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Molecular Characterization of Cotton (*Gossypium hirsutum* L.) Genotypes Using SSR Markers for Fiber Quality Traits

D. K. Sarode[1*], K. M. Sharma[1], N. A. Shinde[1], A. R. Gaikwad[2], R. L. Chavhan[1] and P. A. Pimpale[1]

[1]Vilasrao Deshmukh College of Agricultural Biotechnology, Latur, (M.S.), (Under Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani), India
[2]Cotton Research Station, VNMKV, Nanded, MS, India

**Corresponding author*

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A B S T R A C T

To assess the genetic diversity and relationships among 25 cotton (*Gossypium hirsutum* L.) genotypes were analyzed using morphological and molecular markers. Morphological analysis was performed based on 3 fiber quality traits including fiber length, strength and fineness the mean values of each trait for each genotype were calculated. The results showed that most of the genotypes have long fiber length, very high fiber strength and average fiber fineness. The molecular profiling of 25 cotton genotypes were carried out by using 42 SSR markers reported to be linked to quantitative trait loci (QTLs) for fiber quality traits (Fiber length, Fiber strength and Micronaire). Twenty five SSR markers related with fiber quality traits ware found to be polymorphic produced a total of 53 amplicon with an average of 2.12 alleles per locus ranging from 130 bp to 370 bp products, Markers showing 100% polymorphism with average polymorphism information content (PIC) value of 0.290. The BNL-1059 (PIC-0.481), CIR-244 (PIC-0.538), CIR-354 (PIC-0.660), CIR-381 (PIC-0.784), CIR-246 (PIC-0.749) and JESPR153 (PIC-0.639) marker were found to be most informative which indicates significantly diversity between all genotypes for fiber quality traits. Similarity matrix was used to depict dendrogram through UPGMA cluster analysis based on SSR similarity coefficient ranged from 0.61 to 0.91 indicating the fact that large proportions of genotype were dissimilar. The present study revealed that genotypes BN-1, PH-348, and ARB-908 for fiber quality were found to be the most diverse having a broad genetic base for the concerned fiber traits.

Introduction

Cotton as an annual crop is the world's leading natural fiber crop and an important crop for bio energy production (Lusas and Jividen, 1987; Chen *et al.*, 2007). It belongs to the genus *Gossypium* of the family Malvaceae and consists of approximately 50 species, including 45 diploids (2n =26) and 5 allotetraploids (2n = 52). The four species of cotton cultivated in india are *G. hirsutum* L., *G. arborenum* L., *G. herbaceum* L. and *G. barbadense* L. (Gillham *et al.*, 1995). The geographic center of origin for *G. hirsutum* is

North and Central America and Mexico. Conventional breeding methods generally aim to improve agronomically important traits by combining characters present in different parental lines of cultivated species or their wild relatives. The prediction of genetic similarities among genotypes is very important for crop improvement and accurate selection of parental combinations and the maintenance of sufficient diversity in breeding programs is necessary (Lacape *et al.,* 2010).

Different physical characteristics of cotton fibers are measured ranging from fiber length and length uniformity, strength, elongation (degree of extensibility), maturity (extent of cell wall thickening), micronaire (resistance to air flow across a plug of fibers) and fineness (linear density, a function of diameter and thickness) to color indices (reflectance and yellowness) (Lacape *et al.,* 2010). These mentioned characteristics are associated with the proficient spinning and weaving processes that alter the fiber into fabrics. Therefore, it is very important to improve fiber quality i`n locally dominating cotton genotypes to accomplish the requirements of the growing textile industry, processing and end use (Ali *et al.,* 2008).

Molecular breeding programme in cotton using the molecular marker to improve the efficiency of introgression of fiber quality traits in to the favorable genetic background. This effort relies on, development of genetic map in cotton genome, the identification of fiber quality and the marker assisted introgression of favorable genomic region into an adequate genetic background. SSR markers are very practical for integrating different linkage maps, QTL mapping, and comparative mapping for evolutional study, especially for species with a large genome size like cotton. SSRs can be also employed as anchors to combine linkage maps produced in different laboratories or with different populations to generate a more comprehensive genetic linkage representation. Identification of the molecular markers linked to fiber quality traits may accelerate the selection and breeding of traits.

The aim of the present study was to determine the genetic diversity of commercial cotton genotypes for the fiber length and fiber strength through the molecular markers profiling. The present study evaluated the genetic profile of cotton genotypes using SSR markers linked to fiber quality traits.

Materials and Methods

Parental materials

Twenty five cotton genotypes (*Gossypium hirsutum* L.) were used as experimental material comprised of 19 parents and 6 hybrid (G. hirsutum x *G. hirsutum*) were collected from Cotton Research Station, Nanded (M.S.) under Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani (Table 1).

Morphological analysis

Field experiments

The seeds of 25 genotypes were sown in the field under randomized complete block design with 3 replications during the 2018 and 2019 growing seasons in the field of cotton research station, Nanded (M.S.). All of the agronomic and plant protection practices were followed from sowing to harvesting of the cotton crop.

Fiber quality analysis

The collected boll samples were ginned with a single roller electrical gin on an individual plant basis to obtain lint for fiber analysis. Before fiber quality analysis, lints were

conditioned at 21 ± 1 °C and 65 ± 2% relative humidity for 48 h in a controlled room. An HVI 1000 (Uster, Switzerland) was used to analyze fiber quality traits and the most important cotton fiber properties, i.e. fiber length (mm), fiber strength (g tex–1), fiber fineness (micronaire) were examined.

Molecular analysis

Genomic DNA extraction

The DNA was extracted from young leaves by cetyl trimethyl ammonium bromide (CTAB) extraction method (Doyle and Doyle, 1987) with a few modifications. For each 100 mg of tissue, 500 µL of CTAB isolation buffer (2% hexadecyltrimethylammonium bromide, 1.5 M NaCl, 10 mM EDTA, 100 mM Tris-HCl, 0.2% β-ME, pH 8) was added to each tube, and homogenized. More CTAB extraction buffer (300 µL) was added to each tube and the samples were incubated at 65 °C for 60 min with occasional mixing.

Due to the high content of polyphenolic compounds in cotton tissues, 700 µL of phenol/chloroform/isoamyl alcohol (25:24:1 v/v) was added to each sample and the samples were vortexed and then centrifuged. The supernatants were transferred to a new tube and 500 µL of chloroform/isoamyl alcohol (24:1 v/v) solution was added. Next, 500 µL of ice-cold isopropanol was added to each tube and the tubes were incubated for 30 min at room temperature.

The samples were centrifuged and the supernatants were discarded. The pellets were air-dried and then resuspended in 50 µL of 10 mM Tris, pH 8.0, 1 mM EDTA buffer. Nucleic acids were measured quantitatively and qualitatively by spectrophotometer NanoDrop 2000c (Thermo Fisher Scientific). The extracted DNA was stored at –20 °C.

PCR analysis

The 42 SSR (Microsatellite) primer pairs (BNL, CIR and JESPR primer sets) linked to QTLs for important fiber quality traits as length, strength and fineness were used for PCR analysis (Table 2). Amplification reaction mixture was prepared in 0.2 ml PCR tubes, containing 1 X PCR buffer A (2.5µl), 0.2 mM each dNTP (0.2µl), 1 unit of Taq DNA polymerase enzyme (0.2µl, 5U/µl), 10 picomoles of each primer (0.5µl), 30ng template DNA (2µl) in each 20µl reaction volume. The amplification was carried out on a thermal cycler (Eppendorf, Master cycler gradient). Reactions incubated at 94 °C for 2 min and following 35 amplification cycles (30 s at 95 °C, 50 s at 50–60 °C, and 60 s at 72 °C) were performed. The final PCR products were visualized under UV light after electrophoresis on ethidium bromide-stained 2.5% Agarose gels.

Data analysis

Morphological data analysis was performed based on the three fiber quality traits' HVI measurements, i.e. length (mm), strength (g tex–1) and fineness (mic) and the mean values of each trait for each genotype were calculated. To standardize the assessments of numerical mean values of genotypes, they were scaled based on Bradow and Davidonis' (2000) index and USTER Technologies indices Switzerland, (2010) (Table 3).

For the molecular data analysis, polymorphism information content (PIC) values of molecular markers were calculated according to the following formula: PIC = 1 − Σ P2 i, where Pi is the frequency of the ith allele (Anderson *et al.*, 1993) (Fig. 2). For genetic analysis based on molecular data, each amplified band was scored based on the presence (1) and absence (0) of bands. The binary qualitative data matrix was used to

construct similarity matrices based on Jaccard similarity coefficients (Jaccard, 1908) and to construct dendrograms using UPGMA on NTSYS-pc software (Numerical Taxonomy System, Version 2.02i, Rohlf, 1998) and the polymorphic percentage was calculated by formulae:

$$\text{Polymorphic \%} = \frac{\text{No. of polymorphic amplicons}}{\text{Total No. of amplicons}} \times 100$$

Results and Discussion

Fiber quality characterization

The fiber length analysis, based on Bradow and Davidonis (2000) index, showed that genotype BN 1 has short (22.00 mm) fiber length, and genotypes NH-452, NH-625, PH-93, PH-325, PH-348 and PKV RAJAT have medium (22.0-26.0 mm) fiber length. Moreover, NH-111, NH-545, NH-615, NH-630, NH-635, PH-1009, ARB-757, ARB-908, AC738, AKH-8828, PHULE-0688, PHULE YAMUNA, NHH-44, NHH-206, NHH-225, NHH-250, NHH-324 and PHH-316 has long fiber length (26.1-32.00 mm). According to the USTER Technologies (Switzerland, 2010) the most of genotypes have average fiber strength (average fiber strength with 24.00-26.08 g tex–1). The analysis of fiber fineness showed that Phule Yamuna, AKH 8828, NHH 44, NHH 206 and NH 635 has fine fiber fineness (3.7 mic), while the genotype AC 738 have coarse or average (19 genotypes) fiber fineness (Table 1).

Molecular characterization

SSR marker profiling for fiber quality traits

In the present study total 42 QTL associated simple sequence repeat (SSR) primers of BNL, CIR and JESPR series linked with fiber traits were used to evaluate 25 cotton genotypes for polymorphism. Out of them 25 primers were found polymorphic, 10 were found monomorphic and remaining 7 primers had produced no amplification or did not show clear band.

The polymorphic information content (PIC) value of SSR loci was calculated for the 25 polymorphic primers for fiber traits across 25 cotton genotypes is presented in Table 4. The 25 polymorphic primers amplified a total of 53 alleles.

The average numbers of alleles per marker were found to be 2.12. Amplified alleles ranged from 1 to 4 with PIC value in the range of 0.072 (JESPR220) to 0.784 (CIR381) with an average of 0.290 per primer. These SSR markers with higher PIC values can be used for assessing genetic diversity and selecting parental lines for molecular mapping. Said *et al.*, 2013 reported 721 QTLs distributed across all 26 chromosomes controlling fiber quality traits in tetraploid cotton.

The JESPR-153 primers amplified a higher number of alleles i.e. 4, followed by primers BNL-3627, CIR-354, CIR-413, CIR-320 and JESPR-220 with 3 numbers of alleles (Table 4). While primers BNL-1059, BNL-1395, BNL-1672, BNL-2575, BNL-3255, BNL-2131, BNL-1122, CIR-244, CIR-246, CIR-305, CIR-253, CIR-381, JESPR-127, JESPR-298 and JESPR-307 amplified 2 number of alleles (Table 4) and primer BNL-1047, BNL-1064, BNL-3140, and CIR-307 amplified lowest number of alleles i.e. 2. Primer JESPR-153 amplified 4 allelic bands among 25 genotypes having PIC value 0.639 with 100 per cent polymorphism. However, primers BNL-1059 (PIC-0.481), CIR-244 (PIC-0.538), CIR-354 (PIC-0.660), CIR-381 (PIC-0.784), CIR-246 (PIC-0.749) and JESPR153 (PIC-0.639) as presented in Fig.1. having high PIC value for fiber quality traits

among 25 genotypes with 100 % polymorphism. Similar results were reported in *Gossypium* spp. was observed by few earlier workers (Kumbhalkar, *et al.*, 2018; Ghuge, *et al.*, 2017 and Mishra and Fougat, 2014) and found that primer gave high polymorphism and they are also abundant in both diploid *G. herbaceum* and teraploid *G. hirsutum* genotypes and suggested that it is the best marker for marker assisted selection of cotton genotypes related to fiber quality traits.

Table.1 Mean performance of 25 cotton genotypes for 3 different fiber quality parameters

Sr. No.	Genotype	Source	Fibre quality parameters		
			Fiber length (mm)	Micronaire Values(µ/inch)	Fiber strength (g/tex))
1	NH 111	VNMKV Parbhani	26.40	4.6	**20.60**
2	NH 452	VNMKV Parbhani	25.80	4.1	**24.2**
3	NH 545	VNMKV Parbhani	27.80	4.01	**26.2**
4	NH 615	VNMKV Parbhani	27.8	4.10	**26.8**
5	NH 630	VNMKV Parbhani	26.4	4.00	**24.1**
6	NH 625	VNMKV Parbhani	25.6	4.10	**23.8**
7	NH 635	VNMKV Parbhani	27.00	3.8	**25.8**
8	PH 93	VNMKV Parbhani	24.8	4.1	**23.7**
9	PH 325	VNMKV Parbhani	25.1	4.2	**24.8**
10	PH 348	VNMKV Parbhani	25.9	4.2	**22.7**
11	PH 1009	VNMKV Parbhani	27.5	4.3	**25.3**
12	ARB 757	-	26.8	4.4	**25.0**
13	ARB 908	-	26.8	4.3	**25.1**
14	Phule 0688	MPKV Rahuri	26.7	4.81	**24.4**
15	Phule Yamuna	MPKV Rahuri	26.5	3.90	**24.6**
16	AKH 8828	Dr. PDKV Akola	29.8	3.8	**25.3**
17	PKV Rajat	Dr. PDKV Akola	25.9	5.00	**23.8**
18	BN 1	-	22.00	4.12	**21.8**
19	AC 738	-	26.2	5.10	**22.4**
20	NHH 44	VNMKV Parbhani	27.1	3.8	**29.9**
21	NHH 206	VNMKV Parbhani	26.8	3.9	**23.7**
22	NHH 225	VNMKV Parbhani	27.9	4.1	**26.7**
23	NHH 250	VNMKV Parbhani	27.5	4.4	**26.3**
24	PHH 316	VNMKV Parbhani	26.3	4.3	**25.0**
25	**NHH 324**	**VNMKV Parbhani**	**27.3**	**4.3**	**25.8**

Table.2 Details of SSR Primers used in molecular analysis

SN	Primer Code	Primer sequence (Forward)	Primer sequence (Reverse)
1.	BNL-1047	GCTTGTCATCTCCATTGCTG	TGTATTCTCTTCTTTTCCTTATACTTTT
2.	BNL-1059	CCTTCTCTGACACTCTGCCC	TGTATTCTCTTCTTTTCCTTATACTTTT
3.	BNL-1064	TTTGCGGGTAATCCTATTGC	TGTCTATGGGACATTTCGCA
4.	BNL-1231	TAATAAAAGGGAAAGGAAAGAGTT	TATGGCTCTAGAATATTCCCTCG
5.	BNL-1395	AAGCAGCCAAGAAATACCGA	TCGATAACGGCTACAGTAATCT
6.	BNL-1672	TGGATTTGTCCCTCTGTGTG	AACCAACTTTTCCAACACCG
7.	BNL-2572	GTCCTATTACTAAAATTGTTAATTTAGCC	CGATGTTAAATCAATCAGGTCA
8.	BNL-3140	CACCATTGTGGCAACTGAGT	GGAAAAGGGAAAGCCATTGT
9.	BNL-3255	GACAGTCAAACAGAACAGATATGC	TTACACGACTTGTTCCCACG
10.	BNL-3627	TATGGGCCTGTCCACCTAAG	CAAAGCAACATGCACACACA
11.	BNL1030	TTTGGAGCCATTTACATGCA	AAACCACTTCTGCATCTGGA
12.	BNL1317	AAAAATCAGCCAAATTGGGA	CGTCAACAATTGTCCCAAGA
13.	BNL3994	TTGAGGGCATCCAAATCCAT	CCTCCACCATACACGTGCTA
14.	BNL580	CTATGTTTGGCCTTGGCATT	TAGTGACAGATATCCCCGGC
15.	BNL1421	TGAAGATTTGGAGGCAATTG	GAAATCAAGCCTCAATTCGG
16.	BNL1227	CATCAAGATCTATCTCTCTCTATACCG	TTTACCCTCCGATCTCAACG
17.	BNL3359	TTGTTGTTGGGAATGATGGA	TGACCCTTCACCGACTTTCT
18.	BNL1122	TCGATAACGGCTATAGTAATCTCTC	CAACAAATAAGCAGCCAAGAAA
19.	BNL1017	AGAAAAAAACTTCCTCATGAACC	GTTTCTCTCAGAATTTGTAGGCC
20.	BNL1034	TTGCTTTCAATGGAAAACCC	CGTCGCAAAGTTGAGAATCA
21.	CIR-244	TGGAAGGTGATGTTCTAA	GATCAAAGAGCAAACTAATC
22.	CIR-246	TTAGGGTTTAGTTGAATGG	ATGAACACACGCACG
23.	CIR-305	TTTCCAGCAAAAGAAGT	GAATTTTGAAGTGTCCTG
24.	CIR-307	GACTTGAAAAGATTACACAC	GAATTTGCTGGCTCT
25.	CIR-354	CACAATCCTCAGCCA	AGAGAAGGAAAGAGGAAA
26.	CIR-381	TTTCCATCCTTTTGTGA	AAGGAGAAGAACAAGCAA
27.	CIR-413	TTAAAGCTCACACACACA	CAACAGTAACGAAGAACAAT
28.	CIR320	CCTCCATAAACCCTCTT	TCACATACGAAGACAACC
29.	CIR328	ATCCCTATGCTTGTCATC	ATTACCATTCATTCACCAC
30.	CIR253	CCAACCAAGAAACCAG	GTAAGCATGGGCATTT
31.	CIR45	ACTAGCAGTGCGAATACA	TGGTTAAGGGTTGGG
32.	CIR70	AACCACCAACCATTCA	TGGGACTCGGTCATC
33.	CIR78	TGCATGATGAAGTTAGA	ACATAAATCCCAAGAAC
34.	JESPR65	CCACCCAATTTAAGAAGAAATTG	GGTTAGTTGTATTAGGGTCGTTG
35.	JESPR220	CGAGGAAGAAATGAGGTTGG	CTAAGAACCAACATGTGAGACC
36.	JESPR298	GATGCCCTCGTGTTAAAG	GGACCTTCGGAATAATTACC
37.	JESPR119	CTCAGGGAACTATTTGTAGTAGC	GATCCACAAGAAACTGAAACTAG
38.	JESPR29	CACCGTTTCCAAGTAAGATT	GGTTAATCTTAGTTGAGGTC
39.	JESPR208	CGCAACCAAACATATACTTCACAC	CCCTTTCCATCCATAGAACG
40.	JESPR127	GATTTGGGTAACATTGGCTC	CTGCAGTGTTGTGTTGGGTAGA
41.	JESPR153	GATTACCTTCATAGGCCACTG	GAAAACATGAGCATCCTGTG
42.	JESPR307	CTTGGCCATGTATTCCTTCA	GAAAGACACTAAGCTGAGGC

Table.3 The scale of fiber quality traits used in this study

Fiber length (mm)		*Fiber Strength (g/tex)*			*Fiber fineness (mic) (µg/inch)*	
Short fiber length	0.95-22.0	Average	25.0-27.0		Fine	3.1-3.9
Medium fiber length	22.0-26.0	High	28.0-30.0		Average	4.0-4.9
Long fiber length	26.1-32.0	Very high	>30.0		Coarse	5.0-5.9

Table.4 Characteristics of the amplification products with polymorphic SSR primer

SN.	**Primer Code**	**Allele size (bp)**	**Total number of allele**	**Number of Polymorphic allele**	**Percent Polymorphism**	**PIC**
1.	BNL-1059	190-230	2	2	100	**0.481**
2.	BNL-1395	110-130	2	2	100	**0.078**
3.	BNL-1672	130-150	2	2	100	**0.264**
4.	BNL-2572	110-130	2	2	100	**0.153**
5.	BNL-3255	270-370	2	2	100	**0.375**
6.	BNL-1064	190	1	1	100	**0.073**
7.	BNL-1231	210-290	2	2	100	**0.375**
8.	BNL-3140	230	1	1	100	**0.073**
9.	BNL-3627	150-190	3	3	100	**0.357**
10.	BNL-1047	170	1	1	100	**0.078**
11.	BNL1122	170-210	2	2	100	**0.163**
12.	CIR-244	150-210	2	2	100	**0.538**
13.	CIR-354	150-210	3	3	100	**0.660**
14.	CIR-381	170-190	2	2	100	**0.784**
15.	CIR-413	170-210	3	3	100	**0.073**
16.	CIR-246	150-170	2	2	100	**0.749**
17.	CIR-305	150-170	2	2	100	**0.073**
18.	CIR-307	190	1	1	100	**0.294**
19.	CIR320	150-290	3	3	100	**0.068**
20.	CIR253	110-170	2	2	100	**0.158**
21.	JESPR-153	110-290	4	4	100	**0.639**
22.	JESPR-127	170-290	2	2	100	**0.375**
23.	JESPR-307	210-290	2	2	100	**0.078**
24.	JESPR220	170-210	3	3	100	**0.072**
25.	JESPR298	130-190	2	2	100	**0.240**
Total			**53**	**53**	**2500**	**7.271**
Average			**2.12**	**2.12**	**100**	**0.290**

Table.5 Similarity matrix of SSR marker analysis based on Jaccard's similarity coefficient

	NH-452	NH-545	NH-615	NH-630	NH-635	PH-348	PKV RAJAT	AKH-8828	PHULE 0688	PHULE YAMUN	BN-1	AC-738	NH-625	ARB-757	ARB-908	PH-93	PH-325	NH-111	PH-1009	NHH-44	NHH-206	NHH-225	NHH-324	NHH-250	PHH-316
NH-452	1.0																								
NH-545	0.87	1.0																							
NH-615	0.76	0.79	1.0																						
NH-630	0.77	0.76	0.81	1.0																					
NH-635	0.86	0.83	0.80	0.83	1.0																				
PH-348	0.66	0.71	0.72	0.73	0.65	1.0																			
PKV Rajat	0.79	0.80	0.75	0.80	0.83	0.64	1.0																		
AKH-8828	0.78	0.83	0.80	0.83	0.84	0.70	0.85	1.0																	
PHULE 0688	0.69	0.78	0.75	0.80	0.77	0.67	0.87	0.81	0.1.0																
Phule Yamun	0.74	0.75	0.80	0.81	0.74	0.74	0.75	0.86	0.73	1.0															
BN-1	0.78	0.79	0.78	0.83	0.86	**0.61**	0.83	0.86	0.81	0.80	1.0														
AC-738	0.76	0.79	0.78	0.81	0.84	0.66	0.85	0.84	0.83	0.80	0.88	1.0													
NH-625	0.78	0.75	0.78	0.81	0.84	0.68	0.79	0.84	0.73	0.76	0.80	0.80	1.0												
ARB-757	0.78	0.76	0.80	0.81	0.86	0.66	0.81	0.88	0.77	0.78	0.82	0.84	0.90	1.0											
ARB-908	0.73	0.72	0.73	0.78	0.83	0.64	0.76	0.83	0.72	0.75	0.77	0.79	0.83	**0.91**	1.0										
PH-93	0.74	0.73	0.78	0.77	0.82	0.63	0.71	0.76	0.75	0.72	0.74	0.80	0.78	0.84	0.83	0.1.0									
PH-325	0.77	0.78	0.77	0.76	0.81	0.66	0.70	0.75	0.76	0.71	0.77	0.79	0.71	0.81	0.84	0.85	1.0								
NH-111	0.72	0.75	0.74	0.79	0.80	0.70	0.77	0.78	0.69	0.74	0.78	0.87	0.76	0.82	0.75	0.76	0.79	1.0							
PH-1009	0.77	0.80	0.77	0.78	0.83	0.64	0.78	0.85	0.78	0.77	0.81	0.81	0.79	0.85	0.82	0.85	0.84	0.85	1.0						
NHH-44	0.78	0.81	0.78	0.77	0.78	0.68	0.77	0.82	0.75	0.76	0.74	0.80	0.80	0.86	0.83	0.80	0.77	0.80	0.83	1.0					
NHH-206	0.78	0.85	0.80	0.79	0.82	0.74	0.81	0.88	0.79	0.80	0.78	0.80	0.80	0.88	0.83	0.76	0.77	0.82	0.87	0.88	1.0				
NHH-225	0.70	0.73	0.68	0.73	0.70	0.70	0.69	0.78	0.71	0.72	0.74	0.76	0.74	0.76	0.69	0.66	0.67	0.76	0.75	0.80	0.80	1.0			
NHH-324	0.76	0.81	0.82	0.71	0.72	0.74	0.71	0.78	0.67	0.76	0.70	0.74	0.80	0.80	0.73	0.74	0.75	0.74	0.75	0.82	0.82	0.74	1.0		
NHH-250	0.78	0.81	0.78	0.83	0.78	0.78	0.77	0.80	0.75	0.78	0.76	0.80	0.80	0.84	0.77	0.74	0.77	0.82	0.79	0.84	0.88	0.80	0.82	1.0	
PHH-316	0.77	0.82	0.77	0.80	0.75	0.79	0.76	0.81	0.74	0.79	0.75	0.77	0.73	0.77	0.78	0.67	0.78	0.75	0.72	0.77	0.83	0.77	0.77	0.91	**1.0**
	Maximum: 0.91										**Average: 0.76**													**Minimum: 0.61**	

Fig.1

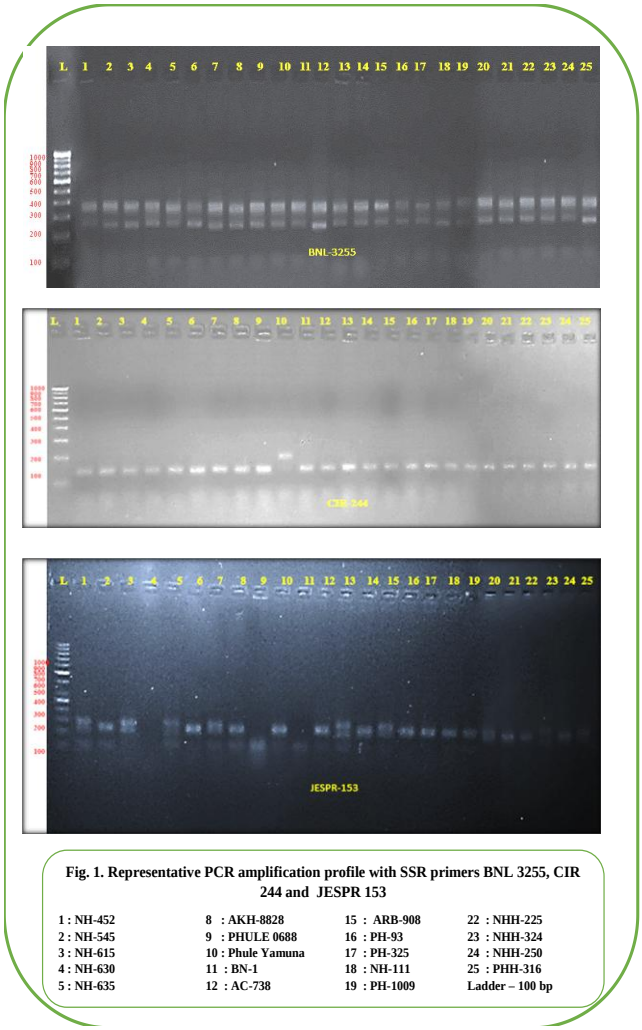


Fig. 1. Representative PCR amplification profile with SSR primers BNL 3255, CIR 244 and JESPR 153

1 : NH-452	8 : AKH-8828	15 : ARB-908	22 : NHH-225
2 : NH-545	9 : PHULE 0688	16 : PH-93	23 : NHH-324
3 : NH-615	10 : Phule Yamuna	17 : PH-325	24 : NHH-250
4 : NH-630	11 : BN-1	18 : NH-111	25 : PHH-316
5 : NH-635	12 : AC-738	19 : PH-1009	Ladder – 100 bp

Fig.2 Frequency of PIC value of all primers for different fibre quality traits

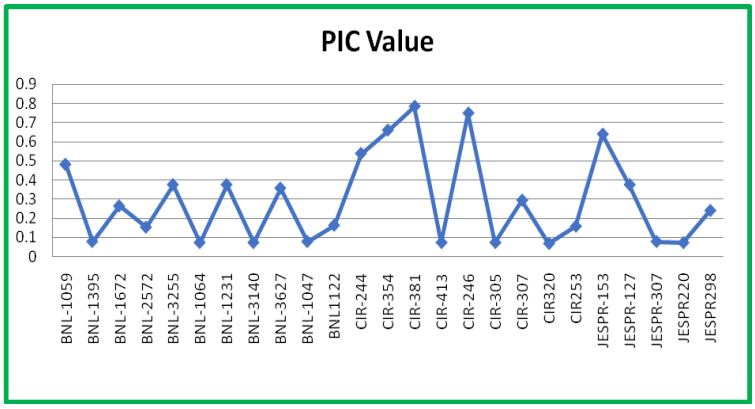
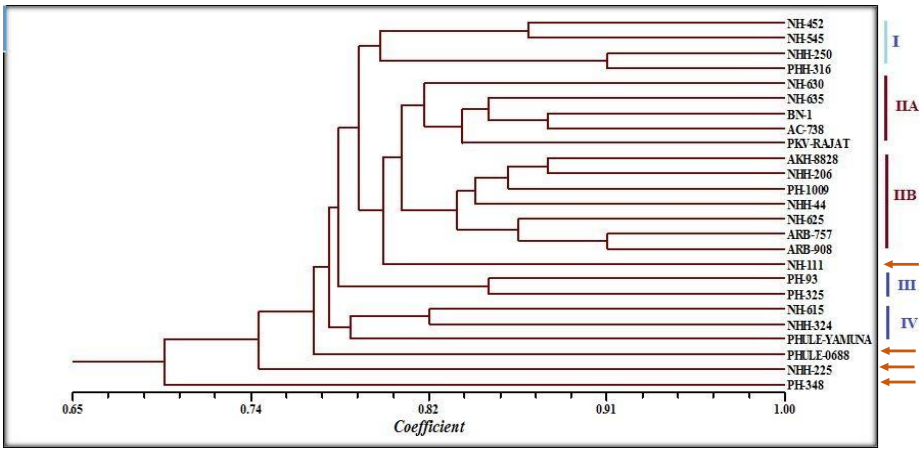


Fig.3 Dendrogram showing clustering pattern in 25 cotton genotypes studied based on SSR analysis



Clustering analysis of cotton genotypes on the basis of SSR analysis

The Jaccard's similarity coefficient showed the genetic relationships among the cotton genotypes. The similarity matrix based on the SSR marker profiling for fiber traits is depicted in (Table 5) and dendrogram depicted in Fig. 3. As concerned to fiber traits, 25 cotton genotypes showed similarity coefficient value ranged from 0.61 to 0.91 indicating more variation in respect of genetic similarity at studied loci. This ultimately means that large range of genetic diversity for fiber traits existed among the studied genotypes. Minimum similarity coefficient i.e. 0.61 was observed in genotypes PH-348 and BN-1. Maximum similarity coefficient of 0.91 was observed in genotype ARB-757, ARB-908, NHH- 2250 and PHH-316.

The cluster analysis revealed that the four cluster contains one major, three minor cluster and three outgroups. The cluster-II being the major cluster with 80% similarity amongst the thirteen genotypes. The Cluster-III was minor cluster with two genotypes having 77% genetic similarity. The cluster-I and cluster-IV contained four and three genotypes sharing 77% and 76% similarity within their respective cluster. The dendrogram also generated three out-group namely, Phule-0688 (sharing 76% similarity with rest of cluster), NH-225 (shearing 75% similarity with rest of cluster) and PH-348 (sharing 69% similarity with rest of cluster). The Major cluster-II was further divided in two subcluster sharing 81% similarity with each other. The two subcluster share 80% similarity with remaining one out group (NH-111). The sub cluster II-A contains 5 genotypes namely (NH-630, NH- 635, BN-1, AC-738 and PKV Rajat) having long fiber length except one genotypes i.e. BN-1 while sub cluster II-B contains 7 genotypes (AKH-8828, NHH-206, PH- 1009, NHH-44, NH-625, ARB-757 and ARB-908) having long fiber length. The NH- 111 was out grouped in this mojor cluster II having long fiber length. The Cluster-III has minor cluster with two genotypes (PH-93 and PH-325) having medium fiber length sharing 77% genetic similarity each other. The cluster-I contained four genotypes (NH-452, NH-545, NHH-250 and PHH-316) having medium to high fiber length they have 77% genetic similarity with each other. The cluster IV containing three genotypes (NH-615, NHH-324 and Phule Yamuna) having long fiber length and sharing 76 % genetic similarity with each other

In conclusion the genotypes BN-1, PH-348 and ARB-908 were found to be the most diverse and dissimilar having a broad genetic base for the concerned fiber traits. Similarly, primers BNL-1059 (PIC-0.481), CIR-244 (PIC-0.538), CIR-354 (PIC-0.660), CIR-381 (PIC-0.784), CIR-246 (PIC-0.749) and JESPR153 (PIC-0.639) showed highest PIC value and greater level of polymorphism 100%, Therefore, these markers provide a basis for future efficient use in marker assisted selection for fiber quality traits in cotton. The SSR was more reliable for in precise identification, genetic divergence analysis and have greater potential to reveal allelic variations. These genotype specific markers can be used as a fingerprint to identify each of them and guide a tool in diagnosis of genetic purity of these particular genotypes. Molecular markers identified in genotypes possessing desired fiber traits needs to be validated in larger segregating population prior to their application in marker assisted selection.

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