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Molecular Polymorphism in Pigeonpea Varieties and Hybrids Using RAPD and SSR Markers

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ABSTRACT

The present investigation was undertaken to assess molecular polymorphism and genetic diversity among 11 pigeonpea (Cajanus cajan) genotypes and hybrids using Random Amplified Polymorphic DNA (RAPD) and Simple Sequence Repeat (SSR) markers. A total of 13 RAPD primers generated 97 scorable bands, of which 56 were polymorphic, resulting in an average polymorphism of 58.31%. The number of bands per primer ranged from 3 to 11, with OPA-16 producing the maximum polymorphic bands (7) and OPQ-12 the minimum (2). The polymorphism percentage varied from 28.57% (OPQ-12) to 83.33% (OPM-14), and the Polymorphic Information Content (PIC) ranged from 0.650 (OPG-12) to 0.897 (OPQ-14). Cluster analysis based on Jaccard's similarity coefficient (ranging from 0.454 to 0.948) grouped the genotypes into two major clusters, revealing the highest genetic similarity between GT-101 and GT-102, and the lowest between GT-100 and CMS GT 288 A. RAPD markers effectively distinguished between parental lines and hybrids, with seven unique bands detected across genotypes. These findings underline the utility of RAPD markers for fingerprinting, diversity analysis, and parental line verification in pigeonpea breeding programs. While SSR markers also showed genetic variability, the RAPD technique proved more informative in this study, providing a robust and cost-effective tool for genetic analysis and varietal identification.

Keywords: Pigeonpea, Genetic Diversity, RAPD Markers, Polymorphism, Molecular Characterization

Introduction

India is the greatest producer of pulses in the world, accounting for 25 percent of the total number of pulses produced worldwide. It is impossible to overstate the significance of pulses as a valuable source of protein in terms of their nutritional value, particularly in a nation like India where the majority of the population is the vegetarian variety. For a diet that is balanced, the daily intake of pulses should range from 85 to 104 grams per person, with the least amount of stimulation being 60 grams per day. It is shockingly low that pulses have such a poor productivity. It is possible that the limited supply of high-quality seed is one of the factors contributing to the low productivity of pulses. The lack of interest shown by private seed farmers in the production of high-quality pulse seeds is primarily responsible for this phenomenon. This lack of interest lies in socio-economic factors. Taking into account the fact that seed is an essential component for production upgrades and that there is a gap between the amount of pulses that are produced and the amount that are consumed in India, the National Food Security Mission (NFSM) has dedicated a significant amount of its ambitious program to the production of high-quality seeds.

The pigeon pea, scientifically known as Cajanus cajan (L.) Millspaugh, is considered to be one of the most significant pulse crops in India. It is ranked second to chickpea in terms of both production and acreage. Redgram, Arhar, and Tur are some of the other names for it. Pigeon pea is produced and consumed in India at a faster rate than any other country in the globe. There are currently 36.3 lakh hectares of land dedicated to the cultivation of pigeon pea, which results in an output of 27.6 lakh tons and a productivity that ranges between 760.33 kg/ha (Anon., 2011a). According to Anon., 2011b, Gujarat is responsible for the cultivation of pigeon pea on approximately 2698 hectares, with an annual yield and productivity of 2590 million tons and 960 kg/ha, respectively. The pigeon pea is a staple food that is high in protein. The amount of protein that it contains is approximately 22 percent, which is nearly three times more than grains. The fact that the protein in pigeon pea is so well-suited to complement the protein in cereals is the best thing about it.

Various genetic markers that are based on DNA polymorphisms have been utilized for the purpose of characterization. These markers include Random Amplified Polymorphic DNA (RAPD), Simple Sequence Repeats (SSR), and Sequence Tagged Sites (STS), Amplified Fragment Length Polymorphism (AFLP), Restriction Fragment Length Polymorphism (RFLP), Sequence Characterized Amplified Region (SCAR), Cleaved Amplified Polymorphism Sequence (CAPS), and DNA Amplification Fingerprinting (DAF). Random Amplified Polymorphic DNA (RAPD), which was separately invented by Welsh and McClelland (1990) and Williams et al. (1990), is the PCR-based approach for DNA fingerprinting that has seen the most widespread application. It is a multilocus marker (Karp et al. 1997) that utilizes the most straightforward and speedy detection technology. Furthermore, it does not entail southern blotting and hybridization, nor does it take into account radioactivity. The majority of RAPD markers are dominant, and they are able to identify variation in any region of the genome, including both coding and non-coding regions. Prior sequence information is not necessary while using RAPD, which is the primary benefit of using this method. According to Klein Lankhorst et al. (1991), it is utilized to target genetic markers of lines that are close to becoming isogenic. Despite the fact that it has an inherent problem with reproducibility, it has been successfully utilized for the purpose of determining the genetic diversity of a number of grain legumes (Ahmed et al., 1996).

In addition to being able to identify high levels of allelic variety, the Simple Sequence Repeats (SSR) markers are extremely helpful molecular tools since they are co-dominant, simple, and inexpensive to assess using polymerase chain reaction (PCR). Odeny et al. (2007) conducted a study to develop microsatellite markers and evaluate their potential for use in pigeonpea genetics and breeding. They are considered ideal for DNA fingerprinting and varietal identification, particularly hybrids, due to their ability to detect a large number of discrete alleles repeatedly, accurately, and efficiently. This ability is particularly useful for hybrids. Pigeonpea, on the other hand, has been found to have results that are less informative when using SSR (Saxena et al., 2010).

Material and Methods

The experimental material was provided from the Centre of Excellence for Research on Pulses, Aanand Gujarat and IIPR Kanpur during 2021-2022.

Table: 1 List of Pigeon pea genotypes

Sr.No.	Varietal designation	Sr.No.	Hybrid/parental designation	
1	GT 100	1	Hybrid	GTH 1
2	GT 101	2	Male	GTR 11
3	GT 102	3	Female	CMS GT 288 A
4	GT 1			
5	AGT 2			
6	Banas			
7	BSMR 853			
8	SKNP 505			

The glassware's and disposable plastic wares that were used were of standard make obtained from Borosil, Axygene and Eppendorf. All the glassware's were thoroughly cleaned with lab wash (Labolene), washed with tap water, rinsed in distilled water and dried in oven before use. Similarly, all the plastic wares viz., micro tips, 2 ml Eppendorf tubes, etc., were also thoroughly cleaned as above and autoclaved before use.

Randomly Amplified Polymorphic DNA (RAPD)

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. (2008) with minor modifications was carried out in 25 µl of reaction mix. The list of RAPD primers used.

Table- 2 List of RAPD primers used in the present study

Sr. No.	RAPD No.	Sequence 5'- 3'	Tm Value
1	OPB 07	GAAACGGGTG	25
2	OPC 05	GATGACCGCC	27
3	OPD 05	TGAGCGGACA	25
4	OPG 12	CAGCTCACGA	25
5	OPM 05	GGGAACGTGT	25
6	OPN 10	ACAACTGGGG	25
7	OPN 13	AGCGTCACTC	25
8	OPQ 12	AGTAGGGCAC	25
9	OPQ 14	GGACGCTTCA	25
10	OPA 16	AGCCAGCGAA	25
11	OPR 14	CAGGATTCCC	37.3
12	OPZ 10	CCGACAAACC	25
13	OPM 14	AGGGTCGTTC	25

The amplification reaction was carried out in 25 µl volume containing 10XTaq Buffer A followed by dNTPs, Taq DNA polymerase, Millipore sterilized water and template DNA in sequence and quantity as given above and finally the primer. The reagents were mixed thoroughly by a short spin using microfuge. The tubes were then placed on the Thermal Cycler for cyclic amplification. PCR products were then subjected to electrophoresis with 100 bp marker DNA of known molecular weight in 1.5 per cent agarose gel.

Simple Sequence Repeats (SSRs)

The SSRs analysis was done following the procedure given by Odenyet al. (2009) with minor modifications. In all 12 microsatellite marker were used that were obtained from Bangalore GeNei, Bangalore.

Table- 3 List of SSR primers used in the present study

Sr. No.	SSR Primer Name	No. of B.P	Primer Sequence	% GC	Tm

1.	CCtta001	20	F TTCTGGATCCCTTTCATTTTT C	50	56.6
		20	R TGACACCCTTCTACCCCATA A	40	59.4
2.	CCtta003	21	F CCAAGAAAAGGTGCTCCAAG	47	59.4
		20	R TTGCTTCTTTTCTCGCTTGC	45	56.4
3.	CCtta004	20	F ACCCATTATTGATTTGGGTA	35	52.3
		19	R CCAAATTTCACCCAAGAAA	36	50.9
4.	CCttc001	22	F CGGGCTTCCTTTTCTTCTCT	50	58.4
		21	R AAAACCCCGAAAACACCATT	40	58.4
5.	CCac001	20	F CTGGGCCTCTAGCATAGCAA	55	60.5
		21	R AAACTTCTGGACGCAAAAA A	38	55.5
6.	CCat001	20	F CTTCCCCCAACTAAGATCCA	50	58.4
		24	R GTTCGTTCTCTTTAATTGAC TGC	37	60.1
7.	CCat002	19	F TTTCCTGAGCCATCAGTCG	52	57.3
		20	R AAGCATCAACGTACCAGCAA	45	56.4
		21	F TGAATTGCTGAGAGGACGTT T	42	57.4

8.	CCat003	20	R CTGTTCCAATTCCACGGTTT	45	56.4
9.	CCat004	20	F CTACAATCCCAGGGAAAAGG	50	58.4
		25	R AACAAACGTAATCTGTGTTGATCTC	36	60.9
10.	CCcat001	20	F TGATAGGGACCACAACGACA	50	58.4
		20	R AGCGTTGACTCCTCCCTCTT	55	60.5
11.	CCggt001	20	F ACGCTTCTGATGCTGTGTTG	50	58.4
		20	R CATCAGCATCATCGTTACCC	50	58.4
12.	CCtacccg001	19	F GTCTTTGAGGGACGGAACC	57	59.5
		19	R GGGGCGGGGAAAGTACATA	57	59.5

As per the above cocktail 10 X PCR buffer (with MgCl2) was added first followed by dNTP mix, Taq DNA Polymerase, forward and reverse primers (10 pmol/µl), Millipore Sterilized Water in sequence and finally the template DNA (50 ng/µl) was added. The reagents were mixed thoroughly by a short spin using a microfuge. The tubes were then placed on a Thermal Cycler (Bio-Rad, USA) for cyclic amplification.

Results and Discussion

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.

OPB – 07

The PCR amplification with OPB-07 primer generated a maximum of 6 DNA fragments with a fragment size ranging from

101 to 859 bp. 3 bands were found to be polymorphic giving 50 % Polymorphism PIC value 0.665. This primer was produced the difference banding patterns in the parental line GTR 11 and GT 288 A among the varities and hybrid.

OPC – 05

OPC-05 amplified maximum of 7 fragments with size ranging from 222 to 1253 bp. Out of which 4 fragments were polymorphic having 57.14 % polymorphism & PIC value 0.831. This primer was produced the difference banding patterns in the parental line GTR 11 and GT 288 A among the varities and hybrid.

OPD – 05

This primer too amplified maximum of 9 DNA fragments. However, the size of fragments varied from 196 to 1262 bp. Six of the nine bands were found to be polymorphic thereby exhibiting 66.66 % polymorphism & PIC value 0.855. This primer was produced the difference banding patterns in the parental line GTR 11 and GT 288 A among the varities and hybrid.

OPG – 12

The PCR amplification with OPG - 12 primer generated a maximum of 3 DNA fragments with a fragment size ranging from 416 to 581 bp. 2 bands were found to be polymorphic giving 66.66 % Polymorphism & PIC value 0.650. This primer was produced the difference banding patterns in the parental line GTR 11 and GT 288 A among the varities and hybrid.

Table 4.: Size, number of amplified bands, per cent polymorphism and PIC obtained by RAPD primers.

RAPD PRIME	Total Band	Monomorphic Band	Polymorphic Band	Unique Band	Shared Band	% P	PIC value
OPB 07	6	1	3	2	3	51.0	0.665
OPC 05	7	1	4	1	2	56.14	0.831
OPD 05	9	1	6	0	3	67.67	0.855
OPG 12	3	1	2	1	1	67.67	0.650
OPM 05	8	2	4	1	2	51.0	0.831
OPN 10	5	2	3	0	1	61.0	0.787
OPN 13	8	2	4	0	2	51.0	0.854
OPQ 12	7	5	2	0	2	28.57	0.856
OPQ 14	11	3	5	0	2	46.45	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**

OPM – 05

OPM-05 amplified maximum of 8 DNA fragments with a fragment size ranging from 186 to 1266 bp. Four of the eight bands were found to be polymorphic thereby exhibiting 50 % Polymorphism & PIC value 0.831. This primer was produced the difference banding patterns in the parental line GTR 11 and GT 288 A among the varities and hybrid.

OPN – 10

The PCR amplification with OPN-10 primer generated a maximum of 5 DNA fragments with a fragment size ranging from 222 to 531 bp. 3 bands were found to be polymorphic giving 60 % Polymorphism & PIC value 0.787. This primer was produced the difference banding patterns in the parental line GTR 11 and GT 288 A among the varities and hybrid.

OPN – 13

The PCR amplification with OPN-13 primer generated a maximum of 8 DNA fragments with a fragment size ranging from 153 to 1185 bp. 4 bands were found to be polymorphic giving 50 % Polymorphism & PIC value 0.854. This primer was produced the difference banding patterns in the parental line GTR 11 and GT 288 A among the varities and hybrid.

OPQ – 12

The PCR amplification with OPQ-12 primer generated a maximum of 7 DNA fragments with a fragment size ranging from 185 to 696 bp. 2 bands were found to be polymorphic giving 28 % Polymorphism & PIC value 0.856. This primer was produced the difference banding patterns in the parental line GTR 11 and GT 288 A among the varities and hybrid.

OPQ – 14

The PCR amplification with OPQ-14 primer generated a maximum of 11 DNA fragments with a fragment size ranging from 133 to 1717 bp. 5 bands were found to be polymorphic giving 45 % Polymorphism & PIC value 0.897. This primer was produced the difference banding patterns in the parental line GTR 11 and GT 288 A among the varities and hybrid.

OPA – 16

The PCR amplification with OPA-16 primer generated a maximum of 9 DNA fragments with a fragment size ranging from 141 to 1595 bp. 7 bands were found to be polymorphic giving 77 % Polymorphism & PIC value 0.884. This primer was produced the difference banding patterns in the parental line GTR 11 and GT 288 A among the varities and hybrid.

OPR – 14

The PCR amplification with OPR-14 primer generated a maximum of 10 DNA fragments with a fragment size ranging from 121 to 1405 bp. 6 bands were found to be polymorphic giving 60 % Polymorphism & PIC value 0.883. This primer was produced the difference banding patterns in the parental line GTR 11 and GT 288 A among the varities and hybrid.

OPZ – 10

The PCR amplification with OPZ-10 primer generated a maximum of 8 DNA fragments with a fragment size ranging from

190 to 944 bp. 5 bands were found to be polymorphic giving 62% Polymorphism & PIC value 0.839. This primer was produced the difference banding patterns in the parental line GTR 11 and GT 288 A among the varities and hybrid.

OPM – 14

The PCR amplification with OPM-14 primer generated a maximum of 6 DNA fragments with a fragment size ranging from 166 to 994 bp. 5 bands were found to be polymorphic giving 83 % Polymorphism & PIC value 0.777. This primer was produced the difference banding patterns in the parental line GTR 11 and GT 288 A among the varities and hybrid.

The development of hybrids in pigeonpea by exploiting cytoplasmic-genetic male-sterility (CGMS) system is an important approach to increase the production which otherwise has remained static for past few decades. Unambiguous characterization of the parental lines in the CGMS system alleviates concerns regarding the seed purity in hybrid seed production. RAPD has been used unambiguously for identification of parental lines and their hybrids in several crops like menthe (Shasany et al. 2005), poplar (Rajora and Rahaman 2003) including closely related species of common bean and soybean (Martin et al. 1996).

The ability of a marker system to resolve genetic relationships among genotypes depends upon the polymorphism content in primers. Fingerprinting of 11 varieties with 13 RAPD primers gave 58 % polymorphic amplicons with a total of 97 unambiguous and scorable bands. The 58 % polymorphism found in the present analysis was among the highest reported with RAPD markers (Souframanien and Gopalakrishna 2004). The size of amplicons ranged from 101-1717 bp and the level of polymorphism from 28 % (OPQ 12) to 83 % (OPM 14). The PIC value ranged from 0.650 (OPG 12) to 0.897 (OPQ 14). OPA 16 primer were found to produce 7 bands, the highest number, while two primers (OPG 12 & OPQ 12) produced only two bands.

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.

Table 5: Unique or rare loci produced by RAPD primer:

Sr. No.	Genotypes	RAPD Primer	Marker Size (bp)
1.	SKNP 505	OPB 07	894
2.	GT 288 A	OPB 07	416
3.	GT 288 A	OPC 05	710
4.	AGT 2	OPG 12	814
5.	GTR 11	OPM 05	245
6.	GT 288 A	OPR 14	483
7.	GT 288 A	OPM 14	473

Table 6: Jaccard's similarity coefficient of 11 pigeonpea genotypes based on RAPD data

	GT 100	**GT 101**	**GT 102**	**GTR 11**	**GTH 1**	**GT 1**	**AGT 2**	**BAN AS**	**BSMR 85**	**SKNP 50**	**GT 288 A**
GT 100	1.000										
GT 101	0.876	1.000									
GT 102	0.845	**0.948**	1.000								
GTR 11	0.711	0.711	0.722	1.000							
GTH 1	0.814	0.876	0.887	0.649	1.000						
GT 1	0.845	0.907	0.918	0.680	0.907	1.000					
AGT 2	0.804	0.866	0.876	0.619	0.866	0.897	1.000				
BANAS	0.866	0.866	0.856	0.680	0.825	0.835	0.856	1.000			
BSMR 853	0.825	0.825	0.835	0.619	0.825	0.856	0.938	0.876	1.000		
SKNP 505	0.763	0.825	0.856	0.639	0.804	0.835	0.897	0.835	0.876	1.000	
GT 288 A	0.546	0.485	0.515	0.711	0.464	0.495	**0.454**	0.515	0.495	**0.454**	1.000

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 cluster 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) 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.

Conclusion

A higher degree of accuracy was achieved through the utilization of molecular markers, such as the RAPD marker, which demonstrated a 94% resemblance between GT 101 and GT 102. Polymorphism in RAPD varied from 28% (OPQ-12) to 83% (OPM-14), with an average percentage polymorphism of 58% across all eleven genotypes. This range was observed in all eleven patients. That RAPD marker is more effective than SSR marker, as stated by the previous sentence. According to the SSR analysis, the similarity coefficient of Jaccard varied from 0.615 to 0.923 overall.

References

Ahmad, M.; McNeil, D.L.; Fautrier, A.G.; Armstrong, K.F. and Peterson, A.M. (1996). Genetic relationships in lens species and parentage determination of their interspecific hybrids using RAPD markers. Theor. Appl. Genet., 92 (8)

: 1091-1098.

Ahmad, S. S.; Nabi, Gh. and Kudesia, R. (2011). Genetic diversity evaluation in pigeonpea [Cajanus Cajan (L.) Millsp.] using protein profiling and rapd. International Journal of Current Research 3 (11) pp: 326-330.

Klein Lankhorst; R.M., Vermont,A.; Weide, R.; Lihorska, T. and Zabel, P. (1991). Single genetic mapping in using haploid DNA mega gametophytes. Biotech. 40 : 686-690.

Odeny, D.A.; Jayashree, B.; Ferguson, M.; Gebhardt, C. and Crouch, J. (2007). Development, characterization and utilization of microsatellite markers in pigeonpea. Pl. Breed., 126 : 130-136.

Odeny, D.A.; Jayashree, B.; Gebhardt, C. and Crouch, J. (2009). New microsatellite markers for pigeonpea [Cajanus cajan (L.) Millsp.]. BMC Res. Notes., 2 : 35.

Ray Choudhary.; Singh, I.P.; George, B.; Verma, A.K. and Singh. N.P. (2008a). Assessment of genetic diversity of pigeonpea cultivars using RAPD analysis. Biologia Planetarium. 52 (4) : 648-653.

Ray Choudhary.; Singh, I.P.; Verma, S.; Singh, N.P. and Kumar, S. (2008b). RAPD markers for identification of cytoplasmic genic male sterile, maintainer and restorer lines of pigeonpea. J. Food Legume., 21 (4) : 218-221.

Saxena, R.K.; Prathima, C.; Saxena, K.B.; Hoisington, D.A.; Singh, N.K. and Varshney, R.K. (2010). Novel SSR Markers for Polymorphism Detection in Pigeonpea (Cajanus spp.). Pl. Breed., 129 : 142-148.

Welsh, J. and McClelland, M. (1990). Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Res. 18 : 7213-7218.

Williams, S. J. K.; Kubelik, A.R.; Livak, K. J.; Rafalski, J. A. and Tingey, S.V. (1990). DNA polymorphism amplified by arbitory primers useful as genetic maker. Nucleic Acids. Res., 18 : 6531-6535.