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Genetic diversity analysis in some elite desi cotton cultivars of *Gossypium herbaceum* and *G. arboreum* and genetic purity testing of their hybrids through microsatellite markers

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SUMMARY

Microsatellite markers were employed to analyse the extent of genetic diversity present in parents and genetic purity testing of inter-specific (*Gossypium herbaceum* and *Gossypium arboreum*) hybrids of desi cotton**.** The SSR analysis with 10 microsatellite markers produced 14 alleles. The average number of alleles per locus were found to be 1.40. The most informative primers were JESPR-223, JESPR-231, JESPR-296 and JESPR-298. No primer amplified two loci in a cultivar. The PIC values varied from 0.00 to 0.80 with an average of 0.30. The repeat markers amplified by a particular JESPR microsatellite primer were compared with the repeats amplified in 'A' genome standard line CMD 09. All ten JESPR series microsatellite primers used in the present study amplified the repeats which were quite near or same in the size to that of amplified repeats in 'A' genome standard line CMD 09. Jaccard's coefficients of similarity revealed that genetic similarities ranged from 0.25 to 1.00. Average genetic similarity among these 16 genotypes was found to be 0.62. Among seven *G. herbaceum* cultivars, range of genetic similarity was found to be between 0.91 to 1.00 with an average of 0.96 and among nine G. *arboreum* cultivars; range of genetic similarities was between 0.50 to 1.00 with an average of 0.75. Dendrogram generated by UPGMA cluster analysis based on jaccard's similarity coefficients grouped the cotton genotypes into two main clusters each cluster including genotypes from respective species only. A total of fourteen loci were amplified by 10 JESPR primers out of which nine were heteroallelic and five homoallelic. Heteroallelic loci were produced by primers JESPR-223, JESPR-231, JESPR-296, JESPR-298 and JESPR-300. These five primers were useful to confirm the hybridity of genotypes.

Key words : Desi cotton, Microsatellite, Genetic diversity

Many cotton (*Gossypium hirsutum* L.) varieties have been developed from crosses between closely related ancestors but so far only limited increase in productivity has been obtained. Pressure for higher productivity in cotton farming has stimulated the search for more exotic germplasm, but although breeding methods have increased the efficiency of transferring alleles from exotic germplasm sources to cotton breeding gene pools but many germplasm sources still remain underused. Van Esbroeck and Bowman (1998) have pointed out that genetic diversity ensures protection procedures against diseases and pests and thus provides a basis for future genetic gains. Molecular markers have been widely used in genetic analyses, breeding studies and investigations of genetic diversity and the relationship between cultivated species and their wild parents because they have several advantages as compared with morphological markers, including high polymorphism and independence from effects related to environmental conditions and the physiological stage of the plant.

Simple sequence repeat (SSR) markers (microsatellites) have been successfully employed in many genetic diversity studies (Liu *et al.*, 2000a; Gutiérrez *et al.*, 2002) and are useful for a variety of applications in plant genetics and breeding because of their reproducibility, multiallelic nature, codominant inheritance, relative abundance and good genome coverage (Powel *et al.*, 1996). The availability and abundance of microsatellite markers throughout the cotton genome coupled with the fact that they are polymorphic, codominant and are based on the polymerase chain reaction (PCR) make them particularly useful in genetic diversity studies of cotton (Reddy *et al.*, 2001), within excess of 1000 microsatellite primers having already been isolated from cotton DNA genome libraries (Nguyen *et al.*, 2004).

The success of hybrid cotton technology depends on the timely production and adequate supply of genetically pure hybrid seed to the farmers. Thus, seed testing or genotype identification is of prime importance for assuring good quality seed. In order to determine the genetic purity,

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Grow Out Test (GOT) is conducted. The GOT is an expensive and time consuming procedure delaying planting and leading to the loss of seed viability. Therefore, alternative techniques that offer efficient, quick and reliable assessment of genetic purity are urgently needed. Isozymes, seed storage proteins and DNA based molecular markers have been used for varietal purity determination, but PCR based DNA markers such as SSR, have proved to be advantageous over the non-PCR markers such as RFLP as they require less time, cost effective, require small quantity of DNA for the analysis.

MATERIALS AND METHODS

Plant material and DNA extraction:

The seeds of 16 parents (collected from Central Institute of Cotton Research, Nagpur and Regional Cotton Research Station, Viramgam and Bharuch) and 32 developed inter-specific hybrids were germinated in blotters and 3-days-old sprouts of each genotype were used for isolating genomic DNA. For genomic DNA isolation Edward *et al*. (1991) procedure was used with some modifications.

1gm of sprouted seedlings were cut into 1 to 2 cm pieces and were ground to a fine powder in liquid nitrogen. The powder was transferred to 10 ml of CTAB-DNA extraction buffer (3% CTAB, 100mM Tris-HCl (pH=8.0), 40 mM EDTA (pH = 8.0), 1.4 M NaCl, 1% 2-mercaptoethanol and 2% PVP) with 30ìl of proteinase K. The solution was mixed thoroughly by vortexing for 30 seconds and centrifuge tubes containing the homogenate were incubated at 60^0 C for 60 minutes in a water bath with occasional mixing. After incubation the tubes were kept for 10 minutes at room temperature to cool down, then equal volume of chloroform:Isoamyl-alcohol (24:1) was added and mixed gently for 2 minutes by inversion and sample tubes were centrifuged at 10,000 rpm for 10 minutes at 4^0C. The upper aqueous phase was transferred to a fresh tube with a wide bore micro-tip. The aqueous phase was re-extracted twice with equal volume of chloroform:Isoamyl-alcohol (24:1) solution. The DNA was precipitated by adding $1/10^{th}$ volume of 3 M sodium acetate (pH = 5.2), mixed thoroughly and later added double volume of chilled ethanol (absolute) and again mixed thoroughly and these tubes were kept overnight at -20^0C. To palletize the DNA, the tubes were centrifuged at 15,000 rpm for 15 min at 4^0C. The supernatant was discarded and the DNA pellet was washed with 70% ethanol twice. The DNA pellet was air dried and dissolved in 200ìl of 1X TE buffer. 5ìl of DNase free RNase A (Fermentas AG) was added to the crude DNA and incubated in water bath at 37^0C for 1 hour. The quality and quantity were estimated by measuring OD at 260/280 nm and 260 nm, respectively in UV spectrophotometer. Intactness of genomic DNA was checked on 0.8% agarose gel.

DNA amplification and gel electrophoresis:

20 JESPR primer pairs of SSR were selected from JESPR series of cotton microsatellites listed at CMD database on the bases of polymorphisum detected by different researchers. All 20 JESPR primer pairs were got synthesized from Operon Tech., California, USA. All of them were screened but only 10 JESPR primer pairs produced easily-detected products which were used for final investigation. Each PCR reaction carried out with 25 µl of reaction mix in a thermal cycler, which consisted of 12.5 µl of PCR master mix (Fermentas); 10pmol of a forward primer; 10pmol of a reverse primer and 60 ng of genomic DNA as a template. DNA was amplified in a Thermal Cycler (Biometra TGradient, Germany), programmed for a first denaturation step of 5 min at 94 °C followed by 35 cycles of 94 °C for 1 min, 56 °C for 1 min and 72 °C for 1 min. After the completion of 35 cycles, a final extension at 72 °C was carried out for 7 min. The completed reactions were then held at 4 °C until electrophoresis.

PCR products were subjected to electrophoresis with marker DNA of known molecular weight (20bp DNA ladder), in 3.5% agarose gel at voltage of 5V/cm using 1X TBE buffer. For staining ethidium bromide was added to the agarose @ 0.5µg/ml of gel. On completion of run, gel was viewed under UV Transilluminator and photographed using AlphaEaseFC4.0.0 Gel Documentation system (Alpha Innotech Corporation, USA).

Scoring and data analysis:

Amplification profiles of genotypes were compared with each other and bands of DNA fragments were scored as present (1) or absent (0). Assembly of all these profiles formed a data matrix. The data matrix was read by NTSYS-pc version 2.2 (Numerical Taxonomy and Multivariate Analysis System for Personal Computers, Exeter Software) developed by F.J. Rohlf, and analyzed by the SIMQUAL (similarity for qualitative data) programme with Jaccard's similarity coefficient.

RESULTS AND DISCUSSION

The results obtained from the present investigation are presented below :

Microsatellite allelic diversity:

Sixteen cultivars, representing two species of cotton

i.e. G. herbaceum and *G. arboretum*, were used for genetic diversity analysis using SSR markers. Out of these16, seven belonged to *G. herbaceum* and nine to *G. arboretum* species.

Ten out of twenty SSRs tested were not exploited for different reasons: (i) ambiguities in allele assignment, (ii) excessive stutter bands, and (iii) poor quality of amplification. From the remaining 10 SSRs the allelic polymorphism at 14 loci was used in further analyses.

The markers JESPR-220, JESPR-228, JESPR-232, JESPR-234, JESPR-236 and JESPR-300 produced single allele and markers JESPR-223, JESPR-231, JESPR-296 and JESPR-298 produced two alleles per locus. For the 16 cotton cultivars evaluated in the present study, it was found that 10 primer pairs amplified 10 loci. No any primer amplified two loci in a single cultivar.

All the primers amplified a total of 14 alleles to give an average of 1.40 alleles per microsatellite locus (Table 1). Similar was reported in cotton by Gutierrez *et al.* (2002) who used 60 pairs of polymorphic primers which amplify 69 loci resulting in a total of 139 alleles and an average of 2 alleles per locus. Bertini *et al*. (2006) used 31 primer pairs to amplify 33 loci resulting in a total of 66 alleles with an average of 2.13 alleles per locus.

The PIC values were calculated to estimate the informativeness of each primer which varied from 0.00 to 0.80 with an average of 0.30 (Table 1), Similar PIC values for SSRs were reported by Liu *et al.* (2000b) who found that PIC values varied from 0.05 to 0.82 with an average value of 0.31. Bertini *et al.* (2006) reported PIC values to vary from 0.18 to 0.62 with an average value of 0.40 in 53 *Gossypium hirsutum* L. cotton cultivars developed and released by public and private institutions in Brazil. The PIC values for the 10 SSRs surveyed in both the species *viz., G. herbaceum* and *G. arboreum* cultivars ranged from 0.00 to 0.92 (average 0.30) and 0.00 to 0.91 (average 0.31), respectively. Lacape *et al.* (2007) found that PIC values varied from 0.08 to 0.89 with an average value of 0.55.

The most informative primers were JESPR-223, JESPR-231, JESPR-296 and JESPR-298. According to molecular maps presented by Song *et al.* (2005), He *et al.* (2007), the primer sites are distributed on chromosomes A02 (JESPR-232), A03 (JESPR-296), D08 and 19 (JESPR-236), 3 and 14 (JESPR-231), 7 and 16 (JESPR-228), 12 and 26 (JESPR-300), 15 (JESPR-298). The other three primers were not mapped (Table 2).

The repeat markers amplified by a particular JESPR microsatellite primer in the present study were compared with the repeats amplified in 'A' genome standard line CMD 09. All ten used JESPR series microsatellite primers amplified the repeats which were quite near to that of amplified repeats in 'A' genome standard line CMD 09 (Table 2). The difference of some base pairs observed was negligible and may be attributed to geographical causes. The genotypes, in present study were collected from varied geographical regions of the India and also during the course of evolution the SSR repeats are known to commonly change in number of repeats. The information pertaining to standard line CMD 09 was retrieved from the cotton microsatellite data base available on web.

The coefficient of similarity was used to calculate the genetic distance between 16 cultivars which were evaluated using microsatellite loci. The values varied from 0.25 to 1.00 with an average of 0.62 (Table 3), indicating that a broad genetic base is present in Asiatic diploid cotton species *viz., G. arboreum* and *G. herbaceum*. Among seven *G. herbaceum* cultivars, range of genetic similarity was found to be between 0.91 to 1.00 with an average of 0.96 and among nine *G. arboreum* cultivars, range of

Table 1 : Allele numbers, repeat type, allele product size and polymorphism information content (PIC) value for SSR loci in 16 cotton genotypes

Sr. No.	Primer	Repeat motif	Product size (bp)	Number of alleles	PIC value
1.	JESPR-220	$(GA)_{20}$	127.58	1	0.00
2.	JESPR-223	$(CT)_{18}$	132.97, 144.77	2	1.00
3.	JESPR-228	$(GA)_{21}$	240.90	1	0.00
4.	JESPR-231	$(GA)_{22}$	156.04, 166.78	2	1.00
5.	JESPR-232	$(CT)_{18}$	143.53	1	0.00
6.	JESPR-234	$(CT)_{18}$	104.51	1	0.00
7.	JESPR-236	$(CT)_{22}$	83.81	1	0.00
8.	JESPR-296	$(TCA)_8(CTT)_{13}$	182.16, 199.84	2	0.46
9.	JESPR-298	$(GAA)_{17}$	126.01, 139.25	2	0.42
10.	JESPR-300	$(CTT)_5(CAT)_6$	206.09	1	0.00
	Total			14	2.88
	Mean			1.40	0.29

Table 2 : Chromosome location and allele product size comparison for SSR loci in 16 cotton genotypes

Sr. No.	SSR primer	Location (Chromosome)	Repeats amplified by primer	
			Present study	'A' genome standard line CMD 09
1.	JESPR-220	-	127.58	126.95
2.	JESPR-223	-	132.97, 144.77	144.26
3.	JESPR-228	7, 16	240.90	238.81
4.	JESPR-231	3, 14	156.04, 166.78	153.22
5.	JESPR-232	A02	143.53	142.52
6.	JESPR-234	-	104.51	103.17
7.	JESPR-236	D08, 19	83.81	81.14
8.	JESPR-296	A03	182.16, 199.84	196.50
9.	JESPR-298	15	126.01, 139.25	140.07
10.	JESPR-300	12, 26	206.09	204.77

Table 3 : Jaccards similarity coefficient values among 16 diploid cotton cultivars using SSR primers

	DLSA – 17	CINA - 315	CINA -316	CINA -318	CINA -329	CINA -343	CINA -344	Jawahar tapti	824	Gvhv – 544	Gvhv – 235	592	G.cot -13	G.cot -21	V - 797	832
DLSA – 17	1.00															
CINA - 315	0.67	1.00														
CINA -316	0.83	0.56	1.00													
CINA -318	0.60	0.90	0.50	1.00												
CINA -329	0.71	0.67	0.57	0.60	1.00											
CINA -343	0.57	0.56	0.67	0.50	0.83	1.00										
CINA -344	0.60	0.90	0.50	1.00	0.60	0.50	1.00									
Jawahar tapti	0.86	0.78	0.71	0.70	0.86	0.71	0.70	1.00								
824	0.60	0.90	0.50	1.00	0.60	0.50	1.00	0.70	1.00							
Gvhv – 544	0.33	0.46	0.25	0.43	0.33	0.25	0.43	0.31	0.43	1.00						
Gvhv – 235	0.33	0.46	0.25	0.43	0.33	0.25	0.43	0.31	0.43	1.00	1.00					
592	0.33	0.46	0.25	0.43	0.33	0.25	0.43	0.31	0.43	1.00	1.00	1.00				
G.cot -13	0.42	0.54	0.33	0.50	0.42	0.33	0.50	0.38	0.50	0.91	0.91	0.91	1.00			
G.cot -21	0.33	0.46	0.25	0.43	0.33	0.25	0.43	0.31	0.43	1.00	1.00	1.00	0.91	1.00		
797	0.42	0.54	0.33	0.50	0.42	0.33	0.50	0.38	0.50	0.91	0.91	0.91	1.00	0.91	1.00	
832	0.33	0.46	0.25	0.43	0.33	0.25	0.43	0.31	0.43	1.00	1.00	1.00	0.91	1.00	0.91	1.00

genetic similarities was between 0.50 to 1.00 with an average of 0.75. A comparison of these values suggested that a higher degree of genetic variation is present at the DNA level among *G. arboreum* accessions as compared to *G. herbaceum* accessions. Therefore, the *G. arboreum* accessions may be an important source of novel genes for genetic improvement of cotton. These data generated trough the present study may also serve as a guide in selecting the core germplasm pool of *G. arboreum*.

The identical results were also reported by Liu *et al.* (2005) for the genetic similarity coefficients among 39 *G. arboreum* accessions and one *G. herbaceum* which was reported to range from 0.58 to 0.99. They also observed similarity coefficient among *G. arboreum* accessions. The coefficients varied from 0.58 to 0.87 with an average of 0.74 which is at par with the present study.

The Jaccard's similarity coefficient matrixes were used to create clusters of cottons with UPGMA clustering method in the program SAHN, and phenograms were built from the cluster matrixes by the TREE program (Fig. 1). The phenograms of SSRs grouped the cotton genotypes into two main clusters each including genotypes from two different species. Similarly, Lacap *et al*. (2007) also observed that clustering of the species and races were strictly conserved.

The first cluster comprised of all *G. arboreum* genotypes and was divided into two sub-groups IA and IB. IA cluster had the genotypes *viz*., DLSA-17, CINA-

Table 4 : Unique male and female parent specific PCR products amplified with SSR primers

Group	Hybrid	Primer																			
		JESPR-220		JESPR-223		JESPR-228		JESPR-231		JESPR-232		JESPR-234		JESPR-236		JESPR-296		JESPR-298		JESPR-300	
		M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
I	G.cot – 13 x CINA – 344	-	-	151	148	-	-	166	156	-	-	-	-	-	-	-	182	-	139	-	-
II	G.cot – 21 x CINA – 318	-	-	151	148	-	-	166	156	-	-	-	-	-	-	199	182	126	139	-	-
	G.cot – 21 x CINA – 329	-	-	-	148	-	-	166	156	-	-	-	-	-	-	199	182	-	139	-	214
	G.cot– 21 x Jawahar tapti	-	-	151	148	-	-	166	156	-	-	-	-	-	-	199	182	-	139	-	214
	G.cot – 21 x DLSA – 17	-	-	151	148	-	-	-	156	-	-	-	-	-	-	199	182	-	139	-	214
III	Gvhv – 544 x CINA – 329	-	-	151	148	-	-	166	156	-	-	-	-	-	-	-	182	-	139	-	214
	Gvhv – 544 x CINA – 343	-	-	-	148	-	-	166	156	-	-	-	-	-	-	-	182	-	139	-	214
	Gvhv – 544 x CINA – 344	-	-	151	148	-	-	166	156	-	-	-	-	-	-	-	182	-	139	-	-
	Gvhv – 544 x Jawahar tapti	-	-	151	148	-	-	166	156	-	-	-	-	-	-	-	182	-	139	-	214
	Gvhv – 544 x 824	-	-	151	148	-	-	166	156	-	-	-	-	-	-	-	182	-	139	-	-
IV	Gvhv – 235 x CINA - 315	-	-	151	148	-	-	166	156	-	-	-	-	-	-	199	182	126	139	-	-
	Gvhv – 235 x CINA – 316	-	-	151	148	-	-	-	156	-	-	-	-	-	-	199	182	-	139	-	214
	Gvhv – 235 x CINA – 318	-	-	151	148	-	-	166	156	-	-	-	-	-	-	199	182	-	139	-	-
	Gvhv – 235 x CINA – 343	-	-	-	148	-	-	166	156	-	-	-	-	-	-	199	182	-	139	-	214
	Gvhv – 235 x CINA – 344	-	-	151	148	-	-	166	156	-	-	-	-	-	-	199	182	-	139	-	-
	Gvhv – 235 x Jawahar tapti	-	-	151	148	-	-	166	156	-	-	-	-	-	-	199	182	-	139	-	214
	Gvhv – 235 x 824	-	-	151	148	-	-	166	156	-	-	-	-	-	-	199	182	-	139	-	-
V	832 x CINA - 315	-	-	151	148	-	-	166	156	-	-	-	-	-	-	199	182	-	139	-	-
	832 x CINA – 316	-	-	151	148	-	-	-	156	-	-	-	-	-	-	199	182	-	139	-	214
	832 x CINA – 318	-	-	151	148	-	-	166	156	-	-	-	-	-	-	199	182	-	139	-	-
	832 x CINA – 329	-	-	-	148	-	-	166	156	-	-	-	-	-	-	199	182	-	139	-	214
	832 x CINA – 343	-	-	-	148	-	-	166	156	-	-	-	-	-	-	199	182	-	139	-	214
	832 x CINA – 344	-	-	151	148	-	-	166	156	-	-	-	-	-	-	199	182	-	139	-	-
	832 x Jawahar tapti	-	-	151	148	-	-	166	156	-	-	-	-	-	-	199	182	-	139	-	214
	832 x 824	-	-	151	148	-	-	166	156	-	-	-	-	-	-	199	182	-	139	-	-
VI	592 x CINA - 315	-	-	151	148	-	-	166	156	-	-	-	-	-	-	199	182	-	139	-	-
	592 x CINA – 329	-	-	-	148	-	-	166	156	-	-	-	-	-	-	199	182	-	139	-	214
	592 x CINA – 343	-	-	-	148	-	-	166	156	-	-	-	-	-	-	199	182	-	139	-	214
	592 x Jawahar tapti	-	-	151	148	-	-	166	156	-	-	-	-	-	-	199	182	-	139	-	214
	592 x DLSA – 17	-	-	151	148	-	-	-	156	-	-	-	-	-	-	199	182	-	139	-	214
	592 x 824	-	-	151	148	-	-	166	156	-	-	-	-	-	-	199	182	-	139	-	-
VII	V – 797 x CINA – 315	-	-	151	148	-	-	166	156	-	-	-	-	-	-	-	182	-	-	-	

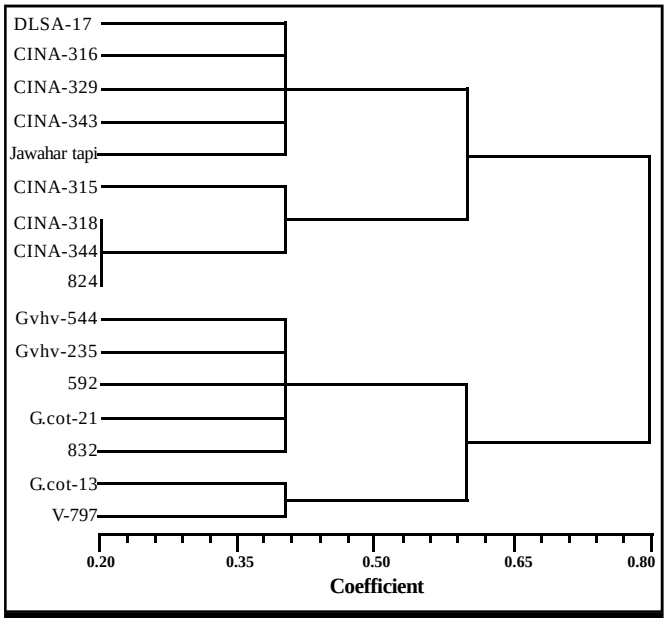
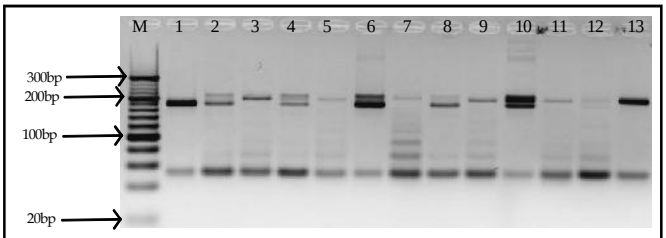
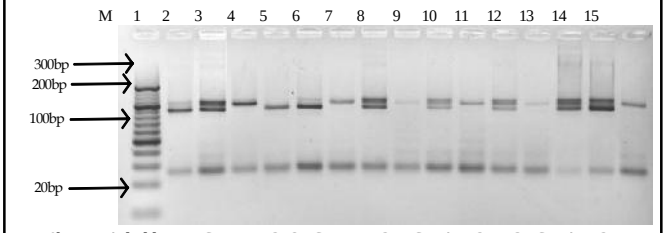


Fig. 1 : Dendrogram of 16 cotton genotypes developed from SSR data based on Jaccard's similarly coefficients using UPGMA as the clustering method



M-20bp DNA ladder, **1**- 592, **2**- 592 x CINA – 315, **3**- CINA – 315, **4**- 592 x CINA – 329, **5**- CINA – 329, **6**- 592 x CINA – 343, **7**- CINA – 343, **8**- 592 x Jawahar tapti, **9**- Jawahar tapti, **10**- 592 x DLSA – 17, **11**- DLSA – 17, **12**- 592 x 824, **13**– 824.



M-20bp DNA ladder, **1**- G.cot – 13, **2**- G.cot – 13 x CINA – 344, **3**- CINA – 344, **4**- G.cot– 21, **5**- G.cot– 21 x CINA – 318, **6**- CINA – 318, **7**- G.cot - 21 x CINA – 329, **8**- CINA – 329, **9**- G.cott – 21 x Jawahar tapti, **10**- Jawahar tapti, **11**- G.cot – 21 x DLSA – 17, **12**- DLSA – 17, **13**- V – 797, **14**- V – 797 x CINA – 315, **15**- CINA – 315.

Plate 1 : SSR profiles of different cotton genotypes belonging to two species and their inter-specific hybrids as amplified by primer JESPR-296

316, CINA-329, CINA- 343 and Jawahar tapti. Sub-group IB contained the genotypes *viz*., CINA-315, CINA-318, CINA-344 and 824. The second cluster contained all *G. herbaceum* cultivars and was divided into two sub-groups IIA and IIB. IIA cluster included the genotypes *viz*., Gvhv-544, Gvhv-235, 592, G.cot-21 and 832. Sub-group IIB contained the genotypes *viz*., G.cot-13 and V-797.

Genetic purity testing:

Total fourteen loci were amplified by 10 JESPR primers out of which nine loci were found to be heteroallelic (JESPR-223, JESPR-231, JESPR-296, JESPR-298 and JESPR-300) and five loci were found to be homoallelic (JESPR-220, JESPR-228, JESPR-232, JESPR-234 and JESPR-236). Since heteroallelic repeats expresses only in either male or female parent, they strongly confirm the hybridity, a typical example with primer JESPR-296 (Plate 1). As shown in Table 4, this study screened SSRs for individual hybrid combination and their parental lines. Thus, SSR markers can be used in testing the genetic purity of hybrid and its parents.

SSR is more reliable for identification and genetic purity testing of cotton hybrids (Dongre and Parkhi, 2005 and Rana *et al.*, 2006). Together with the quick and simple method for DNA extraction, SSR analysis was proved to be a promising approach in variety authentication and purity monitoring. This marker technology will provide corporate managers and breeders with legal evidence of commercial hybrid seeds in the seed market and help to protect plant proprietary rights of the cotton hybrids.

In conclusion, relatively low amount of polymorphism was detected among *G. herbaceum* cultivars by SSR markers which speak of narrow genetic base in this species as compared to *G. arboreum*. Therefore, the *G. arboreum* accessions may be an important source of novel genes for genetic improvement of desi cotton. The data generated trough the present study may serve as a guide in selecting the pool of working germplasm from *G. arboreum*. From the clustering pattern and genetic relationships obtained, using SSR markers can be helpful in developing mapping population and in selection of parents for breeding purpose.

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