Full Length Research Paper

Inheritance studies and quantitative trait loci (QTLs) linked to fibre strength in upland cotton

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Field experiment was conducted during 2005 to 2008 at Indian Agricultural Research Institute (IARI), New Delhi to study genetics of fibre strength in *Gossypium hirsutum* L cotton. Genotypes 'P 56-4' and 'RS 2013' were used to develop 6 generations. Additive × dominance epistasis was found significant. The F_2 of this contrasting cross showed wide range of variation from 18.1 to 33.1 g/tex for fibre strength. Distribution of F_2 population also indicated transgressive segregation for fibre strength. This F_2 population was also used to identify quantitative trait loci (QTLs) linked to fibre strength using Amplified fragment length polymorphism and single sequence repeats (SSR) markers. Linkage map was constructed covering a total of 932.9 cM. The average distance between adjacent markers was 10.11 cM. Six QTLs designated as QTFS-2A.1 to QTFS-2A.6 were detected which explained 9.24 to 36.03% of the phenotypic variation.

Key words: *Gossypium hirsutum,* generation mean, fibre strength, quantitative trait loci (QTLs), amplified fragment length polymorphism (AFLP), single sequence repeats (SSR).

INTRODUCTION

Cotton is an important commercial crop in India and it plays a key role in our national economy and accounts for about 75% of total production of the natural fibers. Amongst the 4 cultivated species, *Gossypium hirsutum* occupies largest area and accounts for about 90% of total cotton production globally. During 2011 to 2012 total cotton production in India was about 35.3 million bales (170 kg each) from an area of about 12.18 million ha and the national average was 493 kg lint/ha. Cotton productivity in India has been very low as compared to the major cotton producing countries of the world and was also lower than the world average of about 750 to 800 kg lint /ha.

Cotton fibre is becoming increasingly important issue in modern textile industry (Muhammad et al., 2008). Adoption of modern ring/open-end spinning system by textile industry has increased demand for high fibre strength cottons as pulls and pressures of high speed ring spinning system reduce the yarn strength by about 20 to 25% during processing (Sreenivasan and Venketakrishnan, 2007). Shen et al. (2005) are also of the opinion that, the present day spinning technology lays more emphasis on fibre strength rather than fibre length and fineness. Fibre quality of cultivar includes fibre length, fibre strength, fineness, maturity and fibre elongation. These individual traits are important for

spinning, weaving and dyeing, etc. (Muhammad et al. 2008). Yarn strength is very important to yarn and textile manufacturers. Most of the presently developed *G. hirsutum* cultivars in India have low fibre strength. Genetic improvement of fibre quality is therefore important to meet the requirements of growing textile industry.

Proper understanding of nature of gene action for yield and fibre quality parameters is therefore important to exploit the available genetic variability. The estimates of components of variance also have a strong bearing on the choice of breeding methods to be followed to achieve the objectives of a breeding programme. Studies have been conducted by various researchers to estimate the nature and magnitude of gene effects in the inheritance of fibre quality traits and both additive and non additive gene effects have been reported (Patel et al., 2007; Asif et al., 2008, Singh et al., 2008; Hussain et al., 2010).

DNA-based molecular markers hold great promise in breeding for quantitative traits like fibre quality (Gupta, 2006: Zeng et al., 2009; Boopathi et al., 2011). Among the DNA markers, amplified fragment length polymorphism (AFLP) and single sequence repeats (SSR) are PCR based markers and may be used for diversity studies, fingerprinting, gene mapping and identification of Quantitative trait loci (QTLs). AFLPs are multi locus and dominant markers, where as SSRs or microsatellites are codominant and easy to use. Both these markers have been widely used in *G. hirsutum* to construct linkage maps and in QTL analysis (Paterson et al., 2003; Shen et al., 2007; Zeng et al., 2009). The present investigation was therefore, taken up to estimate gene effects for fibre strength by using generation mean analysis and to identify QTLs linked to fibre strength.

MATERIALS AND METHODS

Development of six generations

G. hirsutum cottons 'P 56-4' and 'RS 2013' were used as contrasting parents for developing 6 generations, which are: P_1, P_2, F_1, F_2, BC_1, and BC_2. 'P 56-4' is high fibre strength (27.8 g/tex) line developed at Indian Agricultural Research Institute (IARI), New Delhi from the cross 'Bikaneri Narma × Pusa 734'. Variety 'RS 2013' was developed by Agricultural Research Station, Sri Ganganagar, Rajasthan from a three way cross 'RS 20 × (LH 511 × Bombesa)'. It was released for commercial cultivation in Rajasthan state. Its fibre strength was 19.3 g/tex. Crosses were made between 'P 56-4' (P_1) and 'RS 2013' (P_2) during 2005 to obtain F_1. During 2006 to 2007 the F_1 (P 56-4 × RS 2013) was advanced to F_2 generation through selfing and crosses were also made to obtain BC_1 and BC_2 generations, besides making 'P56-4' × 'RS 2013' crosses to obtain F_1. The 6 generations P_1, P_2, F_1, F_2, BC_1, and BC_2 were evaluated in a field trial during 2007 to 2008 at research farm of IARI, New Delhi. Parents (P_1 and P_2) and F_1 were planted in 2 row plots; BC_1 and BC_2 in 4 row plots and F_2 in 8 row plots with 3 replications. The row length was 4.2 m accommodating 15 plants spaced 30 cm apart. The row-to-row spacing was 75 cm.

All the recommended agronomic and plant protection practices were followed to raise a good crop. Data were recorded on randomly selected 10 plants each in parents (P_1 and P_2) and F_1; 20 plants each in back cross generations (BC_1 and BC_2) and 40 plants in F_2 generation in each replication. Observations were recorded on fibre strength by using fully automatic 'High Volume Instrument' (HVI). The generation means were calculated by taking the average over all the replications for each generation. To test the adequacy of the additive-dominance model, the individual scaling tests given by Mather (1949) as well as joint scaling test by Cavalli (1952) were used. All possible models with different combinations of epistatic parameters were tried to identify the best fit model with minimum non-significant value of the chi-square and maximum number of significant parameters as suggested by Mather and Jinks (1982).

Molecular studies

The strain 'P 56-4' showed mean fibre strength of 27.8 g/tex., 2.5% span length of 28.4 mm and micronaire of 3.9 during 5 year period from 2003/2004 to 2007/2008 and was used as high fibre strength parent. In contrast the variety 'RS 2013' showed mean fibre strength of 19.3 g/tex, mean 2.5% span length of 25.4 mm and micronaire value of 4.4 during 2005 to 2007 and was used as low fibre strength parent. For developing mapping population 'P 56-4' and 'RS 2013' were crossed in 2004 to generate F_1 generation, which was advanced to F_2 generation during 2005. The F_2 generation was evaluated during 2006 at IARI, New Delhi. 299 randomly selected single plants from F_2 were phenotyped for fibre strength with fully automatic HVI.

The F_2 population obtained from the contrasting cross 'P 56-4' x 'RS 2013' was used as mapping population to detect QTLs for fibre strength using AFLP and SSR markers. High quality genomic DNA was isolated from young leaves of individual F_2 plants. Extracted DNA was subjected to AFLP and SSR analysis. AFLP analysis was done following Vos et al. (1995) technique in principal. A total of 50 AFLP primer combinations were tested. Data were analysed using Gene Scan 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. A total of 177 Brookheaven National Laboratory (BNL) SSR primers (http.//www.cotton marker.org) were used to detect polymorphism between two parents. 13 AFLP primer combinations showed polymorphism at 33 loci and 4 SSR primers detected polymorphism at 7 loci. 94 F_2 individual plants were genotyped using the above identified polymorphic AFLP and SSR primers. Genotypic data of AFLP and SSR were used to construct molecular genetic maps. Linkage map was constructed using 250 loci MAPMAKER v. 2.0 programme.

RESULTS AND DISCUSSION

Gene effects for fibre strength

The results on mean performance, scaling tests, joint scaling test and best fit model are given in Table 1. The scaling test for fibre strength detected the presence of epistasis as the values for at least one or more scales 'A' and 'B' were significant. After the scaling test failed, the joint scaling test with simple additive-dominance model consisting of mean 'm', additive component [d] and dominance component [h] was tried. Adequacy of the model was tested by the chi-square test.

Significant chi-square value suggested the presence of epistasis. Significance of different components detected by 3 parameters model for fibre strength is of little value

Table 1. Genetic analysis of fibre strength in 6 generations in the cross 'P 56-4' x 'RS 2013' during 2007.

Mean performance					
'P 56-4' (P_1)	'RS 2013' (P_2)	F_1	F_2	BC_1	BC_2
28.7±0.3	21.3±0.2	25.1±0.4	24.9±0.3	25.4±0.4	25.1±0.4
Scaling test					
A	B	C	D		
3.02**±0.55	-3.84**±0.49	0.73±0.82	-0.77±0.43		
Joint scaling test					
m	[d]	[h]	χ^2		
24.96**±0.10	-3.35**±0.10	0.28±0.22	120.88**		
Best fit model					
m	[d]	[h]	[i]	[j]	[l]
24.87**±0.15	0.26±0.31	1.60±0.91	1.55±0.87	-6.86**±0.64	-2.36±1.47

due to inadequacy of the model. When this model failed to explain variation among generation means, successively non-allelic parameters, that is, additive × additive [i], additive × dominance [j] and dominance × dominance [l] were included in the model. The best fit model showed parameters m and [j] (additive × dominance type of epistatsis) as significant for fibre strength. The involvement of additive, non additive and /or epistatic effects in different yield components and fibre quality characters has also been reported by other workers (Ahuja et al., 1999; Patel et al., 2007, Asif et al. 2008, Muhammad et al 2008, Hussain et al. 2010). Singh et al. (2008) have also reported that, additive as well as non additive gene effects were important in the inheritance of seed cotton yield, boll number, boll weight, ginning outturn and 2.5% span length. They also reported that, magnitude of gene effects was different in different crosses. It is therefore, important to select specific breeding strategy for a particular cross to achieve set objectives. The epistatic effects involved in the inheritance of fibre strength as found in the present study may be usefully exploited through hybrid vigour.

Mapping population and molecular studies

299 single plants randomly selected from F_2 population of the cross 'P 56-4' × 'RS 2013' showed wide variation for fibre strength ranging from 18.1 g/tex to 33.1 g/tex. The average fibre strength of 299 plants in F_2 generation was 26.6 g/tex. The distribution of fibre strength in F_2 generation is given in Figure 1. The distribution in F_2 was little skewed towards high fibre strength. As many as 76.5% plants showed fibre strength above 25 g/tex, and 21 plants (7.0%) showed above 28 g/tex which was higher than that of the better parent indicating

transgressive segregation for the trait. Wide range of variation and transgressive segregation observed for fibre strength and other traits may be utilized for combining high yield and superior fibre quality. Percy et al. (2006) also reported transgressive segregation for high and low lint percentage, plant height and fibre strength. They further reported that, 20% lines possessed fibre strength equivalent to high fibre strength parent while 4% exceeded high strength parent.

The F_2 population of the contrasting cross 'P 56-4' × 'RS 2013' was used as mapping population to study detect QTLs for fibre strength using AFLP and SSR markers. Out of 177 SSR primer pairs tested, only 4 (2.26%), which are:. BNL 3537, BNL 3463, BNL 3502, and BNL 840 detected polymorphism between the parents. A similar low level (2.97%) SSR polymorphism was reported by Wang et al. (2006). A total of 103 loci (101 AFLPs and 2 SSRs) was mapped on 8 linkage groups. Length of the linkage groups ranged from 32.8 to 192.0 cM. The remaining 147 markers were found to be unlinked. The resulting linkage groups were numbered 1A to 8A. The map covered a total of 932.9 cM and the average distance between adjacent markers was 10.11 cM. Six QTLs designated as QTFS-2A.1 to QTFS-2A.6 were detected on chromosomes 2A and 3A which explained 9.24 to 36.03% of the phenotypic variation (Table 2 and Figure 2). Mapping populations derived from inter-specific crosses have been used more frequently rather than those derived from intra-*hirsutun* crosses (Preetha and Raveendren, 2008). Ulloa and Meredith (2000) and He et al. (2007) each reported 3 QTLs for fibre strength. Although QTLs have been identified for several yield and fibre quality traits in cotton, nonetheless only a few stable and common QTLs have been reported till now (Preetha and Raveendren, 2008). More studies are therefore needed to identify stable QTLs for use in

Table 2. LOD values, additive values and phenotypic value for fiber strength.

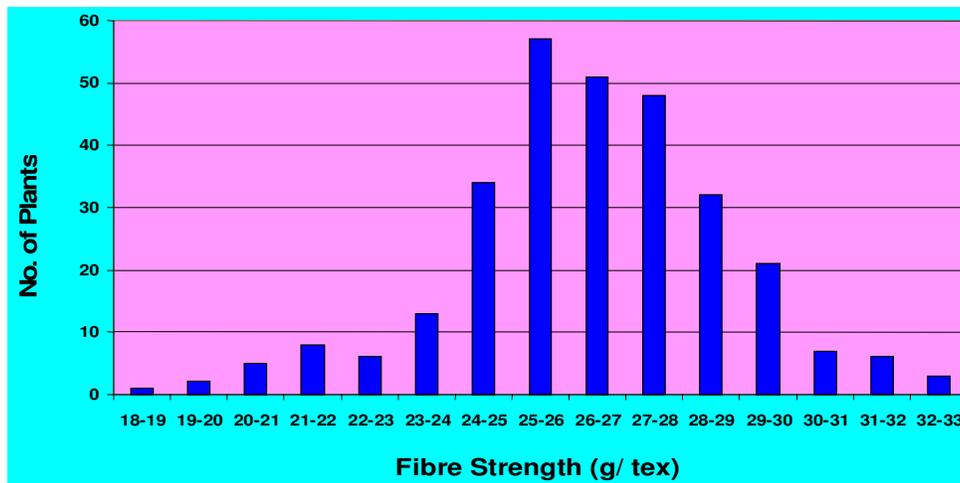| Linkage map	Position	Marker intervals	QTLs	Position (cM)	LOD	A	PVE(%)	Treatment
2A	15	XM63-XM64	QTFS-2A.1	101.61	1.7	-2.1	9.24	T1
			QTFS-2A.2	99.61	3.33	-1.81	12.71	T2
			QTFS-2A.3	99.61	3.56	-2.19	14.66	T3
3A	7	XM97-XM98	QTFS-3A.4	77.81	8.36	-8.92	36.03	T2
			QTFS-3A.5	79.81	2.53	-5.2	19.05	T3
	8	XM98-XM99	QTFS-3A.6	82.01	7.5	-8.92	36.03	T2

Figure 1. Frequency distribution of 299 single plants for fibre strength in F_2 generation.
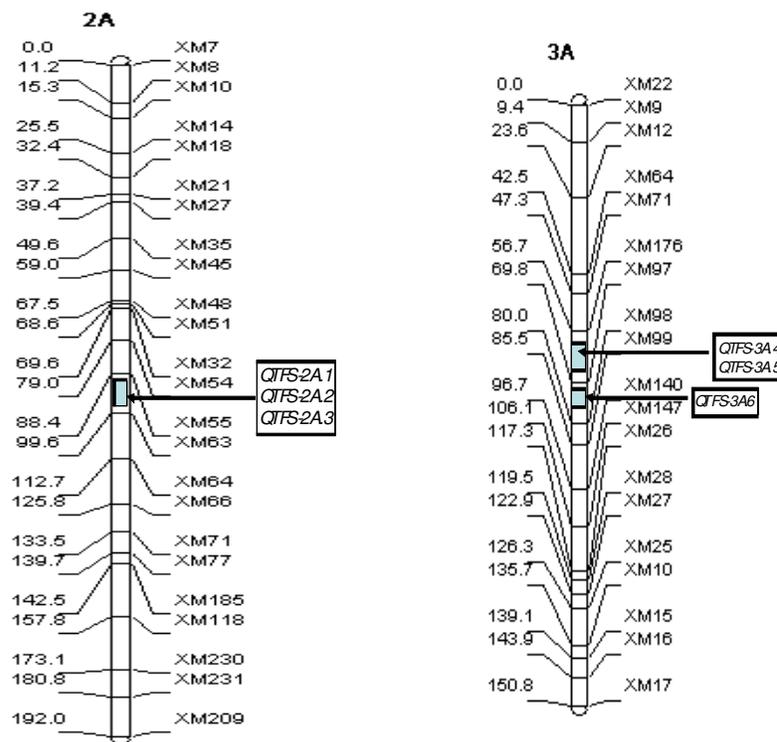
Figure 2. Linkage map showing QTLs for fiber strength.

molecular breeding in cotton.

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