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Estimation of genetic variability of *Vigna radiata* cultivars by RAPD analysis

S. BETAL*, P. ROY CHOWDHURY**, S. KUNDU* and S. SEN RAYCHAUDHURI*,[1]

*Department of Biophysics and Molecular Biology, University of Calcutta, Calcutta-700 009, India**
*BKC College, Calcutta-700108, India***

Abstract

DNA was isolated from 14 cultivars of *Vigna radiata* (L.) Wilczek and subjected to RAPD analysis using 14 random decamer primers. These cultivars revealed polymorphism with respect to RAPD markers and were subjected to hierarchical cluster analysis. A dendrogram was prepared based on these data. Analysis of banding patterns confirmed that two strongly aromatic cultivars IC1, IC4, were closely linked. But another aromatic cultivar, B1, formed a separate cluster. The high yielding cultivars were closely related to B1. The phylogenetic tree constructed by the neighbour joining method showed that RAPD results were correlated with morphological characters like plant height, leaf and seed size, seed colour, *etc*.

Additional key words: dendrogram, mungbean, phylogenetic tree, random amplified polymorphic DNA.

Introduction

Mungbean [*Vigna radiata* (L.) Wilczek], is an important source of proteins and some cultivars possess excellent aroma.

Molecular markers can detect differences in DNA sequence and are less ambiguous than phenotypic markers that require gene expression. The polymerase chain reaction described by Mullis (1986) is an important tool of the molecular biology. One variation of PCR called Random Amplified Polymorphic DNA (RAPD) (Williams *et al.* 1990) involves amplification of random DNA sequence from genomic DNA using single short primers (usually 10 bases long) of arbitrary nucleotide sequence. These primers detect polymorphisms in the absence of specific nucleotide sequence information and the polymorphism functions as genetic markers.

A number of scientists have used RAPD markers successfully to study polymorphism in various plants. A useful population of each cultivar of *Ribes nigrum* was screened for RAPD markers and heterozygous loci were identified by band segregation in contrast with the non-segregation of homozygous loci (Lanham 1996). Xu and Bakalinsky (1996) used specific PCR primers derived from cloned RAPD markers in typing grape rootstocks. Ling *et al.* (1997) clustered nine cultivars into a single group of commercially cultivated poinsettias in accordance with their RAPD data. Cluster analysis based on RAPD markers was preferred to examine pedigree relationships of the cultivars of North American potato by Sosinski and Douches (1996). Although Mediterranean mandarins, like almost all citrus cultivars, are clonally propagated, genetic polymorphism could still be detected in different accessions by RAPD markers while no polymorphism was observed among plants belonging to the same cultivars of *Dioscorea* sp. (Asemota *et al.* 1996). Raghunathachari *et al.* (2000) worked on Indian scented rice "Basmati" found that though a number of genotypes had similar names yet there were no duplicates observed among the 18 rice accessions.

In the present study we provide evidence through RAPD assay for the occurrence of genetic variation in the 14 cultivars of *V. radiata* cultivated in West Bengal. These data will be helpful for producing high yielding and good aromatic cultivars.

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[1] Corresponding author; e-mail: sarmistha25@hotmail.com

Materials and methods

Plants: Dry seeds of 14 different cultivars of mungbean [*Vigna radiata* (L.) Wilczek] were obtained from Baharampur Pulse & Oilseed Research Station. The 14 cultivars were: Sonamung Indigenous Collection 1 (IC1), Sonamung Indigenous Collection 4 (IC4), B-1, Panth mung 1 (PM 1), Pusa Bold 2 (PB 2), ML 5, HUM 2, PDM 84-139, Mgg 332, Narendra mung 1 (NM 1), PDM 11, UPM 921, HUM 9 & Panth mung 4 (PM 4). Of the 14 cultivars, IC1, IC4 and B1 were of excellent aroma while the rest were not so remarkable in their aroma quality.

The seeds of each cultivar were surface sterilized in 10 % commercial sodium hypochlorite solution for 20 min and washed five times with sterile double distilled water in a laminar flow hood. Seeds were then germinated aseptically in sterile glass Petri dish on moist filter paper. The Petri dishes were kept in dark in the culture room maintaining a temperature of 22 - 25 °C and a relative humidity of 55 - 60 %. 7- to 10-d-old etiolated seedlings were used for DNA extraction.

The same cultivars were grown in the field for production of seeds. The characteristic features of the cultivars have been recorded (Table 1). Plant height varied from dwarf (< 22.5 cm) to medium (22.5 to 30 cm) to tall (> 30 cm) and corresponding leaf and seed size were also variable. The colour of seeds ranged from light yellow to green, some seeds showed a shiny and others a dull seed coat.

DNA extraction: Total genomic DNA from young seedlings of the plants was isolated following modified Edwards *et al.* (1991) method. DNA extraction buffer containing 200 mM Tris-HCl pH 7.5, 200 mM NaCl, 25 mM EDTA pH 8 and 0.5 % SDS was added (0.2 cm^3 per seedling) to the tissue and grounded gently in the mortar with a pestle. The homogenate was centrifuged for 5 min at 7 826 *g* at room temperature to remove tissue fragments. The supernatant was extracted twice with phenol-chloroform at 7.826 *g* for 5 min. Finally, the clear upper aqueous phase was collected carefully and 1/10 volume (with respect to the supernatant) of 3 M ammonium acetate and equal volume of isopropanol was added and mixed gently. DNA spooled with sterile capillary was then washed with 70 % ethanol and air-dried. Finally the DNA was dissolved in 0.05 cm^3 of sterile triple distilled water.

The DNA isolated from each cultivar was run in 1 % agarose gel to check the quality and was also scanned in spectrophotometer (*DU640*, *Beckman*, USA) and absorbances (A) were noted at 260 and 280 nm.

RAPD analysis was performed using the genomic DNA isolated from the 14 accessions of *V. radiata*. Fourteen decamer custom oligonucleotides were used as primers to perform RAPD reactions. The amplification reaction mixtures had a final value of 0.025 cm^3 in each PCR tube containing 10 mM Tris-HCl (pH 9), 50 mM KCl [0.0025 cm^3 10× amplification buffer], 100 µM each of dATP, dTTP, dGTP, dCTP [0.0025 cm^3 1 mM dNTP mix], 0.2 µM decamer random primer (0.001 cm^3 of 5 µM primer) (*Bangalore Genei*, Bangalore, India) 25 ng of genomic DNA [0.001 cm^3 diluted DNA], 1.5 mM $MgCl_2$ [0.0015 cm^3 25 mM $MgCl_2$], 3 units of Taq DNA polymerase (*Bangalore Genei*) [0.001 cm^3 Taq DNA polymerase solution] and 0.0155 cm^3 sterile triple distilled water. $MgCl_2$ and Taq DNA polymerase was added after a preheating at 94 °C for 3 min. Finally the PCR tubes were subjected to a denaturation step at 94 °C for 5 min, 45 cycles of 94 °C for 30 s, 35 °C for 30 s, 72 °C for 1 min 30 s and a final elongation at 72 °C for 5 min in a *Perkin Elmer* (USA) thermal cycler. Finally the temperature of amplified products was brought down to 4 °C.

In each set of reactions with a particular primer, along with the 14 different DNA in 14 separate PCR tubes, one tube without any genomic DNA (negative control) was also prepared. The RAPD reaction products were resolved in 1.8 % agarose gel at a constant voltage of 60 V for 7 h and photographs were taken under UV-transilluminator after staining with ethidium bromide.

Data analysis: Positions of clearly visible and scorable RAPD bands were transferred into a binary character matrix, with 1 for the presence and 0 for the absence of a band at a particular position. Proximity matrix was directly computed from Jaccard's coefficient using the *Software Package SPSS-version 9.0*. A dendrogram was obtained (using average linkage within groups) by hierarchical cluster analysis to establish the affinity and relationship of the 14 cultivars of *V. radiata*. Using neighbour joining method (package *Phylip, version 3.57*) (Saitou and Nei 1987) a phylogenetic tree describing the evolutionary relationship among the cultivars of *V. radiata* was constructed.

Results and discussion

14 decamer primers of were used for RAPD analysis of the 14 cultivars of *V. radiata.* The size range of the PCR products varied from primer to primer in a particular cultivar (Table 2). Some bands were found to be unique

Table 1. Characteristic features of the mungbean cultivars studied plant hight (tall >30 cm, medium 22.5 to 30 cm, dwarf < 22.5 cm), leaf size (large > 2.5 cm, medium 2 to 2.5 cm, small < 2 cm), seed size, colour and coat luster.

Cultivar	Plant height	Leaf size	Seed size	Seed colour	Seed coat luster
IC1	medium	medium	small	yellow	dull
IC2	dwarf	small	small	greenish yellow	dull
B 1	medium	large	medium	light yellow	dull
PM 1	medium	large	medium	green	dull
PB 2	tall	large	large	green	shiny
ML 5	tall	large	large	green	shiny
HUM 2	dwarf	small	large	green	dull
PDM 84-139	tall	large	medium	green	shiny
Mgg 332	medium	large	medium	green	dull
NM1	dwarf	medium	large	green	dull
PDM 11	medium	medium	medium	green	shiny
UPM 921	medium	medium	medium	deep green	dull
HUM 9	medium	medium	medium	deep green	shiny
PM 4	dwarf	large	medium	deep green	dull

for a particular cultivar (Figs. 1,2). A total number of 121 bands were obtained with 14 primers of which 76 were polymorphic (63.17 %) and 45 were found to be monomorphic (37.17 %). The primer number 14 with sequence AGGGGTCTTG was found to be most informative showing 19 bands.

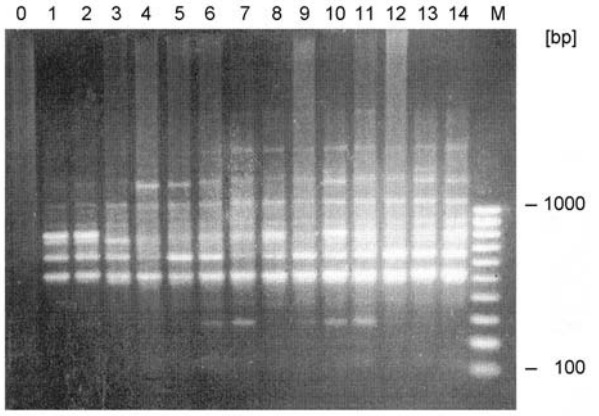

Fig. 1. RAPD profiles from genomic DNA of 14 accessions of *Vigna radiata* using primer AGGGTACCAG. The lanes represent from left: *0* - negative control without any genomic DNA, *1* - Sonamung Indigenous Collection 1 (IC1), *2* - Sonamung Indigenous Collection 4 (IC4), *3* - B-1, *4* - Panth mung 1, *5* - Pusa Bold 2, *6* - ML 5, *7* - HUM 2, *8* - PDM 84-139, *9* - Mgg 332, *10* - Narendra mung 1, *11* - PDM 11, *12* - UPM 921, *13* - HUM 9, *14* - Panth mung 4, *M* - 100 bp ladder (marker).

Pairwise Jaccard's coefficient for genetic similarities (Jaccard 1908, Nei 1972, Nei and Li 1979) was calculated (Table 3). Cluster analysis of the distribution of RAPD bands have been represented as a dendrogram using average linkage (within groups). Jacquard's similarity coefficient ranged from 0.566 to 0.930. It was very interesting to note that the accessions IC1 and IC4 were very much similar with respect to the band position of the RAPD markers. They showed similarity with respect to all but 6 bands with the 14 different primers used. Both of these cultivars have very strong aroma. The cultivar B1, though strongly aromatic was found to form a different cluster (Fig. 3).

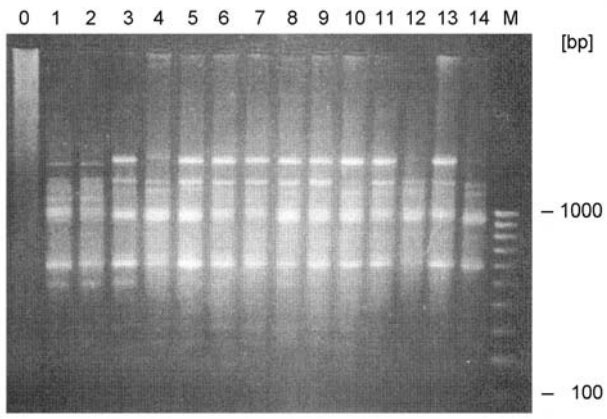

Fig. 2. RAPD profiles from genomic DNA of 14 accessions of *Vigna radiata* using primer CAAACGTCGG. The lanes represent from left: *0* - negative control without any genomic DNA, *1* - Sonamung Indigenous Collection 1 (IC1), *2* -Sonamung Indigenous Collection 4 (IC4), *3* - B-1, *4* - Panth mung 1, *5* - Pusa Bold 2, *6* - ML 5, *7* - HUM 2, *8* - PDM 84-139, *9* - Mgg 332, *10* - Narendra mung 1, *11* -PDM 11, *12* - UPM 921, *13* - HUM 9, *14* - Panth mung 4, *M* - 100 bp ladder.

The dendrogram using average linkage (within groups) (Fig. 3) allowed three main groups to be distinguished. The upper cluster contained IC1, IC4 and PM1. The second cluster contained all other cultivars except HUM 9, which formed the third cluster. It was quite evident from the dendrogram that all the high yielding cultivars with lesser aroma (except PM1) PB2,

ML5, HUM 2, PDM 84-139, Mgg 332, NM1, PDM 11, UPM 921, PM 4, have been found to be closely linked to the aromatic cultivar B1.

Using the value of the proximity matrix for 14 cultivars a phylogenetic tree (Fig. 4) was constructed using the neighbor joining method. The aromatic cultivars IC1 and IC4 were found to be in the same cluster. Three cultivars with lesser aroma namely PDM11, UPM 921 and HUM 9 were found to be in the same group. The morphological features like plant height, leaf and seed size and seed colour were also similar (Table 1). Therefore the genotypic characters as evidenced by the similarity of RAPD bands of these three cultivars were also reflected in their phenotype. Cultivars NM1 and PM4 formed another group. They also showed similarity in external features (Table 1). Similarly B1 and PM1 formed another group and showed similarity in morphological characters.

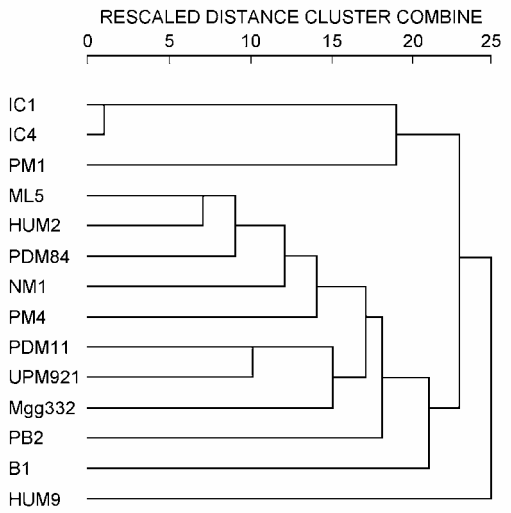

Fig. 3. Dendrogram of 14 cultivars of *V. radiata* based on RAPD bands amplified by 14 arbitrary 10-mer RAPD primers.

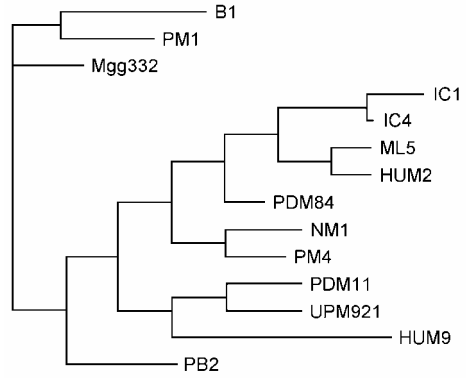

Fig. 4. Phylogenetic tree of the 14 cultivars of *V. radiata* using neighbour-joining method based on the values of the Jaccard's coefficient.

Reports on intraspecific variability of RAPD markers in different cultivars of *V. radiata* are lacking. Sonnante *et al.* (1997) carried out isozyme and RAPD analysis of the genetic diversity within and between *V. luteola*, *V. marina* ssp. *oblonga* and *V. marina* ssp. *marina*. On RAPD analysis 66 bands out of a total of 85 were found to be polymorphic. The dendrogram derived from RAPD data showed three main groups corresponding to the three taxa analysed.

Mung bean linkage map (Menancico-Hautea *et al.* 1992) have been made from RFLP markers using both homologous and heterologous clones and a mapping population derived from the interspecific hybrid between *V. radiata* ssp. *radiata* and *V. radiata* ssp. *sublobata*. Lambrides *et al.* (2000) have reported two new genetic linkage maps for mung bean constructed from RFLP and RAPD markers using F_2 and recombinant inbred lines (RIL) derived from an intersubspecific hybrid between *V. radiata* ssp. *radiata* and *V. radiata* ssp. *sublobata.* These maps were constructed to provide preliminary information on the inheritance and map location of important traits associated with seed quality, pest and disease resistance.

The present investigation on variability of RAPD markers in aromatic and non-aromatic cultivars of *V. radiata* reveals that the RAPD technique is useful to deduce the affinity of these cultivars. The genotypic characters as evidenced by polymorphic DNA bands could be correlated to some extent to the phenotype of different closely associated groups. These results will be beneficial to the farmers and breeders for raising high yielding strongly aromatic cultivars of *V. radiata* in future.

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