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Molecular Characterization and Genetic Relationships among Cluster Bean Genotypes Based On RAPD Analysis

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ABSTRACT

Cluster bean commonly known as Guar is an annual legume plant which has a broad spectrum of industrial value because of an important hydrocolloid present in its endosperm. To determine the level of genetic diversity in relation to geographical origins and morphological characteristics, a total no. of 15 cultivars have been collected from different states of India and were analyzed using RAPD. The 15 decamer primers amplified total 206 fragments, among them 168 loci (81.36 %) were polymorphic. On an average of 10 bands per primer were observed with maximum of 7 bands and minimum of 18. RAPD data were used to generate the similarity coefficients using 'SIMqual' subprogram of software NTSYS-PC. The similarity coefficient values ranged from 0.47 to 0.97, indicating high genetic variability among the selected guar genotypes. This information can be used for identification of varieties and further crop improvement programme.

Keywords: Cluster bean, Genetic diversity, Polymorphic, RAPD.

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INTRODUCTION

Cluster bean [*Cyamopsis tetragonoloba* (L.)] Taub commonly known as guar belongs to leguminoseae family, grown largely in India, Pakistan, United States of America, Italy, Morocco, Spain, France, Greece and Germany. India is the world-leader for cluster bean production as it contributes 80% shares of its total production. Guar seed has an important place in industry because of its galactomannan rich endosperm. Guar as a new crop for western agriculture [4] has been considered as a viable cash crop for industrial gum production.

Effective utilization of any germplasm in a breeding program requires information on genetic variability, heritability and correlation among different characters in the germplasm. Study of genetic diversity and relationships among cluster bean germplasm is essential for the long-term success of breeding programs since a wide range of genetic diversity among parents is essential for hybridization [3]. It also provides support for selection of crossing combinations from bulk parental genotypes and for broadening the genetic basis of breeding programs. Detection and analysis of genetic variation can help us to understand the molecular basis of various biological phenomenons in plants. DNA based molecular markers have been useful in the evaluation of genetic diversity in many crop species [1].

Therefore, it is necessary to study cultivars at the molecular level to distinguish them for their special characters and to differentiate varieties collected from different geographical regions. In this context, the aim of the present study is to determine varietal differences and genetic similarity among guar cultivars collected from different regions of the India.

MATERIALS AND METHODS

Plant Material

Five major guar growing states of India i.e. Rajasthan, Gujarat, Haryana, Punjab, Madhya Pradesh and Utter Pradesh were selected (Table 1) for collection of seeds as the soil and climatic conditions of theses states are favorable for production of guar. Seeds of fifteen cluster bean genotypes were collected from these states. These were grown in Botanical Garden, Banasthali University and healthy leaves were collected for the DNA isolation.

DNA isolation and PCR amplification

DNA extraction was carried out following the CTAB method [2] with slight modifications. The DNA was extracted by using higher concentration of 2-mercaptoethanol, which was subjected to an additional step of purification with chloroform: iso-amyl alcohol treatment followed by precipitation with chilled solution of ethanol-sodium acetate. The RAPD analysis was performed following the methodology given by Williams [13]. PCR mixtures (25 µl) contained *Taq* buffer, dNTPs, primers, *Taq* DNA polymerase, and template DNA. All fifteen random decamer primers were having GC content more than 50% as plant genome contains more GC rich regions so that random decamer primers can easily bind with them. The thermal

cycler was operated by using 44 amplification cycles, annealing temperature 37^0C and final extension step on 72^0C for 5 min. Amplified PCR products were separated on 1.8% (w/v) agarose gel in 1X TBE (pH-8.3) with EtBr.

Data analysis

The frequency of RAPD polymorphisms among 15 guar genotypes was calculated and the data was entered into NTSYS-PC (numerical taxonomy and multivariate analysis system program) [11]. The 0/1 matrix was used to calculate the similarity matrices using 'SIMqual' subprogram of software NTSYS-PC. Dendrogram was built based on the un-weighted pair group method with arithmetic averages (UPGMA).

RESULTS AND DISCUSSION

The application of DNA based molecular markers has been used in a new era of genome wide analysis together with tremendous progress in structural and functional genomics. There is less information available on the existing germplasm diversity in *C. tetragonoloba*; therefore, cataloguing of natural genetic diversity becomes essential for its efficient and sustainable germplasm management. For such an exercise, isolation of purified DNA from plants is challenging because of secondary metabolites, which interacts irreversibly with proteins and nucleic acids [6]. Various modifications in standard CTAB method was done to get purified DNA. Single band purity was obtained by using this method (Fig. 1). Ratio of λ_{260} and λ_{280} was found 1.5-1.9, which indicates the purity of DNA [12] (Table 3). The yield of DNA was ranges from 20 to 61.6-µg/gm tissues.

A total of 15 random decamer primers (Bangalore Genei Pvt. Ltd) were used for RAPD analysis (Table 2). There were 8 primers with 60% GC content and 7 primers with 70% GC content. Each primer generated a varied no. of fragments, ranged from 11-21 (Fig. 2, 3, 4, 5, 6). The maximum no. of bands (13 bands) generated with primer RP-58 where as minimum bands were generated with primer RP-52 (7 bands). The percentage of polymorphism was ranged from 69% to 92.3%. Maximum polymorphism is found in primer RP-48 (92.3%) and minimum polymorphism was obtained with primer RP-49 (Table 2).

Similarity indices was estimated using Jaccard's similarity coefficient (Jaccard 1908), ranges from 0 (all products between evaluated varieties were different) to 1 (all products between evaluated varieties were identical). Similarity between the cultivars varied from 0.47 to 0.97, a maximum similarity value of 0.97 was observed between Swati-55 and Jyoti-555, whereas Pusa selection I and HG-365 were found to be genetically most diverse (0.47) among fifteen genotypes. Punia *et al*. [9] & Rakesh Pathak *et al* [10] also observed a similarity coefficient value ranging between 0.34 and 0.76 among the varieties of cluster bean they selected for their investigation.

The allelic diversity data was used to produce a dendrogram (cluster tree analysis, NTSYS-PC) revealing the genetic relationship among all genotypes (Fig. 7). The dendrogram

separated the accessions collected from 5 different states into 4 major groups. The first group includes all six cultivars from Rajasthan, second group includes cultivars from U.P., third group showed M.P. and Gujarat, and group four included Punjab and Haryana. Group three and four predicts genetic similarity between genotypes of two states. Thus it can be concluded that there is an effective gene flow among those locations.

The moderated to high degree of RAPD polymorphism observed in the present study suggest that RAPD can efficiently be used in cultivar identification of guar, as observed already in crops like tomato [7], pepper [8] etc. Therefore, breeding programs based on selection and hybridization will benefit from the data and the patterns reported in this study.

In future this study opens up a possibility for developing a molecular genetic map that will lead to the application of marker-assisted selection tools to genetic enhancement of cultivated guar. High level of polymorphism among populations suggests that RAPD techniques can be useful for guar systematic and for the maintenance of germplasm banks. From the clustering pattern and genetic relationship obtained using RAPD markers, breeders can identify the diverse genotypes from different clusters and employ them in future breeding programmes.

Table 1 – Guar cultivars collected from different states of India

Sr. No.	Name of Guar Varieties	State
1	RGC-1017	Rajasthan
2	RGC-1003	Rajasthan
3	RGC-1066	Rajasthan
4	RGC-1002	Rajasthan
5	RGC-936	Rajasthan
6	RGC-1031	Rajasthan
7	Pusa-Navbhar	Uttar Pradesh
8	PNB	Uttar Pradesh
9	Neelam-51	Uttar Pradesh
10	Pusa-selection-1	Madhya Pradesh
11	Swati-55	Gujarat
12	Jyoti-555	Gujarat
13	Selection	Punjab
14	Priya-151	Haryana
15	HG-365	Haryana

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Table 2 - Total number of amplified fragments and number of polymorphic fragments generated by PCR using selected random decamers in different varieties of guar.

Sr.No.	Primer	Primer sequence 5'-3'	Tm	% GC	Total no. of amplification product	No. of polymorphic products	% Polymorphism
1	RP 46	GGAGTGGACA	27.1	60	13	9	69.2
2	RP-47	GGACGCTTCA	35.2	60	11	10	90.9
3	RP-48	AGGCTGGGTG	38.3	70	13	12	92.3
4	RP-49	CCCCCTATCA	33.8	60	11	7	63.6
5	RP-50	TCGCCCAGTC	39.7	70	12	8	66.6
6	RP-51	GGGACGATGC	38.7	70	12	10	83.3
7	RP-52	TCTGTCGGTC	27.7	60	13	10	76.9
8	RP-53	GGTCACCTCA	27.1	60	11	10	90.9
9	RP-54	AGTGCGCTGA	36.2	60	11	10	90.9
10	RP-55	CCGCGTCTTG	41.8	70	12	10	83.3
11	RP-56	GAGCGCCTTG	40.5	70	16	11	68.7
12	RP-57	CCCCGAAGGT	41.9	70	15	13	86.6
13	RP-58	CTCCAGCGGA	40.3	70	18	15	83.3
14	RP-59	GGCTAACCGA	35	60	17	15	88.2
15	RP-60	TGTGCCCGAA	42.5	60	21	18	85.7
		Average			12.93	11.2	81.36

Table 3- Yield and purity of DNA extracted from different cultivars of guar

Sr.No.	Varieties	OD_{260}	OD_{280}	$\lambda_{260}/\lambda_{280}$	Conc. (µg/ µl)	DNA yield µg/gm tissue
1	RGC-1017	0.075	0.04	1.87	0.370	61.6
2	RGC-1003	0.072	0.042	1.71	0.360	60.0
3	RGC-1066	0.070	0.041	1.7	0.350	58.3
4	RGC-1002	0.065	0.042	1.54	0.325	21.6
5	RGC-936	0.067	0.035	1.91	0.330	55.0
6	RGC-1031	0.060	0.040	1.50	0.300	20.0
7	Pusa Navbhar	0.069	0.039	1.76	0.345	57.5
8	PNB	0.071	0.039	1.82	0.350	58.3
9	Neelam 51	0.068	0.037	1.83	0.340	56.6
10	Pusa SelectionI	0.065	0.037	1.75	0.320	53.3
11	Swati 55	0.077	0.041	1.87	0.350	58.3

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12	Jyoti 555	0.069	0.04	1.72	0.340	56.6
13	Selection	0.066	0.039	1.69	0.330	55.0
14	HG-365	0.067	0.035	1.91	0.330	55.0
15	Priya 151	0.073	0.04	1.82	0.360	60.0

Table 4- Genetic similarity matrix generated based on Jaccard's similarity coefficient

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
RGC-1017	1.00														
RGC-1003	0.63	1.00													
RGC-1066	0.61	0.78	1.00												
RGC-1002	0.68	0.68	0.55	1.00											
RGC-936	0.60	0.73	0.66	0.63	1.00										
RGC-1031	0.52	0.63	0.65	0.50	0.62	1.00									
Pusa Navbhar	0.53	0.67	0.73	0.51	0.64	0.71	1.00								
PNB	0.52	0.69	0.74	0.53	0.72	0.63	0.73	1.00							
Neelam-51	0.44	0.57	0.56	0.49	0.59	0.51	0.55	0.55	1.00						
Pusa Selection	0.41	0.57	0.61	0.44	0.57	0.52	0.57	0.64	0.44	1.00					
Swati-55	0.56	0.71	0.64	0.60	0.73	0.68	0.67	0.76	0.57	0.63	1.00				
Jyoti-555	0.46	0.50	0.50	0.50	0.50	0.42	0.52	0.49	0.78	0.40	0.51	1.00			
Selection	0.46	0.58	0.56	0.54	0.58	0.50	0.56	0.55	0.93	0.43	0.58	0.80	1.00		
HG-365	0.41	0.47	0.47	0.47	0.49	0.42	0.46	0.45	0.75	0.38	0.47	0.67	0.75	1.00	
Priya-151	0.43	0.55	0.51	0.49	0.53	0.46	0.51	0.50	0.79	0.42	0.56	0.70	0.82	0.74	1.00

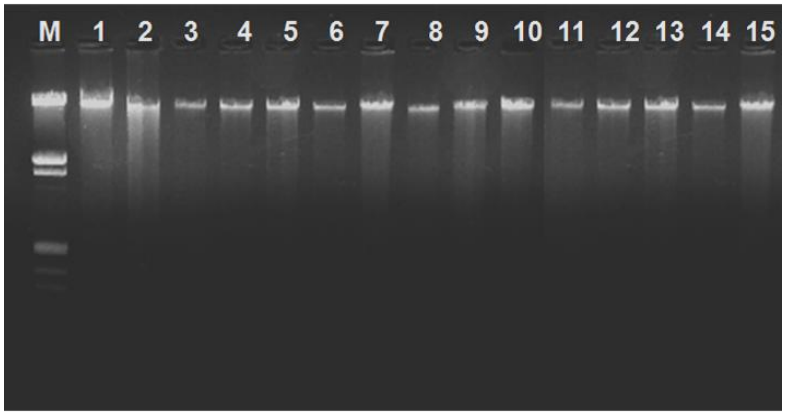

Fig. 1- Genomic DNA of different varieties of guar (Lane 1-15), Lambda DNA *EcoR1/HindIII* double digested (Lane-M)

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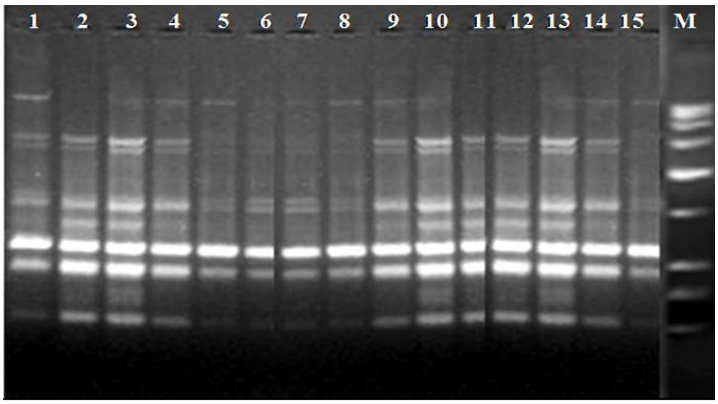

Fig. 2- RAPD profiling of fifteen cultivars of guar with random primer RP-49 (Lane 1-15), DNA 1kb ladder (Lane M).

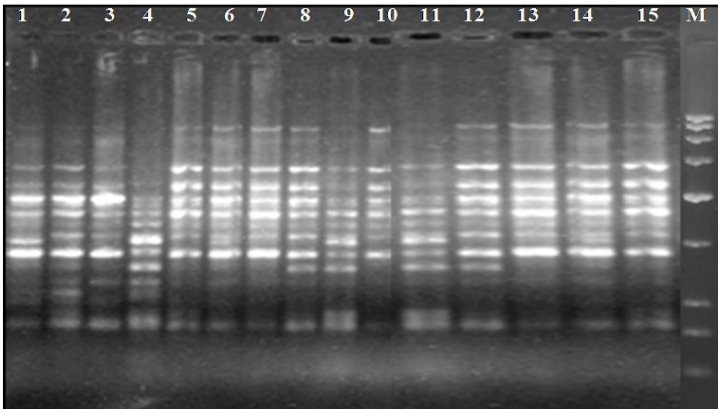

Fig. 3- RAPD profiling of fifteen cultivars of guar with random primer RP-50 (Lane 1-15), DNA 1kb ladder (Lane M).

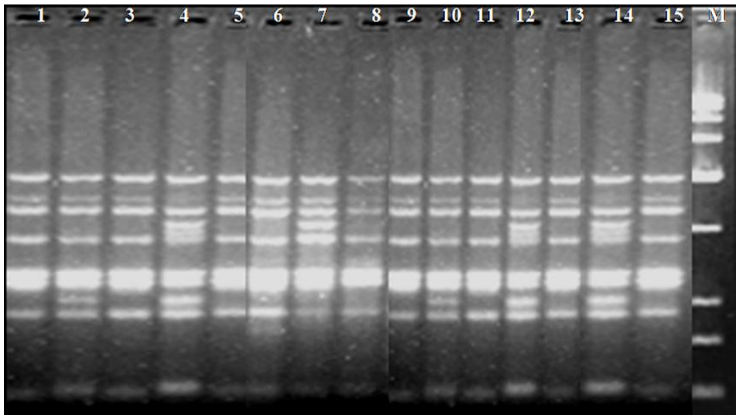

Fig. 4- RAPD profiling of fifteen cultivars of guar with random primer RP-51 (Lane 1-15), DNA 1kb ladder (Lane M).

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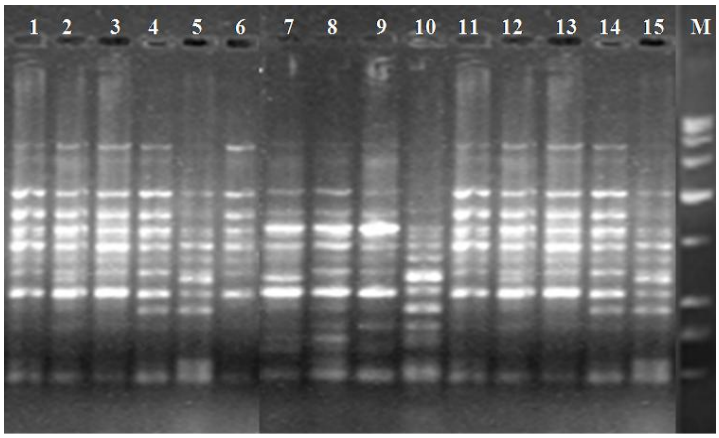

Fig. 5- RAPD profiling of fifteen cultivars of guar with random primer RP-52 (Lane 1-15), DNA 1kb ladder (Lane M).

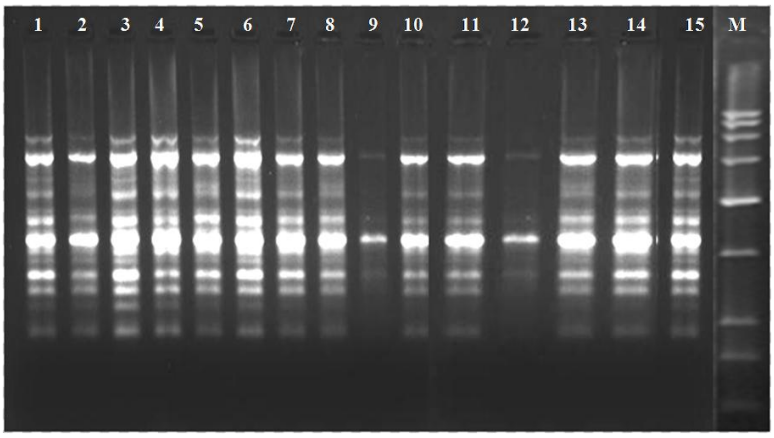

Fig. 6- RAPD profiling of different cultivars of guar with random primer RP-53 (Lane 1-15) DNA 1kb ladder (Lane M).

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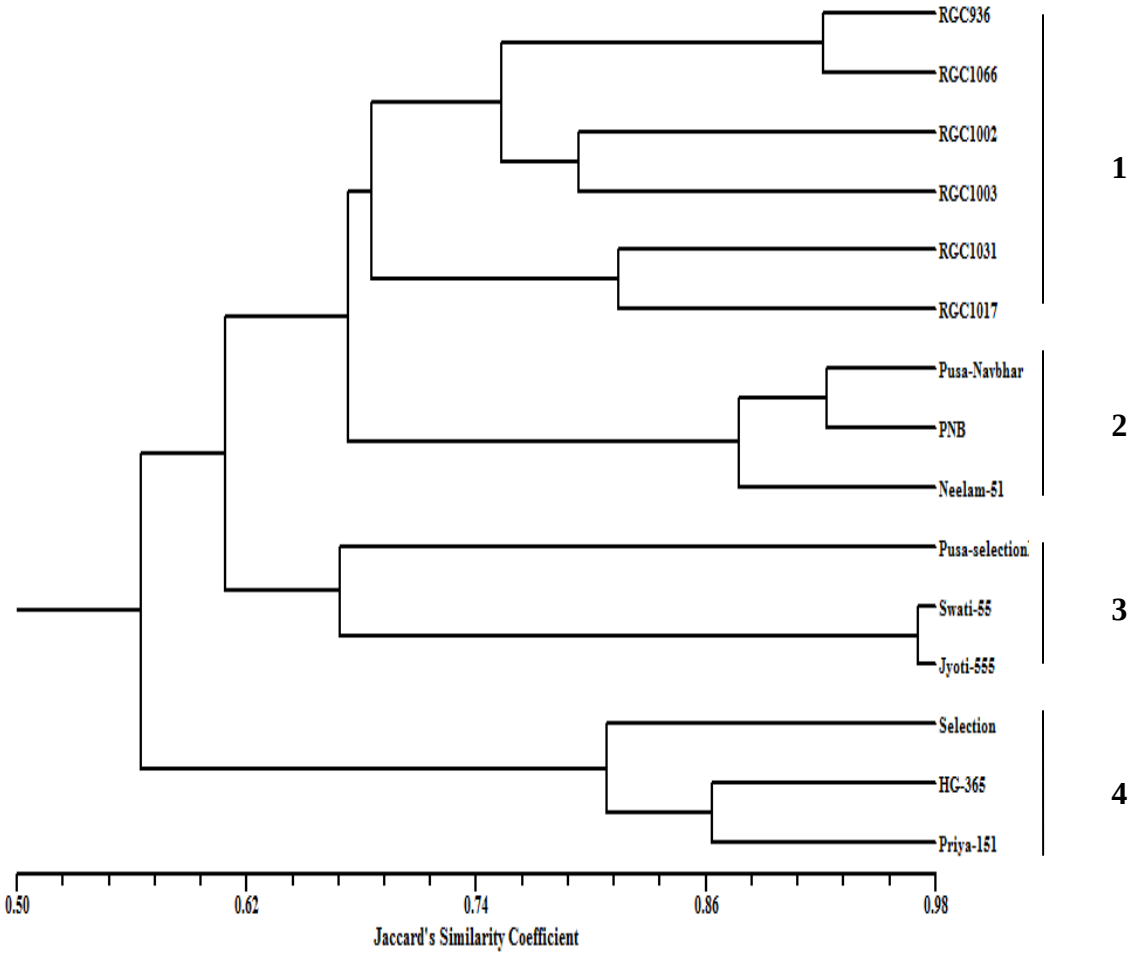

Fig. 7- Dendrogram of fifteen cultivars of cluster bean based on RAPD bands amplified by fifteen arbitrary RAPD primers.

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