Available online at www.ijpab.com

DOI: http://dx.doi.org/10.18782/2320-7051.2459

ISSN: 2320 – 7051

Int. J. Pure App. Biosci. **5 (1):** 674-678 (2017)

Research Article

International Journal of Pure & Applied Bioscience

OPEN ACCESS

Random Amplified Polymorphic DNA (RAPD) Analysis of Released Varieties and Hybrids of Pigeonpea [*Cajanus cajan* (L.) Millspaugh] of Gujarat

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Received: 14.01.2017 | Revised: 25.01.2017 | Accepted: 26.01.2017

ABSTRACT

For diversity analysis of pigeonpea molecular marker used to study 11 diverse genotypes of pigeonpea using 12 each of RAPD primers used to determine varietal/hybrid specific informative molecular markers for precise identification of varieties and hybrid of pigeonpea. DNA was extracted as per CTAB method with minor modification and quality as analysed on nanodrop, the absorbance ratio of DNA at A260/A280 and finally with electrophoresis. Comparatively, RAPD primers generated 97 amplicons with an average 7.46 amplicons per RAPD primers. RAPD primers classified the eleven genotypes of pigeonpea into two different clusters with lot of variation in number and inclusion of genotypes in sub groups. These techniques indicated genotype GT -100 to be distantly apart from genotype GT 288 A, yet comparing other set of genotypes, GT-101 was evinced closer to GT-102 in RAPD analysis.

Key words: RAPD, DNA, *Pigeonpea*, *Hybrids*

INTRODUCTION

Pigeonpea is a protein rich staple food. It contains about 22 per cent protein, which is almost three times than cereals. Pigeonpea [Cajanus cajan (L.) Millspaugh], is one of the most important pulse crops of India and ranks second to chickpea in area and production. At present, pigeonpea is cultivated on 4.09 lakh ha area with 2.89 lakh tons production with productivity 799 lakh kg/ha[3]. Gujarat grows pigeonpea on around 2.76 lakh hecters with an annual production and productivity of 2.72 lakh tons and 986 kg/ha, respectively[4]. It is the only cultivated food crop of the Cajaninae sub-tribe of leguminous tribe Phaseolae. It belongs to the family Leguminosae and sub family Papilionaceae. The chromosome number of all Cajanus species is n=11[2,11]. According to Vavilov[12], genus Cajanus originated in the Hindustan. As per Van Der Maesen[10] also, the centre of origin of the crop in India. There are about 114 recommended varieties in the country for different situation in different maturity groups. In Gujarat early maturing varieties are preferred though mid late maturing varieties are also liked in Suarashtra and Central Gujarat. Lately a CGMS based hybrid, GTH-1 has also been released for cultivation in Gujarat.

Cite this article: Thakar, P., Chauhan, R.M., and Joshi P., Random Amplified Polymorphic DNA (RAPD) Analysis of Released Varieties and Hybrids of Pigeonpea [*Cajanus cajan* (L.) Millspaugh] of Gujarat, *Int. J. Pure App. Biosci.* **5(1):** 674-678 (2017). doi: http://dx.doi.org/10.18782/2320-7051.2459

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Stable varieties of pigeonpea like GT 100, GT 101, GT 102, GT 1, Banas, BSMR 853 (Vaishali), SKNP 505 and AGT 2 have been recommended for cultivation in Gujarat. Among these, GT 100, GT 101, GT 1 and BSMR 853 are frequently indented in the seed production chain. Molecular markers have been tried to characterize different genotypes in different crops to reprieve this problem and conduct the grow out test from DNA extracted from seed or plants right in juvenile stage.

Different genetic markers based on DNA polymorphisms like RAPD, SSR, STS, AFLP, RFLP, SCAR, CAPS have been used for characterization. The most widely applied PCR-based method for DNA fingerprinting is the Random Amplified Polymorphic DNA (RAPD) independently developed by Welsh and McClelland[13] and Williams *et al*[14]. RAPD markers are mostly dominant and detect variation in both coding as well as non coding region of genome. Though it has inherent problem of reproducibility, yet it has been successfully employed for determination of genetic diversity in several grain legumes[1].

MATERIALS AND METHODS

Plant Material:

Eleven genotypes of pigeonpea were grown in pots. The leaf materials were collected for analysis from pigeonpea plants of each genotype.

DNA Extraction:

Total genomic DNA was extracted from the leaves of 9 days seedlings by Cetyltrimethyl ammonium bromide (CTAB) method[5] (Doyle and Doyle, 1990) with some modifications.

1g of leaf tissues was grounded in liquid N_2 with the help of mortar pestle. 4 ml pre-warmed (65^0C) DNA isolation buffer (CTAB DNA extraction Buffer) was added in homogenized leaf material. Transferred homogenized material in capped polypropylene tubes. Incubated for 60 min at 65^0C in water bath with intermittent shaking. Tubes were cooled down to room temperature. Add equal volume of Chloroform :Isomyl-alcohol (24 : 1) was added and mixed gently for 1.5 minutes. The tubes were centrifuged at 15000 rpm for 10 minutes at 4^oC. This was followed by adding 2 µl of DNase free RNase A (Bangalore GeNei Pvt. Ltd.) to the supernatant and kept for one hour at room temperature. The supernatant was re-extracted once more with equal volume of Chloroform :Isomyl-alcohol (24 : 1) solution. Isopropanol 0.6 v/v was added to the supernatant and mixed gently for 2 minutes. Tubes were kept at -20^oC for 120 minutes for better precipitation of DNA. DNA so pelleted was centrifuged at 12000 rpm for 10 minutes at 4^oC. Supernatant was discarded and the pellet was washed thrice by 70 per cent Ethanol. Pellets were air dried and dissolved in 100 µl of Tris-EDTA (TE) buffer by keeping over night at room temperature without agitation. The quality of DNA was checked by Agarose gel electrophoresis and quantification was carried out by Picodrop PET01 using software v2.08 (Picodrop Ltd., Cambridge U.K) and genomic DNA was electrophorated on 0.8 per cent agarose gel.

PCR and RAPD Analysis:

Thirty primers were selected and were used to ascertain polymorphism among varieties and hybrid of pigeonpea released in Gujarat. The Polymerase Chain Reaction (PCR) method given by Ray Choudhary *et al*[8]., with minor modifications was carried out in 25 µl of reaction mixer containing 10X Taq Buffer A followed by 25mM MgCl2, 10 mM each dNTPs (Bangalore GeNei Pvt. Ltd), 3U *Taq* DNA polymerase(Bangalore GeNei Pvt. Ltd), Millipore sterilized water and 50 ng template DNA. Amplification condition were maintained at 94°C for 3 min, 44 cycles at 94°C for 1 min (denaturation), 37°C for 1 min (annealing), 72°C for 2 min (elongation) followed by final extension at 72°C for 7 min. Here, denaturation, primer annealing & extension of annealed primer comprisd of one cycle and the total reaction was carried out for 44 cycles. The PCR products were separated by electrophoresis in a 1.5 % agarose gel containing ethidium bromide (0.5 µg/ml) using 1X TBE (Tris base, Boric acid, EDTA) buffer (pH 8.0) and visualized under UV light. The size of amplification products was determined

in comparison to O' Genei Ruler 50 bp DNA ladder.

RESULTS AND DISCUSSION

Random Amplification of Polymorphic DNA Analysis

RAPD was used for characterization of pigeonpea genotypes for studying genetic diversity and similarity between different genotypes.

In present investigation, 11 pigeonpea genotypes were subjected to RAPD analysis using 13 different primers of Operon series such as OPA, OPB, OPC, OPD, OPG, OPM, OPN, OPQ, OPR and OPZ. Based on the RAPD data, cluster analysis was performed using genetic similarity values and a dendrogram was generated showing genetic relationships among these genotypes. The highest similarity index value of 0.948 was found between GT 101 and GT 102 while the lowest similarity index value of 0.454 was between GT 100 and CMS GT 288 A. The RAPD marker OPA-16 produced maximum number of 7 polymorphic bands, while OPG-12 AND OPQ-12 produced minimum number of 2 polymorphic bands. The details of amplification products are given in (Table 4.1).

Table 4.1: Size, number of amplified bands, per cent polymorphism and PIC obtained by RAPD primers

RAPD PRIMER	Total Band	Monomorphic Band	Polymorphic Band	Unique Band	Shared Band	% P	PIC value
OPB 07	6	1	3	2	3	50.0	0.665
OPC 05	7	1	4	1	2	57.14	0.831
OPD 05	9	1	6	0	3	66.66	0.855
OPG 12	3	1	2	1	1	66.66	**0.650**
OPM 05	8	2	4	1	2	50.0	0.831
OPN 10	5	2	3	0	1	60.0	0.787
OPN 13	8	2	4	0	2	50.0	0.854
OPQ 12	7	5	2	0	2	**28.57**	0.856
OPQ 14	11	3	5	0	2	45.45	**0.897**
OPA 16	9	3	7	0	2	77.77	0.884
OPR 14	10	2	6	1	0	60.0	0.883
OPZ 10	8	1	5	0	2	62.5	0.839
OPM 14	6	0	5	1	2	**83.33**	0.777
Total	**97**	**24**	**56**	**7**	**24**	**-**	**-**
Mean	**7.46**	**1.84**	**4.30**	**0.53**	**1.84**	**58.31**	**0.81**

Pooled RAPD

The data collected from random amplification of polymorphic DNA with 10 arbitrary oligonucleotide primers produced a total 97 DNA fragments, among which 56 fragments were found to be polymorphic. As such the mean number of polymorphic bands per primer among 11 pigeonpea genotypes was found to be 4.30. The size of PCR amplified DNA fragment varied from 101 to 1717 bp. The highest polymorphism (83.33%) was exhibited by primer OPM-14, while the lowest polymorphism (28.57 %) was evinced with OPQ-12. The average polymorphism detected

by the RAPD loci in the present investigation was 58.31 % (Table 4.1). This value was good enough for efficient genetic analysis.

Dendrogram based on NTSYS-pc version 2.1 unbiased measures of genetic distance by UPGMA cluster analysis based on Jaccard's similarity coefficient grouped the 11 genotypes into two major clusters. The first cluster comprised two sub clusters with sub cluster A1 containing one genotype viz; GT 288 A. Sub cluster A2 had one genotype GTR 11. Second cluster contained two sub clusters viz; B1 and B2. Subcluster B1 had sub sub cluster B1(a) and B1(b) contained three genotypes i.e. AGT 2, BSMR 853 & SKNP 505 and four genotypes i.e. GT 101, GT 102, GT 1 & GTH 1 respectively (Figure 4.1).

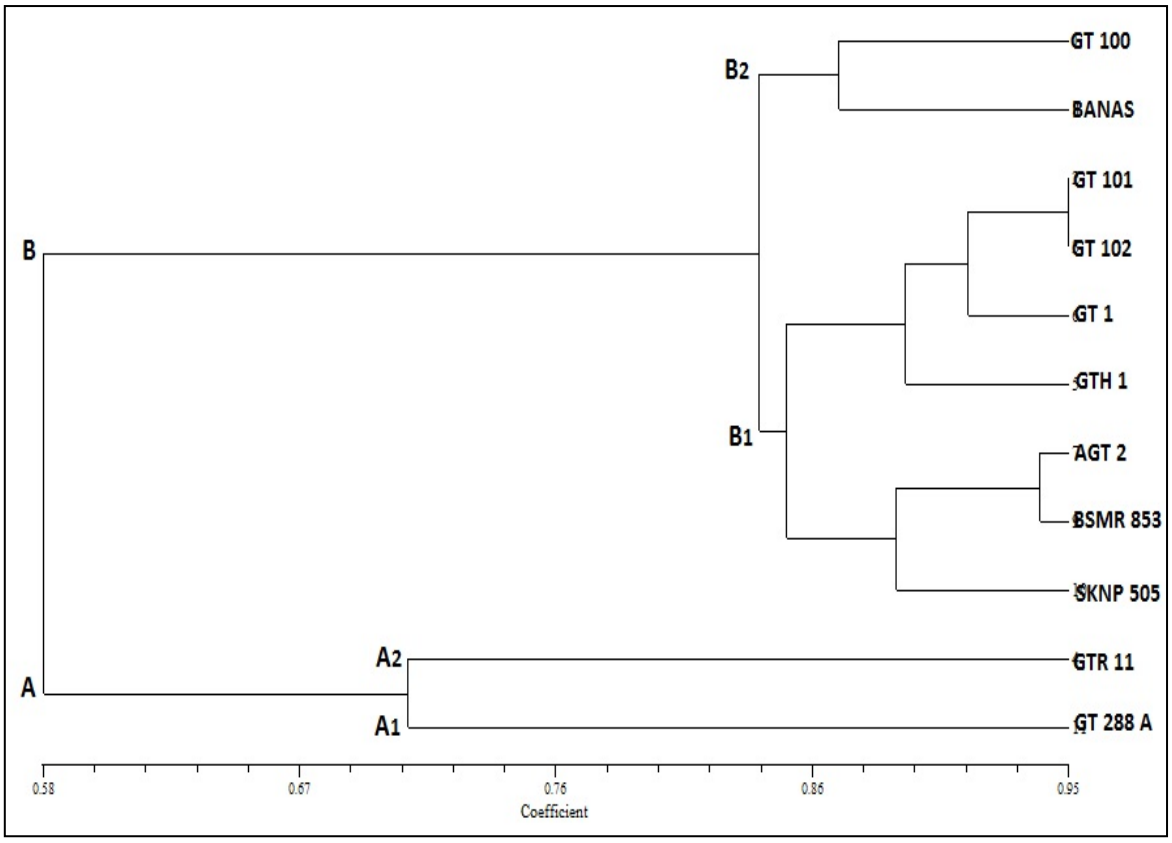

Fig. 4.1

Based on the simple matching coefficient, a genetic similarity matrix was constructed using the RAPD data to assess the genetic relatedness among the 11 accessions. The similarity coefficients ranged from 0.454 to 0.948 for all accessions; the minimum genetic similarity was between GT 288 A and GT 100, the greatest similarity between GT-101 and GT-102 (Figure 4.1). Higher the dissimilarity between the genotypes, better the scope to include them in hybridization. Genetic similarity coefficients indicated the extent of relatedness. The suitability of individual primers for genetic diversity study was determined from the number of polymorphic fragments produced by the different groups of genotypes and the number of recognized fingerprint types. This is similar to the method used by Russell *et al*[9]., and Rajora and Rahman[6]. Out of a set of 19 primers used, all but OPA-11 were consistently repeatable and were useful in detecting polymorphism among the genotypes studied. In our study, 58.3 % fragments were found to be polymorphic with an average of 7.46 bands per primer and is well comparable with the results obtained by Ratnaparkhe *et al*[7]., where 7.93 bands per primer was obtained using 16 polymorphic RAPD primers in 10 pigeonpea cultivars. Moreover, estimated genetic similarity

obtained by the same workers varied from 0.7 to 0.9, whereas in our studies the range widened to 0.454 to 0.948 because of the high variability among the selected genotypes. RAPD has been found to be well correlated with other marker systems.

CONCLUSION

More accurate results obtained by molecular markers as RAPD marker which gave similarity (94%) between GT 101 and GT 102. In RAPD polymorphism ranged from 28% (OPQ-12) to 83% (OPM-14) with an average percentage polymorphism of 58% across all eleven genotypes.

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