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Estimation of genetic divergence among elite mungbean (*Vigna radiata*) genotypes by RAPD analysis

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Abstract

Genetic diversity among 15 mungbean genotypes of Pakistan was assessed through Random Amplified Polymorphic DNA (RAPD) analysis, with 30 random decamer primers using polymerase chain reaction (PCR). A total of 370 bands were observed, with 12.3 bands per primer, of which 91.6% were polymorphic. OPG-08 produced maximum number of fragments while minimum numbers of fragments were produced with primer OPH-05. Cluster analysis by the Unweighted Paired Group Method of Arithmetic means (UPGMA) showed that these 15 genotypes could be classified in five groups with a similarity ranging from 0.48-0.86. Maximum similarity was observed between NM-51, NM-54 and NM-98 (0.86). Interestingly, these mungbean genotypes have been developed at one breeding center, while ML-5 was found the least similar line due to its exotic nature. The analysis revealed that the inter-varietal genetic relationship of several genotypes is related to their center of origin. Most of the mungbean genotypes have a narrow genetic base. These results correspond well with previous reported results on mungbean from other countries. The RAPD analysis indicated that it may be a more efficient marker than morphological marker, isozyme and RFLP technology. Based on present results, these genotypes could be successfully utilized in selecting divergent parents for breeding and mapping purposes in future.

Key words: Cluster Analysis, Mungbean genotypes, Diversity, RAPD, Genetic similarities

1. Introduction

Mungbean (*Vigna radiata* L. Wilczek) 2n=2x=22 is one of the most important pulse crop of Pakistan with an average yield of 546 kg ha^{-1} and is grown over an area of 0.21 million hectares annually (MINFAL, 2007). On the basis of area and production it is the second largest pulse crop after chickpea. It is bi-annually cultivated crop because of its high degree of heat tolerance up to 40°C. India, the central Asian region, is known as the centre of diversity and domestication (Vavilov, 1951; Smartt, 1985).

To identify the useful and effective germplasm for the development of line of an exhaustive characteristic with maximum diversity should be selected. The crosses between parents with maximum genetic divergence are generally the most responsive for bringing up the genetic improvement (Arunachalam, 1981) but this practice resulted in a narrow genetic base for the new mungbean varieties.

Previously morphological markers, with their complex and undeciphered genetic control, were used for individual identification. Morphological features are indicative of genotypes but are represented by only a few loci because there are not a large enough number of characters available. Moreover, they can also be affected by environmental factors and growth pattern. The RAPD technique generates molecular markers for comparative analysis that are quick, effective, easy to use, free from environmental influences, unlimited in number, random but have wide coverage of genome and have a relatively higher level of polymorphism (Newbury and Ford-Lloyd, 1993).

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The RAPD technique provides an unlimited number of markers which can be used for various purposes like cultivar analysis and species identification in most crop plants (Williams *et al*., 1990). (Multani and Lyopreegn, 1995) reported that RAPD markers could be used to distinguish closely related varieties of same species. A few studies have been carried out using RAPD alone or in combinations with Inter-simple sequence repeat (ISSR) for finding out the diversity in mungbean (Roopa *et al*., 2008; Afzal *et al*., 2004; Saini *et al*., 2004; Lakhanpaul *et al*., 2000; Bisht *et al*., 1998; Santalla *et al*., 1998;). Muthusamy *et al*., (2008) studied RAPD and ISSR markers in rice bean and got 719 amplification products from RAPD and 479 from ISSR. Comparing the results of diversity obtained from RAPD with ISSR in the common bean, Galvan *et al.*, (2003) identified that the ISSR is more effective to separate the genotypes on the basis of gene pool. Keeping in view these findings of the earlier researchers, the present research work was, therefore, planned to elucidate the genetic divergence of popular cultivated varieties of *Vigna. radiata* in Pakistan.

2. Materials and Methods

2.1. Plant material

A total of 15 commercial varieties of mungbean, one from India and 14 from Pakistan, (Table 1) were investigated in the present study. Plants were raised in growth chamber under control conditions using vermiculate as growth media for good plant stand and soft leaf tissues.

Table 1. Name, pedigree and centre of origin of 15 mungbean (*Vigna radiata*) genotypes

Genotypes	Pedigree	Center of origin
Ramzan	VC 1482C × NM 92	NIFA, Peshawar, Pakistan
NM-92	VC 2768B × NM 36	NIAB, Faisalabad, Pakistan
NM-98	NM 20-21 × VC 1482E	NIAB, Faisalabad, Pakistan
NM-51	VC1973A x 6601	NIAB, Faisalabad, Pakistan
NM-28	Local Selection	NIAB, Faisalabad, Pakistan
ML-5	No.54 × Hyb.45	PAU, Ludhiana, India
AEM-96	Irradiating 6601	NIA, Hayderabad, Pakistan
NM54	VC1973A × 6601	NIAB, Faisalabad, Pakistan
Pak 22	Local Selection	NIAB, Faisalabad, Pakistan
NM89	NM 20-21 × VC1482E	NIAB, Faisalabad, Pakistan
6601	Local Selection	NIAB, Faisalabad, Pakistan
NM 20-21	Irradiated Pak-22	NIAB, Faisalabad, Pakistan
NM 19-19	Irradiated Pak-22	NIAB, Faisalabad, Pakistan
Chakwal Mung-97 (CM-97)	Local Selection	BARI, Chakwal, Pakistan
AZRI Mung-06	CI/94-4-19	AZRI, Bhakkar, Pakistan

2.2. DNA isolation

Total genomic DNA was isolated with the modified CTAB method (Saghai-Maroof *et al*., 1984) and the DNA quantification was carried out using NanoDrop 1000 Spectrophotometer V3.7.1 Thermo Fisher® Scientific, Inc (Figure 1; Table 2). Approximately 250 mg leaf material was grinded to a fine powder using liquid nitrogen and quickly transferred into 25 ml of pre-warmed (60^0C) isolation buffer in a capped polypropylene tube, incubated for 1 hour at 65^0C in a water bath and mixed by swirling gently with an interval of every 10 minutes. Equal volume of Chloroform: Isoamyl alcohol (CI) was added to these tubes and the contents were hand shaken for 10 minutes.

Figure 1. Thermo Scientific NanoDrop^{TM} 1000 Spectrophotometer used for quantification of genomic DNA

Table 2.Quantification of genomic DNA using NanoDrop 1000 Spectrophotometer software 3.7.1

Sample ID	ng/ul	A260	A280	260/280	260/230	Cursor abs.	340 raw
Ramzan	4521.25	90.425	45.585	1.98	1.93	46.878	4.097
NM-92	3510.52	70.21	35.512	1.98	1.56	44.93	10.33
NM-98	2284.29	45.686	22.297	2.05	1.80	25.349	4.49
NM-51	4272.21	85.444	45.742	1.87	1.35	63.33	13.264
NM-28	2010.67	40.213	20.973	1.92	1.47	27.425	6.066
ML-5	3466.86	69.337	36.458	1.90	1.33	52.02	12.789
AEM-96	3054.09	61.082	31.029	1.97	1.75	34.941	3.678
NM54	2864.69	57.294	29.338	1.95	1.63	35.193	4.884
Pak 22	2024.15	40.483	21.752	1.86	1.46	27.657	6.912
NM89	2449.08	48.982	25.027	1.96	1.63	29.987	7.259
6601	3299.11	65.982	32.777	2.01	1.94	33.966	3.213
NM 20-21	3036.71	60.734	30.326	2.00	1.87	32.51	4.843
NM 19-19	1980.42	39.608	19.884	1.99	1.83	21.594	1.923
CM-97	2174.65	43.493	21.427	2.03	1.92	22.613	2.058
AZRI Mung-06	3042.46	60.849	30.376	2.00	1.88	32.362	3.429

The tubes were centrifuged for 10 minutes at 8000 rpm; the upper aqueous layer was extracted twice with fresh CI and the final aqueous layer was transferred to a centrifuge tube. To these tubes, 0.6 volume of ice-cold isopropanol was added and shaken for several times. By using a glass hook, DNA was spooled out in the form of whitish fibers and transferred to washing solution after drying. DNA was dissolved in an appropriate volume of 1X TE buffer.

For purification, RNase-A was added to the tube (50 μgml^{-1}) and the mixture was incubated for 1 hour at 37^0C. DNA was extracted with CI by centrifuging the tubes at 10,000 rpm for 5 minutes at room temperature. DNA was precipitated with 2 volume of ice cold absolute ethanol and was recovered by centrifuging the tubes at 5000 rpm for 10 minutes. The pellet was washed with 70% ethanol and dissolved in appropriate volume of IX TE.

2.3. RAPD analysis

Thirty random primers (Table 3) of 10-base oligonucleotide of Operon Technologies for the PCR provided by the automated genome facility Southern Illinois University Carbondale (SIUC), USA were used for divergence study in mungbean. PCRs were carried out in 0.05 cm^3 reaction volumes each containing 50 ng of genomic template DNA diluted from master genomic solution (Table 2), 0.2 µM of the particular primer, 100 µM of each dNTP, 2 µl of Taq DNA polymerase 10X buffer, 1 unit Taq polymerase (Perkin Elmer) and 2.5 mM $MgCl_2$. PCR amplification was performed on a Gene AMP PCR System 9700 from PE Applied Biosystems® under the following conditions: Initial denaturation at 95^0C for 5 minutes, followed by 45 cycles of denaturing at 94^0C for 3 minutes, annealing at 32^0C for 1 minute, extension at 72^0C for 1 minute and final extension at 72^0C for 5 minutes. The amplification products obtained from PCR along with 1kb DNA ladder of NEB® were checked on 0.8% agarose gel tray from Maxicell® EC 360M electrophoretic gel system E-C apparatus corporation with electric supplier EC 154 and visualized under UV light using Quantity One® software, following staining with ethidium bromide.

2.4. Data analysis

The frequency of RAPD polymorphism was calculated based on presence (taken as 1) or absence (taken as 0) of common bands (Ghosh *et al*., (1997). The binary data were used to compute pair-wise Jaccard Similarities Coefficient on NTSYS-PC. A dendrogram based on similarity coefficient was generated by using the unweighted pair group of arithmetic means (UPGMA).

Table 3. Primers, their sequences and basic temperature used for mungbean *V. radiata* L. Wilczek) genotypes amplification.

Primer	Sequence	Basic Temperature ^{0}C
OPG-02	GGCACTGAGG	34
OPG-03	GAGCCCTCCA	34
OPG-04	AGCGTGTCTG	32
OPG-06	GTGCCTAACC	32
OPG-07	GAACCTGCGG	34
OPG-08	TCACGTCCAC	32
OPG-09	CTGACGTCAC	32
OPG-10	AGGGCCGTCT	34
OPG-11	TGCCCGTCGT	34
OPG-14	GGATGAGACC	32
OPG-15	ACTGGGACTC	32
OPH-01	GGTCGGAGAA	32
OPH-02	TCGGACGTGA	32
OPH-03	AGACGTCCAC	32
OPH-04	GGAAGTCGCC	34
OPH-05	AGTCGTCCCC	34
OPH-06	ACGCATCGCA	32
OPH-07	CTGCATCGTG	32
OPH-09	TGTAGCTGGG	32
OPH-10	CCTACGTCAG	32
OPH-11	CTTCCGCAGT	32
OPH-12	ACGCGCATGT	32
OPH-13	GACGCCACAC	34
OPH-14	ACCAGGTTGG	32
OPH-15	AATGGCGCAG	32
OPH-16	TCTCAGCTGG	32
OPH-20	GGGAGACATC	32
OPZ-01	TCTGTGCCAC	32
OPZ-05	TCCCATGCTG	32
OPZ-09	CACCCCAGTC	34

3. Results and Discussion

Genomic DNA of 15 mungbean genotypes were amplified with 30 different random primers of Operon Technologies, USA. All 15 genotypes with 30 primers revealed a unique banding pattern. This might be indicative of a wide genetic base of mungbean genotypes studied. Different primers produced a different level of polymorphism among the different varieties (Figure. 2A, 2B, 2C, 2D).

A total of 370 DNA fragments were amplified, with an average of 12.3 RAPD amplification products per primer. Out of 370 amplified fragments, 31 (8.4%) were found to be monomorphic. The remaining 339 (91.6%) were polymorphic in single or multiple bands of the 15 mungbean genotypes. The amplitude of polymorphisms was high even when there was not a single primer (out of 30 studied) which could differentiate clearly between all the mungbean genotypes. The size of the amplified fragments also varied with different primers. The approximate size of the largest fragment produced was 3.0 kb and the smallest fragment produced was approximately 0.25 kb. Out of the 15 genotypes studied, NM-28 (released by NIAB, Faisalabad, Pakistan) produced the maximum number of DNA amplified fragment (248), while ML-5 (released by Punjab Agricultural University, Ludhiana, India) produced 153 bands, which is the minimum number. Other mungbean genotypes produced between 189 and 236 bands in common. The variety NM-54 and NM 19-19 (both released by NIAB, Faisalabad, Pakistan) produced 223 bands, which were maximum common bands for tested genotype. A maximum of 14 fragments were amplified with primer OPG-08 and a minimum of 8 bands with primer OPH-05.

To estimate the genetic similarities of the mungbean genotypes, a similarity matrix obtained using Jaccard coefficients shown in Table 4. These similarity coefficients were used to generate a dendrogram (Figure. 3) by UPGMA analysis in order to determine the grouping of different varieties. Maximum similarity was observed between NM-51, NM-54 and NM-98 (0.86). Interestingly, these varieties have been developed at one breeding center. On the basis of the RAPD data their genetic bases looked very narrow. From the similarity matrix, the least similar genotype was ML-5 as it has been an exotic line of India. Its similarity ranges from 0.48 to 0.69. The coefficient of similarity of most of the

other varieties ranges between 0.48 to 0.83. Using 21 decamer RAPD markers Lakhanpaul *et al*., (2000) got a total of 267 amplification products with an average of 12.71 per primer with an overall polymorphism of 64% using 32 Indian mungbean genotypes. The extent of polymorphism was moderate to low and the Jaccard similarity coefficient values ranged 0.65 to 0.92. Muthusamy *et al*., (2008) assessed genetic diversity among 10 land races of rice bean genotypes and compared the RAPD and ISSR data. In their studies, the RAPD generated more polymorphic loci (70.30%) than the ISSR which were only (61.79%). The pairwise similarity index ranged from 0.530 to 0.782, while Galvan *et al*., (2003) concluded that ISSR is a better tool than RAPDs to identify beans by gene pool of origin though they did not reveal as many differences between individuals as RAPDs.

Cluster analysis using RAPD resulted in five main cluster groups. The dendrogram (Figure. 3) assigned the mungbean genotypes into groups which correspond well with their centers or sub centers of release and or pedigree relationship. In cluster 'B' three genotypes NM-51, NM-54 and NM-98 are more closely related as compared to any other variety. They have high estimates of genetic identity (0.86). In cluster 'A' out of three mungbean varieties Ramzan and NM-92 clustered together indicating that they are more closely related as compared to ML-5 since Ramzan and NM-92 are indigenous while the ML-5 is an exotic line. The cluster 'C' and 'D' comprises three varieties each namely NM 20-21, NM-89, NM 19-19 and NM-28, Pak-22, 6601, with similarities ranges between 0.70- 0.77 and 0.70-0.82, respectively. The variety ML-5 showed a similarity 0.74 with the rest of the varieties of cluster B, C and D. The cluster 'E' comprised of two varieties Chakwal Mung-97 (CM-97) and AZRI Mung-06. The clustering of the varieties might be due to selection of the elite lines from a single population. High level of polymorphism has been observed with RAPD marker, revealing a wide and diverse genetic basis of the germplasm accessions analysed (Roopa *et al*., 2008) and its correlation with agronomic and morphological parameters. Earlier, low to moderate polymorphism was observed while analysing 32 Indian mungbean cultivars using 21 RAPD primers (Lakhanpaul *et al*., 2000). Moreover, breeders mostly crossing the elite lines of other breeding countries in mungbean improvement programs, making the breeding material identical which ultimately result in close kinship of the varieties. The genetic similarity obtained from the analysis will be useful in selecting divergent parents for breeding and mapping purposes.

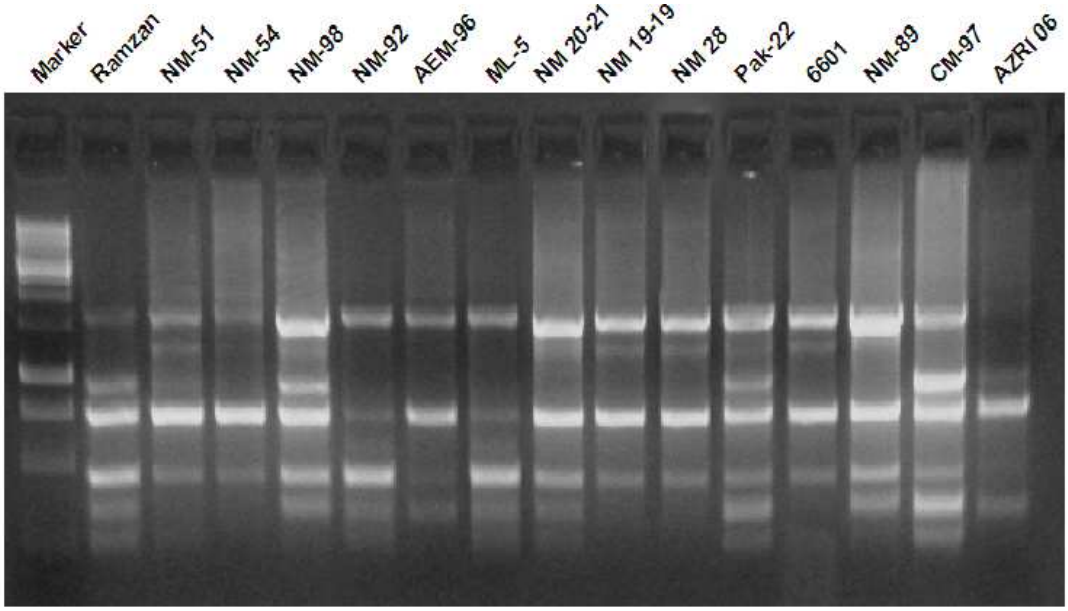

Figure 2A. Amplification products obtained using RAPD Primer OPG-08

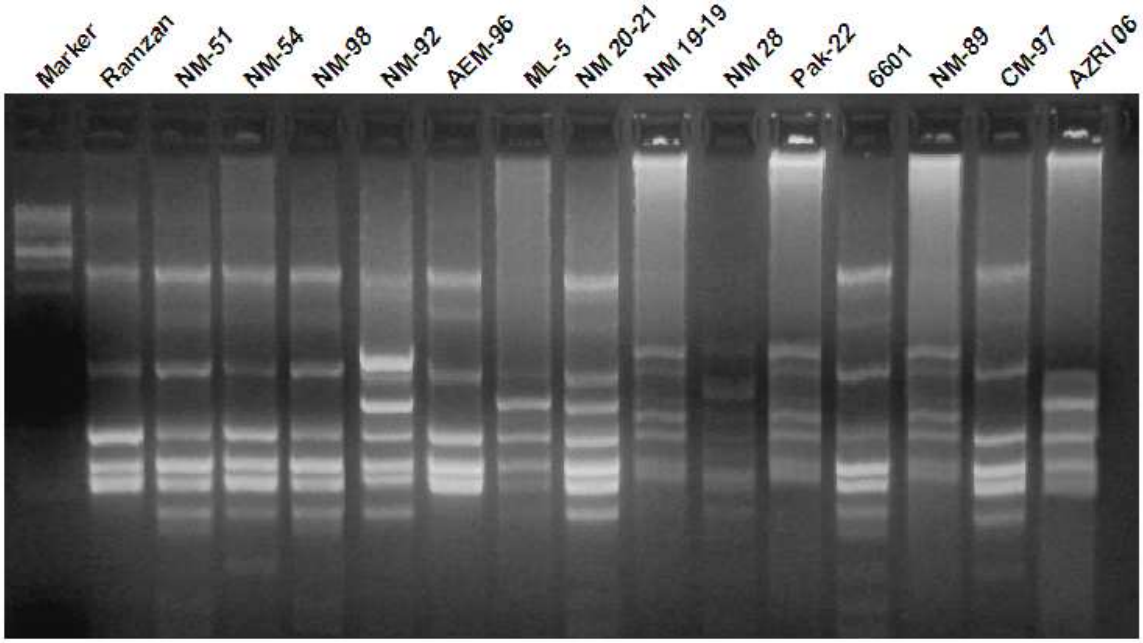

Figure 2B. Amplification products obtained using RAPD Primer OPG-09

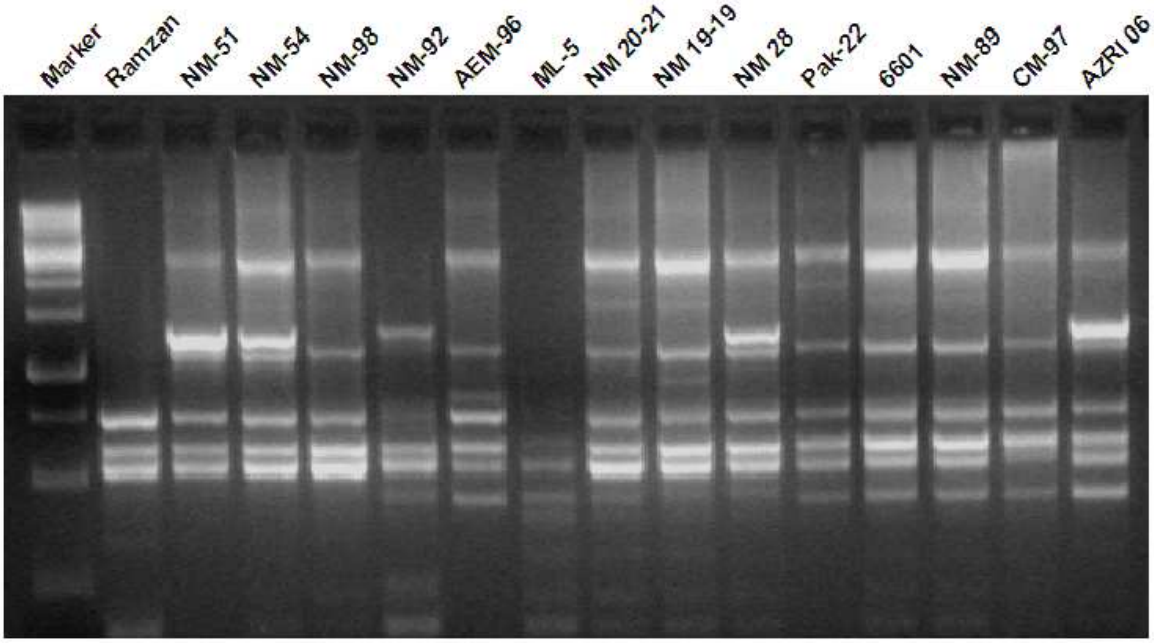

Figure 2C. Amplification products obtained using RAPD Primer OPH-03

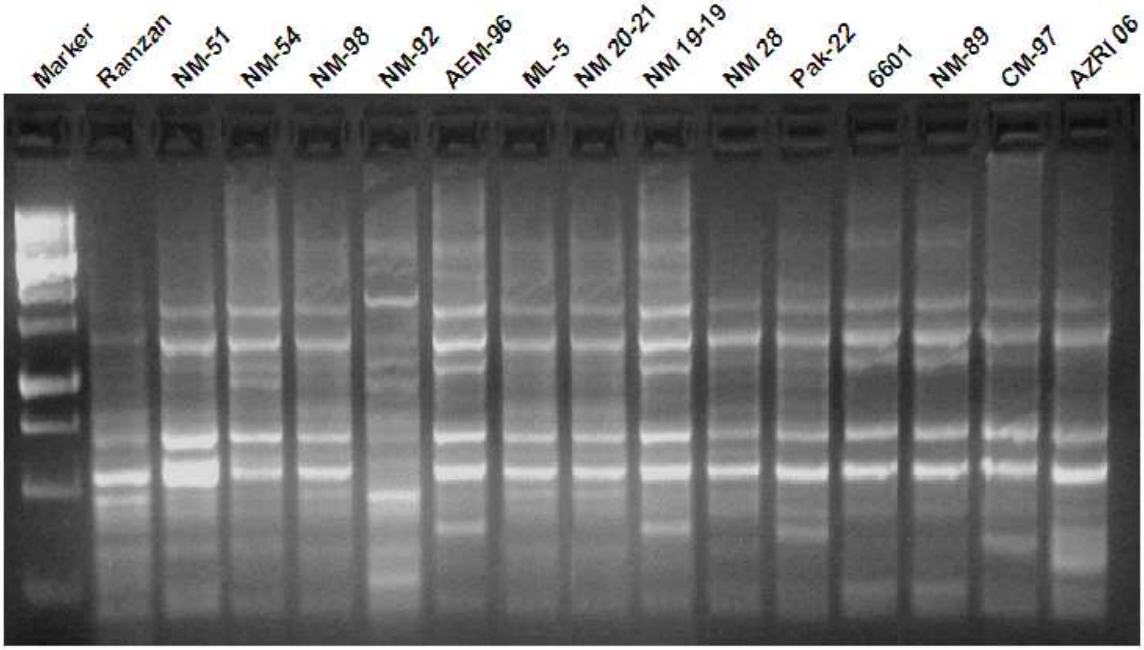

Figure 2D. Amplification products obtained using RAPD Primer OPH-05

Table 4. Similarity matrix for Jaccard coefficients of 15 mungbean genotypes obtained from RAPD marker analysis

	Ramzan	NM-51	NM-54	NM-98	NM-92	AEM-96	ML-5	NM 20-21	NM19-19	NM-28	Pak-22	6601	NM-89	CM -97	AZRI M6
Ramzan	1.00														
NM-51	0.67	1.00													
NM-54	0.65	0.86	1.00												
NM-98	0.65	0.82	0.83	1.00											
NM-92	0.69	0.60	0.55	0.60	1.00										
AEM-96	0.58	0.72	0.74	0.74	0.56	1.00									
ML-5	0.63	0.57	0.53	0.55	0.69	0.50	1.00								
NM 20-21	0.60	0.75	0.75	0.76	0.59	0.74	0.57	1.00							
NM 19-19	0.60	0.76	0.75	0.77	0.53	0.74	0.54	0.77	1.00						
NM-28	0.61	0.75	0.78	0.81	0.58	0.72	0.51	0.76	0.76	1.00					
Pak-22	0.62	0.76	0.75	0.75	0.55	0.74	0.48	0.74	0.77	0.82	1.00				
6601	0.58	0.72	0.71	0.74	0.61	0.72	0.54	0.73	0.75	0.82	0.81	1.00			
NM-89	0.60	0.72	0.72	0.76	0.59	0.71	0.50	0.78	0.76	0.78	0.76	0.76	1.00		
CM-97	0.60	0.70	0.70	0.72	0.58	0.70	0.52	0.70	0.72	0.70	0.72	0.68	0.76	1.00	
AZRI M6	0.63	0.76	0.73	0.74	0.57	0.70	0.55	0.75	0.74	0.74	0.73	0.74	0.77	0.75	1.00

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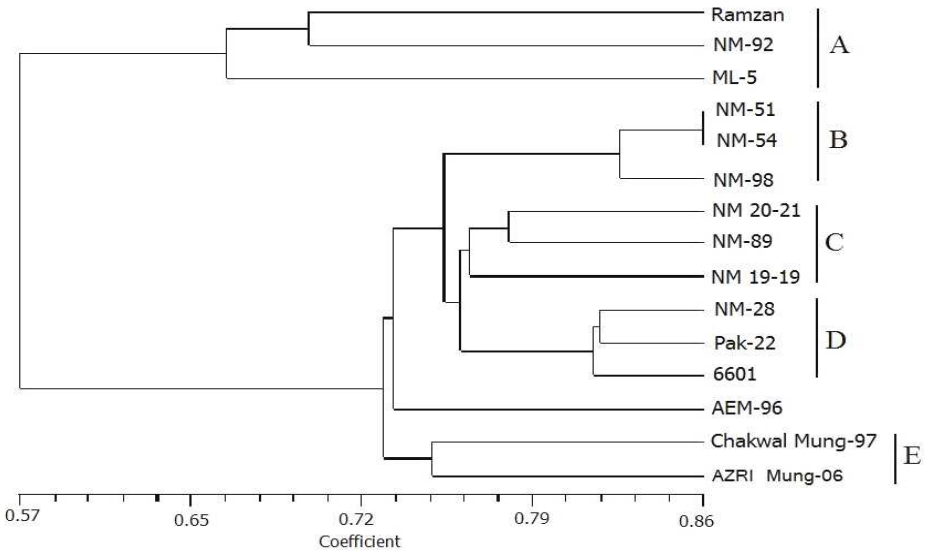

Figure 3. Dendrogram of 15 mungbean genotypes developed from RAPD data using Unweighted Pair Group Method of Arithmetic means (UPGMA).

References

Afzal, M.A., Haque, M.M., 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., 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.

Galvan, M.Z., Bornet, B., Balatti, P.A., Branchard, M. 2003. Inter simple sequence repeat (ISSR) markers as a tool for the assessment of both genetic diversity and gene pool origin in common bean (Phaseolus vulgaris L.). Euphytica 132: 297–301.

Ghosh, S., Karanjawala, Z.E., Hauser, E.R. 1997. Methods for precise sizing, automated inning of alleles and reduction or error rates in large scale genotyping using fluorescently labeled dinucleotide markers. Genome Res. 7: 165-178.

Jaccard, P. 1908. Nauvelles recherches sur la distribution florale. Bull Soc Vaudoise Sci Nat. 44: 223-270.

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

MINFAL, 2007. Agricultural Statistics of Pakistan.

Multani, D.S., Lyon, B.R. 1995. Genetic fingerprinting of Australian cotton cultivars with RAPD markers. Genome. 38: 1005-1008.

Muthusamy, S., Kanagarajan, S., Shanmugasundaram, P. 2008. Efficiency of RAPD and ISSR markers system in accessing genetic variation of rice bean (Vigna umbellata) landraces. Electronic J. Biotech. 3 (11): 1-10.

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

Roopa, L.G., Jyoti, S., Shirish, A.R. 2008. Molecular assessment of genetic diversity in mungbean germplasm. J. Genet. (87) 1: 65-74.

Saghai-Maroof, M.A., Soliman, K.M., Jorgensen, R.A., Allard, R.W. 1984. Ribosomal DNA spacer length polymorphism in barley: Mendelian inheritance, chromosomal location and population dynamics. Proc. Nat. Acad. Sci., USA. 81: 8014-8018.

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

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

Smartt, J. 1985. Evolution of grain legumes III. Pulses in the genus Vigna. Exp. Agric. 21: 87-100.

Vavilov, N.I. 1951. The origin, variation, immunity and breeding of cultivated plants. (Translated by K.S Chester). Chronica Botanica 13: 1-364.

Williams, J.G.K., Kubelik, A.R., Levak, K.J., Rafalski, J.A., Tingey, S.V. 1990. DNA polymorphism amplification by arbitrary primers are useful as genetic markers. Nucleic Acid Res. 18: 6531-6535.

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