

## Intraspecific linkage map construction and QTL mapping of yield and fiber quality of *Gossypium babardense*

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### Abstract

*Gossypium barbadense*, famous for the superior quality of its fibers, is the second most cultivated cotton in the world. In order to determine the genetic basis of its agronomic traits, a linkage map was constructed and QTLs were analyzed. A total of 15,971 markers, including gSSRs, EST-SSRs, SRAPs, and SSCP-SNPs, were used to construct an intraspecific linkage map of *G. barbadense* with 124 F<sub>2</sub> individuals derived from the cross (Hai7124 × 3-79). In the F<sub>2</sub> population, 412 loci showed polymorphism, giving a polymorphic rate of 2.58%. Three hundred and thirty-seven loci were mapped on 52 linkage groups, and 35 groups were assigned to 20 chromosomes. The full length of the linkage map was 2108.34 cM, and the mean distance between adjacent loci was 6.26 cM. Fifty-two loci (12.62%) showed segregation distortion ( $P < 0.01$ ), including 34 loci (65.38%) towards the maternal genotype, 15 (28.85%) towards the paternal genotype, and 3 (5.78%) towards the heterozygote genotype. A total of 33 QTLs for yield and fiber quality traits were detected with three repeats in 2010, including 21 QTLs for yield components and 12 QTLs for fiber quality traits. LOD value ranged from 2.52 to 4.63, and the explanation rates for trait variation ranged from 6.93% to 34.52%. Marker interval of MON\_CGR5376 and NAU2575 was linked to seed cotton weight per boll, lint weight per boll, hundred seeds weight, fiber elongation, and seed index. The main gene action of QTLs was over-dominance.

**Keywords:** Fiber quality trait; *Gossypium babardense*; intraspecific map; QTL mapping; yield trait.

**Abbreviations:** FE\_fiber elongation; FS\_fiber strength; FU\_fiber uniformity; FUHML\_fiber upper-half mean length; HSW\_hundred seeds weight; LI\_lint index; LP\_lint percentage; LW\_lint weight per boll; MV\_micronaire value; SI\_seed index; SW\_seed cotton weight per boll; SRAP\_sequence related amplified polymorphism; SSCP\_single-strand conformation polymorphism.

### Introduction

Cotton is the most important fiber crop in the world, and it plays an irreplaceable role in human lives. Improvements in people's living standards have increased demand for cotton, especially high-quality cotton. Cultivating high-yield varieties with excellent fiber quality is becoming more and more relevant. The most cultivated type of cotton is upland cotton, which accounts for more than 90% of world's cotton production (Wendel et al., 1992). However, in recent years, a bottleneck phenomenon was observed in upland cotton (*G. hirsutum*) (Iqbal et al., 2001). Among many sources of new genes, *G. barbadense* has shown incomparable superiority for its highly desirable fiber properties and strong resistance. The genome of tetraploid cotton is large, about 2.5Gb with 5000cM of genetic distance (Hendrix and Stewart, 2005). There are nearly 5000 molecular markers, producing a map density of 1cM. The development of molecular markers has provided various markers suitable for use in cotton research. These markers have greatly promoted the development of cotton linkage maps. The first RFLP linkage map in cotton was established by Reinisch et al. (1994). Since then, many interspecific maps have been constructed (Ulloa et al., 2000; Rong et al., 2004; Lin et al., 2005; Guo et al., 2007; Yu et al., 2007; Lacape et al., 2009; Yu et al., 2011). However, interspecific introgressions often encountered problems, such as segregation distortion (Jiang et al., 2000), suppression of recombination (Paterson et al., 1990), and linkage drag (Young

and Tanksley, 1998). For this reason, many intraspecific maps of *G. hirsutum* were also constructed (Zhang et al., 2005; Wang et al., 2006; Lin et al., 2009; Zhang et al., 2012). The well-covered intraspecific of *G. hirsutum* contains 978 SSR loci and has a full length of 4184.40 cM. Its mean distance between adjacent loci is 4.30 cM (Zhang et al., 2012). The construction of genetic maps has allowed QTL studies based on these maps. F<sub>2</sub>, F<sub>2:3</sub> and BC populations were widely applied for QTL mapping (Mei et al., 2004; Zhang et al., 2005; Lacape et al., 2005; He et al., 2007). In recent years, more and more recombination inbred lines developed from previous temporary populations have been used for QTL mapping (Chen et al., 2010; Wu et al., 2009; Zhang et al., 2009; Sun et al., 2012; Zhang et al., 2012). Researchers have also compared temporary and permanent populations derived from the same parents (Zhang et al., 2005; Zhang et al., 2009; Sun et al., 2012). Yield components and fiber quality traits are the most researched traits in cotton (He et al., 2007; Chen et al., 2010; Mei et al., 2004; Lacape et al., 2005; Zhang et al., 2008; Zhang et al., 2009; Zhang et al., 2012). Although many QTL studies have been performed on interspecific and intraspecific populations in upland cotton, few have been performed in *G. barbadense*. In order to determine the genetic basis of economic traits of *G. barbadense*, a comprehensive genetic map comprising gSSRs, EST-SSRs, SRAPs, and SSCP-SNPs based on the intraspecific F<sub>2</sub> population derived from the cross of Hai7124 and 3-79 was

**Table 1.** Characteristics of intraspecific map of F<sub>2</sub> population and distribution of segregation distortion (SD) markers on chromosomes.

Chr.	No. LG	Loci	Length	Max distance	Min distance	Mean distance	SD loci
Chr03	1	4	55.46	27.33	9.94	18.49	0
Chr05	1	22	102.08	12.48	0.57	4.64	5
Chr06	1	11	80.34	24.75	2.83	7.3	0
Chr07	1	6	55.6	20.74	5.11	11.12	4
Chr08	2	27	108.47	15.8	0.36	5.42	0
Chr10	1	10	68.4	17.92	1.5	7.6	1
Chr11	3	11	112.41	30.72	4.77	10.22	1
Chr12	1	8	59.64	17.46	1.7	7.46	0
Chr13	2	8	79.31	31.37	6.26	13.22	0
Chr14	1	2	28.38	28.38	28.38	28.38	1
Chr15	4	23	173.44	35.2	2.21	9.13	3
Chr17	3	23	105.81	11.99	0.9	4.6	1
Chr19	2	19	135.41	16.84	1.95	7.13	1
Chr20	1	3	42.61	21.99	20.62	21.3	0
Chr21	3	37	155.73	12.29	0.87	4.21	2
Chr22	1	12	75.53	17.47	0.29	6.29	0
Chr23	2	9	37.77	18.39	2.05	5.4	1
Chr24	2	13	90.22	17.87	6.25	6.94	0
Chr25	1	3	26.5	15.42	11.09	8.83	0
Chr26	2	23	155.34	26.08	0.8	7.4	6
Un	17	63	359.89	34.82	0.25	5.71	6
All	52	337	2108.34	35.2	0.25	6.26	32

“Un” represents the other 26 linkage groups, which were not anchored to any chromosomes.

constructed and used to identify QTLs related to yield and fiber quality as well as their component traits.

## Results

### Polymorphism of markers

A total of 15,971 primer pairs and primer combinations, including 4416 gSSRs, 4096 SRAPs, 1643 EST-SSRs, and 5816 SSCP-SNPs, were used to screen polymorphism of the parents. The polymorphic primers were then genotyped in 124 F<sub>2</sub> individuals, generating 412 polymorphic loci including 165 gSSR loci, 101 SRAP loci, 78 EST-SSR loci, and 68 SSCP-SNP loci. The polymorphic rates were 3.73%, 2.47%, 4.75%, and 1.17%, respectively, and the mean was 2.58%.

### Linkage analysis and map construction

The 412 polymorphic loci were used to construct linkage map, and 337 loci (including 132 gSSRs, 71 SRAPs, 77 EST-SSRs, and 57 SSCP-SNPs) were mapped on 52 linkage groups, and 35 linkage groups assigned to 20 chromosomes (Table 1; Fig. 1). The total length of the linkage map was 2108.34 cM, covering 45.24% of the total length of 4660 cM (Reinisch et al., 1994). Among these groups, the max distance of the adjacent markers was 35.20 cM (Chr15), and the minimum was 0.25 cM (LG08), and the mean distance was 6.26 cM (Table 1). The longest group LG07 (assigned to Chr19) consisted of 15 loci, covering 117.48 cM; and the shortest group LG48 only included 2 loci covering 2.50 cM. In the present map, the At sub-genome contained fewer and shorter loci than the Dt sub-genome. There were 9 chromosomes in the At sub-genome, including 107 polymorphic loci, covering 721.71cM with a mean distance of 6.74 cM. Chr03 (LG42) contained the fewest loci (4 loci). It also had the shortest average length (55.46 cM). Chr08 (LG16/15) contained the most loci (27 loci). However, the longest chromosome was Chr11 (LG29/38/50) at 112.41 cM. There were 11 chromosomes of the Dt sub-genome, including 167 polymorphic loci, covering 1026.74 cM, with a mean distance of 6.15 cM. Chr14 contained the fewest loci (2 loci).

However, the group with the shortest average distance was Chr25 (26.50 cM). Chr21 (LG23/12/13) contained the most loci (37 loci), and the longest chromosome was Chr15 (LG22/41/36/27), which could reach 173.44 cM. In addition to the 35 linkage groups assigned to chromosomes, there were 17 unassigned linkage groups. These consisted of 63 polymorphic loci covering 359.89 cM. The mean distance was 5.71 cM. LG09, which consisted solely of SRAP markers, contained the most loci (20 loci). However, the full length was only 46.98 cM, so the mean distance between adjacent loci was just 2.35 cM.

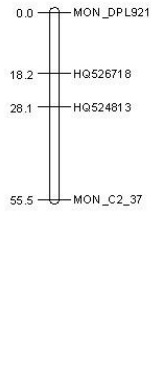
### Characteristics of segregation distortion

For the observed 412 loci, 52 (12.62%) loci deviated from the Mendelian segregation, including 19 gSSRs (11.52%), 15 EST-SSRs (19.23%), 12 SSCP-SNPs (17.65%), and 6 SRAPs (5.94%). Among the 52 distorted segregation loci, 34 (65.38%) skewed towards the “Hai7124” genotype, 15 (28.85%) towards the “3-79” genotype, and 3 (5.78%) towards the F<sub>1</sub> genotype. A total of 32 loci were mapped on the linkage groups including 13 gSSRs, 10 EST-SSRs, 5 SSCP-SNPs, and 4 SRAP loci. In addition, Chr26 contained the most distorted segregation loci (6 loci), at a rate of 26.09%.

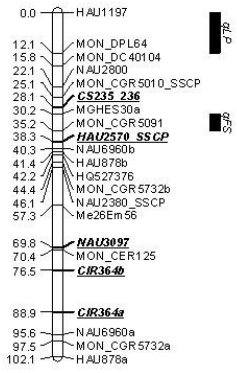
### Correlation analysis among yield and fiber quality traits

In order to determine the correlations among different traits, a Pearson coefficient of yield and fiber quality traits was obtained using SPSS software (Table 2). At a level of significance of 0.05, positive correlations were as follows: LW with HSW, SI and FS; HSW with FUHML; FU with FS. Negative correlations were as follows: LW with FE; LP with FS; FU with FE; MV with FE and FS. At a level of significance of 0.01, positive correlations were as follows: SW with LW, HSW, SI, FUHML and FS; LW with LP; HSW with LI, SI; FUHML with FU and FS. Negative correlations were as follows: SW with FE; LP with FUHML; FUHML with MV and FE; FE with FS.

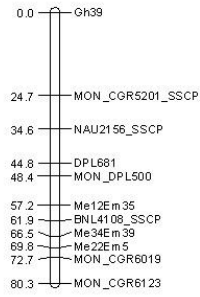
Chr03/LG42



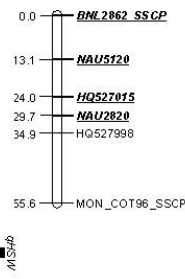
Chr05/LG17



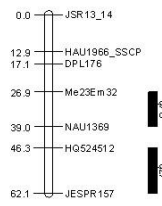
Chr06/LG21



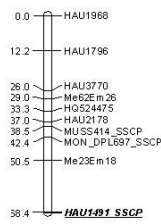
Chr07/LG35



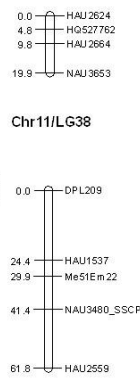
Chr08/LG16



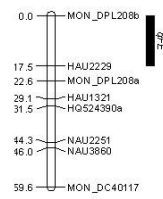
Chr10/LG34



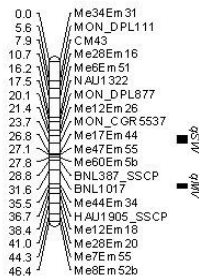
Chr11/LG29



Chr12/LG02



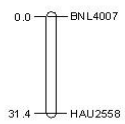
Chr08/LG15



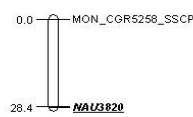
Chr11/LG50



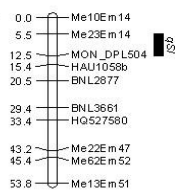
Chr13/LG43



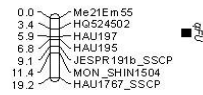
Chr14/LG30



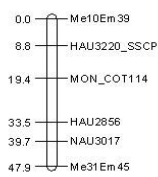
Chr15/LG22



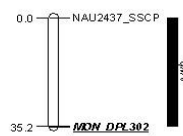
Chr17/LG33



Chr13/LG37



Chr15/LG41



Chr17/LG32

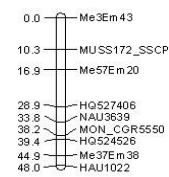
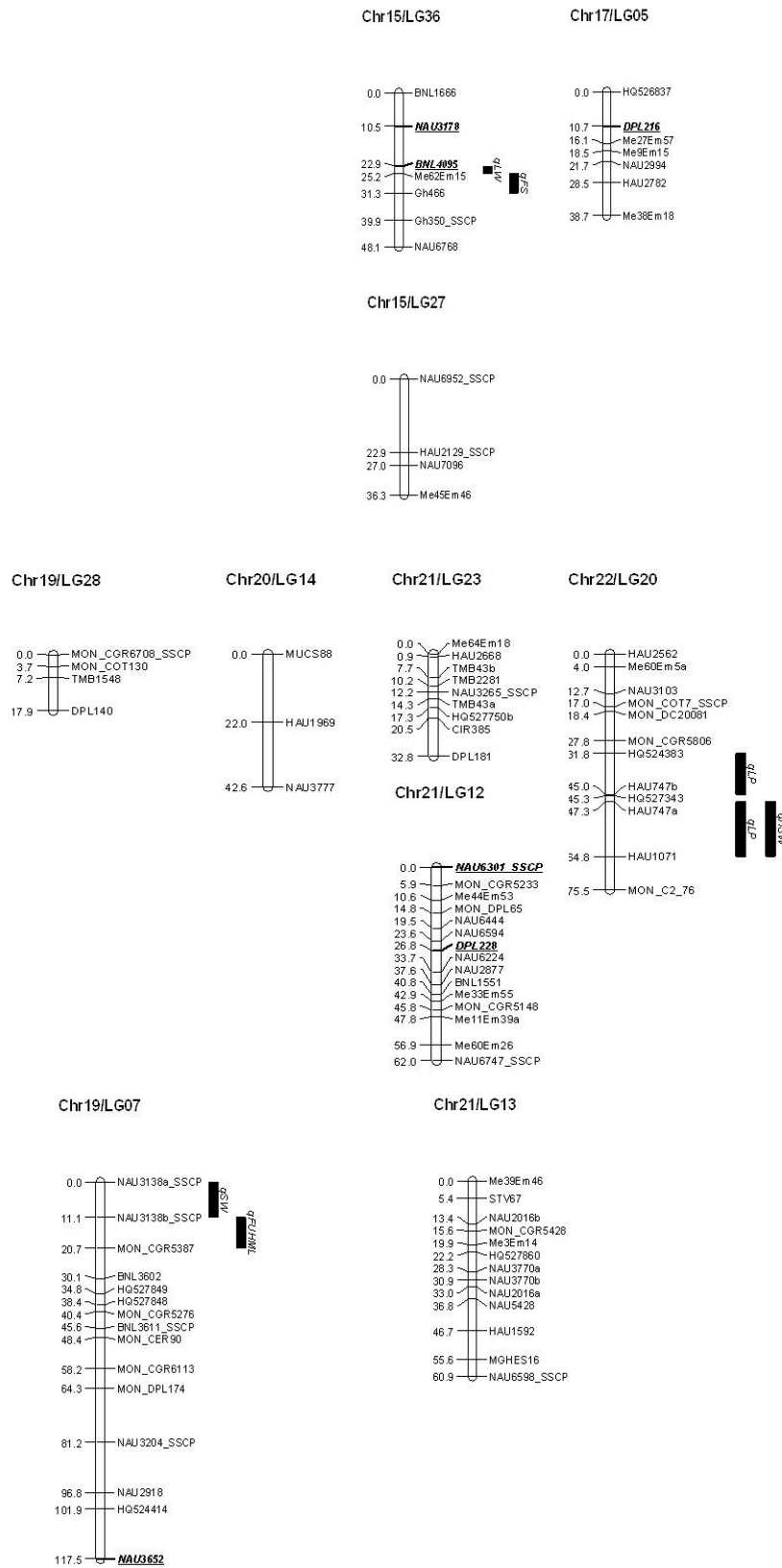
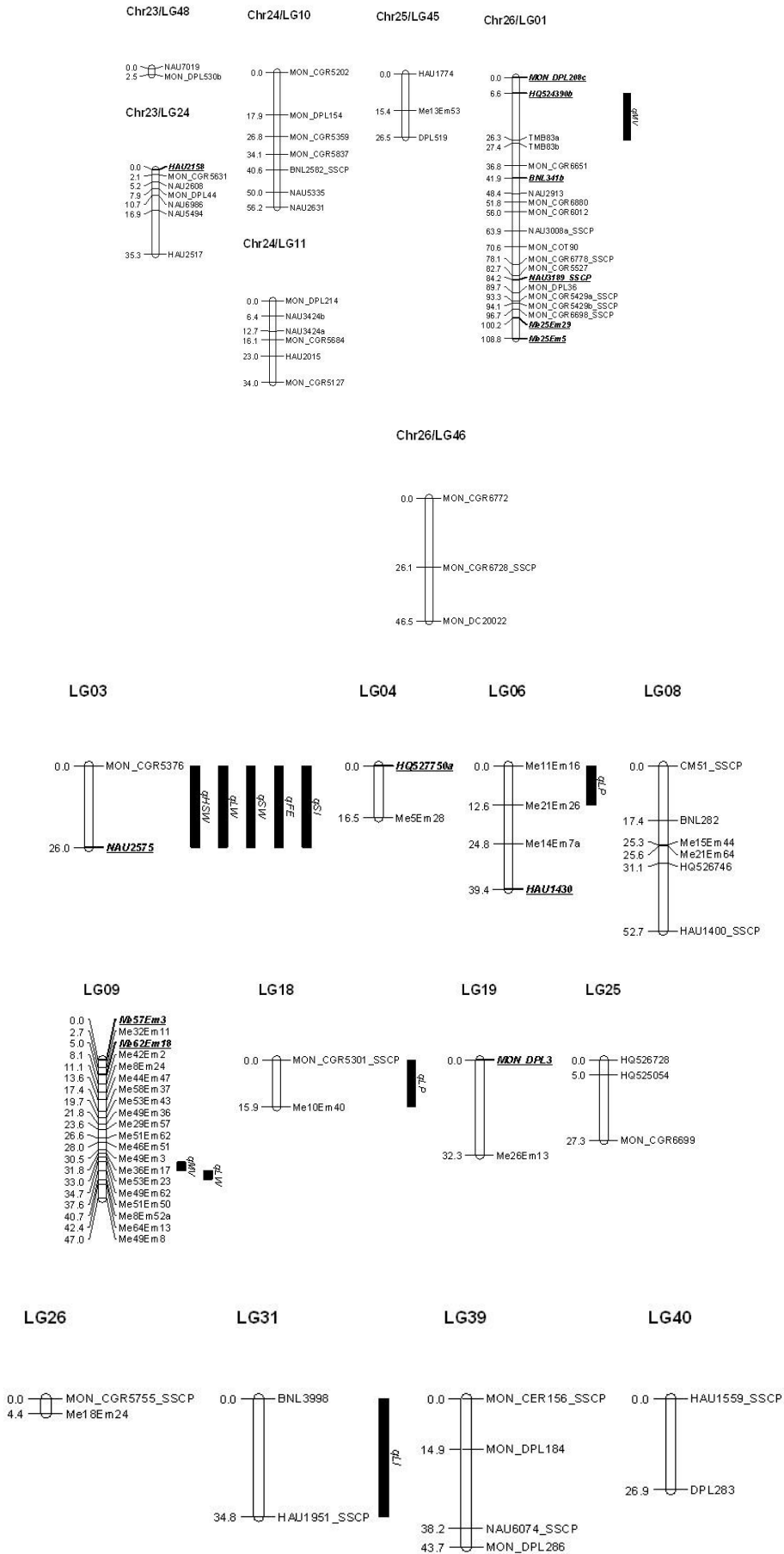


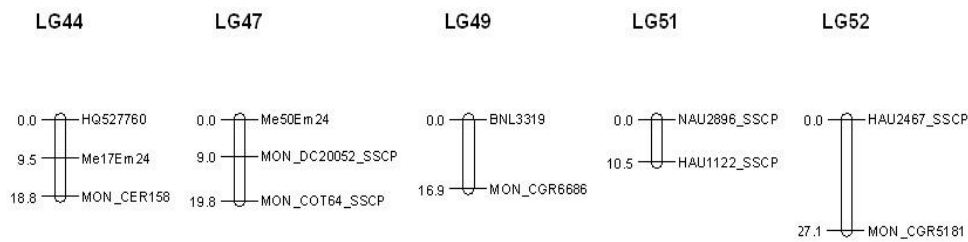
Fig 1. Continued



**Fig 1. Continued**



**Fig 1. Continued**



**Fig 1.** Distribution of QTLs for yield and fiber quality traits in (Hai7124 × 3-79) F<sub>2:3</sub> population. A total of 52 linkage groups containing 335 loci were constructed. Among these, 35 groups were assigned to 20 chromosomes. Black solid bars represent the QTLs for yield and fiber quality traits.

### QTLs for yield and fiber qualities

Based on inclusive composite interval mapping (ICIM) method, 33 QTLs were detected for yield and fiber quality traits in 2010. There were 21 QTLs for yield components and 12 QTLs for fiber quality traits (Table 3; Fig.1). There were 5 QTLs for HSW, 5 for LP, 4 for MV, 3 for FS, 3 for LW, 3 for SW, 3 for FE, 3 for LI, 2 for SI, 1 for FUHML, and 1 for FU. The LOD value ranged from 2.52 to 4.63, and PVE% ranged from 6.93% to 34.52%. Among these QTLs, *qFSchr08* had the highest LOD value (4.63), and *qHSWchr22* had the highest PVE% (34.52%). In addition, the marker interval of HAU747a and HAU1071 was linked to *qHSWchr22* and *qLPchr22-2*. The interval of Me23Em18 and HAU1491\_SSCP was linked to *qLlchr10*, and *qHSWchr10*. The interval of MON\_CGR5376 and NAU2575 was linked to *qHSWlg03*, *qLWlg03*, *qSWlg03*, *qFElg03*, and *qSllg03*. Among the 33 QTLs, 23 QTLs were mapped on chromosomes and 10 were mapped on the other linkage groups. For GA (gene action), 25 showed OD, and the remaining 8 showed A, PD, or D.

### Discussion

It is widely accepted that polymorphism of intraspecific map was lower than interspecific map. Currently, polymorphic rate of the well covered intraspecific map of upland cotton was 6.58% (Zhang et al., 2012); and polymorphic rate of the high-density interspecific map was 15.72% (Yu et al., 2011). For the intraspecific map of *G. barbadense*, the polymorphic rate in our study was 2.58%, and the rate of gSSRs, EST-SSRs, SRAPs, and SSCPs was 3.73%, 4.75%, 2.47%, and 1.17%, respectively. This polymorphic rate was lower than intraspecific map of upland cotton and far lower than interspecific map. The reason for this may be the narrow genetic distance between Hai7124 and 3-79 which was about 0.30–0.50 (Li et al., 2008; Wang et al., 2011). They were selected for construction of the map because they are widely used as parents in many interspecific maps of cotton (Song et al., 2005; Zhang et al., 2008; Li et al., 2010; Yu et al., 2011). The lower polymorphic rate suggested that more effective markers, such as SNPs, should be developed. Most existing linkage maps of cotton are interspecific and intraspecific maps of upland cotton. The linkage map discussed in the present work consisted of 337 loci, spanning 2108.34 cM. It is the first reported integrated interspecific map of *G. barbadense*. Based on this map, thirty-five of the 52 linkage groups were successfully assigned to 20 chromosomes by the anchored markers (Yu et al., 2011; Zhang et al., 2012). However, there were still 17 groups independent of any chromosomes as lacking common markers. NBRI-SSRs and MON-SSRs series markers, which are newly developed, and SSCP-SNPs were

used in the present work, but they have seldom been used in other maps. The present study also showed some chromosomes containing two or three linkage groups. Specifically, Chr08, Chr19, and Chr24 included two groups each, Chr11, Chr17, and Chr21 included three groups each, and Chr15 contained four groups. In order to construct a map comprising 26 chromosomes, covering the whole genome, more types of markers would be needed. The Dt sub-genome contained more loci than the At sub-genome (167 vs 107), and the full length of the Dt sub-genome was found to be longer than that of the At sub-genome (1026.74 cM vs 721.71 cM). These results were similar to those reported by Zhang et al. (2009) and Zhang et al. (2012). LG09 was found to consist solely of SRAP markers. This may be because the parts of the genome that these primer combinations amplify are close to each other. There were many factors affecting map construction, such as number of markers, marker type, and polymorphism between parents. In this study, a comprehensive amplification result was obtained using four molecular markers. gSSRs can amplify any microsatellites in genome, and EST-SSRs can specifically amplify microsatellites in transcription region. The SRAP forward primer can amplify exons, and the target regions of reverse primer were introns and promoters. In this way, these combinations of primers were able to target the whole genome. As a molecular marker, SSCPs can specifically detect variations of even a single nucleotide. The segregation distortion rate can be complex in many different populations. In intraspecific cotton populations, the lowest rate detected was 6.41% of F<sub>2</sub> population in upland cotton (Lin et al., 2005). The highest rate was 77.42%, detected in a F<sub>2</sub> population in Asian cotton (Li et al., 2007). In this study, 52 of the 412 loci showed segregation distortion, a rate of 12.62% ( $P < 0.01$ ). There were many reasons for marker segregation distortion. First, different types of markers can cause different segregation distortion rates (Winter et al., 2000). In the present study, SRAPs showed the lowest rate (5.94%), and the rates of other three types of markers were all over 10%. That may be because SRAPs scan the ORF regions which are seldom affected by factors that can induce segregation distortion (Lin et al., 2005). Second, populations constructed using different parent lines and even the different generations of the same parent line can have different segregation rates (Zhang et al., 2005; Zhang et al., 2009). Third, gametophyte choice is also an important cause of differences in segregation rate (Xu et al., 1997). In this study, 34 of 52 loci (65.38%) deviated towards the maternal genotype, 15 loci (28.85%) deviated towards the heterozygotic genotype, and only 3 loci (5.78%) deviated towards the paternal genotype. The female genome may have a competitive advantage or this may be the influence of maternal cytoplasm. Li et al. (2007) found that the

**Table 2.** Correlation coefficients of yield and fiber quality traits of  $F_{2,3}$ .

	SW	LW	LP	HSW	LI	SI	FUHML	FU	MV	FE
LW	0.82**									
LP	-0.14	0.36**								
HSW	0.27**	0.29*	-0.15							
LI	0.15	0.14	-0.08	0.61**						
SI	0.24**	0.20*	-0.08	0.83**	0.15					
FUHML	0.320**	0.12	-0.34**	0.23*	0.13	0.17				
FU	0.15	0.16	0.11	0.07	-0.02	0.08	0.27**			
MV	0.04	0.14	0.16	0.01	0.05	0.02	-0.30**	0.06		
FE	-0.29**	-0.21*	0.17	-0.03	-0.03	-0.01	-0.41**	-0.20*	-0.19*	
FS	0.37**	0.23*	-0.22*	-0.01	-0.07	0.02	0.58**	0.21*	-0.21*	-0.47**

\* and \*\* represent significance at 0.05 and 0.01, respectively.

**Table 3.** QTLs of yield and fiber quality traits of *G. barbadense*.

QTL	Left marker	Right marker	LOD	PVE%	D/A <sup>a</sup>	GA <sup>b</sup>
<i>qSWchr08</i>	Me12Em26	MON_CGR5537	2.65	13.42	0.65	PD
<i>qSWchr19</i>	NAU3138a_SSCP	NAU3138b_SSCP	3.04	22.88	-18.18	OD
<i>qSWlg03</i>	MON_CGR5376	NAU2575	3.40	8.17	-3.90	OD
<i>qLWchr15</i>	BNL4095	Me62Em15	2.71	24.90	3.64	OD
<i>qLWlg03</i>	MON_CGR5376	NAU2575	3.09	8.87	-2.86	OD
<i>qLWlg09</i>	Me51Em50	Me8Em52a	2.74	12.77	-3.46	OD
<i>qLPchr05</i>	HAU1197	MON_DPL64	4.60	15.45	1.18	D
<i>qLPchr22-1</i>	HQ524383	HAU747b	2.79	23.75	-3.89	OD
<i>qLPchr22-2</i>	HAU747a	HAU1071	3.01	24.74	2.91	OD
<i>qLPlg06</i>	Me11Em16	Me21Em26	3.29	11.02	1.32	OD
<i>qLPlg18</i>	MON_CGR5301_SSCP	Me10Em40	3.56	12.06	0.48	PD
<i>qHSWchr06-1</i>	Me34Em39	Me22Em5	2.67	31.82	29.49	OD
<i>qHSWchr06-2</i>	Me22Em5	MON_CGR6019	2.87	32.43	12.51	OD
<i>qHSWchr22</i>	HAU747a	HAU1071	2.73	34.52	-18.93	OD
<i>qHSWlg03</i>	MON_CGR5376	NAU2575	3.91	24.25	23.95	OD
<i>qHSWchr10</i>	Me23Em18	HAU1491_SSCP	3.29	27.16	4.04	OD
<i>qLlchr08</i>	HQ524512	JESPR157	2.52	11.50	2.71	OD
<i>qLlIg31</i>	BNL3998	HAU1951_SSCP	3.03	30.34	-6.24	OD
<i>qLlchr10</i>	Me23Em18	HAU1491_SSCP	2.55	17.12	5.43	OD
<i>qSlchr15</i>	Me23Em14	MON_DPL504	2.83	16.00	7.55	OD
<i>qSllg03</i>	MON_CGR5376	NAU2575	2.96	10.11	9.24	OD
<i>qFUchr17</i>	HQ524502	HAU197	3.76	13.70	0.16	A
<i>qMVchr08</i>	Me44Em34	HAU1905_SSCP	2.89	13.52	-1.76	OD
<i>qMVchr15</i>	NAU2437_SSCP	MON_DPL302	3.20	9.70	-0.14	A
<i>qMVchr26</i>	HQ524390b	TMB83a	2.99	12.04	18.22	OD
<i>qMVIg09</i>	Me49Em62	Me51Em50	2.70	9.22	3.29	OD
<i>qFEchr11</i>	HAU1756	MGHES74_SSCP	3.29	14.63	0.05	A
<i>qFEchr12</i>	MON_DPL208b	HAU2229	3.00	10.63	-2.62	OD
<i>qFEIg03</i>	MON_CGR5376	NAU2575	2.60	9.56	6.30	OD
<i>qFSchr05</i>	MGHES30a	MON_CGR5091	2.57	6.93	0.60	PD
<i>qFSchr08</i>	Me23Em32	NAU1369	4.63	16.83	-0.34	PD
<i>qFSchr15</i>	Me62Em15	Gh466	2.54	19.96	20.55	OD
<i>qFUHMLchr19</i>	NAU3138b_SSCP	MON_CGR5387	2.56	10.20	1.56	OD

a : D/A Dominance/Additive; b: GA was determined as follows: A (additive), |D/A| 0–0.20; PD (partially dominant), |D/A| 0.21–0.80; D (dominant), |D/A| 0.81–1.20; OD (overdominant), |D/A| over 1.2.

segregation distortion rate could reach 77.42%, and all these loci deviated towards the maternal genotype. This suggested that gamete-specific genes might exist. There were 33 QTLs for both yield components and fiber quality in 2010, and no QTLs common to both the present study and similar, previous studies. However, some common markers were found. NAU1369 was found to be related to FS in this study, but related to FL (fiber length) by Luan et al. (2009). HAU747 was found to be related to HSW and LP in the present study, but it was found to be related to SI by Lin et al. (2009). There may be some reasons for this inconsistency with respect to QTLs. The present study made use of many new markers, such as MON, NBRI, and SSCP series markers. In addition, Chen et al. (2008) found no common QTLs in different linkage maps made using the same parents. This may be because different markers amplify

different regions, or because the density of one or more maps was insufficient. When different parents are used, especially parents of different species, QTLs are also different. In the present study, multiple QTLs were found to be related to same marker intervals. For example, the interval of HAU747a and HAU1071 (Chr22) was linked to *qHSWchr22* and *qLPchr22-2*; the interval of Me23Em18 and HAU1491\_SSCP (LG34) was linked to *qLlIg34* and *qHSWlg34*; and the interval of MON\_CGR5376 and NAU2575 (LG03) was linked to *qHSWlg03*, *qLWlg03*, *qSWlg03*, *qFEIg03*, and *qSllg03*. Many QTLs clustered in the same marker interval. This could be explained by pleiotropic genes or the tight linkage of genes. By analyzing the coefficient of phenotypes (Table2), HSW was found to be positively related to LI, SI, LW, and SW ( $P < 0.01$ ). It is possible that this relationship could explain why QTLs

were linked to the same marker intervals. Three closely linked markers tended to be linked with two QTLs, such as markers on Chr06, Chr15, Chr19, and LG09. The distance between these markers ranged from 1.20 cM to 15.80 cM, with a mean of 8.33 cM. Some researchers have also observed the clustering distribution of QTLs in cotton (Shappley et al., 1998; Wan et al., 2007; Chen et al., 2008). Increasing the number of available markers capable of narrowing these intervals would facilitate the identification of real linked markers.

## Materials and methods

### Plant materials and growth conditions

The parental materials used in this study were *G. barbadense* cv. Hai7124 and acc. 3-79. Hai7124 served as the maternal plant. It was crossed with 3-79 at Huazhong Agricultural University (HZAU), Wuhan, in the summer of 2007; F<sub>2</sub> seeds were obtained from self-pollination of F<sub>1</sub> individuals in the winter of 2007 in Hainan Province. The F<sub>2</sub> population was planted in the summer of 2008 in Wuhan, and 124 individuals were randomly selected for genetic mapping and DNA extraction. Seeds were collected from each individual. In 2010, three replications of F<sub>2:3</sub> and their parents were grown in a randomized plot designed with single-row plots with 100 individuals per row. This was done in Xinjiang Province. The row spacing was (66+10) cm, and individual spacing was 9.5cm, which is the standard cotton cultivation pattern used in agriculture in Xinjiang Province.

### Trait data collection

Yield and yield component traits: In mid-September 2010, 80 bolls were collected from each F<sub>2:3</sub> rows (each row was derived from one F<sub>2</sub> individual). Seed cotton weight per boll (SW), lint weight per boll (LW), lint percentage (LP), hundred seeds weight (HSW), lint index (LI), and seed index (SI) were investigated and measured. Fiber quality traits: 10-15g fibers were collected from middle fruit branches and sent to the department of cotton quality testing center. The test conditions were as follows: temperature 20°C and relative humidity 65%. Test items included fiber length (FL), fiber uniformity (FU), micronaire value (MV), fiber elongation (FE), and fiber strength (FS).

### DNA marker analysis

gSSRs, EST-SSRs, SRAPs, and SSCP-SNPs markers were used to screen for polymorphism between Hai7124 and 3-79. The 4416 gSSRs included BNL, CIR, DPL, JESPR, CS, Gh, MON, and NBRI series markers. Because the names of NBRI series markers were too long, they were substituted by their corresponding Genbank accessions, which are prefixed with HQ. The 1643 EST-SSRs were HAU and NAU prefixed markers. There were also several MUSS, MUSB, MGES and STV markers (Han et al., 2004; Park et al., 2005; Frelichowski et al., 2006; Taliercio et al., 2006; Guo et al., 2007; Zhang et al., 2007). There were 4096 SRAPs primer combinations using 64 forward primers and 64 reverse primers (Lin et al., 2009). Single-strand conformation polymorphism (SSCP) was used to screen SNP polymorphism from monomorphic SSRs. The gSSRs and EST-SSRs analysis procedures were performed as described by Zhang et al. (2008). The SRAP analysis procedures were performed as described by Lin et al. (2005). The SSCP procedure was used as described by Wang et al. (2012).

### Map construction

Linkage analysis and map construction were performed using Jionmap3.0 software (Stam, 1993). Map distances in centi-Morgans (cM) were calculated using the Kosambi mapping function (Kosambi, 1943). A chi-square test was performed to determine whether the genotypic frequencies at each locus deviated from the expected 1:2:1 or 3:1 segregation ratio at a level of significance of 0.01. The resulting linkage map was drawn using MapChart 2.2 software (Voorrips, 2002). Previously identified chromosome-anchored markers and the newly mapped MON and NBRI markers were used in our interspecific map (unpublished data) to assign linkage groups to chromosomes (Yu et al., 2011). The criterion was the presence of at least two markers on the same chromosome.

### Correlation analysis and QTL detection

Correlation analysis of yield and fiber quality traits was carried out using Pearson coefficient of SPSS17.0 software (<http://www-01.ibm.com/software/analytics/spss/>). QTL detection was performed using inclusive composite interval mapping (ICIM) method and QTL IciMapping V3.0 software (<http://www.isbreeding.net>) with a LOD value above 2.5. The ICIM-ADD mode was used to identify QTL action, and probability in stepwise regression was set as 0.001. The degree of dominance of a QTL was estimated as |D/A| value, and the gene action (GA) mode was classified according to Edwards et al. (1987). If the |D/A| value was within the range of 0–0.20, then GA was considered A (additive); if |D/A| was 0.21–0.80, then GA was considered PD (partially dominant); if |D/A| was 0.81–1.20, then GA was considered D (dominant); and if |D/A| was over 1.2, then GA was considered OD (over-dominant). QTL nomenclature was as follows: Individual QTLs were designated “q” followed by the abbreviation of the trait name and the corresponding chromosomes or linkage groups. If more than one QTL affected the same trait, then serial numbers were added.

### Conclusion

In the present study, a comprehensive intraspecific linkage map of *G. barbadense* was constructed using four types of molecular markers with a total of 337 loci and length of 2108.34cM. Thirty-three QTLs related to yield and fiber quality traits were mapped. Marker intervals of MON\_CGR5376 and NAU2575 were found to be related to 5 QTLs. Polymorphism, segregation distortion of markers, and correlations of traits were also analyzed. Enrichment of this map would facilitate further understanding of the genetic characters of *G. barbadense* and allow fine mapping of QTLs.

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