Pak. J. Bot., 48(3): 1273-1277, 2016.

GENETIC DIVERSITY OF MUNGBEAN GENOTYPES IN RELATION TO RESISTANCE AGAINST MUNGBEAN YELLOW MOSAIC VIRUS

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Abstract

Mungbean Yellow Mosaic virus (MYMV) caused by single stranded DNA begomovirus is the most potential and prevailing threat to mungbean worldwide. This study was conducted to determine genetic variation in mungbean genotypes to know whether these genotypes, which differ in field screening results, also vary on a genetic basis or not. One hundred and twenty seven genotypes were screened against MYMV under the field condition. RAPD analysis showed significant amount of genetic diversity within mungbean germplasm. Twenty six primers produced 66% of polymorphism with average 5.36 bands per primer. Minimum similarity was observed among genotypes 8008 and Azri-06, which is 61.15%, while the maximum similarity was observed among genotypes NM-54 and NM-92, which is 91.37%. Three genotypes belonging to resistant category remained unclustered i.e., NM-2011, NM-2006 and Azri-06.Genetic differentiation among mungbean genotypes was similar to the field screening results. RAPD analysis revealed an extensive amount of variation, which could be used for cultivar identification. This information will be helpful in recognition of resistant germplasms and will be utilized for mungbean improvement program.

Key words: Genetic diversity, Mungbean yellow mosaic virus, RAPD

Introduction

Mungbean [*Vigna radiata* (L.) Wilczek] is an important pulse crop grown all over the world. Severe yield losses in mungbean have been caused by the most destructive virus, mungbean yellow mosaic virus (MYMV). Several others legumes crops including urdbean, soybean and cowpea are infected by MYMV (Dhingra & Chenulu, 1985, Qazi *et al*., 2007, Binyamin *et al*., 2011). The disease gain more attention and importance due to its severe destruction of legume crops in Pakistan, Bangladesh, India and Srilanka (Bakar 1981; Malik 1991; Biswass *et al*., 2008; John *et al*., 2008). MYMV causes more than US$ 300 million loss every year in various legume crops (Varma *et al*., 1992). MYMV caused severe destruction of legume crops and this was attributed due to their frequent recombination and transmission caused by *Bemisia tabaci*. Therefore, management of MYMV often linked with the control of *Bemisia tabaci* population which sometimes proves to be ineffective due to high population pressure. The most reasonable, efficient, economical and environment friendly method for the management of MYMV is the host resistance. Molecular markers have been applied to study the genetic variation among the mungbean genotypes. Up to now, the study on genetic diversity of mungbean germplasm in Pakistan on DNA basis was not sufficient. Previous study showed that different DNA markers used in the study of genetic variation of different *Vigna* species were not in enough numbers (Amadou *et al*., 2001). RAPD markers were used for genetic diversity studies because they are dominant markers, simple and inexpensive in use (Harris 1999). Genetic diversity studies of mungbean genotypes were reported by number of different scientists (Dikshit *et al*., 2007; Saini *et al*., 2010; Datta *et al*., 2012; Sony *et al*., 2012).

Identification of disease resistant varieties is regarded as the most durable and economical method for managing plant diseases. A good deal of research has been directed towards genetically diverse and resistant source identification against MYMV. Continuous screening of available varieties and new germplasm is therefore required and which constitutes the basis of this study. The genotypes used in the study are the first Pakistani origin genotypes to be assessed on molecular basis. This will help in development of new cultivars of mungbean with superior properties to meet challenging MYMV threat. This study will contribute basic knowledge in the aspect of their phylogenetic relationships and intra specific diversity. Therefore, 127 mungbean genotypes were evaluated against MYMV in epiphytotic conditions. In the past, only field screening of the mungbean germplasm was performed, which mainly based on disease symptoms. The main focus during the study was to determine genetic variation in mungbean genotypes through RAPD analysis, to know whether these genotypes, which differ in field screening results, also vary on a genetic basis or not.

Materials and Methods

One hundred and twenty seven mungbean genotypes used for disease screening, disease incidence was based on 0-5 arbitrary scale used by Bashir *et al*. (2005) after initiation of first disease symptoms in the field. Ten genotypes were selected on the basis of different field response against MYMV (Table 1). Three genotypes from resistant genotypes, two from moderately resistant, two from moderately susceptible and three genotypes from highly susceptible group were selected randomly. Seeds

of mungbean genotypes were sown in pots. Fresh leaf samples (8-10) from potted mungbean plants were collected and immediately DNA from these samples was extracted following a CTAB method for DNA extraction with certain modifications (Doyle & Doyle, 1990). The concentration of DNA was determined by Gene Quant and working dilutions were optimized on the basis of the best amplification.

Primer sequence of RAPD markers: Total of forty RAPD primers from four different series (I, J, K & L) were used in the study for RAPD analysis and polymorphic primers were selected to amplify the genomic DNA of mungbean germplasm (Table 2).

Optimization of PCR conditions for RAPD: PCR thermal cycler (AG No. 533300839, Germany) was used in the present study. The total reaction was performed in a volume of 25 µL. The components for 25 µL reaction mixture were 2.5 µL of genomic DNA, 0.2 µL *Taq* DNA polymerase (MBI, Fermentas, Vinius, Lithuania), 2.5 µL 10X Buffer, 2.5 µL Gelatin, 3 µL $MgCl_2$ (2.5 mM), 4 µL dNTPs (0.2 mM), 2 µL Primers (15 ng), 8.3 µL d_3H_2O. PCR amplification was performed as follows; initial denaturation at 95°C (5 min.), followed by 40 cycles of 95°C (denaturation for 1 min.), 34°C (primer annealing for 1 min.), 72°C (extension for 2 min.), 72°C (final extension for 10 min.) and at last hold for 4°C.

For molecular characterization bands were counted and data were analyzed by using "popgen32" computer software (ver. 1.44), the genetic variation and similarity were assessed. The genetic relationship among the mungbean was determined by clustering the genotypes.

Results

Ten mungbean genotypes on the basis of their response in field against MYMV were genetically analyzed by using the forty RAPD decamers, out of these twenty six primers were selected for final analysis which showed the distinct and easily detectable bands. Considering all the primers and mungbean genotypes, total of 139 bands were amplified in the PCRs of ten genotypes. Twenty six primers produced 92 polymorphic bands, which showed 66% polymorphism. Maximum percentage of polymorphism was shown by primers GLK-20 and GLI-12 i.e., 85% while primer GLK-08 showed minimum percentage of polymorphism 30% (Fig. 1). Average number of bands produced by twenty six primers was 5.36. Numbers of polymorphic bands per primer were 3.5 bands. The number of bands per genotype ranged from 87 to 128, with an average of 112 bands per genotype. Maximum number of bands were produced by the genotype NM-2011 (128), followed by line 8008 (125), varieties Azri-06 (123), NM-92 (122), NM-54 (118), NM-2006 (115), line 8010 (105), 8011 (102) and variety M-6 (95). Minimum number of bands were produced by genotype C2 94-4-36 (87), (Fig. 2). The number of amplified fragments ranged from 2 to 10, maximum number of fragments were amplified by three primers viz; GLK-09 (10), GLK-16 (10) and GLK-19 (10), while minimum number of fragments amplification was also produced by three primers viz; GLI-20 (2), GLL-08 (2) and GLL-12 (2), (Fig. 1). Bands pattern produced by two primers were shown in Figs. 4 and 5.

Table 1. Mungbean genotypes used for RAPD markers analysis.

Sample No.	Varieties/lines	Resistance level	Sample No.	Varieties/lines	Resistance level
1	NM-2011	R	6	NM-54	MS
2	NM-2006	R	7	M-6	MS
3	Azri-06	R	8	8008	HS
4	NM-92	MR	9	8010	HS
5	C_2 94-4-36	MR	10	8011	HS

R= (Resistance), MR = (Moderately Resistant), MS = (Moderately Susceptible), HS= (Highly Susceptible)

Table 2. Detail of RAPD primers along with their sequences used in the study.

Sr. No.	Primer name	Sequence	Sr. No.	Primer name	Sequence
1.	GL Decamer I-03	CAGAAGCCCA	14.	GL Decamer K-12	TGGCCCTCAC
2.	GL Decamer I-10	ACAACGCGAG	15.	GL Decamer K-15	CTCCTGCCAA
3.	GL Decamer I-11	ACATGCCGTG	16.	GL Decamer K-16	GAGCGTCGAA
4.	GL Decamer I-12	AGAGGGCACA	17.	GL Decamer K-17	CCCAGCTGTG
5.	GL Decamer I-13	CTGGGGCTGA	18.	GL Decamer K-19	CACAGGCGGA
6.	GL Decamer I-14	TGACGGCGGT	19.	GL Decamer K-20	GTGTCGCGAG
7.	GL Decamer I-20	AAAGTGCGGG	20.	GL Decamer L-04	GACTGCACAC
8.	GL Decamer J-15	TGTAGCAGGG	21.	GL Decamer L-05	ACGCAGGCAC
9.	GL Decamer J-19	GGACACCACT	22.	GL Decamer L-07	AGGCGGGAAC
10.	GL Decamer J-20	AAGCGGCCTC	23.	GL Decamer L-08	AGCAGGTGGA
11.	GL Decamer K-08	GAACACTGGG	24.	GL Decamer L-10	TGGGAGATGG
12.	GL Decamer K-09	CCCTACCGAC	25.	GL Decamer L-12	GGGCGGTACT
13.	GL Decamer K-10	GTGCAACGTG	26.	GL Decamer L-13	ACCGCCTGCT

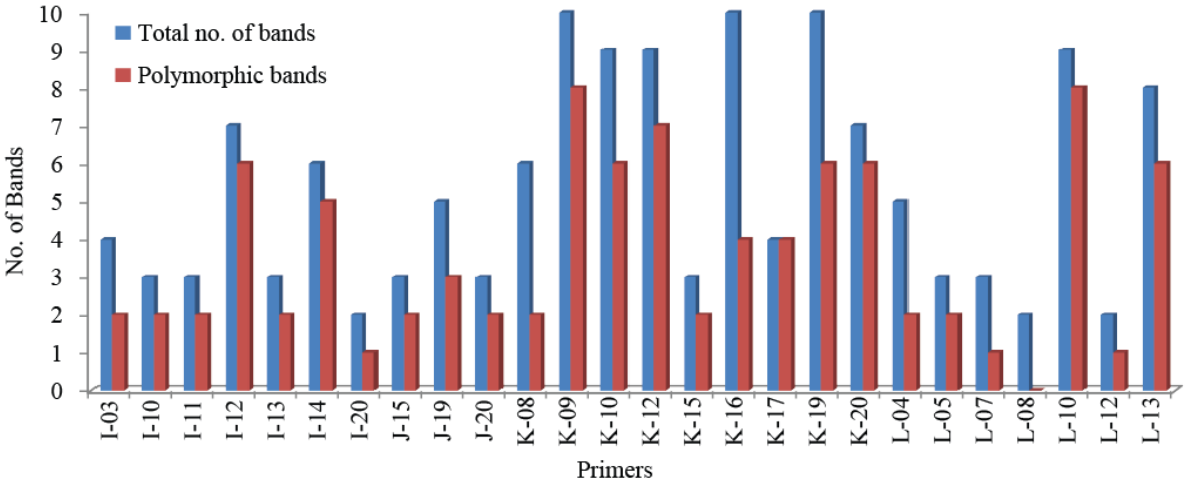

Fig. 1. Number of polymorphic bands per primer.

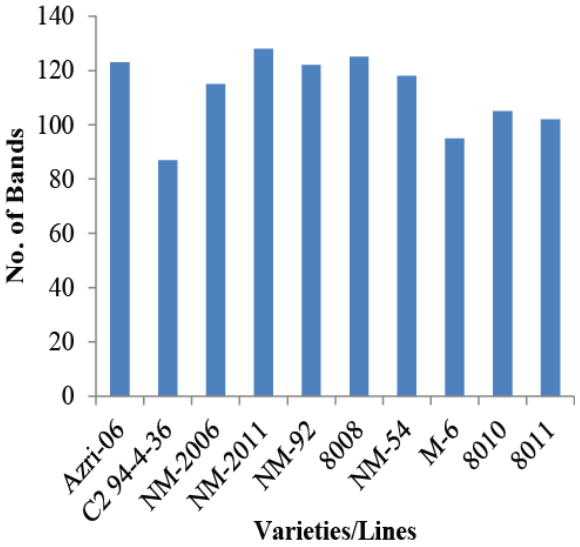

Fig. 2. Number of bands per genotype.

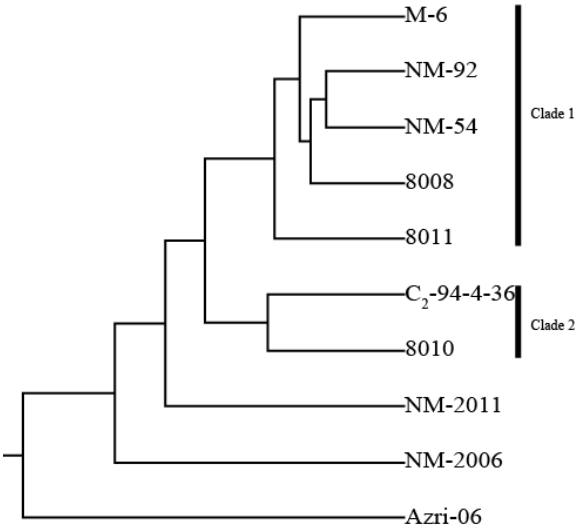

Fig. 3. Dendrogram of genotypes obtained from similarity matrix of RAPD markers.

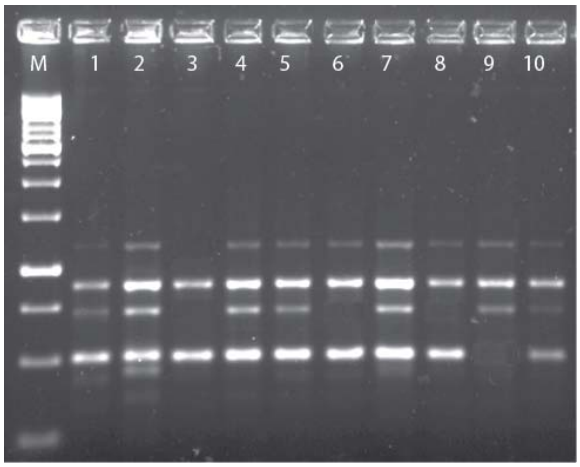

Fig. 4. RAPD-PCR with primer GLK-19 (lanes 1-10 genotypes), (M= 1Kb ladder).

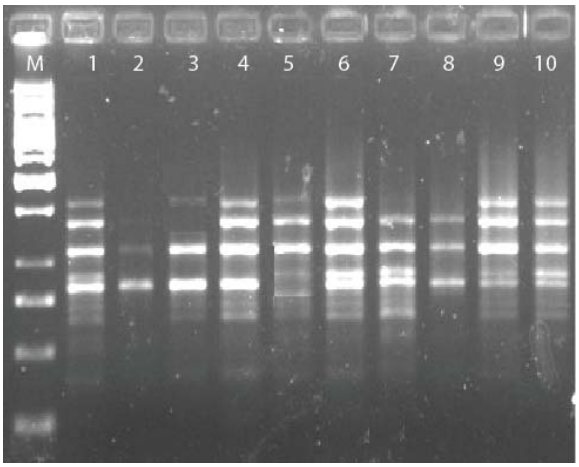

Fig. 5. RAPD-PCR with primer GLI-12 (lanes 1-10 genotypes), (M= 1Kb ladder).

Table 3. Similarity matrix of ten mungbean genotypes through RAPD markers.

Pop ID	M-6	Azri-06	NM-2006	NM-92	8008	NM-54	C2-94-4-36	8011	NM-2011	8010
M-6	****	0.6475	0.7770	0.8777	0.8921	0.8921	0.8273	0.6906	0.8058	0.7914
Azri-06	0.4347	****	0.6259	0.6547	0.6115	0.6403	0.6763	0.7122	0.6259	0.6547
NM-2006	0.2523	0.4686	****	0.7698	0.7410	0.7554	0.7770	0.6403	0.7842	0.7410
NM-92	0.1305	0.4236	0.2616	****	0.8849	0.9137	0.8777	0.7410	0.7554	0.7842
8008	0.1142	0.4918	0.2997	0.1223	****	0.8907	0.8633	0.7266	0.8417	0.8273
NM-54	0.1142	0.4458	0.2805	0.0903	0.0903	****	0.8777	0.7410	0.7842	0.7842
C2-94-4-36	0.1895	0.3912	0.2523	0.1305	0.1470	0.1305	****	0.7482	0.7914	0.8201
8011	0.3701	0.3394	0.4458	0.2997	0.3194	0.2997	0.2901	****	0.7266	0.7554
NM-2011	0.2160	0.4686	0.2431	0.2805	0.1723	0.2431	0.2340	0.3194	****	0.8561
8010	0.2340	0.4236	0.2997	0.2431	0.1895	0.2431	0.1983	0.2805	0.1554	****

Nei's genetic identity (above diagonal) and genetic distance (below diagonal)

Multivariate analysis was conducted to generate a similarity matrix using Popgen 32 software, version 1.44 (Yeh *et al*., 2000) based on Nei's UPGMA to estimate genetic distance and relatedness of mungbean genotypes. Dendrogram drawn for the genetic distances is shown in the Fig. 3 and minimum similarity was observed among 8008 and Azri-06 which is 61.15%, while the maximum similarity was observed among genotypes NM-54 with NM-92 which is 91.37% (Table 3).

Cluster analysis classified the ten mungbean genotypes into two main different clades. Three genotypes remained unclustered, which showed distinct behavior from other genotypes. Clade 1 comprised of five genotypes M-6, NM-92, NM-54, 8008 and 8011, while genotypes NM-54 and NM-92 showed closed similarity between them. Clade 2 consisted of two genotypes C2-94-4-36 and 8010 and showed close similarity. Three genotypes NM-2011, NM-2006 and Azri-06 remained unclustered due to their distinct genetic makeup (Fig. 3).

Discussion

Many commercial mungbean cultivars are susceptible to MYMV. Therefore, requirement for the markers closely linked with MYMV resistance gene increased. So that resistance gene could be transferred to the cultivars having excellent potential yield by using the marker assisted breeding. Breeding programs based on phenotypic and symptomatic analysis are much slower and time consuming, while use of molecular markers for resistance breeding is more authentic and rapid. Varieties having different field response were characterized on molecular basis by employing different markers; the main hypothesis of this study was that, the genotypes that differ in field response against MYMV also differ on molecular basis. Genotypes selected from different reaction groups in response to mungbean yellow mosaic virus were genetically analyzed and they produced different clusters and shown different level of similarity and diversity among themselves. RAPD markers have been used successfully to assess molecular polymorphism in mungbean which is helpful for the estimation of genetic diversity in mungbean germplasm (Santalla *et al*., 1998). Results of the present study when compared with other studies showed following patterns; RAPD primers produced six bands per primer (Afzal *et al*., 2004), which is comparable with the present study results i.e., 5.36 bands per primer. Twenty one RAPD primers were used; primers produced 64% polymorphism (Lakhanpaul *et al*., 2000), while in the present study 66% polymorphism is observed. Saini *et al*. (2010) in India reported that 13.71 bands per primer with polymorphism ranging from 42.85 to 100% by RAPD markers. This showed high level of amplification and polymorphism by the primers than primers used in the present studies. RAPD analysis was used to study the diversity within fifty four varieties of mungbean and found diversity in mungbean genotypes (Lavanya *et al*., 2008), similar to our results. Twenty five RAPD primers were used, which amplified 4.1 bands per primer, with maximum ten number of bands with single primer, with 1.8 polymorphic fragment per primer (Souframanien, 2004), while in current study more number of bands were produced with 3.5 polymorphic band per primer, percentage of polymorphism obtained from both studies was same i.e., 66%. A high polymorphism rate (90%) was also observed in studies on genetic diversity of twenty four genotypes of mungbean (Datta *et al*., 2012) as compared to the 66% reported in our work. Moreover, Karthikeyan *et al*. (2012) also used RAPD markers for the diversity, screening and identification of RAPD markers linked with MYMV resistance in mungbean.

The genetic diversity within the mungbean genotypes was observed on the basis of similarity matrix, and it ranges from 61.15 to 91.37%. Some genotypes have high level of similarity (91.37%) between them while others showed minimum similarity (61.15%). Maximum divergence was showed by two varieties NM-2011 and Azri-06, both of them came from the resistant category of screening results. The maximum diversity in their genetic makeup shows that they have different genes or set of genes that provide resistance against the MYMV. They have either diverse parentage which conferred resistance to the MYMV. Maximum similarity was seen for genotypes (NM-54 and NM-92), NM-54 belonged to moderately susceptible group, and NM-92 belonged to moderately resistant group. These results showed that the two genotypes have close genetic makeup due to common ancestors. Results of the present study are similar to the results obtained by Lakhanpaul *et al*. (2000) who also reported similarity ranged from 65%-92%. Diversity of mungbean germplasm showed similarity range from 0-

48%, suggesting a great level of genetic diversity within the germplasm (Lavanya *et al*., 2008), as compared to germplasm of present study. Genetic similarity between the mungbean germplasm range from 45%-78% (Datta *et al*., 2012), which showed more diversity than present study genotypes. Similarity ranged from 40.8-90.3% during findings of (Saini *et al*., 2010), which showed significant amount of genetic diversity as in the present study.

Cluster analysis classified the ten genotypes into two clades, one having five genotypes NM-92 moderately resistant, M-6 and NM-54 moderately susceptible, 8008 and 8011 highly susceptible groups on the basis of two years screening results obtained from field trail. Second clade consists of two genotypes C2-94-4-36 and 8010, which belong to moderately resistant and highly susceptible groups. Three genotypes remained unclustered, NM-2011, NM-2006, and Azri-06. From cluster analysis results showed by dendrogram, the genotypes NM-92 and NM-54 showed close genetic association. Genotypes C2-94-4-36 and 8010 also showed maximum similarity in their genetic makeup. These genotypes might have common ancestors and have repeated utilization in the breeding program for the mungbean improvement due to their narrow genetic makeup. All three resistant varieties remain unclustered and showed distinct behavior not only from susceptible genotypes but also from each other. This genetic diversity between the resistant genotypes showed that they have different genetic makeup and might have different resistance genes that involved in the resistant behavior of these genotypes. Results from present study are highly productive to look for economical and workable options as no virucide or other chemical has been known for the proper management of MYMV problem in Pakistan.

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(Received for publication 19 January 2015)