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Random Amplified Polymorphic DNA (RAPD) Analysis of Selected Mungbean [*Vigna radiata* (L.) Wilczek] Cultivars

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Abstract: A laboratory experiment was conducted at the Asian Vegetable Research and Development Center, Taiwan to assess the genetic diversity of mungbean [*Vigna radiata* (L.) Wilczek] cultivars using DNA markers. In this study, 21 mungbean cultivars were subjected to RAPD analysis using 34 decamer primers. All the primers produced polymorphic amplification products with some extent of variation. A total of 204 bands were generated with an average of 6.0 per primer and exhibited 75.0% polymorphism. Jaccard's similarity coefficient ranged from 0.54 to 0.85 and concentrated mostly between 0.61 to 0.74. This indicated a rather narrow genetic base of tested cultivars. Genetic similarity obtained in this study may be used for selecting parents for breeding purposes. Clustering of cultivars into four groups showed reasonable variability that may be exploited for yield improvement.

Key words: Mungbean, genetic diversity, RAPD marker

INTRODUCTION

Mungbean [*Vigna radiata* (L.) Wilczek] is a grain legume widely grown in the tropics and subtropics and is an excellent source of dietary protein. It is cultivated in rotation with cereals and therefore important as a nitrogen fixer for maintaining soil fertility. However, the average yield of 384 kg ha^{-1} worldwide is very low[1]. Many biotic and abiotic stresses such as disease, insect pests and drought limit mungbean yields. Despite the efforts of plant breeders during the past few decades, the yield of mungbean has not increased substantially due to lack of sufficient genetic diversity for desirable traits in the germplasm used for improvement[2]. It is imperative to further explore the genetic diversity available in this crop for better utilization of genetic resources in yield improvement.

Several markers may be used to identify and assess the genetic diversity and phylogenetic relationships in plant genetic resources. The traditional method based on morphological traits requires extensive observation of mature plants but cannot serve as unambiguous markers because of environmental influences[3]. Protein and isozyme electrophoresis may be used, but the major limitation of these techniques is insufficient polymorphism among the closely related cultivars. Proteins are the product of gene expression and their amounts vary in different tissues, developmental stages and environments[4]. As an alternative, randomly amplified polymorphic DNA (RAPD) technique developed by Williams *et al.*[5] is reliable, faster and easier for exploiting genetic polymorphism within and among species and populations[16]. RAPD markers have been already successfully used on many other crops[2,4,6-8]. The present study therefore, was undertaken for evaluating the genetic diversity among 21 selected mungbean cultivars using RAPD markers.

MATERIALS AND METHODS

The experiment was carried out at the Asian Vegetable research and Development Center, Taiwan using 21 cultivars (Table 1). The cultivars were chosen to compare the Bangladesh cultivars with some commonly grown Indian cultivars and newly improved lines from the Asian Vegetable Research and Development Center (AVRDC). Seeds were sown on 14 August 2002 in pots under nethouse conditions. Young, healthy leaves pooled from three 20-day-old plants were used for DNA extraction.

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Table 1: Morphological characteristics of selected mungbean cultivars used in diversity analysis

Cultivars	Source	Seed size (mg)	Seed coat color	Surface luster	Hypocotyl color
Barimung-2	Bangladesh	31.4	Green	Shiny	Purple
Barimung-5	Bangladesh	47.4	Green	Shiny	Purple
BUmug-1	Bangladesh	48.8	Green	Shiny	Purple
BUmug-2	Bangladesh		Green	Shiny	Purple
Pusa Baisakhi	India	51.8	Green	Dull	Purple
CN 9-5	AVRDC	60.8	Green	Shiny	Green
VC 6173A	AVRDC	73.0	Green	Shiny	Purple
VC 6153B-20G	AVRDC	66.3	Green	Shiny	Green
VC 6370A	AVRDC	57.4	Green	Shiny	Green
VC 6173B-11	AVRDC	73.0	Green	Shiny	Green
Basanti	India	30.2	Green	Dull	Purple
KPS 2	Thailand	57.7	Green	Shiny	Green
SML 32	India	34.7	Green	Dull	Purple
PDM 11	India	27.8	Brown	Shiny	Purple
Barimung-3	Bangladesh	28.9	Green	Shiny	Purple
Binamung-1	Bangladesh	31.6	Yellow	Dull	Purple
Binamung-3	Bangladesh	36.5	Green	Dull	Green
Binamung-4	Bangladesh	35.9	Green	Dull	Green
Binamung-5	Bangladesh	41.9	Green	Shiny	Green
Sonamung	Bangladesh	20.6	Yellow	Shiny	Purple
Barisal Local	Bangladesh	21.2	Yellow	Dull	Purple

Sample preparation: The harvested leaves were treated with liquid nitrogen and stored at -85°C until total genomic DNA was extracted using the modified protocol CTAB method (6). The DNA concentration was tested by staining DNA with ethidium bromide after electrophoresis in 0.8% agarose gel at 100V for 45 min in 0.5XTBE buffer (0.089M Tris-borate, 0.002 M EDTA, pH 8.0) with known DNA concentration standards (λ DNA, uncut). These results were estimated by AlphaImager program (AlphaImager 2000: AlphaEase TM, Alpha Innotech Corporation).

PCR and agarose gel electrophoresis: PCR reactions were carried out in a Thermolyne Thermal Cycler (Amptitron II). Each 25 µl reaction mixture contained 1X reaction buffer (10 mM Tris-HCl, pH 8.3 and 50 mM KCl), 3 mM $MgCl_2$, 1U Taq DNA polymerase; 100 µM of dNTPs (dATP, dTTP, dCTP and dGTP, all reagents from PROtech Technologies Inc.), 0.2 µM of primer and approximately 25 ng of template DNA. The initial step of PCR amplification consisted of cycles of 1 min at 94°C, 1 min at 40°C and 2 min at 72°C. This was followed by 40 cycles of denaturing at 94°C for 30 sec, primer annealing at 40°C for 30 sec, primer elongation at 72°C for 1 min, followed by an extended elongation step at 72°C for 10 min. Reaction products were mixed with 3.0 µl of 5X loading dye [70% glycerol, 0.02 MEDTA, 0.2% SDS, 0.2% bromophenol blue (w/v]). The amplification products were electrophoresed on 1.5% agarose gel at 100V for 2 h. Gels were stained with ethidium bromide and photographed under ultraviolet light using an AlphaImager Program.

Data analysis: Photography of the gels was analyzed using NTSYS software. Presence or absence (1 or 0) of the band was considered as a single trait and the binary data were used to calculate pair-wise similarity coefficients following Jaccard[9]. This matrix of similarity coefficients was subjected to unweighted pair-group method analysis (UPGMA) to generate a dendrogram using linkage procedure.

RESULTS AND DISCUSSION

Morphological characteristics provide the basic information about the magnitude of genetic variability in crops. Table 1 reveals a wide variability in seed size, seed coat color, surface luster and hypocotyl color in mungbean cultivars of different locations. Such variability is influenced by natural and artificial selection, socio-economic conditions and consumers' preferences within localities[10]. For instance, Sonamung-a small seeded, yellow color, shiny and scented land race of Bangladesh is highly preferred in Kishoregonj. Breeders take such local demand into account when developing improved cultivars of mungbean.

Thirty-four primers were randomly used to detect RAPD markers among the 21 cultivars (Table 2). A total of 204 bands were scored with different primers of which 75% exhibited polymorphism. The number of bands ranged between 2 to 12 with an average of 6.0 per primer. Out of 34 primers, 14 primers showed more than 80% polymorphism. The primer UBC467 produced the maximum number of polymorphic bands. RAPD patterns of cultivars produced by primer UBC467 are shown in Fig. 1. The marker UBC467-1100 is common to all the mungbean cultivars. But the marker UBC467-800 is specific for BUmug-1, UBC467-1350 is specific for Barimung-5 and UBC467-2072 is specific for BUmug-2. Therefore, RAPD

Table 2: List of primers, their sequences and the level of polymorphism obtained in mungbean cultivars

Primer	Primer sequence (5′-3′)	Total bands (No.)	Polymorphic bands (No.)	Polymorphism (%)
UBC125	GCGGTTGAGG	7	6	85.7
UBC173	CAGGCGGCGT	5	4	80.0
UBC176	CAAGGGAGGT	6	3	50.0
UBC194	AGGACGTGCC	5	3	60.0
UBC272	AGCGGGCCAA	4	3	75.0
UBC275	CCGGGCAAGC	5	2	40.0
UBC282	GGGAAAGCAG	3	1	33.3
UBC283	CGGCCACCGT	11	7	63.6
UBC284	CAGGCGCACA	6	5	83.3
UBC287	CGAACGGCGG	4	2	50.0
UBC374	GGTCAACCCT	2	2	100.0
UBC379	GGGCTAGGGT	8	6	75.0
UBC391	GCGAACCTCG	3	2	66.7
UBC399	TTGCTGGGCG	7	7	100.0
UBC412	TGCGCCGGTG	5	5	100.0
UBC414	AAGGCACCAG	2	1	50.0
UBC421	ACGGCCCACC	4	1	25.0
UBC431	CTGCGGGTCA	9	9	100.0
UBC437	AGTCCGCTGC	6	4	66.7
UBC438	AGACGGCCGG	10	7	70.0
UBC467	AGCACGGGCA	12	10	83.3
UBC468	ACGGAAGCGC	8	7	87.5
UBC494	TGATGCTGTC	5	5	100.0
UBC499	GGCCGATGAT	5	4	80.0
UBC584	GCGGGCAGGA	7	5	71.4
UBC592	GGGCGAGTGC	8	8	100.0
UBC594	AGGAGCTGGC	5	3	60.0
UBC599	CAAGAACCGC	3	2	66.7
UBC601	CCGCCCACTG	6	2	33.3
UBC602	GCGAAGACTA	7	6	85.7
OP 01	TGAGGGCCGT	9	6	66.7
OP 03	CATAGAGCGG	8	8	100.0
OP 06	GGGGAAGACA	4	3	75.0
OP 08	CTGGCTCAGA	5	4	80.0

technique is a simpler and quicker method for characterization and analysis of genetic diversity in mungbean cultivars.

Analysis of the relationship based on 204 RAPD markers revealed the genetic diversity among the cultivars, which ranged from 0.54 to 0.85, but mostly concentrated between 0.61 and 0.74. The RAPD cluster pattern is presented in Fig. 2. It showed four main clusters, but Barimung-2 and CN 9-5 each took an independent position. Among the major cluster, Barimung-5 and BUmug-2 were introduced from AVRDC and grouped in one cluster. The major cluster consists of Pusa Baisakhi, VC6173A, VC6173B-11, VC6153B-20G, VC 6370A, Binamung-5, Basanti and KPS 2. In this cluster, Pusa Baisakhi and Basanti are of Indian origin, Binamung-5 is from Bangladesh and others are AVRDC sources. Such clustering of cultivars of different locations ignored the influence of geographic variations within the genetic diversity of mungbean. Bisht *et al.*[11] also reported no correlation between geographic diversity and genetic diversity in mungbean. Manivannan *et al.*[12] ascribed the fact that genetic drift and selection in different environments could cause greater genetic diversity than geographical diversity. In contrast, another small cluster formed by Binamung-1, Binamung-3, Sonamung and Barisal Local in Bangladesh indicated the effect of geographical importance in the genetic similarity of mungbean cultivars. Paredes *et al.*[13] also observed that genotypes originating from a single locality tended to fall within a single branch of the dendrogram, with roughly equal genetic distance occurring among them. The last cluster formed by BUmug-1, PDM 11, Barimung-3, Binamung-4 and SML 32 were very distinct from the others, although PDM 11 and Barimung-3 appear to be close to each other with far distance from SML 32 and Binamung-4.

Genetic similarity of cultivars may originate from the commonness in their parentage. For instance, with few exceptions, most of the AVRDC materials and the cultivars introduced from AVRDC clustered to make one group. But genetic diversity is the resultant of natural selection, spontaneous mutation and artificial selection.

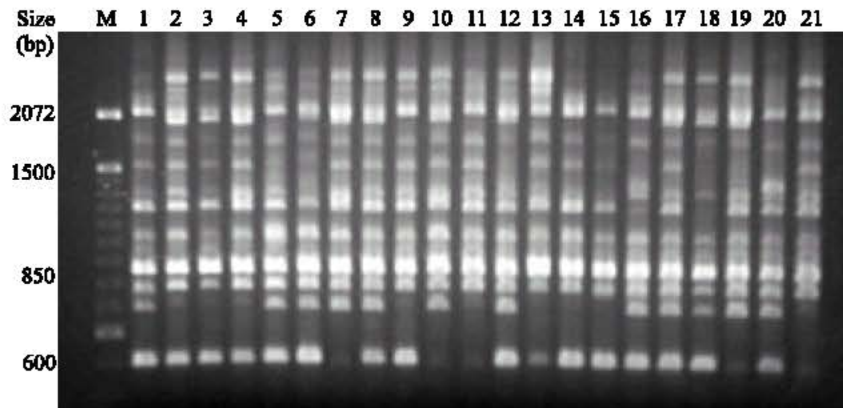

Fig. 1: RAPD profile of 21 mungbean cultivars obtained with UBC467 primer. Lane M: molecular weight standards; the size of fragments in given in base pairs. Lane 1 to 21 correspond to mungbean cultivars listed in Table 1

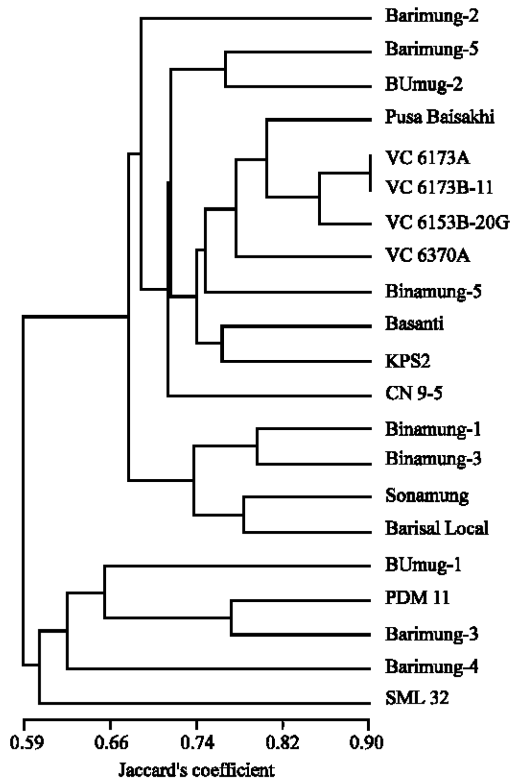

Fig. 2: Dendrogram of 21 mungbean cultivars revealed by UPGMA cluster analysis of Jaccard's similarity coefficients, calculated from 204 bands generated by 34 primers

These may aid in genetic diversity in crops over geographic locations. Barimung-2 was introduced to Bangladesh from the Philippines and SML 32 originated from India. The greater variation among them showed the importance of locations in genetic diversity.

The results revealed that the genetic base among these mungbean cultivars is rather narrow. Collection of diverse germplasm from centers of diversity and acquired from other sources may broaden the genetic base[14]. The genetic base could be broadened through the use of γ radiation, which is a drastic method of mutagenesis resulting in a major reshuffle of the genome[15]. For example, the Nuclear Institute for Agriculture and Biology in Islamabad in Pakistan was able to identify a stable mungbean yellow mosaic virus (MYMV) resistant line through irradiation. Using the identified MYMV resistant source AVRDC was able to successfully develop an improved MYMV resistant mungbean, NM 92. Nevertheless, the reasonable diversity observed in this study may be exploited for further yield improvement.

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