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Genetic Diversity in Cowpea (*Vigna unguiculata* (L.) WALP.), Using RAPD Markers

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Abstract
Random amplified polymorphic DNA (RAPD were assayed to determine the genetic diversity of 13 diverse geneotypes of cowpea cultivated in different regions of India. A total of six random primers were used in the study. Amplification of genomic DNA Indian cowpea genotypes with these RAPD primers yielded 39 fragments that could be scored, of which 34 were polymorphic. Number of amplified fragments with random primers ranged from 1 (OPA 06) to 10 (OPA 1). Percentage polymorphism ranged from 71.42% (OPA 12) to a maximum of 100% (OPA 6, OPA 13 and OPA 19), with an average of 88.5%. The Jaccard's similarity indices based on RAPD profiles were subjected to UPGMA cluster analysis and genotypes grouped in two major groups. five out of 13 genotypes grouped to cluster I and the rest eight to cluster II. The details of diversity analysis of the cowpea genotypes are discussed in the present study.

Keywords: Cowpea, Diversity, RAPD, Polymorphism, Cluster

1. Introduction
Cowpea (*Vigna unguiculata* L. Walp.), an annual legume, is also commonly referred to as southern pea, blackeye pea and crowder pea. Cowpea originated in Africa and is widely grown in Africa, Latin America, Southeast Asia and in the southern United States. It is widespread throughout the tropics and most tropical areas between 40°N to 30°S and below an altitude of 2000 m. It has been grown in 13 m.ha (1) in the world. It is chiefly used as a grain crop, for animal fodder, or as a vegetable. Cowpea seed is a nutritious component in the human diet, as well as a nutritious livestock feed. Cowpeas, are the most productive and heat tolerant legumes around. Cowpea is tolerant of shading and can be combined with tall cereal plants such as sorghum and maize. Cowpea seeds come in bush, vine, tall and short varieties. Great for stopping soil erosion and weed suppression. It is not only versatile and delicious, but also important for human health, offering a number of health benefits, such as their ability to improve digestion, aid sleep disorders, manage diabetes, protect the heart, detoxify the body, promote healthy skin, aid in weight loss, and strengthen circulation. Dual-purpose varieties have been developed in order to provide both grain and fodder while suiting the different cropping systems.

Assessment of genetic diversity in available genotypes has important implications in understanding the progress made in any breeding programme. Morphological markers are routinely used for estimating the genetic diversity, but recently many molecular marker techniques have developed into powerful tools to analyze genetic relationships. Genetic diversity within varieties or germplasm using molecular markers has been studied in several food legume species including *Cicer* [2, 3], Snap bean [4], common bean [5], pea [6], pigeonpea [7], cowpea [8] and soybean [9].

The objective of the present study was to investigate and compare genetic diversity using random amplified polymorphic DNA (RAPD) markers, for assessing the genetic base of cowpea genotypes obtained from different parts of India. The study also aims to generate molecular fingerprints for varietal identification.

2. Materials and Methods
2.1. Plant Materials and DNA isolation
The plant materials used for this study included 13 genotypes of cowpea popularly grown in differ-ent regions of India (Table 1). Cowpea genotypes obtained from Regional Research Station, Vamban, Central Arid Zone Research Institute, Jodhpur, Agricultural College and Research Institute, Madurai, Kerala Agricultural University, Vellayani, Tamilnadu

Agricultural University, Coimbatore, ARS, National Bureau of Plant Genetic Resources, Delhi were used for the present study. DNA was extracted using CTAB method. Leaf bits were transferred into prechilled mortar, frozen using liquid nitrogen and ground to fine powder. 500 µl of warm CTAB extraction buffer (65ºC) was added and transfered to a 2 ml polypropylene centrifuge tube and incubated for 30 min at 80ºC. Cooled for 5 min and 400 µl of chloroform: isoamyl alcohol (24: 1) mixture was added and centrifuged at 12,000 rpm for 20 min. The supernatant was transferred to a new tube and 500 µl phenol: chloroform (1: 1) mixture was added and centrifuged again at 12,000 rpm for 20 min. The supernatant was transferred to a new tube and 600µl of ice cold isopropanol and 100µl of 3M sodium acetate was added and kept at -20 ºC for 1 hr. centrifuged at 12,000 rpm for 20 min and the supernatant was discarded and the pellet was centrifuged again with 70% ethanol and air dried. The DNA pellet was dissolved in 100 µl of TE buffer and stored at -20 ºC.

2.2. PCR Amplifications

RAPD markers were tested for their ability to detect polymorphisms using template DNA and 6 arbitrary 10 bp long oligonucleotides, as primers belonging to OPA series (Operon Technologies, USA). PCR conditions were standardized using varying concentrations of primers and template DNA. After standardization, the rea-ction were carried out in 25 µl volume and contained 2.5 µl of 10× Taq buffer, 2.5 ì µl of 2 mM dNTP mix, 30 nM primer, 1 unit of Taq polymerase and 25 ng of template DNA. The thermal cycling program was carried out in a Thermal cycler. The thermal cycler is programmed as 94 C for 5 min - Initial Denaturation, 94 C for 45 sec-Denaturation, 55 C for 45 sec-Annealing, 72 C for1 min – Extension, 72 C for 5 min-Final extension, 4 C for infinity to hold the sample. Denaturation, annealing and extension were cycled 35 times.

Table 1: Morphological description of 14 cowpea genotypes used in the study.

S. No.	Varieties	Pedigree
1	BDYR-2	Donors
2	K-851	4453 × 3T44
3	PM-4	Type 44× UPU 2
4.	(EGMG 16 × ML3)	
5	MUM-2	Mutant of K 851
6	Pusa Vishal	Selection from NM 92
7	ML-729	Donors
8	Pusa 9072	Pusa 106× 10-215
9	ML 843	Donors
10	ML-682	Donors
11	NM-1	G 65× UPM 79-34
12	IPM-02-03	Pusa Vishal × IPM 99-125
13	Pant Mung-2 Mutant of ML 26	60 - 70

2.3. Scoring and Data Analysis

Digitized gel photograph of RAPD results were analyzed using NTSYS PC Ver.2.0 numerical software package [16]. Data was recorded as 1 (presence) or 0 (absence), each of which were treated as an independent character. The bands which were very faint were not considered for scoring. For each primer, PCR reactions were repeated two times and only reproducible bands were considered for analysis. The primers which did not produce amplification were repeated at least three times before dis-carding them. The pair wise similarity between isolates and polymorphic bands were calculated using Jaccard's coefficient [10], a common estimator of genetic identity, or estimates interspecific relationships. The similarity co-efficients were used to construct a dendrogram for de-termining relationship using unweighted pair group me-thod with arithmetic average (UPGMA).

3. Results

3.1. RAPD Analysis

A total of six RAPD primers from Operon technologies Inc. were used to assess the diversity in the 13 Indian cowpea genotypes. These primers amplified a total of 39 bands (Table 2). Thus the average number of bands amplified per primer was 6.5. six primers showed more than 70% polymorphism. The total number of polymorphic bands amplified was 34 (87.17%). Among the responding primers OPA-1 produces maximum number of bands (10) with 80% polymorphism while primer OPA-06 produces the minimum number of band (1). Percentage polymorphism ranged from 71.42% (OPA-12) to a maximum of 100% (OPA-06, OPA-13 and OPA-19). Figure 1 is the representative of the extent of polymorphism observed among the cowpea cultivars as revealed by OPA 1 and OPA 12.

3.2. Cluster Analysis

The Jaccard's similarity indices based on RAPD profiles were subjected to UPGMA analysis. The dendrogram revealed the genetic similarity among the thirteen varieties of cowpea which ranged from 0.59 to 0.78, but mostly concentrated between 0.71 and 0.76. The RAPD cluster pattern is presented in Figure 2. It showed two main clusters. Data analysis resulted in a dendrogram with two major clusters sharing 59% similarities. Cluster one is further subdivided into four subclusters 1a, 1b, 1c and 1d. Cluster 1a has a similarity coefficient of 64%, 1b has a similarity coefficient of 70.8%, 1c has a similarity coefficient of 73 % and 1d has a similarity coefficient of 78.4%. The cluster two was further subdivided in two sub clusters 2a and 2b. The subcluster 2a has a similarity coefficient of 73% and cluster 2b had a similarity coefficient of 70%. Except the subcluster 1d all the subclusters of the major cluster 1 had single genotypes each. Cluster 1a had GC3, 1b had NBC 13, cluster 1c had CP 43 and cluster 1d had Kanagamoni and COCP 7. Cluster 2a had five genotypes PGCP1, ACM 05-07, CP 18, CP 222 and Vellayani jyothika. Cluster 2b has three genotypes namely V 240, VBN 1 and CP 16.

4. Discussion

In this study, the objective was to examine the relationship among 13 commonly grown cowpea genotypes of India. In recent years a number of studies have been undertaken to assess the genetic diversity and phylogenetic relationship in plant genetic resources. Several workers have reported the usefulness of RAPD technique in studying the diversity of crop genotypes. Markers have been used successfully to assess molecular polymorphism in cowpea [11], Chick-pea [3], pea [6], pigeonpea [7], and in cowpea [8]. The use of appropriate statistical method especially in case of RAPD analysis is very important to make genetic variation more definitive. The UPGMA is based on the assumption that mutation rate among different genotypes is constant and this has been widely used for analysis of genetic variation in plants. This method has been employed in present study during analysis of RAPD polymorphism. Wild relatives, exotics and mutant lines appeared to be good sources for genetic variation. Pre-breeding or genetic enhancement needs emphasis for the

transfer or introgression of genes and gene combinations from unadapted sources into more useable breeding material.

Table 2: Amplification profiles of the RAPD markers on cowpea genotypes.

Primer	Total number of bands	Polymorphic bands	Monomorphic bands	% of Polymorphic bands
OPA-3	10	1	90%	
OPA-4	13	13	0	100%
OPA-5	3	4	1	2
OPA-9	9	8	1	88%
OPA-1315	7	7	0	100%
OPA-19	13	13	0	100%

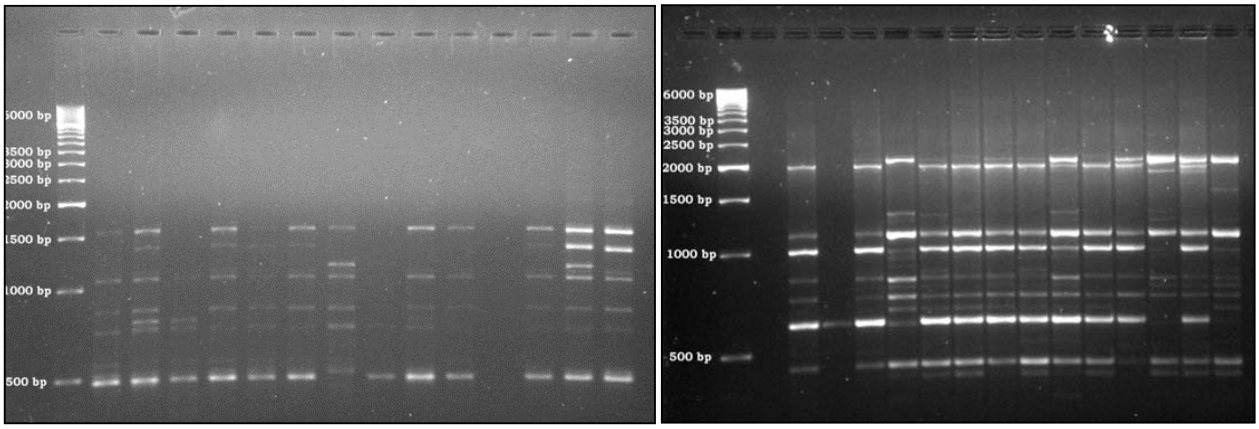

Fig 1: RAPD profile of 13 cowpea varieties using RAPD markers. (a) OPD 1; (b) OPD 12. Lanes 1: Marker DNA, 500 bp ladder. Lanes 1 - 13: Different genotypes of cowpea.

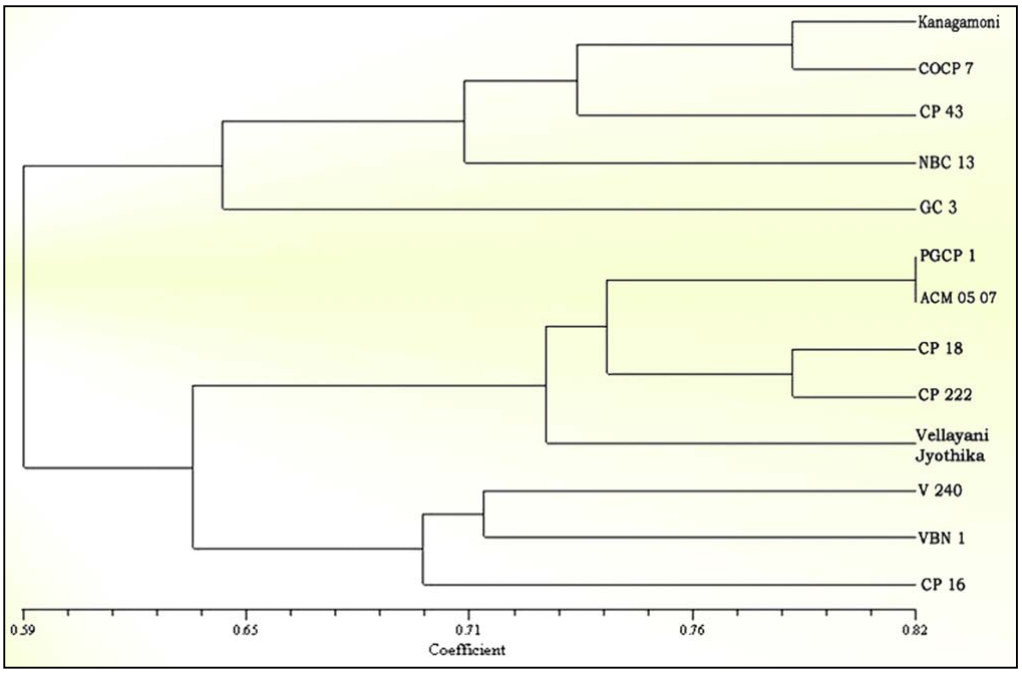

Fig 2: Dendrogram derived from UPGMA cluster analysis showing genetic relationship among Indian cowpea genotypes based on random amplified polymorphic DNA markers.

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