RESEARCH ARTICLE

Molecular assessment of genetic diversity in mung bean germplasm

G. ROOPA LAVANYA[1,*], JYOTI SRIVASTAVA[2] and SHIRISH A. RANADE[2]

[1]*Department of Genetics and Plant Breeding, Allahabad Agricultural Institute-Deemed University, Allahabad 211 007, India*
[2]*PMB (Genomics), National Botanical Research Institute, Rana Pratap Marg, Lucknow 226 001, India*

Abstract

RAPD profiles were used to identify the extent of diversity among 54 accessions of mung bean that included both improved and local land races. Out of the 40 primers screened, seven primers generated 174 amplification products with an average of 24.85 bands per primer. The RAPD profiles were analysed for Jaccard's similarity coefficients that was found to be in the range from 0 to 0.48, indicating the presence of wide range of genetic diversity at molecular level. Cluster analysis was carried out based on distances (1-similarity coefficient) using neighbour-joining method in Free Tree package. The dendrogram resolved all the accessions into two major clusters, I (with 11 accessions) and II (with 43 accessions). However, the cluster was further divided into four subclusters (II A with six, II B with nine, II C with 15 and II D with 13 accessions). The distribution of the accessions in different clusters and subclusters appeares to be related to their performance in field conditions for 10 morphological traits that were scored. This study indicated that the RAPD profiles provide an easy and simple technique for preliminary genetic diversity assessment of mung bean accessions that may reflect morphological trait differences among them.

[Lavanya G. R., Srivastava J. and Ranade S. A. 2008 Molecular assessment of genetic diversity in mung bean germplasm. *J. Genet.* **87**, 65–74]

Introduction

Mung bean (green gram, *Vigna radiata* (L.) Wilczek) is an Asiatic species of the pan-tropical genus *Vigna* with a considerable importance as it is a widely cultivated pulse crop, because of its adaptation to short growth duration, low water requirement, soil fertility and because it can be used in crop rotation practices also. Other properties like easy digestibility and low proportions of flatulence factors also add to its value among the pulse crops. However, a previous assessment by evaluation of morphological traits had established that the germplasm is constrained by low level of diversity in the working gene pool of this crop (Ramanujam 1981). In order that the mung bean gene pool be the best utilized for development of promising or superior varieties, an exhaustive characterization of the various germplasm holdings and collections that constitute the gene pools for the crop need to be fully characterized to identify the useful genetic diversity. The crosses between the parents with maximum genetic divergence are generally the most responsive for genetic improvement (Arunachalam 1981). However, to utilize such parental accessions with maximum genetic divergence, it is necessary to screen and characterize the germplasm for the nature and extent of genetic diversity included in it. Characterization and cataloguing of germplasm have been traditionally carried out by using morpho-agronomic traits, while in the past two decades or so the molecular markers have also been used for germplasm characterization. The molecular markers are proven to be a powerful tools, that can yield significant information that enhances the scope of using germplasm in the crop improvement programmes. Of the several molecular markers possible, those developed using random amplified polymorphic DNA (RAPD) profiles offer a rapid and reliable identification and characterization of genotypes (Williams *et*

*For correspondence. E-mail: lavanya.roopa@gmail.com.

Keywords. genetic diversity; local land races; mung bean; field performance; RAPD.

al. 1990). The RAPD technique generates molecular markers for comparative analysis that are quick, easy to use, free from environmental influences, unlimited in number, random but wide coverage of genome and have a relatively higher level of polymorphism (Newbury and Ford-Lloyd 1993). RAPD profiles have been characterized in case of several crop plant germplasm including coffee (Orozco-Castillo *et al*. 1994), rice (Virk *et al*. 1995) and tea (Wachira *et al*. 1995) to name a few. In case of mung bean also a few previous studies have been carried out using RAPD profiles alone or in combination with ISSR profiles (Bisht *et al*. 1998; Santalla *et al*. 1998; Lakhanpaul *et al*. 2000; Afzal *et al*. 2004; Saini *et al*. 2004; Chattopadhyay *et al*. 2005). In all these studies, only few accessions (12–32) were selected for analysis, with the maximum of 32 being selected by Lakhanpaul *et al*. (2000). It is possible that the smaller number of accessions selected will not be a true representation of the germplasm. Low levels of genetic diversity in mung bean were reported in the above studies. Considering that the germplasm of rung gbean includes a large number of accessions and local land races, it is important to select from a wider range of these accessions for an assessment of the genetic diversity. In this paper we show that the RAPD analysis as carried out for a set of 54 accessions representing a number of local land races and varieties released by the different centres, reveals not only a wider range of diversity but also groups the accessions according to their field performance for some of the morpho-agronomic traits tested.

Materials and methods

Plant materials and DNA isolation

Fifty-four accessions of mung bean were collected from different agri zones of India (table 1). Plants were raised in the field and young leaf tissues were used for DNA extraction. Young leaf tissues were harvested from the plants, washed to free from dirt and dust, and then quickly mopped, dried on blotting sheets. The leaves were deribbed and wrapped in tissue paper inturn wrapped with blotting paper. The packed leaf tissues were kept in zip lock bags along with fine mesh blue silica gel for rapid dehydration. These dry leaf tissues were used for DNA extraction. Genomic DNA was isolated using Plant DNA Isolation Kit (Bangalore Genei, Bangalore), following the manufacturers' instructions. *Bauhinia purpurea* L. was randomly selected as the non-*Vigna* leguminous out group for all studies to test whether our reaction conditions were optimized to resolve it as a distinctly different genotype from the rest of the mung bean accessions.

Table 1. Mung bean accessions used for RAPD analysis and the sources from where these have been obtained are given below.

Sample no.	Name of the genotype	Source
MB1	PDM-84–139	IIPR, Kanpur
MB2	NARENDRAMUNG-1	NDUAT, Faizabad
MB3	LGG-478	Regional Agricultural Research Station, Lam
MB4	EC-30400	AVRDC, Taiwan
MB5	MSO-9	AVRDC, Taiwan
MB6	LGG-499	Regional Agricultural Research Station, Lam
MB7	IPRM-90	IIPR, Kanpur
MB8	T-1	IIPR, Kanpur
MB9	PUSA BOLD-2	IARI, New Delhi
MB10	LM-497	PAU, Ludhiana
MB11	OUM-11-5	OUAT, Bhubaneshwar
MB12	PDM-84-143	IIPR, Kanpur
MB13	HUM-1	BHU, Varanasi
MB14	V-557	AVRDC, Taiwan
MB15	LLR-1	local land race-1
MB16	ML-287	PAU, Ludhiana
MB17	V-4512	AVRDC, Taiwan
MB18	AKM-9601	PKV, Akola
MB19	LAM-M2	Regional Agricultural Research Station, Lam
MB20	ML-583	PAU, Ludhiana
MB21	LGG-491	Regional Agricultural Research Station, Lam
MB22	MH-309	PAU, Ludhiana
MB23	LGG-477	Regional Agricultural Research Station, Lam

MB24	PDM-89-226	IIPR, Kanpur
MB25	K-851	IIPR, Kanpur
MB26	LM-1119	PAU, Ludhiana
MB27	PS-10	IARI, New Delhi
MB28	ML-406	PAU, Ludhiana
MB29	K-2192	Kanpur Local Germplasm
MB30	PUSA-9332	IARI, New Delhi
MB31	MUM-01-1	CCS MU, Meerut
MB32	K-1284	Kanpur Local Germplasm
MB33	EC-398889	AVRDC, Taiwan
MB34	NARP-280	PAU, Ludhiana
MB35	DMG-1103	Kanpur Local Germplasm
MB36	TARM-1	BARC, Mumbai/PKV, Akola
MB37	WGG-37	Regional Agricultural Research Station, Warangal
MB38	SUJATA	OUAT, Bhubaneshwar
MB39	PIMS-11/99	PAU, Ludhiana
MB40	EC-393407	AVRDC, Taiwan
MB41	PDM-11	IIPR, Kanpur
MB42	DMG-1098-1	Kanpur local germplasm
MB43	MARP-280	PAU, Ludhiana
MB44	AKM-8802	PKV, Akola
MB45	TARM-2	BARC, Mumbai/PKV, Akola
MB46	EC-398888	AVRDC, Taiwan
MB47	ML-131	PAU, Ludhiana
MB48	PM-9001	Kanpur Local Germplasm
MB49	OBGG-11	OUAT, Bhubaneshwar
MB50	LM-23	PAU, Ludhiana
MB51	MGG-47	Regional Agricultural Research Station, Madhira
MB52	OBGG-40	OUAT, Bhubaneshwar
MB53	PDM-1	IIPR, Kanpur
MB54	OBGG-52	OUAT, Bhubaneshwar
B2	*Bauhinia purpurea* L.	NBRI, Lucknow

RAPD primers, reactions and agarose gel electrophoresis

The primer kits OP-F and OP-G (20 primers each), were purchased from Operon Technology, USA. The basic reaction included 25 ng template DNA, 0.2 μM primer, 100 μM each of dATP, dGTP, dCTP and dTTP, 2 mM Mg^{2+} concentration and 0.5 U of *Taq* polymerase along with suitable 1× buffer (10 mM Tris-Cl, pH 8.3, 50 mM KCl, 0.001% w/v gelatin) all taken in 25 μl final reaction volume. The reaction was carried out at 94°C for 3 min as predenaturation step, then the reaction was cycled 45 times at 94°C for 45 sec, 35°C for 45 sec and extension at 72°C for 1 min. Additionally a final cycle allowed extension for 5 min at 72°C in PTC 200 Thermal cycler (MJ Research, USA). Care was taken to ensure that the set of accessions to be compared were all analysed in the same machine. The amplified products in all the above trials were separated electrophoretically on 0.8% agarose gels in 0.5× TBE buffer, visualized and photographed over a UV transilluminator after staining with ethidium bromide.

Scoring and analysis of bands

Clear and well marked bands were coded in a binary form by denoting '0' and '1' intended for absence and presence of bands, respectively in each genotype and these data were then used as input for further calculations. To describe genetic relationships among the mung bean accessions, RAPD band data were used to estimate genetic distances, based on Jaccard similarity coefficients computed using the Free Tree (version 0.9.1.50) program (Pavlicek *et al*. 1999). Cluster analysis was carried out based on genetic distances using neighbour-joining (NJ) method in the Free Tree package. The resulting clusters were represented as dendrograms and printed in the program TreeView (version 1.6.5; Page 2001).

Agro-morphological trait analysis using RBD

The experimental design included three replicate RBD (complete) and was carried out in two seasons, kharif 2003 and kharif 2004 at Allahabad. The traits that were scored in this experiment included plant height, number of primary branches, number of clusters per plant, number of pods per cluster, number of pods per plant, number of seeds per pod, pod length, days to maturity, 100 seed weight and seed yield per plant.

Results

RAPD markers have been used for the identification and determination of the genetic relationship among the cultivars in case of several crops. In the present study, seven RAPD primers, among the 40 primers from kits OP-F and OP-G that were screened on a subset of five accessions, resulted in discrete profiles with all the DNAs tested (data not shown). These seven primers namely, OP-F06, OP-F07, OP-F10, OP-G01, OP-G02, OP-G03 and OP-G05 resulted in consistent profiles and were used with the full set of 54 accessions (table 1). Typical RAPD profiles with four primers are shown in figure 1,a–d). A total of 174 amplification products were scored for these seven primers (table 2) in the 54 accessions of mung bean. On an average, 24.85 bands were produced primer^{-1}. The fragments produced from all the 54 accessions varied in size from 450 to 1375 bp, were used as input data for computation of pairwise distances (table 3). The lowest distance was 0.524 between the accessions, LLR-1 and ML-287. The highest distance was 1.0 between the accessions WGG-37 and PDM-1 and between WGG-37 and OBGG-52.

Neighbour-joining method distributed all the 54 mung bean accessions into two broad clusters, I (with 11 accessions) and II (with 43 accessions). The later was further composed of four subclusters (II A with six, II B with nine, II C with 15 and II D with 13 accessions), as shown in figure 2.

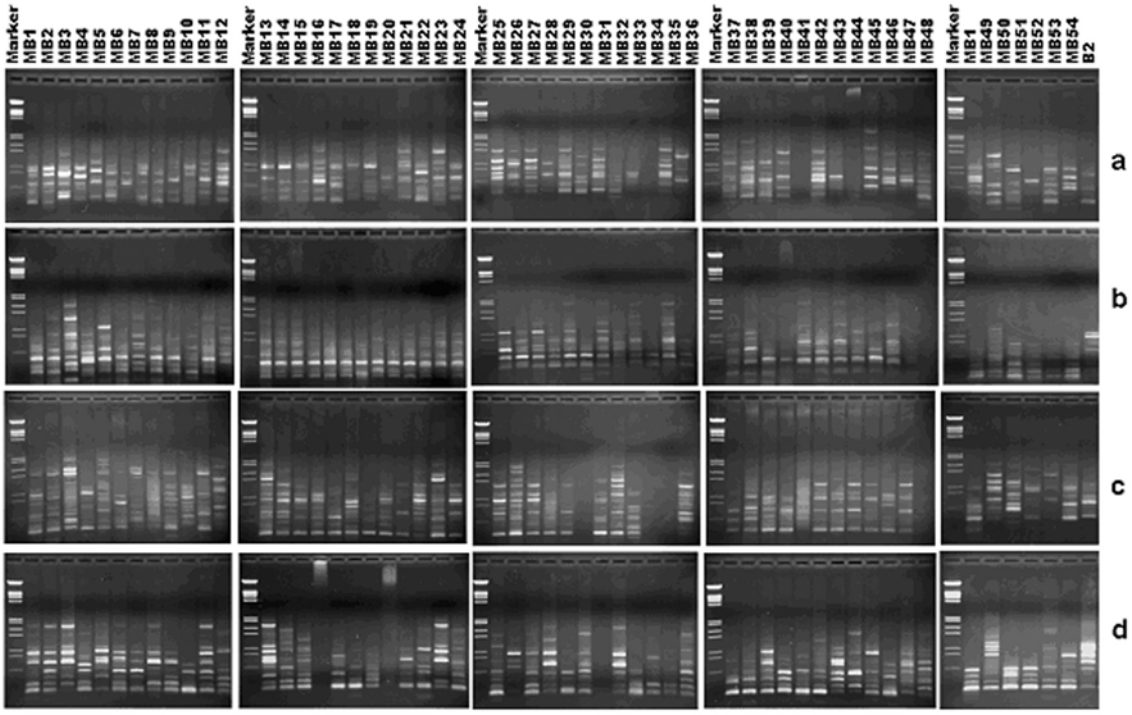

Figure 1. Typical RAPD profiles obtained for the 54 mung bean and outgroup *B. purpurea* DNAs with primers (a), OP-F06; (b), OP-G02; (c), OP-G03 and (d), OP-G05. Lane marked as Marker in each gel panel is for the λ DNA double digested with *Eco*RI and *Hin*dIII as fragment size marker.

Table 2. Primers selected for the present study, the number and size range of RAPD bands produced by these primers among the 54 mungbean accessions.

S. no.	Primer	Sequence (5′ – 3′)	No. of bands	Size range (bp)
1	OP-F06	GGGAATTCGG	26	400–2400
2	OP-F07	CCGATATCCC	26	340–2000
3	OP-F10	GGAAGCTTGG	24	320–1900
4	OP-G01	CTACGGAGGA	17	320–1600
5	OP-G02	GGCACTGAGG	30	320–1850
6	OP-G03	GAGCCCTCCA	27	470–2000
7	OP-G05	CTGAGACGGA	24	340–1900

Table 3. Distance matrix of pairwise distances computed from RAPD data by NJ method using the program Free Tree. The accession names are as listed in table 1.

	MB1	MB2	MB3	MB4	MB5	MB6	MB7	MB8	MB9	MB10	MB11	MB12	MB13	MB14	MB15	MB16	MB17	MB18	MB19	MB20	MB21	MB22	MB23	MB24	MB25	MB26	MB27
MB1																											
MB2	0.56																										
MB3	0.81	0.66																									
MB4	0.72	0.75	0.82																								
MB5	0.69	0.71	0.74	0.73																							
MB6	0.70	0.76	0.73	0.86	0.73																						
MB7	0.80	0.85	0.78	0.82	0.83	0.85																					
MB8	0.70	0.69	0.71	0.75	0.64	0.71	0.78																				
MB9	0.72	0.71	0.79	0.84	0.78	0.71	0.87	0.69																			
MB10	0.83	0.78	0.81	0.79	0.70	0.82	0.86	0.81	0.77																		
MB11	0.79	0.66	0.74	0.84	0.77	0.73	0.80	0.73	0.73	0.78																	
MB12	0.87	0.79	0.77	0.88	0.83	0.86	0.85	0.83	0.80	0.89	0.83																
MB13	0.82	0.77	0.84	0.81	0.80	0.85	0.91	0.87	0.88	0.86	0.84	0.84															
MB14	0.78	0.79	0.80	0.83	0.86	0.78	0.86	0.77	0.84	0.88	0.80	0.80	0.66														
MB15	0.82	0.83	0.81	0.77	0.80	0.77	0.87	0.81	0.80	0.82	0.80	0.80	0.71	0.61													
MB16	0.81	0.86	0.82	0.90	0.89	0.80	0.91	0.84	0.88	0.84	0.79	0.89	0.77	0.69	0.52												
MB17	0.91	0.86	0.75	0.84	0.86	0.79	0.94	0.84	0.88	0.80	0.88	0.90	0.80	0.80	0.67	0.66											
MB18	0.82	0.80	0.81	0.83	0.84	0.73	0.87	0.80	0.84	0.87	0.84	0.86	0.79	0.75	0.79	0.80	0.79										
MB19	0.89	0.90	0.91	0.88	0.88	0.83	0.89	0.90	0.93	0.86	0.85	0.92	0.78	0.85	0.83	0.81	0.73	0.76									
MB20	0.83	0.81	0.86	0.88	0.85	0.85	0.92	0.90	0.85	0.86	0.85	0.88	0.82	0.78	0.80	0.63	0.83	0.79	0.76								
MB21	0.81	0.81	0.89	0.85	0.83	0.77	0.90	0.76	0.80	0.90	0.81	0.86	0.78	0.70	0.78	0.82	0.80	0.62	0.68	0.81							
MB22	0.91	0.85	0.87	0.89	0.85	0.80	0.88	0.87	0.80	0.85	0.76	0.85	0.70	0.70	0.63	0.70	0.72	0.77	0.83	0.89	0.78						
MB23	0.81	0.84	0.80	0.85	0.81	0.80	0.84	0.75	0.88	0.82	0.81	0.82	0.70	0.79	0.70	0.70	0.71	0.75	0.80	0.91	0.78	0.77					
MB24	0.82	0.80	0.87	0.86	0.77	0.84	0.89	0.82	0.88	0.84	0.70	0.88	0.77	0.77	0.81	0.77	0.79	0.81	0.82	0.82	0.76	0.69	0.75				
MB25	0.80	0.82	0.81	0.79	0.82	0.81	0.89	0.80	0.79	0.87	0.80	0.84	0.81	0.76	0.73	0.78	0.81	0.74	0.80	0.77	0.71	0.82	0.76	0.74			
MB26	0.81	0.79	0.77	0.92	0.79	0.84	0.91	0.86	0.86	0.85	0.83	0.79	0.85	0.78	0.84	0.83	0.84	0.80	0.92	0.85	0.86	0.83	0.79	0.80	0.76		
MB27	0.73	0.72	0.76	0.84	0.81	0.79	0.93	0.78	0.83	0.88	0.81	0.77	0.79	0.79	0.75	0.77	0.83	0.82	0.86	0.81	0.79	0.85	0.85	0.80	0.77	0.64	
MB28	0.76	0.75	0.81	0.80	0.79	0.82	0.92	0.83	0.91	0.94	0.85	0.89	0.84	0.78	0.80	0.88	0.82	0.84	0.89	0.86	0.89	0.87	0.88	0.87	0.86	0.72	0.63
MB29	0.84	0.86	0.88	0.91	0.90	0.86	0.93	0.88	0.86	0.84	0.83	0.83	0.88	0.87	0.90	0.85	0.91	0.85	0.84	0.88	0.81	0.91	0.83	0.85	0.83	0.76	0.71
MB30	0.83	0.81	0.83	0.87	0.89	0.87	0.90	0.81	0.95	0.90	0.89	0.89	0.84	0.84	0.85	0.84	0.87	0.89	0.86	0.91	0.89	0.91	0.86	0.85	0.86	0.81	0.63
MB31	0.79	0.84	0.84	0.93	0.86	0.84	0.95	0.91	0.81	0.85	0.82	0.82	0.94	0.91	0.90	0.85	0.89	0.89	0.85	0.82	0.86	0.92	0.92	0.86	0.86	0.74	0.80
MB32	0.76	0.73	0.71	0.84	0.81	0.79	0.81	0.78	0.89	0.91	0.81	0.86	0.85	0.79	0.84	0.87	0.86	0.80	0.89	0.89	0.85	0.91	0.85	0.87	0.85	0.78	0.70
MB33	0.89	0.78	0.85	0.88	0.88	0.85	0.89	0.86	0.88	0.83	0.80	0.85	0.92	0.87	0.90	0.93	0.88	0.79	0.88	0.88	0.77	0.91	0.91	0.88	0.87	0.69	0.81
MB34	0.84	0.91	0.92	0.86	0.91	0.95	0.91	0.89	0.92	0.94	0.96	0.96	0.91	0.90	0.83	0.88	0.89	0.92	0.85	0.93	0.89	0.93	0.90	0.92	0.88	0.91	0.84
MB35	0.89	0.88	0.93	0.88	0.95	0.88	0.93	0.93	0.91	0.93	0.90	0.95	0.90	0.89	0.87	0.90	0.81	0.91	0.76	0.92	0.83	0.95	0.94	0.91	0.87	0.93	0.88
MB36	0.77	0.77	0.77	0.88	0.85	0.83	0.89	0.81	0.87	0.91	0.85	0.84	0.80	0.78	0.82	0.86	0.87	0.87	0.88	0.90	0.84	0.92	0.83	0.87	0.76	0.80	0.68
MB37	0.82	0.87	0.96	0.83	0.84	0.90	0.91	0.89	0.93	0.91	0.87	0.89	0.83	0.92	0.89	0.89	0.95	0.86	0.86	0.90	0.82	0.88	0.90	0.86	0.83	0.89	0.87
MB38	0.90	0.91	0.90	0.89	0.91	0.87	0.85	0.91	0.84	0.88	0.87	0.91	0.84	0.82	0.84	0.86	0.94	0.91	0.86	0.86	0.89	0.80	0.94	0.89	0.88	0.82	0.83
MB39	0.86	0.85	0.89	0.91	0.89	0.87	0.92	0.89	0.87	0.84	0.87	0.89	0.84	0.75	0.67	0.82	0.87	0.89	0.89	0.87	0.87	0.81	0.90	0.89	0.83	0.79	0.76
MB40	0.88	0.89	0.86	0.91	0.91	0.82	0.85	0.87	0.87	0.93	0.80	0.87	0.87	0.90	0.89	0.91	0.92	0.81	0.83	0.89	0.83	0.88	0.90	0.91	0.86	0.82	0.83
MB41	0.91	0.90	0.91	0.92	0.94	0.95	0.96	0.90	0.84	0.90	0.90	0.86	0.95	0.92	0.92	0.94	0.95	0.90	0.93	0.91	0.91	0.91	0.93	0.92	0.88	0.92	0.87
MB42	0.91	0.89	0.87	0.88	0.88	0.93	0.90	0.87	0.91	0.92	0.86	0.86	0.89	0.89	0.88	0.92	0.93	0.95	0.88	0.93	0.93	0.93	0.89	0.95	0.93	0.90	0.81
MB43	0.93	0.91	0.86	0.91	0.91	0.89	0.92	0.87	0.85	0.86	0.87	0.91	0.84	0.83	0.79	0.89	0.87	0.93	0.89	0.94	0.92	0.90	0.88	0.97	0.90	0.89	0.82
MB44	0.84	0.88	0.88	0.92	0.92	0.90	0.89	0.84	0.81	0.87	0.86	0.84	0.90	0.87	0.91	0.92	0.92	0.80	0.85	0.93	0.79	0.95	0.83	0.92	0.83	0.88	0.86
MB45	0.88	0.88	0.92	0.90	0.90	0.93	0.89	0.90	0.90	0.89	0.87	0.92	0.88	0.88	0.87	0.89	0.90	0.98	0.79	0.90	0.94	0.93	0.91	0.90	0.89	0.96	0.88
MB46	0.91	0.88	0.90	0.91	0.86	0.89	0.90	0.91	0.91	0.87	0.84	0.90	0.83	0.89	0.90	0.95	0.93	0.88	0.85	0.91	0.85	0.89	0.93	0.86	0.92	0.90	0.89
MB47	0.78	0.84	0.78	0.85	0.82	0.81	0.89	0.80	0.84	0.84	0.80	0.88	0.86	0.83	0.87	0.83	0.90	0.93	0.80	0.87	0.90	0.90	0.87	0.88	0.83	0.88	0.83
MB48	0.87	0.88	0.94	0.88	0.88	0.91	0.93	0.86	0.89	0.90	0.90	0.92	0.87	0.91	0.90	0.88	0.89	0.86	0.88	0.85	0.85	0.91	0.79	0.88	0.85	0.90	0.87
MB49	0.80	0.75	0.84	0.86	0.89	0.79	0.95	0.80	0.84	0.85	0.80	0.89	0.89	0.92	0.91	0.88	0.91	0.89	0.89	0.86	0.83	0.94	0.88	0.89	0.86	0.84	0.80
MB50	0.80	0.79	0.84	0.84	0.86	0.92	0.95	0.86	0.88	0.91	0.89	0.78	0.78	0.82	0.89	0.93	0.88	0.93	0.90	0.94	0.88	0.90	0.87	0.88	0.91	0.84	0.77
MB51	0.92	0.87	0.86	0.82	0.89	0.90	0.88	0.89	0.85	0.86	0.91	0.85	0.80	0.86	0.82	0.84	0.87	0.92	0.98	0.89	0.95	0.83	0.88	0.87	0.91	0.85	0.85
MB52	0.83	0.83	0.92	0.87	0.92	0.90	0.95	0.83	0.85	0.89	0.92	0.90	0.93	0.96	0.92	0.98	0.98	0.93	0.94	0.90	0.91	0.96	0.96	0.95	0.87	0.92	0.80
MB53	0.90	0.85	0.89	0.90	0.94	0.87	0.97	0.94	0.90	0.92	0.94	0.96	0.88	0.92	0.94	0.95	0.90	0.87	0.94	0.90	0.90	0.95	0.91	0.87	0.91	0.92	0.85
MB54	0.87	0.85	0.87	0.89	0.93	0.91	0.93	0.82	0.84	0.88	0.93	0.91	0.89	0.93	0.91	0.88	0.87	0.96	0.89	0.82	0.92	0.94	0.93	0.91	0.90	0.91	0.80

Table 3 *(contd)*

	MB28	MB29	MB30	MB31	MB32	MB33	MB34	MB35	MB36	MB37	MB38	MB39	MB40	MB41	MB42	MB43	MB44	MB45	MB46	MB47	MB48	MB49	MB50	MB51	MB52	MB53	MB54
MB1																											
MB2																											
MB3																											
MB4																											
MB5																											
MB6																											
MB7																											
MB8																											
MB9																											
MB10																											
MB11																											
MB12																											
MB13																											
MB14																											
MB15																											
MB16																											
MB17																											
MB18																											
MB19																											
MB20																											
MB21																											
MB22																											
MB23																											
MB24																											
MB25																											
MB26																											
MB27																											
MB28																											
MB29	0.81																										
MB30	0.60	0.79																									
MB31	0.82	0.75	0.85																								
MB32	0.59	0.81	0.59	0.82																							
MB33	0.83	0.84	0.84	0.74	0.83																						
MB34	0.90	0.93	0.84	0.97	0.89	0.93																					
MB35	0.89	0.88	0.89	0.88	0.89	0.87	0.75																				
MB36	0.78	0.84	0.69	0.89	0.66	0.90	0.87	0.86																			
MB37	0.85	0.86	0.88	0.83	0.88	0.86	0.87	0.90	0.85																		
MB38	0.92	0.89	0.90	0.90	0.91	0.83	0.91	0.93	0.89	0.81																	
MB39	0.81	0.89	0.84	0.85	0.80	0.84	0.85	0.89	0.83	0.88	0.73																
MB40	0.88	0.89	0.90	0.84	0.83	0.79	0.91	0.85	0.82	0.84	0.79	0.81															
MB41	0.93	0.93	0.91	0.91	0.87	0.93	0.89	0.91	0.87	0.93	0.84	0.78	0.84														
MB42	0.83	0.91	0.89	0.94	0.83	0.86	0.89	0.91	0.90	0.90	0.80	0.79	0.82	0.77													
MB43	0.81	0.92	0.82	0.94	0.80	0.89	0.90	0.89	0.83	0.96	0.83	0.73	0.76	0.78	0.69												
MB44	0.91	0.88	0.89	0.88	0.80	0.85	0.89	0.87	0.83	0.89	0.84	0.80	0.75	0.83	0.73	0.70											
MB45	0.93	0.97	0.96	0.91	0.92	0.97	0.83	0.86	0.93	0.89	0.82	0.83	0.85	0.85	0.72	0.83	0.87										
MB46	0.91	0.89	0.89	0.92	0.85	0.88	0.93	0.88	0.90	0.87	0.87	0.85	0.74	0.83	0.84	0.82	0.83	0.80									
MB47	0.90	0.92	0.82	0.83	0.82	0.90	0.88	0.90	0.80	0.86	0.74	0.83	0.82	0.88	0.75	0.79	0.81	0.73	0.85								
MB48	0.81	0.85	0.82	0.95	0.80	0.91	0.93	0.92	0.85	0.79	0.90	0.87	0.84	0.83	0.89	0.82	0.85	0.91	0.79	0.90							
MB49	0.87	0.83	0.85	0.74	0.84	0.79	0.90	0.85	0.85	0.80	0.90	0.85	0.68	0.84	0.89	0.85	0.86	0.88	0.83	0.81	0.83						
MB50	0.88	0.90	0.94	0.90	0.91	0.90	0.91	0.93	0.91	0.89	0.89	0.85	0.93	0.84	0.84	0.90	0.89	0.87	0.83	0.91	0.88	0.86					
MB51	0.92	0.90	0.94	0.90	0.93	0.95	0.94	0.97	0.91	0.88	0.88	0.86	0.96	0.96	0.96	0.92	0.94	0.89	0.95	0.89	0.93	0.88	0.82				
MB52	0.93	0.91	0.96	0.88	0.95	0.90	0.92	0.91	0.92	0.93	0.92	0.96	0.89	0.82	0.93	0.91	0.95	0.90	0.91	0.89	0.97	0.69	0.84	0.82			
MB53	0.93	0.90	0.98	0.88	0.95	0.90	0.96	0.90	0.93	1.00	0.97	0.93	0.94	0.93	0.98	0.96	0.92	0.93	0.94	0.98	0.94	0.84	0.87	0.78	0.81		
MB54	0.92	0.92	0.94	0.84	0.94	0.92	0.94	0.89	0.92	1.00	0.90	0.88	0.90	0.87	0.89	0.90	0.96	0.88	0.92	0.88	0.98	0.84	0.84	0.76	0.54	0.77	

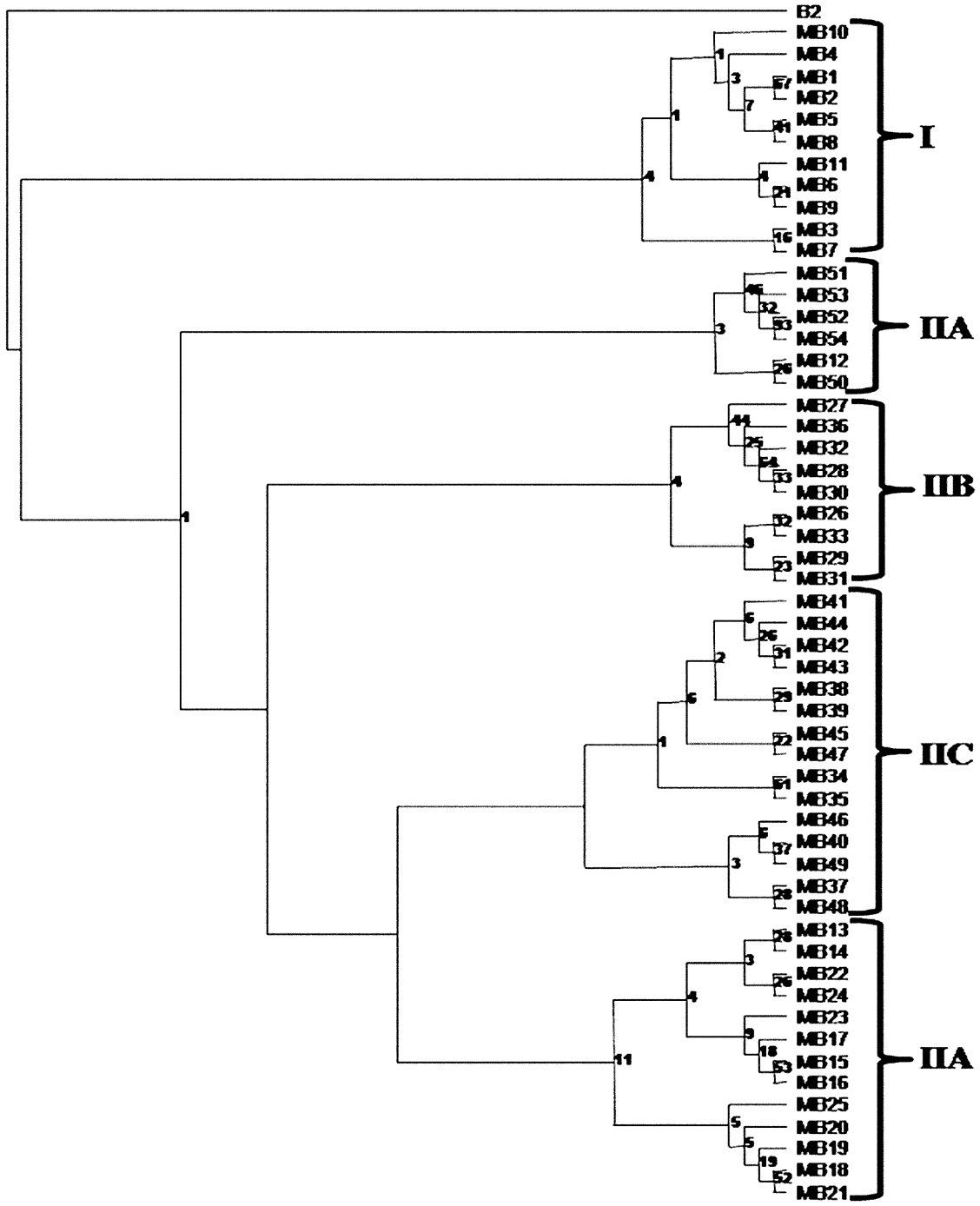

Figure 2. NJ dendrogram for the RAPD band data in case of the mung bean accessions and outgroup DNA. The numbers at the nodes are bootstrap values. OTU labels are to the right of the branches and are as given in table 1. The branch lengths are based on the distances between the genotype pairs. The distribution of the accessions into different clusters and subclusters are indicated to the right of the dendrogram with large parentheses.

The out group genotype, *Bauhinia purpurea* was clearly distinguished from all the mung bean accessions and separated from them in the NJ dendrogram.

The order of occurrence of the accessions in the above clusters based on RAPD profiling was used as a reference for arranging performance values of the accessions for the 10 agro-morphological traits that were measured in field tests over two seasons in RBD. These values are given in table 4. For the sake of clarity, the performance values above the grand trait mean for the entire set of accessions are in boldface. This table thus allows a simultaneous comparison of several traits in

Table 4. Mean performance of the 54 accessions when analysed for the 10 traits. The values in bold are of the accessions with performance parameter value equal to or greater than the trait mean.

Cluster no.[a]	Accessions (sample no.)[b]	Plant height (cm)	No. of primary branches	No. of clusters per plant	No. of pods per cluster	No. of pods per plant	No. of seeds per pod	Pod length (cm)	Days to maturity	100 seed weight (g)	Seed yield per plant (g)
I	LM-497 (MB10)	**58.00**	3.00	11.00	3.00	29.67	**11.00**	6.43	**78.33**	**3.83**	**12.44**
I	EC-30400 (MB4)	**52.00**	3.33	**16.00**	2.33	**41.33**	**9.67**	6.17	75.00	3.67	**11.87**
I	PDM-84-139 (MB1)	**54.67**	**3.67**	**15.33**	2.67	33.33	**9.00**	5.93	71.00	2.73	**13.65**
I	NARENDRA M-1 (MB2)	34.67	**4.33**	**16.67**	**3.67**	**43.00**	7.33	5.63	74.00	3.50	9.96
I	MSO-9 (MB5)	**60.33**	**3.67**	**21.33**	2.33	40.33	**10.67**	**9.73**	**78.00**	**4.90**	**15.82**
I	T-1 (MB8)	**61.00**	**3.67**	12.33	**5.67**	**56.00**	**11.67**	**7.30**	75.00	3.57	10.80
I	OUM-11-5 (MB11)	**51.33**	2.67	11.67	**5.00**	38.33	**9.67**	6.30	**85.00**	3.47	8.51
I	LGG-499 (MB6)	**85.33**	**4.67**	**19.00**	**3.33**	**67.00**	**10.00**	**7.40**	**90.00**	**4.17**	**21.09**
I	PUSA BOLD-2 (MB9)	**62.00**	**3.67**	**20.33**	2.33	36.00	**10.67**	**9.97**	74.00	**5.13**	**15.44**
I	LGG-478 (MB3)	**65.67**	**4.00**	**15.67**	**5.00**	**62.67**	**11.00**	**7.73**	**85.00**	**4.27**	**14.91**
I	IPRM-90 (MB7)	**62.67**	**5.00**	13.67	**5.00**	**57.33**	**10.67**	**6.87**	68.33	**3.93**	**19.05**
II A	MGG-47 (MB51)	38.00	3.33	13.00	3.00	31.00	7.33	6.50	75.33	2.70	7.03
II A	PDM-1 (MB53)	**50.00**	3.33	**21.67**	3.00	**44.00**	**9.67**	**7.13**	68.00	3.40	**12.31**
II A	OBGG-40 (MB52)	36.33	**5.00**	12.33	2.33	20.00	7.00	4.93	69.67	3.27	4.40
II A	OBGG-52 (MB54)	38.67	2.33	10.00	3.00	26.33	7.00	4.73	69.00	2.90	6.25
II A	PDM-84-143 (MB12)	31.67	3.67	**18.67**	**4.00**	**61.00**	6.67	4.97	**80.67**	3.57	**18.15**
II A	LM-23 (MB50)	**48.33**	2.33	13.33	**3.33**	38.67	9.00	**6.67**	75.33	2.93	8.67
II B	PS-10 (MB27)	**53.67**	2.67	**17.33**	**3.33**	29.00	**10.67**	6.77	68.00	3.57	10.52
II B	TARM-1 (MB36)	42.67	**4.00**	12.33	**3.67**	33.00	**10.00**	6.10	**81.67**	**4.07**	5.55
II B	K-1284 (MB32)	37.33	2.00	13.00	2.33	**60.67**	**10.67**	6.53	**88.67**	**4.13**	5.49
II B	ML-406 (MB28)	**48.33**	2.67	13.00	3.00	**47.00**	**10.00**	6.37	65.00	3.67	**11.91**
II B	PUSA-9332 (MB30)	21.33	3.33	8.33	2.67	18.33	**10.33**	6.00	70.33	3.79	5.91
II B	LM-1119 (MB26)	45.33	**4.33**	**23.67**	**5.00**	**61.00**	9.00	5.27	**81.33**	2.87	**12.16**
II B	EC-398889 (MB33)	**69.67**	**5.33**	12.33	2.33	24.00	**11.33**	**9.90**	54.00	**6.33**	**13.84**
II B	KM-2192 (MB29)	27.67	**4.67**	**20.33**	**3.67**	**57.00**	8.00	5.27	**86.00**	**4.07**	**11.76**
II B	MUM-1-1 (MB31)	37.00	3.00	10.00	**4.33**	31.67	8.67	6.27	**83.00**	3.07	**13.09**
II C	PDM-11 (MB41)	**56.00**	**4.67**	15.33	2.67	35.33	**11.00**	**6.87**	69.00	2.43	10.62
II C	AKM-8802 (MB44)	45.00	**4.33**	10.33	**3.67**	36.67	**10.67**	**7.40**	**86.00**	2.90	**14.44**
II C	DMG-1098-1 (MB42)	46.33	**3.67**	10.33	2.67	23.00	7.33	5.83	**85.67**	2.83	6.95
II C	MARP-280 (MB43)	**48.33**	**3.67**	**16.67**	2.33	36.00	8.00	6.03	**80.00**	3.50	9.65
II C	SUJATA (MB38)	**55.00**	3.00	15.00	2.67	39.33	**11.33**	**7.27**	73.00	2.87	10.62
II C	PIMS-11/99 (MB39)	42.33	3.33	12.33	**4.00**	**45.00**	6.67	4.67	**80.33**	3.63	**19.73**
II C	TARM-2 (MB45)	**48.00**	3.33	12.33	3.00	25.00	7.67	**6.73**	73.67	2.77	8.76
II C	ML-131 (MB47)	**60.67**	3.00	**18.67**	**5.00**	**61.00**	**11.67**	**7.83**	**80.33**	**3.83**	**12.35**
II C	NARP-280 (MB34)	**48.33**	3.33	11.67	2.33	26.33	**11.33**	**7.57**	**82.67**	3.40	9.80
II C	DMG-1103 (MB35)	**55.33**	**4.00**	13.33	**3.67**	**42.00**	8.00	5.67	**84.00**	2.83	7.24
II C	EC-398888 (MB46)	**65.33**	**4.67**	12.33	3.00	33.00	**12.00**	**12.80**	67.33	**5.33**	**14.71**
II C	EC-393407 (MB40)	46.00	3.33	**18.33**	2.33	29.33	**12.00**	6.47	**77.67**	**4.21**	7.16

II C	OBGG-11 (MB49)	34.00	2.67	10.00	**4.33**	**42.33**	6.33	6.13	67.67	3.27	10.94
II C	WGG-37 (MB37)	35.67	3.33	13.00	3.00	31.00	6.67	5.40	74.67	**4.03**	**11.67**
II C	PM-9001 (MB48)	**52.67**	3.33	**16.67**	3.00	40.33	8.00	6.10	**77.67**	3.43	9.73
II D	HUM-1 (MB13)	46.67	**3.67**	12.33	**4.33**	**51.33**	7.67	6.37	65.00	3.37	**14.07**
II D	V-557 (MB14)	**65.33**	**4.00**	**17.67**	**3.33**	**46.67**	**11.00**	**7.43**	**78.00**	**4.03**	**14.85**
II D	MH-309 (MB22)	**57.00**	**3.67**	13.33	**3.33**	38.33	**10.67**	**7.20**	70.00	3.80	**11.27**
II D	PDM-89-226 (MB24)	45.67	3.00	10.33	**4.33**	**49.00**	**11.00**	**6.73**	66.33	3.63	**17.85**
II D	LGG-477 (MB23)	**65.67**	**3.67**	**21.33**	2.33	**46.67**	**12.00**	**8.20**	**85.67**	3.57	**12.91**
II D	V-4512 (MB17)	**53.67**	3.33	**19.00**	2.67	39.33	**10.00**	6.07	74.67	2.83	5.56
II D	LLR-1 (MB15)	28.00	3.00	10.00	3.00	22.67	6.67	4.73	**78.00**	1.80	2.52
II D	ML-287 (MB16)	**58.67**	**4.00**	**22.33**	**3.33**	**75.33**	7.33	**6.77**	**81.67**	**3.87**	**16.15**
II D	K-851 (MB25)	38.33	3.33	14.33	2.67	27.33	7.33	5.10	73.33	2.87	6.36
II D	ML-583 (MB20)	**52.67**	**3.67**	**16.67**	2.33	34.67	**10.67**	**7.00**	73.33	**3.90**	**11.24**
II D	LAM-M2 (MB19)	**80.00**	**5.00**	**19.67**	3.00	**51.00**	9.33	**6.73**	**92.33**	3.50	**12.81**
II D	AKM-9601 (MB18)	**52.00**	2.67	13.00	**4.67**	**50.33**	**11.33**	**7.57**	**85.00**	**4.07**	**13.33**
II D	LGG-491 (MB21)	**65.00**	**4.67**	**24.33**	2.67	**57.00**	9.00	**8.10**	**90.00**	**4.53**	**17.81**
	Trait Grand Mean	**47.45**	**3.66**	**15.41**	**3.29**	**40.57**	**9.55**	**6.57**	**77.39**	**3.82**	**11.04**

[a]The accessions are arranged in the order in which they appear in the NJ dendrogram clusters based on the RAPD data.

these accessions not only in relation to each other but also with reference to the position of the accession in the NJ dendrogram.

Discussion

High level of polymorphism has been observed with RAPD marker, revealing a wide and diverse genetic base of the germplasm accessions analysed. Earlier, low to moderate polymorphism was observed while analysing 32 Indian mung bean cultivars using 21 RAPD primers (Lakhanpaul *et al*. 2000). However, as stated earlier, the present study with a larger number of accessions representing more geographical regions is more efficient in resolving the extant genetic diversity among these accessions. The different accessions may have common pedigrees (whole or partial) and may have been subjected to same selection during their breeding but are still distinguishable from each other on the basis of their RAPD profiles. On the basis of similarity coefficients and cluster analysis, accessions WGG-37, PDM-1 and OBGG-52 were found to be quite distinct and they can be used for their desirable characteristics in breeding programmes for mung bean improvement. The genetic similarities obtained from the analysis can also be used for the selection of the parents to generate mapping populations and for selecting parents for breeding purposes.

Clustering of cultivars into five clusters showed reasonable variability that may be exploited for yield improvement (Afzal *et al*. 2004). The dendrogram obtained by the NJ method (figure 2) did not show any significant correlation between the genetic divergence and geographical distribution. The accessions belonging to the same region or developed by the same organization did not show any significant grouping. For example the varieties viz., PDM-84-139, PDM-1, PDM-11 and PDM-89-226 developed by Project Directorate, Kanpur, distributed into clusters I, II A, II C and II D, respectively. In contrast, the varieties developed from different geographical regions are grouped into same cluster. For example cluster II B includes, PS-10, TARM-1, K-1284 and ML-406, and II D includs K-851, ML-583, AKM-9601 and LGG-491. Such clustering of the cultivars of different locations ignored the influence of geographic variations within the genetic diversity of mung bean. Such a lack of correlation between geographic diversity and genetic diversity in mung bean has also been reported by Bisht *et al*. (1998) and Manivannan *et al*. (1998).

An important observation was made for the agronomic performance of the accessions and their distribution in the NJ dendrogram clusters. As it is clear from table 4, when the accessions were arranged in order of their occurrence in the different clusters and their performance for 10 traits recorded, it is apparent that the different clusters have different traits in common among them. Thus all the accessions in cluster I, have plants that are above average regarding plant height and number of seeds per pod. Cluster IIA plants are

distinctly below average in weight of 100 seeds relative to all the other accessions, while those of cluster IIB plants are below average in pod length. Cluster IIC and IID plants are both medium performers but distinguished from each other in that cluster IIC plants show greater plant height, fewer days to maturity and heigher seed yield per plant. While the above average or below average are relative parameters, plants of cluster I are in general showing a superior performance in most of the traits examined except days to maturity where more than half the plants have below average performance. Such a distribution of clusters based on agronomic performance and supported by RAPD profile analyses are a very good starting point for further breeding efforts involving contrasting parental lines, and will further enable tagging of genes and identification of QTLs for these traits with molecular markers. A simple prediction based on the present study will reveal that the plants of the cluster I are in general the superior performing varieties and it is these varieties that should be used not only for commercialization but also as, the starting material for further improvement by breeders.

In a wider context, the speed, efficiency and reliability of the RAPD methodology are important considerations for development of strategies for effective management of germplasm collection in terms of identification of duplicates, the estimation of diversity, monitoring genetic erosion and enhancing the use of these collections. RAPD markers have been used for the identification of cultivars and the genetic relationships among cultivars of other leguminous crops including *Phaseolus vulgaris* (Skroch *et al*. 1992), cowpea (Mignouna *et al*. 1998), *Vigna angularis* (Yee *et al*. 1999) and *Medicago polymorpha* (Paredes *et al*. 2002). The present study has also shown the usefulness of the method not only to assess the range of diversity in the germplasm but also to enable potential clustering of accessions on basis of their affinities to each other in agronomic performance traits.

Acknowledgements

The first author gratefully acknowledges the Hon'ble Vice Chancellor and former Director of Research, Allahabad Agricultural Institute-Deemed University, Allahabad for providing all facilities required during the period of study.

References

Afzal M. A., Haque M. M. and Shanmugasundaram S. 2004 Random amplified polymorphic DNA (RAPD) analysis of selected mungbean (*Vigna radiata* L. Wilczek) cultivars. *Asian J. Pl. Sci*. **3**, 20–24.

Arunachalam V. 1981 Genetic distance in plant breeding. *Indian J. Genet*. **14**. 226–236.

Bisht I. S., Mahajan R. K. and Kawalkar T. G. 1998 Diversity in mungbean (*Vigna radiata* L. Wilczek) germplasm collection and its potential use in crop improvement. *Ann. Appl. Biol*. **132**, 301–312.

Chattopadhyay K., Ali M. N., Sarkar H. K., Mandal N. and Bhattacharyya S. 2005 Diversity analysis by RAPD and ISSR markers among the selected mungbean [*Vigna radiata* (L.) Wilczek] genotypes. *Indian J. Genet. Pl. Breed*. **65**, 173–175.

Lakhanpaul S., Chadha S., Bhat K. V. and Chadha S. 2000 Random amplified polymorphic DNA analysis in Indian mungbean (*Vigna radiata* L. Wilczek) cultivars. *Genetica* **109**, 227–234.

Manivannan N., Murugan E., Viswanathan P. L. and Dhanakodi C. V. 1998 Genetic divergence in greengram. *Legume Res*. **21**, 131–133.

Mignouna H. D., Ng N. Q., Ikea J. and Thotapilly G. 1998 Genetic diversity in cowpea as revealed by random amplified polymorphic DNA. *J. Genet. Breed*. **53**, 151–159.

Newbury H. J. and Ford-Lloyd B. V. 1993 The use of RAPD for assessing variation in plants. *Plant Growth Regulation* **12**, 43–51.

Orozco-Castillo C., Chalmers K. J., Waugh R. and Powell W. 1994 Detection of genetic diversity and selective gene introgression in coffee using RAPD markers. *Theor. Appl. Genet*. **87**, 934–940.

Page R. D. M. 2001 TreeView (Windows 32) version 1.6.5. Distributed by author. http://taxonomy.zoology.gla.ac.uk/rod/rod.html.

Paredes M., Becerra V., Rojo C., Del Pojo A., Ovalle C. and Aronson J. 2002 Ecotypic differentiation in *Medicago polymorpha* L. along an environmental gradient in central Chile. RAPDs studies shows little genetic divergence. *Euphytica* **123**, 431–439.

Pavlicek A., Hrda S. and Flegr J. 1999 Free Tree - Freeware program for construction of phylogenetic trees on the basis of distance data and bootstrapping / jackknife analysis of the tree robustness. Application in the RAPD analysis of the genus *Frenkelia. Folia Biol*. (Praha.) **45**, 97–99.

Ramanujam S. 1981 Biometrical basis for yield improvement in mungbean. In: *Procedings 1st International Mung bean Symposium*, (ed. R. Cowell) pp. 210 AVRDC, Shanhua.

Saini A., Reddy K. S. and Jawali N. 2004 Evaluation of long primers for AP-PCR analysis of mungbean [*Vigna radiata* (L.) Wilczek]: Genetic relationships and fingerprinting of some genotypes. *Indian J. Biotechnol*. **3**, 511–518.

Santalla M., Power J. B. and Davey M. R. 1998 Genetic diversity in mungbean germplasm revealed by RAPD markers. *Plant Breed*. **117**, 473–478.

Skroch P. W., dos Santos J. B. and Nienhuis J. 1992 Genetic relationships among *Phaseolus vulgaris* genotypes based on RAPD marker data. *Annu. Rep. Bean Improv. Coop*. **35**, 23–24.

Virk P. S., Ford-Lloyd B. V., Jackson M. T. and Newbury H. J. 1995 Use of RAPD for the study of diversity within plant germplasm collections. *Heredity* **74**, 170–179.

Wachira F. N., Waugh R., Hackett C. A. and Powell W. 1995 Detection of genetic diversity in tea (*Camellia sinensis*) using RAPD markers. *Genome* **38**, 201–210.

Williams J. G. K., Kubelik A. R., Livak K. J., Rafalski J. A. and Tingey S. V. 1990 DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Res*. **18**, 6231–6235.

Yee E., Kidwell K. K., Sills G. R. and Lumpkin, T. A. 1999 Diversity among selected *Vigna angularis* (Azuki) accessions on the basis of RAPD and AFLP markers. *Crop Sci*. **39**, 268–275.

Received 8 July 2007; accepted 14 November 2007
Published on the Web: 2 April 2008