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Analysis of genetic diversity in pigeon pea (*Cajanus cajan*) by using PCR based molecular marker

Sarika Shende and Anand Raut

Department of Plant Biotechnology, College of Agricultural Biotechnology, M.A.U., Latur, (M.S), India

Abstract
Assessment of genetic diversity and identification of crop genotypes are essential for efficient conservation and utilization of germplasm resources. Random amplified polymorphic DNA (RAPD) was used to determine the genetic relationship among 15 pigeon pea genotypes. A total of 23 amplicons were detected with 5 primers of which 13 were polymorphic. The polymorphism shown by RAPD markers was 56.52%. The average polymorphic information content (PIC) value exhibited by RAPD was found to be 0.127. Genetic similarity values ranged from 22-98% with an average of 67%. A dendrogram based on the Jaccard's similarity values showed distribution pattern of variability between genotypes with different morphological and agronomic traits.

Keywords: Pigeon pea, RAPD, Genetic diversity, Polymorphism.

INTRODUCTION

Pigeon pea is a grain legume belonging to the *Cajaninae* subtribe of the economically important leguminous tribe Phaseoleae (Young *et al*., 2003) and has a diploid genome comprising 11 pairs of chromosome (2n=22) (Greilhuber and Obermayer, 1998). Pigeon pea plays an important role in food security, balanced diet and alleviation of poverty by providing source of food, feed, fodder (Rao *et al*., 2002), fuel wood, rearing *lac* insects (Zhenghong *et al*., 2001), hedges, windbreaks, soil conservation, green manuring and roofing. It is a major source of protein to about 20% of the world population (Thu *et al*., 2003) and is an abundant source of minerals and vitamins (Saxena *et al*., 2002).

The production of pigeon pea has remained static over the last several years (Souframanien *et al*., 2003). The crop's long life cycle and a heterozygous genome structure conserved by out crossing (Saxena *et al*., 1990) make breeding slow and expensive. Wild relatives have now been reported to possess many agronomically important traits such as resistance to pest and diseases (Reddy *et al*., 1996), salinity tolerance (Subbarao *et al*., 1991), high protein content (Saxena *et al*., 1996). Molecular markers and DNA technology is used to assess diversity in the gene pool to identify genes of interest and to develop a set of markers for screening progeny.

MATERIALS AND METHODS

Plant materials

Fifteen genotypes of pigeon pea (*Cajanus cajan*), collected from Agriculture Research Station, Badnapur, Dist. Jalna (M.S) were used in the present investigation (Table 1).

DNA isolation and PCR amplification

The plant genomic DNA was isolated from young fresh leaves of each genotype by following the modified CTAB method (Seghai-Maroof *et al*., 1984; Bhat *et al*., 1999). Purified genomic DNA was subjected to PCR amplification using random primers. A 25µl mixture contained 30ng of genomic DNA, 1U *Taq* DNA polymerase (Genetix), 1XPCR buffer containing 4mM $MgCl_2$, 0.2 mM of each dNTPs (Genetix), 100 pmol of primers. Amplifications were carried out using a 96 thermal cycler (Bio metra) programmed for 40 cycles as follows: initial denaturation at 94^{o}C for 4 min, further denaturation at 94^{o}C for 1 min, annealing at 30^{o}C for 1 min, extension at 72^{o}C for 2 min and final extension for 10 min at 72^{o}C. The amplification products were stored at 4^{o}C until loading. The PCR products were resolved at 100Volts for 4 hours on 1.6% agarose gel prepared in 1xTBE buffer. Gel was photographed using Gel-Documentation system (ALPHAIMAGER TM 2200).

Data Analysis

The amplified product of RAPD was scored for presence (1), absence (0), missing and doubtful cases as (9). All the above analysis were done using NTSYS-PC (version 2.02i) program (Rohlf, 1990). The SIMQUAL programme was used to calculate the Jaccard's coefficient value. Dendrogram was constructed based on UPGMA clustering of a similarity matrix generated by Jaccard's coefficient.

RESULT

Seventeen random 25 decamer *viz*., OPA, OPD and OPM series (MWG Biotech, Banglore) primers were employed for RAPD analysis of 15 pigeon pea genotypes (Table 2). Of these, five primers were selected on the basis of reproducible and scorable amplification

*Corresponding Author

Sarika Shende
Department of Plant Biotechnology, College of Agricultural Biotechnology, M.A.U., Latur, (M.S), India

Email: sarikashende@gmail.com

products for studying the relationships among genotypes. These five random primers generated 23 amplicons of which 13 were polymorphic, at an average of 2.6 polymorphic amplicons per primer. The number of amplified fragments revealed by each primer ranged from 01 (OPD-14) to 04 (OPM-09). The amplification profile of one of the primer is presented in fig. 1. The amplified product size ranged from 120bp (OPD-16) to 1125bp (OPD-14). All these five primers showed polymorphism. The primer OPM-09 exhibited highest polymorphism (100%) amongst all the primers. The average polymorphic information content (PIC) value showed by RAPD primer was found to be 0.127. Primer OPM-7 showed highest PIC value i.e., 0.20.

Diversity Analysis

A dendrogram generated using UPGMA cluster analysis based on Jaccard's similarity coefficient value revealed 67% average similarity among pigeon pea genotypes (Fig. 2). At 73% similarity coefficient value pigeon pea genotypes were divided into two major clusters, A and B. The cluster A comprised of 8 genotypes and further divided into two subclusters A_1 and A_2. The cluster A_1 comprised of single cultivar i.e., ICP-8863, which is selection from Maharashtra landraces. The cluster A_2 further divided into two sub clusters, A_2a and A_2b showing 79% similarity. The A_2a consist of two genotypes *viz*., Vipula, which is selection from farmer participation programme, Rahuri and BDN-2029 from Badnapur are highly close related and showed 98% similarity. They showed similarity in characteristics like indeterminate growth habit, days to maturity (180-185 days), yellow flower and red grain colour. The cluster A_2b consists of five genotypes *viz*., BDN-2, BDN-853, BDN-2001-9 and BDN-2004. All the genotypes in this cluster are from Badnapur and derived from BDN-2 except BDN-2004.

The cluster B contains 4 genotypes and is mostly derived from the parents of ICRISAT except BDN-2001-6 which is from Badnapur. The cluster B is further divided into two subclusters, B1 and B2 showing 78% similarity. Three pigeon pea genotypes *viz*., C-11, AKT-8811 and UPAS-120 being ungrouped and formed separate cluster.

Table 1. List of pigeon pea genotypes and their characteristics.

S.No.	Name of genotype	Pedigree	Flower colour	Grain colour	Growth habit	Days to maturity	Special feature
1	BDN-2	Selection from Bori area Parbhani	Yellow	White	Semi spreading	160-165	Wilt resistant
2	Vipula	Selection from Rahuri	Yellow	-	Indeterminate	175-180	-
3	BDN-2004	-	-	-	-	-	-
4	ICPL-87	T-21x JA277	Yellow	Red	Determinate	120-125	-
5	BSMR-736	ICP-7217xNO.148	Yellow	Red	Indeterminate	180-190	Wilt resistant
6	UPAS-120	Selection from Pantnagar	Yellow	Red	Indeterminate	175-180	-
7	AKT-8811	Mass selection from Akola	Yellow	Red	Indeterminate	165-170	Wilt susceptible
8	BSMR-853	ICP-7336xBDN-1xBDN-2	Red	White	Indeterminate	178-180	Wilt resistant
9	BDN-2001-6	BDN-1xGPS-52	Yellow	-	Semi spreading	165-180	Wilt resistant
10	BDN-2001-9	BDN-2xBWR-370	Yellow	-	Semi spreading	165-180	Wilt resistant
11	ICPL-87119	C-11xICPL-6	Yellow	Red	Indeterminate	175-185	Wilt resistant
12	C-11	BAHRxNP(WR)15	Yellow	Red	Indeterminate	175-180	-
13	BDN-708	BDN-2xICPL-87119	Yellow	Red	Semi spreading	165-165	Wilt tolerant
14	BDN-2029	-	Yellow	Red	Indeterminate	-	-
15	ICP-8863	Selection	Yellow	Red	Indeterminate	165-170	Wilt resistant

Table 2. Details of amplicons produced by RAPD primers in pigeon pea genotypes.

S. No.	Primer	Total number of amplicons	Number of polymorphic amplicons	Percent polymorphism	PIC value
1	OPD-14	4	1	25.00	0.048
2	OPD-16	6	3	50.00	0.18
3	OPD-18	4	3	75.00	0.088
4	OPM-07	5	2	40.00	0.20
5	OPM-09	4	4	100.00	0.12
	Total	23	13	56.52	0.636
	Average	4.6	2.6	--	0.127

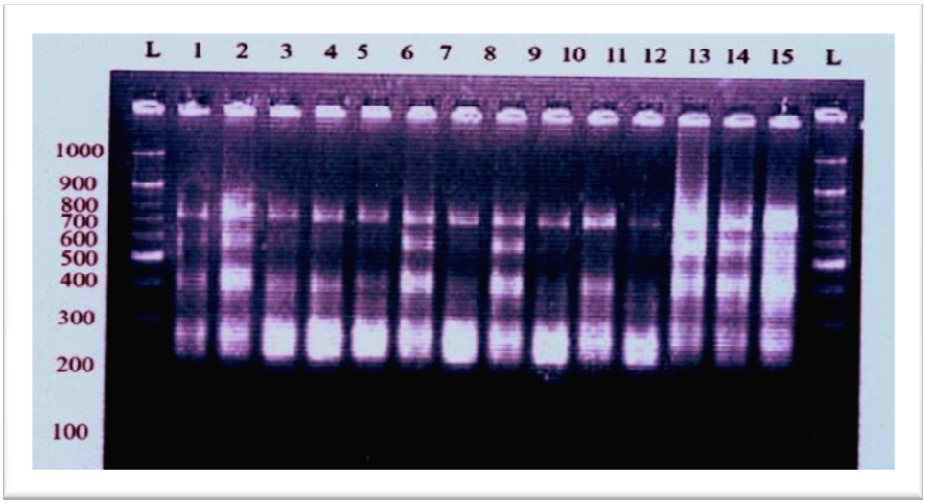

Fig 1. RAPD profile of 15 pigeon pea genotypes generated with primer OPM-07. L: 100bp DNA ladder, Lanes1-15: correspond to pigeon pea genotypes listed in Table 1.

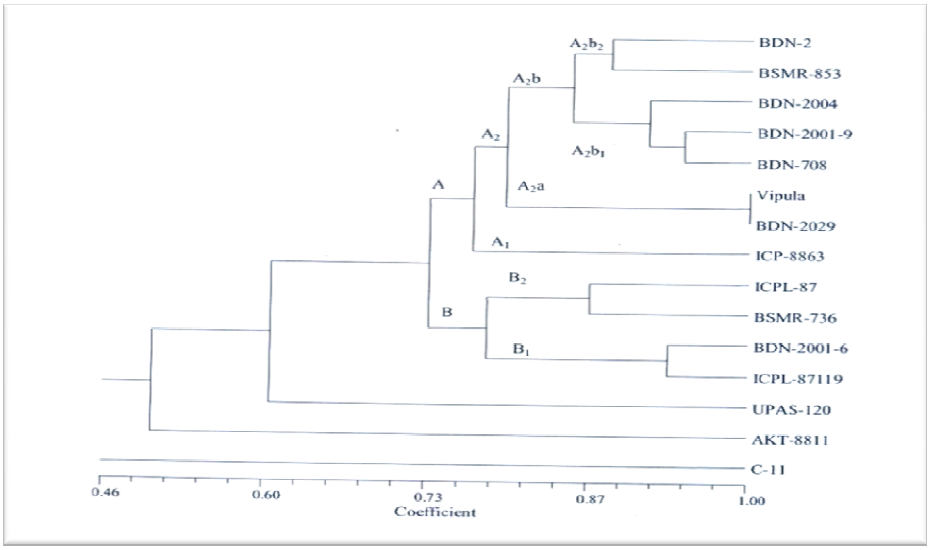

Fig 2. Dendrogram generated through UPGMA analysis showing genetic relationship among the 15 pigeon pea genotypes. Name of the genotypes are given on the termini of branches.

Discussion

The present study revealed moderate level of polymorphism (56.52%) among 15 pigeon pea genotypes and it stands intermediate among the previous studies reported by Lohithaswa *et al*. (2003) 63.46% polymorphism, Gunjanjyoti and Boora (2005) 93.75% polymorphism, Anupama *et al*., (2006) 96.6% polymorphism with RAPD markers whereas low level of polymorphism (21.42%) was reported by Damse (2007) and Ratnaparkhe *et al*. (1995). These medium to high polymorphic results indicates a wide genetic base in pigeon pea accessions and genetic diversity may be due to their characteristics, wide distribution, amplification protocol used or selection of suitable primers as reported by the earlier workers.

The maximum similarity was observed between BDN-2029 and Vipula with 98% similarity coefficient value. The genotypes C-11 and AKT-8811 were found diverse from rest of other genotypes showing 22%minimum similarity, which may be due to out crossing nature of pigeon pea. These diverse genotypes can be used in hybridization programme.

Average similarity coefficient value as determined by Jaccard's similarity coefficient (67%) was in conformity with the results obtained by previous workers as 82% (Damse, 2007), 71% (Anupama, 2006) and 57%-82% (Gunjanjyoti and Boora, 2005).

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