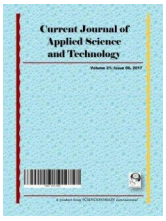

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Molecular Profiling of Cotton Genotypes for Fibre Properties Using Diagnostic Set of Microsatellite (SSR) Markers

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Authors' contributions

This work was carried out in collaboration between all authors. Authors HBK, VLG and SJG designed the experiments, provided the seed material of selected genotype, wrote all protocols and the final version of manuscript. Authors VNW and SRM managed the analysis of study. Author KPI performed the statistical analysis, molecular data compilation and managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Cotton (*Gossypium spp*.) the white gold is the world's leading natural textile fibre and also known as "King of fibres" is one of the best gifts that nature bestowed on mankind. The objective of the present investigation was to identify the diagnostic set of microsatellite (SSR) markers for the estimation of genetic diversity and varietal identification based on genetic distances. The molecular profiling of 15 cotton genotypes were carried out by using 104 simple sequences repeats (SSRs)

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primers (99.26% polymorphism for fibre length and 98.07% polymorphism for fibre strength). The on an average values of polymorphic information content (PIC) were, 0.210 and 0.345, respectively for fibre length and fibre strength. Fifty primers were found polymorphic among 104 SSRs primers. The 27 SSRs primers shown 100 *per cent* polymorphism for the fibre length. Similarly, 23 primers were shown 100 *per cent* polymorphism for fibre strength. A dendrograms for fibre properties (fibre length and strength) were constructed based on the S_{AB} values by adopting the sequential agglomerative hierarchical non-overlapping clustering technique of unweighted Pair Groups Method of Arithmetic Mean comprising 6 main groups for the fiber length and 5 clusters groups for the fiber strength. The study revealed that parents AKH 84635, AKH 10-2 and SURAJ for fibre length and parents AKH 84635, AKH 09-5 and SURAJ for fibre strength were grouped in individual cluster and found to be most diverse and genetically dissimilar parents with broad genetic base for the above fibre properties. The identified polymorphic markers could be used in the marker assisted breeding programme for the development of cotton hybrids having high fibre length and strength through the incorporation of genetically diverse parents.

Keywords: Cotton; SSR markers; molecular diversity; statistical analysis; dendrogram.

1. INTRODUCTION

Cotton is one of the most important cash crop and natural fibre crop in the world. Globally, genus *Gossypium* comprises 50 species [1]. Amongst 50 species, only four species are cultivated *viz*. *Gossypium arboreum* (2n=2x=26) and *Gossypium herbaceum* (2n=2x=26) are diploids while *Gossypium hirsutum* (2n=4x=52) and *Gossypium barbadense* (2n=4x=52) are tetraploid and mostly domesticated because of fibre quality [2]. The fibre quality of cotton plays an important role in world's economy and as raw materials in the textile industries [2]. Factors *viz*. fibre length, fibre strength, fibre color, fibre micronaire predominantly determined the fibre quality [3]. These characteristics associated with the proficient weaving and spinning processes which alter fibre to fabrics. Therefore, it is mandatory to improve the fibre quality in cotton genotypes to meet the ever increasing demand of the textile industries [4]. The main goal of cotton breeding is improving the fibre quality and yield [5]. The narrow genetic base of cotton has been considered as major constraint in cotton improvement [6]. Breeders overexploited the few genetic background that contributed to yield plateau and fibre quality traits [7].

The advent of the molecular marker technology, marker aided breeding is the candidate approach for the improvement of fibre quality through the exploitation of genetic diversity of cotton genotypes [2]. Through the advancement of molecular markers QTLs linked to fibre quality trait can be identified usually effective approach for pyramiding desired traits in a single genotypes. Because of this reason, construction of molecular linkage map using DNA markers have been recognised as an essential tool for plant molecular breeding [8]. Many genetic maps have been developed for *Gossypium* intra-specific and inter-specific populations [9,10,11, 12,13,14,15] and QTLS linked to different fibre quality and yield attributing traits. The development of the molecular markers accelerates the breeding programme for the breeders for the selection and improvement of trait of interest. The DNA polymorphism of the various genes have been found useful for the breeders to disclose the genetic differences among the genotypes to identify the novel genotypes for the successful crop breeding programme [16]. For the association of fibre traits (fibre length and fibre strength) SSR markers employed mostly to tag the fibre quality parameters. The EST-SSR s are used for tagging fibre quality traits because most of EST-SSR markers were developed from the genes expressed at various fibre development stages [17]. Identification of the molecular markers linked to fibre quality (fibre length and strength) 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 fibre length and fibre strength through the molecular markers profiling. The present study evaluated the genetic profile of cotton genotypes using SSR markers linked to fibre quality traits.

2. MATERIALS AND METHODS

2.1 Parental Materials

Fifteen cotton genotypes (Table 1) were used as experimental material comprised of 3 females and 12 males were procured from Cotton

Research Unit, (CRU) Dr. PDKV, Akola and ICAR-Central Institute for Cotton Research (CICR), Nagpur.

2.2 Molecular Analysis Using Microsatellite (SSRs) Primers

A total of 104 simple sequence repeat primers (Table 2) belonging to JESPR, NAU, CIR, BNL and Gh series were chosen randomly across the cotton genome and downloaded from cotton marker databases (CMD) [18] and were synthesised by MWG Biotech Pvt. Ltd, Bangalore, India.

2.3 Molecular Diversity Studies

Genomic DNA was isolated from leaf samples of all 15 cotton cultivars by rapid method [19] using 0.5 gm sample of 10 days old seedling and the quantification along with relative purity were estimated with nanophotometer. The final concentration of each DNA sample was adjusted to 25 ng/µl and 104 SSR primers linked to fibre length and fibre strength were used for the molecular profiling. The polymerase chain reaction (PCR) was performed in a final volume of 20 µl, containing 10X PCR buffer with 25mM $MgCl_2$, 100 mM of each dNTP, 0.4 mM of each primer, 20 ng genomic DNA, and 1 U of *Taq* DNA polymerase. The temperature profile for DNA amplification was 30 s at 94°C for template denaturation, 35s at 55-61°C for primer annealing (vary as per primer annealing temperature), and 30s at 72°C for primer extension for 40 cycles. The PCR reaction was completed with 5 min incubation at 72°C. Finally, the PCR products were separated on 1.2% agarose and amplicons were visualised under gel documentation system (AlfaImager).

Table 1. Parental genotypes with their salient features used for the present investigation

SN	Genotypes	Source	Peculiar features
Females			
1	AKH 84635 (PKV RAJAT)	CRU, Dr. PDKV, Akola	High yielding, high GOT (38-39%), micronaire 4.5 µg/inch, moderately resistant to sucking pests
2	AKH 8828	CRU, Dr. PDKV, Akola	Very high GOT (41-42%), fibre length 27-28 mm, micronaire 4.2 µg/inch
3	AKH 081	CRU, Dr. PDKV, Akola	Early maturity genotype, compact plant canopy, tolerant to sucking pests,
Males			
4	AKH 10-2	CRU, Dr. PDKV, Akola	Dwarf, glabrous and thick leaf, open plant canopy
5	AKH 10-5	CRU, Dr. PDKV, Akola	Dense hairy leaf, medium tall, 2-3 monopodia, tolerant to sucking pests
6	AKH 10-10	CRU, Dr. PDKV, Akola	3-4 monopodial, semi-tall material, slight hairy leaf
7	AKH 11-7	CRU, Dr. PDKV, Akola	Erect type plant, dense hairy leaf, 1-2 monopodia, sucking pest tolerant
8	AKH 2006-2	CRU, Dr. PDKV, Akola	Small, dense hairy leaf, dwarf to bushy type, tolerant to sucking pests
9	AKH 2012-8	CRU, Dr. PDKV, Akola	Highly resistant to sucking pests, broad leaf genotype
10	AKH 2012-9	CRU, Dr. PDKV, Akola	Slightly hairy, medium tall, 2 monopodia, conical boll shape
11	AKH 09-5	CRU, Dr. PDKV, Akola	Big size oval boll 4.0-4.5 gm, dwarf plant type, small to medium leaf
12	AKH 976	CRU, Dr. PDKV, Akola	Glabrous and small leaf, dwarf to bushy type, tolerant to sucking pests
13	AKH 9916	CRU, Dr. PDKV, Akola	High GOT 37-38% , high fibre strength , high yielding, resistant to sucking pest
14	DHY 286	CRU, Dr. PDKV, Akola	Extra dense hairy, tolerant to sucking pests, compact and erect plant type
15	SURAJ	ICAR-CICR, Nagpur	Long staple with high fibre strength , high yielding, tolerant to sucking pests

Table 2. Microsatellite primers associated with fibre length and fibre strength used for the molecular characterization of cotton genotypes

SN	Primer name	Primer sequence (Forward)	Primer sequence (Reverse)
1	NAU1200	CAACAGCAACAACCACAA	CTGCCTCGAGGACAAATAGT
2	JESPR0065	CCACCCAATTTAAGAAGAAATTG	GGTTAGTTGTATTAGGGTCGTTG
3	TMB2557	CCACAACAAACCCAACACAG	AAAAGCCCCTCCCTTTCTTT
4	NAU1037	CACCTTCACCTAACCATCAA	GAAGAATTGCGAGAAGAGGA
5	NAU1197	AGCAAATATCTCTCCCCACA	CATGTTCCACGTCATTTCAT
6	NAU1217	ATCCATATGTGCTGTTGCAG	TGCAGTTGTACAAGCAATCA
7	NAU1262	TTCCTCGTTCCTCATTCTTC	TAAACTCCGATGGTGAAGGT
8	NAU1248	AATGTCAGCTGCCTATTTCC	AAGACAGGCGATGTCATCTT
9	NAU1336	TCGCTAATTCTACCTCCTTATT	GCGGGTAATTGTAGTACATGC
10	NAU3308	AATGGCTCTTCTCCTCCTTT	GCGTATTCCTCGTTACTCGT
11	NAU2687	CTGAGACTGTCCATGTCCAA	ATCTGGGTTTTCCCTTTTTC
12	NAU3481	AGTTGCAGAAACCTGGAGAC	TCCTTCCTTTGTTTCTCTGC
13	NAU3522	AAACCAACGTGCTTTAACAT	GCAAGCAATTGTTTTTCATC
14	NAU5107	CGATGAAGACGATGCTATTG	GTAGCCTTTGGTCTTCGTGT
15	NAU5357	TTCCCTAGCAGTTCAAGGAC	TCAGGCTGAGCATACATCAT
16	NAU5411	GAGTAGAAGGCACCTTGGAA	AGTCCACGTCCACAGACTTT
17	NAU5480	TTAATGGGGTAAACGCAAAT	CTGGTCCTCAACCTTAGCAT
18	TMB0670	GTGTGCTGCGTACTGCTTTA	GAGCTGTAAGAAGGGCCAAC
19	TMB1276	CAATCAACTGATGAGAAAGAGAA	TCAAGTGCTAATGGGAATGC
20	TMB1618	GGGAATTGAACCCAAGACCT	GTGAAAGGGGAGGTTCAACA
21	TMB1838	TCGGTCGTCCTGAGAGAAAT	TGTGACAAGTAGAATGCTTACCTCA
22	BNL2960	TAAGCTCTGGAGGCCAAAAA	CCATTTCAATTTCAAGCATACG
23	Gh277	TACTAAAACCAAGGCAATAAAGTGA	CACCACCTTCCATATATCTTGCTC
24	Gh499	CCACAATAGCATATGAAATCATAATGGG	GTTGCAACCTTGGAAACCATGAAG
25	CIR228	TCCAGGTAAACTCAACAA	TCATCAGTTCAATCACAAG
26	NAU2277	GAACTAGCCACATGATGCAC	TTGTTGAGGCATTAGTTTGC
27	BNL1421	TGAAGATTTGGAGGCAATTG	GAAATCAAGCCTCAATTCGG
28	BNL1521	TGAAGAAAGAAAAAGAGAAAGGG	CTCACCACGTGGCACTTATG
29	BNL1231	TAATAAAAGGGAAAGGAAAGAGTT	TATGGCTCTAGAATATTCCCTCG
30	BNL1227	CATCAAGATCTATCTCTCTCTATACCG	TTTACCCTCCGATCTCAACG

SN	Primer name	Primer sequence (Forward)	Primer sequence (Reverse)
31	MUSS193	GAAAATGAGCACTTCTCCGC	AATGCGAATTGATCCAACAG
32	NAU2894	GGCACGTTGCAAGTGTTAT	AACCTTCCAGAGAAAGCAGA
33	NAU2641	TCTTTTGAGGGTCACCATTT	AACCCAGTTTTTGTTTTCCA
34	NAU1302	ACTCGGCGTATAATTTGGAA	GAGGGAAAACCAAACAGAAA
35	NAU3160	GGTTTTGGGACTTGACTGTT	GGTTAGTGCCGAGAGGTAAA
36	DPL0570	GTGATTGGGTGAATGACAAATG	GAAGTCACATGAGATCAGAAGACG
37	BNL3867	TAATTGAGTTGTTTTCTTACTTGCC	TGCCAATTTAGCAATCACCA
38	BNL3511	TAGAACATAGGGAGGCGTGG	AATGGAGAGACAATGATTTTTCG
39	DPL0600	AGGCACCTCTTTAGTGATACTAATTCC	TTAAGGGTAGCCCTCTCAATCTCT
40	DPL0079	GTAAAGATGAGACTGTGAGGGCTT	TCATTCCAAGAAGCAGAAGACTAAC
41	NAU2640	GTCTTTTGCCATTCTTGGTC	AACATGGGCAATGGTAACTT
42	BNL3580	CTTGTTTACATTCCCTTCTTTATACC	CAAAGGCGAACTCTTCCAAA
43	CIR246	TTAGGGTTTAGTTGAATGG	ATGAACACACGCACG
44	NAU3888	TGTTCCCAGTGATGAACTTG	TTCACTGCATGACCACCTAC
45	NAU3096	CCTGGGTTCTCATCGTATTC	GAAGACAGACTGTTGCGTTG
46	NAU3948	AGAGCTATGGGAAATCATGG	ACATTGGTGTCGAATGTTTG
47	NAU3092	CTATGGCTCCCATTTTGATT	ATCTTTGGGAAGGGTTCTCT
48	Gh471	CAGGCATCAACTAGCATTGAAAACG	ATCTTCTGATCTCTATTAGCTACAACG
49	NAU3426	ACAGACAAGAAATGCAGCAA	TGGCCTTCTTGATGTTGATA
50	NAU3881	AATAGTGATGCTCCCTTTGG	TGCCCACTAAAGAGTTAGCC
51	NAU3084	GATCCTCCTCTTCCTCTTCC	GATGAAAGCGGTGGTTAAGT
52	NAU4047	ATTGGAGCTGTTTGGCTAAG	AATGGCTCCTCAATGGTAAA
53	BNL3790	TTCAGAATGTGTTAGACTAGCTGG	AAAGAAGGAAGTGTGGGCAG
54	BNL3383	GTGTTGTCATCGGCACTGAC	TGCAATGGTTCAGTGGTGAT
55	BNL3359	TTGTTGTTGGGAATGATGGA	TGACCCTTCACCGACTTTCT
56	BNL1122	TCGATAACGGCTATAGTAATCTCTC	CAACAAATAAGCAGCCAAGAAA
57	NAU2658	ACCGGACAATATGGGTAAAA	CAACAAGTACATGCACAGCA
58	NAU2671	TTGCAACCCTAATGTCAATG	AAACGATGGGAAAAGTGGTA
59	CIR305	TTTCCAGCAAAAGAAGT	GAATTTTGAAGTGTCCTG
60	BNL1017	AGAAAAAAACTTCCTCATGAACC	GTTTCTCTCAGAATTTGTAGGCC
61	BNL1064	TTTGCGGGTAATCCTATTGC	TGTCTATGGGACATTTCGCA
62	BNL1034	TTGCTTTCAATGGAAAACCC	CGTCGCAAAGTTGAGAATCA

SN	Primer name	Primer sequence (Forward)	Primer sequence (Reverse)
63	BNL2655	TTGCATAAGTTTTGGGAGGC	GGTTAGACTCTTTATTTTAAACACACG
64	BNL3171	GAAAAATTGAGGAAGGACATACG	GGCCACAACCGAATTTACTG
65	BNL3255	GACAGTCAAACAGAACAGATATGC	TTACACGACTTGTTCCCACG
66	NAU3455	CATCATCACTACCCACCTGA	TTTGTTGAAGGGTTTGGAAT
67	NAU2354	AATATCTCCGTCGCCAATTA	GAAACTTCCTCCTCCTTTCC
68	NAU3791	AGTTTCTGAATCCCATTCCA	TACGTTCCATTTTCATGACG
69	TMB2955	TTTGCATGTTCATCCCAATG	TCTAGTGGACGGTGGAACAA
70	JESPR0220	CGAGGAAGAAATGAGGTTGG	CTAAGAACCAACATGTGAGACC
71	NAU886	TACCACAGACATTCTTGTCA	TGCTTCGATGTTGAGAGGTA
72	JESPR0298	GATGCCCTCGTGTTAAAG	GGACCTTCGGAATAATTACC
73	TMB1750	AAAACTCAGACAAAGAGTGGG	GCTTCTTAGAAGGCAAATAGAC
74	CIR328	ATCCCTATGCTTGTCATC	ATTACCATTCATTCACCAC
75	NAU3825	TCTGTTGGCATCGCAGAG	TTAACGACAATTCCATCGTG
76	NAU2932	CCCCAATAACATGTTCTCAA	GCTGTGGTTGGTGGTAAGAT
77	NAU5120	GCCACCAATAAAGCAACTCT	TGCATCCTGAAGAAGAGACA
78	CIR320	CCTCCATAAACCCTCTT	TCACATACGAAGACAACC
79	NAU2329	TCCATCAAATAATCCCGTGT	GGAGAGGGGAAGACGAGTAT
80	Gh537	GTTGGGTGGCAATTCCTTTTAGATC	AAAGCTAATCCCTATACCTTTTCTTCG
81	NAU2126	AGTCGGAGAGCAGTCCCTA	TAACGGCTGTAACCCTTCTC
82	NAU928	CCCAAGTGAGGGAATACTTG	TAAATTGCCAGGACAAGACC
83	NAU3592	CATCTTCAATTTCACCCACA	TGAAAGGCAACTCCACAATA
84	NAU5189	TGTCCCCCAATCATATTTTC	CAACTTCCCAAGCTCGTATT
85	NAU4034	CGACGGAAAGGGTTATCTTA	ACGCCCTTCATTCAAACAC
86	DPL0752	CACATCACCTAATTACCATTGAAGC	TATCGTGAATATGTATGTGCGTGG
87	Gh107	CATATGTGACTTAACATTCATCGTC	CTCACCCATGTTTGTTCGA
88	Gh273	TTGCTTCGTTTTCTTCCCTGGTG	AAGCAAAGACCAGCTTCTCTTCC
89	JESPR0119	CTCAGGGAACTATTTGTAGTAGC	GATCCACAAGAAACTGAAACTAG
90	NAU5508	GTTTCCCTCGGTTTAGGTTT	TTTGAATTCCTCATCGGATT
91	TMB2068	AAGTTTTCGGCTCCCTCACT	GCTGCTGGGGACTATTCTTG
92	BNL1160	GCTGGGACATTTCTTGCTGT	TGTAAGGGGTAAAACGTCGC
93	NAU1369	TGGCAGAGATGAATGTAAGC	GGTAACGGATGGAAAATCAC
94	NAU2038	GAGACACGAACACAAAAACG	GCGGTGTGTTATACATCCAT

SN	Primer name	Primer sequence (Forward)	Primer sequence (Reverse)
95	NAU2723	CCTAGGTGTTACGGTGGACT	TGGGAAATTGGTTTCTTCAT
96	NAU2836	ATTGGAAGGGTATTGAGCTG	TCCTTTCCCACTTCTGTTCT
97	NAU923	GGAATTCAAGGTTGAAGGAG	CCTCTTCTTTGGCTCTGAAA
98	NAU992	AGAAAAGCGCTGCTGATAGT	GGGTGGCTTGACAAGTCTAT
99	NAU879	AGGAACCGATTCAAAGCTAA	TTTCCCCATTCTTGGTTAAG
100	CIR253	CCAACCAAGAAACCAG	GTAAGCATGGGCATTT
101	NAU3022	AACGGAATGCTTTCTTTTCA	GTTGAATTGGGGTTAGCATT
102	Gh032	GAATCATAGTTTGGTGGTTGAGG	CACATTCACTTCAAAGTCCATCAC
103	Gh039	CCAGTTTATAATAAGAATCATAGTTTGGG	CACATTCACTTCAAAGTCCATCAC
104	Gh428	AAAATTCCCAGTCGTGCTCAACTC	ACAAAGGTTGTCTGTTTGATTCTGAAG

2.4 Statistical Analysis

Polymorphic SSR loci were scored as '1' for presence and '0' for absence. This allowed estimating at each locus of the number of alleles present (NA) and the polymorphic information content (PIC) value. The PIC value of each primer was calculated by the online software (PIC Calc) [20]. The similarity matrix based on SSR profile were subjected to UPGMA (Un-weighted pair group method for arithmetic mean) for cluster analysis and a dendrogram was generated as per the procedure [21]. These computations were performed using the program xlstat software [20] and the polymorphic percentage was calculated by formulae:

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

3. RESULTS AND DISCUSSION

3.1 Molecular Diversity Analysis

Total 104 QTL associated simple sequence repeat primers of NAU, BNL, JESPR, CIR, DPL, TMB, MUSS and GH series associated with fibre properties *viz*., fibre length and fibre strength were used to evaluate 15 cotton genotypes for polymorphism. Out of them 50 primers were found polymorphic (27 primers for fibre length and 23 primers for fibre strength), 35 were found monomorphic and remaining 19 primers had produced no amplification or fuzzy bands and did not show clear amplification.

3.2 Molecular Diversity Analysis for Fibre Length

The polymorphic information content (PIC) value of SSR loci was calculated for the 27 polymorphic primers for fibre length across 15 cotton genotypes is presented in Table 3. The 27 polymorphic primers amplified a total of 81 alleles. The average numbers of alleles per marker were found to be 3.0. Amplified alleles ranged from 2 to 5 with PIC value in the range of 0.002 (NAU3592) to 0.621 (JESPR0065) with an average of 0.210 per primer. The NAU3092 and CIR253 primers amplified a higher number of alleles i.e. 5, followed by primers JESPR0065, CIR228, DPL570 and BNL3255 with 4 numbers of alleles (Table 3). Primers NAU1217, NAU2641, DPL0600, CIR246, NAU1200, NAU3481, NAU3096, NAU3791, JESPR0220, NAU5120, DPL0752, NAU2723 and GH428 amplified 3 numbers of alleles, while, primers NAU2277, NAU1262, CIR305, JESPR0298, NAU3592, GH273, NAU5508 and NAU2038 amplified lowest number of alleles i.e. 2 (Table 3).

Primer CIR253 amplified 5 allelic bands among 15 genotypes for fibre length having PIC value 0.596 with 100 *per cent* polymorphism. Primer NAU3092 as presented in Fig. 3 amplified highest number of allelic bands for fibre length i.e. 5 among 15 cotton genotypes having PIC value (0.382), but it showed lower *per cent* polymorphism i.e. 80 *per cent* (Table 3). However, primers NAU1200 and NAU3481 as presented in Fig. 1 and Fig. 2 amplified 3 allelic bands for fibre length among 15 genotypes. The PIC value was 0.592 and 0.038, respectively with 100 *per cent* polymorphism for fibre length. Similar results were reported [2] and found that primer NAU 1200 gave high polymorphism and they are also abundant in both diploid *G. herbaceum* and *G. arboreum* genotypes and suggested that it is the best marker for marker assisted selection of cotton genotypes related to fibre quality traits.

3.3 Molecular Diversity Analysis for Fibre Strength

The 23 polymorphic primers (fibre strength) amplified a total of 86 alleles. The average number of alleles per marker was found to be 3.74 (Table 4). Amplified alleles ranged from 2 to 9. The primer NAU3308 amplified higher number of alleles i.e. 9 followed by primer NAU1037 with 6 numbers of alleles. Primers TMB2557 and GH499 amplified 5 numbers of alleles. Primers NAU5411, TMB1618, NAU3888, GH471, NAU2932, NAU2126, NAU928 and NAU5189 amplified 4 numbers of alleles. Primers TMB0670, NAU2894, GH277, BNL1064, JESPR0119, NAU923 and GH0032 amplified 3 numbers of alleles, while, primers NAU2687, DPL0079, GH537 and GH0039 amplified lowest number of alleles i.e. 2. The PIC values calculated for these 23 polymorphic primers were in the range of 0.005 (GH537) to 0.848 (NAU3308) with an average of 0.345 per primer (Table 4).

Table 3. Characteristics of the amplification products with polymorphic SSR primers among 15 cotton genotypes (Fibre length)

S. N.	Primers	Total number of amplicons	Monomorphic amplicons	Polymorphic amplicons	*Per cent* polymorphism	Polymorphic Information Content (PIC)
1	JESPR0065	4	0	4	100.00	0.621
2	NAU1217	3	0	3	100.00	0.376
3	CIR228	4	0	4	100.00	0.004
4	NAU2277	2	0	2	100.00	0.018
5	NAU1262	2	0	2	100.00	0.003
6	NAU2641	3	0	3	100.00	0.005
7	DPL0600	3	0	3	100.00	0.020
8	CIR246	3	0	3	100.00	0.164
9	NAU1200	3	0	3	100.00	0.592
10	NAU3481	3	0	3	100.00	0.038
11	NAU3096	3	0	3	100.00	0.152
12	NAU3092	5	1	4	80.00	0.382
13	DPL570	4	0	4	100.00	0.383
14	CIR305	2	0	2	100.00	0.003
15	BNL3255	4	0	4	100.00	0.005
16	NAU3791	3	0	3	100.00	0.593
17	JESPR0220	3	0	3	100.00	0.376
18	JESPR0298	2	0	2	100.00	0.164
19	NAU5120	3	0	3	100.00	0.436
20	NAU3592	2	0	2	100.00	0.002
21	DPL0752	3	0	3	100.00	0.375
22	GH273	2	0	2	100.00	0.020
23	NAU5508	2	0	2	100.00	0.018
24	NAU2038	2	0	2	100.00	0.140
25	NAU2723	3	0	3	100.00	0.164
26	CIR253	5	0	5	100.00	0.596
27	GH428	3	0	3	100.00	0.018
	Total (polymorphic amplicons)	81	1	80	2680.00	5.668
	Average (polymorphic amplicons)	3.0	0.037	2.96	99.26	0.210

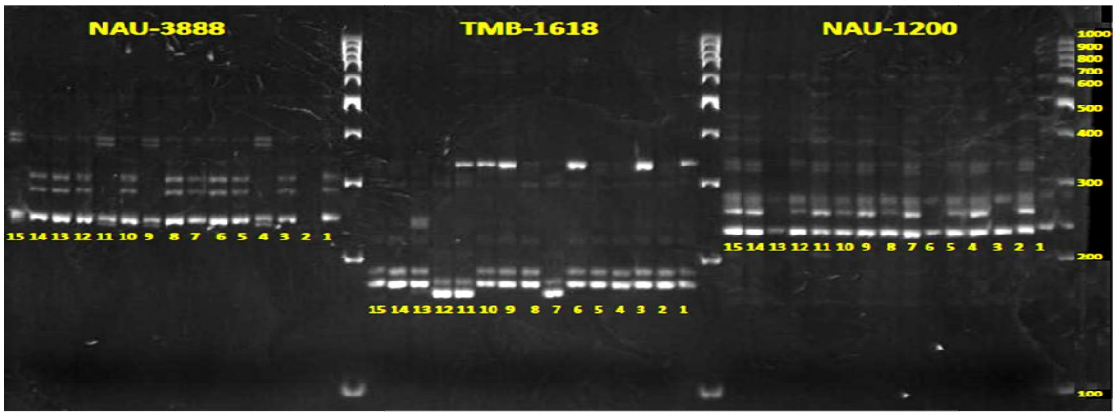

Fig. 1. Representative PCR amplification profile of 15 cotton genotypes with SSR primers NAU3888 (FS), TMB1618 (FS) and NAU1200 (FL) FS= Fibre strength, FS= Fibre length

1.AKH 84635, 2. AKH 8828, 3. AKH 081, 4. AKH 10-2, 5. AKH 10-5, 6. AKH 10-10, 7. AKH 11-7, 8. AKH 2006- 2, 9. AKH 2012-8, 10. AKH 2012-9, 11. AKH 09-5, 12. AKH 976, 13. AKH 9916, 14.DHY 286, 15. SURAJ

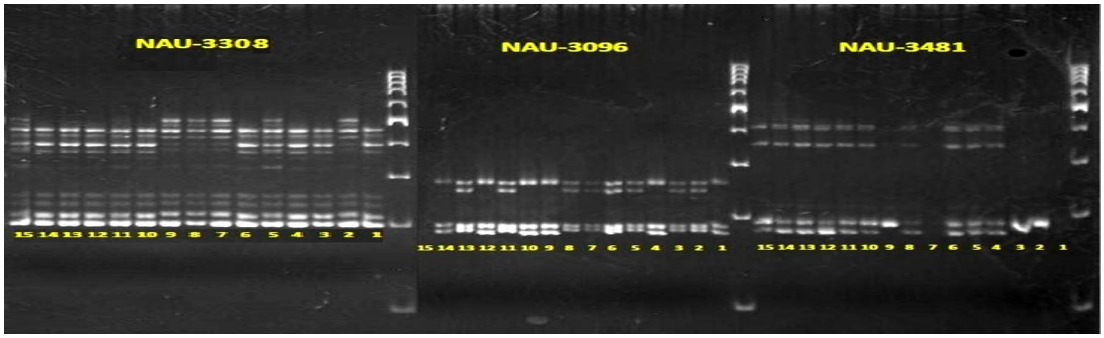

Fig. 2. Representative PCR amplification profile of 15 cotton genotypes with SSR primers NAU3308 (FS), NAU 3096 (FS) and NAU 3481 (FL) FS= Fibre strength, FS= Fibre length

1.AKH 84635, 2. AKH 8828, 3. AKH 081, 4. AKH 10-2, 5. AKH 10-5, 6. AKH 10-10, 7. AKH 11-7, 8. AKH 2006- 2, 9. AKH 2012-8, 10. AKH 2012-9, 11. AKH 09-5, 12. AKH 976, 13. AKH 9916, 14.DHY 286, 15. SURAJ

Table 4. Characteristics of the amplification products with polymorphic SSR primers among 15 cotton genotypes (Fibre strength)

S. N.	Primers	Total number of amplicons	Monomorphic amplicons	Polymorphic amplicons	*Per cent* polymorphism	Polymorphic Information Content (PIC)
1	TMB2557	5	0	5	100.00	0.719
2	NAU5411	4	0	4	100.00	0.390
3	NAU3308	9	4	5	55.55	0.848
4	TMB0670	3	0	3	100.00	0.439
5	NAU1037	6	0	6	100.00	0.732
6	NAU2687	2	0	2	100.00	0.019
7	NAU2894	3	0	3	100.00	0.436
8	GH277	3	0	3	100.00	0.169
9	GH499	5	0	5	100.00	0.374
10	TMB1618	4	0	4	100.00	0.592
11	NAU3888	4	0	4	100.00	0.593
12	GH471	4	0	4	100.00	0.057
13	DPL0079	2	0	2	100.00	0.374
14	BNL1064	3	0	3	100.00	0.151
15	NAU2932	4	0	4	100.00	0.271
16	GH537	2	0	2	100.00	0.005
17	NAU2126	4	0	4	100.00	0.375
18	NAU928	4	0	4	100.00	0.388
19	NAU5189	4	0	4	100.00	0.152
20	JESPR0119	3	0	3	100.00	0.163
21	NAU923	3	0	3	100.00	0.152
22	GH0039	2	0	2	100.00	0.375
23	GH0032	3	0	3	100.00	0.150
	Total (polymorphic amplicons)	86	4	82	2255.55	7.924
	Average (polymorphic amplicons)	3.74	0.17	3.57	98.07	0.345

Primer NAU3308 as presented in Fig. 2 amplified highest number of alleles i.e. 9 among 15 cotton genotypes having maximum PIC value (0.848), but it showed only 55.55 *per cent* polymorphism for fibre strength (Table 4). Similarly, primer GH499 as presented in Fig. 4 amplified 5 allelic bands with calculated PIC value was 0.374 with 100 *per cent* polymorphism. However, primers NAU3888 (Fig. 1), TMB1618 (Fig. 1) and NAU0928 (Fig. 3) amplified 4 numbers of alleles among 15 genotypes. The PIC value was 0.593, 0.592 and 0.388, respectively with 100 *per cent* polymorphism for fibre strength. It was reported [22] that marker NAU923 related to fibre strength

shown highest PIC value but in our present investigation this marker found less PIC value (0.152) compared to others.

3.4 Genetic Distance-based Analysis for Fibre Length

The genetic similarities (GS) were estimated [23] which showed that the genetic relationship among the cotton genotypes. The similarity matrix based on the SSR marker profiling for fibre length is depicted in (Table 5) and dendrogram depicted in Fig. 5. The genotypes showing similarity index of '1' are presumed to be 100 *per cent* genetically similar, while that of '0' are said to be 100 *per cent* genetically dissimilar for the respective trait from each other. In the present study 15 cotton genotypes showed similarity coefficient value ranged from 0.167 to 0.583 indicating more variation in respect of genetic similarity at studied loci across 15 genotypes. This ultimately means that large range of genetic diversity for fibre length existed among the studied genotypes. The highest genetic similarity (0.583) was found between AKH 2006-2 and AKH 10-10 followed by genotype AKH 081 and AKH 8828 with value 0.578. While, the lowest value of genetic similarity (0.167) was found to be observed in genotype SURAJ and AKH 2006-2 which was found to be most diverse parents for fibre length (Table 5). Thus, as similarity index goes on decreasing, the degree of divergence goes on increasing. The degree of divergent or similarity helps to identify genetically diverse genotypes. Thus, this information would be helpful to identify genetically diverse genotypes and high stable performance of genotypes.

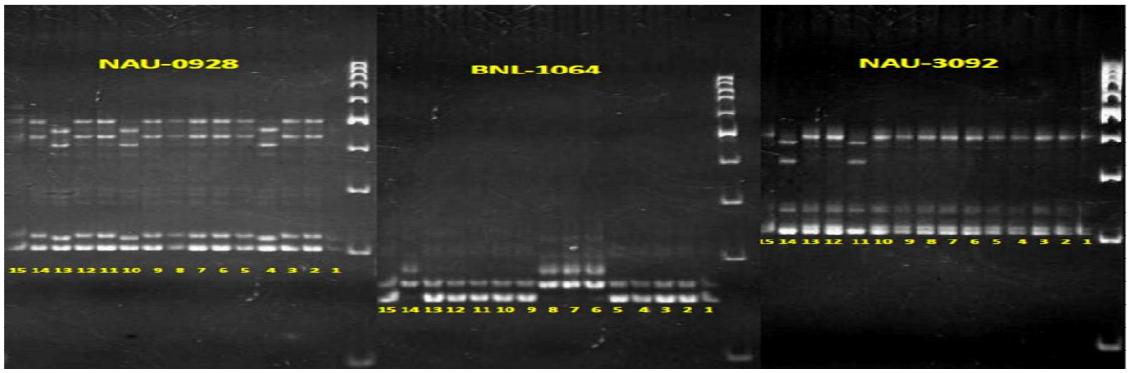

Fig. 3. Representative PCR amplification profile of 15 cotton genotypes with SSR primers NAU0928 (FS), BNL1064 (FS) and NAU3092 (FL) FS= Fibre strength, FS= Fibre length

1.AKH 84635, 2. AKH 8828, 3. AKH 081, 4. AKH 10-2, 5. AKH 10-5, 6. AKH 10-10, 7. AKH 11-7, 8. AKH 2006- 2, 9. AKH 2012-8, 10. AKH 2012-9, 11. AKH 09-5, 12. AKH 976, 13. AKH 9916, 14.DHY 286, 15. SURAJ

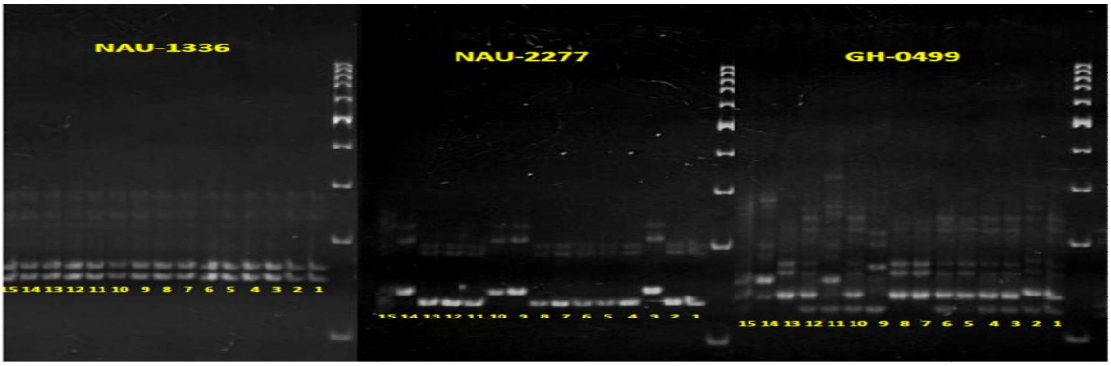

Fig. 4. Representative PCR amplification profile of 15 cotton genotypes with SSR primers NAU5120 (FL), NAU2277 (FL) and GH099 (FS) FS= Fibre strength, FS= Fibre length

1.AKH 84635, 2. AKH 8828, 3. AKH 081, 4. AKH 10-2, 5. AKH 10-5, 6. AKH 10-10, 7. AKH 11-7, 8. AKH 2006- 2, 9. AKH 2012-8, 10. AKH 2012-9, 11. AKH 09-5, 12. AKH 976, 13. AKH 9916, 14.DHY 286, 15. SURAJ

Table 5. Similarity matrix of SSR primer analysis based on Jaccard's similarity coefficient (Fibre length)

	AKH 84635	AKH 8828	AKH 081	AKH 10-2	AKH 10-5	AKH 10-10	AKH 11-7	AKH 2006-2	AKH 2012-8	AKH 2012-9	AKH 09-5	AKH 976	AKH 9916	DHY 286	SURAJ
AKH 84635	1														
AKH 8828	0.333	1													
AKH 081	0.255	0.578	1												
AKH 10-2	0.250	0.385	0.364	1											
AKH 10-5	0.222	0.523	0.400	0.449	1										
AKH 10-10	0.271	0.281	0.382	0.351	0.500	1									
AKH 11-7	0.255	0.479	0.423	0.250	0.429	0.382	1								
AKH 2006-2	0.229	0.392	0.423	0.293	0.429	0.583	0.574	1							
AKH 2012-8	0.209	0.488	0.367	0.388	0.465	0.278	0.340	0.241	1						
AKH 2012-9	0.170	0.345	0.375	0.418	0.460	0.436	0.375	0.400	0.429	1					
AKH 09-5	0.241	0.424	0.450	0.397	0.456	0.391	0.359	0.359	0.356	0.579	1				
AKH 976	0.300	0.351	0.429	0.446	0.382	0.491	0.429	0.455	0.281	0.407	0.476	1			
AKH 9916	0.213	0.438	0.412	0.304	0.447	0.451	0.385	0.385	0.413	0.442	0.417	0.418	1		
DHY 286	0.306	0.462	0.411	0.379	0.442	0.446	0.491	0.580	0.286	0.367	0.438	0.441	0.351	1	
SURAJ	0.206	0.293	0.217	0.295	0.300	0.184	0.191	0.167	0.361	0.229	0.302	0.240	0.200	0.271	1

Table 6. Similarity matrix of SSR primer analysis based on Jaccard's similarity coefficient (Fibre strength)

	AKH 84635	AKH 8828	AKH 081	AKH 10-2	AKH 10-5	AKH 10-10	AKH 11-7	AKH 2006-2	AKH 2012-8	AKH 2012-9	AKH 09-5	AKH 976	AKH 9916	DHY 286	SURAJ
AKH 84635	1														
AKH 8828	0.333	1													
AKH 081	0.475	0.469	1												
AKH 10-2	0.593	0.406	0.587	1											
AKH 10-5	0.473	0.545	0.583	0.542	1										
AKH 10-10	0.397	0.484	0.569	0.420	0.525	1									
AKH 11-7	0.273	0.354	0.485	0.382	0.483	0.524	1								
AKH 2006-2	0.306	0.349	0.439	0.379	0.433	0.550	0.648	1							
AKH 2012-8	0.491	0.566	0.500	0.561	0.556	0.444	0.338	0.400	1						
AKH 2012-9	0.475	0.446	0.793	0.613	0.638	0.569	0.463	0.439	0.500	1					
AKH 09-5	0.448	0.443	0.441	0.492	0.459	0.412	0.415	0.290	0.500	0.400	1				
AKH 976	0.397	0.349	0.532	0.468	0.593	0.603	0.589	0.483	0.448	0.583	0.459	1			
AKH 9916	0.467	0.394	0.645	0.661	0.576	0.471	0.548	0.476	0.468	0.672	0.371	0.576	1		
DHY 286	0.357	0.333	0.525	0.458	0.588	0.443	0.527	0.446	0.339	0.500	0.313	0.473	0.517	1	
SURAJ	0.308	0.412	0.367	0.418	0.521	0.333	0.333	0.352	0.392	0.344	0.462	0.377	0.379	0.333	1

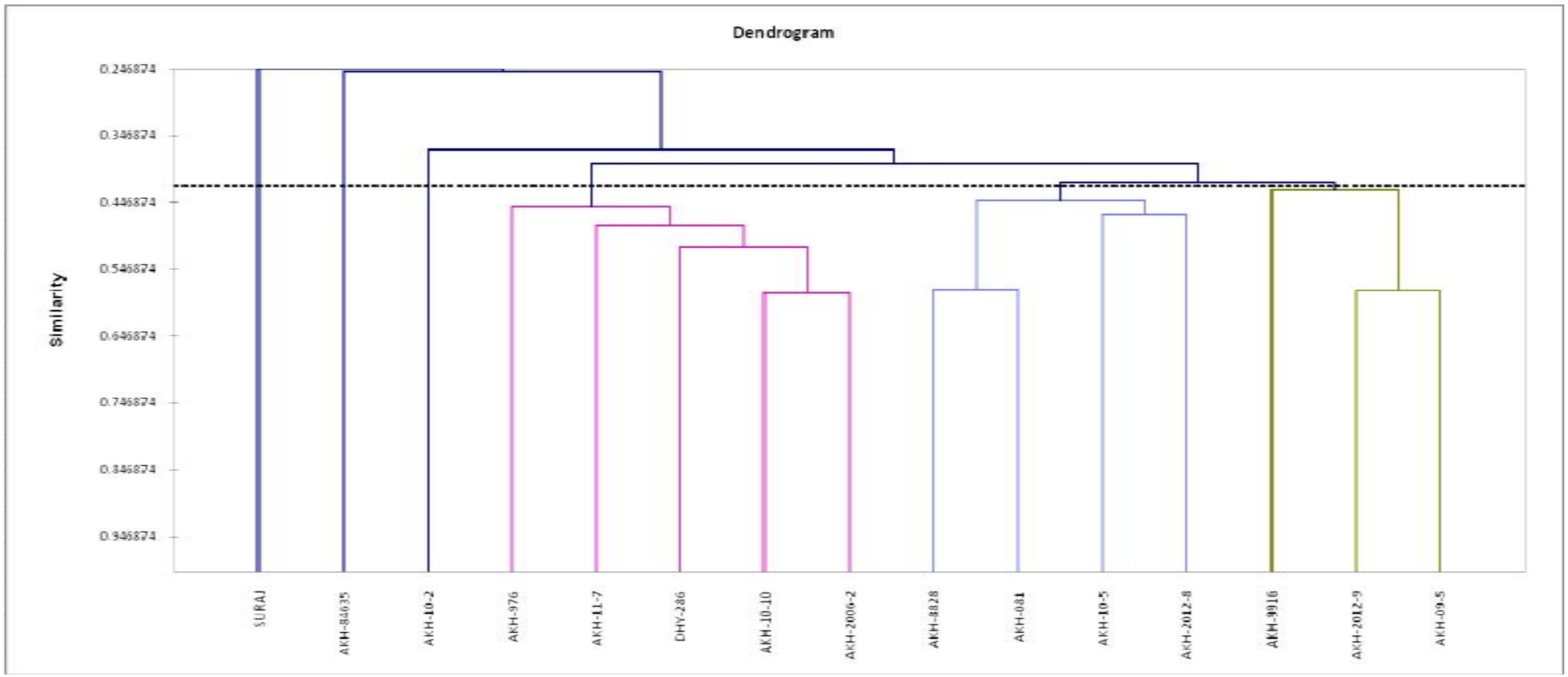

Fig. 5. UPGMA dendrogram based on molecular data from 27 SSR markers associated with fibre length showing relationship among 15 cotton genotypes

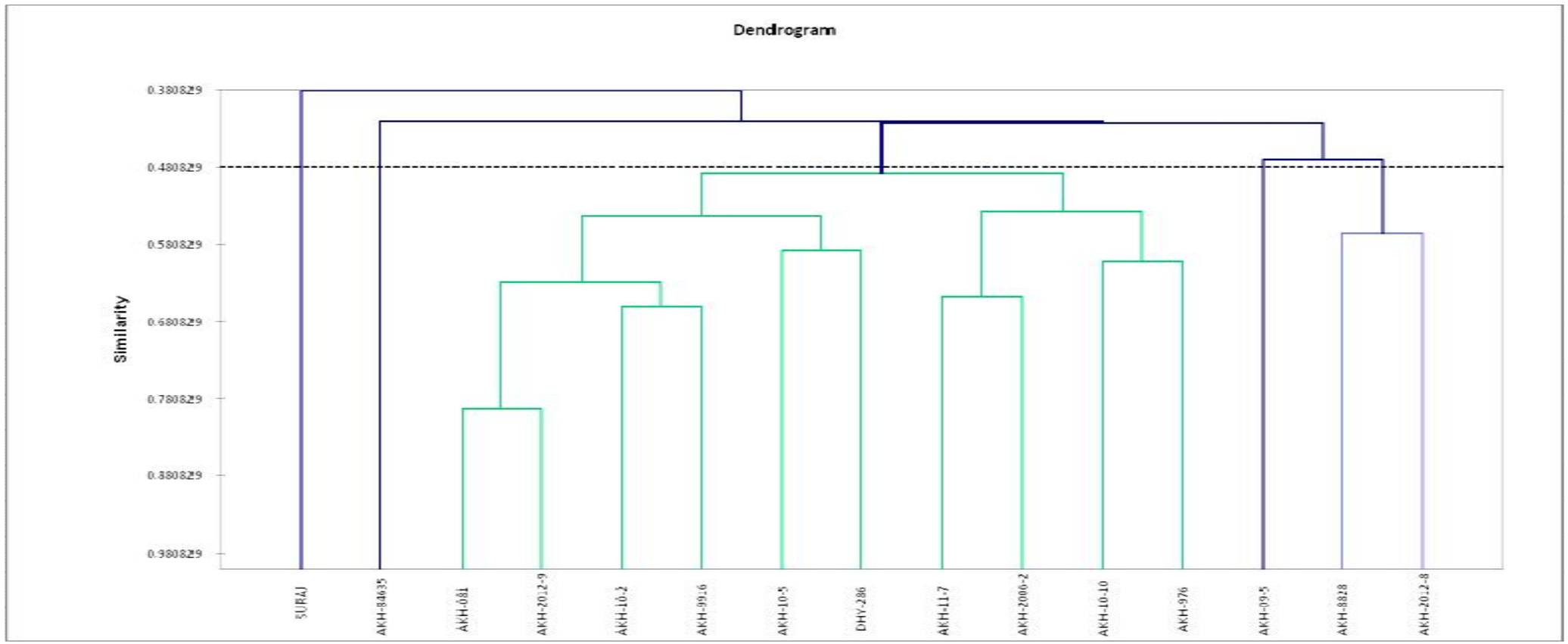

Fig. 6. UPGMA dendrogram based on molecular data from 23 SSR markers associated with fibre strength showing relationship among 15 cotton genotypes

Dendrogram generated for fibre length revealed that the 15 cotton genotypes were grouped into 6 main clusters. Main cluster 'I' comprised of only one genotype AKH 84635. Main cluster 'II' comprised of 4 genotypes *viz.,* AKH 8828, AKH 081, AKH 10-5, and AKH 2012-8. Main cluster 'III' comprised of AKH 10-2 genotype. Main cluster 'IV' comprised of 5 cotton genotypes *viz.,* AKH 10-10, AKH 11-7, AKH 2006-2, AKH 976 and DHY 286. Three genotypes *viz*., AKH 2012-9, AKH 09-5 and AKH 9916 were found to be accumulated in main cluster 'V'. Similarly main cluster 'VI' comprised of only one genotype i.e. SURAJ. Present study reveals that the parents AKH 84635, AKH 10-2 and SURAJ were found to be accumulated in individual cluster and found to be most diverse having broad genetic base for fibre length character (Fig. 5). Similarly, genetic diversity of Turkish commercial cotton varieties for fibre properties with the dendrogram was studied [24]. Maximum diversity between 4 main clusters across 96 cotton genotypes was found between Maydos Yerlisi, Ozbek 142, Delcerro and Giza 70 genotypes based on their medium, long and extra-long fibre quality, respectively.

3.5 Genetic Distance-based Analysis for Fibre Strength

The Jaccard's similarity coefficient showed the genetic relationships among the cotton genotypes. The similarity matrix based on the SSR marker profiling for fibre strength is depicted in (Table 6) and dendrogram depicted in Fig. 6.

As concerned to fibre strength trait, 15 cotton genotypes showed similarity coefficient value ranged from 0.273 to 0.793 indicating more variation in respect of genetic similarity at studied loci. This ultimately means that large range of genetic diversity for fibre strength existed among the studied genotypes. The highest genetic similarity (0.793) was found between AKH 2012-9 and AKH 081 followed by genotype AKH 9916 and AKH 2012-9 with value 0.672. While, the lowest value of genetic similarity (0.273) was found to be observed in genotype AKH 11-7 and AKH 84635 which was found to be most diverse parents for fibre strength (Table 6).

The dendrogram generated for fibre strength revealed that, 15 cotton genotypes were grouped into 5 main clusters. Main cluster 'I' comprised of only one genotype AKH 84635. Main cluster 'II' comprised of 2 genotypes *viz*., AKH 8828 and AKH 2012-8. Main cluster 'III' comprised of 10 genotypes *viz*., AKH 081, AKH 10-2, AKH 10-5, AKH 10-10, AKH 11-7, AKH 2006-2, AKH 2012-9, AKH 976, AKH 9916 and DHY 286. Main cluster 'IV' comprised of only one genotype AKH 09-5. Similarly, main cluster 'V' comprised of single cotton genotype i.e. SURAJ. The study revealed that the parents AKH 84635, AKH 09-5 and SURAJ were accumulated in individual cluster and found to be most diverse and genetically dissimilar parents having broad genetic base for fibre strength (Fig. 6).

4. CONCLUSION

The present investigation revealed that genotypes SURAJ, AKH 10-2 and AKH 84635 for fibre length and SURAJ, AKH 09-5 and AKH 84635 for fibre strength were found to be the most diverse and dissimilar having a broad genetic base for the concerned fibre traits. Similarly, primers JESPR0065, CIR253 and NAU3092 showed greater level of polymorphism for fibre length ranged from 88.00 to 100 *per cent*, whereas, primers NAU3308, NAU3888, TMB1618 and GH499 had shown high polymorphism for fibre strength ranged from 55.55 to 100 *per cent*. Therefore, these markers provide a basis for future efficient use in marker assisted selection for fibre quality traits in cotton.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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