

Original Research Article

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Re-evaluation of the Mungbean [*Vigna radiata* (L.) Wilczek] Genotypes for Resistance to Mungbean Yellow Mosaic Virus (MYMV) under Screen-House Conditions

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ABSTRACT

Mungbean [*Vigna radiata* (L.) Wilczek] is one of the most important grain legumes grown particularly in Asia. The production of mungbean is severely hampered by mungbean yellow mosaic virus (MYMV) caused by begomoviruses transmitted by whitefly, *Bemisia tabaci*. The absence of resistant/tolerant sources against MYMV disease leads to tremendous crop yield losses. To identify the sources of resistance in mungbean against MYMV, 35 genotypes were screened under natural conditions and categorized into different reaction groups using rating scale. In the present study, the screened genotypes were re-evaluated under screen-house conditions in order to confirm the sources of resistance against MYMV disease incidence. Out of them, 2 were categorized as resistant (R), 6 moderately resistant (MR), 12 moderately susceptible, 14 susceptible (S) and 1 as highly susceptible (HS). The resistant and highly susceptible genotypes could be used as parents for the development of mapping population in order to validate the molecular markers to facilitate marker-assisted selection for the development of MYMV resistant breeding lines.

Keywords

Mungbean, *Bemisia tabaci*, MYMV, Screening, Forced feeding method, Resistance

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Introduction

Pulses are the major sources of protein in the vegetarian diet in our country. Besides being a rich sources of protein, they maintain soil fertility through biological nitrogen fixation and thus play a vital role in furthering sustainable agriculture. Among the pulses, mungbean [*Vigna radiata* (L.) R. Wilczek] is one of the most important grain legumes grown particularly in Asia. It belongs to the subgenus *Ceratotropis* in the genus *Vigna* and is a self-pollinating diploid grain legume

(2n=22) with a genome size of 579 Mb (Arumuganathan and Earle, 1991). Due to its protein rich edible seeds, its ability to fix nitrogen, drought tolerance, and early maturity, it is widely planted in various cropping systems (Tangphatsornruang *et al.*, 2009). It is a good source of dietary protein with high contents of folate and iron compared with many other legumes crops (Keatinge *et al.*, 2011). Other properties like easy digestibility and low proportion of flatulence factors also adds to its value among the pulse crops. The seeds and sprouts of mungbean act

as therapeutic agents as they possess antioxidants and have antimicrobial, anti-inflammatory, antidiabetic, antihypertensive and anti-cancerous effects (Tang *et al.*, 2014).

Despite its economic importance, the average yield of this crop is low not only in India but also in the entire tropical and subtropical Asia mainly due to its susceptibility to many diseases such as mungbean yellow mosaic virus (MYMV), powdery mildew, *Cercospora* leaf spot (CLS), bacterial leaf spot (BLS) and leaf crinkle virus (LCV). Among the various diseases of mungbean, MYMV causes significant yield losses and under severe conditions, the yield penalty may reach upto 80 to 100 per cent (Naimuddin, 2001). It is a vital, serious, destructive, open spread and causal component of tremendous yield losses. It is caused by begomoviruses with bipartite genomes transmitted through whitefly, *Bemisia tabaci* (Nariani, 1960). Begomoviruses are the largest and the most important genus within the family *Geminiviridae*. They are plant-infecting single-stranded DNA viruses having characteristic geminate incomplete icosahedral particles. Geminiviruses often occur in disease complexes, and infect individual plant with multiple viruses. The genomes of geminiviruses can undergo high levels of mutation, recombination and reassortment to increase viral diversity (Duffy *et al.*, 2008; Harkins *et al.*, 2009; Martin *et al.*, 2011; Lima *et al.*, 2012). Due to the large populations of whiteflies, and their ability to develop pesticide resistance, vector seclusion is not an ideal way of fighting the spread and damage induced by MYMV. Also, the management of MYMV disease through spray of chemicals and pesticides is not always effective because they can reduce the disease incidence to some extent but complete control is difficult. Further, the recurrent spraying of these chemicals leads to health hazards and creates adverse impact on surrounding environment (Nariani, 1960). Hence, in order to realize a

sustainable agriculture and to get high quality products in terms of safe human health, the use of resistant varieties through conventional breeding appears to be a principal tool to reduce damages caused by pathogens. It would be an economic, durable and feasible approach in alleviation of disease severity as it requires no chemical input and/or plant seclusion and may be stable and long-lasting.

The screening of mungbean germplasm against MYMV disease under diverse environmental conditions is the first step in the identification of resistant donors for evolving mungbean varieties with MYMV resistance and yield. But this approach is time consuming and requires evaluation at 'hot spot' area (Selvi *et al.*, 2006). In fact, spontaneous field-exposure infection has been shown to be largely inefficient, as many plants escape infection, even under heavy inoculation pressure (Vidaysky *et al.*, 1998). To identify plant materials having high level of resistance, screening of genotypes using force feeding methods (Firdaus *et al.*, 2012; Taggar *et al.*, 2013; Sulistyono and Inayati, 2016) and agroinoculation methods (Al Abdallat *et al.*, 2010; Karthikeyan *et al.*, 2011; B Sai *et al.*, 2017) has been exploited in several crops including mungbean. Therefore, it is essential to screen genotypes through forced feeding methods, which can ensure 100% infection rate, and a standardized inoculum pressure. In the present study, 35 genotypes of mungbean screened under natural conditions (Suman *et al.*, 2015) were re-evaluated under screen-house conditions in order to identify the resistant sources to be used further in breeding programs.

Materials and Methods

Artificial screening is most acceptable technique to identifying resistance and susceptible individual or lines (Pico *et al.*, 1998). The experiment was conducted in two parts: field experiment and screen-house

experiment. Thirty five genotypes were used out of which 27 were procured from IIPR, Kanpur and 8 from TCA, Dholi (Table 1).

Field experiment

The test materials were sown in randomized block design (RBD) with three replications during summer. Each test entry was planted in two consecutive rows of 3 meter long with 30 cm row to row distance. One row of the most susceptible check (LGG 450) was planted after every test entry. Two rows of the most susceptible check were also planted all around the experiment to create more disease.

General cultural practices were followed in order to maintain the experiment and no insecticide was sprayed in order to support the population of whitefly for the natural spread of the disease. After germination, the crop was regularly monitored for the presence of whitefly and development of MYMV disease. The genotypes were screened for their resistance to MYMV incidence and evaluated on the basis of score and per cent disease incidence. The genotypes were scored for MYMV disease and grouped into five different categories from resistant to highly susceptible based on 1 to 9 rating scales (Fig. 2 and Table 2) at flowering and pod formation stages following the procedure as used earlier by Singh *et al.*, (1992).

Screen-house experiment

Seeds of all the genotypes were sown in pots (24 X 21cm) in completely randomized design (CRD) with three replications. During the period of experiment, the plants were irrigated weekly. The pots were placed in polyhouse and separated by net of mesh size 0.75 x 0.27 mm with the intention of preventing whiteflies moved to other plant and forced to breed in that plants (Fig.1). White flies were collected from the plants and confined in a susceptible plant showing typical MYMV symptoms for

24 hrs using a small, transparent glass trapper with a spring cap. The same trapper with the trapped flies was then used to inoculate (Polston and Capobianco, 2013) an apparently healthy plant and the viruliferous insects were allowed to feed on the leaf for 24 h. After acquisition feeding, the flies were used for 3-5 transfers for inoculation feeding, thereby allowing the viruliferous flies to transmit MYMV into the plant. Spread of MYMV was recorded at weekly intervals till maximum infection was achieved. The number of genotypes infected per week was calculated. The genotypes were scored on the basis of the degree of incidence of MYMV disease by using 1-9 rating scale for their classification into different infection categories.

Results and Discussion

Field experiment

Yellow mosaic disease appeared on the susceptible check lines about 25-30 days after planting under natural conditions. Small yellow specks of mild intensity were observed on young leaves. Alternate yellow and green patches with irregular margins developed in the first fully formed trifoliate leaf next to the apex after one week. The intensity of disease increased with passage of time. During summer, resistant response against MYMV was observed in the genotypes IPM 02-14 and PDM 139, whereas moderately resistant response was observed in the genotypes Pant Mung 4, Pusa 9531, HUM 1, HUM 12, HUM 16, Meha, DMS 03-17-2, RMG 62 and TMB 37. The genotypes Pant Mung 5, IPM 02-03, PDM 54, SML 134, SML 668, PS 16, MH 96-1, Pusa Vishal, Kopergaon, OUM 11-5, Pusa 032 and Pant Mung 2 displayed moderately susceptible response, while the genotypes AKM 8803, AKM 9910, TARM 2, Pusa 105, T 44, GM 4, LGG 407, Co4, IPM 99-1-6, Pusa 031 and DM 05-74-11 exhibited susceptible response against MYMV.

Table.1 List of mungbean genotypes used in the present study

Sl. No.	Name of the genotype	Source	Developing center
1	Pant Mung 4	IIPR, Kanpur	Pantnagar
2	Pant Mung 5	IIPR, Kanpur	Pantnagar
3	Pusa 9531	IIPR, Kanpur	IARI, New Delhi
4	IPM 02-03	IIPR, Kanpur	IIPR, Kanpur
5	IPM 02-14	IIPR, Kanpur	IIPR, Kanpur
6	PDM 54	IIPR, Kanpur	IIPR, Kanpur
7	PDM 139	IIPR, Kanpur	IIPR, Kanpur
8	HUM 1	IIPR, Kanpur	BHU, Varanasi
9	HUM 12	IIPR, Kanpur	BHU, Varanasi
10	HUM 16	IIPR, Kanpur	BHU, Varanasi
11	AKM 8803	IIPR, Kanpur	PKV, Akola
12	AKM 9910	IIPR, Kanpur	PDKV, Akola
13	SML 668	IIPR, Kanpur	PAU, Ludhiana
14	SML 134	IIPR, Kanpur	PAU, Ludhiana
15	PS 16	IIPR, Kanpur	IARI, New Delhi
16	TARM 2	IIPR, Kanpur	BARC, Akola
17	MH 96-1	IIPR, Kanpur	HAU, Hisar
18	Meha	IIPR, Kanpur	IIPR, Kanpur
19	Pusa 105	IIPR, Kanpur	IARI, New Delhi
20	T 44	IIPR, Kanpur	TNAU, Tamilnadu
21	GM 4	IIPR, Kanpur	S.K. Nagar, Gujarat
22	LGG 407	IIPR, Kanpur	APAU, Lam
23	Pusa Vishal	IIPR, Kanpur	IARI, New Delhi
24	Co 4	IIPR, Kanpur	TNAU, Tamilnadu
25	Kopergaon	IIPR, Kanpur	Local landraces
26	OUM 11-5	IIPR, Kanpur	OUAT, Berhampur
27	RMG 62	IIPR, Kanpur	Durgapura
28	DMS 03-17-2	TCA, Dholi	TCA, Dholi
29	TMB 37	TCA, Dholi	BARC, Mumbai
30	Pant Mung 2	TCA, Dholi	Pantnagar
31	IPM-99-1-6	TCA, Dholi	IIPR, Kanpur
32	Pusa 032	TCA, Dholi	IARI, New Delhi
33	Pusa 031	TCA, Dholi	IARI, New Delhi
34	LGG 450	TCA, Dholi	APAU, Lam
35	DM 05-74-11	TCA, Dholi	TCA, Dholi

Table.2 Rating scale for MYMV disease incidence used in the present study

Rating	Percentage foliage affected	Infection category
1	No visible symptoms or minute yellow specks covering 0.1-5 % leaf area	Resistant (R)
3	Mottling of leaves covering 5.1-15 % leaf area	Moderately resistant (MR)
5	Yellow mottling and discoloration of 15.1-30 % leaf area	Moderately susceptible (MS)
7	Pronounced yellow mottling and discoloration of leaves, pods reducing in leaf size, stunting of plants, 30.1-75 % foliage affected	Susceptible (S)
9	Severe yellow mottling and discoloration of leaves, stunting of plants, failure of flowering and fruit setting 75.1-100 % foliar affected	Highly susceptible (HS)

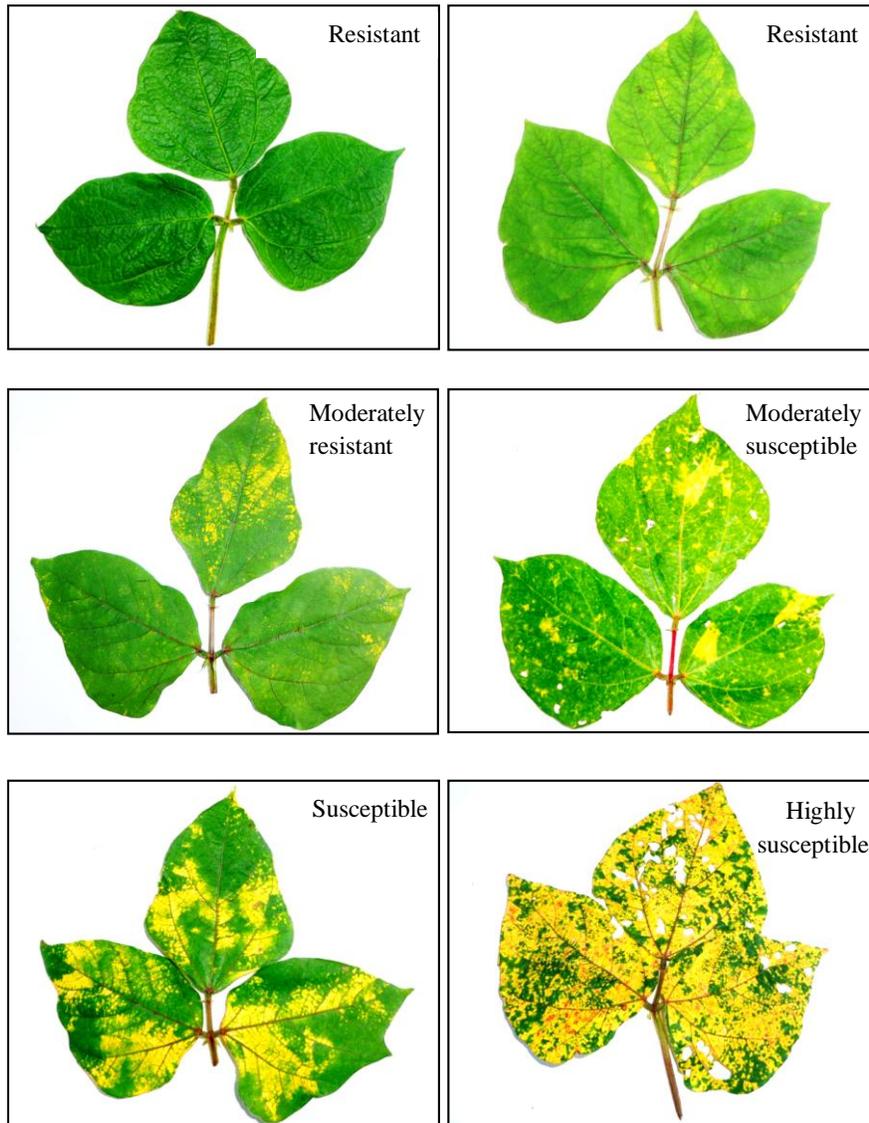
Table.3 Distribution of mungbean genotypes in various infection categories for MYMV disease incidence under screen-house conditions

Disease scale	Rating	No. of genotypes	Genotypes involved
Resistant (R)	1	2	IPM 02-14, PDM139
Moderately resistant (MR)	3	6	DMS 03-17-2, HUM 1, HUM 16, Pant Mung 4, RMG 62, TMB 37
Moderately susceptible (MS)	5	12	Pant Mung 5, Pusa 9531, IPM 02-03, PDM 54, HUM 12, SML 668, SML 134, Meha, Pusa Vishal, MH 96-1, Pant Mung 2, Pusa 032
Susceptible (S)	7	14	AKM 8803, AKM 9910, PS 16, TARM 2, Pusa 105, T 44, LGG 407, Kopergaon, Co 4, GM 4, OUM 11-5, IPM 99-1-6, Pusa 031, DM 05-74-11
Highly susceptible (HS)	9	1	LGG 450

Fig.1 Screening of mungbean genotypes for resistance against MYMV under screen-house conditions



Fig.2 Symptoms of different reaction categories against MYMV disease based on rating scale



The genotype LGG 450 showed highly susceptible reaction against MYMV disease incidence. The degree of disease incidence against MYMV ranged from 2.77 per cent in the resistant genotype IPM 02-14 to 83.33 per cent in the highly susceptible genotype LGG 450. The disease incidence for moderately resistant, moderately susceptible and susceptible response varied from 10 per cent in Pant Mung 4 to 15 per cent in HUM 12, 19.98 per cent in PDM 54 to 29.45 per cent in OUM 11-5 and 32.22 per cent in GM 4 to 41.67 per

cent in LGG 407, respectively (Suman *et al.*, 2016).

Screen-house experiment

Germination was completed within a week. The first appearance of MYMV disease was recorded in several genotypes two weeks after planting. Mild to severe yellow specks were observed on young leaves. Within next two weeks, these specks increased, coalesced and turned into yellow and green patches. The

severity of disease increased with the passage of time. Thirty five days after sowing, all the infected plants turned completely yellow and pods developed mottling symptoms and contained few shriveled seeds. All the genotypes were categorized using disease rating (1-9) scale into resistant (R), moderately resistant (MR), moderately susceptible (MS), susceptible (S) and highly susceptible (HS) infection categories. While assessing the performance of the entries in terms of the number of genotypes involved in different reaction categories it was found that two, six and twelve genotypes showed resistant, moderately resistant and moderately susceptible categories of disease reaction against MYMV, respectively. Amongst the rest of the genotypes under evaluation, fourteen genotypes were grouped under the susceptible category, while a single genotype was placed in highly susceptible group (Table 3).

The reaction pattern of the mungbean genotypes evaluated under screen-house conditions showed slight differences for MYMV disease incidence as compared to the genotypes screened under natural conditions. Three genotypes namely, Pusa 9531, HUM12 and Meha which were screened as moderately resistant under natural conditions were found to be moderately susceptible under screen-house conditions. Similarly, the genotypes namely, PS16, Kopergaon and OUM 11-5 which were rated as moderately susceptible under natural conditions were confirmed as susceptible under screen-house conditions using forced feeding methods. Inconsistency in results of some of the genotypes possibly may be due to the effect of location, disease pressure and virus strain. It is established that initial period of 2-3 weeks is highly critical due to early landing of viruliferous whitefly for the development and spread of MYMV. Increasing incidence and epiphytotic conditions of MYMV depend upon a combination of factors such as high population of viruliferous whitefly, buildup of inoculum potential in some hosts and wide range of favorable environmental conditions (Shad *et al.*, 2006). However, it has also been

reported that certain morphological leaf characteristics and foliar pubescence may affect insect feeding preference on plant genotypes (Taggar and Gill, 2012; Soundararajan *et al.*, 2013; Tamang *et al.*, 2017).

In the present investigation, forced feeding method served as an efficient tool for re-evaluation of the mungbean genotypes screened under natural conditions. The resistant and the highly susceptible genotypes showed similar responses under both natural and screen-house conditions. However, only some of the genotypes in the intermediate categories showed slight variable responses for MYMV disease incidence under screen-house conditions. Forced feeding method has also been used earlier by several workers (Basak *et al.*, 2004; Kundagrami *et al.*, 2009; Maiti *et al.*, 2011) in order to confirm the nature of reaction of parents and segregating mapping populations against MYMV disease. The screening for MYMV disease incidence resulted in the identification of two resistant genotypes namely IPM 02- 14 and PDM 139 and a highly susceptible genotype, LGG 450. These resistant and highly susceptible genotypes could be utilized as parents for the development of mapping population to validate the molecular markers which can facilitate marker-assisted selection (MAS) for the development of MYMV resistant breeding lines.

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