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Genetic diversity assessment of cotton genotypes using RAPD based molecular markers

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Abstract
Knowledge of genetic diversity and relationship among breeding material at molecular level has significant impact on cotton improvement. In the present study genetic variability and relationships among 11 cotton genotypes was studied using RAPD markers. Twenty five RAPD primers generated a total of 123 alleles, of which 71 alleles were found to be polymorphic, resulting in 57.72 % polymorphism. The similarity coefficient based on RAPD markers ranged from 0.44 to 0.87 with an average of 0.71, thus suggesting considerable genetic variation among the cotton genotypes studied. Further, dendrogram generated by UPGMA cluster analysis based on Jaccard's similarity coefficient, grouped 11 parental lines into two distinct clusters. The grouping based on clustering analysis revealed that genetic relationship of several varieties is related to their centres of development. Our result suggests that combined molecular markers data are efficient for measuring genetic diversity, relatedness and also aid in selection of diverse outstanding lines to be used in future breeding programs to develop new cotton hybrids/cultivars.

Keywords: Cotton genotypes, genetic diversity, RAPD, PCR based markers

Introduction
Cotton is the leading natural fibre crop and the second most important oil crop of the world belonging to genus *Gossypium.* India accounts for about 32 per cent of the global cotton cultivation area and contributes 21 per cent of the global cotton produce, second after China. At the global level, declines in cotton yields and quality for the last many years is due to narrow genetic base of cultivated cotton, compounded with lack of innovative tools to effectively exploit the genetic diversity of *Gossypium* species (Mohamed, 2003) [1]. Thus, information about the degree and distribution of genetic diversity and relationships among breeding materials provide a guide for choosing parents and predicting the degree of inheritance, variation and level of heterosis, which are essential for realizing the breeding potential and conservation of genetic resources. To have a reliable estimate of genetic diversity and genetic relationships, recently molecular marker techniques have emerged as powerful tools. Further, molecular evaluation of genetic diversity can provide greater insight into the genetic structure among varieties of different breeding origins; with this information, breeders can select the appropriate plant material to initiate a hybrid breeding programme. The random amplified polymorphic (RAPD) technique of Williams *et al.* (1990) [2] provides an unlimited number of markers which can be used for various purposes like cultivar analysis and species identification in most plants. RAPD technique was the most widely used molecular method owing to its inexpensiveness, technical simplicity and speedy process. DNA fingerprinting studies to assess genetic purity with RAPD have already been conducted in cotton (Patil *et al*., 2007; Chaudhary *et al.*, 2010; Surgun *et al*., 2012; Bakht *et al*, 2017) [3, 7, 8, 4]. Keeping in view of these findings the present work was planned to study the genetic diversity among 11 varities of G*ossypium hirsutum* using PCR based markers to provide essential information for future marker facilitated breeding.

Material and Methods
Plant material: Total 11 diverse genotypes *viz.,* GISV 267, GISV 308, GISV 310, BGDS 1033, CPD 1501, TCH 1716, TCH 1824, CCH 15-1, Suraj, RAH 1069, TCH 321 belonging to *Gossypium hirsutum* species were selected. The seeds of these entries were collected from Main Cotton Research Station, Navsari Agricultural University, Surat. The details of the genotypes with their sources are given in Table 1.

Molecular Analysis: For extraction of the genomic DNA, from each genotype 2-3 young fully expanded leaves were collected and grinded in liquid nitrogen using pestle and mortar.

About 0.5 g of the grinded tissue was transferred in 1.5 ml sterilized eppendrof tube. DNA isolation and purification was carried out using modified cetryl-tetramethyl ammonium bromide (CTAB) method as suggested by Saghai Maroof (1984) [10] with minor modifications. RAPD amplification was performed as described by Williams (1990) [2] using 25 decamer random primers (Bangalore GeNei, India, GeNei). The PCR amplification products were separated on 1.0 % agarose gel for RAPD primers using 1x TBE buffer and visualized by ultraviolet illumination after staining with ethidium bromide.

Data analysis: The banding patterns generated by RAPD were examined to determine the level of polymorphism and the genetic relatedness among the cotton parental genotypes. The presence of band at an amplicon level was scored as 1 and its absence as 0. The binary data was analyzed using standard procedure in NTSYS-PC (Version 2.1; Exeter Biological Software, Setauket, NY) software package (Rohlf, 1998). The data were subjected to the SIMQUAL option to obtain association coefficients using Jaccard's coefficient of similarity to generate a similarity matrix. Clustering analysis was performed with the unweighted pair-group method using arithmetic averages (UPGMA) in the SAHN (sequential, agglomerative, hierarchical and nested clustering method) module of NTSYS-PC.

Results and Discussion

Marker Polymorphism: The amplification profiles of the 11 cotton genotypes produced by 25 arbitrary oligonucleotide primers revealed a total of 123 bands of which 71 were polymorphic. (Table 2 and Figure 1). This corresponds to polymorphism of 57.72 %. Primers viz., RPI 4, RPI 8, RPI 18 and RPI 24 exhibited polymorphism to an extent of 100 per cent. While, primers viz., RPI 9, RPI 11, RPI 13, RPI 20 and RPI 25 were monomorphic. In this respect, Patil *et al*. (2007) [3] also registered 80.83 per cent polymorphism among four cotton genotypes of *Gossypium hirsutum* using 19 random decamer primers. Bharatkumar *et al.* (2014) [5] reported 94.20 per cent polymorphism in cotton genotypes comprised of *G. hirsutum* species using 80 random decamer primer whereas, Minhas *et al*. (2014) [6] detected 41.66 per cent polymorphism in fifteen cotton accessions using 26 RAPD primers. Bakht *et al*. (2017) [4] reported 66.00 percent polymorphism among six lines belonging to *G. hirsutum* using 35 RAPD primers. Thus, these findings support the results obtained in present research programme.

Cluster analysis: Determining true genetic dissimilarity between individuals using molecular markers is an important and decisive point for clustering which provides visual idea about variability presented in studied genotypes in addition to assuring the continued genetic improvement. Jaccard's pairwise similarity coefficient values generated using 25 RAPD primers for 11 cotton genotypes are presented in Table 4. From this result it is obvious that genotypes TCH 1824 and CCH 15-1 showed highest similarity (0.87). On the other hand, least similarity of 0.44 was revealed by GISV 267 and TCH 1824. Varied range of genetic similarities in *G. hirsutum* genotypes were also reported by Patil *et al*. (2007) [3] from 9.68 to 53.29 per cent, Minhas *et al*. (2014) [6] from 68.07 to 96.88 per cent and Bharatkumar *et al*. (2014) [5] from 49.00 to 85.00 per cent. Further all genotypes of showed diversity among themselves indicating that there is a considerable amount of variation which can be exploited through appropriate breeding programme.

Dendrogram generated by UPGMA cluster analysis based on Jaccard's similarity coefficient grouped 11 cotton genotypes into two distinct cluster designated as cluster A and cluster B (Figure 2). Cluster A comprise of three genotypes viz., GISV 267, GISV 308 and GISV 310. While, cluster B includes eight genotypes viz., BGDS 1033, CPD 1501, TCH 1716, TCH 1824, CCH 15-1, Suraj, RAH 1069, TCH 321. In cluster A, genotype GISV 267 and GISV 308 showed closed relationship and in cluster B, TCH 1824 and CCH 15-1 showed closed relationship. The dendrogram assigned the verities into groups which correspond well with their centres of development. Similar results were also obtained by Chaudhary *et al*. (2010) [7], Surgun *et al*. (2012) [8] and Bakht *et al*. (2017) [4].

The present study suggests that combined molecular markers data are efficient for measuring genetic diversity and relatedness, which aid in selection of diverse outstanding lines to be used in future breeding programs to develop new cotton hybrids/cultivars. Further, to address the narrowness of the genetic base of widely grown upland cotton cultivars, there is a urgent need for evaluation of genetic diversity with additional set of genome-wide functional molecular markers.

Table 1: Details of genotype with their source of cotton used in this study.

S. No.	Genotypes	Source
1.	GISV 267	Main Cotton Research Station, Surat
2.	GISV 308	Main Cotton Research Station, Surat
3.	GISV 310	Main Cotton Research Station, Surat
4.	BGDS 1033	University of Agriculture Science, Bengaluru
5.	CPD 1501	University of Agriculture Science, Dharwad
6.	TCH 1716	Tamil Nadu Agricultural University, Coimbatore
7.	TCH 1824	Tamil Nadu Agricultural University, Coimbatore
8.	CCH 15-1	Central Institute for Cotton Research, Coimbatore
9.	Suraj	Central Institute for Cotton Research, Nagpur
10.	RAH 1069	University of Agriculture Science, Raichur
11.	TCH 321	Tamil Nadu Agricultural University, Coimbatore

Table 2: Polymorphism information in parental lines of cotton as revealed by patterns analysis of RAPD markers

S. No.	Name of Primers	Total number of bands	Number of Polymorphic Bands	Per cent polymorphism
1	RPI 1	7	6	85.71
2	RPI 2	6	5	83.33
3	RPI 3	7	6	85.71
4	RPI 4	4	4	100.00
5	RPI 5	7	4	57.14
6	RPI 6	5	4	80.00
7	RPI 7	6	5	83.33
8	RPI 8	3	3	100.00
9	RPI 9	3	0	0.00
10	RPI 10	2	1	50.00
11	RPI 11	3	0	0.00
12	RPI 12	2	1	50.00
13	RPI 13	5	0	0.00
14	RPI 14	3	2	66.67
15	RPI 15	7	3	42.86
16	RPI 16	6	3	50.00
17	RPI 17	4	2	50.00
18	RPI 18	3	3	100.00
19	RPI 19	6	4	66.67
20	RPI 20	7	0	0.00
21	RPI 21	9	4	44.44
22	RPI 22	7	4	57.14
23	RPI 23	7	6	85.71
24	RPI 24	1	1	100.00
25	RPI 25	3	0	0.00
		123	71	57.72

Table 4: Genetic similarity based on Jaccards coefficient between 11 genotypes of cotton according to RAPD banding pattern

Parents	GISV 267	GISV 308	GISV 310	BGDS 1033	CPD 1501	TCH 1716	TCH 1824	CCH 15-1	Suraj	RAH 1069	TCH 321
GISV 267	1.00										
GISV 308	0.76	1.00									
GISV 310	0.67	0.65	1.00								
BGDS 1033	0.56	0.61	0.60	1.00							
CPD 1501	0.49	0.53	0.57	0.67	1.00						
TCH 1716	0.52	0.56	0.66	0.58	0.75	1.00					
TCH 1824	0.44	0.54	0.58	0.69	0.75	0.78	1.00				
CCH 15-1	0.52	0.49	0.58	0.65	0.77	0.73	0.87	1.00			
Suraj	0.54	0.56	0.70	0.55	0.68	0.73	0.70	0.69	1.00		
RAH 1069	0.47	0.54	0.64	0.65	0.83	0.82	0.80	0.78	0.81	1.00	
TCH 321	0.51	0.53	0.63	0.67	0.70	0.75	0.79	0.81	0.74	0.81	1.00

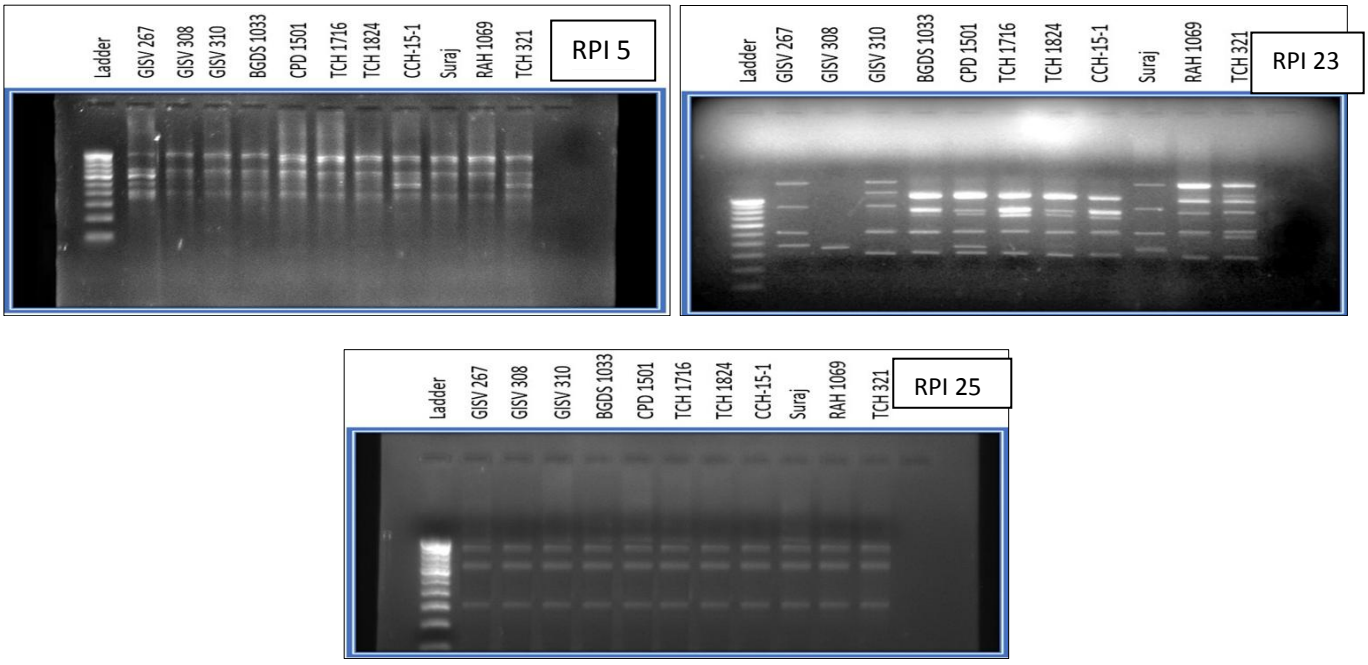

Fig 1: DNA amplification pattern of parental genotypes using RAPD primers viz., RPI 5, RPI 25 and RPI 23.

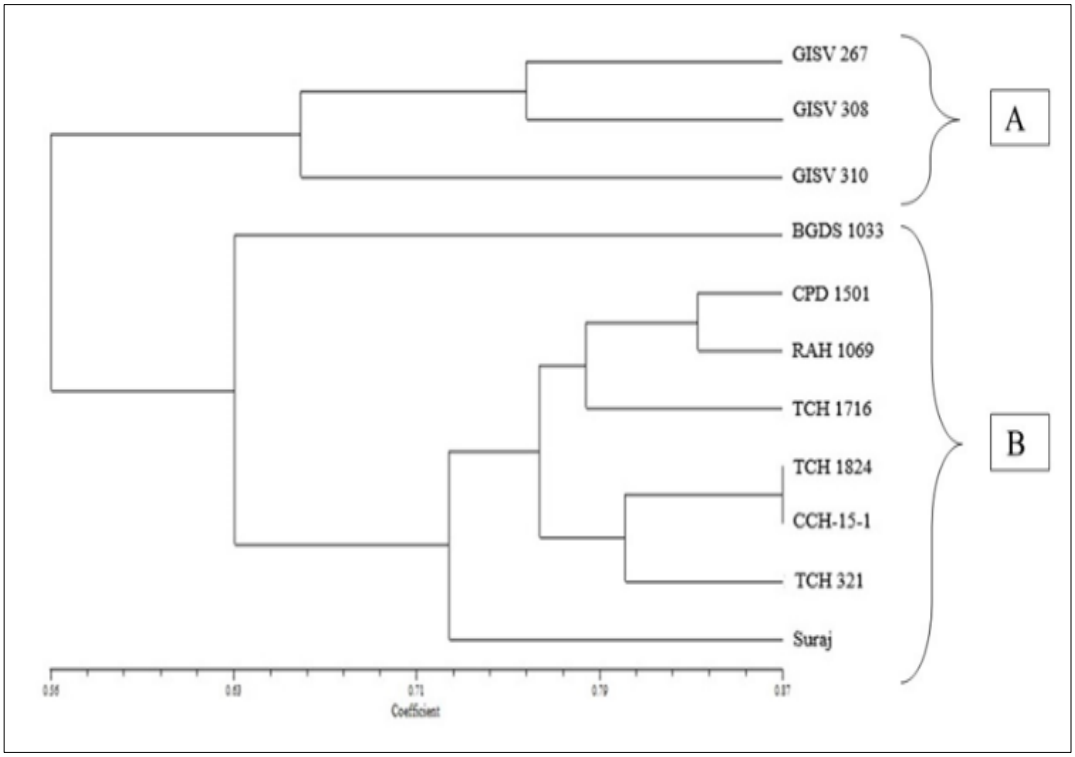

Fig 2: Dendrogram derived from an UPGMA cluster analysis by using Jaccard's coefficient based on RAPD markers in 11 genotypes of cotton

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