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Research Article

Screening of Advanced Breeding Lines (ABLs) of green gram [*Vigna radiata* (L.) Wilczek] in F_6 generation for reaction to Mungbean Yellow Mosaic Virus (MYMV) disease under field condition

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Abstract
Green gram is a store of nutrients and is a hardy pulse crop. It provides huge amount of phytochemicals and antioxidants but production is affected by many biological stress factors and among them MYMV stands first. Keeping this point in view, in the present investigation, 110 ABLs derived from fourteen families of green gram were evaluated for reaction to MYMV during *summer*-2019 at MARS, University of Agricultural Sciences, Dharwad, Karnataka. Amongst the breeding lines evaluated, 2BRD-2, 3BRD-18, 3BRD-9, 4BRD-1, 6BRD-9, 7BRD-7, 7BRD-12, 8BRD-20, E-19, PM-5 recorded resistant reaction and 5BRD-14 and 7BRD-13 recorded moderate resistance to MYMV with less than 10 per cent PDI value. Among 11 checks, TM-96-2 and SML-1815 showed resistance reaction; LGG-460 and RGM-1028 showed highly resistance reaction; MH-421 and IPM-409-4 showed moderately resistance reaction to MYMV with less than 20 per cent PDI value. These ABLs could be further used in resistant breeding programmes.

Key words
Green gram, Resistance, Genotypes, MYMV, Screening

INTRODUCTION

Green gram [*Vigna radiata* (L.) Wilczek], also known as mungu, moong, pachai payaru, golden gram and green soy. It is originated from Indo-Burma region of Hindustan (Vavilov, 1926). Pulses are the major group of food crops that could play an important role in solving the problems of food and nutritional security and also the environmental challenges. It will add about 9-10 per cent to total food grain production and is an inexpensive source of plant derived proteins, minerals and vitamins (Vadivel *et al*., 2019; Pandiyan *et al*., 2018). In some part of the Middle East, it acts as a major ingredient in cuisines. The pods of green gram are directly used as a vegetable in some Indian cookery and dehulled green gram are used as *dal* in India and used for preparation of soups, *usali* in Karnataka. Green gram production is affected by several biotic stresses *i.e.*, pest and diseases. So far 20 diseases of green gram have been documented; among them viral diseases are the most detrimental. Yellow mosaic is the utmost destructive yield reducing viral disease of green gram. The virus, Mungbean Yellow Mosaic Virus (MYMV) is the cause for the disease termed Mungbean Yellow vein Mosaic disease. It was grouped under the family of *Geminiviridae* and genus *Begomovirus*. MYMV was first identified in 1955 (Karthikeyan *et al*., 2014). It is having a single stranded DNA with the genome size of 2.8 kb (Hull, 2004). By using degenerate primers, through PCR and gel-electrophoresis study, 700bp band of DNA-A molecule of Mungbean Yellow Mosaic Virus can be observed. This group of viruses was also termed as “Legumoviruses” (Nair *et al*., 2017). MYMV virus particles were first

observed in plant cells of green gram by Thongmeearkom *et al* (1981). The bipartite begomaviruses are transmitted by special group of sucking insects called white flies (*Bemisia tabaci*, Gennadius), belonging to the order hemiptera. They show circulative persistent manner of virus transmission, where viruses are transmitted through insect stylets (Czosnek *et al*., 2017). Some strains of MYMV showed mechanical transmission in Thailand (Ahmad *et al*., 2017). MYMV could cause up to 85 per cent of yield damage when infection occurs from 4th week of the seeding. So the development and use of disease resistant/tolerant genotypes is the most effective way of crop improvement (Haque *et al*., 2016). Keeping the above details in view, the present exploration was under taken on screening the advanced breeding lines of green gram for reaction to Mungbean Yellow Mosaic Virus (MYMV) disease.

MATERIAL AND METHODS

The 110 advanced breeding lines derived from fourteen families whose parentage is given in parentheses *viz.,* 1BRD-4 to 19 [DGGV-2 × V-02-709], 2BRD-2 to 20 [DGGV-2 × V-02-802], 3BRD-3 to 20 [DGGV-7 × V-02-709], 4BRD-1 to 17 [DGGV-7 × V-02-802], 5BRD-1 to 19 [V-02-802 × DGGV-2], 6BRD-5 to 17 [V-02-802 × DGGV-7], 7BRD-4 to 20 [V-02-709 × DGGV-2], 8BRD-2 to 20 [V-02-709 × DGGV-7], PM-2 to 18 [DGGV-2 × TM-96-2], PTI-2 to19 [DGGV-2 × LGG-460], E-1 to 19 [DGGV- 2 × IPM-409-4], MDR-6 to 20 [DGGV-2 × RGM-1028], MDR-4 to 21 [DGGV-2 × MH-421], MR-1 to 18 [DGGV-2 × SML-1815] in F_6 generation and eleven parental checks *viz*., DGGV-2, DGGV-7, IPM-409-4, IPM-2-14, LGG-460, SML-1815, RGM-1028, MH-421, TM-96-2, V-02-709 and V-02-802 (**Table 1.**) were used to screening the Mungbean Yellow Mosaic Virus (MYMV) tolerance under natural field condition in *summer*-2019, AICRP on MULLaRP, Main Agricultural Research Station (MARS), College of Agriculture, University of Agricultural Sciences, Dharwad, Karnataka. The experiment was laid out in augmented design. Each row was of 4 m with plant to plant spacing of 10 cm and row to row spacing of 30 cm. Each advanced breeding line was sown in three rows in order to minimize the environmental effect on individual ABLs and each row was maintained with 40 plants. The advanced breeding lines were sown along with their resistant, moderately resistant and susceptible checks. All the agronomical practices are followed to raise a good crop except spraying of insecticides to maintain natural whitefly population. The field was regularly monitored to observe the symptoms of MYMV under the natural field

Table 1. The 14 F_6 families with respective Advanced Breeding Lines (ABLs) and the checks used for investigation

Sl. No.	Advanced breeding lines	Number of ABLs	Pedigree
1	1BRD-4 to 19	10	DGGV-2 × V-02-709
2	2BRD-2 to 20	10	DGGV-2 × V-02-802
3	3BRD-3 to 20	10	DGGV-7 × V-02-709
4	4BRD-1 to 17	10	DGGV-7 × V-02-802
5	5BRD-1 to 19	10	V-02-802 × DGGV-2
6	6BRD-5 to 17	10	V-02-802 × DGGV-7
7	7BRD-4 to 20	10	V-02-709 × DGGV-2
8	8BRD-2 to 20	10	V-02-709 × DGGV-7
9	PM-2 to 18	5	DGGV-2 × TM-96-2
10	PTI-2 to19	5	DGGV-2 × LGG-460
11	E-1 to 19	5	DGGV- 2 × IPM-409-4
12	MDR-6 to 20	5	DGGV-2 × RGM-1028
13	MDR-4 to 21	5	DGGV-2 × MH-421
14	MR-1 to 18	5	DGGV-2 × SML-1815
Sl. No.	Checks		
1.	DGGV-2 (Susceptible to MYMV)		
2.	DGGV-7 (Susceptible to MYMV)		
3.	IPM-409-4 (Moderate resistance to MYMV)		
4.	IPM-2-14 (Moderate resistance to MYMV)		
5.	LGG-460 (Moderate resistance to MYMV)		
6.	SML-1815 (Moderate resistance to MYMV)		
7.	RGM-1028 (Moderate resistance to MYMV)		
8.	MH-421 (Moderate resistance to MYMV)		
9.	TM-96-2 (Moderate resistance to MYMV)		
10.	V-02-709 (Resistance to MYMV)		
11.	V-02-709 (Resistance to MYMV)		

condition. After first and second week of sowing there was no symptom of MYMV. After twenty days of sowing, yellowing was noticed in some susceptible lines and then it spread very quickly. The per cent disease incidence (PDI) was calculated by using the formulae given by Basir *et al*. (2005). The disease incidence was observed in the 9th week after sowing. Based on the per cent disease incidence and disease severity index, the populations were categorized into different groups based on 0-5 scale given by Basir *et al*. (2005) (**Table 1.**).

Observations were made by counting the number of plants having MYMV symptoms to the total number of plants in each advanced breeding line. Hence in the present evaluation, PDI value was calculated by counting the diseased plants present in a total of 110 – 120 plants of in each ABL. The symptoms first appeared as minute golden yellow patches on affected trifoliate and severely concentrated to the periphery of the leaf veins. Infected leaves of green gram showed irregular and mixed pattern of green and yellow colors, even the leaf veins also turns into green and yellow patches. As the plant gets older, entire leaves turn into yellow color and it looks like a senesced leaf. The infected plant shows fewer numbers of flowers and pods. At severe infestation the entire pod turns yellow and pods have only few seed. Seeds showed shriveled coat texture with mosaic yellow and green patches. The symptoms are recorded on almost all aerial parts of an affected plant *viz*., stem, petiole, seeds and pods. The symptoms of MYMV disease started appearing after three weeks of sowing, the susceptible genotypes started showing symptoms of MYMV, which progressed with time up to seventh week. Most of the genotypes exhibited these symptoms with relatively similar intensity up to ninth week. Hence recording PDI value between seventh to ninth weeks after sowing will be more reliable than computing mean PDI value at V, VII and IX week. The 110 ABLs were categorized into different classes based on the mean PDI value derived from PDI values recorded at VII and IX weeks after sowing. The 110 ABL's were categorized into different groups based on the standard table given by Basir *et al*. (2005) (**Table 2**.). The formulae for PDI calculation given by Basir *et al*. (2005) is mentioned below.

$$\text{Percent Disease Incidence (PDI)} = \frac{\text{Total number of plants infected in row}}{\text{Total number of plants in a row}} \times 100$$

Table 2. Disease scoring for Mungbean Yellow Mosaic Virus (MYMV)

Disease Scale	Percent infection	Visual symptoms	Category
0	All plants free of virus symptoms	Complete absence of symptoms	Highly resistant
1	1-10% infection	Small yellowish spots scattered on some leaves	Resistant
2	11-20% infection	Yellowish bright spots common on leaves, easy to observe	Moderately resistant
3	21-30% infection	Yellowish bright specks on leaves, easy to observe with larger patches of symptoms	Moderately Susceptible
4	31-50% infection	Bright yellow specks or spots on all leaves, minor stunting of plants and less number of pods	Susceptible
5	50% and more infection	Yellowing or chlorosis of all leaves on whole plants, 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

RESULTS AND DISCUSSION

Improvement in the yield of green gram is becoming tough, mainly due to the existence of pests and diseases. Major biological worries of green gram are mungbean yellow mosaic virus, leaf crinkle virus, anthracnose, powdery mildew, Cercospora leaf spot, gram pod borer, bruchid and whitefly. Plant viral diseases cause severe economic losses in many major crops by reducing seed yield and quality. Mungbean yellow mosaic Virus disease (MYMV) is described to be the harshest viral disease amongst various viral diseases, caused by yellow mosaic virus. Among the numerous diseases, the MYMD was given a special consideration because of severity and ability to cause yield loss up to 85%, which is spreading faster to new areas. Mungbean yellow mosaic virus causes severe yield drop in all green gram growing countries in Asia, including India. The 110 advanced breeding lines derived from fourteen families of green gram had shown differential reaction with varied disease symptoms. No disease symptoms were observed on any of the breeding lines till the crop was three weeks old. The symptoms of MYMV disease started appearing on the leaves of young plants of susceptible breeding lines which became more prominent with time. After three weeks of sowing, the susceptible genotypes started showing symptoms of MYMV; this progressed with time up to seventh week. Most of the genotypes exhibited these symptoms with relatively similar intensity up to ninth week. Hence recording PDI value between seventh to ninth weeks after sowing will be more reliable than computing mean PDI value at V, VII and IX weeks. The disease incidence was recorded on ninth week after sowing to determine the per cent disease

Table 3. Advanced breeding lines (F_6) with resistant reaction to MYMV

Sl. No.	Pedigree	Advanced breeding lines (ABLs)	Per cent Disease Incidence (PDI) (%)	Category
1	DGGV-2 × V-02-802	2BRD-2	4.87	Resistant
2	DGGV-7 × V-02-709	3BRD-18	9.52	Resistant
		3BRD-9	4.76	Resistant
3	DGGV-7 × V-02-802	4BRD-1	8.33	Resistant
4	V-02-802 × DGGV-2	5BRD-14	18.75	Moderately Resistant
5	V-02-802 × DGGV-7	6BRD-9	7.69	Resistant
		7BRD-7	8	Resistant
6	V-02-709 × DGGV-2	7BRD-13	13.33	Moderately Resistant
		7BRD-12	8	Resistant
7	V-02-709 × DGGV-7	8BRD-20	2.85	Resistant
8	DGGV- 2 × IPM-409-4	E-19	3.57	Resistant
9	DGGV-2 × TM-96-2	PM-5	0	Highly Resistant
SL No.	**Checks**		Per cent Disease Incidence (PDI) (%)	**Category**
1	LGG-460 (Resistant Check)		0	Highly Resistant
2	RGM-1028 (Resistant Check)		0	Highly Resistant
3	TM-96-2 (Resistant Check)		10	Resistant
4	SML-1815 (Resistant Check)		10	Resistant
5	V-02-709 (Resistant Check)		18.69	Moderately Resistant
6	V-02-802 (Resistant Check)		19.03	Moderately Resistant
7	IPM-2-14 (Resistant Check)		12.77	Moderately Resistant
8	MH-421 (Resistant Check)		16.66	Moderately Resistant
9	IPM-409-4 (Resistant Check)		14.28	Moderately Resistant
10	DGGV-2 (Susceptible Check)		31.94	Susceptible
11	DGGV-7 (Susceptible Check)		30.91	Susceptible

incidence (PDI) based on the disease scoring scale given by Basir *et al*. (2005). Hence precise disease reaction was attributed to different breeding lines. In the present investigation the per cent disease incidence varied from 0 to 94.11 per cent in *summer*-2019. The mean per cent disease incidence recorded more than 50 for the breeding lines from the families *viz*., 4BRD-1 to 17 [DGGV-7 × V-02-802], 5BRD-1 to 19 [V-02-802 × DGGV-2], 6BRD-5 to 17 [V-02-802 × DGGV-7], 7BRD-4 to 20 [V-02-709 × DGGV-2], 8BRD-2 to 20 [V-02-709 × DGGV-7], MR-1 to 18 [DGGV-2 × SML-1815], PTI-2 to19 [DGGV-2 × LGG-460], E-1 to 19 [DGGV- 2 × IPM-409-4], MDR-6 to 20 [DGGV-2 × RGM-1028] and MDR-4 to 21 [DGGV-2 × MH-421] were classified as highly susceptible families. The remaining four families *viz*., 1BRD-4 to 19 [DGGV-2 × V-02-709], 2BRD-2 to 20 [DGGV-2 × V-02-802], 3BRD-3 to 20 [DGGV-7 × V-02-709] and PM-2 to 18 [DGGV-2 × TM-96-2] were classified as susceptible families. None of the breeding lines showed resistance reaction to MYMV, when we considered mean PDI values of breeding lines from a particular cross as a whole but some of the breeding lines showed resistance response to the MYMV, when we considered PDI value of each progeny row separately. By considering the PDI value for a particular ABL separately, only a few advanced breeding lines derived from nine families showed resistant and moderately resistant reaction to MYMV disease. The advanced breeding line 2BRD-2 derived from the cross DGGV-2 × V-02-802 showed resistant reaction with the per cent disease incidence value of 4.87 per cent. The lines 3BRD-18 and 3BRD-9 derived from DGGV-7 × V-02-709 were observed to be resistant with the least PDI values 9.52 and 4.76 per cent respectively. The cross derivative (4BRD-1) from DGGV-7 × V-02-802 had shown resistance response (PDI= 8.33 %). The progeny rows 5BRD-14 and 6BRD-9 from the crosses V-02-802 × DGGV-2 and V-02-802 × DGGV-7 recorded moderately resistance and resistance response with PDI values, 18.75 and 7.69 per cent, respectively. The three progeny rows, 7BRD-7, 7BRD-13 and 7BRD-12 from V-02-709 × DGGV-2 recorded resistant

Fig. 1. Resistance reaction 2BRD (DGGV-2 X V-02-8 Susceptible reaction DGGV-2 (Check))

(PDI= 8.00 %), moderately resistant (PDI= 13.33 %) and resistant (PDI= 8.00 %) reactions respectively and the progeny rows *viz*., 8BRD-20, E-19 and PM-5 derived from V-02-709 × DGGV-7, DGGV- 2 × IPM-409-4 and DGGV-2 × TM-96-2 showed resistant (PDI= 2.85 %, PDI= 3.57 %) and highly resistant (PDI= 0 %) reactions respectively (**Table 3 and Fig. 1.**). These studies were in agreement with the results of Dharajiya *et al*. (2018), Deepa *et al*. (2017), Ghuge *et al*. (2018), Kingsly *et al*. (2015) and Kabi *et al*. (2017). The resistance reaction of breeding lines 2BRD-2, 3BRD-18, 3BRD-9, 4BRD-1, E-19 and PM-5 could be due to the resistant nuclear genes present in female parents in their respective crosses and the resistant reaction of breeding lines 5BRD-14, 6BRD-9, 7BRD-7, 7BRD-13 and 7BRD-12 could be due to the cytoplasmic genes present in the male parents in their respective crosses. Hence, the advanced breeding lines 2BRD-2, 3BRD-18, 3BRD-9, 4BRD-1, 5BRD-14, 6BRD-9, 7BRD-7, 7BRD-13, 7BRD-12, 8BRD-20, E-19 and PM-5 conferring resistance/moderate resistance reaction to Mungbean Yellow Mosaic Virus (MYMV) should be further screened across locations and under artificial epiphytotic conditions the confirmation of resistance response to MYMV which could be further used in resistance breeding programmes.

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