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ORIGINAL PAPER

QTL mapping of yield and fiber traits based on a four-way cross population in *Gossypium hirsutum* L.

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Abstract Four-way cross (4WC) involving four different inbred lines frequently appears in the cotton breeding programs. However, linkage analysis and quantitative trait loci (QTL) mapping with molecular markers in cotton has largely been applied to populations derived from a cross between two inbred lines, and few results of QTL dissection were conducted in a 4WC population. In this study, an attempt was made to construct a linkage map and identify QTL for yield and fiber quality traits in 4WC derived from four different inbred lines in *Gossypium hirsutum* L. A linkage map was constructed with 285 SSR loci and one morphological locus, covering 2113.3 cM, approximately 42% of the total recombination length of the cotton genome. A total of 31 QTL with 5.1–25.8% of the total phenotypic variance explained were detected. Twenty-four common QTL across environments showed high stability, and six QTL were environment-specific. Several genomic segments affecting multiple traits were identified. The advantage of QTL mapping using a 4WC were discussed. This study presents the first example of QTL mapping using a 4WC population in upland cotton. The results presented here will enhance the understanding of the genetic basis of yield and fiber quality traits and enable further marker-assisted selection in cultivar populations in upland cotton.

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Introduction

Cotton is the world's most-utilized natural textile fiber. This genus is comprised of approximately 50 diploid and tetraploid species (Fryxell 1992), and all tetraploid species exhibit disomic chromosome pairing (Kimber 1961). A tetraploid specie, *Gossypium hirsutum* L. ($n = 26$, AD genome), account for 90% of the world's cotton production (Wendel et al. 1992). Current and obsolete cultivars and strains of upland cotton have been and remain the main sources of cotton breeding programs worldwide (Zhang et al. 2005a, b).

The majority of cotton genetic maps have been developed through interspecific hybridization (Reinisch et al. 1994; Yu et al. 1998; Lacape et al. 2003; Nguyen et al. 2004; Rong et al. 2004; Guo et al. 2007), which currently has little used in a conventional breeding program. The intraspecific genetic linkage maps of upland cotton have been developed and used to identify quantitative trait loci (QTL) for agronomy and fiber quality traits (Shappley et al. 1998; Ulloa and Meredith 2000; Zhang et al. 2003, 2005a, b; Shen et al. 2005, 2006; Wang et al. 2006). Due to lower level of within-species DNA sequence polymorphisms in upland cotton (Lu et al. 2005), most intraspecific maps of upland cotton have included only a relatively small portion of the cotton genome, even a joint map from different mapping populations covered 31% of the cotton genome (Ulloa et al. 2005). If a low coverage ratio map was used to QTL mapping, only a small portion of genome was explored, and a large amounts of QTL information could not be revealed.

Linkage analysis and QTL mapping with molecular markers in cotton has mainly been employed in populations derived from single cross of inbred lines. Although the level of DNA sequence polymorphisms is not high in

upland cotton, some sequence variation among more than two lines could be used for molecular markers. Using four parents of a double cross (i.e., four-way cross: 4WC) has been shown to increase the density of genetic maps (Qin and Zhang 2008). In addition, methods have been developed to use 4WC or analogous population with 4WC in QTL mapping (Xu 1996; Van Ooijen 2004). However, few studies of QTL mapping employing a 4WC population have been reported to date (Xu 1996; Rao and Xu 1998).

In this paper, we reported a linkage map developed in a 4WC population derived from four different inbred lines in upland cotton, and the map and progeny families derived from 4WC population were used to detect QTL influencing yield and fiber quality traits.

Materials and methods

Mapping population

In 2004, a mapping population with 280 individuals, was developed from the 4WC Simian3 (SM3)/Sumian12(SuM12)//Zhong4133(ZH4133)/8891 (Fig. 1). The cotton cultivars SM3, SuM12, ZH4133 and strain 8891 are each, respectively, one of the parents of four elite hybrids, including Wanmian13, Nankang3, Zhongza028, and Xiangzamian2. All 280 individuals were self-mated to develop progeny families in 2004. Seven out of 280 individuals lacked enough self-pollinated seeds, and finally, 273 progeny families, known as 4WC families, were developed to evaluate traits in 2005 and 2006.

Trait evaluation

Two-hundred eighty 4WC individuals were grown and evaluated in Jianpu cotton breeding station of Nanjing Agricultural University, Nanjing, China during 2004. In 2005 and 2006, 273 4WC families were grown in one-row plots and evaluated, also in Nanjing. For the 4WC families field trials, a complete randomized block design with three replications and fifteen individuals per replication was employed. Plot size was 0.8 m wide and 5 m long and plant density approximate 37,500 plants ha^{-1}. Measurements of each yield trait from the four mapping parents and 273 4WC families were averaged over three replicates. The following yield traits were evaluated: number of bolls per plant (NB), boll weight (BW), lint percentage (LP), lint index (LI) and seed index (SI). For each plot, seed cotton yield (SCY) was determined as the sum of the total harvest and lint yield (LY) was determined by multiplying lint percentage by total seed cotton weight. Five individuals nearby each other were randomly picked out to evaluate number of bolls per plant (NB). The following fiber quality traits were evaluated by

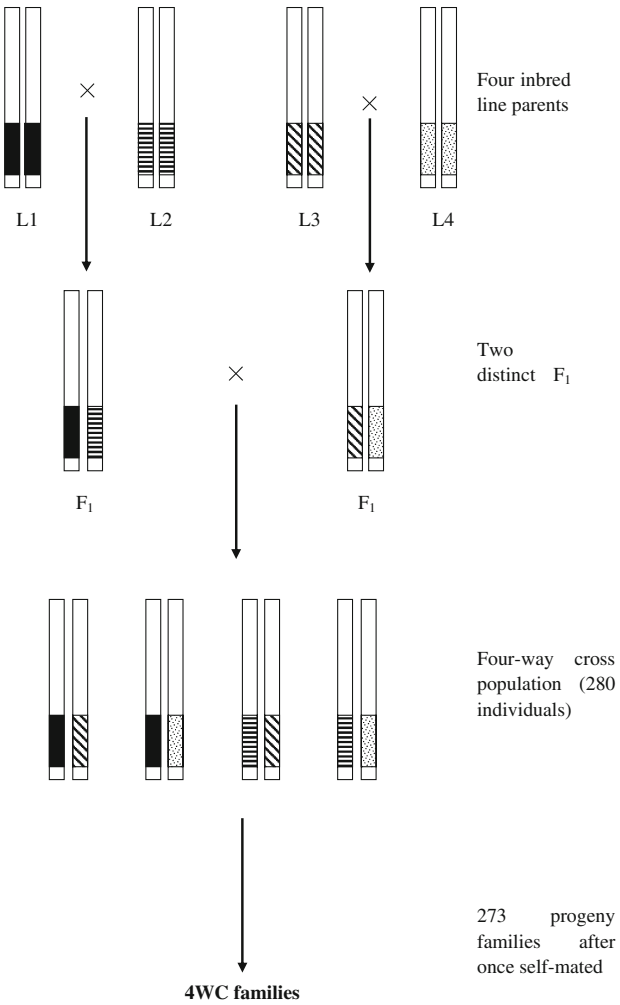


Fig. 1 The production of four-way cross (*4WC*) population and it's progeny families. L_1 SM3, L_2 SuM12, L_3 ZH4133, L_4 8891. *Arrow* indicates that there are four alleles in these four parents

HVI spectrum: 2.5% fiber span length (FL, mm), fiber strength (FS, cN/tex), fiber elongation (FE), micronaire reading (FM), and fiber uniformity ratio (FU).

Data analysis and linkage map construction

DNA was extracted from 280 4WC individuals, two F_1 parents and the four inbred line parents as described by Paterson et al. (1993). To screen for polymorphisms among inbred lines parents, 6,123 simple sequence repeat (SSR) and EST–SSR (eSSR) primer pairs were examined. These SSR and eSSR included BNL, CIR, JSEPR, STV, MUSS, MUCS, NAU, and TM, which were described in deail (Reddy et al. 2001; Nguyen et al. 2004; Han et al. 2004, 2006; Qureshi et al. 2004). Primers sequences can be obtained from Cotton Microsatellite Database (CMD, http://www.cottonmarker.org). Marker nomenclature consisted of a letter that specified the origin of the marker, followed by the primer number. The procedure for SSR

analysis was followed as described by Zhang et al. (2000). In addition, the yellow anther gene, P_1, from 8891 was also surveyed in 4WC individuals and 4WC families.

All SSR primer pairs were used to screen for polymorphisms among SM3, SuM12, ZH4133 and 8891. If one locus screened for polymorphism was homozygous in two of the F_1 parents (*aa_bb*), this locus would be excluded in linkage analysis because the alleles did not segregate in 4WC. The polymorphic markers identified between SM3 and SuM12, or ZH4133 and 8891 were used to survey 280 individuals of 4WC. A Chi-square test for goodness of fit was used to assess Mendelian segregation ratios, including 1:1, 1:2:1, 3:1 and 1:1:1:1 ratios in 4WC.

The genetic configuration of 4WC involving four different inbred lines (L_1, L_2, L_3 and L_4) is similar to that of an outbreed full-sib family. A full-sib family involves a maximum of four alleles (G1, G2, G3, and G4) at each locus. Each parent can be considered as a pseudo F_1 individual and thus a full-sib family is a pseudo 4WC. The only difference between a real 4WC and a regular full-sib family is that the grandparents of a full-sib family cannot be assumed to be inbred (Knott et al. 1997). As a result, the linkage phases of marker loci in the parents of a full-sib family can be ambiguous. Given marker genotypes of a three-generation pedigree, the linkage phases of parents may be inferred fairly accurately. Upon substitution of the true phases by the inferred phases, linkage analysis and QTL mapping in a full-sib family is not different from a 4WC (Rao and Xu 1998). Therefore, the methods of linkage analysis and QTL mapping in a full-sib family of an outbreeding plant species can be applied to a 4WC population without any modification.

JoinMap 3.0 (Van Ooijen and Voorrips 2001) was employed to construct linkage map. Population code was CP, a population resulting from a cross between two distinct heterozygous parents. The recombination frequency was converted to genetic map distance (Morgan, M) using the Kosambi mapping function (Kosambi 1944). Log-of-odds (LOD) scores ≥ 4 were used to determine all linkage groups.

Linkage groups were assigned to chromosomes based on anchored markers in our dense linkage map (Han et al. 2004; Guo et al. 2007). When no subgenome inference was available, the linkage group was designated LGXX, where LG denoted ''linkage group'', and XX referred to its serial number.

QTL analysis was carried out using the program MapQTL 5.0 (Van Ooijen 2004). The implemented QTL mapping procedure is a maximum likelihood approach to the segregation of a mixture of probability distributions. Under the hypothesis a single QTL is segregating, the mixture consists of four distributions. QTL mapping was conducted with four phenotype data sets: two from single environments (2005 and 2006), one from that incorporated the means of two years environments (combined analysis) and one from 4WC individuals. The first three sets of phenotypes are 4WC families. The significance thresholds for LOD scores were calculated by permutation tests in MapQTL 5.0, with a genome-wide significance level of $\alpha < 0.05$, $n = 1{,}000$ as significant QTL and a linkage group-wide significance level of $\alpha < 0.05$, $n = 1{,}000$ as suggestive QTL. The linkage-group specific LODs thresholds are lower than the genome-wide LODs and dependent on marker density (Van Ooijen 1999). QTL position indicated location of the peak. Confidence intervals (95%) associated with QTL location is set as the map intervals corresponding to 1 LOD decline either side of the peak. Furthermore, LOD scores value between 2.0 and 3.0 were used to detect suggestive QTL, as suggested by Lander and Kruglyak (1995). QTL for the same trait across different years (environments) or populations were declared as 'common' QTL if their confidence intervals overlapped. For suggestive QTL detected in combined analysis, only those 'common' QTL were presented in our results.

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, etc.) and the name of chromosome, then followed by the number of QTL affecting the trait on the chromosome.

The measurement of trait value for the *j*th plant ($j = 1,\ldots,n$), Y_j, was indicated by the model (Xu 1996)

$$Y_j = \mu + a_1 X_{1j} + a_2 X_{2j} + dX_{3j} + e_j$$

where μ is the overall mean value for the trait; a_1 is the average allele substitution effects of G_2 substituted by G_1, or the additive effects in the first line cross SM3/SuM12; a_2 is the average allele substitution effects of the G_4 substituted by G_3, or the additive effects in the second line cross ZH4133/8891; d is the overall dominance effect; X_{ij} is a dummy variable; and e_j is the error term with $N(0, \sigma_e^2)$. MapQTL 5.0 only provides four QTL's genotypes value information for CP population that can be defined as followed:

$$G_{13} = \mu + a_1 + a_2 + d$$
$$G_{14} = \mu + a_1 - a_2 - d$$
$$G_{23} = \mu - a_1 + a_2 - d$$
$$G_{24} = \mu - a_1 - a_2 + d$$

where G_{ij} is the value of genotype G_iG_j. So the additive and dominance effects of each QTL can be calculated by the following equations (Xu 1996):

$$a_1 = (G_{13} + G_{14} - G_{23} - G_{24})/4$$
$$a_2 = (G_{13} + G_{23} - G_{14} - G_{24})/4$$
$$d = (G_{13} + G_{24} - G_{14} - G_{23})/4$$

Results

Construction of SSR molecular genetic map in upland cotton

Only 5.5% (339/6123) of the SSR primers revealed polymorphic bands between SM3 and SuM12, or between ZH4133 and 8891. In a 4WC, essentially three distinct polymorphism types were identified comprised of two, three and four alleles (Fig. 2). Our results found that 96.5% (330/342) of the polymorphic loci exhibited two alleles, 3.2% (11/342) three alleles (*ab_ac*), and only one locus showed evidence of four alleles (*ab_cd*). Two allele types were identified within 330 polymorphic loci. At 243 loci, one parent possessed a heterozygous genotype (*ab_aa* or *aa_ab*) and at 85 loci, both parents were heterozygous (*ab_ab*).

The linkage map was constructed from 343 loci generated from 339 markers and the P_1 gene. The map was comprised of 56 linkage groups with 286 mapped loci spanning a distance of 0.37–125.0 cM. The 286 loci covered 2113.3 cM, which was estimated as 42% of the total recombination length of the cotton genome (Stelly 1993). The average genomewide distance between two loci was 7.4 cM. Twenty-four linkage groups assigned to the A-subgenome, contained 126 loci, and spanned 808.4 cM. Twenty-seven linkage groups assigned to the D-subgenome, contained 152 loci, and spanned 1231.6 cM. The average distance between loci was 6.4 cM in the A-subgenome and 8.1 cM in the D-subgenome. Five linkage groups failed to be assigned to any chromosome. Of the 286 mapped loci, 54 (18.9%) were skewed. An excess of heterozygotes were observed in 31 of the 54 skewed loci with no alliance to any of the parental genotypes. Segregation distortion was independent of marker segregation type and was not randomly distributed along the genome.

The genetic and correlation analysis of quantitative traits for yield and fiber traits

Mean values, standard deviation, ranges, skewness, and kurtosis for traits measured in the parents and 4WC families for two years of growing season were shown in Table 1. All traits from four data sets exhibited continuous distribution in the 4WC population, and showed a normal distribution, typical of quantitative traits (data of analysis in each year not shown). ANOVA analysis showed that there were significant differences ($P < 0.05$) for all 12 traits among the four parents and in the population (data not shown).

Correlations among yield and fiber traits based upon 4WC family means of two years were given in Table 2, and agree with previous studies (Meredith 1992; Shappley et al. 1998; Ulloa and Meredith 2000). Most traits were correlated with one or more other traits. Lint yield was correlated positively with FE and all yield components traits but SI, and negatively with FL, FS. Lint percentage

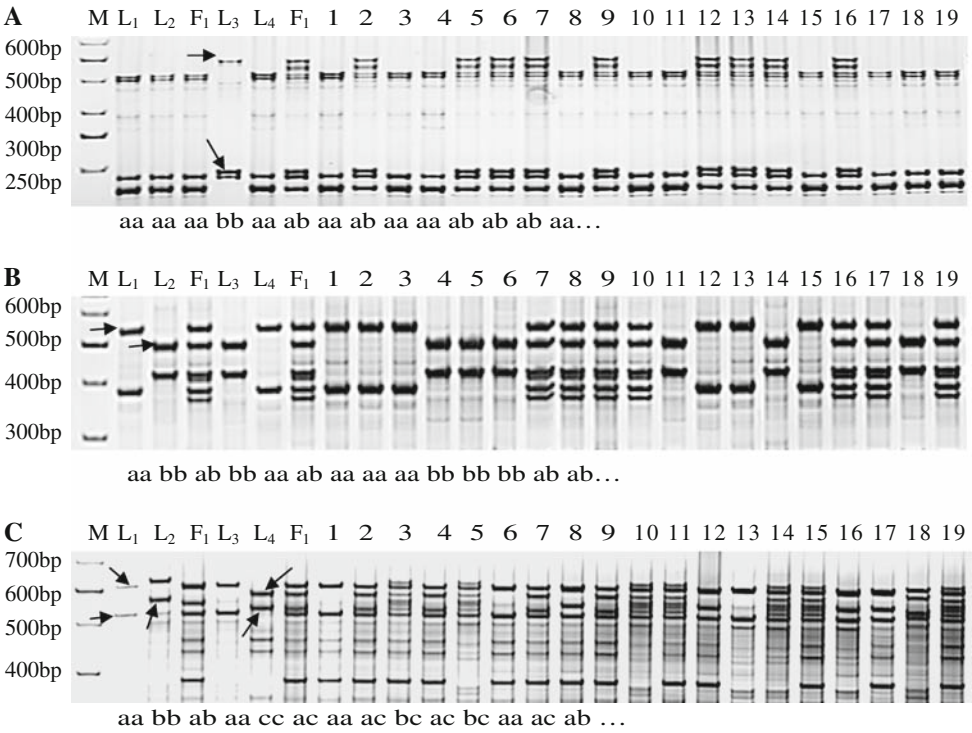


Fig. 2 SSR profile of primer NAU828(*aa_ab*) (**a**), primer NAU2820(*ab_ab*) (**b**) and primer NAU3499(*ab_ac*) (**c**) in 4WC. In **a**, **b** and **c**, *Lane* L_1, L_2, F_1, L_3, L_4 and F_1 were SM3, SuM12, SM3 × SuM12, Zhong4133, 8891 and Zhong4133 × 8891, respectively. *Lanes 1–19* were individuals of 4WC. M, DNA size marker. *Arrows* point the characterized bandings. The scored of four inbred line parents, two F1 parents and 1–8 individuals of 4WC were shown underneath the gel images

Table 1 Phenotypic variation of 15 traits involved yield and fiber quality in 273 four-way cross families and the four parent lines

Traits[a]	Parent lines				Four-way cross families				
	SM3	SuM12	ZH4133	8891	Minimum	Mean	Maximum	Skewness	Kurtosis
NB	26.40 ± 0.64	23.80 ± 1.24	20.70 ± 1.32	19.90 ± 1.63	13.85	18.06 ± 1.67	23.61	0.23	−0.04
BW	4.83 ± 0.13	4.89 ± 0.12	5.06 ± 0.14	5.70 ± 0.19	4.58	5.15 ± 0.26	5.95	0.19	−0.14
LP(%)	42.8 ± 0.46	41.3 ± 0.8	34.8 ± 0.82	42.8 ± 0.33	35.4	40.6 ± 1.5	44.4	−0.09	−0.15
LI	7.46 ± 0.22	7.04 ± 0.23	5.07 ± 0.26	8.43 ± 0.30	5.78	6.88 ± 0.41	7.87	−0.03	−0.09
SI	10.03 ± 0.30	10.02 ± 0.22	9.55 ± 0.25	11.27 ± 0.13	8.38	9.63 ± 0.43	10.90	0.14	0.06
SCY	49.5 ± 3.9	57.0 ± 4.83	50.0 ± 1.65	57.8 ± 4.47	40.0	59.2 ± 6.4	76.7	0.03	0.22
LY	21.3 ± 1.9	23.6 ± 2.08	16.5 ± 1.32	24.5 ± 2.71	15.8	25.4 ± 3.0	34.4	0.07	0.39
FL	29.97 ± 0.42	28.60 ± 0.39	31.56 ± 0.38	27.79 ± 0.39	27.53	29.39 ± 0.74	31.46	−0.02	−0.33
FS	30.97 ± 0.96	29.20 ± 0.88	34.25 ± 0.18	29.99 ± 0.72	28.13	31.36 ± 1.19	34.57	0.11	−0.25
FM	5.32 ± 0.11	4.51 ± 0.29	4.63 ± 0.14	5.24 ± 0.17	4.62	5.18 ± 0.22	5.83	0.02	0.05
FU(%)	84.37 ± 0.46	82.51 ± 0.64	82.87 ± 0.63	84.27 ± 0.65	81.98	83.79 ± 0.61	85.47	−0.19	0.03
FE(%)	5.94 ± 0.24	6.24 ± 0.25	5.05 ± 0.09	6.07 ± 0.21	4.85	5.64 ± 0.31	6.48	−0.00	−0.17

[a] Number of bolls per plant (*NB*), boll weight (*BW*), seedindex (*SI*), lint percent (*LP*), lintindex (*LI*), seed cotton yield per plant (*SCY*), lint yield per plant (*LY*), fiber length (*FL*), fiber strength (*FS*), micronaire reading (*FM*) fiberelongation (*FE*) and fiber uniformity ratio (*FU*)

Table 2 Correlation coefficients among all traits involved yield and fiber quality in 273 four-way cross families

Traits[a]	NB	BW	LP	SI	LI	SCY	LY	FL	FS	FM	FU
BW	−0.13†										
LP	0.06	−0.03									
SI	−0.13†	0.47‡	−0.44‡								
LI	−0.01	0.29‡	0.66‡	0.15†							
SCY	0.57‡	0.05	0.11†	0.05	0.17‡						
LY	0.55‡	0.03	0.36‡	−0.11†	0.33‡	0.90‡					
FL	0.02	−0.09	−0.5‡	0.22‡	−0.41‡	−0.06	−0.20‡				
FS	−0.10†	0.11‡	−0.43‡	0.34‡	−0.22‡	−0.10†	−0.21‡	0.49‡			
FM	0.00	0.14†	0.29‡	0.09	0.36‡	0.12†	−0.21‡	−0.45‡	0.04		
FU	−0.06	−0.06	−0.18‡	0.19‡	−0.06	−0.04	−0.08	0.26‡	0.62‡	0.14†	
FE	0.04	−0.11	0.39‡	−0.30‡	0.24‡	0.02	0.16‡	−0.51‡	−0.74‡	0.07	−0.3‡

†,‡ Indicate that the correlation is significant at 0.05 and 0.01 probability levels, respectively

[a] See Table 1 for abbreviations

was correlated with most yield and all fiber traits, and negatively with FL, FS, positively with FM. Among five fiber traits, significantly correlation was observed between all trait pairs except for FM and FS, FM and FE.

We also evaluated narrow-sense heritability (h^2) (Table 3) for our own data by performing 4WC family/4WC regressions (Smith and Kinmam 1965). All measures of fiber quality showed moderately high and highly significant heritability, ranging from 0.20 to 0.44, generally consistent with the literatures. The four yield components traits, BW, LP, LI, SI, also showed moderately high and highly significant heritability, ranging from 0.18 to 0.47. The narrow-sense heritability of two yield traits SCY, LY, and yield components traits NB were low (in fact nonsignificant heritability), and were shown to be a characteristic highly influenced by the experimental environment and difficult to select. In addition, when dealing with multiple crossings, it should be remembered that these estimates may be influenced by non-additive variances (Cahaner and Hillel 1980; Burton 1987), and/or interactions existing in population that supposedly are not in Hardy-Weinberg equilibrium (Luís and Natal 2004).

QTL mapping of yield traits and fiber qualities

A total of 31 QTL with 5.1–25.8% of the total phenotypic variance explained were detected in combined analysis. Location and confidence interval were shown in Fig. 3. A

Table 3 Estimates of heritability for yield and fiber quality traits using four-way cross (4WC) family/4WC regression

Cases	Traits[a]											
	NB	BW	LP	LI	SI	SCY	LY	FL	FS	FM	FU	FE
2005	−0.11	0.18†	0.32‡	0.26‡	0.34‡	−0.17	−0.03	0.40‡	0.42‡	0.34‡	0.22‡	0.33‡
2006	0.01	0.20‡	0.47‡	0.25‡	0.22‡	0.03	0.03	0.44‡	0.26‡	0.20‡	0.27‡	0.37‡
Mean	−0.03	0.22‡	0.42‡	0.29‡	0.28‡	−0.02	0.03	0.41‡	0.36‡	0.27‡	0.26‡	0.30‡

†,‡ Indicate that the regression is significant at 0.01 and 0.001 probability levels, respectively

[a] See Table 1 for abbreviations

summary of all QTL detected in combined analysis, including the position and LOD score, the mean value of four different genotypes, percentage of the phenotypic variance explained by the QTL (PVE), additive effects of a_1 and a_2 and overall dominance effects d was shown in Table 4. The summary of QTL detected more than one trial was shown in Table 5.

For NB, LP, SI and LY, a total of six significant QTL detected in combined analysis were also detected in the analysis of both environments (in 2005 and 2006) as significant or suggestive QTL. For BW, LI, SCY and LY, five suggestive QTL detected in combined analysis were also detected in the analysis of one (or both) environments as suggestive QTL except for *qLY-A6-1* and *qSCY-A6-1*, which were detected as significant QTL in 2006 with LOD score 4.6 and 4.3 but not detected even as suggestive QTL in 2005. Nine common QTL detected with almost same position and same direction as a_1, a_2 and d except for a_2 of *qBW-D2-1*(in 2006) and a_2 of *qSI-D3-1*(in 2005). In all QTL involved in LP, LI and SI, five with positive a_1 meant SM3 contributed alleles leading to an increase in these traits. Three QTL with minus a_1 indicated SuM12 contributed three alleles leading to an increase in LP, SCY and LY. ZH4133 contributed seven alleles leading to an increase in NB, BW, LP, LI, SCY and LY, which was suggested by that seven QTL involved these traits were detected with positive a_2. 8891 contributed three alleles leading to an increase in LI and SI (with minus a_2).

For all five fiber quality traits, a total of nine significant QTL and 11 suggestive QTL were detected in combined analysis. Out of them, 15 were detected as common QTL in the analysis of both environments(in 2005 and 2006) with almost same position and same direction as a_1, a_2 and d except for a_1 of *qFS-D13-1*(in 2006), d of *qFL-D13-1* and *qFS-A10-1*. Four were detected as significant QTL in one environment (2005 or 2006) with LOD score 4.1, 4.8, 5.2 and 4.4 and not in another environment. One suggestive QTL, *qFL-A11-1* was also detected as *qFL-A10-2* by Wang (2006). In all 20 QTL, 16 and 12 alleles leading to an increase in all five fiber quality traits conferred by SM3 (positive a_1) and ZH4133 (positive a_2), respectively. SuM12 (minus a_1) contributed five alleles leading to an increase in FL, FM and FE. 8891 (minus a_2) contributed seven alleles leading to an increase in FL, FS, FM and FE.

Stability of QTL

Twenty-four QTL were detected across environments (Table 5). Twelve of the twenty-four common QTL were also detected with data collected from 4WC individuals. These results suggested these QTL had little interaction with the environment and showed high stability, therefore might be of value for a MAS program.

Six QTL were detected across different populations with common markers. *qSI-D2-1* and *qLP-D2-1* were also detected in a RILs population derived from the F_2 of SM3 and Carmen with single marker analysis (Zhang et al. 2005); *qFL-D2-1*, *qFM-D2-1* and *qFL-A11-1*were detected in a RILs population derived from the F_2 of XZM2 (Wang et al. 2006), and *qFL-D9-1* was detected in a RIL population derived from the F_2 of 7235 and TM-1 (Shen et al. 2006). We had tried to compare the QTL in the present report with those in previous studies conducted by other research groups, but it seemed very difficult, because a few common markers existed between their data and ours.

Six QTL, *qLY-A6-1*, *qSCY-A6-1*, *qFM-D2-1*, *qFM-D4-1*, *qFM-A5-1*, and *qFS-A11-1* were detected as significant QTL only in one year with high LOD score, but not detected even as suggestive QTL in another year. The results implied a significant interaction with the environment and they may be difficult to reproduce.

Discussion

Four-way crosses are important in their own right

The 4WC involves four inbred lines (L_1, L_2, L_3 and L_4). The polymorphism markers identified between L_1, L_2, or L_3 and L_4 can be employed to develop a genetic linkage map. If only two parents were employed to mapping, in our results, about 37% of all polymorphic markers (a half of aa_ab) would be homogeneous and could not be used to

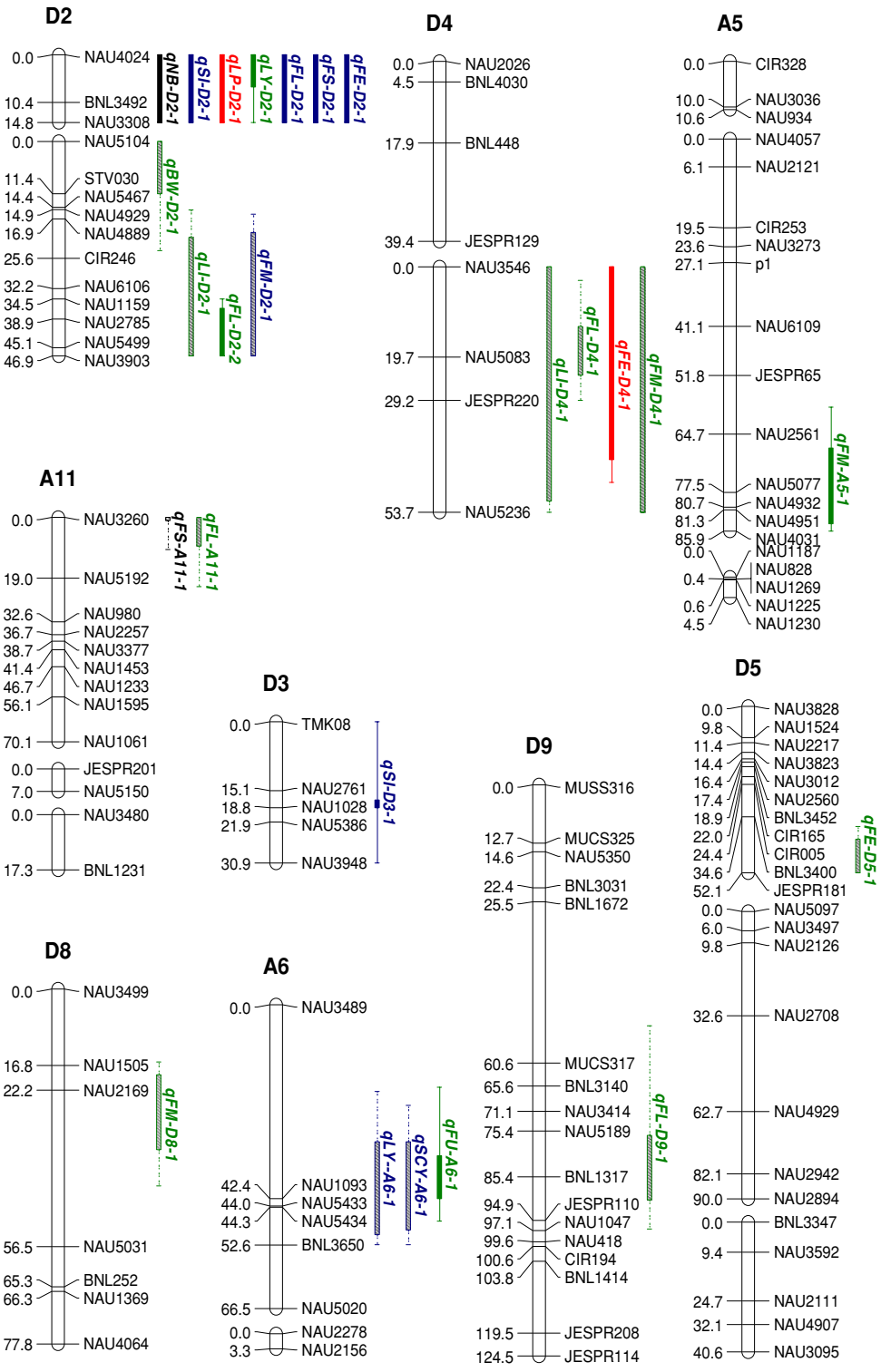
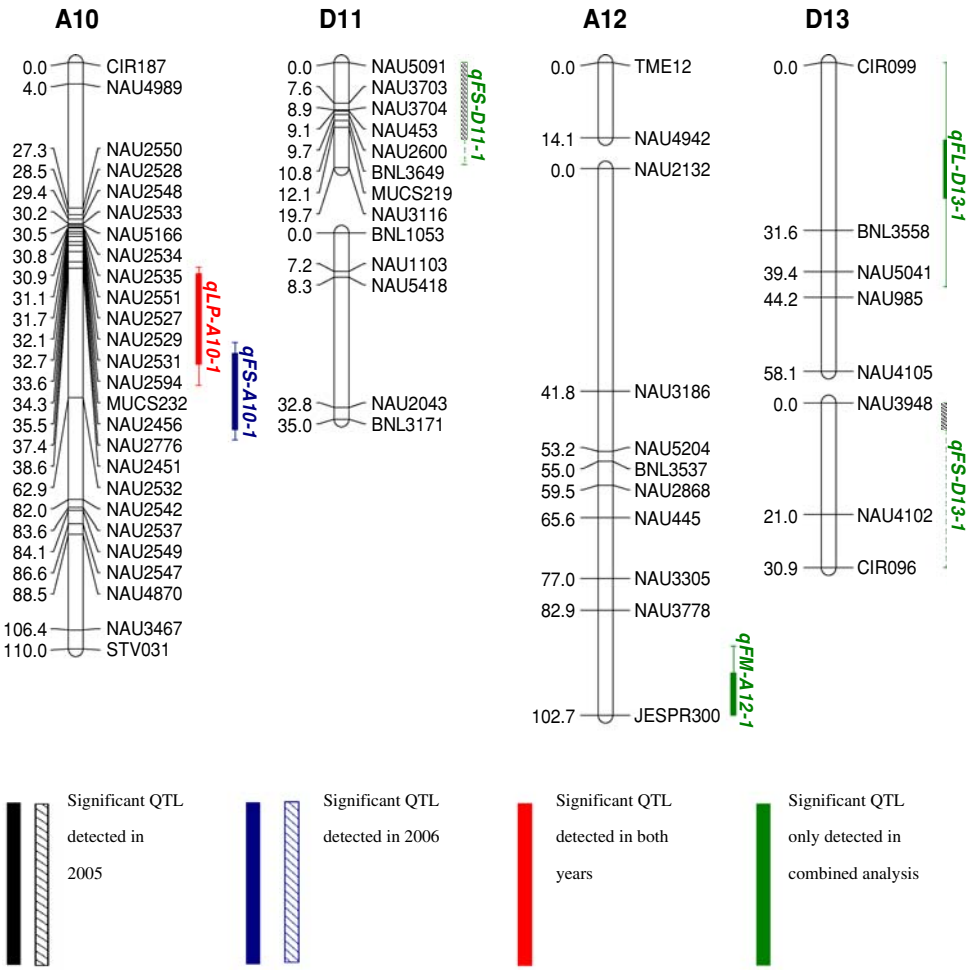


Fig. 3 Location for QTL (detected in combined analysis) associated with yield and fiber quality traits in the population derived from the 4WC of SM3/SuM12//ZH4133/8891 in upland cotton (*Gossypium hirsutum* L.). Positions of loci are given in centi-Morgan. *Bars and lines* indicate 1 LOD (tenfold) and 2 LOD (100-fold) likelihood intervals. The *solid Bars and lines* indicate significant QTL, and empty *Bars and dashed* suggestive QTL. Thirty-one QTL are shown as number of bolls per plant (*NB*), boll weight (*BW*), seedindex (*SI*), lint percent (*LP*), lintindex (*LI*), seed cotton yield per plant (*SCY*), lint yield per plant (*LY*), fiber length (*FL*), fiber strength (*FS*), micronaire reading (*FM*), fiber elongation (*FE*), and fiber uniformity ratio (*FU*)

linkage analysis. Therefore, utilizing a 4WC can increase the density and coverage of the linkage map and, to some degree, counteract the lower levels of polymorphism in upland cotton. Wang et al. (2006) had constructed an intraspecific linkage map based on XZM2-derived recombinant inbred lines. They detected 122 polymorphic loci with 4106 SSR primer pairs. Plus 26 polymorphic loci from AFLP, RAPD, SRAP and P_1 gene, they developed a map of 132 loci covered 865.20 cM, which is approximately 18.57% of the total length of the cotton genome. Compared with what can be achieved in single cross populations, our map doubles coverage of cotton genome. In addition, we

Fig. 3 continued



have also compared the map with a dense map derived from interspecific (Guo et al. 2007), our map was good collinear with dense map as other linkage maps (Shen et al. 2005; Wang et al. 2006) from single cross populations (data not shown). The results suggested 4WC was useful to develop linkage map in upland cotton characterized as low polymorphism in DNA level.

For QTL mapping, some advantages in using 4WC over a simple line cross have been discussed by Xu (1996). A 4WC $(L_1 \times L_2) \times (L_3 \times L_4)$ is analogous to a two-way ANOVA experiment. It provides three tests simultaneously: one for QTL segregation between L_1 and L_2, one for segregation between L_3 and L_4, and one for the interaction (dominance) effects. In contrast, a backcross population $(L_1 \times L_2) \times L_1$ or $(L_3 \times L_4) \times L_3$ is analogous to a one-way ANOVA which can only test segregation between $L_1$and L_2, or between L_3 and L_4. If alleles of one QTL was heterogeneous between $L_1$and L_2 and homogeneous between L_3 and L_4, alleles of another QTL was homogeneous between $L_1$and L_2 and heterogeneous between L_3 and L_4, only one QTL would have been detected when one single cross population derived from $L_1 \times L_2$ or $L_3 \times L_4$ had been used for mapping. The other QTL are not detectable because the parent chosen for mapping happens to be homozygous for this locus. Therefore, using 4WC can potentially reduce the type II error caused by a random sampling of parents and increase the probability of detecting QTL if they segregate in one line cross but not in the other (Xu 1996). In our results, for *qFM-A12-1*, the value of G_{13} was 5.12, equal to G_{14}, and the value of G_{23} was 5.25, equal to G_{24}, which indicated alleles of *qFM-A12-1* was homogeneous between P3(zhong4133) and P4(8891). If a single cross population derived from P3 (zhong4133) and P4 (8891) had been used for mapping, the QTL of *qFM-A12-1* is not detectable. Other QTL, such as *qNB-D2-1*, *qBW-D2-1* and *qFE-D4-1*, the d and one of two additive effects, a_1 or a_2, were not zero, but nearly equal to zero and their magnitude were far less than that of the other additive effect, we presume that allele of those QTL were also only heterogeneous in one F_1 parent and is homogeneous in another F_1 parent. If one single cross population was employed for mapping, only some of those QTL should be detected and another should be not.

Table 4 Summary of the location and the effects of QTL using interval mapping method in combined analysis

Group	QTL[a]	Position	Nearest marker	LOD	LOD[b]	LOD[c]	G_{13}[d]	G_{14}^{d}	G_{23}^{d}	G_{24}^{d}	PVE(%)[e]	a_1^{f}	a_2 [g]	d[h]
D2(1)	*qNB-D2-1*	12.4	BNL3492	4.85*	4.2	2.2	17.49	17.56	18.64	18.55	10.20	−0.53	0.01	−0.04
	qSI-D2-1	14.8	NAU3308	6.15*	4.1	2.2	9.69	9.79	9.42	9.63	10.00	0.11	−0.08	0.03
	qLP-D2-1	13.4	NAU3308	8.04*	4.1	2.4	40.01	40.26	41.61	40.58	14.40	−0. 48	0. 19	−0. 32
	qLY-D2 − 1	4.0	NAU4024	4.16*	4.1	2.4	25.33	24.37	26.73	25.22	8.00	−0.56	0.62	−0.13
	qFL-D2 - 1	14.8	NAU3308	5.75*	4.1	2.3	29.53	29.67	29.07	29.30	9.70	0.21	−0.09	0.02
	qFS-D2 - 1	14.8	NAU3308	8.12*	4.2	2.2	31.75	31.78	30.69	31.19	14.40	0.41	−0.13	0.11
	qFE-D2 - 1	14.8	NAU3308	6.64*	4.1	2.2	5.57	5.52	5.79	5.69	11.70	−0.10	0.04	−0.02
D2(2)	*qBW-D2 - 1*	3.0	NAU5104	3.54	4.2	2.7	5.24	5.05	5.21	5.09	9.60	0.00	0.08	0.02
	qLI-D2 - 1	46.9	NAU3903	3.88	4.1	2.8	6.93	6.88	6.97	6.68	6.80	0.04	0.09	−0.06
	qFL-D2-2	25.6	CIR246	3.08	4.1	2.7	29.24	29.41	29.30	29.72	5.10	−0.09	−0.15	0.06
	qFM-D2-1	28.6	CIR246	3.59	4.1	2.7	5.22	5.15	5.23	5.08	6.80	0.01	0.06	−0.02
D3	*qSI-D3-1*	18.1	NAU1028	4.33*	4.1	2.4	9.78	9.66	9.45	9.66	7.40	0.08	−0.02	0.08
D4(2)	*qLI-D4-1*	35.2	JESPR220	3.83	4.1	2.3	6.78	7.07	6.76	6.86	9.90	0.06	−0.10	−0.05
	qFL-D4-1	19.7	NAU5083	2.95	4.1	2.4	29.46	29.14	29.40	29.64	6.10	−0.11	0.02	0.14
	qFM-D4-1	15.0	NAU5083	2.94	4.1	1.8	5.18	5.27	5.14	5.12	7.30	0.05	−0.02	−0.11
	qFE-D4-1	4.0	NAU3546	10.17*	4.1	2.5	5.50	5.80	5.52	5.75	18.90	0.01	−0.13	−0.02
D5(1)	*qFE-D5-1*	47.6	JESPR181	3.48	4.1	2.1	5.71	5.76	5.63	5.49	10.90	0.09	0.02	−0.05
A5(2)	*qFM-A5-1*	77.5	NAU5077	4.63*	4.1	2.7	5.27	5.15	5.13	5.14	8.00	0.04	0.03	0.03
A6(1)	*qSCY-A6-1*	44.0	NAU5433	3.99	4.1	2.8	56.78	58.48	62.67	58.87	10.70	−1.57	0.53	−1.37
	qLY-A6-1	44.0	NAU5433	2.91	4.1	2.1	24.54	25.08	26.93	25.12	8.50	−0.61	0.32	−0.59
	qFU-A6-1	38.0	NAU1093	4.16*	4.1	2.4	83.87	84.00	83.44	83.80	11.90	0.83	0.22	0.52
D8	*qFM-D8-1*	22.2	NAU2169	3.34	4.1	2.1	5.18	5.10	5.25	5.21	5.60	−0.04	0.03	0.01
D9	*qFL-D9-1*	85.4	BNL1317	3.16	4.1	2.2	29.62	29.33	29.45	29.16	5.20	0.08	0.15	0.00
A10	*qLP-A10-1*	48.6	NAU2451	4.97*	4.1	2.6	41.34	40.50	39.68	40.39	14.50	0. 44	0. 03	0. 39
	qFS-A10-1	61.6	NAU2532	5.28*	4.2	2.7	30.72	32.24	31.62	31.06	23.90	0.07	−0.24	−0.52
A11(1)	*qFL-A11-1*	0.0	NAU3260	2.42	4.1	2.2	29.43	29.66	29.54	28.94	13.80	0.15	0.09	−0.21
	qFS-A11-1	0.0	NAU3260	2.64	4.2	2.6	30.79	32.13	31.71	30.72	25.80	0.12	−0.09	−0.58
D11(1)	*qFS-D11-1*	0.0	NAU5091	2.58	4.2	2.1	31.57	31.32	31.58	30.90	5.00	0.10	0.23	−0.11
A12(2)	*qFM-A12-1*	98.7	JESPR300	4.42*	4.1	2.2	5.12	5.12	5.25	5.25	8.80	−0.07	0.00	0.00
D13(1)	*qFL-D13-1*	20.6	BNL3558	4.12*	4.1	2.5	29.66	29.34	29.50	29.05	10.30	0.11	0.19	−0.03
D13(2)	*qFS-D13-1*	9.0	NAU3948	3.91	4.2	2.7	31.87	31.13	31.42	30.87	9.70	0.18	0.32	0.05

* Significant QTL

[a] See Table 1 for abbreviations

[b,c] The genome-wide and linkage group LOD significance thresholds by permutation test

[d] The mean value of genotype

[e] Percentage phenotypic variation explained

[f,g] a_1, a_2-the additive (or average allele substitution) effects

[h] The overall dominance effects

In contrast to a simple line cross in which only two alleles are involved, a 4WC can have a maximum of four alleles. Because of this, the additive and dominance effects in a 4WC are defined differently from a simple cross to accommodate different inbred cross designs (Xu 1996; Rao and Xu 1998). When only two different alleles exist among four inbred parents, the additive and dominance effects of alleles have common mean with that of alleles identified in a single cross population. If allele of one parent differs from other three parents on one locus, a 4WC population is analogous to a conventional BC population. According to the mixture model, one of two additive effects, a_1 or a_2, and d would be zero. The effects of QTL would have common mean with that of identified in BC population. Such as those QTL mentioned previous, *qFM-A12-1*, *qNB-D2-1*, *qBW-D2-1* and *qFE-D4-1*. If alleles were heterozygous in both F_1 parents, a 4WC population is analogous to a conventional F_2 population. The effects of QTL would

Table 5 Summary of QTL detected in more than one trial

QTL	Group	Position			LOD			LOD[d]			a_1[e]			a_2[f]			d[g]		
		04[a]	05[b]	06[c]	04[a]	05[b]	06[c]	04[a]	05[b]	06[c]	04[a]	05[b]	06[c]	04[a]	05[b]	06[c]	04[a]	05[b]	06[c]
qNB-D2-1	D2(1)	ND	12.4	2.0	ND	2.2	3.9	ND	2.2	2.2	ND	−0.48	−0.53	ND	0.03	0.09	ND	−0.07	−0.05
qBW-D2 -1	D2(2)	ND	0.0	14.9	ND	3.3	2.8	ND	2.8	2.7	ND	−0.02	0.00	ND	0.10	0.06	ND	0.01	0.01
qSI-D2-1	D2(1)	3.0	14.8	14.8	3.6	3.2	7.7*	2.2	2.2	2.2	0.15	0.11	0.10	−0.16	−0.03	−0.12	0.10	0.00	0.08
qSI-D3-1	D3	ND	18.8	3.0	ND	3.5	4.1*	ND	2.4	2.4	ND	0.07	0.13	ND	0.02	−0.06	ND	0.10	0.09
qLP-D2-1	D2(1)	3.0	14.4	14.4	4.0	10.0*	4.2*	2.3	2.3	2.3	−0.28	−0.53	−0.45	0.28	0.36	0.07	−0.39	−0.50	−0.16
qLP-A10-1	A10	44.6	44.6	48.6	4.6	4.5*	4.5*	2.8	2.8	2.8	0.36	0.45	0.50	0.22	0.09	−0.13	0.38	0.44	0.30
qLI-D2-1	D2(2)	46.9	46.9	46.9	1.9	3.1	3.0	2.7	2.8	2.7	0.01	0.05	0.03	0.06	0.11	0.06	−0.10	−0.03	−0.08
qLI-D4-1	D4(2)	ND	32.2	39.2	ND	2.8	2.9	ND	2.3	2.2	ND	0.05	0.08	ND	−0.09	−0.10	ND	−0.08	−0.07
qLY-D2-1	D2(1)	ND	7.0	0.0	ND	2.3	2.4	ND	2.2	2.2	ND	−0.52	−0.62	ND	0.44	0.85	ND	−0.27	−0.04
qFL-D2 -1	D2(1)	0.0	14.8	14.8	6.8	3.5	6.1*	2.2	2.2	2.2	0.30	0.21	0.20	−0.29	−0.06	−0.13	−0.07	−0.04	−0.01
qFL-D2 -2	D2(2)	14.4	25.6	25.6	2.1	2.7	2.8	2.7	2.7	2.6	0.17	−0.12	−0.07	−0.13	−0.15	−0.14	0.00	0.08	0.04
qFL-D4 -1	D4(2)	53.7	50.2	53.7	3.1	2.4	3.4	2.3	2.3	2.2	−0.05	−0.01	−0.23	0.22	0.06	0.11	0.28	0.37	0.11
qFL-D9 -1	D9	85.4	85.4	85.4	3.9	2.6	2.5	2.4	2.2	2.2	0.05	0.08	0.08	0.29	0.16	0.14	−0.05	0.00	0.00
qFL-D13-1	D13(1)	ND	58.1	37.5	ND	3.5	3.3	ND	2.5	2.5	ND	0.08	0.12	ND	0.31	0.17	ND	0.09	−0.06
qFS-D2 -1	D2(1)	0.0	14.8	14.8	5.2	2.2	10.8*	2.4	2.2	2.2	0.65	0.26	0.55	−0.63	−0.06	−0.22	0.02	0.02	0.21
qFS-A10-1	A10	ND	62.9	62.9	ND	3.5	4.5*	ND	2.8	2.8	ND	0.16	−0.06	ND	−0.21	−0.22	ND	−0.37	−0.63
qFS-D11-1	D11(1)	ND	0.0	7.6	ND	2.1	2.4	ND	2.1	2.1	ND	0.05	0.15	ND	0.25	0.26	ND	−0.04	−0.08
qFS-D13-1	D13(2)	ND	10.0	8.0	ND	2.8	3.5	ND	2.5	2.1	ND	0.30	0.08	ND	0.28	0.38	ND	−0.09	0.14
qFM-D8 -1	D8	ND	22.2	27.2	ND	3.1	3.5	ND	2.1	2.1	ND	−0.05	−0.04	ND	0.01	0.07	ND	0.02	0.00
qFM-A12-1	A12(2)	ND	0.0	4.0	ND	3.6	3.3	ND	2.1	2.1	ND	−0.06	−0.07	ND	0.00	0.00	ND	0.00	0.00
qFE-D2 -1	D2(1)	14.4	14.8	14.8	7.1	3.3	9.0*	2.3	2.2	2.2	−0.13	−0.10	−0.10	0.09	0.01	0.06	−0.05	−0.01	−0.02
qFE-D4 -1	D4(2)	5.0	0.0	9.0	3.2	11.6*	4.9*	2.7	2.5	2.5	0.02	0.00	0.02	−0.12	−0.16	−0.10	−0.04	−0.03	−0.01
qFE-D5 -1	D5(1)	ND	47.6	48.6	ND	2.8	2.7	ND	2.7	2.7	ND	0.10	0.07	ND	0.01	0.03	ND	−0.05	−0.03
qFU-A6 -1	A6(1)	42.4	29.0	42.4	2.8	3.8	3.1	2.6	2.6	2.6	0.25	0.20	0.15	−0.16	−0.21	−0.05	0.12	0.22	0.00

ND not detected

* Significant QTL

[a–c] Separate analysis with data derived from F_2 individuals in 2004(04), $F_{2:3}$ lines in 2005(05) and 2006(06)

[d] The linkage group wide LOD significance threshold by permutation test

[e,f] a_1,a_2-the additive (or average allele substitution) effects

[g] The overall dominance effects values with underline detected with different direction from that in other environments (years)

have common mean with that of identified in F_2 population. The absolute value of two additive effects, a_1 and a_2 would be same. *qSI-D2-1*, *qLY-D2-1*, *qFM-A5-1*, *qFM-D8-1* and *qFS-A11-1* might be classified to this type. In addition, the genetic construction also has the potential to dissect multiple alleles derived from different inbred lines. In our results, those QTL with larger magnitude of *d*, and the absolute value of two additive effects, a_1 and a_2, were evidently different, may be comprise multiple alleles although we can not confirm it with enough evidence.

Traditional selection methods have low efficiency and the cotton germplasm possesses a narrow genetic base, therefore cultivar improvement in cotton has slowed in the USA over the last 10–15 years (Zhang et al. 2005a, b). The same situation now exists in China. To pyramid traits distributed in different lines, 4WC were often employed in breeding programs. In fact, a great deal of the varieties released in China is derived from 4WC hybrid breeding programs (Huang 1996). If one QTL is identified in one segregating population and that result is applied to MAS in another breeding population, the efficiency of MAS is often greatly reduced because of the issue of epistasis. Therefore, the direct application of information of dissecting QTL in breeding populations, especially to multiple cross breeding populations, is very helpful to select "ideal genotype" or "best combination of alleles" with the assistance of markers associated with QTL. QTL mapping in a 4WC population can provide more direct and applicable information for MAS in a 4WC breeding program of cotton.

Molecular mechanism of trait correlation and linkage drag—QTL clusters

The phenomenon of QTL cluster has previously been reported in cotton (Shappley et al. 1998; Ulloa and Meredith 2000; Ulloa et al. 2005; Mei et al. 2004; Wang et al. 2006) and in many other organisms (Lin et al. 1995; Chen et al. 1999; Peng et al. 2003). This phenomenon was also present in our results. Several QTL that influence yield, components of yield and the quality of cotton fiber were detected in the same genomic regions. A total of six intervals were found to be involved in the control of two or more yield or fiber traits and located on D2(1), D2(2), D4(2), A6(1), A10 and A11(1). The universal existence of QTL clusters explains why so many traits were highly correlated each other. Likewise, QTL clusters explain why linkage drag is often observed in breeding practice aiming at improvement on fiber quality. In the NAU3308–NAU4024 interval located on D2(1), *qFL-D2-1*, and *qFS-D2-1* influenced fiber quality and shared the same direction of additive effects as a_1, a_2; *qSCY-D2-1*, *qLY-D2-1* and *qNB-D2-1* influenced yield and also shared the same direction of additive effects as a_1, a_2. In regards to the additive effects a_1 or a_2, the QTL influencing fiber quality and the QTL influencing yield, both located on the NAU3308-NAU4024 interval are said to operate in opposite directions. That is to say, in the NAU3308-NAU4024 interval, one inbred parent line contains some QTL that increase yield, and the other QTL decreases fiber quality. QTL influencing yield and fiber quality traits with the opposite effects direction can also be seen in other map intervals harbored QTL cluster.

The cause of QTL cluster has been discussed by Ulloa et al. (2005) and Wang et al. (2006). The NAU3308–NAU4024 interval on D2(1) harbored seven significant QTL and influenced different traits including LY, NB, SI, LP, FL, FS and FE. Especially, all those QTL were detected in different environments, and some were detected across generation and populations. Considering the high correlation between some traits, coupling tight linkage and pleiotropy could better explain the data (Peng et al. 2003). In-depth evidence for that need to provide by QTL analysis based on more saturated genetic map.

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