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RESEARCH PAPER

Genetic diversity analysis of cowpea [*Vigna unguiculata* (L.) Walp] genotypes using RAPD markers

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Abstract : Genetic diversity among 21 released varieties of cowpea [*Vigna unguiculata* (L.) Walp] was analysed using RAPD markers. A total of forty primers of two series (OPF01-20 and OPAG01-20) were used to screen the polymorphic primers and the profiles generated by three such primers were used for analysis. The genetic similarity co-efficients among genotypes varied between 0.44 to 0.82. Based on UPGMA and SAHN clustering cowpea genotypes grouped into two main clusters. Cluster II comprised of genotypes CO-7 and GC 3, while other nineteen genotypes were grouped under cluster I. However, the primers OPF 2, OPF 20 and OPAG15 were most polymorphic that can be used to fingerprint cowpea varieties.

Key Words : Diversity, Genetic similarity, RAPD markers, Cowpea

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INTRODUCTION

Cowpea [*Vigna unguiculata* (L.) Walp] is an important multipurpose grain legume extensively cultivated in arid and semiarid regions of Africa and Asia. Whether utilized as a pulse, or green pod vegetable or as a forged crop, it forms an important component of farming systems from semi-arid to the humid tropics. Its grain and leaves are rich of high quality protein and vitamins, which provide an excellent supplement to the lower quality cereal protein (Kitch *et al.*, 1998). Cowpea varieties/ accessions can be distinguished phenotypically from one another by their growth habit, time to maturity, yield and seed size and colour. A clear characterization of accessions is a necessary first step to guide efforts to conserve biodiversity and to facilitate breeding efforts. Data on genetic diversity of Indian cultivars is lacking. The main objective of this study was to determine the pattern and extend RAPD marker variation between cowpea populations from different agro ecological zones, and to determine the degree of genetic relationships among different varieties released in India. The degree of polymorphism generated by PCR based techniques ensures the higher level of differentiation of plant varieties in comparison to morphological and biochemical markers.

It has been demonstrated that cultivated cowpea had lower genetic diversity than many other crops, especially legume crops (Pasquet, 2000). So far, most characterization data of cowpea accessions in genebanks are based on morphological characters. Knowledge of genetic distance and diversity at the molecular level among landrace materials is important for a more profound characterization and to identify gene flow among populations. While numerous studies have evaluated molecular diversity of common bean, recent reports related to cowpea genetics have focused on linkage map and genome analysis. Information on molecular diversity of cowpea is limited (Nkongolo, 2003) and data on molecular variation at the DNA level of Indian landraces of cowpea is lacking.

The morphological characters have been used both for

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plant variety identification and for the diversity analysis. The use of morphological descriptors has been criticized because of environmental and epistatic interactions and the unknown genetic control of the traits. The use of biochemical markers involving seed storage protein and isozymes have been questioned due to poor genomic sampling. Random amplified polymorphic DNA (RAPD) is one of the several multiple arbitrary amplicon profiling technique used to characterize plant varieties at the molecular level. It uses a single primer of arbitrary nucleotide sequence to generate relatively complex DNA profile (Williums *et al.*, 1990).

MATERIALS AND METHODS

Plant material :

Twenty-one released cultivars of cowpea [*V. unguiculata*. (L.) Walp] were used for the present study. Seeds were obtained from the collection maintained at Seed Unit, University of Agricultural Sciences, Dharwad, Tamil Nadu Agricultural University Coimbatore and IARI New Delhi. The plant material includes the varieties bred by different centers of cowpea breeding.

DNA isolation :

DNA was isolated from 7 day-old seedlings germinated in pots. Young leaf tissues (1-2g) were ground in liquid nitrogen and mixed immediately with 15 ml of pre-heated CTAB extraction buffer (1.4 M NaCl, 100 mM Tris HCl; PH 8.0, 20 mM EDTA, 0.03% mercaptoethanol, 2% CTAB). The samples were incubated at 65° C for 30 min. Equal volume of Chloroform: Isoamylalcohol (24:1) was then added and mixed gently by inverting the tubes for 10 min. The aqueous phase was recovered after centrifugation at 8000 rpm for 10 min. This was followed by DNA precipitation in 0.7 volume of isopropanol. The precipitated DNA was then spooled out and transferred to 1.5 ml Eppendorf tube. DNA was washed twice in 70 per cent alcohol and dried under vacuum. The DNA pellet was dissolved in 10:1 TE (10 mM Tris-HCl, 1 mM EDTA; pH 8.0) solution. This solution was treated with RNase A to the final concentration of 100 µg /ml. The samples were incubated at 37°C for 1 hour. Equal volume of Phenol: Chloroform: Isoamylalcohol (25:24:1) was added and mixed gently and aqueous phase was recovered after centrifugation at 14,000 rpm for 5min. DNA was reprecipitated by adding 0.5 volume of 7.5 mM NH_4OAc and two volumes of absolute ethanol. DNA was collected and washed, followed by drying under vacuum and dissolving in TE. The samples were then stored at- 20 ^{0}C.

PCR reaction :

For amplification of random genomic sequences in reproducible way, the reaction mix was rigorously optimized (750mM Tris HCl pH8.8, 200 mM $(NH_4)SO_4$, 0.5% Tween 20, 2.5 m mols each of $dNTP_s$, 15 mM $MgCl_2$, 5 pM primer, 1 U Taq DNA polymerase and 20-50 ng template DNA in a total volume of 25µL). The PCR reactions were carried out in Primus Thermal Cycler programmed for initial denaturation at 94^{0}C for 7 min, followed by 40 cycles of 1 min. at 94^{0}C, 1 min. at 36^{0}C and 2 min. at 72^{0}C. After the last cycle, the samples were kept at 72^{0}C for an additional 10 min for final extension and then cooled to 4^{0}C. The amplified products were electrophoresed in 1.2 per cent Agarose gel with loading buffer (BPB) prestained with Ethidium bromides at a constant voltage (5 V/cm gel). The gel was visualized using uv-illumination and documented. Two series of oligonucleotide primers of Operon were screened and the primers giving the good polymorphic and reproducible banding pattern were selected for screening.

RAPD profiles were visually scored for the presence or absence of bands across the lanes. The combined data of all the primers were used to calculate the similarity co-efficient for all possible pair-wise combinations. These similarity co-efficients were used for cluster analysis to depict the relationship existing among the genotypes using unweighted pair group method for arithmetic average (UPGMA). All the computation and statistical analyses were performed using NTSys spc. 2.0 programme.

RESULTS AND DISCUSSION

In previous studies, cultivated cowpea showed a very low level of genetic diversity at the isozyme level (Pasquet, 2000) and chloroplast DNA level (Vaillancourt and Weeden, 1992). In the present study RAPD methodology was employed to study cowpea cultivars of India reaction parameters including $MgCl_2$ concentration and DNA concentration influenced yield and types of amplification products

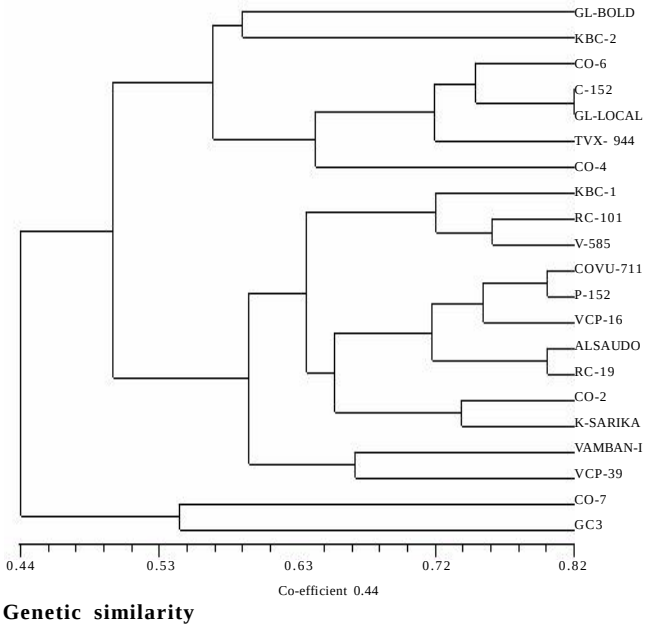

Fig. 1: Dendrogram based on UPGMA and SAHN cluster analysis of cowpea genotypes using OPF2, 20 and OPAG15 RAPD marker combinations

synthesized. Best RAPD products were obtained with 40 cycles.

Each RAPD analysis was repeated in separate experiments from two to four times and was always found to be reproducible. In total 228 bands were obtained using most polymorphic primers (OPF2, OPF20 and OPAG15). Combination of these primers was sufficient enough to distinguish all the cultivars. RAPD markers were able to distinguish all cultivars including those with similar morphological and agronomic traits. OPF 20can distinguish Gulburga bold, P-152, KBC-1, C152, Vamban-1, VCP-39, CO-2 and OPAG15 can distinguish COVU711 with the presence of unique banding pattern. Dendrogram was constructed based on UPGMA and SAHN cluster analysis of cowpea genotypes shows two main clusters, CO-7 and GC-3 come in one cluster and rest is subdivided in another cluster into thirteen sub clusters (Fig. 1). Genetic similarity varies between 0.44 to 0.82 and picture show complete distinguishing characters.

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