

Original Research Article

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Assessment of Genetic Diversity by Using RAPD Markers of *Vigna unguiculata* cultivars in Telangana State, India

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ABSTRACT

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In the present study genetic diversity based on RAPD markers was carried for five high yielding cultivars of *Vigna unguiculata* from Telangana region. A total 16 primers were used for the detection of polymorphism. Out of 230 bands formed 109 bands were found to be polymorphic. However the extent of polymorphism varied with each primer present and an average of 91.1% polymorphism was found among the cultivars in *Vigna*. Dendrogram constructed based on UPGMA analysis showed two clusters with first cluster bifurcating into two subclusters a and b. Dendrogram and similarity index clearly indicated that Gangothri and Pusakomal are in the same cluster a, and two cultivars Navrathan and Gowthami in cluster b, of the first cluster representing two genetically similar groups. Gomchi found to be genetically much distant and formed a second cluster indicating that this cultivar is much variant from others.

Introduction

Cowpea is also commonly referred to as southern pea or black-eyed pea. Cowpea is one of several species of the widely cultivated genus *vigna*. It is chiefly used as a grain crop; for animal fodder, or as a vegetable. Cowpea is one of the principal pulses of common use in India. The crop appears to have spread from India to china and other south East Asian countries. Vavilov (1939) recognized India as the main centre of origin of this crop. Cowpea is particularly valuable component of low input farming systems of resource for poor farmers because of its productivity and ability of the crop to enhance soil fertility for succeeding cereal grown on rotation.

The present study was undertaken to evaluate the pattern and existence of genetic variability and relatedness among cultivars for genetic improvement of cowpea using RAPD markers. RAPD (Random Amplified polymorphic DNA) analysis can be used to characterize DNA variation patterns within species and among closely related taxa (Williams *et al.*, 1990). Within grain legume crops alone, RAPD markers have been widely used for the identification of genetic relationships among cultivars (Sonnante and Pignone 2001); among wild forms (Freyre *et al.*, 1996; Cattan-Toupance *et al.*, 1998), or between cultivars and wild forms (Mimura *et al.*, 2000; Raina *et al.* 2001). To date, few

studies have been performed in cowpea using RAPDs (Menendez *et al.*, 1997; Mignouna *et al.*, 1998).In 2004 Fana Sylla and Paul have reported genetic diversity in cowpea (*Vigna unguiculata*) as revealed by RAPD markers. To characterize DNA variation patterns among closely related taxa and within species in *Vigna*, RAPD (Dikshit *et al.*, 2007) and SSRs (Dikshit *et al.*, 2007) have been adopted.

Materials and Methods

The plant material used in the present study consisted of five cultivars of cowpea (Navrathna, Pusakomal, Gnagothi, Gowthami, and Gomchi) collected from National Seeds Corporation, Hyderabad, Telangana. Genomic DNA from *vigna unguiculata* leaves was extracted with the modified CTAB (Cetyl Tri Methyl Ammonium Bromide) method (Saghai-marooof *et al.*, 1984). About 150-300mg of fresh leaf sample was surface sterilized and crushed with 2XCTAB extraction buffer with 2% PVP and 10 mM of β -mercaptoethanol (Doyle and Doyle1987). Sample is collected in 1.5ml tube and incubated at 65°C for 30 minutes in a water bath and mixed gently with swirling. To these tubes equal volume of Chloroform: Iso-amyl alcohol (24:1) are added and mixed properly. The tubes were centrifuged at 8000 rpm for 15 minutes. The final aqueous layer was transferred to a centrifuge tube and equal volume of chilled isopropanol is added and mixed thoroughly. This is incubated at -20°C for 1 hour and centrifuged at 8000 rpm for 15 min. The supernatant is discarded and the pellet is air dried for overnight. The pellet is resuspend in 30-50 μ l TE buffer. The sample is incubated at 60°C for 1 hour and stored at 0°C for further use.

DNA samples were first suspended in 100 μ l of tris EDTA buffer and then diluted to 4 ng/ μ l with water. The PCR reaction mixture, contained a total reaction volume of 25 μ l, including 32 ng of template DNA, Mgcl₂ to a final concentration of 1.9 mM, 25 mM of each Dntps and 33 ng of decamer primer (Bioserve, India). DNA sequences were amplified using eppendorff thermo cycler. The parameters of the PCR cycle were as follows: 1 cycle at 94° c for 2 min: 40 cycles of 94° c for 1 min, 35° c for 1 min and 72° c for 2 min: 1 cycle at 72° c for 5 min; and 1 cycle at 30° c for 30 min. After amplification, 3 μ l of gel loading buffer was added to each sample. A sample of 10 μ l of PCR product was separated by electrophoresis at 100 volts and stained with (0.5 μ l ethidium bromide per ml of buffer) before visualizing the banding patterns under UV light and scanning the images. A 1.5 kb DNA ladder was used for sizing the RAPD bands. Primers were selected from those chosen by Menendez et al (1997) out of a total of 16 primers.

Results and Conclusion

Genomic DNA was extracted from five different cultivars of *vigna*. A total of 12 primers were used for RAPD analysis in *vigna* cultivars. Among 12 primers, four primers did not show any amplification, 4 primers showed only monomorphic bands, four primers OPA -1, OPA -4, OPB- 10, OPB- 15 showed polymorphism in all the samples, however the extent of polymorphism varied with each primer. A total of 230 loci (109 polymorphic ranging between 100-1350bp) were amplified. On an average a total polymorphism percent in *vigna* was recorded as 91.1% among the cultivars under study.

Table.1 List of 4 RAPD primers, polymorphism and banding patterns of genotypes of *Vigna*

Primer name	Primer Sequence	TM	GC	Max.B	TB	PB	MB	%P	Range of band	PIC.
OPA1	CAGGCCCTTC	32	60%	8	17	16	1	94.1	150-1350bp	0.8
OPA4	AATCGGGCTG	32	60%	11	31	28	3	90.3	150-600bp	0.664
OPA10	GTGATCGCAG	32	60%	13	40	36	4	90	200-1200bp	0.7532
OPA15	TTCCGAACCC	32	60%	13	32	29	3	90	100-1350bp	0.598

Fig.1 RAPD PCR based polymerase chain reaction fingerprints pattern for genomic DNA of *Vigna* genotypes from the Telengana State.

L: LADDER G1: GANGOTHRI G2: PUSA G3: NAVRATHAN G4: GOWTHAMI G5: GOMCHI

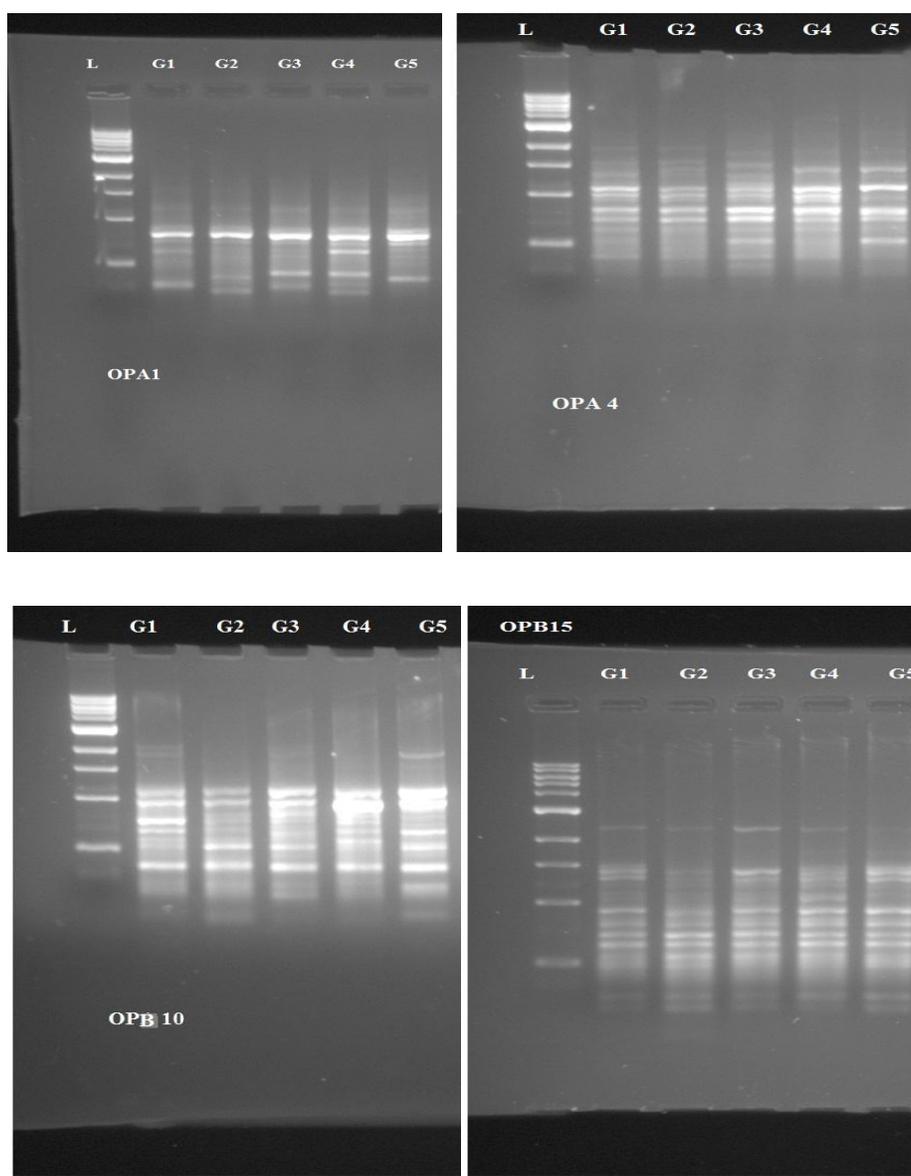


Fig.2 UPGMA deprogram showing relatedness among the different local cultivars of *vigna* using RAPD primers.

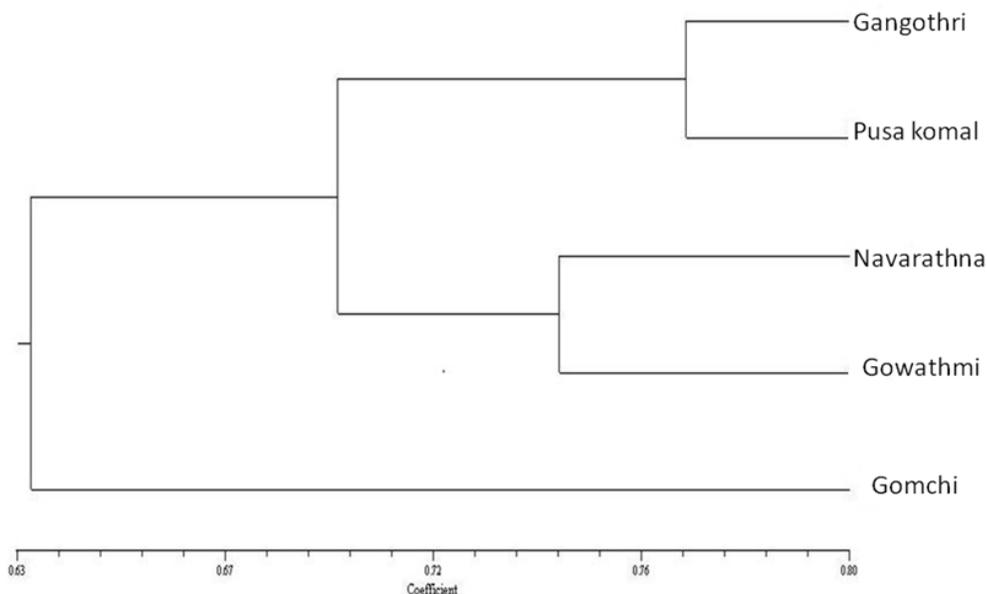


Fig.3 Table.3: Dice coefficients of similarity based on RAPDs showing the relationship between *Vigna* cultivars

	Gangothri	Navarathna	Pusakomal	Gowthami	Gomchi
Gngothri	1.0000000				
Navarathna	0.7000000	1.0000000			
Pusakomal	0.7666667	0.7142857	1.0000000		
Gowthami	0.6774194	0.7407407	0.6896552	1.0000000	
Gomchi	0.6774194	0.6206897	0.6333333	0.6000000	1.0000000

Polymerase chain reaction (PCR) based markers using arbitrary primers, such as RAPD, have been widely used for investigating Gene relatedness and diversity in plant population and cultivars. Markers are polymorphic in nature. DNA-based markers can be applied for this purpose because they can be used for assessing precisely the genotype of a plant. Several DNA marker systems are now commonly used in diversity studies of plants.

Based on UPGMA (un-weighted pair group of arithmetic means) analysis, dendrogram

was constructed. This Dendrogram and similarity index clearly indicates the presence of two main clusters, cluster 1 and cluster 2. Cluster 1 is sequential cluster. It is further divided into cluster a and cluster b. Cluster a contains two cultivars Gangothri and pusakomal, these are genetically similar with a genetic distance of 0.77. Cluster b contain two cultivars Navratan and Gowthami that are genetically similar, with genetic distance of 0.74. Cluster 2 with only Gomchi cultivar is genetically much variant from both clusters, cluster a and Custer b, with genetic distance of 0.80 indicating that

Gomchi is genetically much variant from other cultivars.

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