markers used in the F_2 population on this RI population, to further fine map these QTLs.

Exact QTL genotypes for a mapping population are usually unknown; however, marker information (marker allele size) is observable. With the help of statistical computation, QTL effects and positions can be determined. Thus, marker assisted selection procedure used for improvement of traits of interest really depends on the availability of QTL information (QTL positions and effects) and flanking marker information (flanking marker positions and allele sizes). However, such genetic information is usually not reported in many scientific papers. The listings of QTL positions, effects, and allelic sizes of flanking markers in this study should help cotton breeders determine not only which markers but also which marker alleles will be used for improving single or multiple traits.

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