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ORIGINAL PAPER

SSR marker-assisted improvement of fiber qualities in *Gossypium hirsutum* using *G. barbadense* introgression lines

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Abstract

Key message **This study demonstrates the first practical use of CSILs for the transfer of fiber quality QTLs into Upland cotton cultivars using SSR markers without detrimentally affecting desirable agronomic characteristics.**

Abstract *Gossypium hirsutum* is characterized by its high lint production and medium fiber quality compared to extra-long staple cotton *G. barbadense*. Transferring valuable traits or genes from *G. barbadense* into *G. hirsutum* is a promising but challenging approach through a traditional interspecific introgression strategy. We developed one set of chromosome segment introgression lines (CSILs), where TM-1, the genetic standard in *G. hirsutum*, was used as the recipient parent and the long staple cotton *G. barbadense* cv. Hai7124 was used as the donor parent by molecular marker-assisted selection (MAS). Among them, four CSILs, IL040-A4-1, IL080-D6-1, IL088-A7-3 and IL019-A2-6, found to be associated with superior fiber qualities including fiber length, strength and fineness QTL in Xinjiang were selected and backcrossed, and transferred these QTLs into three commercial Upland cotton cultivars such as Xinluzao (XLZ) 26, 41 and 42 grown in Xinjiang. By backcrossing and self-pollinating twice, five improved lines (3262-4, 3389-2, 3326-3, 3380-4 and 3426-5) were developed by MAS of background and introgressed segments. In diverse field trials, these QTLs consistently and significantly offered additive effects on the target phenotype. Furthermore, we also pyramided two segments from different CSILs (IL080-D6-1 and IL019-A2-6) into cultivar 0768 to accelerate breeding process purposefully with MAS. The improved lines pyramided by these two introgressed segments showed significant additive epistatic effects in four separate field trials. No significant alteration in yield components was observed in these modified lines. In summary, we first report that these CSILs have great potential to improve fiber qualities in Upland cotton MAS breeding programs.

Abbreviations

RIL	Recombinant inbred line
DH	Doubled haploid
SSR	Simple sequence repeat
QTL	Quantitative trait locus/loci
CSIL	Chromosome segment introgression line
MAB	Marker-assisted backcross
BC	Backcross

Introduction

Conventional breeding approaches that strive to improve fiber quality through interspecific introgression have been hindered by complex antagonistic genetic relationships between important fiber and agronomic traits (Culp et al. 1979). *Gossypium hirsutum* is characterized by high lint

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production (>90 %) and medium fiber quality compared to extra-long staple cotton *G. barbadense*. *G. barbadense* is far less desirable relative to *G. hirsutum* because of low yield and its limited adaptation to conditions in most cotton growing areas. Interspecific introgression projects that target essentially any multigenic trait, irrespective of the performance of the donor species, can be used to enhance multigenic traits of economic importance.

Modern molecular quantitative genetics have shown that cotton fiber quality traits are quantitative and are affected dramatically by the complex genetic background (Kohel et al. 2001; Ulloa and Meredith 2000). Nevertheless, the application of molecular marker-assisted selection (MAS) breeding remains scarce, in particular, efforts devoted to better understanding the molecular basis of cotton fiber quality have lagged behind similar studies in other crop species. Still, there are many reports of fiber quality-related quantitative trait loci (QTL) from studies of *G. hirsutum* × *G. barbadense* crosses determined using different populations (F_2, BC and RIL) (Jiang et al. 1998; Kohel et al. 2001; Paterson et al. 2003, Rong et al. 2007). However, it is difficult to verify the repeatability and stability of these QTLs. Chromosome segment introgression lines (CSILs) are extremely useful in reducing the complexity of polygenic traits as these separate the loci underlying all agronomically important traits into a set of monogenic loci, and the 'immortal' lines containing interesting QTLs can be phenotyped repeatedly (Eshed and Zamir 1996, Peleman and Voort 2003). Therefore, CSILs are ideal for genome research and for MAS breeding programs. It has become an important method for permitting and characterizing bidirectional target region exchanges between *G. hirsutum* and *G. barbadense*. The heritability of cotton fiber properties is moderate to high (Meredith and Bridge 1984; May 1999), indicating that these traits can be manipulated in early segregating generations.

One hundred and seventy-four CSILs containing 298 introgression segments were identified to understand the genetics underlying fiber qualities in extra-long staple *G. barbadense* (Wang et al. 2012). We chose four CSILs showing superior fiber qualities in Xinjiang as the donor parent and three extra-early Upland cotton cultivars (*G. hirsutum*) being grown in this area as the recurrent parents. The first and second backcrosses to the recurrent parent were used for QTL detection, and the target genotype individuals were identified using simple sequence repeat (SSR) markers linked to specific target QTL in the BC_2S_1 populations. The results obtained in this present study demonstrate that these CSILs related to superior fiber quality properties can make a significant contribution to cotton breeding under field trial conditions in four and/or five distinct environments. The present study reports for the first time a maker-assisted breeding strategy that combines the genetic information for cotton with marker-assisted backcrossing using CSILs in cotton.

Materials and methods

Plant materials and crossing scheme

Among 174 CSILs in the genetic background of TM-1 (Wang et al. 2012), four, IL080-D6-1, IL088-A7-3, IL040-A4-1 and IL019-A2-6 giving superior fiber quality performance in Xinjiang, were selected as the donor parents to improve the fiber qualities of Upland cotton cultivars grown in this area. The cultivars Xinluzao (XLZ) 26, XLZ41, XLZ42 and 0768 were used as recurrent parents as these varieties are used extensively in Xinjiang.

Molecular marker analysis

DNA was isolated using the cetyl trimethyl ammonium bromide method. Amplified products were resolved using 10 % non-denaturing polyacrylamide gel electrophoresis. DNA fragments were visualized by staining with silver nitrate (Wang et al. 2012). For selection of the target introgressed region (foreground), SSR markers known to be linked to major QTLs were used (Wang et al. 2012) and these can be found in supplementary Table 1.

Foreground selection

Four CSILs (donor parents) were crossed with four cultivars (recipients) to obtain F_1 seeds (Fig. 1). F_1 plants were backcrossed twice with the corresponding recurrent parent

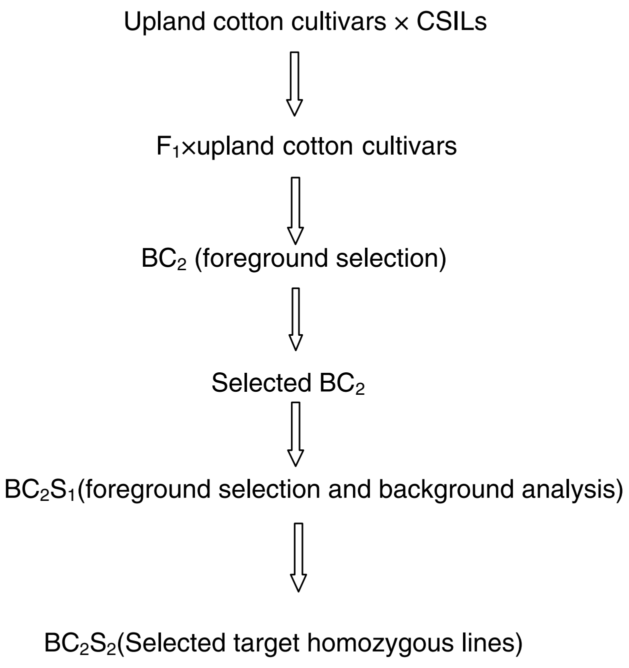

Fig. 1 Development of the target genotype line

to obtain a large number of BC_2 seeds, and these were then self-pollinated to produce homozygous lines. In each backcross generation (BC_n), the target region was monitored by SSR markers linked to the QTLs (supplementary Table 1). F_1s were backcrossed with the corresponding recipient parent to produce about 30 BC_1 plants. Approximately 7–14 BC_1 individuals that were heterozygous at the target locus were identified by anchored SSRs and used to backcross with the corresponding recipient parent. This generated 40 BC_2 plants for reducing the population size for further crossing (foreground selection). Moreover, 10–17 BC_2 plants from different crosses and identified to be heterozygous in the introgressed target region were selfed to produce BC_2S_1 seeds. Individually planted BC_2S_1 plants were screened for the homozygous Hai7124 introgressed segments for subsequent field trials (supplementary Figure 1).

Background selection

To identify improved lines with greatest genetic similarity with the recipient parent, 630 SSR markers with an average intermarker distance of 5 ~ 10 cM and evenly spaced across the 26 chromosomes of Upland cotton were selected based on an earlier linkage map (Guo et al. 2008). These markers were used to screen for polymorphisms between TM-1 and the four recurrent parents (Table 1). The polymorphic markers were used to conduct background screening of the BC_2S_1 plants to identify the greatest genetic background similarity (GBS) compared to the recipient. The genetic composition of the target genotype plants from the various levels of the CSILs was calculated using the cotton genetic standard TM-1 (as the recurrent parent) and the long staple cotton Hai7124 (as the donor parent introgression), and according to the formula $GBS = N/S \times 100\ \%$ (Zhao et al. 2010), where S is the number of polymorphic markers between the donor parent and recipient parent and N is the number of monomorphic markers between the selected plants and the recurrent parent.

Field evaluation and data analysis

BC_2S_1 populations were grown in Sanya (Hainan province, the southern cotton growing region of China) during 2009. During 2010, the agronomic performance of fiber yield and quality for the target genotype lines were further evaluated in field trials in Shihezi (Xinjiang, the Northwestern cotton growing region of China) according to a randomized complete block design with two replicates for each target genotype line and corresponding recurrent parent. For the BC_2S_2 families, plot row size was 0.5 m wide and 5 m long (10 m^2/plot) while plant density was approximately 10 plants/m^2. In October 2010, integrating the evaluation of the agronomic performance with molecular marker analysis, we selected one or two elite lines per cross for further field trial evaluation. In the Sanya field trial, the rows were 0.5 m wide and 5 m long (10 m^2 per plot) while plant density was approximately 3 plants/m^2. The April 2011 and 2012 Shihezi field trial were carried out using the same randomized complete block design as for the 2010 Shihezi trial. For the 2012 field trial, a larger plot size (20 m^2 per plot) was used. The field trials were also conducted for improved lines in Nanjing (Jiangsu, the Yangtze River cotton growing region of China) using a row size of 0.5 m wide and 5 m long (10 m^2 per plot) and a plant density of approximately 3 plants/m^2. The Nanjing trial was performed in three successive years (2010–2012).

The following yield traits were scored: number of bolls per plant (NB), boll weight (BW) and lint percentage (LP). For each plot, ten individuals near to each other were randomly picked out to evaluate NB. A 50-boll sample was hand-harvested from the first position bolls near the middle nodes of the plants in each plot. Each sample was weighed

Table 1 Polymorphism of SSR between TM-1 and the recurrent parents, number of BC_2F_2 plants and target genotype plants, and the background recovery percentage

Cross	No. analyzed markers	No. polymorphic SSRs	Percentage of polymorphic SSRs (%)	No. BC_2S_1 plants	No. target genotype plants	Background recovery percentage (%)	
						Mean	Range
IL040-A4-1[a] × XLZ26	630	81	12.9	121	22	73.6	65.7 ~ 85.2
IL 088-A7-3 × XLZ41	630	54	8.5	117	17	83.4	62.9 ~ 92.6
IL040-A4-1 × XLZ42	630	67	10.6	109	18	77.1	62.7 ~ 85.1
IL019-A2-6 × XLZ42	630	67	10.6	116	16	82.1	59.7 ~ 86.6

[a] IL040-A4-1 contained target interval between NAU6003 and NAU3127; IL088-A7-3 contained target interval between NAU3028 and NAU2186, and IL019-A2-6 contained target interval between NAU3149 and NAU2277

and ginned to determine BW and LP, respectively. The following fiber quality traits were evaluated according to the HVI spectrum: 2.5 % fiber span length (FL, mm), fiber strength (FS, cN/tex) and fiber micronaire (FM). ANOVA was conducted and performed using the SAS/STAT software (SAS Institute 1994).

Analysis of variance (ANOVA) was conducted using the SAS/STAT software (SAS Institute 1994). PROC ANOVA was used to analyze the data for a randomized complete block design in one environment, and PROC GLM with genotype as fixed effect and environment as random effect was used to analyze the data in multiple environments. Broad sense heritabilities for the homozygous lines on an entry mean basis were calculated by dividing the genotypic variance by the phenotypic variance (Hallauer and Mirando 1981).

Evaluation of genetic effects

The additive effect was half of the difference between each introgressed line (IL) and the control, and its significance level was determined by comparison of the IL to the control. The epistatic effect was estimated as (control + ILab) − (ILa + ILb) and the percentage epistatic effect was equal to (epistatic effect/control phenotypic value) × 100 % (Eshed and Zamir 1995, 1996).

Results

Microsatellite-assisted backcrossing to improve fiber qualities

To improve the fiber qualities of Upland cultivars grown in Xinjiang, the Northwestern cotton growing region of China, we screened our CSILs in Aksu, Xinjiang during 2009 and 2010. Four CSILs, IL040-A4-1, IL080-D6-1, IL088-A7-3 and IL019-A2-6, were found to be associated consistently and significantly with superior fiber quality QTL including fiber length, strength and fineness in Xinjiang (supplementary Table 1). Heritability for fiber quality and yield traits was high except NB (supplementary Table 2). Therefore, these four CSILs were backcrossed with various commercial Upland cotton cultivars being grown in Xinjiang. The BC_2S_1 plants were individually planted to initiate MAS screening of the homozygous *G. barbadense* Hai7124 introgressed segments (supplementary Figure 1). The number of BC_2S_1 plants and target genotype plants containing the target interval SSR markers that were screened, and the background recovery percentage (mean and range) data are shown in Table 1. Seventeen plants with *G. barbadense* introgressed segments were identified from 117 (IL088-A7-3 × XLZ41) BC_2S_1 individuals using the target region SSR markers (NAU3028 and NAU2186). The background analysis for these homozygous plants using 54 polymorphic markers between TM-1 and XLZ41 showed that the mean recovery percentage was 83.4 %, ranging from 62.9 to 92.6 %.

Sixteen plants with a *G. barbadense* introgressed segment were identified from 116 (IL019-A2-6 × XLZ42) BC_2S_1 individuals using the target region SSR markers (NAU6003 and NAU3127). The background analysis for these homozygous plants using 67 polymorphic markers between TM-1 and XLZ42 showed that the mean recovery percentage was 82.1 % and this ranged from 59.7 to 86.5 %.

Twenty-two plants with a *G. barbadense* introgressed segment were identified from 121 (IL040-A4-1 × XLZ26)BC_2S_1 individuals using the target region SSR markers (NAU3419 and NAU2277). The background analysis for these homozygous plants using 81 polymorphic markers between TM-1 and XLZ 26 showed that the mean recovery percentage was 73.6 % and this ranged between 65.7 and 85.2 %.

Eighteen plants with a *G. barbadense* introgressed segment were identified from 109 individuals in the BC_2S_1 population resulting from the cross of IL040-A4-1 × XLZ42 using the target region SSR markers. The background analysis for these homozygous plants using 67 polymorphic markers between TM-1 and XLZ42 showed that the mean of recovery percentage was 77.1 % and this ranged between 62.7 and 85.1 %.

Diverse field evaluations of the fiber quality-improved lines

By the BC_2S_2 generation, those homozygous plants with high background recovery percentage and best similar to their corresponding recurrent parents were selected for further field trials on the basis of fiber quality tests and yield components. The combined analysis of variance (ANOVA) for the results of four or five field trials confirmed significant genotype effects on fiber length, fiber strength and micronaire value (supplementary Table 3). The combined ANOVA also showed that there is no significant genotype × environment interaction for fiber length, fiber strength and micronaire value except for fiber micronaire in 3326-3 (supplementary Table 3).

In the five environments field trials, the two homozygous lines (3326-3 and 3380-4) selected from the cross of IL088-A7-3 × XLZ41 showed superior fiber qualities and shared 87.0 and 83.3 % similarity to XLZ41, respectively. The 3326-3 and 3380-4 plants had significantly ($p < 0.01$) greater FL (31.94 and 30.11 mm, respectively) and FS (30.70 and 30.08 cN/tex, respectively) values than XLZ41 (29.20 mm and 29.36 cN/tex), but lower FM (4.03 and 4.29, respectively) than XLZ41 (4.72) (Table 2).

Table 2 Mean values for fiber quality and yield traits of superior fiber length lines and the recurrent parents in 4 or 5 environments

Selected lines/controls	FL (mm)	Dif[a]	FS0 (cN/tex)	Dif	FM	Dif
3326-3	31.94 ± 0.22	2.74**	30.70 ± 0.05	1.34**	4.03 ± 0.11	−0.69**
3380-4	30.11 ± 0.64	0.91**	30.08 ± 0.37	0.72**	4.29 ± 0.40	−0.43*
XLZ41	29.20 ± 0.28		29.36 ± 0.23		4.72 ± 0.06	
3426-5	30.48 ± 0.40	1.71**	29.79 ± 0.40	1.75**	3.92 ± 0.21	−0.61**
XLZ42	28.77 ± 0.58		28.04 ± 0.17		4.52 ± 0.13	
3262-4	30.66 ± 0.78	1.75**	30.12 ± 0.78	1.11**	4.73 ± 0.08	0.37**
XLZ26	28.91 ± 0.07		29.01 ± 0.28		4.36 ± 0.03	
3389-2	31.10 ± 0.49	2.31**	29.93 ± 0.28	1.52**	4.27 ± 0.11	−0.40**
XLZ42	28.79 ± 0.07		28.41 ± 0.35		4.67 ± 0.21	

Environments: Sanya (Oct 2009), Shihezi (Apr 2010), Sanya (Oct 2010), Shihezi (Apr 2011) and Shihezi (Apr 2012) are Env1, Env2, Env3, Env4 and Env5, respectively

[a] The difference between each improved homozygous line for *G. barbadense* allele and the corresponding recipient parent

*, ** Significance from the corresponding recipient parent at 0.05 and 0.01 levels, respectively

In the four environments field trials, the homozygous line 3426-5 selected from the cross IL019-A2-6 × XLZ42 showed superior fiber qualities and shared 83.6 % similarity to XLZ42. Compared to XLZ42, the 3426-5 plants had significantly ($p < 0.01$) greater mean FL (30.48 vs 28.77 mm) and FS (29.79 vs 28.04 cN/tex), and significantly ($p < 0.01$) lower mean FM (3.92 vs 4.52) (Table 2).

In the five environments field trials, the homozygous line 3262-4 derived from the cross IL040-A4-1 × XLZ26 showed superior fiber qualities and shared 82.7 % similarity to XLZ26. The 3262-4 line had significantly ($p < 0.01$) greater mean FL (30.12 mm) and FS (30.66 cN/tex) compared to XLZ26 (29.01 mm in FL and 28.91 cN/tex in FS),and significantly ($p < 0.01$) greater mean FM (4.73) than XLZ26 (4.36) (Table 2). In addition, the homozygous line 3389-2 from the same cross IL040-A4-1 × XLZ42 showed superior fiber qualities and shared 83.6 % similarity to XLZ42. Moreover, the 3389-2 plants had significantly ($p < 0.01$) greater mean FL (29.93 mm) than XLZ42 (28.41 mm) and FS (31.10 cN/tex) than XLZ26 (28.79 cN/tex), and significantly ($p < 0.01$) lower mean FM (4.27) compared with XLZ42 (4.67) (Table 2). It is clear that this introgressed segment has a consistency effect in improving the fiber qualities in different backgrounds.

The mean BW, LP, BN, seed cotton yield/plot and lint cotton yield/plot of each line did not differ significantly ($p > 0.05$) from values observed for the recurrent parents (data not shown).

Microsatellite-assisted pyramiding of CSILs to improve fiber qualities

(IL080-D6-1 × 0768) F_1 and (IL019-A2-6 × 0768) F_1 progenies were each backcrossed with 0768. In the BC_2 generation, the plants possessing a heterozygous *G. barbadense* introgression chromosome segment from each cross were inter-crossed to pyramid two *G. barbadense* introgression chromosome segments. Then the pyramiding plants were selfed and individually planted for MAS of the segregation plants containing homozygous double *G. barbadense* introgression chromosome segments.

Twelve homozygous lines containing *G. barbadense* introgression chromosome segments from IL080-D6-1, ten homozygous lines containing *G. barbadense* introgression chromosome segments from IL019-A2-6, and eight homozygous lines pyramiding two *G. barbadense* introgression chromosome segments were identified from 200 individual plants from the pyramiding F_2 population. Three improved lines (S1, S2 and D1) were selected on the basis of fiber quality tests and yield parameters, and these contained a *G. barbadense* introgression chromosome segment from IL080-D6-1, or IL019-A2-6, or pyramiding double *G. barbadense* segments from IL080-D6-1 and IL019-A2-6, respectively. Background surveying showed that S1, S2 and D1 shared 82.4, 85.4 and 87.1 % similarity to the 0768 line, respectively. The mean values of fiber quality and yield traits of these improved lines (S1, S2 and D1) and the recurrent parent in the four distinct environments are presented in Table 3. S1, S2 and D1 had significantly ($p < 0.01$) greater mean FLs (31.36, 30.99 and 32.49 mm, respectively) than 0768 (30.43 mm), significantly ($p < 0.01$) greater mean FSs (36.20, 36.60 and 37.25 cN/tex, respectively) compared with 0768 (35.86 cN/tex) and significantly ($p < 0.01$) greater mean FMs (4.32, 4.57 and 4.01, respectively) than 0768 (4.82) (Table 3).

The additive effects on FL, FS and FM of two single segment introgression lines (S1 and S2) and the epistasis effect of one double segment pyramiding line (D1) in the 0768 background are presented in Table 4. The FL mean additive effects in S1 (substitution segment *i*) and S2 (substitution

Table 3 Comparison of fiber quality and yield components of the single segment introgression lines, the two segments pyramiding line and the control during four field trials

Env.	Selected lines/ control	FL (mm)	Dif	FS (cN/tex)	Dif	FM	Dif	Lint (%)	NB	Boll weight (g)
1	S_i	31.07 ± 0.44	0.93^{**}	35.2 ± 0.18	−0.1	4.60 ± 0.04	-0.20^{*}	39.4 ± 1.12	21.3 ± 0.42	5.3 ± 0.17
	S_j	30.73 ± 0.73	0.59^{**}	39.9 ± 0.23	4.6^{**}	4.70 ± 0.26	−0.10	39.1 ± 0.94	20.7 ± 0.22	5.4 ± 0.28
	D_{ij}	32.16 ± 0.21	2.02^{**}	38.6 ± 0.23	3.3^{**}	4.60 ± 0.05	-0.20^{*}	41.1 ± 1.41	21.3 ± 0.51	5.2 ± 0.43
	0768	30.14 ± 0.37		35.3 ± 0.08		4.80 ± 0.11		40.5 ± 1.21	22.1 ± 0.48	5.3 ± 0.10
2	S_i	31.50 ± 0.76	1.07^{**}	38.7 ± 0.45	3.0^{**}	4.30 ± 0.12	-0.40^{**}	39.1 ± 0.72	20.1 ± 0.82	5.4 ± 0.14
	S_j	31.12 ± 0.43	0.69^{**}	36.4 ± 0.50	0.7^{*}	4.30 ± 0.16	-0.40^{**}	38.8 ± 0.79	21.5 ± 0.39	5.3 ± 0.22
	D_{ij}	32.99 ± 0.22	2.56^{**}	39.6 ± 0.52	3.9^{**}	4.10 ± 0.14	-0.60^{**}	39.4 ± 0.83	20.8 ± 0.52	5.5 ± 0.32
	0768	30.43 ± 0.40		35.7 ± 0.85		4.70 ± 0.04		38.8 ± 0.92	21.4 ± 0.76	5.3 ± 0.21
3	S_i	31.37 ± 0.03	1.08^{**}	36.9 ± 0.14	-2.0^{**}	4.41 ± 0.06	-0.98^{**}	39.7 ± 0.84	24.1 ± 0.55	5.3 ± 0.43
	S_j	30.77 ± 0.07	0.48^{**}	35.2 ± 0.75	-3.4^{**}	4.75 ± 0.09	-0.64^{**}	39.7 ± 0.53	23.5 ± 0.74	5.3 ± 0.28
	D_{ij}	32.42 ± 0.11	2.13^{**}	38.90 ± 0.43	0.3^{**}	4.13 ± 0.01	-1.26^{**}	39.9 ± 0.93	22.1 ± 0.92	5.4 ± 0.35
	0768	30.29 ± 1.26		38.61 ± 0.46		5.39 ± 0.04		40.8 ± 0.49	24.5 ± 0.56	5.5 ± 0.29
4	S_i	31.50 ± 0.07	0.64^{**}	34.10 ± 0.06	0.1	3.95 ± 0.21	-0.44^{**}	39.8 ± 1.21	7.4 ± 0.26	5.3 ± 0.21
	S_j	31.32 ± 0.37	0.46^{**}	34.90 ± 0.08	1.0^{**}	4.53 ± 0.04	0.14	40.7 ± 0.84	7.8 ± 0.57	5.8 ± 0.33
	D_{ij}	32.40 ± 0.76	1.58^{**}	31.90 ± 0.43	-2.0^{**}	3.19 ± 0.03	-1.2^{**}	39.7 ± 0.98	7.5 ± 0.62	6.1 ± 0.27
	0768	30.86 ± 0.13		33.90 ± 0.77		4.39 ± 0.02		42.1 ± 1.21	7.7 ± 0.47	5.9 ± 0.39
Mean	S_i	31.36 ± 1.21	0.93^{**}	36.20 ± 0.22	0.34^{*}	4.32 ± 0.64	-0.5^{**}	39.5 ± 0.89	18.2 ± 0.43	5.3 ± 0.21
	S_j	30.99 ± 1.02	0.56^{*}	36.60 ± 0.55	0.74^{**}	4.57 ± 0.73	-0.25^{*}	39.5 ± 1.19	18.3 ± 0.39	5.5 ± 0.17
	D_{ij}	32.49 ± 0.49	2.06^{**}	37.25 ± 0.62	1.39^{**}	4.01 ± 0.76`	-0.81^{**}	40.0 ± 0.83	17.9 ± 0.29	5.6 ± 0.34
	0768	30.43 ± 0.43		35.86 ± 1.11		4.82 ± 0.82		40.6 ± 0.74	18.9 ± 0.87	5.5 ± 0.38

Environments: Nanjing (Apr 2010), Nanjing (Apr 2011), Nanjing (Apr 2012) and Shihezi (Apr 2011) were designated as Env1, Env2, Env3 and Env4, respectively

S_i: single segment introgression lines with introgression segment *i* (NAU3677–NAU1454)

S_j: single segment introgression lines with introgression segment *j* (NAU3419–NAU2277)

D_{ij}: double segment pyramiding line with *i* (NAU3677–NAU1454) and *j* (NAU3419–NAU2277)

segment *j*) were 0.47 and 0.28 mm, respectively. The mean epistatic effect of additive-by-additive interaction on FL, FS and FM between substitution segments *i* and *j* of D1 were 0.59 mm, −0.42 cN/tex and −0.13, respectively. In the four field trials the mean percentage change of the epistatic effect on the FL, FS and FM of 0768 were 3.4, −1.2 and −1.2 %, respectively. The additive effects and additive-by-additive effects of a QTL interfered with each other during the four field trials, and this confirmed that the magnitude and directions of the additive effects and additive-by-additive effects were stable.

Discussion

MAB breeding using CSILs

Traditional plant breeding approaches to improve fiber quality through interspecific introgression have been hindered by complex antagonistic genetic relationships (Culp et al. 1979). Attempts to incorporate genes from *G. barbadense* into Upland cotton generally have not achieved stable introgression of the *G. barbadense* fiber properties (Stephens 1949; McKenzie 1970). Associated with these attempts at introgression have been the poor agronomic qualities of the progeny, distorted segregation, sterility, mote formation, and limited recombination due to incompatibility between the genomes (Reinisch et al. 1994).

CSILs are a series of near-isolines with one or a few homozygous chromosome segments derived from the donor parent, but the rest of the genome is the same as the recipient parent. These lines are ideal for identifying additive and epistatic QTL for FL, FS and FM, and for identifying stable QTL and their corresponding CSILs that can then be used in MAS for improving fiber qualities in cotton varieties. In this present breeding program, a set of interspecific CSILs in the genetic standard Upland cotton line TM-1 were developed by backcrossing and MAS with *G. barbadense* cv. Hai7124. Then these CSILs were adopted as breeding materials to overcome interspecific introgression obstacles. Of these, four CSILs (IL019-D6-1, IL019-A2-6, IL088-A7-3 and IL040-A4-1) containing fiber quality QTLs were

Table 4 Epistasis effects of QTLs for double segments in 0768 ($p < 0.01$)

Traits	Env	a_i (NAU3677–NAU1454)	a_j (NAU3419–NAU2277)	a_{ij}	a_{ij} %
FL (mm)	1	0.47	0.30	0.50	1.7
	2	0.54	0.35	0.80	2.5
	3	0.32	0.23	0.48	1.6
	4	0.54	0.24	0.57	1.9
	Mean	0.47	0.28	0.59	3.4
FS (cN/tex)	1	−0.05	2.30	−1.20	−3.4
	2	1.50	0.35	1.45	−0.8
	3	0.15	0.70	1.15	9.3
	4	0.60	1.00	−4.2	−12.8
	Mean	0.54	0.74	−0.42	−1.2
FM	1	−0.10	−0.05	0.10	2.1
	2	−0.20	−0.20	0.20	4.3
	3	−0.49	−0.32	0.36	6.7
	4	−0.22	0.07	−0.90	−20.5
	Mean	−0.25	−0.13	−0.06	−1.2

a_i: Additive effect of introgression segment *i*

a_j: Additive effect of introgression segment *j*

a_{ij}: Epistatic effect of additive-by-additive interaction between introgression segments *i* and *j*

a_{ij} %: Percentage of the epistatic effect compared to the fiber qualities of 0768

used to improve fiber quality of four Upland cultivars (XLZ 26, XLZ 41, XLZ42 and 0768). The cotton yield and resistance levels of these recipient parents are expected to deliver economic benefits when grown in large areas around Xinjiang (the Northwestern cotton growing region of China).

Classical breeding studies have shown that these properties tend to be moderately to highly heritable (Meredith and Bridge 1972). Previous studies showed that variations in fiber qualities are controlled mainly by minor genes (Wilson and Wilson 1975; Tang et al. 1993; May and Green 1994), and additive effects may play similar roles in other genetic backgrounds (Tanksley and Nelson 1996; Bernacchi et al. 1998). We believe that the *G. barbadense* fiber quality QTLs in the CSILs could help to improve fiber quality of Upland cultivars.

Individuals sharing 100 % similarity with the recurrent parent were expected to be identified in the BC_2S_1 population, but the background analysis showed that there was not one such example detected in the segregating populations. Therefore, it was necessary in the breeding scheme to enlarge the number of individuals in the segregating population to permit the detection of homozygous plants possessing the same genetic background as the recipient parent. In five environment field trials, the fiber qualities of the improved lines were influenced by the growing climate, however fiber quality tests confirmed that these lines in each environment always significantly outperformed the corresponding recurrent parent.

Identifying epistatic interaction of fiber quality QTLs using CSILs

The total genetic effect can be divided into additive, dominant and epistatic effects (Fisher 1915). Mather and Jinks (1971) divided epistatic effects into additive × additive, additive × dominant and dominant × dominant. Epistasis is considered to be an important factor in the genetic composition of quantitative traits (Yu et al. 2002; Zhuang et al. 2002; Jiang et al. 2004; Zhao et al. 2009), and it is necessary to understand the interaction pattern and epistasis effect when using MAS for pyramid breeding (Zhao et al. 2009). In this present study, the IL019-A2-6 and IL080-D6-1 lines were backcrossed with 0768 to pyramid two substitution segments in the 0768 background for improving fiber qualities and for studying gene interactions. It was found that fiber quality QTL pairs had differing effect strengths and acted in opposing directions at each locus, which indicated that the gene pyramiding may have resulted in various interaction patterns, indicating its complex in fiber quality improvement. These results are important for improving fiber qualities by MAS breeding.

The stability of fiber quality QTLs and relationships with other yield traits

Within each environment, fiber quality-related traits are influenced substantially by the conditions in which the plants grown. Nevertheless, the improved lines always outperformed the corresponding recurrent parent line in each environment. Our findings showed that FL and FS tended to correlate positively with each other. These results are consistent with classical quantitative genetic studies (Kloth 1998; May 1999). In the present study, the consecutive field trials demonstrated that the target fiber quality-related traits were consistent across multiple growing seasons and this included no significant changes in yield traits. Thus, our efforts have overcome the unfavorable association between fiber yield and fiber quality traits.

This present study demonstrates the practical use of SSR markers for the transfer of fiber quality QTLs into Upland cotton cultivars (*G. hirsutum* L.) without detrimentally affecting desirable agronomic characteristics, and the magnitude and directions of the additive effects and additive-by-additive effects were found to be stable. In conclusion, the MAB strategy is an effective means for introgression of *G. barbadense* QTLs into a set of popular cultivars.

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Conflict of interest The authors declare that they have no competing interests.

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