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Molecular characterization in F₂ segregating population for improving fibre properties of cotton (*Gossypium hirsutum*)

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Abstract

“Molecular characterization of F₂ segregating population for fibre properties of cotton” was attempted using phenotypically diverse parentages from Dr. PDKV, Akola and their molecular characterization for fibre quality parameters using simple sequence repeat (SSR) markers. Two F₂ populations were developed [cross-I (PKV-Rajat x MCU-5) and cross-II (AKH-081 x MCU-5)]. Staple length (SL) and staple strength (SR/Sst) were recorded on selected 40 F₂ populations in both the crosses. Polymorphism was observed for SL and SR/Sst in both the populations. In case of molecular markers studied a total of 64 SSR markers found good polymorphic between the parents on 26 chromosomes out of 116. The polymorphism *percent* varies from chromosome to chromosome. Highest was found on chromosome 22, 23 and 24 (100%), whereas lowest was obtained on chromosome 13 and 20 (25%). The range of polymorphism was 25 to 100%. Tri-nucleotide repeats of microsatellite markers found polymorphic than di and polynucleotide. 12 SSR markers were consistently linked with different traits studied. Three SSR NAU769, BNL2656 and BNL226 linked with number of staple length/plant on chromosome 3, with LOD 2.61-2.62, PVE 0.15-19.52%. Two SSR BNL2920 and BNL3920 were linked with staple strength on chromosome 15, with LOD 7.54-3.00, PVE 0.69-6.18%.

Keywords: Cotton, Fibre quality, hirsutum, microsatellite, SSR, seed cotton yield

Introduction

Cotton (*Gossypium spp.*) is the most preferred natural fibre in the world and plays a major role in the economy of agriculture and industry. In case of yield enhancement of lint, fibre qualities i.e. staple length, staple strength and micronair are very important for crop improvement.

Cotton breeders have traditionally relied on lengthy methods to breed cotton varieties with high-quality fibre. Current genetic information and plant breeding methods cannot lead to quick improvement of fibre quality to meet the rapid advances in the textile industry to ensure high productivity. In traditional breeding to develop superior quality fibre properties, the quality of fibre could determined only after harvesting and testing of the fibre. As a result, it is difficult, expensive and time consuming to develop cotton cultivars with superior quality fibre by these methods. Technological advancements in the field of genetics and plant breeding have provided the breeders with new tools. DNA-based molecular markers hold great promise in breeding for qualitative traits like fibre quality (Gupta 2006, Zeng *et al.* 2009, Boopathi *et al.* 2011)^[1]. Among the DNA markers ‘microsatellite’ also known as ‘Simple Sequence Repeats’ (SSRs), is an ideal polymerase chain reaction (PCR)-based DNA marker, which has played an important role in the dramatic progress of cotton genetics and genomics. The development of abundant cotton SSR markers has stimulated more efforts in molecular characterization of cotton germplasm around the world (Blenda *et al.* 2006).

SSR markers have been used as tools in genotype identification and variety protection, seed purity evaluation, germplasm characterization, diversity studies, gene and quantitative trait locus (QTL) analysis, pedigree analysis and marker assisted breeding. Being both co-dominant and multi-allelic, microsatellites are highly reproducible and informative genetic markers (Morgante *et al.* 2002, Turkoglu *et al.* 2010)^[2].

Materials and methods

In the present experiment fibre property was the prime objective in which F₂ plants showing promising results of fibre qualities were only advanced for further morphological and molecular analysis. Molecular markers allow breeders to dissect complex traits without having to measure the phenotype every time, thus reducing the need for extensive field testing over time and space and avoid unreliable phenotypic assays and accelerate accurate identification

of genotypes. Therefore cross-I and cross-II giving good results for fibre properties were used and advanced for further evaluation and Cross III and IV showing poor results for quality was not used in further study. The other purpose for advancement of first two crosses were that maximum exploitation of the promising parents were obtained and found in cross-I and II, whereas cross number III and IV are intraspecific *hirsutum* cross involving narrow genetic base of the parents, resulting into low level of variability among the F₁ and F₂ populations. Hence it was worthwhile to use and advance first two crosses to capture maximum variability and genetic information involving parents PKV Rajat and AKH-081 with superior parent of fibre quality MCU-5. This was resulted into saving of the resources and laboratory expenses

required for the genotyping of the large F₂ population. In Cross-I and Cross-II 40 transgressive segregants were selected on the basis of low, medium and high categories as proposed by Singh *et al.* (2004)^[3].

A segregating population consisted of 40 F₂ populations from both the crosses between i.e. PKV Rajat x MCU-5 and AKH 081 x MCU-5 the genetic. The F₂ plants were maintained in the field as living specimens to produce seed, fiber, and leaf tissue for this molecular characterizations. The parental genotypes for experimental study populations are used for fibre properties in cotton were obtained as experimental material during the present study with a view to developed advance generations.

Table 1: Following diverse parents are used to generate different crosses.

Particulars	MCU – 5	PKV Rajat	AKH 8828	AKH 081
Staple length (mm)	Long 31-32	Short 22-24	Long 29-30	Short 23-24
Strength (g/text)	30-32	26-27	29-30g	27- 28

Using these popular and diverse genotypes for fibre and agronomical traits were used as parental for attempting the crosses, accordingly based on their genetic diversity of trait of interest (fibre and yield) were used to make the F₁ crosses (Table: 1). The F₂ population was obtained by selfing the F₁ population. The plant selection was carried out on the basis of fibre properties recorded in F₁ and F₂ generations.

Measurement of fiber quality properties

Individual cotton plants usually do not produce enough fiber for routine fiber measurements. To overcome the shortage of fiber, individual F₂ plants were grown as perennials in the field and fiber samples large enough for analysis were obtained over multiple reproductive cycles and fiber measurements from field. Sampling of fibers over time from the greenhouse does not present a greater problem than any other form of replications in space. Bundle fiber quality properties for fiber strength, length, and fineness of the F₂ lint samples were determined by stelometer methodologies. The stelometer method of measurement was used over high volume instrumentation (HVI) methods to provide more precise measurements.

Assays of DNA markers

Cotton DNA was isolated from fresh young leaves. High levels of polyphenolics and other secondary compounds in cotton cells were removed with cetyltrimethyl-ammonium bromide (CTAB). Purified genomic DNA was first evaluated for quality and quantity, and then used for analyses of two marker systems. Total 64 SSR primers were used for the present study. The synthesis of following primers was done from Genaxy Scientific Pvt. Ltd., India. The primers used for amplification of the DNA with their sequences that gave satisfactory amplification and band resolution were chosen for the study.

Results and Discussions

The LOD and threshold curve showing the association of SSR markers linked with staple length of F₂ population of cross-I (PKV Rajat × MCU 5), where SSR maker BNL3535 and BNL 3347 located on chromosome number 19 on left and right side of the sequence regions exhibiting staple length at 187.50cM with 3.52 LOD and PVE% 1.30, hence the region between these two markers are associated with the fibre length in cotton and another SSR maker BNL4024 and BNL

3502 located on chromosome no 14 on left and right side of the sequence regions exhibiting staple length at 187.49 and 261.76 chromosomal region. In cross-II (AKH-081 × MCU5) only one associated marker was shown on chromosome number 3 at 185.4cM position with the markers BNL2656 on left side and BNL226 on right side with LOD 2.62 and total phenotypic variance expression to the tune of 19.52 % for staple length.

Similar findings observed by Rong *et al.* (2008) while studying quantitative trait loci for fibre quality observed on seven Dt chromosome or the linkage group chromosome numbers 14, 17, 20, 22,23 had found more fibre related QTL than their homologous partners, with the largest difference between linkage group D08 (21 QTL) and Chr.4/5 (8 QTL). Similarly in present study the fibre quality trait marker was linked on Chr 14 and 23 for fibre length and strength trait. Rakshit *et al* (2010) showed that the similar finding for yield and fibre quality trait with AFLP and SSR markers using F₂ and F₃ population for Marker-trait associations were studied for eight agronomic and five fibre quality traits through simple and multiple regression analysis. Simple linear regression analysis identified 30 markers for at least one of the 13 traits. They observed SSR marker BNL 3502 was consistently identified to be in association with fibre strength. In this study BNL 840, BNL 3502 showed the association for monopodia, staple length, staple strength and no of boll.

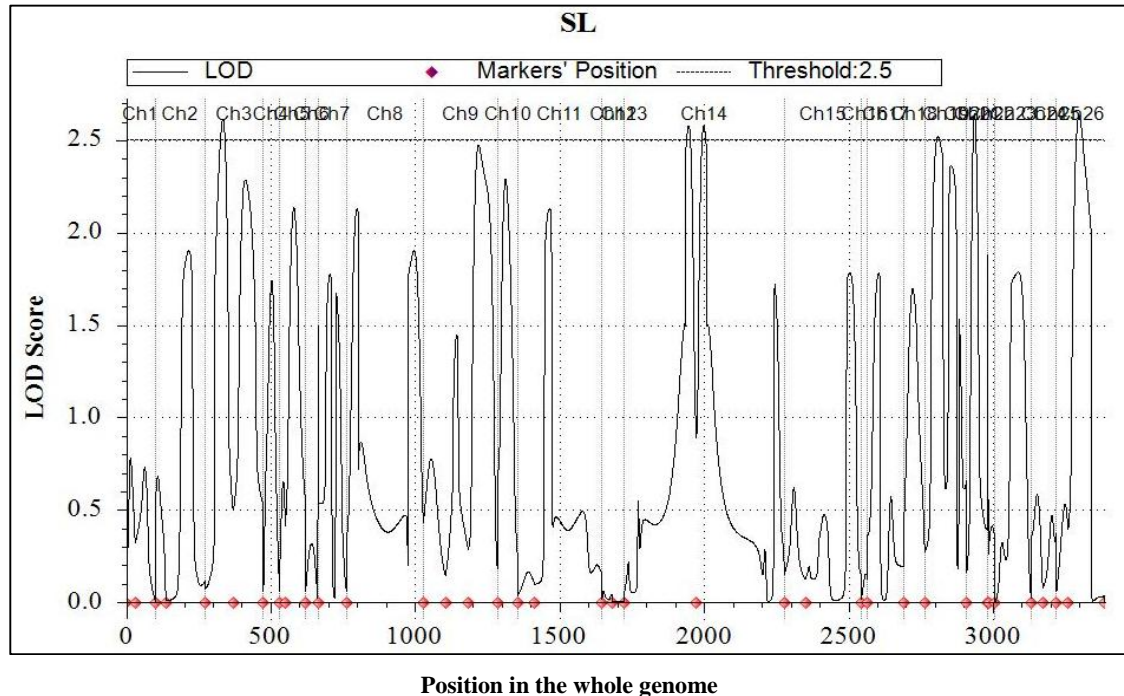
Similar study was done by He *et al* (2008), they developed recombinant inbred lines of population consisting of 121 F₆ RIL crossing *Gossypium hirsutum* cv Handan 208 and *G. barbadense* cv Pima 90 via single- seed descent. Three significant loci were found for fibre length at BNL3646 on chromosome no 20 and BNL 1404 on linkage group D02. The most significant in association that explained 10% of the variance and was associated with the long fibre allele from Pima 90.

Staple strength The association of SSR markers linked to staple strength of F₂ population of Cross-I (PKV Rajat × MCU 5), where the five makers BNL3580 and BNL 2440, NAU1200 and BNL 3569, NAU 4024 and BNL3502, BNL2920 and BNL3920, BNL3031 and BNL 840, located on chromosome no 2, 5, 14, 15, 26 respectively on left and right side of the sequence /regions exhibiting fibre strength. Hence the regions between these two markers are associated with the fibre strength.

In cross-II (AKH081 × MCU-5) for the trait of staple strength on chromosome no 15 and 17 showed the association on 269.7cM and 128.1cM location with the association with marker BNL2920 on left marker BNL3920 right marker with LOD 3.00 total phenotypic variation expression was 6.18 and another BNL2471 left marker and BNL3590 right marker with LOD 2.76 with PVE % 6.12.

Rakshit *et al* (2010) showed the similar findings for yield and fibre quality trait with AFLP and SSR markers using F₂ and

F₃ population for marker-trait association were studied for eight agronomic and five fibre quality traits through simple and multiple regression analysis. Simple linear regression analysis identified 30 markers for at least one of the 13 traits. They observed SSR marker BNL 3502 was consistently identified to be associated with fibre strength. In this study BNL 840, BNL 3502 Show the associated for monopodia, staple length, staple strength and no of boll.



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