RESEARCH ARTICLE

Association of AFLP and SSR markers with agronomic and fibre quality traits in *Gossypium hirsutum* L.

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Abstract

Molecular markers linked to QTL contributing to agronomic and fibre quality traits would be useful for cotton improvement. We have attempted to tag yield and fibre quality traits with AFLP and SSR markers using F_2 and F_3 populations of a cross between two *Gossypium hirsutum* varieties, PS56-4 and RS2013. Out of 50 AFLP primer combinations and 177 SSR primer pairs tested, 32 AFLP and four SSR primers were chosen for genotyping F_2 individuals. Marker-trait associations were studied for eight agronomic and five fibre quality traits through simple and multiple regression analysis (MRA) using a set of 92 AFLP polymorphic loci and four SSR markers. Simple linear regression analysis (SLRA) identified 23 markers for eight different traits whereas multiple regression analysis identified 30 markers for at least one of the 13 traits. SSR marker BNL 3502 was consistently identified to be associated with fibre strength. While all the markers identified in SLRA were also detected in MRA, as many as 16 of the 30 markers were identified to be associated with respective traits in both F_2 and F_3 generations. The markers explained up to 41 per cent of phenotypic variation for individual traits. A number of markers were found to be associated with multiple traits suggesting clustering of QTLs for fibre quality traits in cotton.

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Introduction

Worldwide, the textile industry depends largely on cotton (*Gossypium* sp.) fibres. Spinnable fibres are obtained from the allotetraploid species *G. hirsutum* and *G. barbadense* ($n = 2x = 26$) (AD genome) and the diploid species *G. herbaceum* and *G. arboreum* ($n = x = 13$) (A genome) (Wendel *et al*. 1992). Among these species, *G. hirsutum* and *G. barbadense* possess long and fine fibre. Fibre yield and quality, and the key factors that determine its economic value, are the most important targets of cotton breeding programmes across the world. The fibre quality demands of the textile industry are changing with changes in spinning technology. Consequently, the fibre quality parameters targeted for breeding are dictated by industrial demands. For example, current spinning technology lays greater emphasis on fibre strength rather than fibre length and fineness (Shen *et al*. 2005). Although borne on seed, fibre is a maternal tissue and hence fibre properties of the seed are not reliable indicators of the performance of the progeny. Further, the fibre quality can be assessed only after the harvest of the crop and hence breeding for fibre quality is a challenge. *G. hirsutum* is widely cultivated in India (85% of the total area worldwide). However, genetic polymorphism for fibre quality traits among *hirsutum* cotton is quite low, and this further restricts its quality improvement (Lu and Myers 2002; Wu *et al*. 2007).

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DNA-based molecular markers hold great promise in breeding for improvement of complex traits such as fibre quality that are controlled by several genes (Gupta 2006; Zeng *et al*. 2009). Among various DNA markers, amplified fragment length polymorphism (AFLP) and simple sequence repeats (SSR) are PCR-based markers with potential application in diversity analysis, fingerprinting, gene mapping and quantitative trait locus (QTL) identification. AFLPs are multilocus and dominant markers while SSRs or microsatellites are codominant and easy-to-use. SSRs and AFLPs have been widely employed in *G. hirsutum* to construct linkage maps and in QTL analysis (Paterson *et al*. 2003; Zhang *et al*. 2003; Mei *et al*. 2004; He *et al*. 2005, 2007; Lacape *et al*. 2005; Lin *et al*. 2005; Shen *et al*. 2005, 2006; Ulloa *et al*. 2005; Zhang *et al*. 2005; Shen *et al*. 2007; Zeng *et al*. 2009). Though map-based QTL analysis is efficient in detecting QTL, it is time consuming and laborious. On the other hand, single-marker analysis (SMA), based on association of a marker with the phenotype, is the simplest method and does not require a complete linkage map. SMA based on regression is computationally less demanding and hence can find association between a trait and marker faster than other methods. In simple linear regression analysis (SLRA)-based QTL analysis, the marker loci in the individuals of a mapping population are coded as 0 and 1 (independent variable) and phenotype (dependent variable) is regressed onto the marker. Significance of the regression coefficient implies association between the marker and the QTL (Hackett 2002; Gupta and Kulwal 2006). Similarly, multiple regression analysis (MRA) gives estimates and test of significance of the parameters of multiple linear regression equations. It also provides the coefficient of determination (R^2) which indicates the proportion of variability of a dependent variable that can be explained by a linear function of independent variables (Gomez and Gomez 1984). Single marker analysis has been used for QTL study in many crops including cotton (Roy *et al*. 2006; Wu *et al*. 2007).

Here, we report association of AFLP and SSR markers with five fibre quality and eight agronomic traits in cotton following linear and multiple regression analysis. A total of 30 markers were identified, each associated with at least one of the 13 traits and of these; 23 markers were detected in both simple and multiple regression analyses.

Materials and methods

Plant materials and mapping population

Two accessions, P56-4 and RS2013 were identified as the most diverse (table 1) out of six *G. hirsutum* parents based on Mahalanobis D^2 cluster analysis (data not shown), and were crossed in 2005 Kharif season to obtain F_1 seeds. F_1 generation was raised during 2005 Rabi season and selfed to obtain F_2 seeds. F_1 and 94 F_2 parents were grown in the field of Indian Agricultural Research Institute, New Delhi, India, during Kharif 2006. Each F_2 plant was sequentially numbered and selfed to obtain F_3 seeds. These F_3 families were grown along with the parents in complete randomized block design with three replications during May–October 2007.

Table 1. Details of cotton genotypes used in Mahalanobis D^2 cluster analysis.

Genotype	Parents	UR (%)	STR (g/tex)	ELONG (%)
P 56-4	BN×Pusa 734	54.4	28.4	6.3
P 56-6	BN×Pusa 734	54.7	27.6	6.3
P95-27-2P1	Selection from P595	53.7	25.5	6.1
Bikaneri Narma	Local selection	54.0	20.5	5.9
RS810	RS 644×Khandwa 3	52.8	21.2	5.8
RS2013	RS20×LH-511-Bombasa	51.9	19.9	5.8

Phenotyping

Data were recorded on each F_2 plant and on five plants of each F_3 family for eight agronomic traits viz., plant height (PH), number of monopodia per plant (MONO), number of sympodia per plant (SYMPO), seed cotton yield (SCY), boll weight (BW), ginning out turn (GOT), seed index (SI) and lint index (LI). Upon maturation, bolls from individual F_2 plant and F_3 families were collected. Approximately 20 g bolls were analysed for fibre quality traits using HVI900 (Zellweger USTER, Uster, Switzerland). Analysis was carried out under controlled temperature of 20°C and relative humidity of 65%. The fibre quality traits determined included 2.5% span length (SL), uniformity ratio (UR), micronaire value (MICRON), fibre strength (STR) and elongation (ELONG).

Genotyping

High-quality genomic DNA was isolated from young leaves using Qiagen mini kit (Qiagen, Hilden, Germany). Extracted DNA was subjected to AFLP and SSR analysis. AFLP analysis was carried out with 500 ng of DNA per reaction. Restriction digestion, ligation of adapters, preamplification and selective amplification were carried out according to manufacturer's instructions (Applied Biosystems, Carlsbad, USA), which principally followed Vos *et al*. (1995). A total of 50 AFLP primer combinations were tested. PCR products were resolved on 4% PAGE using ABI Prism^{TM} 377 DNA sequencer (Applied Biosystems, Carlsbad, USA) using dROX (dichlororhodamine dye) as an internal size standard. Data were analysed using GeneScan analysis software version 3.2.4 (PE Biosystems, Foster City, USA) and Genotyper analysis software version 2.5 (PE Biosystems, Foster City, USA) following manufacturer's instructions. AFLP markers were designated with the name of the respective *Eco*RI and *Mse*I primers used in selective amplification, followed by the allele size. The details of *Eco*RI and *Mse*I primers are given in table 2.

A total of 177 BNL (Brookheaven National Laboratory) SSR primers (sequences available at cotton SSR database;

http://www.cottonmarker.org), were tested to detect polymorphism between the parents, P56-4 and RS2013. Amplification was carried out in 20 μL reaction with 50 ng genomic DNA, 2.5 mM $MgCl_2$, 25 mM dNTPs, 10 μM of each primer and 0.5 units of *Taq* DNA polymerase in 1× *Taq* Buffer A (Bangalore Genei, Bangalore, India). The amplification profile consisted of an initial denaturation at 94°C for 7 min (step 1), followed by 9 cycles (step 2) of 94°C for 15 s, 65°C for 30 s and 72°C for 60 s with touch down by 1°C in each cycle from 65°C to 56°C followed by 40 cycles (step 3) of 94°C for 15 s, 55°C for 30 s and 72°C for 60 s. Final extension cycle was carried out at 72°C for 7 min (step 4). PCR amplification was carried out in MJ Thermal Cycler PTC-200 (MJ Research, Watham, USA). The amplified products were resolved on 3.5% Metaphor Agarose (Cambrex) gel with ethidium bromide. The PCR products were electrophoresed in 1× TAE at constant voltage (80 V) in a Hu 25 horizontal gel electrophoresis system (Severn Biotech, Worcestershire, UK). A 50-bp DNA ladder (0.5 μg/μL, Fermentas, Burlington, USA) was run alongside the amplified products to determine their approximate size. The electrophoresis band patterns were recorded using a gel documentation system (Alpha Innotech, San Leandro, USA).

Data analysis

The bands on auto-generated AFLP output and SSR analysis were scored in a 0-1 binary format (0 for absence, 1 for presence). The binary data were used in regression analysis with the phenotypic data of 13 different traits recorded on 94 F_2 individuals (2006), their corresponding F_3 families (2007) and on the mean of two years data (pooled). F_2 data were obtained from single plant, while the F_3 family data were averaged over replication. The association analysis was carried out using both SLRA and MRA. For SLRA, data on individual phenotypic trait were regressed on whole 0-1 binary marker data for each individual markers using Excel data analysis (regression) programe. MRA was conducted using 'backward' method of 'linear regression analysis' option of SPSS version 13.0. Markers showing significant regression values were considered as associated with the trait under consideration. F_2 individuals were grouped for the traits based on the marker showing association.

Results and discussion

SSR and AFLP polymorphism

Considering their ease of use, we first tested SSR markers for tagging of traits in cotton. However, out of 177 SSR primer pairs tested, only four (2.26%), viz., BNL3537, BNL3463, BNL3502 and BNL840 detected polymorphism between the parents (figure 1). A similar low level (2.97%) of SSR polymorphism was reported by Wang *et al*. (2006) who tested 4106 markers in a *G. hirsutum* RIL population for QTL mapping of fibre quality traits. In fact, low level of intraspecific polymorphism is the major bottleneck in profitable use of SSR markers for mapping and tagging of traits in *G. hirsutum* (Jiang *et al*. 2000; Abdalla *et al*. 2001; Brubaker and Wendel 2001; Lu and Myers 2002; Zhang *et al*. 2005). Further in majority of cases, SSRs can scan only one locus at a time (Liu *et al*. 2000; Shen *et al*. 2005). Therefore, we used AFLP technique that allows examination of several loci in one scan, for further analysis. Out of 50 AFLP primer combinations tested, 32 (64%) gave good amplifications. These 32 primer combinations amplified a total 1505 bands with an average of 47.0 bands per combination (table 2). Representative AFLP output showing polymorphism between the parents is presented in figure 2. Among 32 primer combinations, 28 detected polymorphism between the parents with an average of 12.3 polymorphic bands per primer combination. The proportion of polymorphic bands in these 28 primer combinations ranged from 1.7% (E7M8) to 80.7% (E3M2). The level of AFLP polymorphism detected in this study is comparable to those reported by Lacape *et al*. (2003) and Myers *et al*. (2009). Thus four SSR markers and six AFLP primer combinations (E3M2, E3M6, E3M7, E4M2, E4M7 and E5M6) with a total 272 polymorphic AFLP bands between parents were used to genotype 94 F_2 individuals.

Regression analysis

Segregation of markers was studied in F_2 and only those showing 3:1 segregation were used for regression analysis.

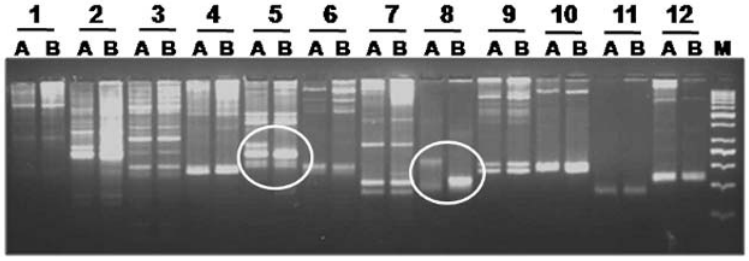

Figure 1. SSR profile of (A) P56-4 and (B) RS2013 with (1) BNL2805, (2) BNL2884, (3) BNL1160, (4) BNL2570, (5) BNL3463, (6) BNL3452, (7) BNL3535, (8) BNL3502, (9) BNL3436, (10) BNL3590, (11) BNL3510 and (12) BNL3511 primers. M 50-bp ladder.

Table 2. Summary of AFLP polymorphism detected between the parents with different primer combinations.

Nomenclature	Primer pairs		Total	Number of bands	
	*Eco*RI	*Mse*I		Polymorphic	Polymorphic%
E1M6	eAAC	mCTC	35	10	28.6
E1M8	eAAC	mCTT	46	12	26.1
E3M1	eAGC	mCAA	30	6	20.0
E3M2*	eAGC	mCAC	62	50	80.7
E3M6*	eAGC	mCTC	63	47	74.6
E3M7*	eAGC	mCTG	83	61	73.5
E4M1	eACA	mCAA	42	10	23.8
E4M2*	eACA	mCAC	31	22	70.9
E4M7*	eACA	mCTG	77	40	51.9
E5M2	eACT	mCAC	30	9	27.0
E5M4	eACT	mCAT	30	2	6.7
E5M5	eACT	mCTA	35	3	8.6
E5M6*	eACT	mCTC	60	42	70.0
E5M7	eACT	mCTG	32	8	25.0
E5M8	eACT	mCTT	25	3	12.0
E6M1	eAAG	mCAA	50	2	4.0
E6M2	eAAG	mCAC	59	0	0.0
E6M3	eAAG	mCAG	60	0	0.0
E6M4	eAAG	mCAT	58	2	3.5
E6M5	eAAG	mCTA	61	2	3.3
E6M6	eAAG	mCTC	55	3	5.5
E6M7	eAAG	mCTG	55	1	1.8
E6M8	eAAG	mCTT	56	0	0.0
E7M1	eACG	mCAA	40	2	5.0
E7M2	eACG	mCAC	30	1	3.3
E7M3	eACG	mCAG	35	0	0.0
E7M4	eACG	mCAT	45	1	2.2
E7M5	eACG	mCTA	40	1	2.5
E7M8	eACG	mCTT	60	1	1.7
E8M1	eAGG	mCAA	35	1	2.9
E8M4	eAGG	mCAT	40	1	2.5
E8M5	eAGG	mCTA	45	2	4.4

*These combinations were used to screen F_2 individuals.

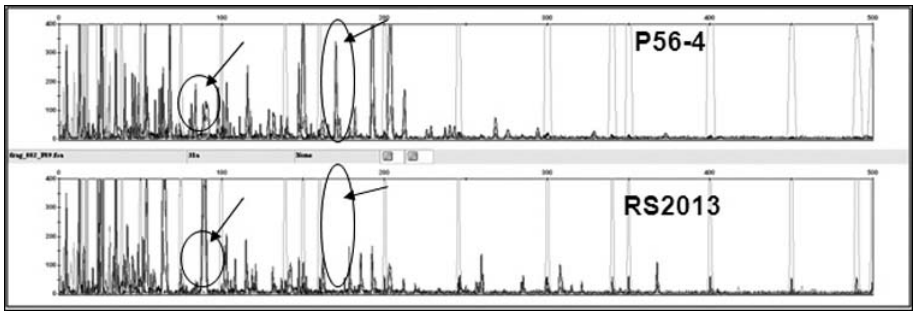

Figure 2. AFLP patterns of P56-4 and RS2013 using mCTG+eACA primer combination.

Thus a total of 92 markers were used in further analysis. Among these 92 markers, 43 were from RS2013 and remaining 49 from P56-4. Deviation from Mendelian segregation is often encountered in several crops including cotton (Ulloa and Meredith 2000; Lin *et al*. 2005). Data of each 13 phenotypic traits were separately regressed on each of the 92 polymorphic markers. Through SLRA, we could identify a total 23 markers associated with eight traits (table 3). Of these, 15 markers were from the parent RS2013 and eight were from P56-4. While most of the markers were associated with a

Table 3. Marker-trait associations identified through simple linear regression analysis in different generations.

Trait	R^2 value (%)			Marker*
	F_2	F_3	Pooled	
PH	9.3	–	4.9	13
	4.7	–	–	10
	4.3	–	–	19
MONO	5.5	–	7.1	18
	–	5.6	5.1	21
	–	4.1	–	19
	–	5.0	–	20
SCY	4.1	4.0	–	4
BW	7.1	–	5.0	2
SL	–	9.7	6.5	15
	–	5.0	3.4	17
	–	6.5	5.7	18
	4.9	–	–	11
	–	6.1	–	5
	–	5.6	–	14
	–	4.7	–	20
MICRON	4.5	–	–	3
	6.6	–	–	8
STR	6.1	8.2	7.2	1
	6.3	–	4.5	9
ELONG	4.0	–	4.0	3
	5.3	–	4.0	12
	–	7.2	3.9	6
	–	1.0	5.6	7
	6.4	–	–	10
	–	6.2	–	16
	–	4.6	–	17
	–	7.2	–	22
	–	–	5.7	23
	–	5.0	–	19

*1, BNL3502; 2, E3M6L21; 3, E3M6L33; 4, E3M6L80; 5, E3M6L111; 6, E3M6L126; 7, E3M6L131; 8, E3M6L132; 9, E3M6L152; 10, E3M7L71; 11, E4M7L18; 12, E4M7L43; 13, E4M7L82; 14, E5M6L17; 15, E5M6L19; 16, E5M6L26; 17, E5M6L31; 18, E5M6L34; 19, E5M6L42; 20, E5M6L55; 21, E5M6L72; 22, E5M6L105; 23, E5M6L114. (The underlined markers are from the parent RS2013).

single trait, each of six markers showed association with two traits. For example, in F_2, marker E3M7L71 was associated with PH and ELONG, and marker E3M6L33 with MICRON and ELONG. Likewise in F_3, marker E5M6L55 was associated with MONO and SL, E5M6L31 with SL and ELONG, and E5M6L42 with MONO and ELONG. Marker E5M6L55 had a positive effect on MONO but negative effect on SL. In F_2, 12 markers were found to be associated with eight traits namely, PH, MONO, BW, SCY, MICRON, ELONG, SL and STR, whereas in F_3, we detected 14 markers associated with five traits viz. MONO, SCY, ELONG, SL and STR. Use of pooled data (i.e. F_2 and F_3) led to detection of 13 markers associated with six traits. Thirteen markers were detected to be associated with the same trait in different generations (F_2, F_3 or pooled), which indicated consistency of their association. The SSR marker BNL3502 was found to be associated with fibre strength in F_2, F_3 and pooled analysis, and explained on an average 7.1% of the variance for fibre strength. The phenotypic variance explained by a single marker for any trait ranged from 1.0 to 9.7. Maximum number of markers (10) was associated with ELONG followed by SL (7) and MONO (4). Only one marker each was associated with BW and SCY.

Stepwise backward multiple regression analysis for 13 traits were carried out using 92 markers. A total of 30 markers, 17 belonging to the parent RS2013 and 13 belonging to P56-4 were found to be associated with at least one of the 13 traits (table 4). These included all the markers that were detected in SLRA. Thus MRA identified more markers than SLRA and found marker-trait association for all the 13 traits studied. The number of markers showing significant association with individual traits varied between generations and ranged from 1 to 14 (table 4). A maximum of 14 markers were associated with SL. The markers could explain 3% to 41% variance for individual traits. A higher R^2 values were obtained for traits SI, SCY, SL and UR. Marker E3M6L33 associated with SI had a positive contribution to the trait. Presence of this marker increased value of SI. For eight traits namely, SCY, BW, SI, SL, UR, MICRO, STR and ELONG, 1 to 6 markers were consistently detected in F_2, F_3 and pooled analysis which suggested their close association with traits.

Heritability of a trait has a bearing on consistency of marker-trait association over generations. If the heritability is low, marker-trait associations may not be found consistently. On the other hand, a consistent association of a marker with a trait suggests that the associated QTL influencing the trait is less affected by the environment. Hence, markers showing association with a trait over years are sought for marker-assisted selection. Several studies have reported high broad-sense heritability for fibre quality traits such as UR, MICRO, SL, STR etc. (Hendawy *et al*. 1999; Ulloa 2006). We also found high broad-sense heritability (> 0.8) for STR, UR, SL, SI, MICRO and ELONG (data not shown). Marker-trait associations were found more often for these traits than other traits with low heritability such as PH, MONO, SYMPO, BW and SCY. A maximum of seven common markers were associated with SI in both F_2 and F_3 , followed by UR (six markers), STR (four markers), SL and MICRON (three markers each). These markers which account for a significant proportion of variance for the respective traits are of potential value for marker-assisted breeding.

Majority of the markers were found to be associated with more than one trait, particularly in MRA. Such an association may arise due to pleiotropic effect of the linked QTL on different traits (Miller and Rawlings 1967; Meredith and Bridge 1971; Culp *et al*. 1979). Closely linked QTLs affecting different traits may also lead to a single marker showing association with multiple traits which would be reflected in correlations between such traits. In cotton, several studies have detected correlations between agronomic traits and fibre quality, particularly fibre strength and lint yield, and also among various fibre quality measurements

Table 4. Marker-trait associations detected in different generations through multiple regression analysis.

Trait	Generation	Associated markers*	R^2 (%)
PH	F_2	9, 10, 13, **19**, 22	20.0
	F_3	6, 8, 15, 16, 17, **19**, 20, 21, 23, 29	33.0
	Pooled	13, 16, 21, 22, 27	18.0
MONO	F_2	8, 15, 18, 19, 27	16.0
	F_3	2, 6, 7, 12, 16, 21 24, 28	24.0
	Pooled	6, 7, 12, 21	19.0
SYMPO	F_2	7, 13, 16	13.0
	F_3	10, 14, 13	10.0
	Pooled	10, 12	8.0
SCY	F_2	**4**, 5, 6, 9, 10, 16, 17, 18, **23**, 25	39.0
	F_3	**4**, 15, 19, 22, **23**, 24, 26, 29	24.0
	Pooled	9, 10, 15, 16, 19, 22, 23, 24, 26, 29	31.0
BW	F_2	2, 24, 29	14.0
	F_3	16, 30	6.0
	Pooled	2, 24, 29	13.0
GOT	F_2	11	3.0
	F_3	2, 4, 9, 10, 13, 16	19.0
	Pooled	2, 13, 16	12.0
SI	F_2	**3, 4, 8, 11, 27, 28, 29**	39.0
	F_3	**3, 4, 8, 11, 27, 28, 29**	36.0
	Pooled	3, 4, 8, 12, 6, 27, 29	37.0
SL	F_2	**2**, 4, 11, 12, 13, **18, 21**, 22, 23, 24, 28	41.0
	F_3	**2**, 5, 6, 16, **18, 21**	19.0
	Pooled	2, 6, 10, 11, 12, 13, 15, 16, 18, 21, 22, 23, 24, 28	37.0
UR	F_2	**3, 4, 8**, 10, **11, 27, 28**	28.0
	F_3	**3, 4, 8**, 9, **11**, 14, 17, **27, 28**	35.0
	Pooled	3, 4, 8, 9, 11, 14, 17, 27, 28	35.0
MICRON	F_2	3, **4, 8**, 10, 11, 27, **28**, 29	31.0
	F_3	**4**, 6, **8**, 23, 26, **28**	19.0
	Pooled	3, 4, 6, 8, 23, 26, 28	26.0
STR	F_2	**1, 9, 12**, 23, **27**, 28	16.0
	F_3	**1**, 3, 7, 8, **9**, 11, **12**, 13, **27**, 29	39.0
	Pooled	1, 3, 7, 9, 27	20.0
ELONG	F_2	3, **7**, 10, 12, 29	22.0
	F_3	**7**, 13, 22, 23	22.0
	Pooled	3, **7**, 12, 13, 18, 20, 23	27.0
LI	F_2	4, 6, 8, 10, 14, 18, 20	18.0

*Details of markers 1–23 are given in table 3 and the rest are as follows: 24, E3M2L74; 25, E3M6L9; 26, E3M6L135; 27, E3M6L156; 28, E4M7L28; 29, E5M6L6; 30, E5M6L43 (markers found in both F_2 and F_3 are shown in bold and those underlined in the list are from the parent RS2013).

(Miller and Lee 1964; Ulloa 2006; Kantartzi and Stewart 2008). In the present study also, we found significant correlation between various agronomic and fibre quality traits (table 5). For example, STR showed significant positive correlation with SL, UR, ELONG, MICRON, PH, MONO, LI, BW and SI. This correlation was also evident in shared associated markers for these traits. For example, in MRA, seven common markers were found to be associated with MICRON, STR and SI. Kantartzi and Stewart (2008) found BNL1030 associated with QTLs for ELONG, MICRO and maturity. Similarly, He *et al*. (2007) reported six QTLs on chromosome 14 for lint yield, seed index, elongation and micronaire. It is noteworthy that the BNL3502 which was detected to be associated with fibre strength in the present study is also located on chromosome 14. Clustering of QTLs for different fibre quality traits is reported in several studies (Ulloa and Meredith 2000; Zhang *et al*. 2005; Wang *et al*. 2006).

This study has identified one highly reliable SSR marker BNL3502 linked with fibre strength which can be used in breeding for high fibre strength. Other markers such as E3M7L71, E5M6L34, E5M6L72, E3M6L33, E4M7L82 and E5M6L42 which are consistent and shared by different

Table 5. Correlation matrix of morphological traits, fiber quality and yield in cotton.

	SL	UR	MICRO	ELONG	SCY	PH	MONO	SYMPO	LI	GOT	BW	SI
SL	1.00											
UR	0.32	1.00										
MICRO	0.08	0.03	1.00									
ELONG	0.76**	0.68**	0.10	1.00								
SCY	−0.31	0.17	−0.05	−0.10	1.00							
PH	0.45	0.39	0.63**	0.55*	−0.33	1.00						
MONO	0.02	0.51*	0.12	0.28	0.02	0.45	1.00					
SYMPO	−0.55*	0.23	0.31	−0.21	0.65**	−0.02	0.51*	1.00				
LI	0.88**	0.09	0.08	0.51*	−0.23	0.28	−0.13	−0.57*	1.00			
GOT	−0.43	−0.74**	−0.09	−0.73**	0.18	−0.62**	−0.51*	0.10	−0.20	1.00		
BW	0.51*	0.37	0.48*	0.66**	−0.05	0.73**	0.53*	0.11	0.42	−0.52*	1.00	
SI	0.91**	0.40	0.14	0.74**	−0.29	0.54*	0.11	−0.52*	0.88**	0.63**	0.57*	1.00
STR	0.89**	0.63**	0.02	0.87**	−0.18	0.51*	0.39	−0.29	0.73**	−0.68**	0.69**	0.64**

*Significant at $P = 0.05$, **significant at $P = 0.01$.

quality fibre traits may also be relevant for breeding quality cotton varieties. However, they need to be converted to more readily usable markers such as SCARs. In cotton SSR marker polymorphism is found mostly at the interspecific level but their utility in intraspecific level appears to be limited. However, breeding work generally involves intervarietal crosses. Hence there is an urgent need for identification of new SSR markers suitable for detecting polymorphism at the intraspecific level. Further, most of the QTL studies in cotton have used interspecific crosses. However, as QTL detection depends on genotype background, the significance of such QTLs in standard *G. hirsutum* backgrounds needs to be validated. Further, correlations among traits appear to be due to clustering of QTLs and molecular markers will be critical for breaking undesirable linkages between quality and yield related traits.

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