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RAPD ANALYSIS AMONG PIGEON PEA [*CAJANUS CAJAN* L. MILL SP.] CULTIVARS FOR GENETIC DIVERSITY

GANGURDE S.S.*[1], GHORPADE B.B.[2], DINESH A.[3], BARAMAPPANAVARA M.[3], SAVANT A.R.[2], AUTADE R.H.[2]

[1]Biotechnology Centre, Dr. Panjabrao Deshmukh Agicultural University, Akola 444104, Maharashtra, India
[2]College of Agricultural Biotechnology, Loni, Pravaranagar, 413736, Maharashtra, India
[3]Department of Genetics and Plant Breeding, Agricultural University, Raichur, 584104
*Corresponding Author: Email- sgangurde40@gmail.com

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Abstract- Pigeon pea (*Cajanus cajan* L.) is major pulse crops of the tropics, and is important in small-scale farming in mainly developing, semi-arid regions. The RAPD analysis of genomic DNA by 3 RAPD primers showed good polymorphism in 5 pigeon pea cultivars namely as Vipula, ICPL-87, BSMR-853, BSMR-736, Rajeshwari. Total 14 amplicons were generated by 3 primers (OPC-11, OPC-9 and OPC-1). Out of them 12 amplicon were found to be polymorphic showing 85.71% polymorphism, the Jaccard similarity coefficient among all genotypes ranged from 0.35 to 0.77. The highest genetic similarity to an extent of 77% was recorded between Vipula and ICPL-87 isolates followed by 64% similarity between BSMR-736 and BSMR-853. The dendrogram based on Jaccard similarity coefficient produced 2 main clusters A & B. Cluster A divided into two sub clusters A1 and A2. The sub cluster A1 shows two genotype Vipula, ICPL-87 and sub cluster A2 to contains only BSMR-853. The cluster B comprised of two genotype BSMR-736 and Rajeshwari showing the similarity about 51%.

Keywords- Polymorphism, Dendrogram, Jaccard Similarity coefficient.

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Introduction

Pigeon pea (*Cajanus cajan*(L.) Mill sp.) belongs to the family *Cajaninae* sub-tribe of leguminous tribe *Phaseoleae.* Pigeon pea is widely cultivated in India and Australia. The origin of this crop is West Africa. The chromosome number is (2n = 22) with physical size 0.853 pg [1]. The seeds are rich in Iron, iodine, and essential amino acids like Lysine, Cystine and Arginine. It is a major source of protein to about 20% of the world population [2,3]. It is an abundant source of minerals and vitamins [4]. However, specific cultivar improvement has been difficult due to the limited knowledge on the inheritance of important traits and lack of understanding on the level of inter and intra-specific genetic diversity. Molecular marker technology helpful for determination of the number, chromosomal location and interactive effects of genes that control traits. Random Amplified Polymorphic DNA analysis is a simple, easy to understand, quick technique, requires a very small amount of DNA for analysis [5].

The major advantage with RAPD is that prior sequence information is not required. It is used to target genetic markers of near isogenic lines [6] and also used for creation of high-density genetic maps [7]. It is a dominant marker and finally it is such as bulk sergeants and interval mapping [8] have further extended the importance of technique, especially as a step in a map based cloning [9] and fingerprinting [10].

Earlier studies showed that randomly amplified polymorphic DNA (RAPD) markers were used for the identification of pigeon pea [*Cajanus cajan* (L.) Mill sp.] cultivars and their related wild species. The level of polymorphism among the wild species was extremely high, while little polymorphism was detected within *Cajanus cajan* accessions [11-15]. Genetic diversity in F_2 populations of Pigeon pea derived from contrasting parents was also studied using RAPD markers [16]. The aim of present study is assess the genetic diversity among the pigeon pea cultivars by using RAPD markers.

Materials and Methods

Plant materials

Five cultivars of Cajuns Cajun were produced from All India co-ordinated Pulse Improvement Project (AICPIP) MPKV, Rahuri having different agronomic characters and phenotypic variation as [Table-1].

Sample collection

The Seeds of pigeon pea seeds were germinated in pots in green house in control environmental condition Young fresh leaves of pigeon pea seedlings were used for DNA extraction.

DNA Isolation

The plant genomic DNA was extracted by CTAB method as described by [17]. DNA was extracted from young leaf sample of 5 genotype briefly approximately 2 gm of fresh leaves from field grown plant were collected and crushed in liquid nitrogen (-196^{0}c). The crushed powder was added to a tube containing 10 ml of extraction buffer (CTAB (10 %),4 M NaCl, 1 M Tris HCl (pH 8.0),0.5 M EDTA (pH 8.0), pre-warmed to 65^{0}c for 30 min. After heating 2 % B-mercapto ethanol and 0.3 % PVP was added .the tube were incubated at 65^{0}c for 45 min, after incubation a equal volume of chloroform: isoamylalcohol (24:1 v/v) was added to the tube and mixed well. The tube was then centrifuged at 13000 rpm for 11 min at 4^{0}c. The aqueous phase containing the nucleic acids was transferred to fresh tube and equal volume of chilled isopropanol was added and mix well and DNA was allowed to precipitate at -20^{0}c for overnight .the tube was centrifuged at 13000 rpm for 11 min .after centrifugation supernatant was discarded and pellet was washed by adding 1 ml 70 % ethanol and centrifuge at 7000 rpm for 5 min then the supernatant was discarded and dried. The pellet re-suspended in 200-250 ul TE buffer and kept overnight for complete dissolution. The extracted DNA was treated with RNase 2µl /200 of DNA and incubated at 37° 0 c for 30 min. This DNA sample was stored at -20° c.

Quantification of DNA

The quantity of DNA was checked by using 1 µl of DNA of all 5 cultivars by using Nano drop (ND-1000 spectrophotometer) machine [Table-2] at state level Biotechnology centre MPKV Rahuri. Stock DNA was diluted in TE buffer to make a working solution of 30 ng/µl for PCR reaction. A part of DNA sample was diluted with appropriate quantity of TE buffer to yield a working concentration of 30 ng/µl and stored at -20^{O}C for further work until PCR amplification.

Table-1 *List of Pigeon pea Genotype and their characteristics*

Sr. No	Varieties	Pedigree	Flower colour	Grain colour	Growth habit	Days to maturity	Special feature
1	Vipula	Selection from Rahuri	Yellow	_	Indeterminate	175-180	_
2	ICPL-87	T-21XJA277	Yellow	Red	Determinate	120-125	_
3	BSMR-853	ICP-7336xBDN-1xBDN-2	Red	White	Indeterminate	78-180	Wilt resistant
4	BSMR-736	ICP-7217XNO.148	Yellow	Red	Indeterminate	175-180	Wilt resistant
5	Rajeshwari	_	_	_	_	_	_

Table-2 *Quantification of DNA sample*

SN	Genotype Name	Concentration of DNA ng/ µl	260/280
1.	Vipula	520.7	2.03
2.	ICPL-87	320.1	2.20
3.	BSMR-853	411.3	2.30
4.	BSMR-736	602	1.99
5.	Rajeshwari	535.2	2.13

PCR Reaction

Random primers already available were used for RAPD analysis of 5 Pigeon pea cultivars lines. For these, three decamer primers were used.

Table-3 *The components of PCR reaction mixture*

Components	Volume for one tube (µl)	Final concentration
10X PCR buffer (10x)	1.5	1.5X
dNTPs (10mm each)	0.7	0.46mM (each)
$MgCl_2$(50 mm)	1.5	4.5 mM
Primer (100 pmol) Genei^{Tm} Bangalore	1	5pmol
Sample DNA (30 ng/µl)	1	30 ng
*Taq*DNA polymerase (50/ul)	0.2	1 U
Sterile double distilled water	9.1	---
Total	**15**ul	---

The PCR reaction mixture was made 15 ul as mentioned in [Table-3]. In PCR tubes were then placed in thermal cycler (Gradient, Germany) for amplification of the genomic DNA as per the standardized protocol for Pigeon pea, which is enumerated below [Table-4].

Temperature profile

Table-4 *Temperature profile for PCR amplification*

Sr. No.	Step	Temperature (^{0}C)	Duration (min.)	Number of cycles
1.	Initial Denaturation	94	10	1
2.	Denaturation	94	1	40
3.	Annealing	37	1	
4.	Extension	72	2	
5.	Final extension	72	10	1
6.	Hold temperature	4		

Resolution of amplified product

The amplified products were resolved on 1.5 % agarose gel at 100 V for 2 hours. The gel was stained with ethidium bromide (5µl/100 ml). After electrophoresis, the gel was carefully taken out of the casting tray and the photograph was taken on a Gel Documentation System (Bioera, Pune).

Data scoring and analysis

The amplified products of RAPD from agarose gel images the DNA bands were scored for the presence (1), absence (0), missing and doubtful cases were scored as 9. Band size was determined by comparison with 100 bp and 1 kb DNA ladder (Himedia) Data analysis was performed using NTSYS-PC (Numerical Taxonomy System, Version 2.02,) [18]. The SIMQUAL programmed was used to calculate the Jaccard's coefficient. Dendrogram was constructed using un weighted pair group method for arithmetic mean (UPGMA) based on Jaccard's coefficient.

Results and Discussion

Three RAPD primers were generated 14 amplicons with an average 4.6 amplicons per primer [Table-5]. Out of total 14 amplicons, 12 amplicons were found polymorphic. They showed 85.71% polymorphism and the average number of polymorphic bands per primer was 4 [Table-5]. Different primers produced a different level of polymorphism among the different genotypes.

The primer OPG-01 was the most informative as it generated the highest number of polymorphic amplicons i.e., 5 polymorphic amplicons [Fig-1], the primer OPB-09 and OPC-11 stood less informative primer than OPB-09 by generating 4 polymorphic bands. The average size fragments were between 100 to 900 bp. The data obtained by RAPD marker were analysed by NTSYS-PC and dendrogram was generated by using the Jaccard similarity coefficient. Dendrogram was presented in [Fig-1]. To estimate the genetic similarity of the pigeon pea cultivars a similarity matrix is shown in [Table-6], obtained by Jaccard similarity coefficient. The dendrogram clearly indicated two clusters of *Cajanus cajan* and again sub clusters were found. The maximum similarity was observed between Vipula and ICPL-87 [Fig-2]. These two genotypes were found highly similar with similarity coefficient 0.77.

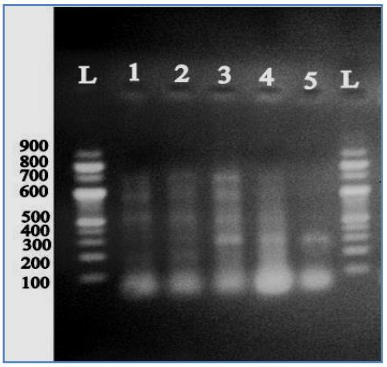

Fig-1 OPG-01

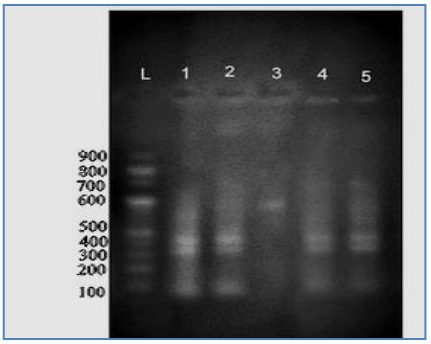

Fig-2 OPC-11

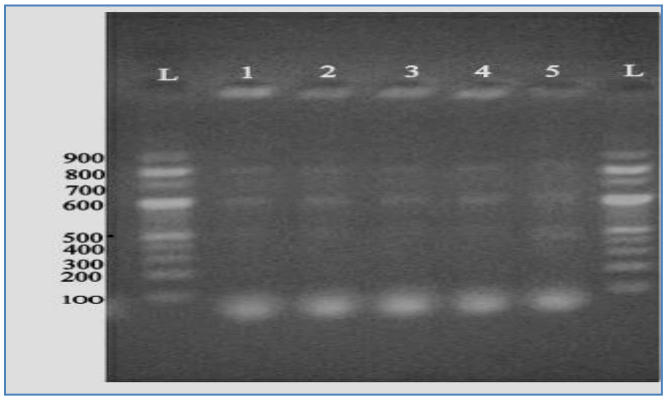

Fig-3 OPB-09

Given
L-Ladder
1-vipula
2-ICPL-87
3-BMR-853
4-BSMR-736
5-Rajeshwari

Table-5 *List of RAPD primers and polymorphic amplicons generated*

Sr. No.	Primer code	Total No of amplicons	No. of polymorphic amplicons	Percent polymorphism
1	OPG-01	5	5	100
2	OPB-09	5	4	80
3	OPC-11	4	3	75
TOTAL		14	12	85.71

Binary similarity matrix for RAPD analysis

A binary similarity matrix of combined data from 3 primers for five genotypes of *Pigeon pea (Cajanus cajan* L.) was prepared by scoring bands for presence or absence. DNA bands of same mobility were assumed to be identical.

The cluster analysis was carried out by using Genetic Similarity estimate (Jaccard's coefficient) based on RAPD banding pattern to study the genetic relationship in the form of dendogram Jaccard's coefficient value for five of Pigeon pea genotype are presented in [Table-4].

Table-6 *Binary matrix of each genotype obtained by RAPD Marker*

	Vipula	ICPL-87	BSMR-853	BSMR-736	Rajeshwari
Vipula	1.00				
ICPL-87	0.77	1.00			
BSMR-853	0.57	0.64	1.00		
BSMR-736	0.50	0.56	0.43	1.00	
Rajeshwari	0.55	0.52	0.35	0.50	1.00

In present study, the similarity coefficient value ranged from 0.35 to 0.77 across five cultivars isolates indicating high degree of genetic variation.

This ultimately means high range of genetic diversity among the isolates studied. The highest genetic similarity to an extent of 77 % was recorded between Vipula and ICPL-87 isolates followed by 64 % similarity between BSMR-853 and ICPL-87 isolates. Least genetic similarity 35 % was observed in between BSMR-736 and BSMR-853.

Cluster analysis of RAPD marker

The dendrogram RAPD analysis shows that cultivars can be grouped into two major clusters *viz.* A and B. The cluster A and B had 0.49% genetic similarity. Cluster A divided into two sub clusters namely cluster A1 and A2. Sub cluster A1 shows two cultivars, Vipuls, ICPL-87, and sub cluster A2 to contain only BSMR-853. Out of these, Vipula and ICPL-87 shows 0.77 similarity, whereas BSMR-853 shows 0.58 similarity coefficient. .The cluster B distinct from cluster A containing two genotype BSMR786 and Rajeshwari 0.51 similarity.

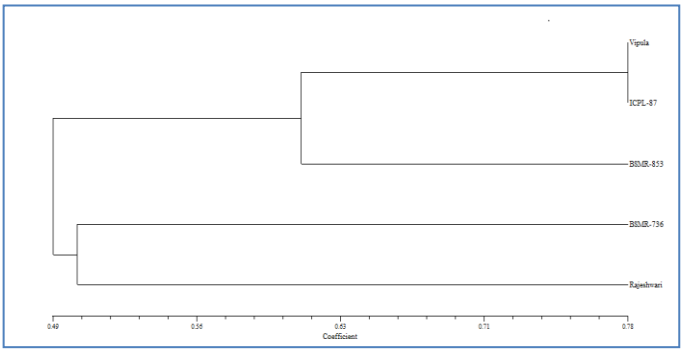

Fig-4 Dendrogram showing results of RAPD analysis of Pigeon pea cultivars

The roots of plant breeding are found in genetic diversity. A full understanding of the amount of genetic variation that exists between the available cultivated and wild population is important for enhancing genetic potential [19]. Plant genetic diversity also reveals the relationship among genotypes in a population, which guides the researcher to understand their evolution, indicates the centers of origin [20], ot also provides prediction for heterosis and helps establish a core collection for maximum genetic variation in a minimum number of accessions [21].

Conclusion

In pigeon pea, the main advantage of using molecular markers is introgression of resistance genes into the cultivars by accessing genetic diversity data given by the RAPD profile. The estimate of genetic relationship is useful for the selection of parents for hybridization and reducing the number of accession needed to ensure broad range of genetic variability. The Dendrogram obtained by RAPD marker showed similarity coefficient values in the range of 0.35 to 0.77 between pigeon pea cultivars. In the RAPD profile two pigeon pea genotypes Vipula and ICPL-87 showed highest similarity i.e 0.77%. BSMR-853 showed least similarity of 0.35 % in RAPD marker analysis. RAPD primer OPC-09 generated maximum number of amplicons i.e. 5 and OPC-11 polymorphism i.e.75% and OPC-01 primer showed highest (100%) variation.

Conflict of Interest: None declared

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