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Evaluation of genetic diversity in Indian tetraploid and diploid cotton (*Gossypium* spp.) by morphological characteristics and RAPDs

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Abstract

Genetic diversity was evaluated among 7 diploid (*G. arboreum*) and 15 tetraploid (*G. hirsutum*) cotton cultivars using Random Amplified Polymorphic DNA (RAPD) and morphological markers. RAPD markers were efficient and detected 88% polymorphism. The diploid and tetraploid cultivars could be divided into separate clusters at 30% similarity with RAPD and 20% similarity with morphometric markers. Classification of the cultivars based on the two markers showed a high degree of agreement with a correlation of (+) 0.90.Both markers revealed higher diversity among diploids than among tetraploids.

Key words: Cotton, genetic diversity, RAPD, morphological characters

Introduction

Cotton (*Gossypium* spp.) the most important natural source of fiber is comprised of about 50 species . Commercial cotton fiber is produced from only four species: two diploids *G. arboreum* L. (n = 13, A_2 A_2) and *G. herbaceum* L. (n = 13, A_1A_1) and two tetraploids *G. barbadense* L. (n = 26, AA D_2D_2), and *G. hirsutum* L. (n = 26, AA D_1D_1).

India is the largest cotton growing country of the world but has a productivity of only 300 kg/ha that is far lower than the world average of 544 kg/ha. In the past 50 years the Indian subcontinent has witnessed a shift from diploid cotton to one dominated by tetraploids, rendering the cultivation of diploid cottons obsolete . Diploid cottons have several useful traits that could be transferred to present day tetraploid cottons [1]. Selecting useful traits for inter specific transfer depends on the genetic diversity in the parental material [2]. In this context reliable assessment of diversity in Indian diploid and tetraploid cottons at the molecular (RAPD) and morphological levels assumes substantial importance.

Morphological and isozyme patterns have been used for estimating genetic relatedness but are few and lack adequate levels of polymorphism in *Gossypium*. Among the several molecular techniques, RAPD is simple and widely used, efficient and relatively inexpensive. Among these RAPD markers have been successfully used to discriminate intra and interspecific genotypes in cotton [3-5]. However studies on cultivated Indian cotton genotype are limited [6] and no reports were available for Indian tetraploid cottons at the time that this study was started.

The objectives of this study were to (1) investigate genetic diversity of Indian diploid and tetraploid cotton cultivars using morphological and RAPD markers and (2) compare genetic similarity estimated from RAPDs and morphological characteristics.

Materials and methods

Plant Material: The material consisted of 15 cultivated tetraploid American Cotton (*G. hirsutum*) and 7 diploid Asiatic cotton (*G. arboreum*) cultivars, representing all the cotton growing states in India (Table 1).

DNA extraction and RAPD analysis: DNA was isolated from 5g of a bulked sample of leaves from 10 plants of each cultivar, following the protocol of Saghai-Maroof [7]. A total of 26 arbitrary decamer primers (Operon, USA) were selected for final RAPD-PCR analysis from 50 that were initially screened. Amplification was carried out in 25µl reaction volume (1 × PCR assay buffer, 200 mM dNTP mix, 20 ng primer, 2U Taq DNA polymerase (Banglore Genei,India) and approximately 50 ng template DNA). Amplification was carried out in a thermal cycler (Perkin Elmer, Model 9600, USA) under the following conditions: (1) 94°C for 3 min, (2) 36°C for 1 min (3) 72°C for 1 min, (40 cycles) (4) 72°C for 4 min and then held

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Table 1. List of *Gossypium* cultivars used for the present study

Cultivars	Source	Pedigree
G. arboreum		
C402W	Bulanshahar	Local land race
HD107	HAU, Hisar	Selection from local germplasm
G27	PAU, Ludhiana	Selection from Sanguineum cotton
LD327	PAU, Ludhiana	G57 × (G27 × LD124)
Shyamli	Kanpur	35-1 × CJ73
DLSA17	UAS, Dharwad	PA140 (*G. arboreum*) × Purnima (*G. hirsutum*)
AK235	PKV, Akola	H420 × H487
G. hirsutum		
LH1556	PAU, Ludhiana	(LH886 × LH 901) × LH952
H1123	HAU, Hisar	Selection from local germplasm
F846	PAU, Faridkot	452 × LH223-481
RS2013	ARS, Sri Ganganagar	RS20 × (LH-511 × Bombasa)
Pusa953	IARI, New Delhi	Selection from Bikaneri Narma (Rajasthan)
BN	ARS, Sri Ganganagar	Selection from hirsutum mixture
RST9	ARS, Sri Ganganagar	Bikaneri Narma × PS 10-27-1
GA	ARS, Sri Ganganagar	Reselection of RS 89-166
LRA5166	AV, Coimbtore	Laxmi × (Reba B50 × AC122)
GH17	AV, Surat	1762 × Yerli - 197-2
Rajat	PDKV, Akola	(*G. hirsutum* × *G. thurberi*) × *G. anomalum*
L604	ANGRAU, Guntur	MCU5 × (L389 × SRT1)
RS875	ARS, Sri Ganganagar	C-1412 × Delta Pine 66-69-7
Pusa4515	IARI, New Delhi	Pusa 959B × BJR734
Pusa8-6	IARI, New Delhi	Pusa 959B × BJR734

at 4°C until electrophoresis. Amplicons were electrophoresed in 1.5 % agarose.

Morphological analysis: Seventeen morphological traits (Table 2) were measured in field experiments. The cultivars were sown in a randomized block design with three replications in a three row plot of row length of 4.2 m, row spacing of 75 cm and plant spacing of 30 cm. Data for morphological characters were recorded as an average of five plants per plot.

Data analysis: The RAPD products were scored as presence (1) or absence of band (0) for each primer-genotype combination. Data was entered into a binary data matrix and Jaccard's coefficient of similarity was measured [8] (data not shown). The matrix was subjected to Unweighted Pair Group Method using

Table 2. Morphological characters of the diploid and tetraploid cotton cultivars.

S.No.	Cultivars	Plant yield (g)	Plant height (cm)	Nodes /plant	Inter node length (cm)	No. mon-opodia /plant	Boll wt. (g)	Fiber wt./ boll (g)	Seed wt./ boll (g)	Ginning out turn%	Seed index	Lint index	2.5% span length	50% span length	Uniformity	Micronaire	g/tex	Elongation
1	C402W	51.7	172.7	45.3	3.81	0.10	2.46	0.74	1.71	30.3	5.4	2.9	23.2	19.3	83.1	7.0	34.0	6.0
2	HD107	101.9	152.8	46.7	3.28	0.90	2.91	1.03	1.88	35.3	4.9	2.8	19.5	15.4	79.3	7.7	1.6	7.9
3	G27	76.0	198.7	50.8	3.91	0.80	2.80	1.07	1.73	38.1	4.9	2.9	19.9	15.7	78.6	7.6	23	8.1
4	LD327	72.0	186.8	56.3	3.33	0.30	3.35	1.33	2.02	39.7	5.2	2.9	18.1	14.3	79.0	8.0	22.1	4.6
5	Shyamli	87.1	182.5	47.3	3.86	0.90	3.03	0.95	2.07	31.5	5.8	3.1	21.0	16.8	80.1	7.3	28.4	6.4
6	DLSA17	40.8	190.5	49.7	3.83	1.30	2.53	0.78	1.75	30.8	5.8	3.1	26.9	22.1	82.2	5.3	32.2	6.2
7	AK235	50.8	198.7	46.7	4.25	0.90	2.49	0.82	1.67	33.0	5.5	3.0	24.1	19.3	80.1	6.2	31.4	6.1
8	LH1556	61.5	114.0	36.0	3.17	0.60	4.18	1.24	2.94	29.7	8.9	4.5	28.1	23.1	82.4	5.1	32.4	5.7
9	H1123	72.7	121.2	39.3	3.09	1.10	4.39	1.29	3.09	29.5	8.6	4.5	24.3	19.9	81.9	5.5	30.4	5.9
10	F846	68.4	122.2	37.7	3.24	0.40	4.51	1.33	3.18	29.5	10.1	4.2	26.8	22.4	83.4	4.9	33.1	6.1
11	Rs2013	54.1	108.0	33.5	3.22	0.20	4.69	1.52	3.16	32.5	7.9	3.8	25.3	21.1	83.5	4.6	29.4	6.0
12	Pusa953	35.6	134.7	38.0	3.55	0.90	4.88	1.60	3.28	32.8	9.4	4.6	24.2	20.1	83.1	4.9	9.6	6.3
13	BN	67.6	112.5	35.7	3.15	1.40	4.24	1.33	2.91	31.3	7.8	4.2	24.6	20.8	84.4	5.0	8.3	5.5
14	RST9	46.5	107.7	32.7	3.29	0.50	4.64	1.53	3.11	33.0	8.7	4.6	23.1	18.8	81.3	5.7	30.5	5.7
15	GA	52.6	122.2	35.8	3.41	0.30	4.62	1.44	3.18	31.2	8.0	4.4	26.3	21.5	81.8	4.9	33.1	6.1
16	LRA5166	66.9	112.0	36.0	3.11	0.80	4.16	1.34	2.82	32.2	7.0	3.6	26.7	22.2	82.9	4.7	1.8	6.2
17	GH17	64.8	110.7	36.0	3.08	0.60	5.10	1.65	3.45	32.3	9.3	4.1	27.2	22.5	82.8	5.0	30.2	5.9
18	Rajat	65.2	118.8	36.8	3.23	1.20	4.74	1.47	3.27	31.0	9.2	4.7	27.3	22.7	83.4	4.7	33.4	6.0
19	L604	61.6	103.3	34.2	3.02	1.20	4.37	1.21	3.16	27.6	9.4	4.0	26.3	22.3	84.7	4.4	32.7	5.8
20	RS875	51.1	108.0	39.5	2.73	0.70	4.47	1.45	3.02	32.4	8.7	4.5	24.9	20.5	82.6	5.6	28.9	5.7
21	Pusa4515	47.3	134.7	38.8	3.47	1.10	4.82	1.46	3.36	30.3	9.5	4.6	26.8	21.9	82.4	4.6	30.2	6.1
22	Pusa8-6	70.1	133.7	40.2	3.32	1.10	5.01	1.52	3.49	30.3	9.3	4.4	27.7	23	82.9	4.5	33.9	5.7

Arithmetic Mean (UPGMA) to generate a dendrogram using NTSYS-pc. The similarity matrix was obtained with all of the 26 primers using the MXCOMP subprogram in NTSYS-pc. The average similarity index for all pair wise comparisons ($\bar{x}_D$) were calculated and used to establish the probability of DNA fingerprints of two cultivars being identical by chance as described by Ramakrishana *et al.* [9], where, $\bar{x}_D$ = average similarity index and n = average number of amplified products per cultivar.

Statistical analysis of the morphological data was conducted using the NTSYS-pc version 2.01 [8]. The means of the observations were normalized prior to cluster analysis. Standardization of data was achieved in the present study, as per the standard practice of subtracting the mean of each character among all genotypes from the value of the character for a given genotype followed by division with the standard deviation of that character over all genotypes. Pair wise genetic similarities (C_{ij}) between all cultivars using SPSS version 10.0 were calculated as

$$C_{ij} = \sum_k x_{ki} x_{kj} / \sqrt{\sum_k x_{ki}^2 x_{kj}^2}$$

where C_{ij} is the genetic similarity between the ith and jth cultivar for the kth character. This similarity coefficient is based on interval measure data collected for the morphological traits. Cluster analysis was conducted on the similarity matrix with the UPGMA.

Results and Discussion

Genetic diversity analysis based on RAPD markers: The 26 decamer primers generated 371 bands of which 325 (88%) were polymorphic (Table 3). The RAPD technique was successful and efficient in discriminating all the 22 cotton cultivars. The number of bands per primer ranged from 5 for OPA 16 and 3, to 21 for OPA 19 with an average number of 14.3 bands per primer. OPA 17 and 19 were most informative with 19 polymorphic bands while OPA 3 and 16 were least informative with only 4 polymorphic bands. Average polymorphism per primer was 12.5. The probability of identical match of RAPD pattern of any two cultivars by chance was calculated to be 1.6×10^{-46}.

Overall similarity indices ranged from 0.25 to 0.95. *G. arboreum* cultivar C402W derived from a land race was least similar to all others while *G. hirsutum* cultivars Pusa 8-6 and Pusa 4515, derived from the same parental cross, were most similar to each other. Genetic similarity values were used to construct UPGMA based dendrogram (Fig. 1) to effectively quantify patterns in genetic distances. Diploid and tetraploid cottons separated into two major groups at 30% similarity. Among the 7 diploids, cultivar C402W was distinct

Table 3. Summary of RAPD amplicons and polymorphism detected by 26 random decamer primers among 22 Indian *Gossypium* cultivars.

	Primer code	Sequence (5' to 3')	Number of fragments		Percent polymorphism
			Total	With polymorphism	
1	OPA 01	CAGGCCCTTC	14	13	93
2	OPA-02	TGCCGAGCTG	13	12	92
3	OPA-03	AGTCAGCCAC	5	4	80
4	OPA-06	GGTCCCTGAC	11	9	82
5	OPA-07	GAAACGGGTG	12	10	83
6	OPA-11	CAATCGCCGT	17	16	94
7	OPA-14	TCTGTGCTGG	16	13	81
8	OPA-15	TTCCGAACCC	8	7	88
9	OPA-16	AGCCAGCGAA	5	4	80
10	OPA-17	GACCGCTTGT	20	19	95
11	OPA-19	CAAACGTCGG	21	19	90
12	OPA-20	GTTGCGATCC	7	6	86
13	OPB-01	GTTTCGCTCC	15	13	87
14	OPB-02	TGATCCCTGG	18	16	89
15	OPB-03	CATCCCCCTG	12	11	92
16	OPB-04	GGACTGGAGT	13	10	77
17	OPB-05	TGCGCCCTTC	18	17	94
18	OPB-07	GGTGACGCAG	14	11	79
19	OPB-08	GTCCACACGG	20	16	80
20	OPB-09	TGGGGGACTC	13	12	92
21	OPB-10	CTGCTGGGAC	9	7	78
22	OPB-19	ACCCCCGAAG	16	15	94
23	OPC-02	GTGAGGCGTC	19	17	89
24	OPC-07	GTCCCGACGA	20	15	75
25	OPC-09	CTCACCGTCC	17	16	94
26	OPC-11	AAAGCTGCGG	18	17	94

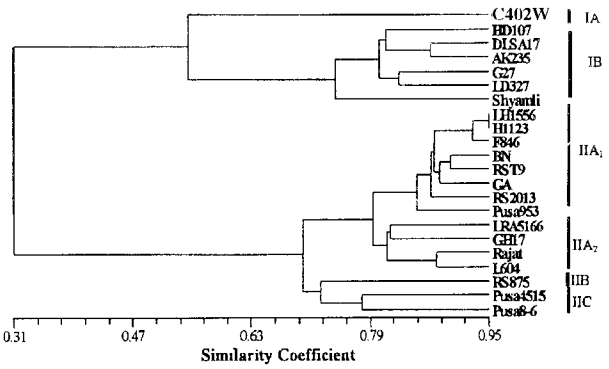

Fig. 1. UPGMA dendrogram showing clustering of 7 diploids, *G. arboreum* (I) and 15 tetraploids, *G. hirsutum* (II) cotton cultivars. The dendrogram is derived from pairwise comparison using RAPD (26 primers, 371 bands) data

from the rest of the group. Tetraploids could be split into two groups of 3 and 12 cultivars at 70 % similarity. The diploid *G. arboreum* is endemic and well adapted to the Indian sub continent and possesses several valuable traits. In this study the diploids showed lesser average similarity (0.72), and thereby greater diversity, than tetraploids (0.79).

Morphological analysis: The similarity indices based on morphological characters ranged from 0.01 to 0.99 and was higher than that observed with RAPDs. As seen with RAPD markers, the tetraploid cultivars showed higher average similarity (0.88) among themselves than diploids (0.67). Lowest genetic similarity (0.01) was seen between *G. hirsutum*, F845 and *G. arboreum*, G27 while highest similarity (0.99) was between *G. hirsutum*, L 604 and Rajat.

UPGMA dendrogram based on similarity values classified the 22 cultivars into diploids (group I) and tetraploids (group II) at 19% similarity (Fig. 2). Diploids were further divided into sub-groups IA and IB. Subgroup IA consisted of three diploid cultivars of which two cultivars DLSA17 and AK235 showed 94% similarity. Cultivar, C402W, in sub-group IA stood out from the rest of the diploids as was also observed with RAPD analysis. Tetraploids (Group II) could be split into three clusters IIA, IIB and IIC of 6,5 and 3 cultivars respectively at 91% similarity. The grouping of cultivars was largely consistent with what is known about their breeding history.

Correspondence between RAPD and morphological marker systems: Similarity matrices based on RAPD and morphological markers had identical mean values of 0.61. Correlation between RAPD and morphological similarity matrices was also high (r±0.90). The genetic variation detected among the cultivars using morphological characters was lower (0.61) than that detected with RAPDs (0.88), although morphometric markers detected a greater range of diversity than RAPDs within the diploid and tetraploid groups. Also, when classified using morphometric markers, the cultivars overlapped in their range of similarity. This was not observed with RAPD markers. RAPDs could discriminate diploids from tetraploids more sharply than morphometric markers. Morphological characters are highly influenced by the environment and by the genetic background making them weaker indicators of genetic variation compared to environmentally benign RAPD markers. The greater discriminatory power of RAPD markers has been documented in several previous reports with cotton [3,5,6,11].

Low genetic diversity among G. hirsutum: Both RAPD and morphological markers detected greater diversity among the 7 diploids of this study when compared to the tetraploids. The average similarity based on morphological data among *G. hirsutum* (0.88) was higher than that among *G. arboreum* (0.67), which is twice the average distance between G.hirsutum and *G. arboreum* (0.30). Similar observations were also made on RAPD based similarity. Similar observations have very recently been made on 7 diploid (*G. arboreum*) and 20 (*G. hirsutum*) tetraploid cultivars from India [11] using a different set of 32 decamer primers in an RAPD analysis. Two *G. hirsutum* and one *G. arboreum* cultivars are common to these two studies. The RAPD based diversity analysed in the two studies however represents different parts of the cotton genome since the oligomers have different sequences. Tetraploid cultivars that are relatively recent introductions to the Indian subcontinent are grown over much greater area than diploids and have undergone excessive process of breeding. Yet the variation among them is not high

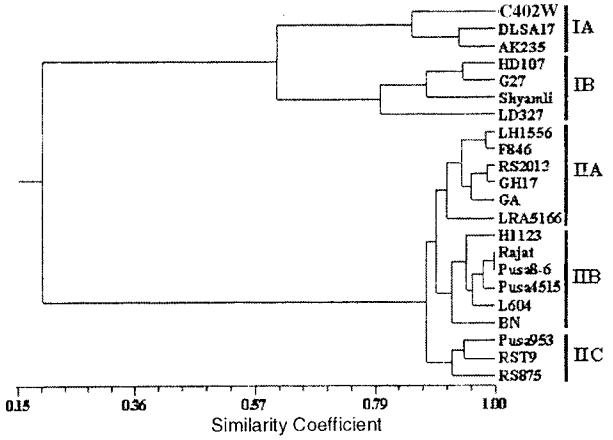

Fig. 2. UPGMA dendrogram of 7 diploids, *G. arboreum* (I) and 15 tetraploids, *G. hirsutum* (II) cotton cultivars derived from pairwise comparison of 17 morphological characters

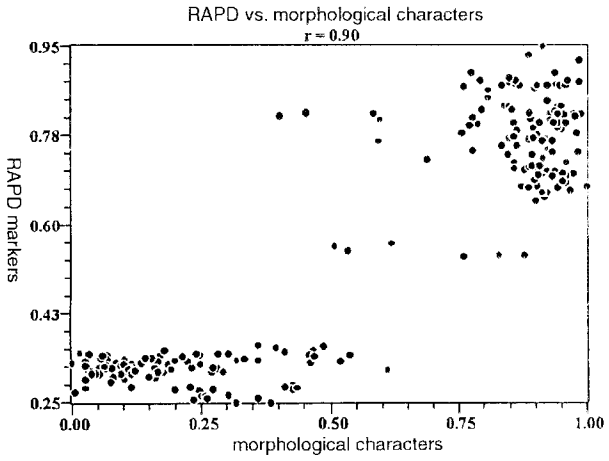

Fig. 3. Matrix correlation between RAPD and morphological classification

as compared to diploids. *G. arboreum* diploid cottons, on the other hand, are endemic to India and reflect the larger innate genetic variability prevalent among them. Greater diversity among diploids, as compared to tetraploids has been documented earlier [10,11]. Multani and Lyon [4] also found high genetic similarity (92.1-98.9%) among nine Australian cotton cultivars. Similarly Iqbal *et al.* [3] found 81.5-93.5% genetic similarity among 17 *G. hirsutum* cultivars. The number of genetic mutants reported for diploid cotton varieties are also as numerous as those of tetraploid cotton varieties, which have twice the genome size of diploids [12].

The following conclusions can be drawn from this study. RAPD markers are an extremely efficient and reliable tool for estimating genetic diversity and should be used on a continuing basis to document the available variability in the cotton germplasm as a first step. There is greater diversity among diploid cottons and lower diversity among tetraploids currently being cultivated in India. This strongly implies the importance of collecting, conserving and utilizing endemic diploid species to widen the genetic base of future cotton breeding programmes.

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