Molecular Breeding (2005) 15: 169–181
DOI 10.1007/s11032-004-4731-0

Molecular mapping of QTLs for fiber qualities in three diverse lines in Upland cotton using SSR markers

Xinlian Shen[1], Wangzhen Guo[1], Xiefei Zhu[1], Youlu Yuan[1], John Z. Yu[2], Russell J. Kohel[2] and Tianzhen Zhang[1,*]
[1]*National Key Laboratory of Crop Genetics & Germplasm Enhancement, Cotton Research Institute, Nanjing Agricultural University, Nanjing 210095, P. R. China;* [2]*USDA ARS, Southern Plain Agriculture Research Center, Crop Germplasm Research Unit, College Station, TX 77845, USA; *Author for correspondence (e-mail: cotton@njau.edu.cn; phone/fax: +1-0086-25-84395307)*

Received 15 June 2004; accepted in revised form 11 October 2004

Key words: Common QTL, Cotton, Fiber qualities, Marker-assisted selection (MAS), Molecular tagging, Quantitative trait locus (QTL)

Abstract

The improvement of cotton fiber quality is extremely important because of changes in spinning technology. The identification of the stable QTLs affecting fiber traits across different generations will be greatly helpful to be used effectively in molecular marker-assisted selection to improve fiber quality of cotton cultivars in the future. Using three elite fiber lines of Upland cotton (*Gossypium hirsutum* L.) as parents, three linkage maps were constructed to tag QTLs for fiber qualities using SSR markers. There were 39 QTLs, 17 significant QTLs, LOD ≥ 3.0 and 22 suggestive QTLs, $3.0 >$ LOD ≥ 2.0, detected by composite interval mapping for fiber traits, in which 11 QTLs were for fiber length, 10 for fiber strength, 9 for micronaire and 9 for fiber elongation. Out of 17 significant QTLs, 5 QTLs with high logarithm of odds (LOD) score value and stable effect could be found in both F_2 and $F_{2:3}$ segregating populations, showing a great potential for molecular-assisted selection in improving fiber quality. At least three common QTLs could be identified in two populations. These common QTLs detected in different populations suggested that there existed elite fiber genes and possibly of the same origin. In addition, we found three pairs of putative homoeologous QTLs, *qFL-7-1c* and *qFL-16-1c*, *qFS-D03-1a*, *qFS-A02-1b* and *qFS-A02-1c*, and *qFE–D03-1a* and *qFE-A02-1c*. Our results provided a better understanding of the genetic factors of fiber traits in AD tetraploid cottons.

Abbreviations:: MAS – marker-assisted selection; QTL – quantitative trait locus; SSR – simple sequence repeat

Introduction

Cotton is an important cash crop. A long-term challenge facing a cotton breeder is the simultaneous improvement of yield and fiber quality to meet the demands of the cotton producer as well as the textile industry. In recent years, improvement of cotton fiber quality has been extremely

important because of changes in spinning technology. However, a negative association between lint yield and fiber quality is still present after many years of exhaustive breeding for improved fiber properties. Conventional breeding procedures are difficult for further improving fiber quality because of high costs, long duration, and low selective efficiency.

Studies in other crop plants have demonstrated that biotechnology promises to provide powerful tools for enhanced genetic improvement of quantitative traits. Advances in the identification of DNA markers for fiber quality QTLs have been reported. Many QTLs for fiber traits were identified from four interspecific populations of *Gossypium hirsutum* and *Gossypium barbadense* (Jiang et al. 1998; Kohel et al. 2001; Mei et al. 2004; Paterson et al. 2003). In intraspecific populations, more than a hundred QTLs for fiber traits were detected (Shappley et al. 1998; Ulloa and Meredith 2000; Zhang et al. 2003). Comparison of QTLs from different studies revealed poor consistency among populations (Jiang et al. 1998; Kohel et al. 2001; Paterson et al. 2003; Mei et al. 2004). Few stable QTLs and common QTL have been reported up to now due to non-replicated experiments and difficulty in assignment of the linkage groups to corresponding chromosomes. By use of F_2 individuals and $F_{2:3}$ family lines derived from a cross between 7235 and TM-1, a widely used genetic standard line of Upland cotton, a major QTL for fiber strength was identified. The QTL was detected in different locations such as Nanjing and Hainan in China, and Texas in the USA. Marker assisted-selection (MAS) revealed that DNA markers linked to this QTL could be used in MAS to increase fiber strength of commercial cultivars in early segregating breeding generations (Zhang et al. 2003).

Microsatellite, also known as simple sequence repeats (SSRs), is an ideal polymerase chain reaction (PCR)-based DNA marker for genetic mapping and MAS breeding because of their abundance, wide dispersion in diverse genomes, and co-dominantly inherited characteristic. In this study, we selected three Upland cotton lines with superior fiber quality from different breeding programs as mapping parents and constructed three independent linkage maps using SSR markers. Assignment of linkage groups to subgenomes and chromosomes was made with monosomic and mono-telosomic stocks and referenced to the latest published maps of cotton genome (Lacape et al. 2003; Rong et al. 2004).

The objective of this research was to screen the stable QTLs affecting fiber traits across different generations, to determine the presence of the same QTLs associated with a given trait among different populations so that these QTLs can be used effectively for MAS during the process of improving fiber quality in the future. The development of DNA markers linked to the fiber quality QTLs would allow cotton breeders to trace this very important trait in early plant-growing stages or early segregating generations. The use of these DNA markers increases the prospect for streamlining the cotton breeding programs to improve fiber quality while maintaining fiber yield.

Materials and methods

Mapping populations

Three high fiber quality parents, 7235, HS427-10, and PD6992 were chosen for molecular tagging of QTLs for fiber qualities. Their fiber properties are presented in Table 1. A *Gossypium anomalum* introgression line 7235 (Qian et al. 1992) was kindly made available from Jiangsu Academy of Agricultural Sciences. PD6992 (*G. hirsutum* L.) was developed from the triple hybrid introgression from the cross of two F_1 hybrids (SC − 1 × PD8619)× (Coker 310 × PD7396) (Culp et al. 1985). The pedigree of HS427-10 (*G. hirsutum* L.) is unknown. HS427-10, PD6992, and TM-1 were kindly made available from USDA-ARS, Southern Plains Agriculture Research Center, Texas, USA. TM-1 is a genetic standard in Upland cotton (Kohel et al. 1970) and was used as parent to construct the linkage groups in our laboratory (Zhang et al. 2002). Simian 3 (SM3) (*G. hirsutum* L.), a Chinese commercial variety, was a widely planted variety with high yield, but with relatively poor fiber properties before transgenic Bt hybrid cottons were extensively grown in the Yangtze River cotton growing region in China.

The three high fiber quality lines, 7235, HS427-10, and PD6992 were crossed with TM-1 and SM3 to produce mapping populations such as (7235 × TM-1) F_2(Population A or Pop A),

Table 1. The performance of fiber properties for parents, F_2 and $F_{2:3}$.

Population	Source (year)	Fiber length (mm)	Fiber strength (cN/tex)	Micronaire	Elongation
Pop A, 7235 × TM-1	7235(1998/1999)	35.10/33.60	40.53/35.4	4.14/3.89	5.10/5.98
	TM-1(1998/1999)	30.48/29.90	28.97/27.56	5.00/4.63	5.78/6.59
	F_2(1998)	32.72 (28.30–36.70)	31.58 (25.61–41.86)	4.26 (3.20–5.20)	5.45 (4.50–6.50)
	$F_{2:3}$(1999)	32.09 (28.90–35.40)	29.94 (21.58–37.44)	3.73 (2.10–4.90)	5.11 (4.00–6.40)
Pop B, HS427-10 × TM-1	HS427-10(2000/2001)	30.60/31.20	41.2/39.6	5.10/5.30	5.40/5.90
	TM-1(2000/2001)	30.40/30.90	30.1/31.2	5.00/5.20	6.40/6.80
	F_2(2000)	30.92 (27.90–33.60)	33.36 (25.35–42.12)	4.94 (3.00–6.10)	5.34 (4.50–6.70)
	$F_{2:3}$(2001)	31.34 (29.14–33.50)	33.07 (28.56–39.52)	4.90 (3.97–5.54)	6.01 (5.09–6.71)
Pop C, PD6992 × SM3	PD6992(2002/2003)	34.10/32.60	37.5/32.1	5.23/4.00	4.70/6.90
	SM3(2002/2003)	30.90/32.30	30.4/29.2	5.49/4.70	6.70/6.50
	F_2(2002)	31.99 (29.40–34.80)	33.63 (25.5–43.6)	5.17 (4.15–5.73)	6.27 (5.10–7.70)
	$F_{2:3}$(2003)	31.97 (30.00–34.30)	30.54 (26.1–34.9)	4.54 (3.50–5.40)	6.61 (5.50–8.80)

Note: The data in parentheses shows the range of the trait in F_2 and $F_{2:3}$ populations.

(HS427-10 × TM-1) F_2(Pop B), and (PD6992 × SM 3) F_2(Pop C). These F_2 segregating populations and their corresponding $F_{2:3}$ family lines were grown in Jiangpu Experiment Station, Nanjing Agricultural University (NAU) respectively, in 1998 and 1999 for Pop A, in 2000 and 2001 for Pop B, and in 2002 and 2003 for Pop C. The reason to use SM3 as parent for Pop C was for the purpose of breeding to develop the same high yield as SM3, but transferred elite fiber quality genes from PD6992. Each individual plant was harvested for the fiber tests from all three F_2 and $F_{2:3}$ populations. Ten to twelve plants per $F_{2:3}$ family lines were evaluated for corresponding F_2 individual. Fiber samples were tested in the Supervision, Inspection, and Test Center of Cotton Quality, Ministry of Agriculture in China. Fiber quality traits included fiber length (FL), fiber strength (FS), micronaire (FM), fiber elongation (FE), fiber uniformity ration (FUR), and fiber short index (FSI).

There were low temperatures and excessive rain in Nanjing during the fiber development in 1999 and 2003, and fiber did not develop very well, and fiber properties were greatly influenced in mapping populations, especially for strength and micronaire values.

Assay of DNA markers

DNA from individual plants of the three F_2 populations was extracted as described by Paterson et al. (1993). A total of 1378 SSR primer pairs available in our laboratory were used to screen polymorphism. These SSR primers were separately obtained from the following sources: BNL primers from Research Genetics Co. (Huntsville, AL, USA, http://www.resgen.com); JESPR from Reddy et al. (2001) sequences; TM from Dr John Yu, USDA-ARS, Crops Germplasm Research Unit, Texas, USA, and EST from Dr S. Saha, USDA-ARS, Crop Science Research Laboratory, Mississippi, USA. NAU SSR primers were EST-SSR surveyed the gene bank EST and cDNA sequences (Zhang, unpublished data). Nomenclature of markers is the letter in each marker describes the origin of marker, followed by the primer number. The procedure for SSR analysis was reported (Zhang et al. 2000, 2002). DNA bands of SSRs were developed with silver staining and recorded with SX-image system (Sixing Biological Technology Co. Shanghai, China).

Data analysis and QTL mapping

Linkage maps were made using MAPMAKER/Exp Version 3.0b (Lander et al. 1987). A logarithm of odds (LOD) threshold of 5.0 was used in Pop A because it revealed severe segregation distortion. A default LOD score of 3.0 and a 50 cM maximal distance were used in Pop B and Pop C. QTL were identified by composite interval mapping (Zeng 1994) using Windows QTL Cartographer 2.0 (Basten et al. 2001). A stringent LR threshold of ≥13.8 (equal to LOD score 3.0) was used to declare significant QTLs in order to keep the likelihood of even one false positive below 5% in the large genome of cotton (Jiang et al. 1998). Meanwhile, a

LR scores value between 9.2 and 13.8 (equal to LOD score 2.0–3.0) were used to detect suggestive QTL, as suggested by Lander and Kruglyak (1995). Effects and percent of phenotypic variance (PV) explained by a single QTL (R^2) were estimated with Windows QTL Cartographer 2.0 at highest probability peaks. Negative additive effect indicates that 7235, HS427-10, and PD6992 alleles improved the traits. A χ^2 test was performed to determine if the allele frequency at each individual locus segregated normally.

Confidence intervals (90–95%) associated with QTL locations are set as the map interval corresponding to a 1 LOD decline either side of the peak. QTL for the same trait across different generations were declared as 'common' QTL when their confidence intervals overlapped.

Assignment of linkage groups to chromosomes and QTL nomenclature

Assignment of linkage groups to subgenomes and chromosomes was made based on our linkage maps (Zhang et al. 2002). When no chromosome inference was available, the 'A' or 'D' designation of the linkage group was described from that of Lacape et al. (2003) and Rong et al. (2004) after aligning groups with common SSR loci.

QTL nomenclature was adapted according to the method in rice (McCouch et al. 1997), starting with 'q', followed by an abbreviation of the trait name (for example FL for fiber length, FS for fiber strength, FM for micronaire, and FE for fiber elongation) and the name of chromosome or linkage group, then followed by the number of QTL affecting the trait on the chromosome or linkage group. Additionally, a/b/c stands for the name of the population in the present study.

Results

Performance of fiber qualities of parents and populations

The fiber quality performance of parents and their F_2 and $F_{2:3}$ segregating populations are shown in Table 1. The differences in fiber length, strength, micronaire, and elongation between parents were obvious and the wide genetic diversities were represented in these parents. Segregation data for fiber traits in all populations were initially tested for normal distribution. To determine if traits were normally distributed, skewness and kurtosis values were calculated for all traits (data not shown). Fiber short index and fiber uniformity ratios did not fit normal distributions in Pop B and Pop C, so these two traits were not mapped in the present research. There were positive and negative transgressive segregations for fiber length, fiber strength, and micronaire in all three populations. A negative transgressive segregation in Pop A and Pop B for fiber elongation was observed, while the bidirectional transgressive segregation occurred in Pop C.

Table 2. Basic characteristics of the three individual genetic maps.

Item	Pop A	Pop B	Pop C
Total SSR loci	127	77	96
Number of mapped loci	86	56	73
Number of individuals	163	169	142
Number of linkage groups	21	17	22
Number of unlinked loci	41	21	23
Map length (cM)	666.7	557.8	588
Total number of skewed loci	56	9	9

Construction and characterization of intraspecific linkage maps

The basic characteristics of the three maps are given in Table 2. There were 127, 77, and 96 polymorphic SSR loci between parents 7235 and TM-1, HS427-10, and TM-1, and PD6992 and SM3, respectively, amplified from 1378 SSR primer pairs. The genetic map for Pop A (Map-A) generated by MAPMAKER/Exp 3.0 included 86 loci covering 666.7 cM, approximately 14.8% of the total recombinational length of the cotton genome (Ulloa and Meredith 2000). The Map-B for Pop B included 56 loci covering 557.8 cM, approximately 12.4% of the total recombinational length of the cotton genome. The Map-C for Pop C included 73 loci covering 588 cM, approximately 13.1% of the total length of the cotton genome. Genotypic frequencies deviating from the expected segregation ratio of 1:2:1 for codominant locus or 3:1 for dominant locus were detected in three populations (see Table 2). Pop A showed

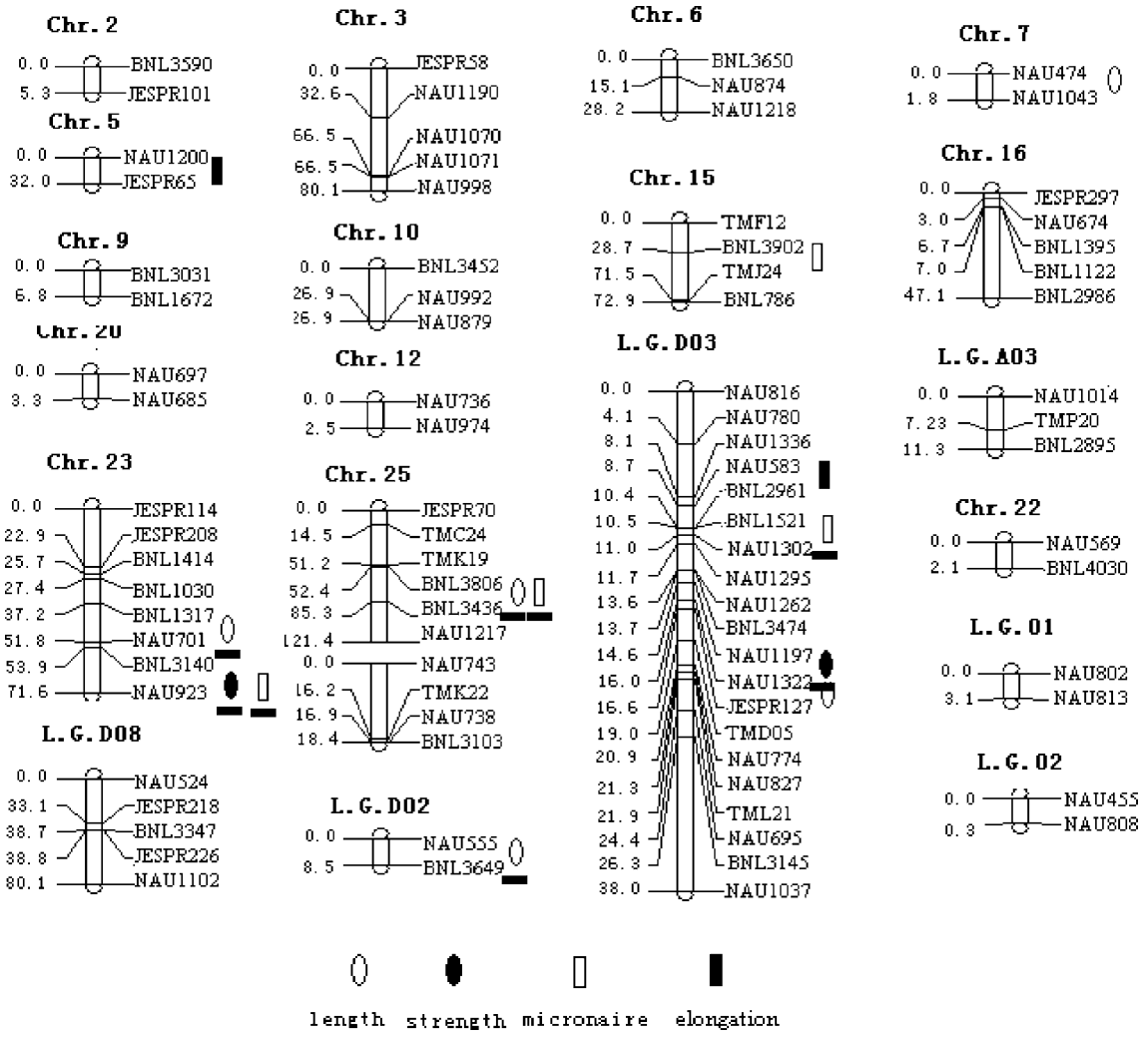

Figure 1. Genetic linkage map and QTLs location from Pop A (7235 × TM-1) F_2 and $F_{2:3}$. The gaps indicate the linkage distance > 50 cM. ■ indicate significant QTLs.

severe segregation distortion by 44.1% in 56 out of 127 SSR markers. There were 23 common loci between Map-A and Map-B since they had a common parent. The Map-B and Map-C, Map-A and Map-C shared 16 and 15 common SSR loci, respectively. All common loci produced identical order on linkage groups (Figures 1–3).

QTL mapping for fiber qualities

Pop A

The list of the QTLs identified in Pop A are presented in Table 3. Their most likely positions on the linkage map are shown in Figure 1. Altogether, eight QTLs significant for fiber properties were detected. Among them, three QTLs were for fiber length, two for fiber strength and three for micronaire, respectively.

Among three QTLs affecting fiber length, the QTL in Chr. 25 could be detected in both F_2 and $F_{2:3}$, which were mapped in the neighboring interval. Their confidence interval (data not shown) overlapped each other and regarded as common QTL. It could be explained 14.1 and 13.1% of PV in F_2 and $F_{2:3}$, respectively. At this QTL, the 7235 allele increased fiber length by 0.577–0.808 mm. The other two QTLs could be identified only in F_2

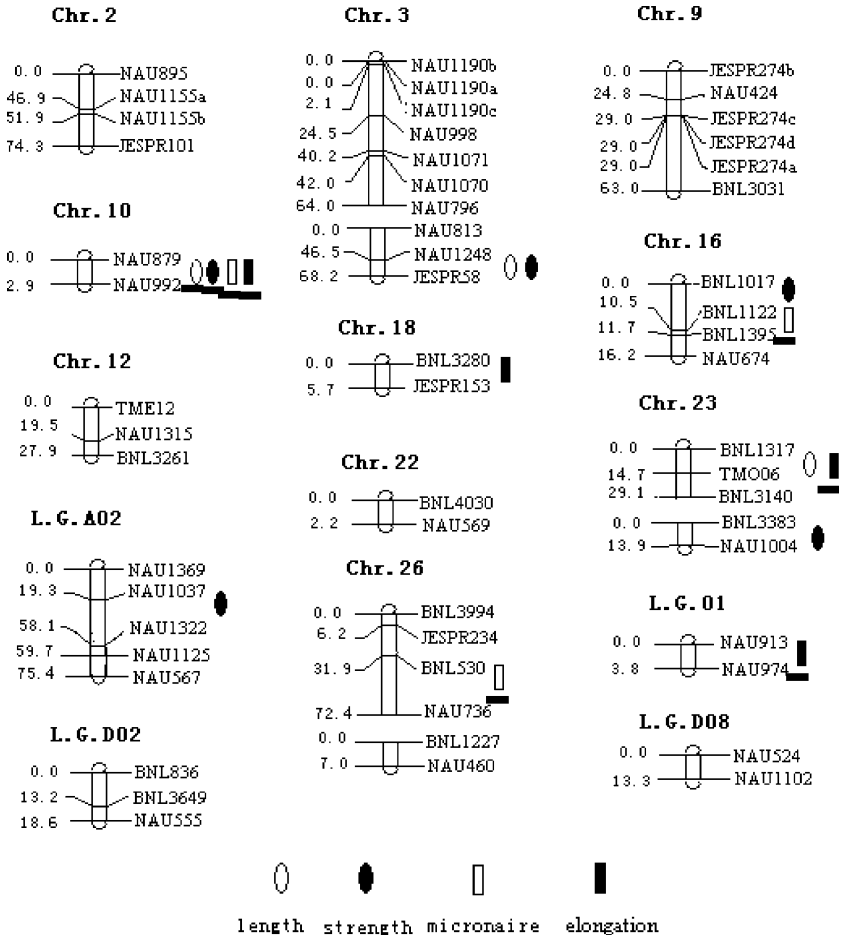

Figure 2. Genetic linkage map and QTLs location from Pop B (HS427-10 × TM-1) F_2 and $F_{2:3}$. The gaps indicate the linkage distance > 50 cM. ■ indicate significant QTLs.

or in $F_{2:3}$, not both, and the proportion of PV explained by these QTL was fairly low. In addition, two suggestive QTLs (LOD score fell between 2.0 and 3.0) on Chr. 7 and LGD03 were identified.

Two significant QTLs for fiber strength were detected in this population on LGD03 and Chr. 23. A major QTL for fiber strength, QTLfs1.1 anchored on Chr. 10, had been detected with bulk segregant analysis (BSA) by interval mapping in our laboratory (Zhang et al. 2003). From our monosomic tests, we mapped SSR markers linked with this major QTL on Chr. 10, however, using SSR markers such as BNL2449, BNL2961, and BNL1521 primer pairs in this group were used to screen the (Telo10Lo × 3-79) F_1 and (Telo10sh × 3-79) F_1 monotelodisomic DNA, the parental heterozygous band was found in their DNA amplification product, i.e., these SSR markers were not in either deleted arm of Chr.10 in Telo10Lo and Telo10sh (Zhang, unpublished data). Therefore, we cannot ascertain which chromosome this linkage group is associated with. At this report, the linkage group was named as LGD03 after aligning groups with common SSR loci reported by Lacape et al. (2003) and Rong et al. (2004).

We used the same data to further unravel distribution of fiber quality QTL in this cotton genome region with a linkage map using more SSR markers. The common QTL, *qFS-D03-1a*, was detected and explained 18.2 and 12.8% of total PV in F_2 and $F_{2:3}$ in Nanjing by composite interval mapping. It shows less effect than with interval

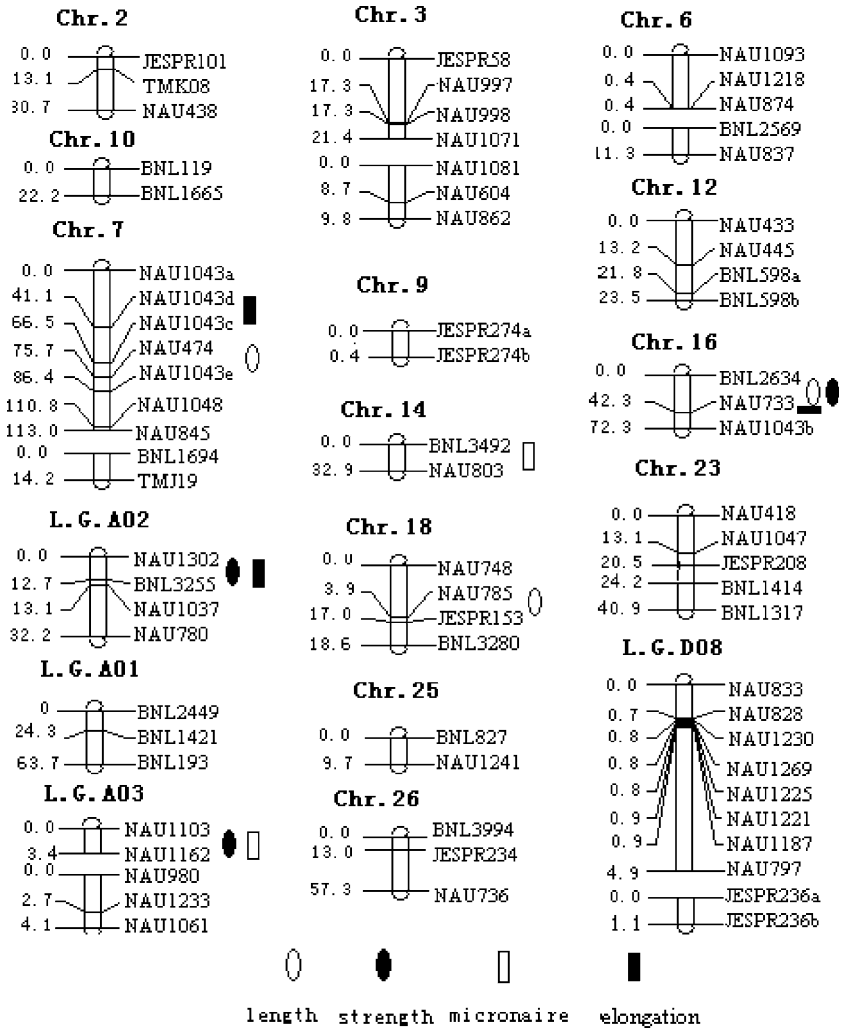

Figure 3. Genetic linkage map and QTLs location from Pop C (PD6992 × Simian 3) F_2 and $F_{2:3}$. The gaps indicate the linkage distance > 50 cM. ■ indicate significant QTLs.

mapping (Zhang et al. 2003). A possible cause is that there are more than one QTL for fiber strength on LGD03. QTL analyses by composite interval mapping revealed two close neighboring peaks (Figure 4), so, the possibility of one or two closely linked QTLs existed in the region remains to be tested.

Three significant QTLs for micronaire were identified, *qFM-23-1a*, *qFM-25-1a*, and *qFM-D-3-1a*. *qFM-25-1a* located Chr. 25 could be detected in both F_2 and $F_{2:3}$, which explained 20.8 and 21.0% of PV in F_2 and $F_{2:3}$, respectively. *qFM-15-1a* on Chr. 15 was a suggestive QTL. These four QTLs had the same direction of additive effect originated from TM-1, explained 50.7–63.9% of PV in total.

No significant QTL for fiber elongation was detected. But two suggestive QTLs on LGD03-1a and Chr. 5-1 were detected in one or two generations (Table 3 and Figure 1).

Pop B

In this population, a total of seven significant QTLs were identified (Table 3 and Figure 2), one for fiber length, one for fiber strength, two for micronaire, and three for fiber elongation. QTL influencing fiber length on Chr. 10 was detected in both F_2 and $F_{2:3}$ with positive additive effects originating from TM-1. Two suggestive QTLs for fiber length on Chr. 3 and Chr. 23 were also detected.

Table 3. Characteristics of QTLs affecting fiber properties in three populations.

Population	QTL	Generations	Chr. or LG	Interval	LR	*a*	*d*	R^2 (%)	Direction
(7235 × TM-1)	*qFL-25-1a*	F_2	Chr. 25	BNL3806–BNL3436	14.47	−0.808	0.2288	14.1	7235
$F_2/F_{2:3}$		$F_{2:3}$	Chr. 25	BNL3436–NAU1217	22.82	−0.577	−0.3242	13.1	7235
	qFL-23-1a	F_2	Chr. 23	BNL1317–NAU701	15.94	0.667	0.2848	8.6	TM-1
	qFL-D02-1a	$F_{2:3}$	LGD02	NAU555–BNL3649	15.81	−0.4905	0.4935	8.3	7235
	qFL-7-1a	$F_{2:3}$	Chr. 7	NAU474–NAU1043	12.52	−0.148	−0.6488	6.3	7235
	qFL-D03-1a	$F_{2:3}$	LGD03	NAU1322–JESPR127	13.57	−0.472	0.226	7.6	7235
	qFS-D03-1a	F_2	LGD03	NAU1197–NAU1322	34.57	−1.2337	0.0589	18.2	7235
		$F_{2:3}$	LGD03	BNL3474–NAU1197	14.15	−1.128	−0.4193	12.8	7235
	qFS-23-1	$F_{2:3}$	Chr. 23	BNL3140–NAU923	17.97	−0.7317	−0.9664	11.2	7235
	qFM-23-1a	F_2	Chr. 23	BNL3140–NAU923	11.06	0.0433	0.2278	6.2	TM-1
		$F_{2:3}$	Chr. 23	BNL3140–NAU923	33.24	0.1423	0.4356	19.2	TM-1
	qFM-25-1a	F_2	Chr. 25	BNL3806–BNL3436	22.3	0.2426	0.3077	20.8	TM-1
		$F_{2:3}$	Chr. 25	BNL3806–BNL3436	13.93	0.1808	0.2081	21.0	TM-1
	qFM-D03-1a	F_2	LGD03	BNL1521–NAU1302	20.71	−0.2273	0.0745	12.2	TM-1
	qFM-15-1a	F_2	Chr. 15	BNL3902–TMJ24	11.15	0.2057	−0.131	11.6	TM-1
	qFE-D03-1a	F_2	LGD03	NAU583–BNL2961	9.63	0.1364	0.0508	5.4	TM-1
		$F_{2:3}$	LGD03	BNL2961–BNL1521	12.19	0.194	0.132	7.2	TM-1
	qFE-5-1a	$F_{2:3}$	Chr. 5	NAU1200–JESPR65	11.74	−0.0614	0.269	8.6	7235
(HS427-10 × TM-1)	*qFL-10-1b*	F_2	Chr. 10	NAU879–NAU992	15.41	0.4316	0.0809	8.5	TM-1
$F_2/F_{2:3}$		$F_{2:3}$	Chr. 10	NAU879–NAU992	17.69	0.3821	0.0607	10.4	TM-1
	qFL-3-1b	F_2	Chr. 3	NAU1248–JESPR58	11.17	−0.4342	−0.5333	15.1	HS427-10
		$F_{2:3}$	Chr. 3	NAU1248–JESPR58	10.99	−0.4303	−0.381	16.9	HS427-10
	qFL-23-1b	F_2	Chr. 23	BNL1317–TMO06	9.72	0.0852	−0.463	5.2	TM-1
		$F_{2:3}$	Chr. 23	BNL1317–TMO06	10.3	0.1783	−0.3555	20.2	TM-1
	qFS-10-1b	F_2	Chr. 10	NAU879–NAU992	17.65	1.027	−0.0833	9.5	TM-1
		$F_{2:3}$	Chr. 10	NAU879–NAU992	19.35	0.7207	0.4469	10.0	TM-1
	qFS-16-1b	F_2	Chr. 16	BNL1017–BNL1122	12.91	−0.9794	−0.6547	10.2	HS427-10
		$F_{2:3}$	Chr. 16	BNL1017–BNL1122	11.57	−0.7273	−0.8167	12.0	HS427-10
	qFS-23-1b	$F_{2:3}$	Chr. 23	BNL3383–NAU1004	10.22	−0.988	−1.0319	9.0	HS427-10
	qFS-3-1b	$F_{2:3}$	Chr. 3	NAU1248–JESPR58	12.01	−0.8251	−0.5051	11.9	HS427-10
	qFS-A02-1b	$F_{2:3}$	LGA02	NAU1037–NAU1322	11.03	−0.7023	−0.4879	9.2	HS427-10
	qFM-26-1b	F_2	Chr. 26	BNL530–NAU736	11.27	−0.1278	−0.0911	6.0	HS427-10
		$F_{2:3}$	Chr. 26	BNL530–NAU736	14.18	−0.124	0.0715	9.2	HS427-10
	qFM-10-1b	F_2	Chr. 10	NAU879–NAU992	12.96	−0.1272	−0.1353	7.3	HS427-10
		$F_{2:3}$	Chr. 10	NAU879–NAU992	25.14	−0.1565	0.01	12.5	HS427-10
	qFM-16-1b	$F_{2:3}$	Chr. 16	BNL1122–BNL1395	17.43	0.2715	−0.008	8.3	TM-1
	qFE-23-2b	$F_{2:3}$	Chr. 23	BNL1317–TMO06	13.85	0.0984	−0.0418	5.1	TM-1
	qFE-LG01-1b	F_2	LG01	NAU913–NAU974	12.01	0.091	0.1402	7.1	TM-1
		$F_{2:3}$	LG01	NAU913–NAU974	36.47	0.0194	0.348	29.5	TM-1
	qFE-23-1b	F_2	Chr. 23	BNL1317–TMO06	10.07	−0.032	−0.1562	14.9	HS427-10
	qFE-18-1b	$F_{2:3}$	Chr. 18	BNL3280–JESPR153	10.05	0.0896	−0.0143	3.7	TM-1
	qFE-10-1b	$F_{2:3}$	Chr. 10	NAU879–NAU992	30.93	0.1642	−0.01	11.8	TM-1
(PD6992 × SM3)	*qFL-16-1c*	F_2	Chr. 16	BNL2634–NAU733	22.99	−0.552	0.585	18.4	PD6992
$F_2/F_{2:3}$	*qFL-7-1c*	F_2	Chr. 7	NAU474–NAU1043	9.63	−0.725	−0.634	12.9	PD6992
	qFL-18-1c	$F_{2:3}$	Chr. 18	NAU785–JESPR153	9.67	0.3795	0.5257	5.5	Simian 3
	qFS-16-1c	F_2	Chr. 16	BNL2634–NAU733	9.93	−0.485	−1.36	7.3	PD6992
	qFS-A03-1c	F_2	LGA03	NAU1103–NAU1162	9.96	−0.6903	1.221	7.2	PD6992
	qFS-A02-1c	$F_{2:3}$	LGA02	NAU1302–BNL3255	10.38	−0.689	−0.089	7.0	PD6992
	qFM-14-1c	F_2	Chr. 14	BNL3494–NAU803	11.57	−0.096	−0.127	8.8	PD6992
	qFM-A03-1c	F_2	LGA03	NAU1103–NAU1162	12.66	0.0866	−0.1469	8.7	Simian 3
	qFE-A02-1c	F_2	LGA02	NAU1302–BNL3255	10.32	0.2095	0.0714	7.1	Simian 3
		$F_{2:3}$	LGA02	BNL3255–NAU1037	13.36	0.2044	0.1874	8.3	Simian 3
	qFE-7-1c	F_2	Chr. 7	NAU1043d–NAU1043c	10.78	0.3279	0.7748	46.4	Simian 3

Note: 1. FL for fiber length, FS for fiber strength, FM for micronaire and FE for elongation; 2. LR = likelihood ratio; 3. *a* = additive effect; 4. *d* = dominance effect; 5. R^2 = phenotypic variation explained by a single QTL; and 6. Direction = the parent improving trait.

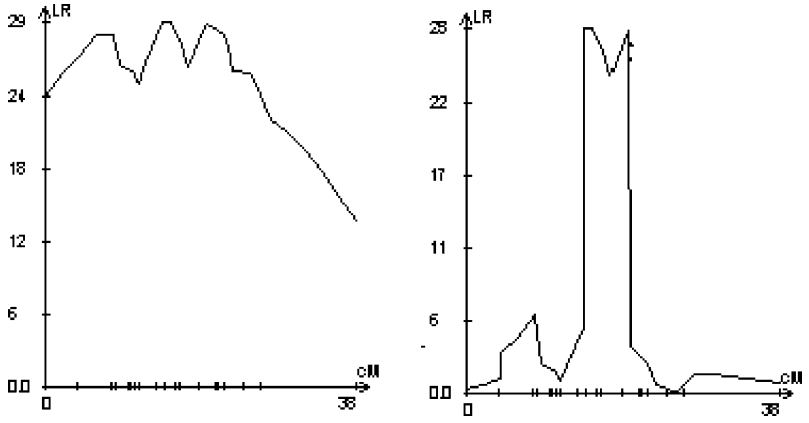

Figure 4. The distribution of LR score for fiber strength QTL on LGD03 by two QTL mapping methods. LR: likelihood ratio; Left: interval mapping; Right: composite interval mapping.

Only one significant QTL affecting fiber strength was detected on Chr. 10 in both F_2 and $F_{2:3}$, in which, TM-1 allele increased fiber strength by 0.72–1.03cN/tex. Moreover, four suggestive QTLs were identified on Chr. 3, Chr. 16, Chr. 23, and LGA02, respectively. *qFS-16-1b* has similar LOD score and phenotypic variation in two generations.

A total of three significant QTLs influencing micronaire were detected on Chr. 10, Chr. 16, and Chr. 26 only in $F_{2:3}$. Increased micronaire value was conferred by HS427-10 allele at the *qFM-10-1b* and *qFM-26-1b*, and TM-1 allele at *qFM-16-1b*.

A total of three significant QTLs influencing fiber elongation were detected on Chr. 10, Chr. 23, and LG01 only in $F_{2:3}$ generation. Increased fiber elongation was conferred by TM-1 allele at all these loci. In addition, two suggestive QTLs for fiber elongation were detected on Chr. 18 and Chr. 23.

The interval NAU879–NAU992 on Chr. 10 contained four significant QTLs for all fiber traits analyzed in this study with higher LR score. The direction of additive effect at the loci all originated from TM-1, indicating there was likely a gene with pleiotropic effects in this chromosome region (Table 3 and Figure 2).

Pop C

Only one significant QTL for fiber length was identified on Chr. 16 in F_2 in this population, which explained 18.4% PV. The allele in PD6992 contributed to increase fiber length. In addition, two suggestive QTLs for fiber length, three QTLs for fiber strength, two QTLs for micronaire and two for fiber elongation were identified.

Compared with Pop A and B, fewer QTLs were detected in Pop C, especially in $F_{2:3}$. Only one QTL was a significant QTL. Different weather conditions between 2002 and 2003 were probably the main cause of the difference (Table 3 and Figure 3).

Comparison of QTLs across different populations

In this study, some QTLs (significant or suggestive QTLs) were found in at least two populations. For fiber length, a common QTL was found on Chr. 23 in both Pop A and Pop B, as supported by the bridge marker BNL1317. The direction of additive effects was the same from a common parent, TM-1. Two suggestive QTLs (*qFL-7-1a* and *qFL-7-1c*) from Pop A and C were located in the same marker interval, NAU474–NAU1043. The direction of additive effect originated from high fiber quality parents, 7235 and PD6992, so they were likely a common QTL. Another putative common QTL for fiber strength on LGA02 was found in both Pop B and Pop C based on the neighboring interval, which had the same additive direction from high fiber quality parents. These three pairs of QTLs should be common and have possibly the same origin. In addition, *qFS-23-1a* and *qFS-23-1b*, *qFS-16-1b*, and *qFS-16-1c* for fiber strength on Chr. 23 and Chr. 16 tagged in Pop A and Pop B resided at the different interval. Although these QTLs cannot be confirmed to be common by bridge marker or interval, they have the same

Table 4. QTLs located in the same chromosome from three different populations.

Trait	QTL	Population	Chr. or LG	interval	LR	*a*	*d*	R^2 (%)
Fiber length	*qFL-23-1a*	Pop A/F_2	Chr. 23	BNL1317–NAU701	15.94	0.6670	0.2448	8.6
	qFL-23-1b	Pop B/F_2	Chr. 23	BNL1317–TMO06	9.72	0.0852	−0.4630	5.2
		Pop B/$F_{2:3}$	Chr. 23	BNL1317–TMO06	10.30	0.1783	−0.3555	20.2
Fiber length	*qFL-7-1a*	Pop A/$F_{2:3}$	Chr. 7	NAU474–NAU1043	12.52	−0.1480	−0.6488	6.3
	qFL-7-1c	Pop C/F_2	Chr. 7	NAU474–NAU1043e	9.63	−0.7250	−0.6340	12.9
Fiber strength	*qFS-A02-1b*	Pop B/$F_{2:3}$	LGA02	NAU1037–NAU1322	11.03	−0.7023	−0.4879	9.2
	qFS-A02-1c	Pop C/$F_{2:3}$	LGA02	NAU1302–BNL3255	10.38	−0.6890	−0.0890	7.0
Fiber strength	*qFS-23-1a*	Pop A/$F_{2:3}$	Chr. 23	BNL3140–NAU923	17.97	−0.7319	−0.9664	11.2
	qFS-23-1b	Pop B/$F_{2:3}$	Chr. 23	BNL3383–NAU1004	10.22	−0.9880	−1.0319	9.0
Fiber strength	*qFS-16-1b*	Pop B/F_2	Chr. 16	BNL1017–BNL1122	12.91	−0.9794	−0.6547	10.2
		Pop B/$F_{2:3}$	Chr. 16	BNL1017–BNL1122	11.27	−0.7273	−0.8167	12.0
	qFS-16-1c	Pop C/F_2	Chr. 16	BNL2634–NAU733	9.93	−0.4850	−1.3600	7.3

Note: 1. FL for fiber length, FS for fiber strength, FM for micronaire and FE for elongation; 2. LR = likelihood ratio; 3. *a* = additive effect; 4. *d* = dominance effect; 5. R^2 = phenotypic variation explained by a single QTL; and 6. Direction = the parent improving trait.

direction of additive effect and express recessively, therefore, they are supposed to be common QTL (Table 4).

Homoeology among cotton fiber QTLs

According to Endrizzi et al. (1985) and Crane et al. (1994), eight pairs of homoeologous chromosomes have been determined in the tetraploid cotton genome; namely, Chr. 1 and 15, Chr. 2 and 14, Chr. 4 and 22, Chr. 6 and 25, Chr. 7 and 16, Chr. 9 and 23, Chr. 10 and 20, and Chr. 12 and 26. Five new pairs of homoeologous chromosomes were suggested by molecular mapping of the cotton genome (Lacape et al. 2003; Rong et al. 2004), namely, Chr. 5 and LGD08, LGA02 and LGD03, LGA03 and LGD02, LGA01 and Chr. 18, Chr. 3 and 17. In this report, we found three pairs of putative homoeologous QTLs. There exist two QTLs for fiber length mapped to the interval NAU474–NAU1043e on Chr. 7 and BNL2634–NAU733 on Chr. 16 in Pop C. The QTL interval on Chr. 16, BNL2634–NAU733, is near a bridge marker NAU1043b. The direction of additive effect all came from PD6992; therefore they are possible homoeologous QTLs. Two QTLs for fiber strength, *qFS-D03-1a* in Pop A and *qFS-A02-1b(c)* in Pop B and Pop C are located on a pair of homoeologous chromosomes, LGD03 and LGA02. The direction of additive effect originated from high fiber quality parents, 7235, HS427-10, and PD6992. Two QTLs for fiber elongation also mapped on homoeologous chromosomes, LGD03 and LGA02 (Table 5). The direction of additive effect came from low fiber quality parents, TM-1 and SM3. Jiang et al. (1998) and Paterson et al. (2003) also identified two pairs of homoeologous QTLs for fiber elongation and fiber strength on LGA02 and LGD03. But they could not be confirmed to be common due to lack of bridge markers.

Discussion

Implication of segregation distortion

Segregation distortion has been reported in the cotton genome (Wang et al. 1995; Ulloa et al. 2002; Lacape et al. 2003). In this study, Pop A showed significant segregation distortion with 56 (44.1%) distorted marker of 127 markers scored. Among them, 34 loci (64.3%) had an excess of 7235 alleles, 6 loci (10.7%) had an excess of TM-1's and 16 loci (28.6%) had an excess of heterozygous one. Loci with skewed segregation tended to cluster on Chr. 3, Chr. 22, Chr. 25, LGA03, and LGD03. A marker dense skewed segregation region was detected on LGD03, in which 20 loci covered 38.0 cM with a mean distance of 1.9 cM. All codominant loci favored heterozygous allele and homozygote genotype are deficient for 7235. One possible reason for the severe skewed segregation attributed to high level of divergence of the

Table 5. Putative homoeologous QTLs for fiber traits among three populations.

Traits	QTLs	Pop	Chr.	LR	*a*	*d*	R^2 (%)
Fiber length	*qFL-7-1c*	Pop C/F_2	Chr. 7	9.63	−0.7250	−0.6340	12.9
	qFL-16-1c	Pop C/F_2	Chr. 16	22.99	−0.5520	0.5850	18.4
Fiber strength	*qFS-D03-1a*	Pop A/F_2	LGD03	34.57	−1.2337	0.0589	18.2
		Pop A/$F_{2:3}$		14.15	−1.1234	−0.4193	12.8
	qFS-A02-1b	Pop B/ $F_{2:3}$	LGA02	11.03	−0.7023	−0.4879	9.2
	qFS-A02-1c	Pop C/$F_{2:3}$	LGA02	10.38	−0.6890	−0.0890	7.0
Fiber elongation	*qFE-D03-1a*	Pop A/F_2	LGD03	9.63	0.1364	0.0508	5.4
		Pop A/$F_{2:3}$		12.19	0.1940	0.1320	7.2
	qFE-A02-1c	Pop C/F_2	LGA02	10.32	0.2095	0.0714	7.1
		Pop C/$F_{2:3}$	LGA02	13.36	0.2044	0.1874	8.3

Note: 1. FL for fiber length, FS for fiber strength, FM for micronaire and FE for elongation; 2. LR = likelihood ratio; 3. *a* = additive effect; 4. *d* = dominance effect; 5. R^2 = phenotypic variation explained by a single QTL; and 6. Direction = the parent improving trait.

parents since 7235 (Qian et al. 1992) is a *G. anomalum* introgression line. A significant level of genetic diversity was detected between 7235 and TM-1 as compared with Pop B, Pop C (Table 2), and other intraspecies mapping population in our laboratory. In terms of genetic factors, genetic mechanisms for preferential segregation include pollen tube competition, pollen lethal, preferential fertilization and selective elimination of zygotes (Lu et al. 2002). From this report, a strong indication that distorted markers on LGD03 were deficient for homozygote and closely linked to each other suggested the presence of major recessive deleterious genes in 7235 lines. The recessive deleterious gene might affect pollen fertility, and result in markers with skewed segregation linked to it. The fact that chromosome containing distorted marker bore more QTLs (46.2%), suggests we must carefully deal with distorted marker. Higher LOD score combined with chromosome assignment was suitable for mapping distorted markers.

Comparison of QTLs across generations and populations

A high agreement between QTL position and effect across generations are essential for MAS because QTLs are usually identified in early generations and their flanking markers are used for selecting lines during advanced generations. In this study, only five significant QTLs were detected in both F_2 and $F_{2:3}$ generations simultaneously due to using a stringent LOD threshold of ≥3.0. Although, the number of stable QTLs detected is limited, these QTLs have stable and large genetic effect, showing a great potential for MAS. If a suggestive LOD score value of 2.0 was used, another nine QTLs would be detected in both F_2 and $F_{2:3}$ generations simultaneously. Many factors could influence adequate statistical significance in one or two environments, including QTL effect, sample size, the genomic size, the genetic map density and environment effect. Employing these QTLs in MAS could produce poor effect or even no effect. But these candidate QTLs will be helpful to analyze QTL expression in different environments and different backgrounds, and QTL distribution in the genome.

In Pop C, only one significant QTL could be detected. Different weather conditions were probably the main cause of the difference. During growing seasons of Pop A ($F_{2:3}$) in 1999 and Pop C ($F_{2:3}$) in 2003, low temperatures and excessive rain delayed fiber development and resulted in immature fiber. Harvesting was delayed by 1 month compared to normal years. Means and variation of fiber strength and micronaire gave significant difference between F_2 and $F_{2:3}$ generations (Table 1). Larger non-genetic variance reduced efficiency and accuracy of QTL mapping. This underlines that reliable phenotypic data are of crucial importance in QTL mapping studies.

Three populations derived from three different original parents with high fiber quality were used to detect QTLs for fiber traits. The comparison of QTLs (significant and suggestive QTL) across genetic populations may provide a better

understanding of the genetic factors of fiber traits. A total of 39 QTLs (17 significant QTLs and 22 suggestive QTLs) affecting fiber traits were found in these three populations. At least three QTLs were common from different genetic backgrounds, supported by bridge molecular marker (interval). QTLs for fiber length on Chr. 23 were found in Pop A and Pop B, which were linked to a bridge marker, BNL1317. Pop C also contained this SSR marker, but the QTL was not detected in Pop C. The reason is that the additive effect at this QTL originated from common parent, TM-1, which was not used as parent in Pop C. Another QTL for fiber strength on LGA02 was detected in Pop B and Pop C. In Pop A, there were no polymorphic markers on LGA02, but there was a stable major QTL responsible for fiber strength on its homoeologous chromosome, LGD03, indicating it may be a conserved QTL. The detection of a common QTL across different populations suggests a high fiber quality gene might have the same origin. Most genes of elite fiber properties originated from an introgression of a triple hybrid as well as the introgression of segment of DNA from *G. barbadense*. PD6992 experienced triple hybrid introgression evidently. Although the detailed pedigree of HS427-10 is not clear, it is from an Acala background (S. Oakley, pers. comm.). Upland cotton 7235 is a *G. anomalum* introgression line, but two high fiber-strength strains, Acala 3080 and PD4381, were used to develop this line, thus it is not surprising that they may have the same fiber quality gene.

Three linkage maps constructed here only covered 12.4–14.8% of the recombinational length of the cotton genome due to low polymorphisms in intraspecific tetraploid hybrids. Further saturation of cotton genetic map will find more stable common QTLs across different populations if we have more polymorphic markers.

Putative homoeologous QTLs

We found three pairs of putative homoeologous QTLs. Jiang et al. (1998) detected that two QTLs for fiber elongation on LGA02 and LGD03 were a pair of homoeologous QTLs. Paterson et al. (2003) found six pairs of fiber quality QTLs appearing to map homoeologous locations, fiber fineness QTLs on Chr. 2–Chr. 14, Chr. 9–Chr. 23, and Chr. 6–Chr. 25; fiber strength QTLs on LGA02–LGD03; and fiber yellowness QTLs on Chr. 6–Chr. 25. Interspecific populations of *G. hirsutum* and *G. barbadense* were used in these two reports. The fact that A subgenome QTL corresponded to one D subgenome QTL in two interspecific populations suggested some A subgenome favorable alleles had already been fixed before polyploidization (Jiang et al. 1998; Wright et al. 1998; Paterson et al. 2003). Two of three parents with fine fiber quality used in this study, 7235 and PD6992, might contain DNA segment of wild species, B- and D-genome. Although seldom does direct evidence indicated that introgression has taken place, it is undoubted fact that triple hybrid was the contributor of the enhanced fiber properties. Our finding that modern superior fiber strains contain homoeologous QTLs may be explained by a recombinational event occurring randomly in A subgenome or D subgenome homoeologous region during the process of introgression. Because homologous recombination was the main mechanism of chromosome exchanges, QTL correspondence may be the outcome of introgression taking place in A subgenome and D subgenome simultaneously.

Detection of common and homoeologous QTLs provides an opportunity for breeders to make full use of QTL information in one population to design and execute breeding research in other populations, avoiding the repetition of mapping experiments. This is the first attempt to tagging QTLs responsible for fiber properties from different genetic backgrounds in Upland cotton. Characterization of stable QTL from different generations, common QTL from different populations and homoeologous QTLs promises to increase information on the genetic base and distribution of valuable QTL in the cotton genome and facilitating application of MAS in improving fiber quality.

Acknowledgements

This program was supported financially by National High-tech Program (2004AA211172), National Science Foundation in China (30070483, 30270806), the Key Project of Chinese Ministry of Education (10418), Jiangsu High-tech Project (BG2002306) and IAEA (12846).

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