The Pharma Innovation Journal 2023; 12(2): 605-608

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; 12(2): 605-608

www.thepharmajournal.com
Received: 01-11-2022
Accepted: 04-12-2022

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Assessment of genetic diversity in cowpea (*Vigna unguiculata* L.) genotypes by using RAPD markers

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DOI: https://doi.org/10.22271/tpi.2023.v12.i2h.18484

Abstract
The present study was carried out at the department of Plant Biotechnology, College of Agricultural Biotechnology Latur, which aimed to study or assess the genetic diversity in eight different Cowpea genotypes by using the RAPD markers. This is important for cowpea genetic improvement and for the overall management of cowpea germplasm. Eight Cowpea genotypes namely GDVC-2, GC-3, GC-4, GC-6, Gangotri, Pusa Komal, Arka Garima, and VU-5 were assessed by using two RAPD markers (RPI-07 and RPI-08). In RAPD analysis, total of 20 amplicons were generated, among them 19 were polymorphic with 88.8% average polymorphism. RPI-7 and RPI-8 showed 88.8% and 100% polymorphism respectively. The similarity coefficient values ranged from 0.350% to 0.950% indicating high degree of genetic variation. The highest genetic similarity i.e. 0.857% was recorded between Pusa Komal and GC-6 and least genetic similarity i.e. 0.125% was observed in between VU-5 and GC-4. Two screened primers are found to be polymorphic (94.4%) with high polymorphic information content (PIC) which efficiently discriminating the varieties at molecular level.

Keywords: Genetic diversity, cowpea, RAPD markers

Introduction
Vigna unguiculata L. is commonly known as Cowpea, crowder-pea, southern pea, black-eyed pea, or lobia and is considered as "Vegetarian Meat". It is an annual herbaceous, tropical grain legume from the genus *Vigna*, family Leguminaceae. It is originated from Sub-Saharan Africa and widely distributed in sub-Saharan Africa, Asia, central & south America as well as the southern part of Europe (Vavilov N. I., 1939) [13]. Due to their high protein content (20-25%), cowpea plays a major role in human nutrition (Singh & Emechebe, 1997) [14]. It tolerates low-fertility soil due to its high rate of nitrogen fixation (Prashanti L. *et al.*, 2012) [10]. Genetically cowpea is a complex crop with major economic importance in tropical and sub-tropical countries (Singh *et al.*, 2011) [11]. Due to the shading effect of its wide, droopy leaves, cowpea is known for its drought-resistant nature. It has multiple uses like food, feed, forage, fodder, green manuring, and vegetable.

The estimation of genetic diversity between different varieties is important in plant breeding programmers and also essential for constituting a linkage map. Genetic diversity can be studied by using molecular markers are preferred as they are based on the varieties of an individual, shows a genetic variation on a more detailed level, and are often free from environmental effects/factors. Genetic diversity plays an important role in the success of any breeding program (Ali *et al*., 2008) [1].

Among the various DNA markers, PCR-based RAPD, SSR, ISSR, and AFLP markers are very important and popular and can be used in fingerprinting. Several studies have investigated the genetic diversity, variation, and genetic distance among cowpea genotypes; according to molecular markers such as Amplified Fragment Length Polymorphism (AFLP) (Coulibaly *et al*., 2002; Tosti & Negri, 2002) [3, 12], Random Amplified Polymorphism DNA (RAPD) (Fall *et al*., 2003; Prasanthi *et al*., 2012) [6, 10], Restriction Fragment Length Polymorphism (RFLP) (Fatokun *et al*., 1993), and Microsatellite or SSR markers (Badiane *et al*., 2004; Li *et al*., 2001) [2, 9]. So, the application of DNA markers helps to determine the diversity level and genetic relationship among Cowpea varieties. Molecular markers have been used to answer questions related to the management of genetic variation, identity, and relationship in breeding and production population. It can be used from any tissue at any time during plant growth, thus expediting the process of variety identification and breeding, and helping in overcoming the

limitations of traditional methods. The selection of RAPD was based on their relatively technically simple and fast, analysis of large numbers of samples, the level of polymorphism they detect, cost-effectiveness, easily applicable to any plant species, and target those sequences which are abundant throughout the genome, and rapidly evolved. Despite questions about its reproducibility, its utility in diversity analysis, mapping and genotype identification has been exploited in many plant species. (Badiane *et al*., 2004) [2].

The present study was aimed to identify the diversity in eight different Cowpea genotypes by using the RAPD marker. This is important for cowpea genetic improvement and for the overall management of cowpea germplasm.

Material and Method

The present investigation was carried out at the department of Plant Biotechnology, College of Agricultural Biotechnology Latur. Eight genetically diverse genotypes (Table 1) i.e. GDVC-2, GC-3, GC-4, GC-6, Gangotri, Pusa Komal, Arka Garima, and VU-5 were used in this study. Cultivars selected from the field located at Latur region, College of Agriculture, Latur. Genomic DNA was isolated from leaves by modified CTAB extraction method described by Doyle and Doyle (1987; 1990) [4, 5].

RAPD analysis

A total of ten 10-mer oligonucleotides (Table 2) were used. The PCR reaction mixture consisted of 20-50 ng genomic DNA, 1x PCR buffer, 2.0 mmol/L MgCl2, 100 µmol/L of each dNTP, 0.1 µmol/L primer and 3U Taq polymerase in a 25 µL volume. The amplification protocol was 94 ºC for 5 min to predenature, followed by 45 cycles of 94 ºC for 1 min, 36 ºC (for RAPD analysis) for 1 min and 72 ºC for 1 min, with a final extension at 72 ºC for 10 min. Amplification products were fractionated on 1.5% agarose gel (for RAPD analysis).

Data Analysis

RAPD data were scored (1) for presence and (0) for absence, each band was regarded as a locus. Two matrices, one for each marker, were generated. Based on the similarity matrix, a dendrogram showing the genetic relationships between genotypes, was constructed using the unweighted pair group method with arithmetic average (UPGMA) 24 through the software NTSYs-pc version 2.11.

Result and Discussion

Ampffication of DNA and selection of primers

Preliminary amplification trials were conducted on eight genotypes selected to standardize the DNA amplification conditions on cowpea. They were (Table 1) GDVC-2, GC-3, GC-4, GC-6, Gangotri, Pusa Komal, Arka Garima, and VU-5. Different concentrations of DNA, $MgCl_2$, nucleotides and Taq DNA polymerase were tested using primer RPI-7 (Fig. 1) and RPI-8 (Fig. 2), in order to obtain the most reproducible and reliable DNA amplification profiles. Final optimal conditions which showed clear and reproducible DNA amplification fragments were used for analysis.

Identification and evaluation of RAPD markers for diversity estimates

In RAPD analysis, two primers of arbitrary nucleotide sequences were used to amplify DNA Segments from eight cowpea genotypes. The RAPD primers generated a total number of 20 amplicons in 8 genotypes. Amongst these, 19 amplicons were found to be polymorphic with an average polymorphism 88.8 percent. The RAPD primer RPI-8 showed 100% polymorphism, while RPI-7 primer showed only 88.8 percent polymorphism. The similarity coefficient value ranged from 0.350% to 0.950% across eight genotypes indicating high degree of genetic variation. This ultimately means high range of genetic diversity among the varieties study. The highest genetic similarity to an extent of 0.857% was recorded between Pusa Komal and GC-6. Least genetic similarity 0.125% was observed in between VU-5 and GC-4.

Genetic diversity analysis

The Jaccard's binary similarity matrices of combined data from 2 primers for eight varieties of Cowpea were prepared by scoring bands for presence or absence. DNA bands of same mobility (molecular weight) were assumed to be identical. In present study the similarity coefficient value ranged from 0.350% to 0.950% across eight genotypes indicating high degree of genetic variation. This ultimately means high range of genetic diversity among the varieties study. The highest genetic similarity to an extent of 0.857% was recorded between Pusa Komal and GC-6. Least genetic similarity 0.125% was observed in between VU-5 and GC-4.

A cluster analysis (UPGMA) was used to generate a dendrogram based on the genetic identity of RAPD between eight varieties. NTSYS-PC 2.2 software (Numerical Taxonomy and Multivariate Analysis System, version 2.0) was used to perform cluster analysis on the data in the similarity matrix with UPGMA.

The dendrogram produced of cowpea samples (Fig.3) shows two main clusters. The major cluster A consists of samples GDVC-3, Gangotri, GC-4, and major cluster B consists of samples GC-3, GC-6, Pusa Komal, Arka Garima and VU-5. The major cluster A consists of 3 varieties with 2 sub-clusters in that sub cluster A1 shows 2 varieties GDVC-3, Gangotri and sub cluster A2 shows 1 variety GC-4. The major cluster B consists of 5 varieties with 2 sub sub clusters in that sub cluster B1 consists of 1 variety GC-3 and sub cluster B2 consists of 4 varieties GC-6, Pusa Komal, Arka Garima and VU-5. Out of these Pusa Komal and GC-6 shows 0.875% similarity coefficient whereas VU-5 and GC-4 shows 0.125% similarity coefficient.

The major cluster-A distinct from major cluster-B containing single variety GC-3 with 0.35% similarity coefficient.

Two screened primers are found to be polymorphic (94.4%) with high polymorphic information content (PIC) which efficiently discriminating the varieties at molecular level.

Table 1: List of Cowpea genotypes used for genetic diversity analysis

Sr. No.	Cowpea genotypes	Source
1.	GDVC- 2	COA, Latur
2.	GC-3	
3.	GC-4	
4.	GC-6	
5.	Gangotri	
6.	Pusa Komal	
7.	Arka Garima	
8.	VU-5	

Table 2: List of RAPD primers used for genetic diversity study

Sr. No.	Primer Name	Sequence(5'-3')	Number of bases
1.	RPI-7	ACATCGCCCA	10
2.	RPI-8	ACCACCCACC	10

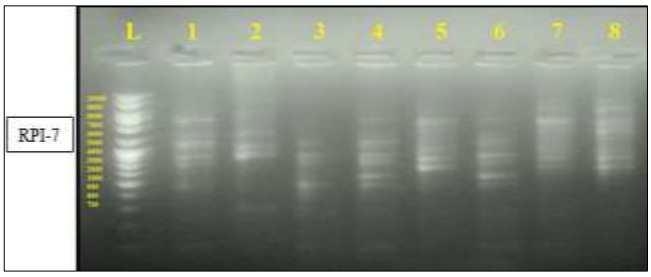

Fig 1: Amplification of Cowpea varieties by using RAPD Primer RPI-7, 1. GDVC-2, 2. GC-3, 3. GC-4, 4.GC-6, 5. Gangotri, 6. Pusa Komal, 7. Arka Garima, 8. VU-5

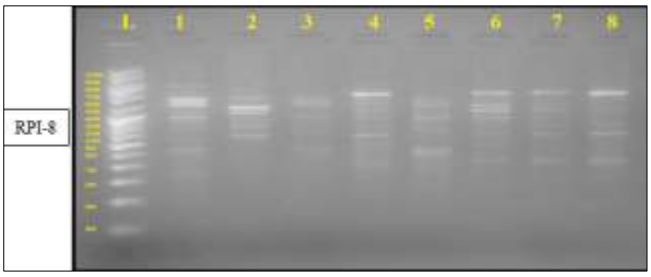

Fig 2: Amplification of Cowpea varieties by using RAPD Primer RPI-8, 1. GDVC-2, 2. GC-3, 3. GC-4, 4. GC-6, 5. Gangotri, 6. Pusa Komal, 7. Arka Garima, 8. VU-5

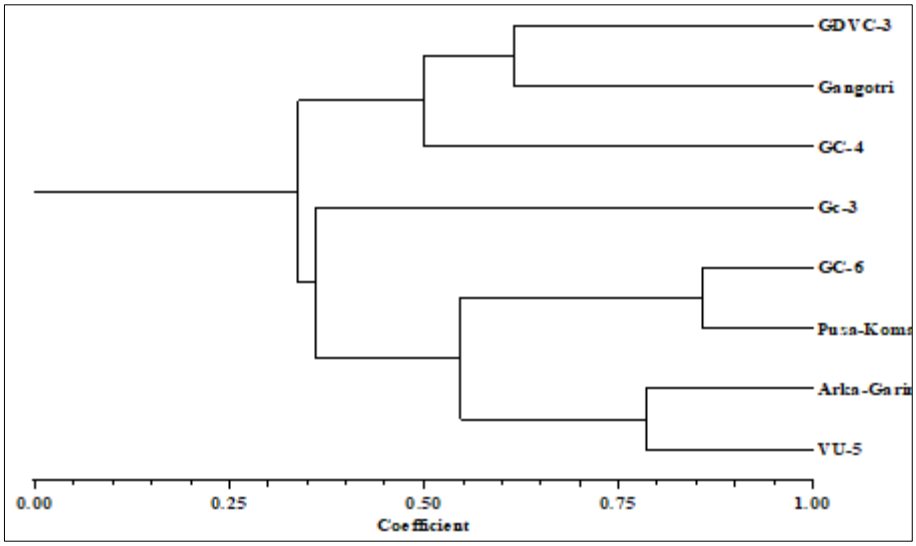

Fig 3: Dendrogram of Cowpea varieties revealed by UPGMA cluster analysis based on Jaccard's genetic similarity index estimates derived from RAPD fingerprints

Conclusions

The present study could explore the use of RAPD to allowed for the exploration of current divergence with a reasonably extensive genetic basis of breeding material. The selection process by collecting and using the landraces breeding programme will be facilitated by such a positive core of markers associated with high genetic diversity. Thus, the molecular data uncovered by the current study combined with the field evaluation results in future assistance to cowpea breeders in the development of high yielding inbreeds and hybrids to improving productivity by enabling them in the selection, saving them time, money, and labor during empirical cowpea improvement. The prospective cowpea genotypes were screened out in this study using RAPD-based molecular analysis according to their genetic distance. This information could be used to choose cowpea accessions from various genera according to how genetically distant or similar they are.

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