Molecular Plant Breeding 2020, Vol.11, No.15, 1-11
http://genbreedpublisher.com/index.php/mpb

Research Article Open Access

QTL Mapping and Genetic Analysis of Fiber Quality Traits in Hybrid Cotton 'Ji1518'

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Molecular Plant Breeding, 2020, Vol.11, No.15 doi: 10.5376/mpb.2020.11.0015
Received: 14 Jul., 2020
Accepted: 19 Jul., 2020
Published: 07 Aug., 2020

Preferred citation for this article:
Zhang S.J., Zhou X.D., Tang L.Y., Li X.H., Wang H.T., Liu C.J., Cai X., Zhang X.Y., and Zhang J.H., 2020, QTL mapping and genetic analysis of fiber quality traits in hybrid cotton 'Ji1518', Molecular Plant Breeding, 11(15): 1-11 (doi: 10.5376/mpb.2020.11.0015)

Abstract 'Ji1518' is a new hybrid cotton variety suitable for mechanized harvesting, the two parents of 'Ji228' and 'Ji567' were crossed to established F_2, $F_{2:3}$ and $F_{2:9}$ (recombinant inbred lines RILs) population. Simple sequence repeats (SSR) was performed to construct two different genetic maps based on the F_2 and $F_{2:9}$ populations respectively. The QTL mapping of five fiber quality traits was performed in three populations above. A genetic map was constructed by F_2 population, which contained 15 loci in 4 linkage groups, with a full-length coverage of 237.10 cM. While the other linkage map was constructed by $F_{2:9}$ populations, which contained 45 loci in 11 linkage groups, with a full-length coverage of 554.42. Based on the inclusive composite interval mapping method with QTL IciMapping 4.1, 15 QTLs related to upper half mean length, fiber strength, the micronaire value, the elongation, and the uniformity were both detected in F_2 and $F_{2:3}$ segregating populations, among them, the QTL locus *qFM-4-2* related to the micronaire value explained the highest phenotypic variation rate at 21.10%. QTLs with dominant or super-dominant effects accounted for 66.7% of the total, which showed that dominant genes were the main source of fiber quality heterosis in 'Ji1518'. Meanwhile, 6 QTLs related to the above five traits of fiber quality were detected in the RIL ($F_{2:9}$) population, and the contribution rate was between 5.10% and 10.26%. QTL loci related with FS and MIC were detected near HAU2349 in all three populations, and QTL loci related with FU were detected near HAU2710 in all three populations, and the markers above were linked on A6 chromosome. These stable and common QTLs are beneficial to the MAS breeding, which could improve the breeding efficiency.
Keywords Ji1518; SSR; Fiber quality; QTL mapping

Upland cotton (*G. hirsutum* L.) is the most important natural fiber crop in the world (Fang et al., 2017a). The fiber quality of Chinese cotton is the middle level in the world, and the high-end raw cotton still relies on imports. The fiber quality cannot meet the needs of the rapid development of Chinese textile industry (Tan, 2018). Therefore, it is necessary to carry out in-depth research on cotton fiber quality.

Cotton fiber quality is a complex quantitative trait controlled by multiple genes (Fang et al., 2017b). Due to the narrow genetic background of current upland cotton varieties and the negative genetic correlation between yield and fiber quality (Zhang et al., 2011; Clement et al., 2012; Gapare et al., 2017), the traditional breeding method is inefficient and time-consuming. If the stable major quantitative trait locus (QTL) of fiber quality were applied to practice, the breeding years would be effectively shortened.

Simple sequence repeat (SSR) markers are widely used in phenotypic trait mapping and related gene identifying. In recent ten years, breeders have used different parental segregation populations to locate cotton fiber QTLs (Zhang et al., 2005; Shen et al., 2007; Wang et al., 2007; Lacape et al., 2010; Said et al., 2013; Tang et al., 2015; Yang et al., 2015; Zhang et al., 2015). According to the statistical results of Cotton QTLdb database (Said et al., 2013; Said et al., 2015a; Said et al., 2015b), 1 607 QTLs related to 5 fiber quality indexes (fiber length, fiber strength, fiber micronaire value, fiber uniformity, fiber elongation) have been preliminarily identified. However,

due to the significant environmental impact on cotton fiber quality, the corresponding genotypes could not be determined by phenotypic values, which increased the difficulty of mapping of QTLs for fiber quality and identification of candidate genes. So far, the fine mapping of cotton fiber quality QTLs is still relatively slow. Here are some fine mappings QTLs for fiber quality using linkage mapping in recent years.

Cao et al. (2015) fine mapped the fiber length QTL (*qFL-chr. 7*) and fiber strength QTL (*qFS-chr. 7*) to 0.36 cM on chromosome 7, and the fiber micronaire value QTL (*qFM-chr. 7*) to 0.44 cM. Liu et al. (2016) used F_2 secondary segregation population (Yumian1 × RIL118) to fine map the QTL for fiber density, fiber strength, fiber micronaire value and fiber uniformity on chromosome 6 within the 0.28 cM interval. Fang et al. (2017b) mapped the QTL for fiber strength of chromosome 7 in a 62.6 kb region using a F_2 population consisting of 2 484 individuals and a $F_{2:3}$ population with 2 571 individuals. The interval included four genes. LRR RLK was identified as the most likely candidate gene by gene expression and comparative cloning. Xu et al. (2017) fine mapped the fiber length QTL (*qFL-chr1*) on chromosome 1 of sea-island cotton (*Gossypium barbadense*) within the 0.9 cM interval using near isogenic introgression line (NIIL) populations (BC_4F_2 and BC_4F_3), and identified two gene expression levels were significantly positively correlated with fiber length, which may be candidate genes for fiber length QTL. Chen et al. (2018) constructed two secondary recombinant inbred lines (RILs), in which 20 and 27 QTLs related to fiber quality were detected, and further QTL clusters related to fiber length and strength were obtained on chromosome 7. Li et al. (2019) identified 103 QTLs related to fiber quality using sea-land chromosome segment introgression lines, most were distributed on subgroup D, and focused on six chromosome segments related to fiber quality (Seg-A02-1, Seg-A06-1, Seg-A07-2, Seg-A07-3, Seg-D07-3, Seg-D06-2), proposed that more attention should be paid to the above-mentioned QTLs in fine QTL mapping. Ijaz et al. (2019) proposed that QTL mapping combined with multi-omics approaches such as, RNA sequencing datasets to identify differentially expressed genes have benefited the improvement of fiber quality, which helps to accelerate validate candidate genes and to use marker assisted selection (MAS) on fiber quality in breeding programs. Shi et al. (2020) used two land-sea backcross lines (CCRI36, CCRI45) to test the stability of three QTLs (length, strength, micronaire value) under multiple environments. 39 and 79 stably expressed QTLs were found at least two environments in the CCRI36 and CCRI45, respectively.

Previous studies have used molecular markers to locate QTLs for fiber quality traits in upland cotton, finding marker sites and genes, which laid a foundation for further study on the genetic mechanism of cotton fiber quality traits. However, there are still few major QTLs with multi environment stability (Tan, 2018). The number of molecular markers that can be used in breeding practice is obviously insufficient, so it is still necessary to identify more new major stably expressed QTLs for fiber quality in different environments for gene mapping and assisted breeding.

In order to explore the source of heterosis of fiber quality traits, and to obtain molecular markers closely linked to fiber quality, so as to provide guidance for the breeding of new hybrid cotton varieties. 'Ji1518', which was cultivated by Institute of Cotton, Hebei Academy of Agriculture and Forestry Sciences, and its two parents ('Ji228' and 'Ji567') were used to establish F_2, $F_{2:3}$ and $F_{2:9}$ population to carry out QTL mapping and genetic analysis of fiber quality traits in this study.

1 Results and Analysis

1.1 Variation analysis of Fiber quality traits

Analyzed the fiber quality traits of P_1, P_2, F_1, F_2, $F_{2:3}$ and RIL ($F_{2:9}$) populations, we found that the fiber upper half mean length and strength of the two parents were significantly different. The average of the five fiber quality indexes of 'Ji1518'(F_1) were between the parents, showed a mid-parent heterosis. The fiber strength of 'Ji1518' was significantly higher than that of the two parents' average. The absolute values of skewness and kurtosis coefficient of five fiber quality indexes in F_2, $F_{2:3}$ and RIL ($F_{2:9}$) populations were less than or slightly greater than 1, which was consistent with the genetic characteristics of quantitative traits controlled by multigenic. Over parent

lines appeared in F_2, $F_{2:3}$ and RIL($F_{2:9}$) populations (Table 1), and some families showed the same fiber quality during purification, which indicted that there may be stably expressed QTLs expression in these families.

Table 1 Fiber quality traits of parents and generations

Trait	Parents/ Generations	Min	Max	Average	Coefficient of variation (%)	Standard deviation	Skewness	Kurtosis
Fiber upper half mean length (mm)	P_1			31.24		0.13		
	P_2			28.20		0.10		
	F_1			30.80		0.12		
	F_2	26.63	32.76	29.81	4.90	1.46	-0.27	-0.22
	$F_{2:3}$	28.01	33.23	30.69	3.26	1	-0.04	-0.36
	$F_{2:9}$	27.56	33.32	29.61	3.51	1.04	1.03	0.38
Fiber strength (cN.tex^{-1})	P_1			33.50		0.13		
	P_2			28.90		0.11		
	F_1			33.08		0.12		
	F_2	24.8	33.7	29.36	6.27	1.84	-0.18	-0.5
	$F_{2:3}$	28.5	37.5	32.09	4.92	1.58	0.15	-0.06
	$F_{2:9}$	24.7	36.8	31.21	6.18	1.93	-0.05	0.1
Fiber micronaire value	P_1			4.43		0.01		
	P_2			4.94		0.04		
	F_1			4.68		0.01		
	F_2	3.6	6.04	5.05	8.12	0.01	-0.81	0.99
	$F_{2:3}$	3.48	5.71	4.78	7.74	0.37	-0.23	0.37
	$F_{2:9}$	4.42	6.17	5.46	5.49	0.30	-0.39	0.02
Fiber elongation (%)	P_1			6.18		0.01		
	P_2			6.90		0.06		
	F_1			6.63		0.02		
	F_2	5.7	7.1	6.32	3.96	0.25	0.40	0.05
	$F_{2:3}$	5.7	6.7	6.23	2.73	0.17	-0.02	0.21
	$F_{2:9}$	5.4	8.6	6.83	7.76	0.53	0.15	-0.09
Fiber uniformity (%)	P_1			85.03		0.20		
	P_2			82.76		0.11		
	F_1			83.02		0.13		
	F_2	81.7	87.5	85.48	1.10	0.94	-0.57	0.81
	$F_{2:3}$	80.6	88.5	84.72	1.51	1.28	-0.28	0.23
	$F_{2:9}$	80.7	87.1	84.49	1.25	1.06	-0.21	0.13

1.2 Correlation coefficients among fiber quality traits

Analyzed the correlation coefficients among fiber quality traits of F_2, $F_{2:3}$ and RIL ($F_{2:9}$) populations (Table 2), the results showed that the fiber upper half mean length is significantly correlated at $p<0.01$ with the fiber strength in each generation. The correlation coefficient was the highest in F_2 generation, which was 0.719, and the lowest in $F_{2:9}$, was 0.209. It was negative correlated significantly with micronaire value and elongation, and positive correlated significantly with uniformity in two generations. Fiber strength was positive correlated significantly and negative correlated significantly with uniformity and elongation, respectively, in two generations. And it was negative correlated significantly with micronaire value in generation. Micronaire value was positive correlated significantly with uniformity in two generations. And it was negative correlated significantly with elongation in generation.

The correlation analysis of the same fiber quality index in different populations (Table 3) showed that the fiber upper half mean length, strength and micronaire value were significantly correlated (p<0.01) among F_2, $F_{2:3}$ and $F_{2:9}$ populations. Elongation was significantly correlated (p<0.01) among F_2 and $F_{2:3}$, correlations coefficient was 0.201, and was not significantly among $F_{2:9}$, and F_2, $F_{2:3}$. Uniformity was not significantly correlated among different populations.

Table 2 Correlations coefficients among fiber traits in the same generation

Trait	Populations	Fiber upper half mean length	Fiber strength	Fiber uniformity	Fiber elongation
Fiber strength	F_2	0.719**			
	$F_{2:3}$	0.558**			
	$F_{2:9}$	0.209**			
Fiber uniformity	F_2	0.310**	0.446**		
	$F_{2:3}$	0.103	-0.024		
	$F_{2:9}$	0.296**	0.281**		
Fiber elongation	F_2	-0.800**	-0.806**	-0.437**	
	$F_{2:3}$	-0.563**	-0.468**	-0.118	
	$F_{2:9}$	0.045	-0.112	0.142*	
Fiber micronaire value	F_2	0.091	0.113	0.367**	-0.262**
	$F_{2:3}$	-0.289**	-0.637**	0.283**	-0.008
	$F_{2:9}$	-0.475**	-0.086	-0.129*	-0.119

Note: **: Significantly correlated at p<0.01; *: Significantly correlated at 0.01<p<0.05

Table 3 Correlation analysis of the same fiber quality traits in different generations

	Fiber upper half mean length	Fiber strength	Micronaire value	Fiber elongation	Fiber uniformity
$F_2/F_{2:3}$	0.401**	0.373**	0.310**	0.201**	0.095
$F_{2:3}/F_{2:9}$	0.367**	0.265**	0.505**	-0.125	-0.019
$F_2/F_{2:9}$	0.253**	0.181**	0.350**	-0.093	-0.005

Note: **: Significantly correlated at p<0.01

1.3 Construction of linkage map

$F_{2:9}$ population parents and F_1 were used to select 3 989 pairs of SSR primers, and obtained 33 pairs of polymorphic primers, which were used to carry out genotyping for 244 individuals in F_2 population, obtained 36 polymorphic markers. Then Joinmap 3.0 was used to construct genetic maps (Figure 1A). There were 15 loci in 4 linkage groups, with a full-length coverage of 237.10 cM. The longest linkage group was 112.40 cM, the shortest was 1.30 cM, and the average of each linkage group was 59.28 cM. Each linkage group contained 2~7 markers, with an average of 3.75 markers. The average genetic distance between markers was 15.8 cM.

In order to increase the marker density of linkage map, we continued to use the DNA of parents and F_1 to screen polymorphic markers. A total of 4 912 SSR primers and 58 polymorphic primers were screened, accounting for 1.18% of the total primers. The genotypes of 244 $F_{2:9}$ families were detected by differential primers, and 55 polymorphic markers were obtained. The linkage relationship of molecular markers was determined by QTL IciMapping 4.1 (Figure 1B). There were 45 loci in 11 linkage groups, with a full-length coverage of 554.42 cM. The longest linkage group was 89.24 cM, the shortest was 21.82 cM, and the average of each linkage group was 46.20 cM. Each linkage group contained 2~10 markers, with an average of 4.09 markers. The average genetic distance between markers was 12.60 cM.

1.4 Chromosome mapping of linkage groups

According to the information of CottonGen database (www.cottongen.org) (Yu et al., 2014a) and previous mapping results (Yu et al., 2011; Yu et al., 2014b; Chen et al., 2015), linkage groups, corresponding to A1, D10 and A6 chromosomes, were constructed with F_2 population (Figure 1A). Linkage groups, which were constructed with RIL (F2:9), corresponding to A1, A5, A6, A7, A9/D9, A12, A13, D1, D3, D10 chromosomes, respectively (Figure 1B).

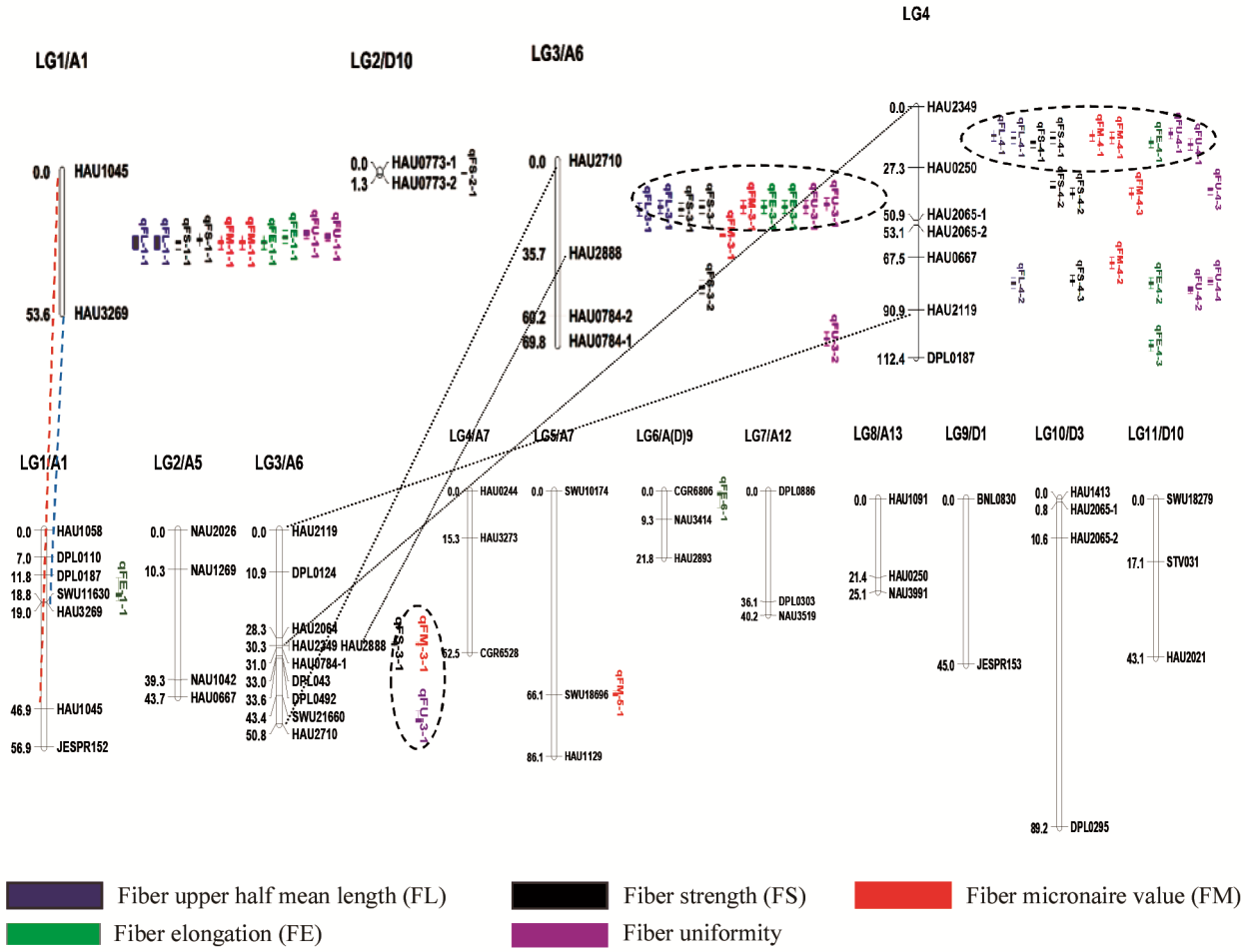

Figure 1 The QTL detected with fiber quality in F_2 population, $F_{2:3}$ population (A) and RIL ($F_{2:9}$) (B) population

1.5 QTL mapping of fiber quality traits

Based on the inclusive composite interval mapping method with QTL IciMapping 4.1, we analyzed the fiber quality traits in F_2 and $F_{2:3}$ segregating populations. A total of 28 QTLs correlated with fiber quality traits were mapped (Figure 1; Table 4). The additive effect of QTL was -0.62~0.29, and the dominant effect was -2.47~1.01, which could explain 0.77% ~21.10% phenotypic variation. Among them, 4 QTLs were correlated with the Fiber upper half mean length, 7 were correlated with the fiber strength, and 5 were correlated with the micronaire value. 5 and 7 QTLs were correlated with elongation and uniformity, respectively. 15 QTLs could be detected in F_2 and $F_{2:3}$ populations at the same time.

Table 4 The QTL detected with fiber quality in F_2 population and $F_{2:3}$ population

Traits	QTL name	Gene-ration	Linkage group/ Chromosome	Position	Left marker	Right marker	LOD	Additive effect	Dominant effect	Contribution rate (%)
Fiber upper	*qFL-1-1*	F_2	1/A1	27	HAU1045	HAU3269	5.42	-0.20	0.66	5.35
half		$F_{2:3}$	1/A1	27	HA7U1045	HAU3269	5.16	0.03	-0.23	2.92
mean length	*qFL-3-1*	F_2	3/A6	19	HAU2710	HAU2888	4.67	-0.22	0.30	3.35
		$F_{2:3}$	3/A6	18	HAU2710	HAU2888	4.39	-0.05	0.42	2.94
	qFL-4-1	F_2	4	13	HAU2349	HAU0250	2.79	-0.16	0.14	1.66
		$F_{2:3}$	4	14	HAU2349	HAU0250	2.68	0.08	0.26	1.61
	qFL-4-2	F_2	4	79	HAU0667	HAU2119	2.94	-0.20	0.08	2.19
Fiber strength	*qFS-1-1*	F_2	1/A1	27	HAU1045	HAU3269	5.05	-0.04	0.31	1.49
		$F_{2:3}$	1/A1	26	HAU1045	HAU3269	5.42	-0.09	0.65	2.93
	qFS-2-1	F_2	2/A10	1	HAU0773a	HAU0773b	3.08	-0.61	0.08	6.01
	qFS-3-1	F_2	3/A6	49	HAU2710	HAU2888	4.04	-0.13	-0.31	1.87
		$F_{2:3}$	3/A6	18	HAU2710	HAU2888	4.36	0.14	0.20	1.89
	qFS-3-2	F_2	3/A6	49	HAU2888	HAU0784b	2.80	-0.24	-0.95	6.36
	qFS-4-1	F_2	4	16	HAU2349	HAU0250	3.45	-0.24	0.74	4.87
		$F_{2:3}$	4	14	HAU2349	HAU0250	2.88	0.10	0.64	3.33
	qFS-4-2	F_2	4	36	HAU0250	HAU2065a	3.12	-0.19	0.94	6.21
		$F_{2:3}$	4	39	HAU0250	HAU2065a	4.87	-0.41	0.76	9.76
	qFS-4-3	F_2	4	78	HAU0667	HAU2119	6.20	-0.62	1.01	13.14
Fiber	*qFM-1-1*	F_2	1/A1	27	HAU1045	HAU3269	5.59	-0.05	0.24	5.77
micronaire		$F_{2:3}$	1/A1	27	HAU1045	HAU3269	5.18	-0.01	0.02	2.10
value	*qFM-3-1*	F_2	3/A6	29	HAU2710	HAU2888	6.97	0.04	-0.80	19.69
		$F_{2:3}$	3/A6	18	HAU2710	HAU2888	4.07	0.01	-0.01	1.03
	qFM-4-1	F_2	4	13	HAU2349	HAU0250	2.52	0.00	-0.12	1.55
		$F_{2:3}$	4	14	HAU2349	HAU0250	3.15	-0.01	-0.19	5.01
	qFM-4-2	F_2	4	70	HAU0667	HAU2119	4.00	-0.01	-0.97	21.10
	qFM-4-3	$F_{2:3}$	4	39	HAU0250	HAU2065a	5.67	0.12	-0.13	10.65
Fiber	*qFE-1-1*	F_2	1/A1	27	HAU1045	HAU3269	5.53	0.05	-0.12	4.38
elongation		$F_{2:3}$	1/A1	25	HAU1045	HAU3269	5.57	0.01	-0.10	6.67
	qFE-3-1	F_2	3/A6	18	HAU2710	HAU2888	3.97	0.02	0.01	2.39
		$F_{2:3}$	3/A6	18	HAU2710	HAU2888	4.29	-0.01	-0.03	1.01
	qFE-4-1	$F_{2:3}$	4	16	HAU2349	HAU0250	2.83	-0.01	0.05	2.60
	qFE-4-2	$F_{2:3}$	4	79	HAU0667	HAU2119	2.89	-0.01	0.09	4.09
	qFE-4-3	$F_{2:3}$	4	107	HAU2119	DPL0187	3.69	-0.06	0.04	8.98
Fiber	*qFU-1-1*	F_2	1/A1	25	HAU1045	HAU3269	5.38	-0.21	-0.55	6.61
uniformity		$F_{2:3}$	1/A1	26	HAU1045	HAU3269	5.22	0.08	-0.16	0.77
	qFU-3-1	F_2	3/A6	18	HAU2710	HAU2888	4.40	0.02	-0.89	8.13
		$F_{2:3}$	3/A6	17	HAU2710	HAU2888	4.89	-0.12	0.40	3.80
	qFU-3-2	F_2	3/A6	69	HAU0784b	HAU0784a	2.56	-0.15	-2.47	14.74
	qFU-4-1	F_2	4	12	HAU2349	HAU0250	3.02	-0.23	-0.16	2.51
		$F_{2:3}$	4	17	HAU2349	HAU0250	3.20	0.22	0.25	4.40
	qFU-4-2	F_2	4	83	HAU0667	HAU2119	3.92	-0.29	-1.55	23.96
	qFU-4-3	$F_{2:3}$	4	37	HAU0250	HAU2065a	3.88	0.29	0.50	11.33
	qFU-4-4	$F_{2:3}$	4	79	HAU0667	HAU2119	2.60	0.03	-0.35	1.93

The contribution rate of 4 QTLs correlated with the fiber upper half mean length was between 1.61% and 5.35%. According to the |D/A| effect, *qFL-1-1*, *qFL-3-1* were super-dominant. *qFL-4-1* showed dominant and super-dominant in F_2 and $F_{2:3}$ segregation population, respectively. *qFL-4-2* showed partial-dominant in F_2 segregation population. The contribution rate of 7 QTLs correlated with the fiber strength was 1.49%~13.14%.

qFS-2-1 showed additive effect in F_2, and others showed super-dominant. The contribution rate of 5 QTLs correlated with the micronaire value was 2.10%~21.10%. All of them showed super-dominant or dominant. The contribution rate of 5 QTLs correlated with the elongation was 1.01%~8.98%. *qFE-3-1*, *qFE-4-3* showed partial-dominant, and others were super-dominant. The contribution rate of 7 QTLs correlated with the uniformity was 0.77%~23.96%. All of them showed super-dominant or partial-dominant. In a word, among the QTLs controlling fiber quality, 66.7% of the QTLs were dominant or super-dominant phenotypes, 26.6% were partial-dominant and 6.7% were additive effects. Dominant gene is the main source of fiber quality heterosis in 'Ji 1518'.

Based on the inclusive composite interval mapping method with QTL IciMapping 4.1, we analyzed the fiber quality traits in RIL ($F_{2:9}$) populations. A total of 6 QTLs correlated with fiber quality traits were mapped (Figure 1B; Table 5). Among them, 1 QTL was correlated with fiber strength, 2 QTLs were correlated with micronaire value, and elongation, 1 QTL was correlated with uniformity. They were located on chromosomes A1, A6, A7 and A9/D9, respectively. The genetic effect was mainly additive effect because the later generations of RIL population tend to be homozygous (Chen et al., 2015). The obtained QTL additive effect was between -0.52 and 0.17. Contribution rate was between 5.10% and 10.26%. *qFS-3-1* additive effect was -0.52, additive gene was from female parent 'Ji228', contribution rate was 5.10%. Additive effect of *qFM-3-1* and *qFM-5-1* were 0.07 and 0.08, respectively. Both additive genes were from male parent 'Ji567'. Contribution rate were 5.29% and 7.51%, respectively. *qFU-3-1* additive effect was -0.30. Additive gene was from female parent 'Ji228'. *qFE-6-1* additive effect was -0.24. Additive gene was from female parent 'Ji228'. Contribution rate was 7.42%. *qFE-1-2* additive effect was 0.17. Additive gene was from male parent 'Ji567'. Contribution rate was 10.26%.

Table 5 The QTL detected with fiber quality in RIL($F_{2:9}$) population

Traits	QTL name	Generation	Linkage group/ Chromosome	Position	Left marker	Right marker	LOD	Additive effect	Contribution rate (%)
Fiber strength	*qFS-3-1*	RIL ($F_{2:3}$)	3/A6	30	HAU2064	HAU2349	2.75	-0.52	5.10
Fiber micronaire value	*qFM-3-1*	RIL ($F_{2:3}$)	3/A6	30	HAU2064	HAU2349	2.54	0.07	5.29
	qFM-5-1	RIL ($F_{2:3}$)	5/A7	66	SWU10174	SWU18696	4.55	0.08	7.51
Fiber uniformity	*qFU-3-1*	RIL ($F_{2:3}$)	3/A6	50	SWU21660	HAU2710	3.33	-0.30	6.38
Fiber elongation	*qFE-6-1*	RIL ($F_{2:9}$)	6/A(D)9	1	CGR6806	NAU3414	3.86	-0.24	7.42
	qFE-1-1	RIL ($F_{2:9}$)	1/A1	17	DPL0187	SWU11630	5.65	0.17	10.26

2 Discussion

2.1 Multi generation consistency analysis of QTLs for fiber quality traits

Cotton fiber quality has complex genetic background and is susceptible to environmental influence (Zhang, 2015). Stably expressed QTL existed in multiple generations indicated that genetic background more strongly impacts on fiber quality traits. And it was valuable to be used in molecular marker assisted breeding (Lacape et al., 2009). Comparing the mapping results between the primary segregation population and the secondary population, QTLs related to the five fiber quality indexes could be repeatedly detected in the section between HAU2710 and HAU2888, HAU2349 and HAU0250, HAU0667 and HAU2119, which are marked by SSR in F_2 and $F_{2:3}$. After using $F_{2:9}$ to reconstruct the map, the above segments were located in the same linkage group on chromosome A6. In this segment, loci related to fiber quality (F_2 or $F_{2:3}$: *qFL-4-1*, *qFS-4-1*, *qFM-4-1*, *qFU-4-1*, *qFL-3-1*, *qFS-3-1*, *qFM-3-1*, *qFE-4-1*, *qFU-3-1*; $F_{2:9}$: *qFS-3-1*, *qFM-3-1*, *qFU-3-1*) were detected near HAU2349 and HAU2710 in F_2, $F_{2:3}$ and secondary RIL ($F_{2:9}$) populations. These loci are highly stable independent of generations and may be valuable for marker assisted selection.

Besides, QTLs related to five fiber quality indexes were detected in HAU1045 and HAU3269 segments of A1 chromosome in F_2 and $F_{2:3}$ segregation populations. After adding markers, QTLs related to elongation (*qFE*-1-1) were detected on A1 chromosome near HAU1045-HAU3269 segment with the help of $F_{2:9}$ population.

On the whole, the QTLs for fiber quality in F_2 and $F_{2:3}$ populations of lower generation had better repeatability, and multiple QTLs related to fiber quality traits showed cluster distribution. This also exists in other studies with primary group mapping (Liu et al., 2016; Qiao et al., 2019). This phenomenon may be more obvious due to the low density of genetic map constructed by the primary population in this study. In the secondary mapping population, QTL loci related to a fiber quality index could be located near the QTL cluster related to fiber quality. The results showed that stably expressed QTL existed in the QTL cluster, which is greatly affected by genotype. Meanwhile, compared with the mapping results of the primary population, the number of loci mapped by the secondary RIL population decreased, which may be due to the weakening or disappearance of some non-major loci in the process of multi generation breeding.

2.2 Consistency analysis of reported loci

Liu et al. (2016) used 6 975 F_2 to map QTLs correlated with fiber length, strength, micronaire value, and uniformity at the same time on chromosome A6 of upland cotton. SSR markers on both sides were HAU2119 and SWU2302. In this study, QTLs correlated with fiber length, strength, micronaire value and uniformity (F_2 or $F_{2:3}$: *qFL-4-2*, *qFS-4-3*, *qFM-4-3*, *qFE-4-3*, *qFU-4-4*) were also located near HAU2119 in F_2 or $F_{2:3}$ segregation population, inferred it was same or similar with QTLs mapped by Liu et al. (2016). And QTLs correlated with fiber quality were detected in F_2, $F_{2:3}$ and secondary RIL ($F_{2:9}$) populations. While in the RIL ($F_{2:9}$) populations, the distance between *qFS-3-1*, *qFM-3-1* and HAU2349 was 0.31 cM, and that of between *qFU-3-1* and HAU2710 was 0.84 cM. The above QTLs and HAU2119 were in the same linkage group, but no common markers were found in the CottonGen database (Yu et al., 2014a). It was inferred that these QTLs were new linked to Liu et al. (2016). These loci or genes related to fiber quality control have strong linkage relationship, which is worthy of further study.

In addition, after comparison, it was found that the QTL loci *qFE-4-1* found in $F_{2:3}$ population was partially coincident with that correlated with elongation found by Tang et al. (2015). The QTL loci correlated with micronaire value and elongation were mapped on chromosomes A1, A7 and A/D9 by subpopulation. Due to the lack of common markers with previous mapping results, this QTL loci may be newly detected in this study.

In conclusion, QTL mapping for fiber quality traits was carried out in primary population and secondary population, and stable genetic loci were obtained for multiple generations in this study. Through the consistency analysis with reported loci, we not only mapped QTLs or loci consistent with previous results, but also mapped new QTL loci, which can reflect the reliability and innovation of this study.

3 Materials and Methods

3.1 Experiment materials

'Ji1518' is a hybrid cotton variety (cotton 2014005, cotton 20189001) suitable for mechanized harvesting, which was crossed by the two parents of 'Ji228' and 'Ji567'. The female parent 'Ji228' (cotton 2008003) (P_1) was a high-quality fiber trait, and its strength and upper half mean length were outstanding. The male parent 'Ji567'(P_2) was a high-quality fiber trait bred by Ji668×GK12. 'Ji1518' F_1 seeds were obtained by crossing two parents in Xiaoanshe, Shijiazhuang in the summer of 2008. In 2010, F_2 genetic population was constructed and strictly self-crossed. In 2011, 244 $F_{2:3}$ lines were planted. After multiple generations of self-crossed, each family was relatively stable ($F_{2:9}$). In 2017, 244 recombinant inbred lines (RILs) were obtained. $F_{2:3}$ and RIL ($F_{2:9}$) populations were completely randomized design, single line, length of 7.5 m, line distance of 0.8 m, plant distance of 18~20 cm. And carry out routine test management.

3.2 Fiber quality detection and data statistics

Determined the fiber quality data of F_1, F_2, $F_{2:3}$ and RIL ($F_{2:9}$) populations. In the middle of September, 30 open bolls were randomly harvested in the middle of each strain and sent to CIQ of Hebei Province for testing. Each sample was tested three times. Testing index include the fiber upper half mean length (FL), fiber strength (FS), fiber micronaire value (FM), fiber elongation (FE), and fiber uniformity (FU). Analyze-Descriptive statistics-Descritives of SPSS 19.0 was used to carry out descriptive statistical analysis of fiber quality traits.

3.3 Construction of genetic linkage map

Genomic DNA was extracted from young cotton leaves by improved CTAB method (Parerson et al., 1993). SSR primer sequences were obtained from CottonGen (http://www.com.cottongen.org/) database (Yu et al., 2014a) and Cotton Marker Database (http://www. cottonmarker.org/). Conventional amplification method was used to separate RCR products by 8% polyacrylamide gel electrophoresis, and silver staining was carried out with reference to Zhang et al. (2000). Refer to the method of Tang et al. (2018) for statistical marker band type. Genetic map construction: Joinmap4.0 was used to construct molecular marker linkage maps in F_2 groups. The lowest LOD value of linkage was 3.0. QTL IciMapping 4.1 was used to construct molecular marker linkage maps in $F_{2:9}$ groups, LOD was selected as 3.0, the algorithm is nnTwoOpt and the standard is SARF. ICIM-ADD method of QTL IciMapping 4.1 was used to map QTLs correlated with fiber quality in F_2, $F_{2:3}$, and RIL ($F_{2:9}$) populations. LOD ≥ 2.5 was used as a threshold to screen QTLs with additive or dominant effects. According to the method of Stuber et al. (1992), the dominant potential was used to determine the gene effect type of each QTL, and the Map Chart 2.2 was used to draw the distribution map of QTL on the linkage group. QTL was named according to QTL (q) + trait name (abbreviation) + linkage group (number) + QTL number (number).

Authors' contributions

ZSJ and ZXD designed and carried out this experiment. TLY and LXH performed the statistical analysis. WHT, LCJ, CX, and ZXY participated in the design of the study and results analysis. ZJH conceived of the study, and guide its design, data analysis, draft, and revision of the manuscript. All authors read and approved the final manuscript.

Acknowledgments

This study was supported by Major State Research Development Program (2016YFD0101415), Innovation Project of Hebei Academy of Agriculture and Forestry Sciences (2019-3-7-1; 2019-3-7-4), and Open Project of State Key Laboratory of Cotton Biology (CB2018A15). Thanks to Shu Jinshai, associate researcher from Institute of Vegetables and Flowers, Chinese Academy of Agricultural Sciences.

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