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ORIGINAL PAPER

Using three overlapped RILs to dissect genetically clustered QTL for fiber strength on Chro.D8 in Upland cotton

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Abstract Fiber strength is an important trait among cotton fiber qualities due to ongoing changes in spinning technology. Major quantitative trait loci (QTL) for fiber quality enable molecular marker-assisted selection (MAS) to effectively improve fiber quality of cotton cultivars. We previously identified a major QTL for fiber strength derived from 7235 in Upland cotton. In the present study, in order to fine-map fiber strength QTL, we chose three recombinant inbred lines (RIL), 7TR-133, 7TR-132, and 7TR-214, developed from a cross between 7235 and TM-1 for backcrossing to TM-1 to develop three large mapping populations. Phenotypic data for fiber strength traits were collected in Nanjing (JES/NAU) and Xinjiang (BES/XJ) in 2006 and 2007. Three simple sequence repeat (SSR) genetic linkage maps on Chro.24(D8) were constructed using these three backcrossed populations. The SSR genetic maps were constructed using 907 individuals in (7TR-133 × TM-1)F_2 (Pop A), 670 in (7TR-132 × TM-1)F_2 (Pop B), and 940 in (7TR-214 × TM-1)F_2 (Pop C). The average distance between SSR loci was 0.62, 1.7, and 0.56 cM for the three maps. MapQTL 5 software detected five-clustered QTL ($2.5 < LOD < 29.8$) on Chro.D8 for fiber strength following analysis of three RIL backcrossed $F_2/F_{2:3}$ progenies at JES/NAU and BES/XJ over 2 years. Five QTL for fiber strength exhibited a total phenotypic variance (PV) of 28.8–59.6%.

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Introduction

Cotton is an important economic crop worldwide, and cotton fiber is a basic raw material used in the textile industry. In recent years, changes in spinning technology have resulted in the need for unique and often increased cotton fiber quality, especially strength, for processing (Benedict et al. 1999; Deussen 1992). Advances in the use of DNA markers for marker-assisted selection (MAS) have shown promise in streamlining plant-breeding programs. The development of DNA markers linked to fiber quality QTL would allow cotton breeders to identify this important trait at early plant-growing stages or early segregations.

Quantitative trait loci (QTL) conferring fiber quality traits have been described and mapped using molecular markers in inter-specific populations from crosses between *Gossypium hirsutum* and *G. barbadense* (Jiang et al. 1998; Kohel et al. 2001; Paterson et al. 2003; Mei et al. 2004; Lacape et al. 2003; Lacape et al. 2005; Park et al. 2005), and in intra-specific *G. hirsutum* populations (Shappley et al. 1998; Ulloa and Meredith 2000; Zhang et al. 2003; Shen et al. 2005; Ulloa et al. 2005; Shen et al. 2007). More than 100 agronomic and fiber trait QTL have also been mapped in an intra-specific population in *G. hirsutum* (Shappley et al. 1998).

In our laboratory, we employed bulk segregant analysis to identify a major QTL on Chro.24 (D8) for fiber strength

using an F_2 segregating population and $F_{2:3}$ family lines derived from a cross between 7235 and TM-1 in Upland cotton (Zhang et al. 2003). The QTL was detected in diverse environments, including Nanjing and Hainan in China, and Texas in USA, which had an average phenotypic variance (PV) of 18.5–53.8%. The QTL was later further confirmed in (7235 × TM-1) F_2, and $F_{2:3}$ family lines, and (7235 × TM-1) RILs (Shen et al. 2005, 2007). The 95% confidence interval (CI) of this major fiber strength QTL (*qFS-D8-1*) was identified by Shen et al. (2007) in a recombinant inbred line (RIL) derived from 7235 and TM-1. The 95% CI ranged from 39.1 to 53.9 cM between SSR marker BNL2961 and CIR070a. MAS results revealed that DNA markers linked to this QTL could be used to increase fiber strength of commercial cultivars in early segregating breeding generations (Zhang et al. 2003; Guo et al. 2005). This region was densely populated with markers and QTL, with 8 QTL for 8 traits detected within a chromosome region of 33.4 cM, including 36 SSR loci in Chro.D8 (Shen et al. 2007). In the present study, three recombinant inbred lines (RIL), 7TR-133, 7TR-132, and 7TR-214, developed from a cross between 7235 and TM-1, were chosen and backcrossed with TM-1 to develop three mapping populations to fine-map the major fiber strength QTL derived from 7235 to lay a basis of map-based cloning of this major QTL in cotton.

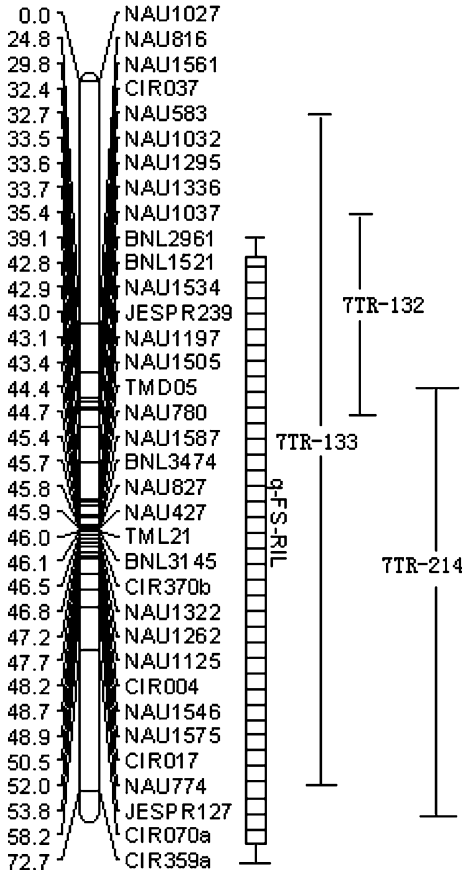

Fig. 1 Linkage group (Shen et al. 2007) and chromosome positions of three RILs in Chro.D8

Materials and methods

Plant materials

Three RILs, 7TR-133, 7TR-132, 7TR-214, derived from a cross between 7235 and TM-1 using a bulk-selfing technique (Shen et al. 2007) were used to fine-map the major fiber strength QTL previously identified from 7235 (Zhang et al. 2003), an introgressive line exhibiting high fiber quality (Qian et al. 1992). These three RIL lines, 7TR-133 from NAU583 to NAU774 within a 19.3 cM region, 7TR-132 from NAU1037 to NAU 780 within an 9.3 cM region, and 7TR-214 from TMD05 to JESPR127 within a 9.4 cM region, had different intervals, but overlapped to cover the major fiber strength QTL region from BNL 2961 and NAU774 in Chro.D8 detected in the (7235 × TM-1) RIL (genetic linkage map shown in Fig. 1) (Shen et al. 2007). The RIL lines were backcrossed as maternal parents with TM-1, Upland cotton genetic standard (Kohel et al. 1970), to generate three mapping populations.

Individual plants from 7TR-133,7TR-132 and 7TR-214 were backcrossed with TM-1 in 2005 and their F_1 seeds were sent to Hainan Island to produce F_2 seeds. In 2006, the F_2 seeds were grown for fiber strength scoring, QTL tagging, and self-pollination to produce F_3 family seeds in Jiangpu Experiment Station, Nanjing Agriculture University (JES/NAU). In 2007, three F_3 family line seeds were divided into two groups, one group planted at JES/NAU, the Yangtze River cotton growing region in China, and the other at the Baotou Lake Experiment Station, Xinjiang Academy of Agriculture and Reclamation Sciences (BES/XJ), China's Northwest cotton growing region. Nanjing is situated at longitude 118°46′, and latitude 32°03′; and Baotou Lake Experiment Station at longitude 86°07′, latitude 41°46′. It is important to note that cultivation practices differ between these two cotton-growing regions. At JES/NAU, cotton seeds are typically planted in nursery pot seedbeds, and transplanted to the field when seedlings exhibit 3–4 leaves; seedlings are planted 45 cm apart. At BES/XJ, cottonseeds are planted directly in the field under plastic film with plants spaced 15 cm apart. Drip irrigation under film is usually employed at BES/XJ to conserve water. However, at JES/NAU adequate rainfall eliminates the need for irrigation.

Because an inadequate number of seeds were harvested from some F_2 individuals to plant F_3 progeny at both locations simultaneously, and/or the harvested fiber from the F_2 individuals was not enough to conduct fiber tests, different plants and family lines represented the F_2 and $F_{2:3}$ lines at

both locations (Table 2). The (7TR-133 × TM-1) F_2, (7TR-132 × TM-1) F_2, and (7TR-214 × TM-1) F_2 included 907, 670, and 940 individuals to be used to construct the linkage map and to produce their corresponding F_3 family lines generated by self-pollinating the corresponding F_2 individuals, but only 764, 663, and 813 individuals were used in QTL mapping in their F_2s because no enough fiber sample was harvested to conduct fiber quality test. Both F_2 and $F_{2:3}$ seeds were first planted in nursery pots and transplanted to the field at JES/NAU during 2006 and 2007; however, $F_{2:3}$ seeds were directly planted at BES/XJ in 2007. A randomized incomplete block design was employed in the field trials. The traditional cotton growing practices were used in two locations, at JES/NAU, plots were 5 m long with plants spaced 45 cm apart, and at BES/XJ plants were spaced 15 cm apart in 2 m long plots. Fiber samples from F_2 individuals at JES/NAU in 2006, and from $F_{2:3}$ family lines at BES/XJ and JES/NAU in 2007 were collected from bolls in the interior middle of the plant. In 2006 and 2007, fiber quality was tested by HVICC at the Henan Test Center of Cotton Quality, China.

Assay of DNA markers

DNAs were extracted from individual plants of the three F_2s as described by Paterson et al. (1993). In the present research, we employed 6123 SSR primer pairs including BNL, JESPR, CRI, etc., presently available in cotton (http://www.cottonmarker.org). Shen et al. (2005) screened the SSR primer pair polymorphisms between the two parents 7235 and TM-1. We subsequently screened the remaining SSR primers from NAU2500 to NAU6123 developed in our lab in this study. The sources of these NAU SSR primers were reported in our prior work (Han et al. 2004; Han et al. 2006; Wang et al. 2006; Guo et al. 2007). SSR–PCR amplifications were performed using a Peltier Thermal Cycler-225 (MJ Research), and PCR product electrophoresis was conducted as described by Zhang et al. (2000, 2002).

Data analysis and QTL mapping

Trait means were calculated using SAS (SAS institute 1989) and linkage maps were constructed with JoinMap Version 3.0 (Van Ooijen and Voorrips 2001). The Multiple-QTL model (MQM) using MapQTL5 (Van Ooijen. 2004) was chosen to determine the QTL likelihood map, gene action, and phenotypic variance (PV) explained by individual QTL. In addition, 1,000 permutation analysis was applied to each putative QTL. Chi-square was used to determine if the allele frequency at each individual locus showed normal segregation patterns. Confidence intervals (90–95%) associated with QTL locations were set as the map interval corresponding to one LOD decline on either side of the peak.

QTL nomenclature was adapted according to the method developed in rice (McCouch et al. 1997), starting with 'q', followed by a trait abbreviation of FS designating for fiber strength, followed by $F_2/F_{2:3}$ population, and an abbreviation of the cultivation location (JES/NAU designates Jiangpu Experiment Station, and BES/XJ Baotou Lake Experiment Station, Xinjiang Academy of Agriculture and Reclamation Sciences), and followed by the number of QTL affecting the trait on Chro.D8. Additionally, a/b/c stands for the population names, respectively, for 7TR-133, 7TR-132, and 7TR-214.

Results

Phenotypic values for fiber strength of parents, F_2 and $F_{2:3}$

Significant differences in fiber strength were detected between these RILs and their backcrossed parent TM-1 at JES/NAU and BES/XJ in 2006 and 2007 (Table 1). Fiber strength phenotypic data for the F_2 and $F_{2:3}$ populations at JES/NAU and BES/XJ during 2006 and 2007 are presented in Table 2. F_2 and $F_{2:3}$ fiber strength distributions at JES/NAU and BES/XJ were generated using SAS (SAS institute 1989). To determine if fiber strength were normally distributed, skewness and kurtosis values were calculated for all mapping populations. The skewed values were between 0.24 and 0.94 (Table 2; Fig. 2), indicating they fit normal distributions and can be used to conduct QTL mapping for fiber strength. Low temperature during the late growing stages when the fiber maturation at BES/XJ in 2007 results in approximately 3 cN/tex fiber strength lower at BES/XJ than that at JES/NAU (Table 2; Fig. 2).

Construction of the three linkage maps

Of the 6123 SSR markers employed in this study, 170 polymorphic SSR markers were detected between 7235 and TM-1, and a total of 25, 12, and 21 polymorphic SSR loci detected between 7TR-133, 7TR-132, 7TR-214, and TM-1 on Chro.D8. The three genetic maps for (7TR-133 × TM-1) F_2, (7TR-132 × TM-1) F_2, and (7TR-214 × TM-1) F_2 were generated by JoinMap3.0, using a LOD = 3.0 (Fig. 3). The SSR genetic map was constructed using 907 individuals from (7TR-133 × TM-1)F_2 (Pop A) and included 22 loci covering 13.7 cM, which represented approximately 12.3% of the total 111.7 cM recombinational length of cotton Chro.D8 (Guo et al. 2007). The map constructed from 670 individuals in (7TR-132 × TM-1)F_2 (Pop B) included 11 loci covering 19.1 cM, approximately 17.1% of the recombinational length of cotton Chro.D8, and the map constructed using 940 individuals from (7TR-214 × TM-1)F_2 (Pop C) included 18 loci covering 10.1 cM, approximately

Table 1 Distribution of fiber strength (cN/tex) of F_2 and $F_{2:3}$ in three populations at JES/NAU and BES/XJ

Generation	Population size	Range	Mean	SD	Skewness
(7TR-133 × TM-1)F_2 at JES/NAU	764	23.80	34.15	2.79	−0.94
(7TR-133 × TM-1)$F_{2:3}$ at JES/NAU	907	21.00	33.50	3.08	0.65
(7TR-133 × TM-1)$F_{2:3}$ at BES/XJ	890	12.70	30.39	1.85	0.28
(7TR-132 × TM-1)F_2 at JES/NAU	663	22.10	33.14	2.97	−0.24
(7TR-132 × TM-1)$F_{2:3}$ at JES/NAU	670	19.90	34.02	3.03	0.42
(7TR-132 × TM-1)$F_{2:3}$ at BES/XJ	649	12.90	31.28	2.44	0.42
(7TR-214 × TM-1)F_2 at JES/NAU	813	22.90	32.44	2.97	−0.62
(7TR-214 × TM-1)$F_{2:3}$ at JES/NAU	940	23.80	32.81	3.00	0.70
(7TR-214 × TM-1)$F_{2:3}$ at BES/XJ	858	11.00	29.88	1.65	0.62

JES/NAU the location of Jiangpu Experiment Station, Nanjing Agriculture University, *BES/XJ* the location of Baotou Lake Experiment Station, Xinjiang Academy of Agriculture and Reclamation Sciences

Table 2 Fiber strength performance of four parents at JES/NAU and BES/XJ

Parents	Strength (cN/tex)[a]	Parents	Strength (cN/tex)[a]	Parents	Strength (cN/tex)[a]
7TR-133	36.9	7TR-132	34.72	7TR-214	33.05
TM-1	29.08	TM-1	29.08	TM-1	29.08
Difference	7.82**	Difference	5.64**	Difference	3.97*

* Significance at $P = 0.05$ and **$P = 0.01$, respectively

[a] Data presented were the means of two locations ("JES/NAU", "BES/XJ") and 2 years (2006 and 2007). *JES/NAU* the location of Jiangpu Experiment Station, Nanjing Agriculture University, *BES/XJ* the location of Baotou Lake Experiment Station, Xinjiang Academy of Agriculture and Reclamation Sciences

9.4% of the recombinational length of cotton Chro.D8. Most SSR markers in (7235 × TM-1)RIL and (7235 × TM-1)F_2 on Chro.D8 (Shen et al. 2005, 2007) were linked in the present genetic maps (Fig. 3). Eight, 2 and 10 new SSR loci were added to Pops A, B and C, respectively. The average distance between two SSR loci was 0.62 cM in Pop. A, 1.7 cM in Pop B, and 0.56 cM in Pop C. Except for three seriously distorted segregation SSR loci, which interspersed with other loci arrangement in linkage groups, in Pop A, 1 in Pop B, and 3 in Pop C, other distorted SSR markers were included in linkage map construction. Sixteen, 7 and 10 distorted SSR loci were detected in Pops A, B, and C. The percentage of skewed segregation ratios were 72.7, 63.6, and 55.6%, respectively, a great deal of decrease from 100% in (7235 × TM-1)RIL (Shen et al. 2007).

QTL tagging for fiber strength

Fiber strength QTL tagging was conducted by MapQTL 5 software. For Pop A, 764 F_2 individuals, and 907 $F_{2:3}$ family lines at JES/NAU, and 890 $F_{2:3}$ family lines at BES/XJ were used for fiber strength QTL tagging; for Pop B, 663 F_2 individuals, and 670 $F_{2:3}$ family lines at JES/NAU, and 649 family lines at BES/XJ were used; and for Pop C, 813 F_2 individuals, and 940 $F_{2:3}$ family lines at JES/NAU, and 858 in BES/XJ were used for the analysis (Table 2). We found five QTL ($2.5 < LOD < 29.8$) for fiber strength on Chro.D8 in these three populations.

QTL tagging in (7TR-133 × TM-1) F_2 and $F_{2:3}$

Two QTL were detected in (7TR-133 × TM-1)F_2, and three QTL in (7TR-133 × TM-1)$F_{2:3}$ in this chromosome region (Fig. 3a; Table 3). The *qFS-F_2-JES-1a* and *qFS-$F_{2:3}$-JES-1a* were detected in the same interval range from TMD05 to BNL3145 in F_2 and $F_{2:3}$, both grown at JES/NAU in 2006 and 2007, and *qFS-F_3-BES-1a* from TMD05 to NAU3207 in $F_{2:3}$ grown at BES/XJ in 2007. Therefore, this is a very stable and common QTL, and we proposed it to be named *qFS-1*. Another QTL (*qFS-F_2-JES-2a*) from NAU2665 to NAU3605 was detected in (7TR-133 × TM-1)F_2 at JES/NAU in 2006. However, this QTL was further dissected into two QTL at this region in $F_{2:3}$ at JES/NAU and BES/XJ. Both *qFS-$F_{2:3}$-JES-2a* from NAU1534 to NAU3605 and *qFS-$F_{2:3}$-BES-2a* from NAU1534 to NAU3499 were shown to overlap over a large region, which suggested a new QTL. We proposed *qFS-2* as the name for this QTL. In addition, *qFS-$F_{2:3}$-JES-3a* from NAU4099 to NAU1534 and *qFS-$F_{2:3}$-BES-3a* from BNL1521 to NAU1534 were identified at JES/NAU and BES/XJ in 2007, and overlapped with three loci, NAU4099, NAU2665. and NAU1534, in (7TR-133 × TM-1)$F_{2:3}$. Consequently, it suggested that this should be a third QTL, and we proposed to name this QTL as *qFS-3*. QTL *qFS-1* exhibited a phenotypic variance of 5.8–13.6% and a CI of 1.35–1.82 cM with 6–8 SSR markers, and the largest LOD value of 28.82 was detected in a $F_{2:3}$ grown at BES/XJ. QTL *qFS-2* exhibited a phenotypic variance of 6.5–13.4% and a CI of 1.16–2.11 cM with 2–4 SSR markers. The largest LOD value was as high as 29.76 for this QTL in a $F_{2:3}$ grown at BES/XJ. QTL *qFS-3*

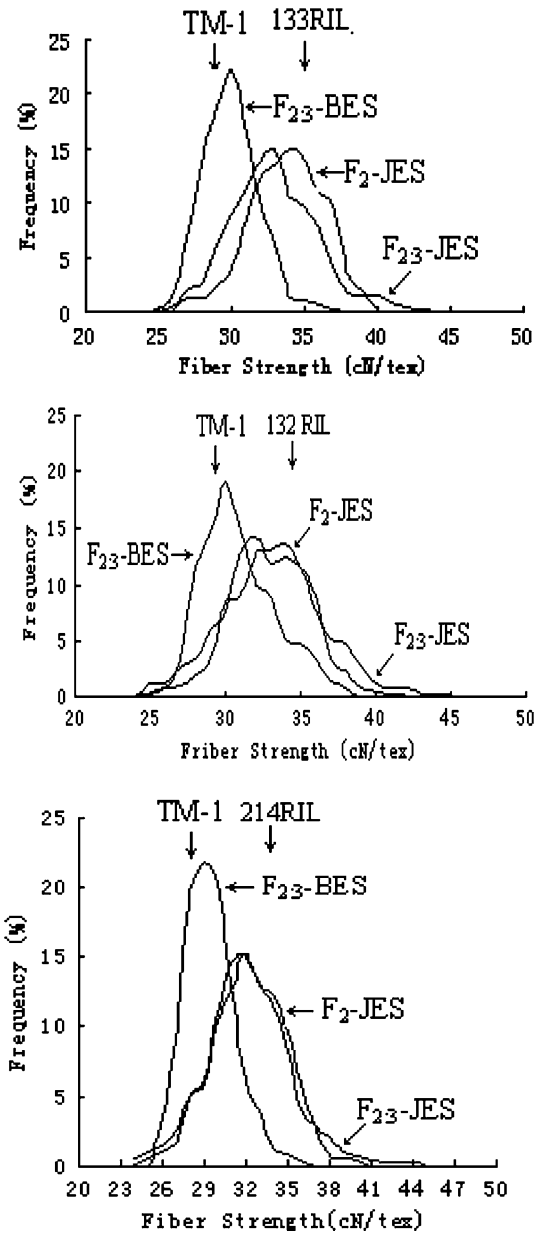

Fig. 2 The distribution for fiber strength in F2 and F2:3 in three populations in two locations

was detected in the $F_{2:3}$ at JES/NAU and BES/XJ, showing a phenotypic variance of 7.4–14.8%. The CI of *qFS-3* was 0.11–0.66 cM from 3 to 4 SSR markers, and the largest LOD value was 25.32 in the $F_{2:3}$ from BES/XJ.

QTL tagging in (7TR-132 × TM-1) F_2 and $F_{2:3}$

Three QTL for fiber strength were further detected in Pop B (Fig. 3b; Table 3). Among them, both *qFS-F_2-JES-1b* and *qFS-$F_{2:3}$-JES-1b* from NAU1197 to NAU780, and *qFS-$F_{2:3}$-BES-1b* from NAU3201b to TMD05 were detected in the most regions overlapped in both F_2 and $F_{2:3}$ grown at JES/NAU and BES/XJ in 2006 and 2007. These QTL were anchored in the same position as *qFS-1* and bridged with TMD05 locus in (7TR-133 × TM-1) F_2 and $F_{2:3}$(Fig. 3a). Therefore, we considered these QTL as *qFS-1*. Another QTL, *qFS-F_2-JES-2b* from BNL2961 to NAU1534 was detected at JES/NAU in 2006. However, we subsequently detected three QTL, *qFS-$F_{2:3}$-JES-2b* from BNL2961 to NAU1295 and *qFS-$F_{2:3}$-BES-2b* from BNL2961 to NAU3562 and *qFS-$F_{2:3}$-BES-3b* from BNL1037 to BNL1521 in this region. It is likely that QTL, *qFS-F_2-JES-2b* and *qFS-$F_{2:3}$-JES-2b* detected at JES/NAU in 2006 and 2007, was dissected into two at BES/XJ in 2007. The first two QTL overlapped in most regions and bridged with BNL1521 and NAU1534. Therefore, we considered these two QTL are the same as *qFS-2*. The third QTL, *qFS-$F_{2:3}$-BES-3b* from BNL1037 to BNL1521 was only detected in (7TR-132 × TM-1)$F_{2:3}$ at BES/XJ in 2007 and may be the same QTL as *qFS-3*. *qFS-1* exhibited a phenotypic variance (PV) of 6.8–12.9%, a CI of 1.82–3.78 cM with three SSR markers, and the largest LOD value detected was 14.5 in F_3 from BES/XJ. *qFS-2* showed a phenotypic variance of 5.0–11.2%, and a CI of 1.92–3.86 cM covered by 3–6 SSR markers. The largest LOD value was 14.8 in F_3 grown at BES/XJ. *qFS-3* was only identified in F_3 grown at BES/XJ, and exhibited a phenotypic variance of 10.5%.

QTL tagging in (7TR-214 × TM-1) F_2 and $F_{2:3}$

7TR-214 covered most of the chromosome region where *qFS-1* was anchored. However, three QTL were detected in this region (Fig. 3c; Table 3). qFS-F_2-JES-1c ranged from NAU5379 to BNL3474, qFS-$F_{2:3}$-JES-1c from NAU5379 to NAU2926 and qFS-F_2-BES-1c from NAU5379 to TMD05 were identified almost in the same interval in both F_2 and $F_{2:3}$ from JES/NAU and BES/XJ in 2006 and 2007. They are bridged with SSR marker TMD05 with Pop A and B, which supported that the QTL might be *qFS-1*. However, both qFS-F_2-JES-2c and qFS-$F_{2:3}$-BES-2c were identified in the same interval ranged from BNL3474 to NAU1322 in F_2 and $F_{2:3}$ and are bridged with SSR markers NAU827, NAU3207, TMI21 and BNL3145 anchored for *qFS-1* in Pop A. The remaining QTL identified in F_2 at JES/NAU in 2006 and $F_{2:3}$ at BES/XJ in 2007 had not previously been detected; however, still included in the CI of *qFS-1* in Pop A. It is clear that *qFS-1* identified in Pop A and B could be further dissected into three QTL and we propose the names *qFS-1, qFS-4*, and *qFS-5*. *qFS-1* exhibited a phenotypic variance (PV) of 4.2–9.4%, a CI of 2.31–3.16 cM with 3–5 SSR markers, and the largest LOD value was 12.46 in $F_{2:3}$ at BES/XJ. *qFS-4* and *qFS-5* were detected in F_2 (JES/NAU) and $F_{2:3}$ grown at BES/XJ, but not in $F_{2:3}$ at JES/NAU. *qFS-4* showed a phenotypic variance of 6.5–8.0% and *qFS-5* a phenotypic variance of

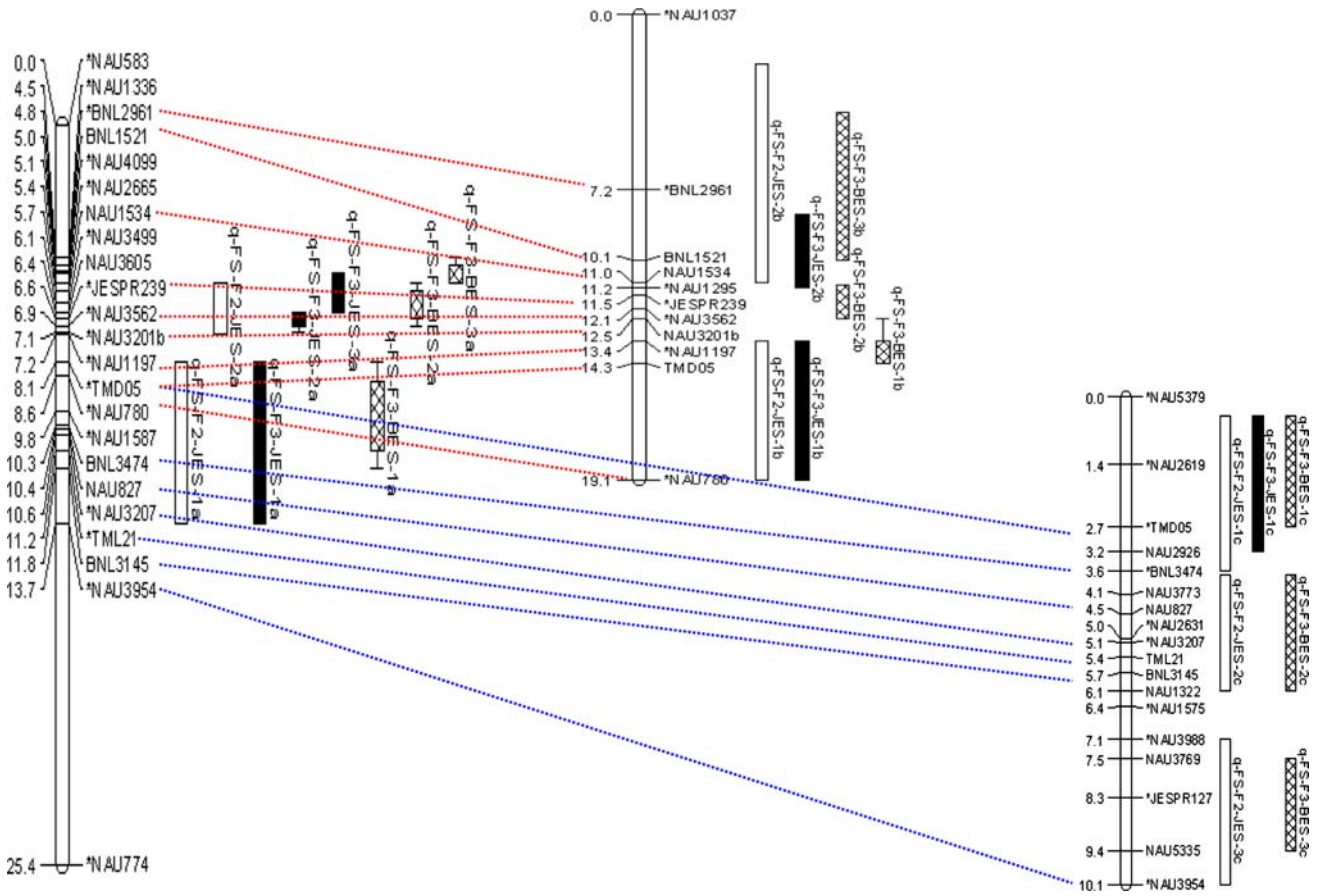

Fig. 3 Linkage groups and QTL position in three linkage groups

5.7–9.8%. The CI of qFS-4 was 2.41 cM with eight SSR markers, and the largest LOD value was 12.08 in the $F_{2:3}$ population at BES/XJ. The CI of qFS-5 was 1.95–3.0 cM with 3–5 SSR markers, and the largest LOD value was 11.48 in the $F_{2:3}$ cultivated at BES/XJ.

Discussion

In our previous reports, one major QTL for fiber strength was identified in (7235 × TM-1)F_2/$F_{2:3}$ and (7235 × TM-1)RILs (Shen et al. 2005, 2007). In the present research, five tightly linked and/or clustered QTL were identified on Chro.D8 in three populations generated using three RIL lines, which overlapped with our previously identified major QTL region (Fig. 1), although slight differences in QTL tagging positions were detected in the data collected at JSE/NAU and BES/XJ, most likely due to their differences in the growing environments and cotton planting practices. QTL *qFS-1*, identified in all mapping populations of F_2 and $F_{2:3}$ at JES/NAU and BES/XJ, showed a bridge SSR marker-TMD05; therefore, *qFS-1* was regarded as a common QTL. The *qFS-1* allele increased fiber strength by 0.85–1.13 cN/tex in 7TR-133, 0.9–1.40 cN/tex in 7TR-132 and 0.82–1.06 cN/tex in 7TR-214. *qFS-2* had a bridge SSR marker-NAU1534 detected in F_2 and $F_{2:3}$ at JES/NAU and BES/XJ in Pops A and B, respectively. The *qFS-2* allele increased fiber strength by 0.78–1.14 cN/tex in 7TR-133, and by 0.88–1.27 cN/tex in 7TR-132. *qFS-3* was identified in (7TR-133 × TM-1)$F_{2:3}$ at JES/NAU and BES/XJ and (7TR-132 × TM-1)$F_{2:3}$ grown at BES/XJ. Our data suggested that *qFS-3* at JES/NAU and BES/XJ might be the same because their confidence intervals overlapped. The *qFS-3* allele increased fiber strength by 0.80–1.27 cN/tex in 7TR-133, and 1.16 cN/tex in 7TR-132. The *qFS-4* allele identified as qFS-F_2-JES-2c at JES/NAU and *qFS-$F_{2:3}$-BES-2c* at BES/XJ increased fiber strength by 0.76–1.02 cN/tex. The *qFS-5* identified as *qFS-F_2-JES-3c* at JES/NAU, and *qFS-$F_{2:3}$-BES-3c* at BES/XJ increased fiber strength by 0.63–0.95 cN/tex. Five QTL for fiber strength exhibited a total phenotypic variance of 28.8–59.6%.

Three overlapped RIL backcrossed progenies and the large mapping populations used for the fiber strength QTL tagging most likely resulted in the five clustered QTL identified for the same trait. The CI of the QTL identified in the

Table 3 Characteristics of QTL affecting fiber strength in three populations

Populations	QTL		Confidence interval	Flanking markers	Position[a] (cM)	LOD[b]	Additive[c]	Dominent[d]	PV (%)[e]	AC[f]
7TR-133 × TM-1(Pop.A)	qFS-F_2-JES-1a	*qFS-1*	7.38–9.20	TMD05–BNL3145	8.97	4.49	0.9972	0.3012	5.8	2.1
	qFS-$F_{2:3}$-JES-1a		7.38–9.20	TMD05–BNL3145	8.97	5.25	0.8526	-0.0332	7.8	2.0
	qFS-$F_{2:3}$-BES-1a		7.38–8.73	TMD05–NAU3207	8.42	28.82	1.1309	0.1383	13.6	2.1
	qFS-F_2-JES-2a	*qFS-2*	3.55–5.66	NAU2665–NAU3605	4.56	4.84	0.8909	0.3690	6.5	2.1
	qFS-$F_{2:3}$-JES-2*a*		3.97 –5.66	NAU1534–NAU3605	4.56	6.11	0.7809	0.4866	7.0	2.0
	qFS-$F_{2:3}$-BES-2a		3.97–5.13	NAU1534–NAU3499	4.28	29.7 6	1.1418	0.1324	13.4	2.1
	qFS-$F_{2:3}$-JES-3*a*	*qFS-3*	3.31–3.97	NAU4099–NAU1534	3.55	5.76	0.7983	0.1433	7.4	2.0
	qFS-$F_{2:3}$-BES-3a		3.15–3.97	BNL1521–NAU1534	3.36	25.32	1.2673	0.1543	14.8	2.1
7TR-132 × TM-1(Pop B)	qFS-F_2-JES-1b	*qFS-1*	13.37–17.15	NAU1197–NAU780	14.30	4.02	0.9944	-0.0882	7.5	1.9
	qFS-$F_{2:3}$-JES-1b		13.37–17.15	NAU1197–NAU780	14.30	3.85	0.8965	0.1675	6.8	1.9
	qFS-$F_{2:3}$-BES-1b		12.48–14.30	NAU3201b–TMD05	12.88	14.5	1.3995	0.1619	12.9	2.0
	qFS-F_2-JES-2b	*qFS-2*	7.15–11.01	BNL2961–NAU1534	10.15	3.44	0.8769	-0.3789	5.4	1.9
	qFS-$F_{2:3}$-JES-2b		8.15–11.23	BNL2961–NAU1295	10.15	3.48	0.9373	0.0831	5.0	1.9
	qFS-$F_{2:3}$-BES-2b		10.15–12.07	BNL2961–NAU3562	11.01	14.8	1.2720	0.1178	11.2	2.0
	qFS-$F_{2:3}$-BES-3b	*qFS-3*	4.00–9.15	NAU1037–BNL1521	8.15	13.4	1.16225	0.0987	10.5	2.0
7TR-214 × TM-1(Pop C)	qFS-F_2-JES-1c	*qFS-1*	0.40–3.56	NAU5379–BNL3474	2.71	8.36	0.9651	0.4745	6.3	2.0
	qFS-$F_{2:3}$-JES-1c		0.40–3.25	NAU5379–NAU2926	2.71	2.52	0.8238	0.1834	4.2	1.8
	qFS-$F_{2:3}$-BES-1c		0.40–2..71	NAU5379–TMD05	1.71	12.46	1.0629	0.0091	9.4	2.1
	qFS-F_2-JES-2c	*qFS-4*	3.72–6.13	BNL3474–NAU1322	5.41	8.64	1.0219	0.4054	6.5	2.0
	qFS-$F_{2:3}$-BES-2c		3.72–6.13	BNL3474–NAU1322	5.01	12.08	0.7562	0.0461	8.0	2.1
	qFS-F_2-JES-3c	*qFS-5*	7.06–10.06	NAU3988–NAU3954	8.26	7.54	0.9562	0.3248	5.7	2.0
	qFS-$F_{2:3}$-BES-3c		7.47–9.42	NAU3769–NAU5335	8.42	11.48	0.6340	0.0332	9.8	2.1

[a] Peak position in centiMorgans

[b] Maximum LOD

[c] Additive effect, a positive value indicates the genotype from the P1 parent (7TR-132/7TR-133/7TR-214) toward increase the value, a negative value indicates the genotype from the p2 parent (TM-1) toward increase the trait value

[d] Dominance effect

[e] Percentage of phenotypic variation explained by the QTL

[f] The significant LOD threshold for the trait at $P = 0.05$ determined by 1,000 permutation test

present research was decreased, most likely due to the large populations, the RIL backcrossed method and a randomized incomplete block design, might eliminate the influence of the blocks. Such clustered QTL for fiber qualities and yield have been reported in Upland Cotton (Ulloa et al. 2000; Yin et al. 2002).

Skewed segregation ratios have frequently been reported in cotton (Ulloa et al. 2002, 2005; Lacape et al. 2003; Mei et al. 2004). Furthermore, high segregation distortion frequency (49–80%) has been observed in inter-specific crosses, most likely due to divergence between species (Paterson et al. 1988). The SSR markers on Chro.8D for (7235 × TM-1) RIL all exhibited skewed distribution patterns (Shen et al. 2007). In the present research, 55.6–72.7% of the markers in the three genetic maps constructed using TM-1 as recurrent parent were skewed. Furthermore, the frequency of distorted ratios was high, but greatly decreased from 100% in (7235 × TM-1) RIL (Shen et al. 2007). The higher distorted segregation ratio further reflects 7235 introgression from *G. anomalum* (B-genome). 7235 was developed via a cross between *G. hirsutum* cv. 86-1 and *G. anomalum*, followed backcrossing to Xumian 6, Acala 3080 (a *G. barbadense* introgressed line), and PD4381 (a *G. thurberi* introgressed line) (Qian et al. 1992). Therefore, distorted markers clustered on Chro.D8 may further indicate that this chromosome contains *G. anomalum* and *G. barbadense* introgressed segments, which contribute DNA and/or QTL for fiber qualities.

From a series of QTL tagging experiments using parents 7235 and TM-1, we found 5-clustered QTL on Chro.D8 were major stable QTL. The major fiber strength QTL located on Chro.D8 was used effectively to pyramid QTL by MAS (Guo et al. 2005). Future fine positioning of the clustered QTL for fiber strength, cloning, and sequencing of this region will aid in the investigation of the genetic mechanisms of high fiber strength QTL.

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References

Benedict CR, Kohel RJ, Lewis HL (1999) Cotton fiber quality. In: Smith WC (ed) Cotton: origin, history, technology, and production. Wiley, New York, pp 269–288

Deussen H (1992) Improved cotton fiber properties: the textile industry's key to success in global competition. In: Proc cotton fiber cellulose: structure, function and utilization conference. Memphis, National Cotton Council of America, Tennessee, pp 43–64

Guo WZ, Zhang TZ, Ding YZ, Zhu YC, Shen XL, Zhu XF (2005) Molecular marker assisted selection and pyramiding of two QTL for fiber strength in Upland cotton. Acta Genetic Sinica 32(12):1275–1285

Guo WZ, Cai CP, Wang CP, Han ZG, Song XL, Wang K, Niu XW, Wang C, Lu KY, Ben S, Zhang TZ (2007) A microsatellite-based, gene-rich linkage map reveals genome structure, function and evolution in *Gossypium*. Genetics 176:527–541

Han ZG, Guo WZ, Song XL, Zhang TZ (2004) EST derived microsatellites from diploid A-genome *Gossypium arboreum* and their genetic mapping in AD allotetraploid cotton. Mol Gen Genet 272:308–327

Han ZG, Wang CB, Song XL, Guo WZ, Guo JY, Li CH, Chen XY, Zhang TZ (2006) Characteristics, development and mapping of *Gossypium hirsutum* derived EST-SSR in allotetraploid cotton. Theor Appl Genet 112:430–439

Jiang CX, Wright RJ, El-Zik KM, Paterson AH (1998) Polyploid formation created unique avenues for response to selection in *Gossypium* (cotton). Proc Natl Acad Sci USA 95:4419–4424

Kohel RJ, Richmond TR, Lewis CF (1970) Texas marker-1. Description of a genetic standard for *Gossypium hirsutum* L. Crop Sci 10:670–671

Kohel RJ, Yu J, Park YH, Lazo GR (2001) Molecular mapping and characterization of traits controlling fiber quality in cotton. Euphytica 121:163–172

Lacape JM, Nguyen TB, Thibivilliers S, Bojinnov TB, Courtois B, Cantrell RG, Burr B, Hau B (2003) A combined RFLP-SSR-AFLP map of tetraploid cotton based on a *Gossypium hirsutum* × *Gossypium barbadense* backcross population. Genome 46:612–626

Lacape JM, Nguyen TB, Courtois B, Belot JL, Giband M, Gourlot JP, Gawryziak G, Roques S, Hau B (2005) QTL analysis of cotton fiber quality using multiple *Gossypium hirsutum* × *Gossypium barbadense* backcross generation. Crop Sci 45:123–140

McCouch SR, Cho YG, Yano PE, Blinstrub M, Morishima H, Kinoshita T (1997) Report on QTL nomenclature. Rice Genet Newslett 14:11–13

Mei M, Syed NH, Gao W, Thaxton PM, Smith CW, Stelly DM, Chen ZJ (2004) Genetic mapping and QTL analysis of fiber-related traits in cotton (*Gossypium*). Theor Appl Genet 108:280–291

Park YH, Alabadt MS, Ulloa M, Sicker B, Wilkins TA, Yu J, Stelly DM, Kohel RJ, El-Shihy OM, Cantrell RG (2005) Genetic mapping of new cotton fiber loci using EST-derived microsatellites in an interspecific recombinant inbred line cotton population. Mol Genet Genomics 274:428–441

Paterson AH, Lander SM, Hewitt JD, Peterson S, Lincoln SE, Tanksely SD (1988) Resolution of quantitative traits into Mendelian factors by using a com plete linkage map of restriction fragment length polymorphism. Nature 335:721–726

Paterson AH, Brubaker CL, Wendel JF (1993) A rapid method for extraction of cotton (*Gossypium* spp.) genomic DNA suitable for RFLP or PCR analysis. Plant Mol Biol Rep 11(2):122–127

Paterson AH, Saranga Y, Menz M, Jiang CX, Wright RJ (2003) QTL analysis of genotype × environment interaction affecting cotton fiber quality. Theor Appl Genet 106:384–396

Qian SY, Huang JQ, Peng YT, Zhou BL, Ying MC, Shen DZ, Liu GL, Hu TX, Xu YJ, Gu LM, Ni WC, Chen S (1992) Studies on the hybrid of *G. hirsutum* L. and *G. anomalum* Wawr. & Peyr. and application in breeding. Sci Agric Sinica 25:44–51

SAS institute (1989) SAS/STAT user's guide version 6, 4th edn. SAS Institute, Cary

Shappley ZW, Jenkins JN, Zhu J, McCarty JC (1998) Quantitative trait loci associated with agronomic and fiber traits of Upland cotton. J Cotton Sci 2:153–163

Shen XL, Guo WZ, Zhu XF, Yuan YL, Yu JZ, Kohel RJ, Zhang TZ (2005) Molecular mapping of QTL for fiber qualities in three diverse lines in Upland cotton using SSR markers. Mol Breed 15:169–181

Shen XL, Guo WZ, Lu QX, Zhu XF, Yuan YL, Zhang TZ (2007) Genetic mapping of quantitative trait loci for fiber quality and yield trait by RIL approach in Upland cotton. Euphytica 155:371–380

Ulloa M, Meredith WR (2000) Genetic linkage map and QTL analysis of agronomic and fiber quality traits in an intraspecific population. J Cotton Sci 4:161–170

Ulloa M, Cantrell RG, Pency RG (2000) QTL analysis of stomatal conductance and relationship to lint yield in an interspecific cotton. J Cotton Sci 4:10–18

Ulloa M, Meredith WR, Shapplet ZW, Kahler AL (2002) RFLP genetic linkage maps from four $F_{2:3}$ population and a joinmap of *Gossypium hirsutum* L. Thero Appl Genet 104:200–208

Ulloa M, Saha S, Jenkin N, Meredith WR, McCarty JC, Stelly DM (2005) Chromosomal assignment of RFLP linkage groups harboring important QTL on an intraspecific cotton (*Gossypium hirsutum* L.) joinmap. J Hered 96:132–144

Van Ooijen JW (2004) MapQTL version 5.0: software for the mapping of quantitative trait loci in experiment population. Plant Research International, Wageningen

Van Ooijen JW, Voorrips RE (2001) Software for the calculation of genetic linkage maps. Plant Research International, Wageningen

Wang CB, Guo WZ, Cai CP, Zhang TZ (2006) Characterization, development and exploitation of EST-derived microsatellites in *Gossypium raimondii*. Ulbrich Chin Sci Bull 51:557–561

Yin JM, Wu YT, Zhang J, Zhang TZ, Guo WZ, Zhu XF (2002) Tagging and mapping of QTL controlling lint yield and yield components in upland cotton (*Gossypium hirsutum* L.) using SSR and RAPD markers. Chin J Biotechnol 18:162–166

Zhang J, Wu YT, Guo WZ, Zhang TZ (2000) Fast screening of SSR markers in cotton with PAGE/silver staining. Cotton Sci Sinica 12:267–269

Zhang J, Guo WZ, Zhang TZ (2002) Molecular linkage map of allotetraploid cotton (*Gossypium hirsutum* L. × *Gossypium barbadense* L.) with a haploid population. Theor Appl Genet 105:1166–1174

Zhang TZ, Yuan YL, Yu J, Guo WZ, Kohel RJ (2003) Molecular tagging of a major QTL for fiber strength in Upland cotton and its marker-assisted selection. Theor Appl Genet 106:262–268