Indian J. Genet., **63**(4): 329-330 (2003)

Short Communication

Assessment of genetic divergence in some pigeonpea [*Cajanus cajan* (L.) Millsp.] genotypes using RAPD markers

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(Received: January 2003; Revised: October 2003; Accepted: October 2003)

Pigeonpea [*Cajanus cajan* (L.) Millsp.] is an important protein rich pulse crop in several parts of the semi-arid tropics. Genetic diversity in the pigeonpea has been well documented based on phenotypic characterization. Recently, molecular markers such as radom amplified polymorphic DNA (RAPD), which is simple and fast, is widely used for the identification of genotypes, gene mapping and QTL analysis. However, reports on divergence study in pigeonpea using RAPDs are few. In a crop like pigeonpea, in which no other markr systems are designed, RAPD technique would be useful method that enables to rapidly identify many markers and estimate the relationships and genetic diversity of populations.

Eleven genotypes of pigeonpea maintained at Agricultural Research Station, Gulbarga *viz.*, ICP-8863, TS-3, WRP-1, WRP-248, ICPL-87119, GS-1, BSMR-736, TAT-9621, ICPL-94063 and PT-221 were used in the study. Total genomic DNA was isolated by CTAB method [1] using frozen leaf tissue. Fifteen decamer oligonucleotide primers (OPBB-15, OPBB-19, OPBE-08, OPBE-12, OPBE-17, OPBF-14, OPBD-10, OPBD-11, OPAE-02, OPAE-03, OPAE-08, OPAE-16, OPAF-05, OPAF-13, OPAB-6) were initially screened to identify the most promising primers for detecting polymorphism. Eight primers OPBB-15, OPBB-19, OPBE-08, OPBE-12, OPBE-17, IPBF-14, OPBD-10 and OPBD-11 were selected to screen the eleven pigeonpea entries. Each 20 µl of dNTPs, 2 µl of reaction buffer and 0.25 µl Taq polymerase enzyme. The polymerize chain reaction was carried out in a Eppendorf Master Cycler Gradient. The following cycle was repeated 40 times: denaturing at 94oC for 20 seconds, annealing at 36oC for 1 minute and elongation at 72oC for 1 minute followed by final elongation at 72oC for 8 minutes. Amplification products were separated electrophoretically in 2% (w/v) agaroge gel using 1 × TBE buffer. Banding patterns were visualized on a UV-transilluminator after staining the gels with ethidium bromide at a concentration of 0.5 µg/ml. Statistica programme (version 4.1) was used to calculate the genetic similarities (squared Euclidian distances) and cluster analysis (Unweighted pair group average method) based on [2].

A total of 52 DNA bands were detected from the 11 entries using eight random primers. Of 52 levels, 63.46% (33 bands) were polymorphic. The total number of bands produced by each primer varied from a minimum of five amplified by OPBE-08, OPBD-11 and OPBE-12 primers to a maximum of 10 bands generated by OPBE-14. Average genetic distances between genotypes are presented in Table 1. ICPL-87 and TS-3 and GS-1 and TS-3 had high genetic diversity between

Table 1. Squared Euclidean distances between 11 pigeonpea genotypes

Genotypes	TS-3	WRP-1	WRP-248	ICPL-87119	ICPL-87	GS-1	BSMR-736	TAT-9621	ICPL-94063	PT-221
ICP-8863	18	13	13	12	16	12	16	11	11	11
TS-3	-	9	13	16	24	24	22	21	21	15
WRP-1	-	-	12	13	19	17	21	18	16	12
WRP-248	-	-	-	11	17	17	15	14	16	12
ICPL-87119	-	-	-	-	16	14	16	11	15	9
ICPL-87	-	-	-	-	-	12	14	15	19	17
GS-1	-	-	-	-	-	-	16	13	15	17
BSMR-736	-	-	-	-	-	-	-	9	15	11
TAT-9621		-	-	-	-	-	-	-	6	6
ICPL-94063	-	-	-	-	-	-	-	-	-	8
PT-221										

them. ICPL-94063 and TAT-9621 and PT-221 and TAT-9621 showed the least genetic distance. The arbitrary primers used in the study were useful for discriminating varieties of distinct characters. The primer OPBB-15 produced unique banding pattern specific to different varieites. Whereas, the primer OPBB-19 produced specific banding profiles in ICP-8863 and GS-1. Similar results were reported by Ratnaparkhe *et al.* [3] using RAPD markers for the identification of pigeonpea cultivars and eight of their related wild species. The use of single primers of arbitrary nucleotide sequence resulted in the selective amplification of DNA fragments that were unique to individual accessions. The level of polymorphism among the wild species was extremely high, while little polymorphism was detected within *C. cajan* accessions. Tyagi [4] also reported that the use of the single primers of arbitrary nucleotide sequence resulted in the selective amplification of DNA fragments that were unique to parents, F_1 and F_2 progeny using two strains of pigeonpea.

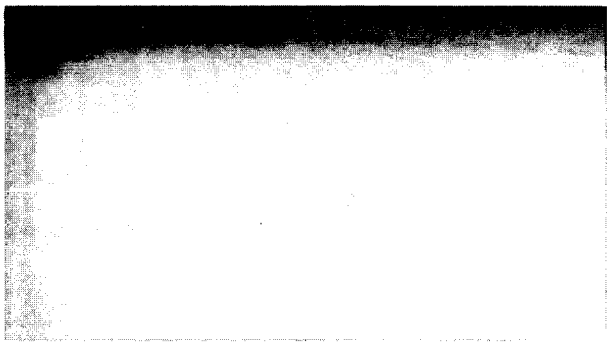

Fig. 1. Agarose gel electrophoresis of PCR products using the primer OPBB-19 amplified with DNA from 11 pigeonpea genotypes

groups. In addition, only eight primers generated as many as 33 RAPDs in pigeonpea. Therefore, RAPD analysis can be a powerful technique as well as time and cost saving one due to its single and fast operation to evaluate and characterize genetic diversity,

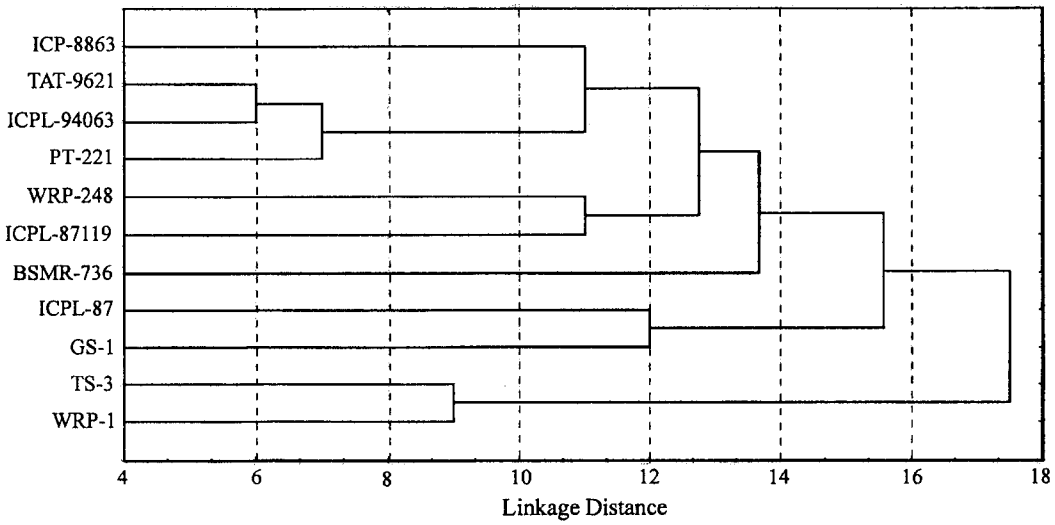

Fig. 2. Tree diagram for 11 variables based on Squared Euclidean distance (Unweighted pair-group average)

The dendrogram constructed by the unqeighted paired group method (UPGMA) is shown in Fig. 1. Eleven genotypes were grouped into two major clusters at a linkage distance of 16. Two genotypes TS-3 (white and bold seeded variety, maturing in 190-195 days) and WRP-1 (white and medium bold seeded, wilt resistant variety, maturing in 160-165 days) forming one cluster and the remaining entries in the second major cluster. Two subclusters were branched out from the major cluster (at a genetic distance of 14) with genotypes ICPL-87 (short duration variety maturing in 120-125 days, wilt susceptible with determinate growth habit) and GS-1 (white and medium bold seeded variety, wilt susceptible, maturing in 160-165 days) in one subcluster and the rest in another subcluster. RAPD markers clustered all the tested entries into different relationship and genetic finger printing of varieties in pigeonpea.

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