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Research Article

Microsatellite marker based DNA fingerprinting of cotton (*Gossypium spp.*) hybrids and their parents

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Abstract
Cotton production in India by the vast majority comes from cotton hybrids whose genetic purity is of great significance in the seed production chain and trade. Therefore, there is a need to develop a rapid, reliable and reproducible technique to assess the genetic purity of cotton hybrids as the traditional, morphological traits-based 'Grow-Out Test' is resource intensive, time consuming, tedious and not an infallible procedure. In this regard, a study was planned to understand the genetic diversity among the hybrids and their parents and also to identify SSR markers for confirmation of genetic purity or hybridity. One intra-*arboreum* hybrid, CICR2 (DS 5 GMS × LD 327 Sel.), four intra-*hirsutum* hybrids *viz.,* CSHH198 (CSH 19 × CSH 8), CSHH238 (SH 2379 9Y × PIL 8 Sel.), CSHH243 (CSH 2013 × CSH 43), CSHH1862 (GMS 16A × CB 33) and one *hirsutum* × *barbadense* hybrid, Phule 388 (RHC-006 × RHCb-001) along with their respective parental lines were selected for molecular characterization. Of the total 215 SSR markers surveyed, 60 markers conveyed polymorphism. The information conveyed by the polymorphic SSR markers was utilized to assess the molecular divergence among the study material. Maximum genetic dissimilarity of 0.66 was noted between Phule 388 and LD 327 (Sel.), and between RHC-006 and DS 5 (GMS). Minimum genetic dissimilarity of 0.07 was observed between CSHH1862 and CB 33, followed by 0.11 between CICR2 and DS 5 (GMS). SSR markers were highly efficient in capturing both intra-species and inter-species level diversity. The clustering and factorial analysis were in congruence with the species of *Gossypium*. The diploid species genotypes were clustered separately and distinctly from the rest of the genotypes. All the *hirsutum* hybrids and their respective parents were found closely clustered. The inter-specific hybrid, Phule 388 along with its parents was found grouped closely. The genetic purity of the hybrids was confirmed using identified SSR markers [*GH486*, *BNL1421*, *BNL3594*, *JESPR151* for *G. hirsutum* hybrids, CSHH198; *GH486*, *BNL2449*, *JESPR151*, *TMB0436* for *G. hirsutum* hybrids, *CSHH238*; *BNL2449*, *JESPR151*, *JESPR152* for *G. hirsutum* hybrid, CSHH243 and *GH527*, *BNL3812*, *TMB1484*, *TMB1645*, *NAU1190*, *BNL3816* for inter-specific *G. hirsutum* × *G. barbadense* hybrid Phule 388]. The SSR markers were efficient in the analysis of hybrid seed purity. The information generated in the present study about genetic diversity and genetic purity testing will greatly facilitate quality seed production of these cotton hybrids and thus, better cotton production.

Keywords: Cotton, DNA fingerprinting, Hybrids, Microsatellites, SSR markers, Diversity

INTRODUCTION

Cotton is the world's most important source of natural textile fibre and a significant oilseed crop. India leads the world in area and production of cotton with an estimated area of ≈13 million hectares with a production estimate of ≈37 million bales (https://cotcorp.org.in/statistics.aspx). Cotton production in India by the vast majority comes from cotton hybrids. Hybrids have an advantage of heterosis by the virtue of genomic heterozygosity and are produced

through hybridization between two genetically diverse pure lines (near homozygous and homogenous) having better trait complementation. These hybrid seeds then need to be authenticated for genetic purity, parentage and quality potential before they are released for cultivation by the farmers. Mechanical handling, outcrossing and ecological adaptation and at times, mutations may deteriorate the identity and purity of the hybrid seeds. The success of hybrid cotton production depends upon timely production and an adequate supply of genetically pure hybrid seeds to the farmers. It is estimated that for every 1% impurity in the hybrid seed, there will be a yield reduction of 100 kg per hectare (Mao *et al*., 1996). Thus, the genetic purity of hybrids is of great significance in the seed production chain and trade.

The genetic purity of the hybrid is assessed traditionally by Grow-Out Test (GOT) which is based on morphological traits (Tatineti *et al*., 1996). This procedure is resource intensive, time consuming, tedious and not infallible procedure. These morphological traits are sensitive to environmental variations and prone to subjective assessment. Owing to this, it can be difficult to distinguish the morphological differences between true hybrids and off types, especially when the parents share a closer pedigree (Selvakumar *et al*., 2010). The resource demanding and time consuming GOT may lead to delay in planting and resultantly can affect seed viability (Ali *et al*., 2008). Therefore, it is necessary to develop a rapid, reliable and reproducible technique to assess the genetic purity of cotton hybrids. Earlier studies have explored DNA marker systems such as RFLP (Pendse *et al*., 2001), RAPD (Mehetre *et al*., 2007), AFLP (Rana and Bhat, 2004), SSR (Saravanan *et al*., 2007; Selvakumar *et al*., 2010; Menka *et al*., 2016) and ISSR (Rana *et al*., 2006) to rapidly screen the genetic purity of hybrid seed. These molecular markers precisely assess the genotype, and not the phenotype (Sundaram *et al*., 2008). Among these markers, SSR markers are widely preferred for genetic purity testing (Saravanan *et al*., 2007; Selvakumar *et al*., 2010; Menka *et al*., 2016, Bora *et al*., 2016; Ben Romdhane *et al*., 2018), DNA fingerprinting (Santhy *et al*., 2019; Santosh *et al*., 2020), genetic diversity analysis (Abd El-Moghny *et al*., 2017) apart from other plant breeding applications owing to their reproducibility, co-dominant inheritance, genome-wide presence, robustness, higher polymorphism and analytical simplicity (Rakshit *et al*., 2011). Therefore, this study was planned to understand the genetic diversity among the hybrids and their parents and to identify SSR markers for confirmation of genetic purity or hybridity.

MATERIALS AND METHODS

The study material included one intra-*arboreum* hybrid, CICR2 (DS 5 GMS × LD 327 Sel.), four intra-*hirsutum* hybrids *viz.,* CSHH198 (CSH 19 × CSH 8), CSHH238 (SH 2379 9Y × PIL 8 Sel.), CSHH243 (CSH 2013 × CSH 43), CSHH1862 (GMS 16A × CB 33) and one *hirsutum* × *barbadense* hybrid, Phule 388 (RHC-006 × RHCb-001) and their respective parental lines. The detailed information about the study material is provided in **Table 1**. The pure seeds of these hybrids and their male and female parent were received from their respective breeders/institutions. The freshly opened young leaves of each of the hybrid and their parents were taken for extraction of genomic DNA and maintained in ice cold conditions. The genomic DNA was extracted using the quick Cetyl Trimethyl Ammonium Bromide method (Paterson *et al*., 1993). Extracted DNA was quantified on 0.8% agarose gel and quality was assessed using a spectrophotometer. The genomic DNA of these 18 genotypes (6 hybrids and their respective parents) was profiled to identify DNA polymorphisms using 215 genomic SSR markers and polymorphic markers were identified. The information conveyed by the polymorphic SSR markers was utilized to assess the molecular divergence among the study material. PCR amplification was carried out in 15µl reaction using the touchdown PCR protocol in Veriti® 96 well Fast Thermal Cycler (Applied Biosystems). The PCR amplification programme (Rakshit *et al*., 2010) consisted of an initial denaturation step at 94°C for 7 min (step-1), followed by 9 cycles (step-2) of 94°C for 15s, 65°C for 30s and 72°C for 60s with touch down by 1°C in each cycle from 65°C to 56°C followed by 40 cycles (step-3) of 94°C for 15s, 55°C for 30s and 72°C for 60s. The final extension was carried out at 72°C for 7 min (step-4). The PCR amplicons were electrophoresed on 4% agarose gel stained with ethidium bromide and visualized under UV transillumination. The molecular profiles were visually scored based on the product size in comparison with the standard 50bp DNA ladder (Thermo Scientific). The allelic data was converted into 1 (presence) – 0 (absence) binary matrix to estimate the genetic dissimilarity indices based on Jaccard's similarity coefficient. The genetic dissimilarities among the genotypes were utilized for clustering of the genotypes using the Neighbour Joining method and factorial analysis employing DARwin 6.0 software (Perrier *et al*., 2003). In order to confirm the hybridity or genetic purity, the genomic DNA of hybrid vis-à-vis its male and female parent was assayed with polymorphic SSR markers. The SSR markers clearly distinguishing the male and female parent of each of the hybrid was identified among the 60 polymorphic SSR markers. The markers producing multiple bands with heterozygosity were excluded for genetic purity analysis. SSR markers which revealed different, homozygous alleles in parents with a distinct difference in allele size were identified for each of the hybrids. The genetic purity was confirmed in each of the hybrids using identified markers distinctly polymorphic between respective parents.

RESULTS AND DISCUSSION

Of the total 215 SSR markers employed for polymorphism survey among the hybrids and their parents, 60 markers

Table 1. List of hybrids along with their parentage and salient features

S. No.	Name	Species*	Year of release	Institution	Pedigree	Crop maturity (days)	Average yield (q/ha)	Ginning percent	Fibre length (mm)	Bundle strength (g/tex)	Miccronaire value (µg/inch)	Release for states
1	CICR2 (CISSA2)	A×A	2005	CICR, Sirsa	DS 5 (GMS) × LD 327 (Sel.)	160-170	21	38.4	19.2	16.3	7.0	Punjab, Haryana and Rajasthan
2	CSHH198 (Shresth)	H×H	2005	CICR, Sirsa	CSH 19 × CSH 8	160-165	22	32.8	26.7	25.7	4.5	Punjab, Haryana and Rajasthan
3	CSHH238 (Hybrid Kalyan)	H×H	2007	CICR, Sirsa	SH 2379 9Y × PIL 8 (Sel.)	150-160	21	33.5	27.6	22.5	4.5	Punjab, Haryana and Rajasthan
4	CSHH243	H×H	2008	CICR, Sirsa	CSH 2013 × CSH 43	165-170	34	33.7	27.5	23.5	4.6	Punjab, Haryana and Rajasthan
5	CSHH1862	H×H	2011	CICR, Sirsa	GMS 16A × CB 33	160-170	21	34.5	27.8	21.9	4.2	Punjab, Haryana and Rajasthan
6	Phule 388 (RHB –388)	H×B	2002	MPKV, Rahuri	RHC-006 × RHCb-001	170-180	17-20	34	35	23.8	3.4	Maharashtra

* H – *Gossypium hirsutum*; B – *Gossypium barbadense*; A – *Gossypium arboreum*

conveyed a polymorphism of 27.9 per cent. The list of polymorphic SSR markers and their details is provided in **Table 2**. The higher polymorphism per cent observed in the present study might be due to inclusion of different species of cotton in the study. In our earlier diversity studies, 16 and 27 per cent SSR polymorphism was observed in *G. arboreum* (Santosh *et al*., 2020) and tetraploid cotton (Santhy *et al*., 2019), respectively. Earlier, Selvakumar *et al*. (2010) observed 30 per cent polymorphism during genetic purity analysis of three cotton hybrids using SSR markers. Menka *et al*. (2016) noted 20 per cent polymorphism while studying hybrid purity in two cotton hybrids. Marker polymorphism depends on many factors such as breeding behaviour of the species, genetic diversity in the study material, sample size, sensitivity of genotyping method and location of primers in the genome used for study. The information conveyed by the polymorphic SSR markers was utilized to assess the molecular divergence among the study material.

The genetic dissimilarity among the genotypes under study is presented in **Table 3**. Maximum genetic dissimilarity of 0.66 was noted between inter-specific H × B hybrid, Phule 388 with *G. arboreum* line LD 327 (Sel.) and between *G. hirsutum* line RHC-006 with *G. arboreum* line DS 5 (GMS). The dissimilarity of 0.65 was observed between inter-specific H × B hybrid, Phule 388 and intra-*arboreum* hybrid, CICR2 and its female parent DS 5 (GMS), between *G. hirsutum* line CSH 43 and *G. arboreum* line LD 327 (Sel.), between *G. hirsutum* line RHC-006 and *G. arboreum* line LD 327 (Sel.). The lesser genetic diversity was observed between the hybrids and its parents. The minimum genetic dissimilarity of 0.07 was observed between intra-*hirsutum* hybrid, CSHH1862 and its female parent CB 33, followed by 0.11 between intra-*arboreum* hybrid, CICR2 and its female parent DS 5 (GMS). The genetic similarity of 0.85 was noted between intra-*hirsutum* hybrid, CSHH243 and its male parent CSH 2013, and between intra-*hirsutum* hybrid, CSHH1862 and its female parent GMS 16A, and between GMS 16A and CB 33. The SSR markers were highly efficient in capturing both intra-species and inter-species diversity (Abd El-Moghny *et al*., 2017; Santhy *et al*., 2019; Santosh *et al*., 2020) as they revealed higher genetic diversity between different species and lesser diversity within species or between hybrids and their parents.

The information on genetic dissimilarity among the genotypes was utilized for clustering and factorial analysis. Both clustering based on unweighted Neighbour Joining (**Fig. 1**) and factorial analysis (**Fig. 2**) depicted a pattern of genetic diversity and the grouping of genotypes was in congruence with the ploidy of the species. The diploid species (*G. arboreum*) hybrid, CICR2 along with their parents [DS 5 (GMS) and LD 327 (Sel.)] were clustered separately and distinctly from the rest of the genotypes. All the *hirsutum* genotypes (Hybrids CSHH198, CSHH238, CSHH243, CSHH1862 and their respective parents were also found closely

Table 2. List of polymorphic markers, their repeat motif and sequence information

S. No.	Marker	Repeat motif	Forward primer	Reverse Primer
1	BNL0852	(CA)13	TGCTTTCAGCCAATGACTTG	AACAATGCCCCCAATATTCA
2	BNL0861	(AC)21	AAGATGGTAGTGGCTTGAACG	GTTCCTTCTTACTTCCATGTGC
3	BNL1045	(AG)16, (CA)10	GGCAATCAACTTTAGGCTGC	TGGTGAAGATCCCCATTTTC
4	BNL1227	(AG)15	CATCAAGATCTATCTCTCTCTATACCG	TTTACCCTCCGATCTCAACG
5	BNL1317	(AG)14	AAAAATCAGCCAAATTGGGA	CGTCAACAATTGTCCCAAGA
6	BNL1421	(AG)29, (AG)14	TGAAGATTTGGAGGCAATTG	GAAATCAAGCCTCAATTCGG
7	BNL1604	(AG)25	AGAGGGAGTAAAGATTTGGGG	TCCAGTTCTTTTTGCCTTGG
8	BNL2449	(GA)16, (TC)16	ATCTTTCAAACAACGGCAGC	CGATTCCGGACTCTTGATGT
9	BNL2544	(AG)11	GCCGAAACTAAAACGTCCAA	TCCTTACTCACTAAGCAGCCG
10	BNL2634	(AG)11	AACAACATTGAAAGTCGGGG	CCCAGCTGCTTATTGGTTTC
11	BNL2725	(AG)28	AGCATTAGCAGGCACCTTTATA	ACATTTGGTTCGTTTTCTCTTTAA
12	BNL2741	(GA)15, (TC)15	TGTGGAGTTGTTTGTCTCGC	GTCAACAGTCCTCTGCTGCA
13	BNL3031	(AG)27	AGGCTGACCCTTTAAGGAGC	AACCAACTTTTCCAACACCG
14	BNL3090	(AG)31	GAAATCATTGGAAGAACATATACTACA	TTGCTCCGTATTTTCCAGCT
15	BNL3383	(AG)10, (CT)11	GTGTTGTCATCGGCACTGAC	TGCAATGGTTCAGTGGTGAT
16	BNL3442	(CA)14(TA)5	CATTAGCGGATTTGTCGTGA	AACGAACAAAGCAAAGCGAT
17	BNL3594	(TC)37	AGGGATTTTGATTGTTGTGC	TGAATTCAAAACAAATGTTAGCC
18	BNL3644	(TC)13	GTGCTGTTTGGGCCTTACAT	TAAGCGCATTGACACACACA
19	BNL3806	(TG)18, (AG)18, (AC)9+N+(CA)7+C+N+(CA)2+(AT)5	GACAGGCCAGACCAGAACAT	TCAAACAAAGCACATATATAATACACA
20	BNL3812	(CA)6+C+N+(CA)11	AACCACCCCAATTTGATGAT	GGGTTTCTCCTTCCCTGTTC
21	BNL3816	(TG)15, (TG)5TA(TG)15	GTTAGCCACGTGTTAGTTCTATG	ATCGATCACTTGCTGGTTCC
22	BNL3992	(TC)26, (GA)26	CAGAAGAGGAGGAGGTGGAG	TGCCAATGATGGAAAACTCA
23	BNL3995	(AC)16	ATATTTTATTCTTTTAATAGCTTTATTCCC	TTGGAAAAACCCATGGTGAT
24	BNL4061	(CA)26	TAGTAGGTGTCCCCTGTGCC	TGAAGCACCAGATGAAAACA
25	BNL4071	(GT)7+(GA)23	CATTTCAGAAGTTGACATTTTCG	CACTGCCCCTAAGAAGTTGC
26	BNL4096	(GT)8+(GA)17	TGTGGTGGGTTTCACTTTCA	GACACGGATCCTACTGAGCC
27	BNL0946	(GA)14	GCTGTTGCTCCACATCTCCT	GGGCAAACAGATAGGCAGAA
28	CM0043	(TC)20	GCGCAGATATTATTATCACAGC	TATATAAATTTGCATCAGTTGGC
29	CM0066	(CT)14	GGATACGTAGGCCTCCACATATTC	GCTGCCTGCTGTTGAATGCTG
30	DPL0600	-	AGGCACCTCTTTAGTGATACTAATTCC	TTAAGGGTAGCCCTCTCAATCTCT
31	GH288	GT(22)	CTATTCCACAAGCTTCATTCTGCAG	GGAGCACAATGAGGAAGTATACTG
32	GH434	AGA(18)	AGAGCTAGTAGGTGGCTTAAAGAG	GTGGATGAATTATCTAAGTCGACCG
33	GH470	CT(19)	ACATCAACTTTCAAACCGTTCAACC	CTGGAAGCTAAATATACAGAGCAAG
34	GH486	TCT(20)	TTGTCACCCTAAATTACATTGAATGGC	GTAAAGGCTTTGACTACAAGCACC
35	GH527	TCT(6)	AGCTGGAGGATTTCAGCTTGATTC	ATGCCAGTTAACTTACCACGTTGG
36	GH539	AC(13)	AGTTCGTGCCTTTGATACTGAAGG	CAAACGAAGTGAATGTTAGTCTATTCG
37	JESPR101	(TA)3(GT)15	CCAAGTCAAGGTGAGTTATATG	GCTCTTTGTTACTGAAATGGG
38	JESPR151	(GAA)9(Y)4(GAA)10	CTGGACTAAAAACCTTAACTGG	CTCGATTCTAACTCAATCACG
39	JESPR152	(GAA)50	GATGCACCAGATCCTTTTATTAG	GGTACATCGGAATCACAGTG
40	JESPR197	(TAC)11	CAATACCTGGAACATAGACAAATG	CTTGAGGCTTGCAAAAAATG
41	JESPR215	(GA)22	CGAGAAGATGAGATTGGAGGAG	CCCTTCTGAGTTTTCTTTGG
42	JESPR220	(GA)20	CGAGGAAGAAATGAGGTTGG	CTAAGAACCAACATGTGAGACC
43	JESPR224	(GA)22	GGGGAGCAACGAAAACTTAGC	CCACCATTCTCTTTCATTTTCTCC
44	JESPR0065	(GAA)25	CCACCCAATTTAAGAAGAAATTG	GGTTAGTTGTATTAGGGTCGTTG
45	MUCS557	GTT(2)CTG(4)	GGCATCTAGTTGAGGGAAGG	TGGAACATGCACTTTAATCACC
46	MUCS566	AAC(2)GAC(4)	CAGAGGAGAGAAGAGAAGAGAGG	GGATTTTGAAGAGCCTCAACC
47	MUCS620	TTA(2)TA(6)	CGAAGATGGGAAGAGAAAGG	CAAAAGCTAGCAACATTACAACTCC
48	MUSB0100	(AT)5	TTCTGTTCCCACATTTCAAGC	AAAGGGGTGCTGGTTTCG
49	MUSS161	GGA(4)	AGAGGAATCGGACAATGACG	CCCAAGAATCTGAAGCATCC
50	MUSS397	GCT(4)	ACAAGCTAACGGTGACTGGC	CTTCTCCTCGGGTTTCTTCC
51	NAU1070	(AGG)10	CCCTCCATAACCAAAAGTTG	ACCAACAATGGTGACCTCTT
52	NAU1190	(GGC)6	CCATGTCCGTATCCATGTTA	TAAGGCAAGATAGGGTCAGG
53	NAU2083	GAC(9)	AGAAGAGGTTGACGGTGAAG	TGAGTGAAGAACCTGCACAT
54	NAU4073	(ATGT)6	CCCACCCTTTTCTTCTTTTT	GCTGCCAAATTTCATCTCTT
55	NAU5046	(CATC)6	CTTCCCTCCTCTGTCTCTCA	GAGAGAGGGGAAAGTTAGGG
56	NAU5189	(TTC)8	TGTCCCCCAATCATATTTTC	CAACTTCCCAAGCTCGTATT
57	TMB0436	(GA)5+(GA)17+(GAA)4	TGTGGCACAACCTTCCAAT	CGTGTTCTCCATTTGATTCAT
58	TMB1427	(CA)26	TGTTTTTGGGTACAGTTTTGACA	TTCTCTTCAAAGGGGAGTGTTT
59	TMB1484	(CA)18	ACCACCCCAATTTGATGATT	GGGTTTCTCCTTCCCTGTTC
60	TMB1645	(GA)36+(GA)12	AAATCCATTAGAATGTATAGGG	TCAGTTCTTCCGGCTGTAG

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Table 3. Genetic dissimilarity among the genotypes under study

Dissimilarity co-efficient	LD 327 (Sel.)	DS 5 (GMS)	CICR2	CSH 8	CSH 19	CSHH 198	PIL 8 (Sel.)	SH 2379 9Y	CSHH 238	CSH 43	CSH 2013	CSHH 243	CB 33	GMS 16A	CSHH 1862	RHCb-001	RHC-006
DS 5 (GMS)	0.25																
CICR2	0.17	0.11															
CSH 8	0.61	0.59	0.58														
CSH 19	0.64	0.63	0.61	0.40													
CSHH198	0.61	0.59	0.57	0.24	0.18												
PIL 8 (Sel.)	0.59	0.57	0.56	0.28	0.44	0.32											
SH 2379 9Y	0.64	0.62	0.62	0.45	0.20	0.27	0.42										
CSHH238	0.60	0.58	0.56	0.31	0.29	0.18	0.26	0.22									
CSH 43	0.65	0.64	0.62	0.35	0.45	0.31	0.20	0.45	0.27								
CSH 2013	0.62	0.61	0.59	0.29	0.29	0.26	0.33	0.29	0.25	0.33							
CSHH243	0.61	0.58	0.57	0.26	0.36	0.21	0.24	0.34	0.18	0.18	0.15						
CB 33	0.58	0.57	0.55	0.30	0.32	0.19	0.27	0.36	0.24	0.29	0.30	0.22					
GMS 16A	0.57	0.58	0.54	0.30	0.37	0.28	0.32	0.40	0.32	0.35	0.36	0.31	0.15				
CSHH1862	0.55	0.53	0.51	0.31	0.34	0.23	0.28	0.38	0.22	0.31	0.32	0.26	0.07	0.15			
RHCb-001	0.63	0.63	0.63	0.54	0.55	0.52	0.55	0.57	0.55	0.59	0.55	0.54	0.55	0.55	0.55		
RHC-006	0.65	0.66	0.63	0.37	0.40	0.34	0.39	0.45	0.37	0.45	0.31	0.36	0.39	0.46	0.40	0.54	
Phule 388	0.66	0.65	0.65	0.46	0.53	0.43	0.50	0.55	0.48	0.53	0.50	0.47	0.50	0.53	0.51	0.18	0.39

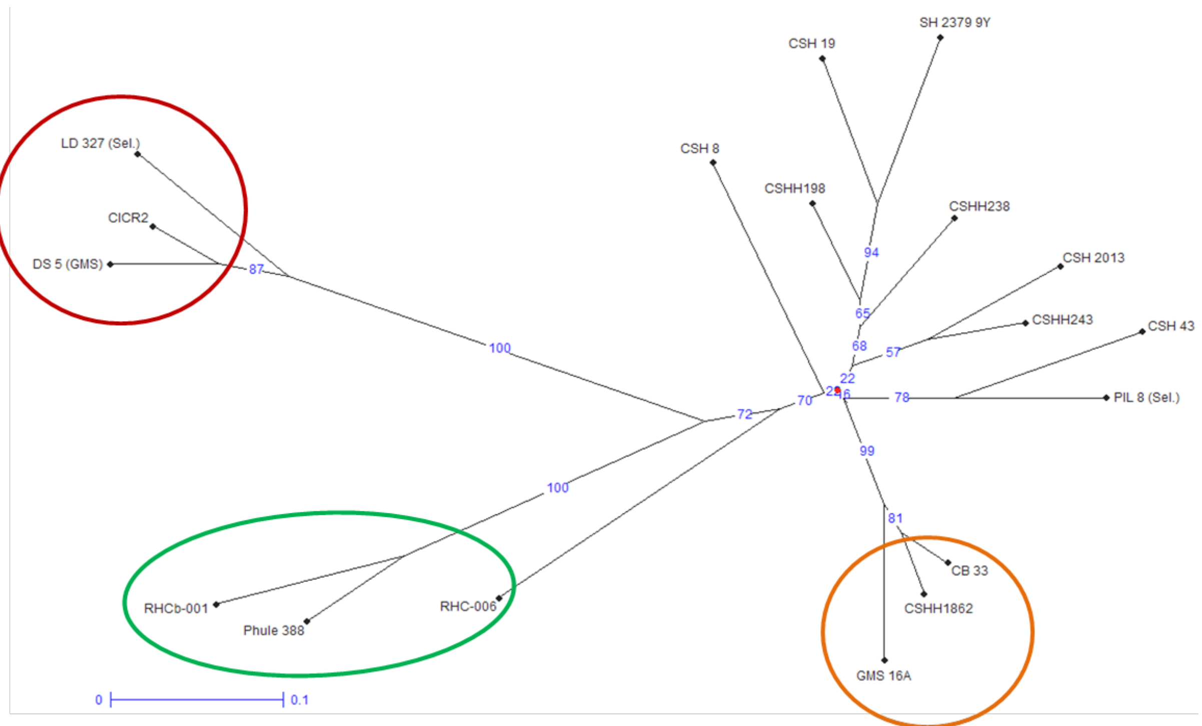

Fig. 1. Clustering of genotypes as revealed by polymorphic SSR markers

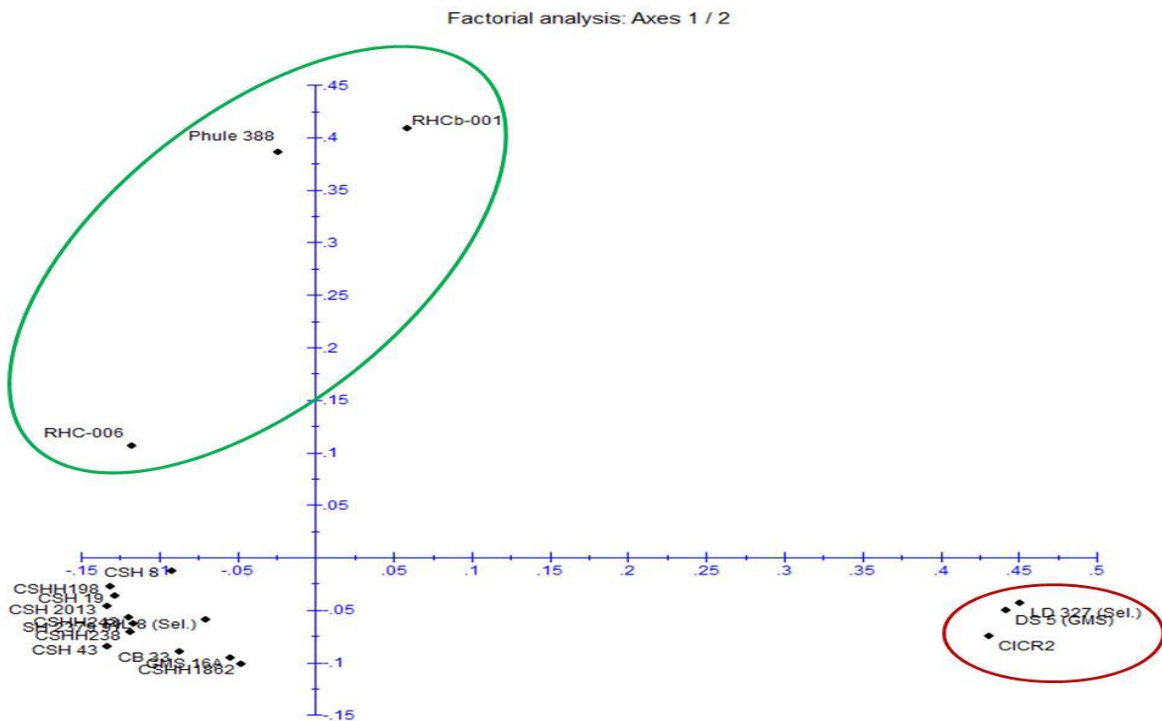

Fig. 2. Factorial analysis of cotton genotypes

clustered. Similarly, the inter-specific hybrid, Phule 388 along with its *G. hirsutum* (RHC-006) and *G. barbadense* (RHCb-001) parent formed a distinct group. The factorial coordinate analysis provides an overall representation of diversity while, clustering tends to faithfully represent the individual relations (Santosh *et al.,* 2017). Clustering based on molecular makers revealed that a particular hybrid and its parents were grouped together as a cluster and the hybrid was positioned in a near midway between its two parents (Rana *et al*., 2006; Chauhan *et al*., 2016). A similar pattern of distinct grouping was observed for the hybrids Phule 388, CSHH1862 and CICR2.

Out of the 215 SSR markers surveyed, 60 were observed as polymorphic among the material included in the present study. Polymorphic markers which clearly differentiated the male and female parent of each of the hybrids were identified from the 60 polymorphic markers. The genetic purity was confirmed in each of the hybrids using identified markers that differentiated male and female parents of each hybrid by clear, scorable and unambiguous amplified fragments. The markers producing multiple bands with heterozygosity were excluded for genetic purity analysis. Microsatellite markers in cotton are known to reveal multiple banding patterns per locus (Rudmann-Maurer *et al*., 2007; Rana *et al*., 2006; Selvakumar *et al*., 2010; Chauhan *et al*., 2016), which may be the result of polyploidy or amplification of repetitive sequences or due to pollen contamination.

The markers *GH486*, *BNL1421*, *BNL3594* and *JESPR151* differentiated the parents (CSH19 and CSH8) of *G. hirsutum* hybrid, CSHH198 and confirmed the genetic purity of the hybrid by producing alleles from both the parents (**Fig. 3**). The SSR markers *viz., GH486*, *BNL2449*, *JESPR151* and *TMB0436* produced parent-specific alleles in the SH2379-9Y and PIL8 Sel. and hybridity was confirmed in *G. hirsutum* hybrid, CSHH238 by producing both the parental alleles (**Fig. 4**). The parents (CSH2013 and CSH43) of *G. hirsutum* hybrid, CSHH243 produced genotype specific alleles for the markers *BNL2449*, *JESPR151* and *JESPR152* (**Fig. 5**). These markers produced heterozygous bands specific to male and female parents of the hybrid, thus confirming the hybrid purity. The parents of inter-specific hybrid, Phule 388 were found to be homozygous for different alleles of *GH527*, *BNL3812*, *TMB1484*, *TMB1645*, *NAU1190* and *BNL3816* (**Fig. 6**). The hybrid produced

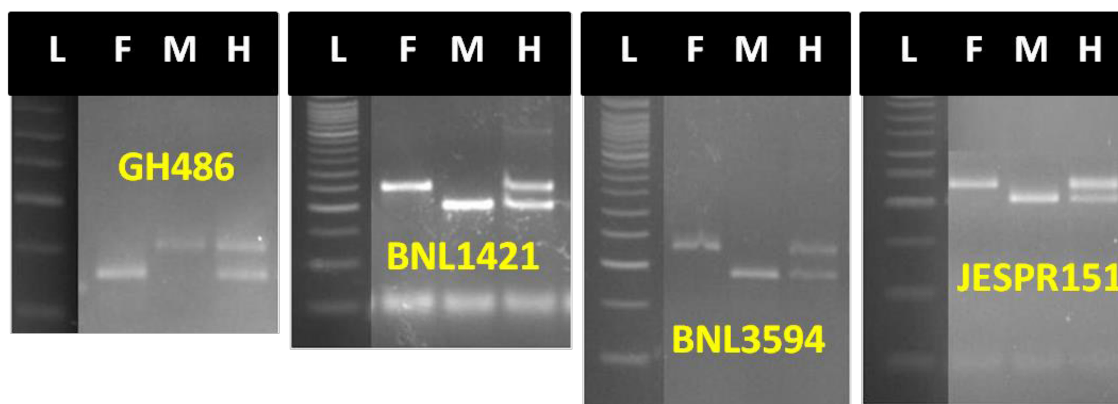

Fig. 3. DNA fingerprinting of *G. hirsutum* hybrid CSHH198

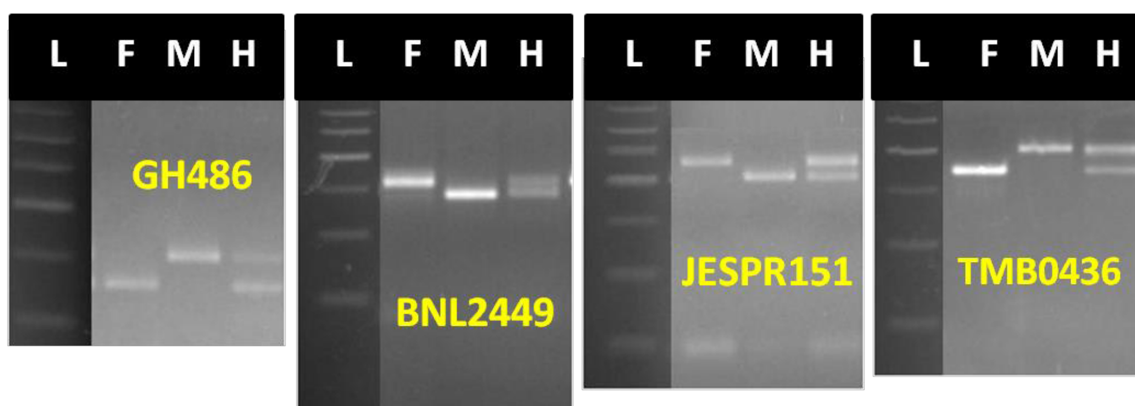

Fig. 4. DNA fingerprinting of *G. hirsutum* hybrid CSHH238

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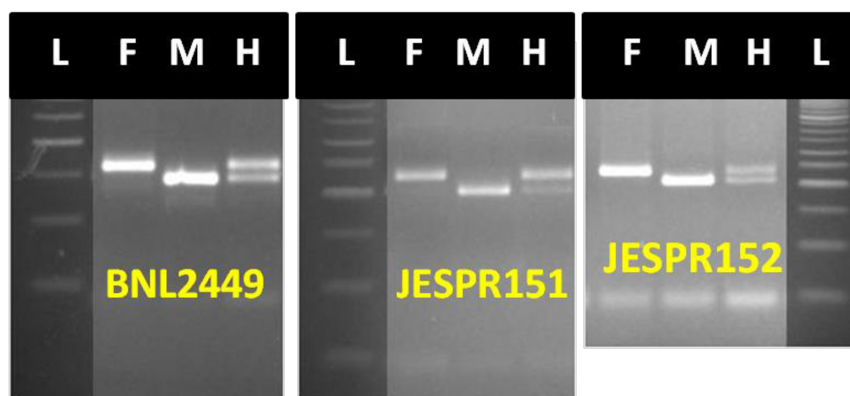

Fig. 5. DNA fingerprinting of *G. hirsutum* hybrid CSHH243

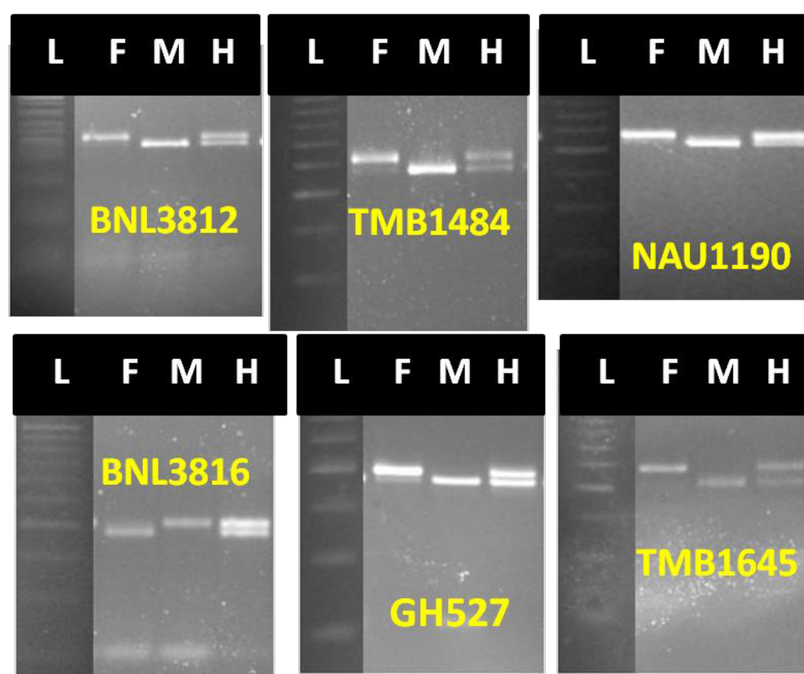

Fig. 6. DNA fingerprinting of *G. hirsutum* × *G. barbadense* interspecific hybrid Phule 388

both *G. hirsutum* and *G. barbadense* parent specific alleles for each of these markers, thus confirming genetic purity of the hybrid. Markers distinctly differentiating the parents of intra-*arboreum* hybrid, CICR2 and intra-hirsutum hybrid CSHH1862 and also unambiguously confirming the genetic purity of these hybrids were not observed in the study. SSR markers are known for their efficiency in genetic purity analysis and were utilized for genetic purity testing of different cotton hybrids (Rana *et al*., 2006; Selvakumar *et al*., 2010; Rao *et al*., 2015; Chauhan *et al*., 2016; Menka *et al*., 2016).

Phenotyping based on morphological traits is very important as they represent the expressed part of the genome. Since, most of these morphological traits are quantitative in inheritance and environmentally influenced, more often, there exists a risk of categorising genetically different cultivars as similar or vice-versa owing to subjective assessment (Santhy and Meshram, 2015). The SSR markers can be used in the efficient analysis of hybrid seed purity since this technique is simple to use, more accurate and not affected by the environment when compared with GOT. Moreover, SSR based clustering is known to have a better correlation with the pedigree than the dendrogram from morphological data (Giancola *et al*., 2002). Pattanaik *et al*. (2018) carried out the comparison of traditional grow-out test and DNA-based PCR assay to estimate F_1 hybrid purity in cauliflower and proposed that molecular marker-based hybrid purity assessment may serve as an effective substitute to traditional GOT. A combination of SSR markers and morphological descriptors is proposed for comprehensive

and unambiguous cultivar identification and differentiation (Santhy *et al*., 2019; Santosh *et al*., 2020). The present study has identified polymorphic SSR markers which can be used in hybrid purity testing. The information generated in the study about genetic diversity and genetic purity testing will greatly facilitate the seed production of these cotton hybrids. The polymorphic SSR markers identified in the study will facilitate their robust identification and thus, their licensing and commercialization.

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REFERENCES

Abd El-Moghny, A.M., Santosh, H.B., Raghavendra, K.P., Sheeba, J.A., Singh, S.B. and Kranthi, K.R. 2017. Microsatellite Marker based genetic diversity analysis among cotton (*Gossypium hirsutum*) accessions differing for their response to drought stress. *Journal of Plant Biochemistry and Biotechnology,* **26**(3): 366–370. [Cross Ref]

Ali, M.A., Seyal, M.T., Awan, S.I., Niaz, S., Ali, S. and Abbas, A. 2008. Hybrid authentication in upland cotton through RAPD analysis. *Australian Journal of Crop Science,* **2**: 141–149.

Ben Romdhane, M., Riahi, L., Jardak, R., Ghorbel, A. and Zoghlami, N. 2018. Fingerprinting and genetic purity assessment of F_1 barley hybrids and their salt-tolerant parental lines using nSSR molecular markers. *3 Biotech,* https://doi.org/10.1007/s13205-017-1080-3. [Cross Ref]

Bora, A., Choudhury, P. R., Pande, V. and Mandal, A. B. 2016. Assessment of genetic purity in rice (*Oryza sativa* L.) hybrids using microsatellite markers. *3 Biotech* ,https://doi.org/10.1007/s13205-015-0337-y. [Cross Ref]

Chauhan, S. B., Gupta, R.K., Choudhury, P. R. and Mandal, A. B. 2016. DNA Fingerprinting of cotton (*Gossypium* L.) hybrids and their parental lines using SSR markers and their use in genetic purity assessment. *Advances in Life Sciences,* **5**(8): 2999-3007.

Giancola, S., Poltri, S. M., Lacaze, P. and Hopp, H. E. 2002. Feasibility of integration of molecular markers and morphological descriptors in a real case study of a plant variety protection system for soybean. *Euphytica,* **127:** 95–113. [Cross Ref]

Mao, C. X., Virmani, S. S. and Kumar, I. 1996. Technological innovations to lower the cost of hybrid rice seed production. *In:* Advance in hybrid rice technology. Proceedings of Third International Symposium on Hybrid Rice, Directorate of Rice Research, Hyderabad, India.

Mehetre, S. S., Pardeshi, S., Pawar, S., Gahukar, S. and Chavan U. 2007. *In ovulo* embryo cultured hybrid between *Gossypium hirsutum* and *Gossypium arboreum*: hybridity confirmation. *Journal of Cotton Research and Development,* **21**: 131–139.

Menka, Kumar, A. and Yadav, S. K. 2016. Identification of suitable SSR marker for testing of genetic purity of cotton hybrids, *International Journal of Bio-resource and Stress Management,* **7**(4) Special: 668-672.

Paterson, A. H., Brubaker, C. L. and Wendel, J. F. 1993. A rapid method for extraction of cotton (*Gossypium* spp.) genomic DNA suitable for RFLP or PCR analysis. *Plant Molecular Biology Reporter,* **11**: 122–127. [Cross Ref]

Pattanaik, A., Reddy, D.C.L., Ramesh, S. and Chennareddy, A. 2018. Comparison of traditional grow-out test and DNA-based PCR assay to estimate F1 hybrid purity in cauliflower. *Current Science,***115** (11): 2095- 2102. [Cross Ref]

Pendse, R., Malhotra, S., Pawar, S. and Krishna, T. 2001. Use of DNA markers for identifying inbreds and hybrid seeds in cotton (*Gossypium hirsutum* L.). *Seed Science and Technology,* **29**: 503–508.

Perrier, X., Flori, A. and Bonnot, F. 2003. Data analysis methods. *In:* P. Hamon (ed) Genetic diversity of cultivated tropical plants. Science Publishers, France, pp 43–76.

Rakshit, A., Rakshit, S., Santhy, V., Gotmare, V. P., Mohan, P., Singh, V.V., Singh, S., Singh, J., Balyan, H.S., Gupta, P.K. and Bhat, S.R. 2010. Evaluation of SSR markers for the assessment of genetic diversity and fingerprinting of *Gossypium hirsutum* accessions. *Journal of Plant Biochemistry and Biotechnology*, **19**(2): 153-160. [Cross Ref]

Rakshit, S., Santosh, H.B., Sekhar, J.C., Nath, R., Shekhar, M., Chikkappa, G.K., Gadag, R.N. and Dass, S. 2011. Analyses of genetic diversity among maize inbred lines differing for resistance to pink borer and post-flowering stalk rot. *Journal of Plant Biochemistry and Biotechnology,* **20**(2): 173–181. [Cross Ref]

Rana, M. K. and Bhat, K.V. 2004. A comparison of AFLP and RAPD markers for genetic diversity and cultivar identification in cotton. *Journal of Plant Biochemistry and Biotechnology,* **13**: 19–24. [Cross Ref]

Rana, M. K., Singh, S. and Bhat, K.V. 2006. RAPD, STMS and SSR marker for genetic diversity and hybrid purity testing in cotton. *Seed Science and Technology,* **35**: 709–721. [Cross Ref]

Rao, P. S., Aruna, P., Anuradha, G. and Keshavulu, K. 2015. Fingerprinting of cotton (*Gossypium* Spp.) hybrids and their parental lines using microsatellite markers and their utilization in genetic purity assessment. *Journal of Agricultural Engineering and Biotechnology,* **3**(3): 120-124. [Cross Ref]

Rudmann-Maurer, K., Weyand, A. and Fischer, M. 2007. Microsatellite diversity of the agriculturally important alpine grass *Poaalpina* in relation to land use and natural environment. *Annals of Botany,* **100**:1249-1258. [Cross Ref]

Santhy, V. and Meshram, M. 2015. Widening the character base for distinctness in cotton plants. *Current Science,***109**(11):1913.

Santhy, V., Meshram, M., Santosh, H.B. and Kranthi, K.R. 2019. Molecular diversity analysis and DNA fingerprinting of cotton varieties of India. *Indian Journal of Genetics and Plant Breeding,* **79**(4): 719-725. [Cross Ref]

Santosh, H.B., Bharadwaj, C., Santosh, S., Raghavendra, K.P. and Kumar, J. 2017. Genetic diversity analysis for productivity enhancement through desi-kabuli introgression breeding in chickpea. *Electronic Journal of Plant Breeding,* **8**(1): 125-133. [Cross Ref]

Santosh, H. B., Meshram, M., Santhy, V. and Waghmare, V.N. 2020. Microsatellite marker based diversity analysis and DNA fingerprinting of Asiatic cotton (*Gossypium arboreum*) varieties of India. *Journal of Plant Biochemistry and Biotechnology, https://doi.org/10.1007/s13562-021-00675-z.* [Cross Ref]

Saravanan, N., Ram, S., Thiruvengadam, V., Ravikesavan, R. and Raveendran, T. 2007. Production and fertility restoration of an interspecific Hybrid between *Gossypium hirsutum* L. and *G. raimondii* U. *Cytologia,***72**: 195–203. [Cross Ref]

Selvakumar, P., Ravikesavan, R., Gopikrishnan, A., Thiyagu, K., Preetha, S. and Boopathi, M.N. 2010. Genetic purity analysis of cotton (*Gossypium* spp) hybrids using SSR markers. *Seed Science and Technology,* **38**: 358–366. [Cross Ref]

Sundaram, R., Naveenkumar, B., Biradar, S., Balachandran, S., Mishra, B., Ilyas, A.M., Viraktamath, B., Ramesha, M. and Sarma, N. 2008. Identification of informative SSR markers capable of distinguishing hybrid rice parental lines and their utilization in seed purity assessment. *Euphytica,***163**: 1–10. [Cross Ref]

Tatineti, V., Cantrell, R.G. and Davis, D. D. 1996. Genetic diversity in elite cotton germplasm determined by morphological characteristics and RAPDs. *Crop Science,* **36**: 186–192. [Cross Ref]