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Molecular analysis through RAPD markers in greengram genotypes

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Abstract
The objective of the study is to determine the genetic relationships and diversity among 20 genotypes of greengram. Ten randomly chosen decamers in total were screened, and all of them were amplified. There were 1071 amplified bands in total, 110 of which were polymorphic, 10.27 percent polymorphism was the average. The band size of the final PCR-amplified products varied from 100 bp to 1000 bp. According to the presence or absence of unique alleles were found in sixteen genotypes of greengram. The values of the Jaccard's similarity coefficient for RAPD primers ranged from 0.0 to 0.5. The majority of the genotypes were divided into six major clusters based on the dendrogram produced by using the UPGMA method. The genetic distance between genotypes VRM-1 and MH 421was the smallest, while the genetic distance between genotypes CO 1 and CO 8, VBN 3 and CO 2 (99%) was the greatest.Hence, these findings provides valuable information for molecular classification and marker-assisted breeding for crop improvement in the *Vigna* germplasm pool.

Keywords: DNA amplification, Genetic diversity, Greengram, RAPD, Polymorphism, Similarity coefficient

INTRODUCTION

Greengram (*Vigna radiata*. L)Wilczek, (2n=22) it is a self-pollinated and short duration crop. It belongs to the family *Leguminaceae*, is a large pan tropical genus with 104 species (Lewis, 2005). It is a small, annual herbaceous drought-tolerant crop. In India, greengram is widely grown on loamy to sandy loamy soil, where it increases soil fertility by fixing atmospheric nitrogen. Greengram are known in Ayurveda as Sattvic food, which means wholesome food, and are easy to digest due to their 24 per cent protein content (Mohammed *et al.*, 2020). Greengram contains vitamins and minerals as well as the nutrients folate and beta-carotene.

India is the world's top producer of greengram accounting for about 75% of all pulses. It contributes 10% to the total production of pulses and is grown on about 4.5 million hectares, producing 2.5 million tonnes at a productivity of 548 kg/ha. The third advance estimates from the Government of India put the production of greengram at 2.64 million tonnes in 2020-21. (http://www.indiastat.com).However, the yield per hectare of mungbean in the nation is still low, and there is a need for increase in the yield, to fill the gap of the protein shortage. Estimating genetic diversity in current cultivars is essential to determining whether the lack of genetic diversity may be a limiting factor given that yields of greengram have remained low throughout subtropical and tropical Asia (Lakhanpaul *et al.*, 2000 and Patel *et al.*, 2021).

Crop breeding and plant genetics are the base for most of the fundamental knowledge of genetics. The study of plants has produced a plethora of information on development as well as significant insights into biological processes that are substantially preserved.

Right now, agriculture is in a good position to benefit from genomics to the fullest extent possible (Aljanabi, 2001). The techniques for identifying and measuring genetic diversity have evolved over time, moving from the analysis of morphological traits to molecular traits. Historically, morpho-agronomic traits have been used to characterise and catalogue germplasm as they are unstable due to environmental influences. The DNA sequence based molecular markers are more varied and reliable as they are not affected by environment. The morphological markers don't accurately depict true genetic links and weren't quite enough to reveal the genetic diversity. The range and dependability of molecular markers based on DNA sequences is greater. They are unaffected by the environment and discernible at all developmental stages.

Molecular markers are strong tools that can produce important information in crop improvement programmes, for the investigation of genetic diversity, trait mapping, and clinical diagnosis numerous DNA-based genetic markers has been evolved over past 20 years. The identification of molecular marker is connected to certain characters of genetic diversity, variations, and linkages, hybrid detection, genetic mapping and soma clonal variation analysis. RAPD have been used in numerous applications across various organisms in recent years. (Ali and Ahmed, 2001; Ali *et al*., 2002; Soliman *et al*., 2003; Ahmed *et al*., 2004; El-Zaeem *et al*., 2006). The practise of genetics has been fundamentally altered by the use of DNA-based genetic markers. Since plant genetic resources are involved in conservation and management of different germplasm from a variety of molecular approaches, the plant breeders use these techniques in selecting of beneficial crops that are resistant to pests and diseases (Ranade *et al*., 2001; Rao, 2004).

MATERIALS AND METHODS

The molecular experiments for the current study were carried out for a total of 20 greengram genotypes, Department of Genetics and Plant Breeding, Annamalai University, Chidambaram. The list of genotypes used was represented in **Table 1**. A set of ten greengram specific RAPD markers were used for the molecular analysis. Fresh leaves of 12 days old plants were collected and the CTAB method was followed for DNA extraction. The extracted DNA was treated with RNase to avoid RNA contamination present in the sample. The quality of DNA was checked by using 1.8 per cent agarose gel electrophoresis. The list of RAPD primers used in the study is presented in **Table 2**. A total number of 10 RAPD primers (Biogene Life Science Pvt. Ltd. Chennai, India) that produced a more number of polymorphic and reproducible fragments were selected to amplify genomic DNA. PCR amplification was carried out in a thermal cycler and a final volume of 25 µl, the thermo cycler was programmed containing 2.0 µl template DNA, 1.0 nM each of the four dNTPs, 1.0 µm/ µl of primers,1 mM $MgCl_2$, 2.5 µl Taq buffer and 0.34 Unit Taq DNA polymerase were utilized.

Table 1. List of greengram genotypes used for study

Genotypes	Name of the genotypes	Geographical location
G1	Chidambaram Local-1	Chidambaram, TamilNadu
G2	Maruvathur Local-1	Thanjavur, TamilNadu
G3	Kambam Local-1	Theni, TamilNadu
G4	Paiyur 1	Regional Research Station, Paiyur
G5	ADT 2	TNAU, Coimbatore
G6	ADT 3	TNAU, Coimbatore
G7	VBN 2	National Pulse Research Centre,Vamban
G8	VBN 3	National Pulse Research Centre,Vamban
G9	CO 1	TNAU, Coimbatore
G10	CO 2	TNAU, Coimbatore
G11	CO 7	TNAU, Coimbatore
G12	CO 8	TNAU, Coimbatore
G13	VRM 1	TNAU, Coimbatore,
G14	KM 2	Kolliyanur block, Villupuram
G15	DGGV 2	UAS, Dharwad
G16	DGGV 7	UAS, Dharwad
G17	Pusa Vishal	IARI, New Delhi
G18	AKM 8803	Pulse Research Unit, Akola
G19	TAP 7	Pulse Research Unit, Akola
G20	MH 421	HAU, Haryana

The thermo cycler was programmed for an initial denaturation at 94° C for 5 minutes followed by 40 cycles each of denaturing at 94°C for 1 min, annealing at 36°C (for different primers different annealing temperatures were used) for 1.2 minute and extension at 72°C for 1 minutes, followed by one final extension at 72°C for 10 minutes and at last the hold temperature was of 4°C. The amplified products as developed by the primers were separated by agarose (1.8 per cent) gel electrophoresis and documented in BIO RAD gel documentation unit. The scoring of bands in RAPD analysis was indicated as presence and absence (presence=1 and absence=0) of character for subsequent analysis using the software NTSYS-pc version 2.02. (Rohlf, 1998). The dendrogram was built using scoring data in the form of binary values. The SIMQUAL programme of NTSYS-pc software, version 2.02, was used to create the similarity matrix (Rohlf, 1998). The similarity coefficients were used for cluster analysis, and the dendrogram was built using (Sneath and Sokal, 1973), Unweighted Pair-Group Method Arithmetic Mean (UPGMA).

RESULTS AND DISCUSSION

In the traditional breeding approach, for the morphological markers the breeders spent a lot of time because a clear link between the desired characteristics was never established, such morphological markers was also impractical because; (i) many morphological markers had unfavourable pleiotropic effects on plant phenotypes; and (ii) it was impossible to score multiple morphological mutant traits in a single segregating population (Ranade *et al*., 2001).

The ten RAPD primers pairs *viz*., OPA-01, OPA-02, OPA-03, OPA-04, OPA-05, OPA-06, OPA-07, OPA-08, OPA-09, OPA-10 were used for analysis in twenty genotypes of greengram (**Table 2**).Ten RAPD primers generated 1071 alleles for the assessment of genetic variability between the genotypes studied. Thirty alleles were as produced by the primer OPA-10 and 182 alleles was produced by the primer OPA-02 among the twenty genotypes studied. Out of 1071 RAPD alleles amplified, only 110 alleles were polymorphic which had resulted 10.27 per cent polymorphism. All the ten primers produced polymorphic markers; however, the genotypes showed a varying degree of genetic diversity based on their amplification profile. The average percentage of polymorphism was 11.0. A low level of polymorphism was observed among the 20 greengram genotypes studied. The similar and contradictory research findings were reported by several researchers. Abdul *et al.* (2012) reported that among 53 primers produced 36 polymorphic fragments in blackgram and out of these 72% were polymorphic.

Based on the similarity matrix, the value of similarity coefficient ranged from 0 to 0.05.The greengram genotypes CO1 with CO 8 showed maximum similarity (100%) followed by VBN 3 with CO 2 (99%) among the ten genotypes, while the genotypes VRM 1 with MH 421 showed least pair wise similarity (82%) followed by VRM 1 with Pusa Vishal (83%) among the 20 greengram genotypes given in **Table 3.**

The genetic similarity between the genotypes could be explained due to the high degree of commonness in their pedigrees. The narrow genetic base of the greengram cultivars revealed the need to exploit the large germplasm collections having diverse morpho agronomic traits in cultivar improvement programs. The high degree of similarity in their pedigrees could explain the close genetic similarity between the cultivars.

As per the cluster analysis, the dendrogram was constructed for 20 genotypes of greengram using

Table 2. List of ten RAPD primers and their sequence with their band's characteristics generated on twenty greengram genotypes

Primers	Sequences (5'-3')	Band size	Total number of bands	Number of polymorphic bands	Percentage Polymorphism
OPA-01	GTGTCTCAGG	100-1000	148	15	10.13
OPA-02	GTGGGCTGAC	100-1000	182	13	7.14
OPA-03	GTCCATGCCA	180-1000	156	12	7.69
OPA-04	ACATCGCCCA	200-800	137	10	7.29
OPA-05	GTGGTCCGCA	180-1000	127	14	11.02
OPA-06	TCCCGCCTCA	150-920	44	11	25.0
OPA-07	AACGCGTCGG	400-980	84	4	4.76
OPA-08	AAGGGCGAGT	320-930	77	7	9.09
OPA-09	GGAAGCCAAC	300-800	86	8	9.41
OPA-10	GGCTTGGCCT	140-890	30	16	53.3
		Total	1071	110	
		Average	107.1	11.0	

Table 3. Genetic similarity among the 20 greengram genotypes through 10 RAPD markers

	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12	G13	G14	G15	G16	G17	G18	G19	G20
G1	1																			
G2	0.94	1																		
G3	0.85	0.89	1																	
G4	0.91	0.95	0.92	1																
G5	0.87	0.91	0.90	0.94	1															
G6	0.87	0.91	0.90	0.94	0.97	1														
G7	0.87	0.91	0.90	0.94	0.97	1.00	1													
G8	0.87	0.91	0.90	0.94	0.97	0.98	0.98	1												
G9	0.87	0.91	0.90	0.94	0.97	0.98	0.98	1.00	1											
G10	0.87	0.91	0.90	0.94	0.97	0.98	0.98	0.99	1.00	1										
G11	0.86	0.90	0.89	0.93	0.96	0.98	0.98	0.97	0.97	0.97	1									
G12	0.87	0.91	0.90	0.94	0.97	0.98	0.98	0.99	1.00	1.00	0.97	1								
G13	0.79	0.84	0.83	0.86	0.85	0.85	0.85	0.84	0.84	0.84	0.84	0.84	1							
G14	0.84	0.89	0.89	0.91	0.90	0.90	0.90	0.90	0.90	0.90	0.89	0.90	0.88	1						
G15	0.91	0.96	0.88	0.94	0.90	0.90	0.90	0.90	0.90	0.90	0.89	0.90	0.83	0.88	1					
G16	0.86	0.91	0.89	0.93	0.94	0.94	0.94	0.94	0.94	0.94	0.93	0.94	0.84	0.89	0.90	1				
G17	0.85	0.89	0.88	0.92	0.93	0.93	0.93	0.93	0.93	0.93	0.92	0.93	0.83	0.88	0.88	0.96	1			
G18	0.87	0.91	0.90	0.94	0.95	0.95	0.95	0.94	0.94	0.94	0.94	0.94	0.84	0.89	0.90	0.98	0.98	1		
G19	0.83	0.88	0.88	0.90	0.89	0.89	0.89	0.89	0.89	0.89	0.88	0.89	0.85	0.90	0.87	0.88	0.87	0.88	1	
G20	0.85	0.89	0.88	0.92	0.91	0.91	0.91	0.90	0.90	0.90	0.89	0.90	0.82	0.87	0.88	0.90	0.89	0.90	0.87	1

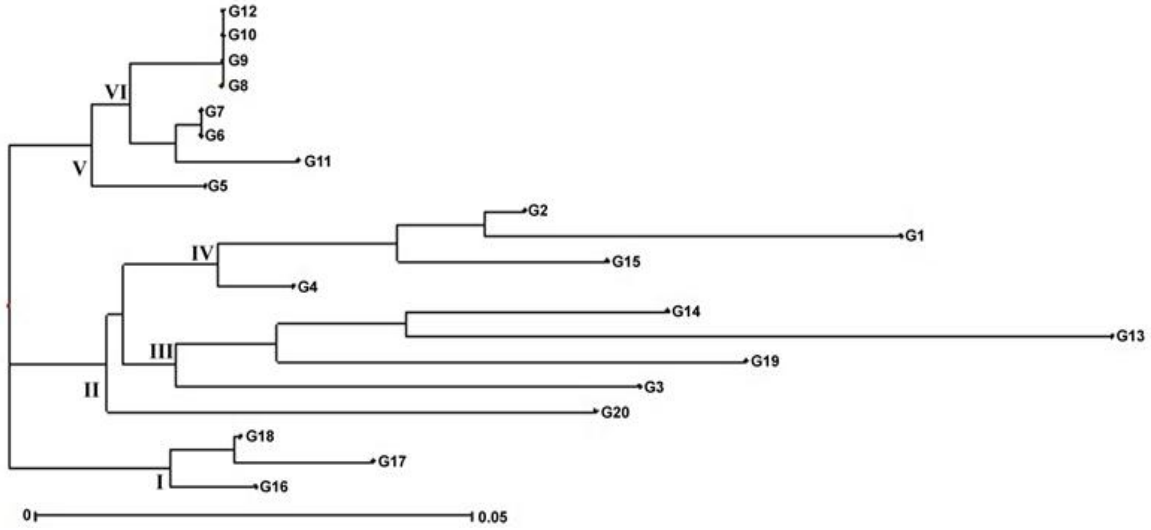

Fig. 1. Dendrogram construction for 20 greengram genotypes through Jaccard's similarity

G1	- Chidambaram Local-1	G6	- ADT 3	G11	- CO 7	G16	- DGGV 7
G2	- Maruvathur Local-1	G7	- VBN 2	G12	- CO 8	G17	- Pusa Vishal
G3	- Kambam Local-1	G8	- VBN 3	G13	- VRM 1	G18	- AKM 8803
G4	- Paiyur 1	G9	- CO 1	G14	- KM 2	G19	- TAP 7
G5	- ADT 2	G10	- CO 2	G15	- DGGV 2	G20	- MH -421

Table 4. Identification of unique bands in greengram genotypes by RAPD primers

MARKERS	PRESENT/ABSENT	GENOTYPES IDENTIFIED	Base pairs
OPA-01	+	Pusa Vishal	630 bp
		TAP- 7	300 bp,620 bp
OPA-02	+	Maruvathur Local-1	440 bp
		Pusa Vishal	420 bp
		Kambam Local-1	430 bp
		VRM -1	900 bp
OPA-03	+	Pusa Vishal	940 bp,270 bp,500 bp
		DGGV- 2	380 bp
		ADT -3	400 bp,280 bp
		VRM- 1	450 bp
OPA-04	+	DGGV - 7	460 bp
		Pusa Vishal	470 bp
		VRM -1	700 bp
OPA-05	+	ADT- 2	700bp
		CO -1	710 bp,690 bp
		CO- 8	720 bp
OPA-07	+	VBN- 3	350bp
OPA-O8	+	DGGV -2	690 bp
		ADT- 3	810 bp
		Chidambaram Local-1	710 bp
OPA-10	+	CO-1	500 bp
		TAP- 7	450 bp
		KM -2	120 bp

UPGMA method (Swati Sharma *et al*., 2018).The 20 genotypes of greengram were grouped into six clusters given in **Fig. 1**. The Cluster I consisted of three genotypes (DGGV- 7, Pusa Vishal, AKM 8803), cluster II also consist of one genotype (MH 421) and Cluster III consist of four genotypes (Kambam Local-1, VRM 1, KM 2, TAP- 7). The cluster IV consist of four genotypes namely (Chidambaram Local-1, Maruvathur Local-1, Paiyur 1, DGGV -2), cluster V consist of ADT 2 genotype and cluster VI consist of seven (ADT- 3, VBN 2, VBN 3, CO -1, CO -2, CO- 7, CO- 8) genotypes (**Fig. 1**). Unique alleles were located across all the primers that individually identified each of the genotypes. About sixteen genotypes of greengram were identified based on the presence or absence of unique alleles as given in **Table 4.**

RAPD analysis is said to have an advantages over other techniques because it is a reasonably simple technique to utilise and the number of loci that may be analysed is limitless. In many cases, identifying polymorphisms within a species just requires a few primers. The characterisation and identification of greengram genotypes were aided by RAPD markers. The genotypes CO 1 with CO 8 showed the most similarity (100%) followed by VBN 3 with CO 2 (99%) among the twenty genotypes, while genotypes VRM 1 with MH 421 exhibited the lowest pair-wise similarity (82%) followed by VRM 1 with Pusa Vishal (83%) among the twenty genotypes of greengram. Due to the genetic material's limited genetic base, ten RAPD primers yielded a relatively low level of polymorphism percentage (10.27 percent). The genotypes MH 421 and ADT 3 were found in separate clusters throughout the six-cluster cluster analysis because they are genetically distinct and highly variable genotypes. The current investigation discovered a few distinctive primers for determining greengram genotypes. OPA-07, a RAPD primer, produced the distinctive bands VBN 3.

The RAPD approach demonstrated a more noticeable effect of allozymes, making it easy to identify genetic differences in the populations (Mamuris *et al*., 1999). To find the genetic diversity and similarity in a variety of organisms using the different primers. It can be used for resolving taxonomic issues in many organisms (Kjolner *et al*., 2004), as a useful tool for identifying species (Guo *et al*., 2001, Bernardi and Talley 2000). The present study highlights the importance of utilising large germplasm collections with diversified morphological traits in varietal improvement programmes due to the constricted genetic base of the greengram genotypes. Therefore, utilising other molecular approaches, RAPD assays can be expanded to more precisely analyse features and get knowledge

about certain genes and genetic networks, sequencing of these genotypes and to identify polymorphic bands by RAPD experiments and do further research to identify the genetic relationships among the various species.

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