International Journal of Plant & Soil Science

33(7): 39-45, 2021; Article no.IJPSS.67776
ISSN: 2320-7035

Screening of Mungbean (*Vigna radiata* L. Wilczek) Genotypes for Resistance to Mungbean Yellow Mosaic Virus under Field Condition

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Authors' contributions

This work was carried out in collaboration among all authors. Authors YG designed the study and wrote the first draft of the manuscript. Author MNS supervised the study and finalized the manuscript. Authors AS and SL managed the field data collection and literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJPSS/2021/v33i730448
Editor(s):
(1) Dr. Muhammad Shehzad, The University of Poonch Rawalakot AJK, Pakistan.
Reviewers:
(1) Maria Luisa Izaguirre, Universidad Tecnica de Manabi, Ecuador.
(2) Aparna B. Gunjal, Dr. D.Y. Patil, Arts, Commerce & Science College, India.
Complete Peer review History: http://www.sdiarticle4.com/review-history/67776

Original Research Article

Received 14 February 2021
Accepted 28 April 2021
Published 28 April 2021

ABSTRACT

Greengram [*Vigna radiata* (L.) Wilczek] is an economically important grain legume crop next to chickpea, pigeon pea and urad gram. Among the biotic factors, *Mungbean Yellow Mosaic Virus* (MYMV) is reported to be the most destructive viral diseases, which may reduce the seed quality as well as the yield losses up to 100 per cent. It is transmitted through whitefly (*Bemisia tabaci*) in a persistent manner throughout Asia, including India. A set of forty-two diverse mungbean genotypes were sown in two replications using a Randomized Block Desigh (RBD) during the summer season of 2015. The infector row method was adopted to evaluate a set of mungbean genotypes to know the difference in the level of resistance against MYMV infection under field condition during summer, 2015. Percent Disease Incidence (PDI) was calculated at 30 DAS and 50 DAS respectively. It varied from 2.18 to 64.77% and 5.38 to 76.87% at 30 DAS and 50 DAS respectively in summer, 2015. On the basis of disease severity recorded, the mungbean genotypes were

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classified in to five disease infection categories. Out of the forty-two mungbean genotypes, thirteen genotypes viz., Pusa 0672, IPM 205-7, HUM 8, KM 2245, IPM-2-03, ML 1464, KM 2241, PDM-139, TARM-1, HUM 26, Meha, HUM 16 and IPM 409-4 were found to be resistant and may provide the source of resistance against MYMV to develop mapping population for molecular breeding, development of molecular markers, QTL identification for MYMV resistance, as well as development of MYMV resistant varieties.

Keywords: Mungbean; screening; yellow mosaic virus; percent disease incidence.

1. INTRODUCTION

In the traditional vegetarian diet of Indian population, pulses occupy second place next to cereals and act as the main source of protein, carbohydrates, minerals, vitamins. Because of its nutritional importance to human food, they are known as “poor man’s meat”. Besides nutritional importance, it also fixes atmospheric nitrogen in the root nodules and thus plays a vital role in sustainability of agricultural production system [1]. Among several pulses grown, mungbean or greengram [*Vigna radiata* L. Wilczek. 2n=22], is an economically important short duration grain legume crop which can be grown as sole or intercrop for grain and green manure in different environments across three crop seasons viz., *Kharif, Rabi* and Summer in various parts of the country. Globally, mungbean yellow mosaic virus (MYMV) remains a main constraint of mungbean production and management of this lethal disease is still the major challenge. Thus, finding ways to manage MYMV including identification and development of mungbean varieties possessing resistance against MYMV is a research priority for mungbean crop [2].

Mungbean is grown in an area of 4.2 Million hectares with a total production of 2.01 Million tonnes, with an average productivity of 472 kg/ha [3] which is very low as compared to other important pulse crops of our country. An improvement in the yield of mungbean is becoming difficult, mainly due to vulnerability to several biotic (mungbean yellow mosaic virus, powdery mildew, Cercospora leaf spot, leaf crinkle virus, anthracnose, gram pod borer, bruchid and whitefly) and abiotic (temperature, drought, salinity, water logging etc.) stresses [4-6]. Among the biotic factors, Mungbean Yellow Mosaic Virus (MYMV) is reported to be the most destructive viral disease and limit the mungbean production throughout Asia, including India [7,8].

MYMV belong to Geminiviridae family and genus Begomovirus which is characterized by the bi-partite genome (DNA-A and DNA-B) or monopartite genomes and is transmitted by the insect vector, white fly (*Bemisia tabaci*) and not through seed, soil or mechanical inoculation [8,9]. Geminiviruses are circular single-stranded DNA viruses that infect a wide range of plant species including many important crops like mungbean [10]. The impact of geminiviruses is widespread and destructive. The family Geminiviridae has nine genera based on viral genome structure and insect vectors. In the case of begomoviruses, genomes can be mono- or bipartite, with each circular DNA (~2.5 Kb) packaged in a twinned icosahedral particle [11]. Severe MYMV infestation may reduce the seed quality as well as the yield losses up to 100 per cent [12]. The incidence and management of the MYMV disease depend on the vector (whitefly) population, which in turn depends on environmental conditions [13]. The MYMV control is often based on reducing the vector population with application of insecticides, though, spraying of insecticide do not give effective control of MYMV under sever whitefly infestations. Therefore, the more efficient and environmentally safe long-term strategy is the development of MYMV resistant cultivars.

Keeping this background under consideration, the present investigation was envisaged with the objective to identify the mungbean resistant genotypes based on the field screening to evaluate its expediency in breeding for MYMV resistance.

2. MATERIALS AND METHODS

A total of 42 diverse mungbean [*Vigna radiata* (L.) Wilczek] genotypes used in the present investigation were procured from Department of Genetics and Plant Breeding, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, India.

2.1 Field Evaluation of Mungbean Genotypes Against MYMV

The experiment comprising of forty-two mungbean genotypes was conducted in randomized block design (RBD) with two

replications at Agricultural Research Farm, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, India, during summer season, 2015 for screening of the genotypes against MYMV infestation. Each plot consisted of single row of three meter length with row to row and plant to plant distances being 30 and 10 cm, respectively.

The infector row technique was adopted in which one infector row of Co 5 was raised after every two rows of the test entries besides growing infector row around the border sites of the experimental materials to evaluate MYMV infection. All the recommended cultural practices were followed to express the full genetic potential of the genotypes without insecticide sprays so as to maintain optimum whitefly (vector) population for high inoculum pressure of MYMV pathogen.

The crop was regularly monitored for the development of disease symptoms and observations were recorded on 10 randomly selected plants from each genotype in each replication at an interval of 30 and 50 days after sowing (DAS), respectively. During the observation, the leaves showing clear symptoms (venial yellowing and scattered bright yellow spots) of randomly selected plants were counted and Percent Disease Incidence (PDI) was calculated by using the formula [14]. Accordingly, the genotypes were grouped into five different categories based on 0-5 arbitrary scale (Table 1), as suggested by [15,16].

The equation for the PDI is as follows:

Percent Incidence (PI)= Total number of infected leaves/ Total number of leaves observed×100

Percent Disease Incidence (PDI)=Sum of numerical rating/(Total number of leaves observed x Max.grade) ×100

3. RESULTS AND DISCUSSION

In the present study, a set of 42 mungbean genotypes were screened to know the differences in the level of resistance against MYMV infection under field condition during Summer, 2015. In general, screening of mungbean for YMD resistance is mostly performed at the MYMV hot spots as mechanical transmission of this virus is not possible. However, screening using agroinoculation technique and viruliferous whiteflies which are more precise are on the rise. Infector row technique was used in the present study to transfer the viral particles. The infector rows technique wherein highly susceptible check, Co 5 was planted after every two rows of the test entries and around the border of the experimental site (without insecticide spray), could ensure enough whitefly population (5-10 whiteflies per plant), for spreading of disease and thus limiting the chances of disease escape. At the end of the experiment, the infector check turned completely yellow, showing maximum disease severity, ensuring a good evaluation of mungbean genotypes against the yellow mosaic disease. Some studies were also conducted earlier to determine the exact mechanism of seed-borne nature of YMVs in addition to confirm the role of vector, whitefly in transmission of MYMVs. Where in, PCR amplicons sequencing and confocal microscopy confirmed the presence of MYMV in the seed coat, cotyledon and embryonic axes against to whitefly transmission of virus. Except for one report, there was no other report confirming (or) validating the seed borne nature of YMVs in any other Vigna species. However, seeds from infected plants also showed abnormalities like shrinking, discoloration, poor filling of pods and misshapen appearance. Detailed review was presented on genetics of MYMV resistance in mungbean, blackgram, and interspecific crosses by [2].

On the basis of disease severity recorded, the genotypes were classified in to five categories (Table1). The PDI of each genotypes was worked out at two different intervals *i.e*., at 30 and 50 DAS which varied from 2.18 (Pusa 0672) to 64.77(Co 5) and 5.38 to 76.87 per cent, respectively (Table 2). Out of 42 mungbean genotypes, 13 genotypes viz., Pusa 0672, IPM 205-7, HUM 8, KM 2245, IPM- 2-03, ML 1464, KM 2241, PDM-139, TARM-1, HUM 26, Meha, HUM 16 and IPM 409-4 were found resistant; four genotypes *i.e*., ML 1465, IPM 02-17, PUSA 9531 and ML 1296 as moderately resistant whereas, nine genotypes namely, COGG 912, IPM 2-19, SML 1082, MH 2-15, MHG 3-18, PUSA 0871, HUM 1, HUM 7, and ML 717 were moderately susceptible. However, six genotypes namely, PUSA 95-31, ML 712, MH 521, DGG 1, AKH 9904 and ML 5 were observed to be susceptible and 10 genotypes (HUM 12, LG 460, K 851, Pusa Vishal, COGG 902, MH 84-1, SML 1455, China mung, Kopergaon and PUSA RATNA) exhibited highly susceptible reaction to MYMV infestation (Table 2).

Table 1. Disease scoring (0-5 Scale) for MYMV based on the percentage of disease incidence (PDI)

Disease scale	Percent infection	Visual symptoms	Infection category	Reaction group
0	All plants free of virus symptoms	Complete absence of symptoms	Highly Resistant	HR
1	1-10 % infection	Small yellowish spots scattered on some leaves	Resistant	R
2	11-20% infection	Yellowish bright spots common on leaves, easy to observe	Moderately Resistant	MR
3	21-30 % infection	Yellowish bright specks common on leaves, easy to observe with larger patches of symptoms	Moderately Susceptible	MS
4	30-50 % Infection	Bright yellow specks or spots on all leaves, minor stunting of plants and less number of pods	Susceptible	S
5	>50 % infection	Yellowing or chlorosis of all leaves on whole plant, shortening of internode, severe stunting of plants with no yield or few flowers and deformed pods produced with small, immature and shriveled seeds	Highly Susceptible	HS

Table 2. Reaction of mungbean genotypes against MYMV in the field during Summer, 2015

S.N.	Genotypes	PDI at 30 DAS	PDI at 50 DAS	Y2+Y1	(Y2+Y1)/2	T 2-t 1	Area Under Disease Progress Curve AUDPC (%) Y2+Y1)/2*t 2-t 1	Disease Scale (50 DAS)	Host Reaction
1	Pusa 0672	2.18	5.38	7.56	3.78	20	75.60	1	R
2	IPM 205-7	2.46	5.66	8.12	4.06	20	81.20	1	R
3	HUM 8	2.47	5.86	8.33	4.165	20	83.30	1	R
4	KM 2245	3.04	6.40	9.44	4.72	20	94.40	1	R
5	KM 2241	3.15	6.78	9.93	4.965	20	99.30	1	R
6	IPM 2-03	3.20	6.85	10.05	5.025	20	100.50	1	R
7	PDM 139	3.45	7.54	10.99	5.495	20	109.90	1	R
8	ML 1464	3.65	7.95	11.6	5.8	20	116.00	1	R
9	HUM 26	3.70	8.65	12.35	6.175	20	123.50	1	R
10	Meha(IPM99-125)	3.76	8.94	12.7	6.35	20	127.00	1	R
11	TARM 1	4.65	9.12	13.77	6.885	20	137.70	1	R
12	HUM 16	5.38	9.5	14.88	7.44	20	148.80	1	R
13	IPM 409-4	6.36	9.76	16.12	8.06	20	161.20	1	R
14	ML 1465	10.23	13.54	23.77	11.885	20	237.70	2	MR

S.N.	Genotypes	PDI at 30 DAS	PDI at 50 DAS	Y2+Y1	(Y2+Y1)/2	T 2-t 1	Area Under Disease Progress Curve AUDPC (%) Y2+Y1)/2*t 2-t 1	Disease Scale (50 DAS)	Host Reaction
15	IPM 02-17	10.28	13.65	23.93	11.965	20	239.30	2	MR
16	ML 1296	11.67	15.85	27.52	13.76	20	275.20	2	MR
17	PUSA 9531	15.9	19.75	35.65	17.825	20	356.50	2	MR
18	COGG 912	20.78	23.58	44.36	22.18	20	443.60	3	MS
19	IPM 02-19	20.95	23.86	44.81	22.405	20	448.10	3	MS
20	SML 1082	23.16	26.56	49.72	24.86	20	497.20	3	MS
21	MH 2-15	23.65	26.87	50.52	25.26	20	505.20	3	MS
22	HUM 1	23.85	27.05	50.9	25.45	20	509.00	3	MS
23	ML 717	24.26	27.46	51.72	25.86	20	517.20	3	MS
24	HUM 7	25.37	28.37	53.74	26.87	20	537.40	3	MS
25	MH 3-18	26.21	29.25	55.46	27.73	20	554.60	3	MS
26	PUSA 0871	26.83	29.39	56.22	28.11	20	562.20	3	MS
27	PUSA 95-31	35.62	40.26	75.88	37.94	20	758.80	4	S
28	ML 712	36.24	41.68	77.92	38.96	20	779.20	4	S
29	MH 521	37.45	42.56	80.01	40.005	20	800.10	4	S
30	DGG 1	38.61	43.16	81.77	40.885	20	817.70	4	S
31	AKM 9904	41.27	47.72	88.99	44.495	20	889.90	4	S
32	ML 5	43.67	49.58	93.25	46.625	20	932.50	4	S
33	HUM 12	45.78	58.45	104.23	52.115	20	1042.30	5	HS
35	K 851	54.28	59.35	113.63	56.815	20	1136.30	5	HS
34	LGG 460	53.47	59.85	113.32	56.66	20	1133.20	5	HS
36	Pusa Vishal	55.85	61.45	117.3	58.65	20	1173.00	5	HS
37	COGG 902	55.65	62.75	118.4	59.2	20	1184.00	5	HS
38	MH 84-1	56.35	63.21	119.56	59.78	20	1195.60	5	HS
39	SML 1455	57.51	65.47	122.98	61.49	20	1229.80	5	HS
40	CHINA MUNG	57.85	65.58	123.43	61.715	20	1234.30	5	HS
41	KOPERGAON	61.45	72.53	133.98	66.99	20	1339.80	5	HS
42	PUSA RATNA	62.98	74.69	137.67	68.835	20	1376.70	5	HS
43	CO 5*	64.77	76.87	141.64	70.82	20	1416.40	5	HS

**Co 5 genotype of urdbean was used as the infector row; DAS= Days after Sowing; Y1= Percentage of Disease Incidence (PDI) at 30 DAS; Y2= Percentage of Disease Incidence (PDI) at 50 DAS*

It may be emphasized here that, none of the genotypes were found to be highly resistant against MYMV infestation, showing consistent occurrence of disease in the field. However, 13 genotypes, appeared as resistant which indicated existence of small amount of resistance is present in mungbean genotypes against MYMV. Similar studies conducted by Bashir in 2003 evaluated 276 mungbean genotypes under natural condiction and found 10 lines exhibited as resistance against MYMV [18]. However, nine lines, out of 83 mungbean genotypes, appeared as resistance under field condition screended by Awasthi and Shyam in 2008 [19]. Similarly, Nainu and S. Murugan were screened 81 mungbean genotypes under field condition for resistance against MYMV and found seven genotypes as resistance [20]. However, the resistance nature of the genotypes, IPM 02-03, PDM-139, Pusa 0672, and HUM 16 have been also reported by few previous works [21-28].

4. CONCLUSION

The resistant mungbean genotypes identified may be used in further breeding programme to develop mapping population for molecular breeding, development of molecular markers, QTL identification for MYMV resistance, as well as development of MYMV resistant varieties. Based on present investigation, it is suggested that large number of genotypes should be screened in different agro-climatic conditions over years for identifying stable resistant genotypes against MYMV for developing high yielding coupled with resistant varieties.

ACKNOWLEDGEMENTS

Authors are highly thankful to Institute of Agricultural Sciences, Banaras Hindu University, Varanasi for providing necessity facilities and support for carried out the present experiment on which the manuscript was written.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/67776