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Molecular chracterisation of cotton genotypes using SSR, ISSR and RAPD markers in relation to fiber quality traits

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ABSTRACT : In the present study, twenty five cotton (*Gossypium* sp.) genotypes comprising of *six G. hirsutum, three G. arboreum, one G. anomalam and one G. barbadense genotype,* three intraspecific hybrids (*G. hirsutum x G. hirsutum), two* intraspecific hybrids (*G. hirsutum x G. barbadense) and nine* F_6 *lines of trispecific cross (G. arboreum GMS x G. anomalam x G. barbadense)* were genotyped. Eighteen SSR markers reported to be linked to fiber quality QTLs, seven ISSR and six RAPD markers were used to assess genetic diversity and identification of markers associated with fiber quality traits. On pooled data analysis all genotypes were distinguished as separate clusters with similarity index range of 0.58-0.97 into eight broad groups. Highest divergence was observed in *G. anomalum*; three *G. arboreum* genotypes, introgression line I-197, *G. barbadense* RHCb-001 and *G. hirsutum* Phule688. In both dendrogram and 2-D scatter plot, *G. hirsutum* genotypes grouped into two groups as per their contrasting fiber qualities. Low level of divergence was observed in genotypes with medium fiber quality traits that were grouped along with *G. hirsutum* x *G. barbadense* interspecies hybrids having extra-long fiber, good strength and fineness. Three, seven and twelve SSR markers were found to be highly correlated with fibre length, strength and fineness respectively.

SSR markers exhibiting highly significant correlation for fibre quality traits will assist breeders in transferring and maintaining valuable fibre quality traits during cultivar development.

Key words : Fiber traits, genetic variability, *Gossypium*, molecular markers

Cotton is one of the most important fiber crop of the world, with a global production projected to increase to 24.9 million tonnes and cultivated under 31.7 million ha area in 2017-2018. Among forty five diploid species and five tetraploid species of *Gossypium,* two allotetraploid species, *G. hirsutum* and *G. barbadense,* and two diploid species, *G. herbaceum* and *G. arboreum* have been extensively cultivated around the world (Jamshed *et al.,* 2016). *G.hirsutum* covering more than 90 per cent of the total world production however, its low quality fibre requires improvement to meet human demands (Sun *et al.,* 2017). *G. barbadens* provides high quality extra long fiber accounting 5 per cent of the annual world cotton production (Liu *et al.,* 2015).

The major challenge is to combine improved fiber quality traits with high yield, early maturity, as fiber quality traits governed by many quantitative trait loci (QTLs), environment sensitive and are negatively correlated with yield and early maturity traits (Su *et al.,* 2016). Interspecific and intraspecific hybridisation can prove very important for improvement of fiber quality traits. For improvement of fiber qualities in *G. hirsutum* L., superior fiber properties of *G. barbadense* can provide novel variations. A successful breeding program depends on the complete knowledge and understanding of the genetic diversity within and among genetic

resources of the available germplasm. It enables plant breeders to choose parental sources that will generate diverse populations for selection. The lack of genetic diversity is implicated in the slowing of progress in developing new cotton cultivars with improved yield and quality potential as well as stress resistance. Further, correct genotype identification for obtaining truthful seed is of prime importance in cotton seed production. Molecular markers can be used, as they offer a quick and reliable technique, for precise genotyping as compared to morphological scoring.

Molecular markers are useful to define the genetic factors affecting fiber quality (Tang *et al.,* 2015). DNA profile may come in use in future for characterization of genotypes; and providing valuable information for selection and crossing parents or to minimize linkage drag associated with introgression during back crossing, and also for selection of traits that are difficult to score by conventional phenotypic assays (Salunke *et al.,* 2012). Molecular markers specifically SSR markers linked to fibre trait QTLs may help in generating their DNA fingerprints, evaluation of genetic variability as well as their validation for association with particular trait. The objective of the present

Table 1. Details of cotton genotypes used in the present study

S.no.	Name of genotype	Species details	Details
1.	RHC 006	*G. hirsutum*	Female parent of Phule 0388
2.	RHC 003		Female parent of Phule 492
3.	RHC 004		Male parent of Phule 492
4.	JLH 168		Cultivar
5.	Phule 688		Cultivar
6.	LRA 5166		Cultivar
7.	Y 1	*G. arboreum*	Cultivar
8.	JLA 794		Cultivar
9.	*MPKV GMS*		Genetic male sterile line
10.	*G. anomalum*	*G. anomalum*	Wild diploid species from Africa
11.	RHC-b 001	*G. barbadense*	Male parent of Phule 0388
12.	Rasi 2	*G. hirsutum* × *G. hirsutum*	*Bt* cotton hybrids
13.	Mallika	Intraspecific hybrid	
14.	Phule 492		Non *Bt* cotton hybrids
15.	Kashinath	*G. hirsutum* × *G. barbadense*Interspecific	*Bt* cotton hybrids
16.	Phule 0388	(HxB) hybrid	Non *Bt* cotton hybrid
17.	I-41	2(*G. arboreum* GMS × *G. anomalum*) ×	Introgressed material of F6 families
		G. barbadense	from trispecies cross
18.	I-46		
19.	I-61		
20.	I-103		
21.	I-151		
22.	I-171		
23.	I-188		
24.	I-193		
25.	I-197		

study was to evaluate genetic divergence and relationship between 25 cotton genotypes using fibre trait linked SSR; unlinked ISSR and RAPD markers.

MATERIALS AND METHODS

Twenty five cotton (*Gossypium* sp.) genotypes comprising of six *G. hirsutum,* three *G. arboreum,* one *G. anomalam* and one *G. barbadense* genotype, three intraspecific hybrids (*G. hirsutum x G. hirsutum),* two intraspecific hybrids (*G. hirsutum x G. barbadense*) and nine F_6 lines of trispecific cross (*G. arboreum GMS x G. anomalam x G. barbadense)* were used in the present study. All the experimental material were obtained from the Cotton Breeder, Cotton Improvement Project, Mahatma Phule Krishi Vidyapeeth, Rahuri (Table 1).The genomic DNA was isolated from leaves of seedlings of these cotton genotypes as per HiPura Plant Genomic DNA Miniprep kit (m/s Himedia Ltd.) after grinding them in liquid nitrogen. The genomic DNA was quantified by spectrophotometer and integrity checked by 0.8 per cent agarose gel electrophoresis.

PCR amplification was performed using 18 microsatellite markers reported to be highly polymorphic and linked to fiber quality QTLs in Cotton Microsatellite Database (http://

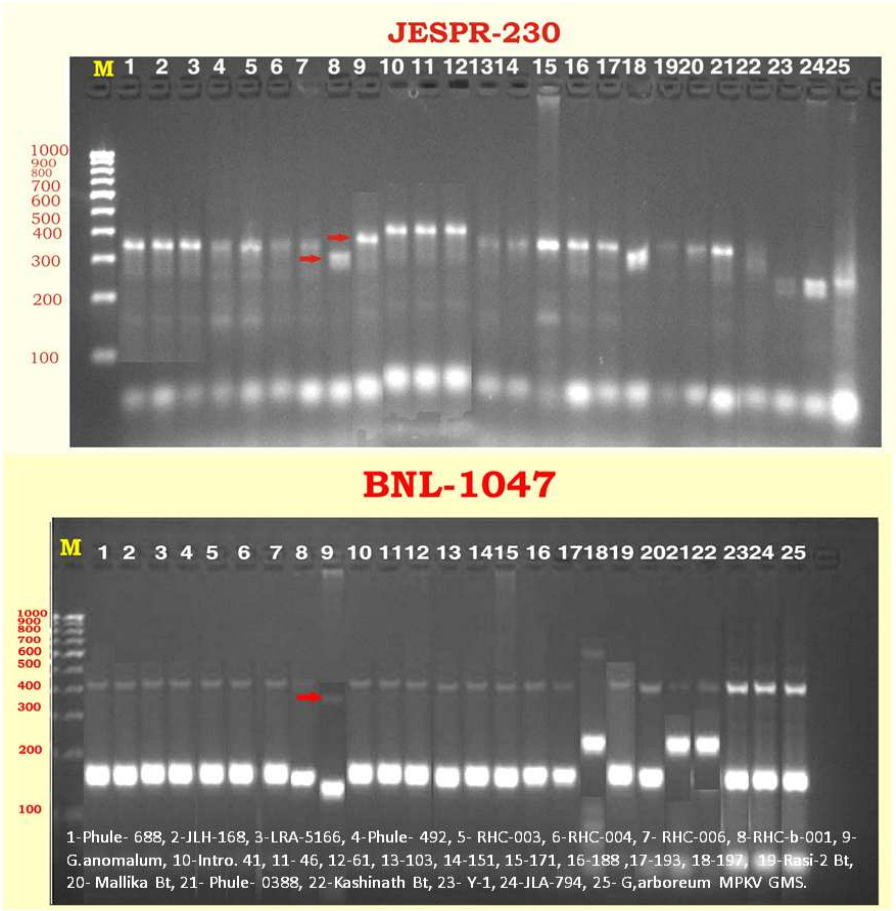

Fig. 1. Representative PCR amplification profile with SSR primers JESPR230 and BNL1047

www.cottonssr.org; http:/www.cottongen.org), 7 ISSR and 6 RAPD primers (Table 2). They were custom synthesized from m/s Bangalore Genei Pvt. Ltd., Bangalore. Amplification reaction mixture was prepared in 0.2 ml PCR tubes, containing 1 X PCR buffer A (100 mM Tris pH 9.0; 500 mM KCl; 15 mM $MgCl_2$ and 0.1 per cent gelatin), 0.2 mM each dNTP, 1 unit of *Taq* DNA polymerase enzyme, 20 picomoles of each primer, 20 ng template DNA in each 20µl reaction volume. The amplification was carried out on a thermal cycler (Eppendorf, Master cycler gradient).

SSR, ISSR and RAPD primer amplification involved an initial denaturation step of 5 min at 94°C. SSR amplification regime consisted 40 cycles at 94°C for 30 sec, annealing for 30 sec at different temperatures for different primers (varying from 49°C to 60°C; Table 2) min and 72°C for 30 sec min. ISSR and RAPD amplification reactions were cycled 40 times at 94°C for 1 min, annealing for 1 min. (at 36 °C for RAPD primers and different temperatures varying from 45 to 55°C for ISSR primers) and 72°C for 1 min. In all three amplification processes, a final elongation was allowed for 10 min at 72°C. For SSR analysis, amplified products were resolved by 2.5 per cen agarose gel electrophoresis while ISSR and RAPD markers were resolved using (1.2%) agarose gel electrophoresis. Resolved gels were then observed under UV transilluminator in gel documentation system (m/s FlorChem™ Alpha Innotech) and image was captured.

The clearly resolved PCR amplified bands of cotton species with 18 different SSR primers and 7 ISSR and 6 RAPD primers were scored manually for their binary data. Pooled data was analyzed using the computer package NTSYSpc 2.02i. The similarity matrix was constructed from binary data with Dice similarity coefficients, while. for clustering analysis Unweighed Pair Group Method Using Arithmetic Averages (UPGMA) was employed. Principal Co-ordinate analysis (PCO) was performed to estimate the genetic distance between each group of the varieties using NTSYS software. The polymorphism Information Content (PIC) value was calculated as PIC = 1- "P^2_{ij} where P_{ij} is the frequency of the j^{th} allele for the i^{th} marker locus and summation extends over n alleles. Correlations were calculated to detect relationships between the banding pattern data with the three fibre traits *i.e.* fibre length, strength and fineness.

RESULTS AND DISCUSSION

SSR markers based profiling : In the present study on SSR analysis with 18 primers reportedly linked with different fiber trait amplified a total of 43 bands (Table 3, Fig. 1). Out of the 43 bands nine bands were monomorphic, eight were unique and rests of them were polymorphic amongst samples studied. The SSR primers, CIR244 and CIR354 produced maximum of five polymorphism alleles. PIC values ranged from 0.14 to 0.867, with an average of 0.558. BNL 3435 showed the highest PIC value (0.967) and BNL3147 the lowest value (0.14) was founds. 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.

Among eight unique SSR bands (Table 4),

Table 2. Details of SSR, ISSR and RAPD Primers used in molecular analysis.

SN.	Primer		Sequence of primers (5' to 3')	T_{ann} (°C)	PIC value	Fibre QTLs linked
SSR Primers						
1.	BNL 1047	F	GCTTGTCATCTCCATTGCTG	53.8	0.505	qMV8-chr22
		R	TAGCCCGGTTCATGTTCTTC			
2.	BNL 1059	F	CCTTCTCTGACACTCTGCCC	60	0.433	qFL1+qMV1+qMV2-chr14
		R	TGTATTCTCTTCTTTTCCTTATACTTTT			
3.	BNL 1672	F	TGGATTTGTCCCTCTGTGTG	53.8	0.77	qFL3-chr09+qLY-chr23
		R	AACCAACTTTTCCAACACCG			
4.	BNL 3090	F	GAAATCATTGGAAGAACATATACTACA	58.1	0.96	qFL4-chr01
		R	TTGCTCCGTATTTTCCAGCT			
5.	BNL 3147	F	ATGGCTCTCTCTGAGCGTGT	58.1	0.14	qFL3-chr09
		R	CGGTTCAGAGGCTTTGTTGT			
6.	BNL 3435	F	CGTGGATTTAAGCACCGATT	53.8	0.967	qLY_N-chr26
		R	TAAGAAATGGTGTTGCAATTACC			
7.	BNL 3510	F	GCACCAGTGCTCAGACACACA	56.3	0.87	qLY_N-chr26
		R	ATNTGAGTTGAAATCTGCCGTAA			
8.	BNL 3580	F	CTTGTTTACATTCCCTTCTTTATACC	53.8	0.174	qFL4-chr01
		R	CAAAGGCGAACTCTTCCAAA			
9.	BNL 3627	F	TATGGGCCTGTCCACCTAAG	53.8	0.884	qMV7+qFS2-chrA02b
		R	CAAAGCAACATGCACACACA			
10.	BNL 3867	F	TAATTGAGTTGTTTTCTTACTTGCC	56.3	0.194	qLY_N-chr26
		R	TGCCAATTTAGCAATCACCA			
11.	JESPR 134	F	GTCAGAGTCTTCGGGTTGTC	58.0	0.548	qSI-chr26+qLY_N-chr05a
		R	GTAACAGCAGAGAAGTCGGTG			
12.	JESPR 151	F	CTGGACTAAAAACCTTAACTGG	56.3	0.538	qFS2-chr23
		R	CTCGATTCTAACTCAATCACG			
13.	JESPR 230	F	GGGACTAAAGAAGTAATTATGCC	53.8	0.293	qFL3-chr09
		R	GAAACCCTTGGCCATGAG			
14.	JESPR 289	F	CATTGCATTTTGCCCC	58.1	0.482	qFL4chr01
		R	AATCTAGCGCACAAGGGC			
15.	CIR 089	F	CTCCATTCCTCGTTTG	49	0.223	qFL4chr01+ qSI-chr29
		R	AGATTTCGTTTCCCATT			
16.	CIR 244	F	TGGAAGGTGATGTTCTAA	56.3	0.799	qFS1-chrA02
		R	GATCAAAGAGCAAACTAATC			
17.	CIR 354	F	CACAATCCTCAGCCA	56.3	0.361	qFSN-chrA02+ qFLN-chrA02
		R	AGAGAAGGAAAGAGGAAA			
18.	CIR 413	F	TTAAAGCTCACACACACA	49	0.906	qMV-chrD03
		R	CAACAGTAACGAAGAACAAT			
ISSR Primers						
1	IS 8		CACACACACACACAGC	47	0.327	
2	IS 12		GTGTGTGTGTGTGTTG	55	0.206	
3	IS 13		GTGTGTGTGTGTGTGTCA	45	0.154	
4	ISSR 827		ACACACACACACACACG	46	0.471	
5	ISSR 834		AGAGAGAGAGAGAGAGYT	52	0.116	
6	ISSR 841		GAGAGAGAGAGAGAGAYG	48	0.389	
7	ISSR 857		ACACACACACACACYC	52	0.327	
RAPD Primers						
1.	OPE 9		CTTCACCCGA	36	0.341	
2.	OPE 10		CACCAGGTGA	36	0.329	
3.	OPE 11		GAGTCTCAGG	36	0.436	
4.	OPE 18		GGACTGCAGA	36	0.472	
5.	OPE 20		AACGGTGACC	36	0.286	
6.	OPC 16		CACACTCCAG	36	0.310	

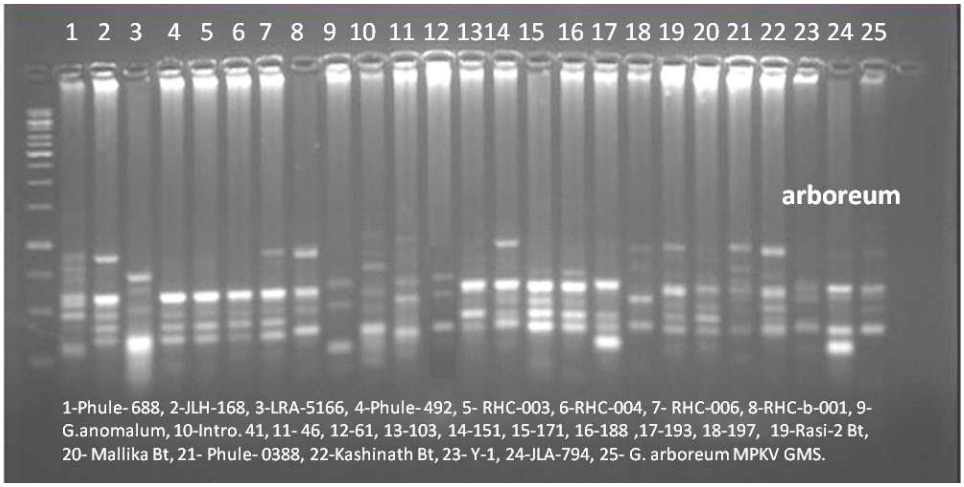

Fig. 2. Representative PCR amplification profile with ISSR-857 Primer

BNL 1047_{196bp}, JESPR $151_{450bp,}$ JESPR 289_{165bp} amplified specifically in wild *G. anomalum*. BNL1059_{160bp} was present in Phule 0388 (interspecific cotton hybrid between *G. hirsutum x G. barbadense).* JESPR 134_{178bp} was observed in *G. arboreum*. Unique allele JESPR 230_{325bp}, CIR 354_{290bp} and CIR-089_{155bp} present in RHC-b-001 a *G.barbadense* male parent of cotton hybrid Phule-0388 and can be used in its identification.

Table 3. Amplification details of SSR, ISSR and RAPD markers

Observations	SSR analysis	ISSR analysis	RAPD analysis
Marker used	18	10	07
Markers yielding amplification	18	07	06
Polymorphic markers	14	07	06
Total alleles	43	28	32
Total monomorphic bands	09	02	04
Total polymorphic bands	34	26	28
Total unique bands	08	03	04
Average bands produced/marker	2.38	04	5.33
Average polymorphic bands produced	1.88	3.71	4.67

Markers polymorphic between *Ga*, *GH* and *Gb* are suitable for interspecific mapping. Higher numbers of alleles were observed among *G. arboreum* and *G. anomalum* than *G. hirsutum*. Dongre *et al.,* (2011) observed that during genetic purity testing of *G. hirsutum* hybrid Phule 388, JESPR151 and JESPR152 primers were heteroallelic for parents.

Correlation of SSR markers with fiber quality traits : Fiber length, strength, and fineness are key determinant of cotton yield and fibre quality. In intraspecific segregating populations of upland cotton many QTLs associated with fiber quality traits have been identified using molecular markers (Su *et al.,* 2016). In the present study high correlation with fibre length, strength and fineness data was observed for three markers *i.e.* BNL1672-1, BNL3510-1 and CIR354-5 were (Table 5). In the present study, BNL1672_{190bp} marker got amplified in twelve genotypes (including 10 of 11 with highest strength) with average fibre strength 23.1 g/tex. BNL1672-1_{130bp} marker was present in all fine fiber genotypes with fineness

<3.8 except JLH168; while it was absent in all the genotypes with coarse fiber having fiber fineness >4.2. Five of ten genotypes with intermediate fiber fineness ranging from 3.9-4.2 had the same marker. $BNL3510_{245bp}$ marker got amplified in all six genotypes with fiber length <28.6mm except *G. anomalum*. It also got amplified in three medium length genotypes *viz.,* I-197 (FL=28.6mm); I188 (FL=28.5); and RHC 003 (FL=27.6).

For fibre length, high correlation was observed with BNL1672-1 (+0.613; average fibre length 30.78 mm), BNL3510-1 (-0.667; average fibre length 26.6 mm) and CIR345-5 (-0.531; average fibre length 28.5 mm) markers (Table 3). BNL1672 and CIR354 are known to amplify alleles linked with fibre length quantitative trait loci on chromosome 3 and A02, respectively (http:/www.cottongen.org). $JESPR230_{120bp}$ marker reportedly linked with fiber length QTL got amplified in twenty genotypes other than four genotypes with long fiber length (RHC001; I-197; Mallika and I151).

For fibre strength, highly significant positively correlation was observed with two SSR markers [BNL1672-1 (+0.672; average fibre strength 23.1 g/tex) and JESPR151-1 (+0.571; present only in *G. anomalum*)]. These SSR markers associated with *G. anomalums* will assist breeders in transferring and maintaining valuable fibre quality traits from *G. anomalums* during cultivar development. Newaskar *et al.,* (2013) had also reported a JESPR151-407 bp *G. anomalum* specific marker in their studies. *G. anomalum* is an excellent source for transferring high fibre strength to cultivated *G. hirsutum* (Mehetre, 2010). Negative correlation was observed with five SSR markers *i.e.* CIR354-5(-0.652), BNL3510-1 (-0.613; average strength 20.1 g/tex), CIR354-2 (-0.571; absent only in *G. anomalum*), JESPR151-2 (-0.571; absent only in *G. anomalum*) and BNL3627 (-0.566; average strength 19.97 g/tex). JESPR151, BML3627 and CIR354 are known to amplify markers linked to fibre strength from chromosome23, A02B and A02 (http:/www.cottongen.org). $BNL3580_{400bp}$ marker was present in nine of ten genotypes (except Rasi 2) with coarse fiber>4.0.

Similarly, for fibre fineness highly significant positive correlation was observed with eight SSR markers *i.e.* BNL3510-1; BNL3435-1 (present only in *G. arboreum* and I197); CIR354-1 (present only in *G. arboreum*); CIR354-5; CIR413-1 (present in *G. arboreum* + Rasi2+RHC003+RHC006); CIR244-1 (present in *G. arboreum*+RHC003+RHC006); CIR244-4 and JESPR134-2. Highly significant negative correlation with fineness was observed with four markers *i.e.* BNL1672-1 (average micronaire value 3.67); JESPR230-1 (absent only in *G. arboreum*), JESPR134-1 (absent only in *G. arboreum*) and CIR244-5 (absent only in *G.*

Table 4. Polymorphic markers for identification of cotton genotypes/species

Genotypes/species	Unique molecular markers
RHC-b- 001 *(G. barbadense)*	JESPR $230_{320\ bp}$, CIR $354_{290\ bp}$, CIR $089_{155\ bp}$,OPE-$9_{900\ bp}$, OPE-$20_{1250\ bp}$
Phule 0388 Interspecific (HxB) hybrid	BNL 1059_{160bp}
G. anomalum	BNL $1047_{196\ bp}$, JESPR $151_{450\ bp}$, JESPR $289_{165\ bp}$, IS 13 $_{375\ bp}$, ISSR 841 $_{640\ bp}$
(*G. arboreum*) MPKV GMS	JESPR $134_{178\ bp}$
RHC-006*(G. hirsutum)*	ISSR 834 $_{475\ bp}$

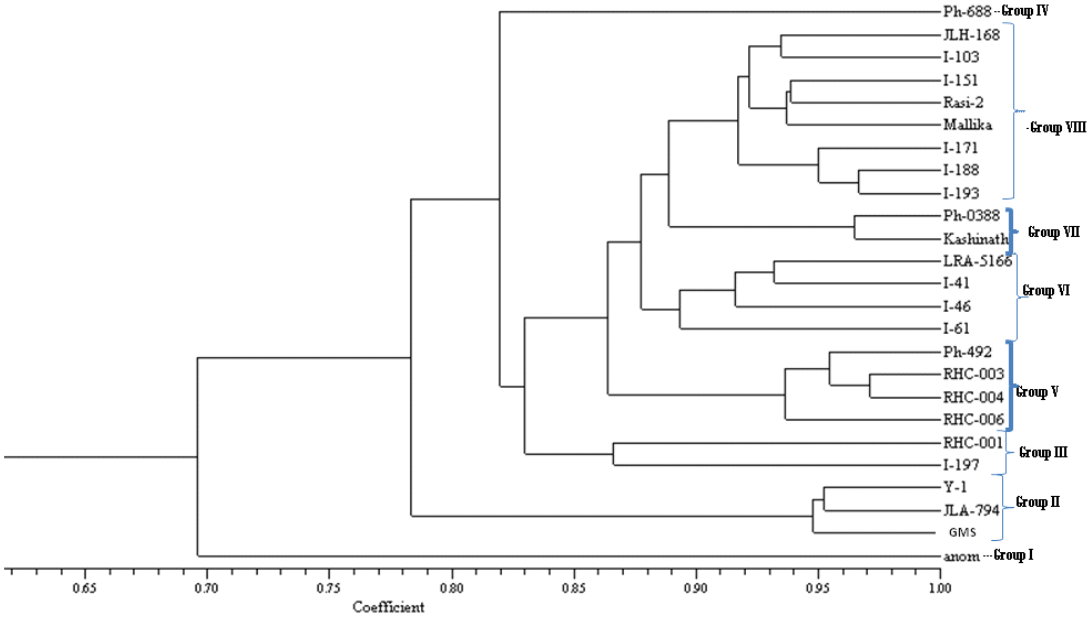

Fig. 3. Dendrogram showing clustering pattern in cotton genotypes studied based on pooled data of SSR, ISSR and RAPD analysis.

arboreum and PH688). BNL1672 and CIR243 are known to amplify markers linked to fibre fineness present on chromosome-23 and D03 (http:/www.cottongen.org). Newaskar *et al.*, (2013) while studying interspecies hybrids and their parents reported that *G. arboreum* associated $BNL3435_{200\ bp}$ marker was parent specific; whereas *G. arboreum* associated $CIR244_{726\ bp}$ and$CIR354_{142\ bp}$ markers were further observed in the interspecific hybrid and its derivates.

ISSR and RAPD marker based profiling: Molecular characterization of cotton genotypes was carried out using 7 ISSR and 6 RAPD primers. ISSR primers amplified 28 fragments of which 25 were polymorphic thus produced on an average 3.71 polymorphic bands (Table 3; Fig. 2). The number of bands generated by each primer varied from 2 (ISSR 13) to 5 (ISSR 12, ISSR 857) with an average of 4 fragments/ primer. Three unique bands were observed which may be species specific. The ISSR markers, IS 13 and ISSR 841 amplified each one unique band separately and present in a wild cotton parent, *G. anomalum*. Six RAPD primers amplified 32 fragments of which 28 were polymorphic thus produced on an average 4.67 polymorphic bands.

Pooled data based clustering analysis : On pooled data of SSR, ISSR and RAPD analysis similarity coefficient values ranged from 0.58 in most diverse combination (*G. anomalum* and JLA794) to 0.97 in three most similar combinations (RHC004 and RHC003/Phule 492 as well I-193 to I-188). Highest mean similarity coefficient value 0.883 was recorded in I-193. *G. anomalum* similarity coefficient values with tetraploid genotypes ranged from 0.63 (with I-197) to 0.75 (with I-103); while three *G. arboreum* genotypes similarity coefficient values with

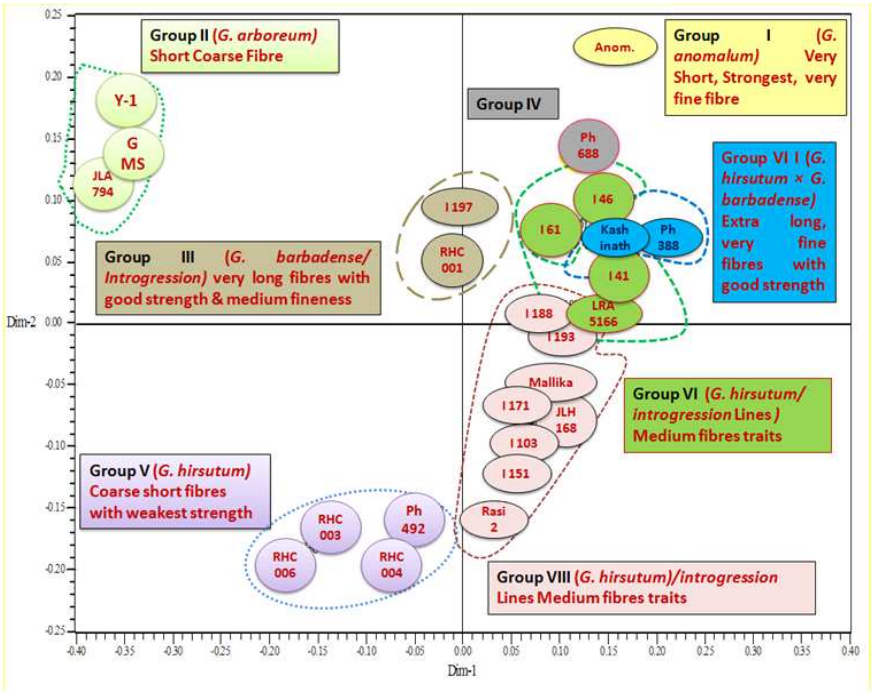

Fig. 4. 2D Scatter plot showing divergence in cotton genotypes studied

tetraploid genotypes ranged from 0.70 (with Phlue388) to 0.88 (with RHC003).

On pooled data of SSR, ISSR and RAPD analysis as well as individual SSR and ISSR analysis all genotypes could be classified into eight distinct groups (Fig. 3). Most divergent Group I comprised of *G. anomalum* which produces cotton with highest fiber strength (27.1 g/tex) and fineness (3ug/Inch) but smallest fiber (15mm). Next most divergent group II comprised of three *G. arboreum* genotypes (*viz.,* Y1, JLA794 and MPKV GMS) showing species specific identity with most coarse fiber (4.9-5.1 ug/Inch) with short fiber length (26.2-26.6 mm) and poor strength (20.3-21.5 g/tex). Group III comprised of two genotypes (*G. barbadense* RHC001 and I-197) with very long fibers (31.9-32.9 mm) having good strength (23.5-24.2 g/tex) and medium fineness (3.8-3.9 ug/inch). Group IV had a single genotype Phule688. Group V comprised of four genotypes (Hybrid Phule492 with its parents RHC003 and RHC004 along with RHC006) having fibers with weak strength (18.0-20.0 g/tex), coarse (4.1-4.9 ug/inch) and short length fiber (25.8-27.6 mm).

Group VI to VIII are more closely tied together. Group VI comprised of four genotypes (I-41, I-46, I-61 and LRA5166) having medium fiber length (28.3-29.0 mm), strength (21.0-21.7 g/tex) and fineness (4.1-4.9 ug/Inch). Group VII had two interspecies (*G. hirsutum* × *G. barbadense*) cross hybrids (Kashinath and Phule 388) with desired fiber qualities like extra long (33.9-35.7), finest (2.9-3.3 ug/Inch) and strongest fibers (except *G. anomalum*24.2-25.0g/tex). Group VIII comprised of rest 8 genotypes with fiber length (28.3-31.3 mm), strength (20.8-23.0 g/tex) and fineness (3.6-4.5 ug/inch).

Table 5. SSR Markers showing correlation with fibre length, strength and fineness traits

S. No.	Marker	Length	Strength	Fineness	Samples present	Fibre trait QTL for which it is previously reported to be linked
1	BNL 1047-3	-0.402	-0.483*	0.394	12	qMV8-chr22
2	BNL 1672-1	0.613**	**0.672****	-0.636**	12	qFL3-chr09+qLY-chr23
3	BNL 3090-1	-0.027	0.069	0.442*	5	qFL4-chr01
4	BNL 3435-1	-0.191	-0.063	0.528**	4	qLYN-chr26
5	BNL 3510-1	**-0.667****	-0.613**	**0.727****	9	qLYN-chr26
6	BNL 3627-1	-0.374	-0.566**	0.287	7	qMV7+qFS2-chrA02b
7	BNL 3627-2	-0.160	-0.421*	0.191	12	qMV7+qFS2-chrA02b
8	CIR 354-1	-0.350	-0.182	0.625**	3	qFSN-chrA02+ qFLN-chrA02
9	CIR 354-2	—-	-0.571**	0.382	24	qFSN-chrA02+ qFLN-chrA02
10	CIR 354-4	-0.462*	-0.347	0.445*	22	qFSN-chrA02+ qFLN-chrA02
11	CIR 354-5	-0.531**	-0.652**	0.573**	20	qFSN-chrA02+ qFLN-chrA02
12	CIR 413-1	-0.369	-0.383	0.629**	6	qMV-chrD03
13	CIR 413-2	-0.483*	-0.26	0.515*	9	qMV-chrD03
14	CIR 244-1	-0.433*	-0.361	0.587**	5	qFS1-chrA02
15	CIR 244-2	-0.49*	-0.136	0.207	5	qFS1-chrA02
16	CIR 244-4	-0.484*	-0.406	0.591**	11	qFS1-chrA04
17	CIR 244-5	0.379	0.175	-0.528**	21	qFS1-chrA04
18	JESPR134-1	0.35	0.182	-0.625**	22	qSI-chr26+qLYN-chr05a
19	JESPR134-2	-0.466*	-0.417*	0.606**	9	qSI-chr26+qLYN-chr05a
20	JESPR151-1	—-	0.571**	-0.382	1	qFS2-chr23
21	JESPR151-2	—-	-0.571**	0.382	24	qFS2-chr23
22	JESPR230-1	0.350	0.182	-0.625**	22	qFL3-chr09
23	JESPR230-2	-0.447*	-0.248	0.047	20	qFL3-chr09

** Significant at P = 0.01;*Significant at P = 0.05

Pooled data based 2D scatter plot analysis : In 2-D PCO scatter plot analysis, grouping pattern was clearly reflected (Fig. 4). First component (X axis) distinguished Groups II/III/V from Groups I/IV/VI/VII/VIII; while the second component (Y axis) distinguished Groups I/II/III/IV/VI/VII from groups V/VIII. *G. anomalum* (Group I) was placed distinctly along with Group IV (Phule 688), VI (I-41, I-46, I-61 and LRA5166) and Group VII (H x B hybrids) sharing a quarter with same components (+/+). However these overlapping groups (*i.e.* IV/VI/VII) were clearly distinguished in 3-D scatter plot by the 3rd component (Z axis; results not presented). Distinct Group II (*G. arboreum*) shared another quarter along (-/+) with Group III (long fibered *G. barbadense* RHC001 and I-197). Group V (RHC006/003/004and Phule 492) genotypes with weak coarse fiber were present together in a same quarter (-/-). Six out of eight Group VIII genotypes (except I-188/I-193 present just across X axis) were clearly distinguished being placed in component quarter (+/-). Rasi2 having coarse (4.5ug/Inch) but long fiber (30.0 mm) was present closer towards coarse fiber Group V.

Sapkal *et al.,* (2011) analyzed genetic diversity amongst 91 upland cotton accessions, and three wild species *viz., G. aridum*, *G. thurberi*

and *G. anomalum* using SSR and RAPD markers and reported moderate level of genetic diversity in upland cotton.

Both the SSR and ISSR markers in combination were faster, 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. Comparison of genetic diversity among cultivars provided foundation for marker-assisted breeding for fiber quality trait and understanding the effect of selection on fiber quality improvement. 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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