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Screening of mungbean germplasm for resistance to mungbean yellow mosaic virus under natural condition

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Abstract
Mungbean Yellow Mosaic Virus (MYMV) is one of the most important diseases of Mungbean. It is transmitted through whitefly (*Bemisia tabaci*). The present investigation aimed to identify stable MYMV resistant lines through screening under natural condition. The experimental material consisted of two parents *viz*., MYMV resistant mungbean cultivar - Meha and agronomically superior but MYMV susceptible variety GM-4 as well as their 165 F_5 RILs. Screening for MYMV resistance was done by planting infector rows along with the test entries. Results revealed that most of the genotypes studied were categorized as resistant to highly susceptible. None of the test entries appeared to be immune. It was observed that the two parents Meha and GM-4 showed uniform expression of resistance and susceptibility, respectively. Infector rows of GM-4 were heavily infected, while resistant parent Meha remained uninfected till maturity. RIL population showed various reactions *viz*. Resistant (R), moderately resistant (MR), moderately susceptible (MS), susceptible (S) and highly susceptible (HS) based on disease score. In spite of the variable response to MYMV, out of 165 RILs, 78 RILs exhibited resistant reaction, 6 RILs moderately resistant, 7 RILs moderately susceptible, 37 RILs susceptible and 37 RILs highly susceptible. These genotypes would be utilized as donors to develop MYMV resistant lines. Heterozygous Inbred Lines may be identified from this population for molecular mapping and dissection of MYMV resistance in mungbean utilizing genomics tools.

Keywords: Recombinant inbred lines, mungbean yellow mosaic virus, screening, yellow mosaic disease

Introduction
Green gram (*Vigna radiata* (L.) R. Wilczek) (2n=22), is an important self-fertilizing pulse crop, ranks third in production after chickpea and pigeon pea. The estimated genome size of mungbean is 579 Mb (Kang *et al*., 2014) [9]. Among several constraints for mungbean production, Mungbean Yellow Mosaic Virus (MYMV) disease occupies supreme position and is the most destructive and devastating viral disease. It was first reported in India in (1955) and is transmitted by the insect vector, White fly *(Bemisia tabaci*). MYMV infects mungbean, soybean, mothbean, cowpea, urdbean and some other leguminous hosts (Dhingra and Chenululu, 1985, Qazi *et. al*. 2007) [4, 13]. MYMV belongs to genus Begomovirus of the family Geminiviridae (Bos, 1999). The virus has geminate particle morphology (20 x 30 nm) and the coat protein encapsulates spherical, single stranded DNA genome of approximately 2.8 Kb (Hull, 2004) [7]. In *Bemisia tabaci*, which transmits MYMV persistently, the adult females are 3 times more proficient transmitters than males. The white fly nymphs obtain the virus from diseased leaves. Whitefly-transmitted plant viruses are found in the humid and sub-humid countries.

The virus causes uneven yellow and green specks or patches on the leaves which finally turn entire yellow. Affected plants generate fewer flowers and pods, which also develop mottling and remain small and contain fewer, smaller and shrunken seeds. Yield loss up to 80% was reported in susceptible cultivars by During sever infestation at reproductive stage, pods also turn yellow and becomes curvy at the tips. Controlling MYMV incidence is only possible by the way of reducing the vector *viz*., whitefly population using insecticides which are ineffective under severe infestations.

Chemical management of the vector is not economic since numerous sprays of insecticides are required to control vector population. Recurrent sprayings also lead to health hazards and ecological disturbance. On the contrary, use of virus resistant variety is the most efficient approach to alleviate the occurrence of MYMV disease in economic and ecological terms. Screening mungbean germplasm against MYMV for the identification of resistant genotypes is very much essential. A good quality of research efforts have been directed towards screening mungbean germplasm against MYMV for the identification of resistant sources under diverse

environmental conditions and a number of resistant lines have been reported by some workers Ghafoor *et. al*. 1992; Bashir, 2002; Shad *et. al*. 2006; Pandiyon *et. al*. 2007) [5, 2, 15, 12]. With a view to screen MYMV resistant lines, F_5 RILS population obtained from a cross between phenotypic extremes Meha and GM-4 were screened against MYMV under natural hot spot at Navsari Agricultural University, Navsari, Gujarat, India during Summer, 2017, when higher population density of viruliferous white fly is always present due to warm and humid environment.

Materials and Methods

The experimental material in the present study was developed from the cross between the early as well as high yielding susceptible variety GM-4 as female parent and resistant variety Meha as a pollen parent. F_5 RILs were developed from F_2 population of this cross by single seed descent method. Total 165 F_5 RILs were grown at hot spot with GM-4 as an infector row by keeping one infector row after every two rows of RILs for screening against MYMV infection at College Farm, N. M. College of Agriculture, Navsari Agricultural University, Navsari during Summer, 2017 which is a nature hot spot for MYMV screening due to warm and humid climate which favours white fly vector population.

All the recommended agronomic practices were followed. No insecticidal spray was given in order to allow the whitefly population build up for severe spread of the disease. The crop was regularly monitored for the presence of whitefly and development of disease symptoms. Whitefly started landing on the plants soon after germination and the disease made its first appearance during 3rd week after sowing. The test material was scored after 80% of the plants in spreader rows showed MYMV incidence. The following rating scale suggested by Singh *et al*. (1988) [16] was adopted to characterize trifoliate leaves (Table 1).

Table 1: MYMV Disease rating scale (Singh *et al*., 1988) [16]

Sr. No.	Rating scale	Percentage of plants foliage affected	Reaction
1.	1	No visible symptoms (or) minute yellow specks covering 0.1–5.0% of the leaf area	Resistant (R)
2.	3	Mottling of leaves covering 5.1–10.0% of the leaf area	Moderately resistant (MR)
3.	5	Mottling and yellow discolouration of 10.1–25.0% of the leaf area	Moderately susceptible (MS)
4.	7	Mottling and yellow discoloration of leaves on 25.1–50% of the leaf area	Susceptible (S)
5.	9	Yellow mottling on more than 50% and up to 100% of the leaf area	Highly susceptible (HS)

Results

Evolution of resistant varieties is considered to be the most feasible and durable solution of controlling MYMV disease. Screening mungbean germplasm against MYMV disease under natural condition is the first step in identifying the resistant donors for evolving the mungbean varieties with MYMV resistance and seed yield. In the present study, RILs were phenotyped as resistant and susceptible based on the field evaluation by using rating scale. Two progenitor of RIL population Meha and GM-4 depicted uniform expression of resistance and susceptibility, respectively. Spreader rows of GM-4 were heavily (90–100%) infected, while resistant parent Meha remained uninfected till maturity. The RIL population showed various reactions *viz*. Resistant (R), Moderately resistant (MR), Moderately susceptible (MS), Susceptible(S) and Highly susceptible (HS) based on disease score (Table 1). The RILs with resistant and moderately resistant reactions showed consistent reaction, that is, either R or MR, while the RILs with MS, S and HS reactions were either S or HS. Hence, RILs were grouped into two categories *viz*. resistant (R) and susceptible (S). Out of these 165 RILs, 85 lines showed resistant reaction, while 80 showed susceptible reaction (Table 2).

Table 2: Reaction of mungbean F_5 RILs against MYMV at hotspot

Disease reactions	Reaction of Individual RILs	No. of RILs	Final classification of F_5 RILs on the basis of MYMV infection
Resistant	1, 5, 8, 11, 14,16, 20, 24, 27, 28, 29, 30, 33, 34, 38, 39, 40, 42, 45, 47, 50, 54, 55, 56, 59, 61, 62, 68, 72, 74, 76, 79, 83, 84, 85, 89, 86, 88, 90, 91, 92, 95, 97, 99, 103, 105, 106, 108, 109, 111, 113, 114, 115, 119, 121, 124, 125, 127, 130, 134, 135, 136, 138, 141, 142, 143, 148, 151, 152, 153, 156, 158, 159, 161,	78	85 (Resistant RILs)
Moderately resistant	12, 26, 63, 81, 102, 129, 160	7	
Moderately susceptible	3, 7, 18, 22, 60, 118	6	80 (Susceptible RILs)
Susceptible	2, 9, 15, 19, 21, 25, 31, 32, 36, 37, 43, 46, 48, 52, 57, 64, 69, 71, 75, 80, 87, 93, 94, 98, 101, 107, 116, 120, 126, 132, 140, 146, 149, 150, 157, 162,	37	
Highly susceptible	4, 6, 10, 13, 17, 23, 35, 41, 44, 49, 51, 53, 67, 70, 73, 77, 78, 82, 96, 100, 104, 110, 112, 118, 122, 128, 129, 131, 133, 137, 139, 145, 147, 155, 160, 163	37	

Resistant | Moderately resistant | Moderately susceptible

Susceptible | Highly susceptible

Fig 1: Typical symptoms of MYMV in F_5 RILs of mungbean

Discussion

Viral diseases including MYMV drastically reduce the yield of numerous legume crops. Screening of mungbean genotypes in response to MYMV under field conditions determines the greater susceptibility of genotypes to MYMV, which may perhaps be associated to favourable ernvironmental conditions for the disease development owing to the presence of enormous vector population in the field. The susceptible check lines after every two test entries resulted in enhanced vector population. The MYMV vector, whitefly (*Bemisia tabaci*) appeared to inhabit plant soon after the emergence and remained till maturity and with the passage of time, disease severity increased significantly. In summer season, high temperature along with humid conditions supported vector dispersal which provided opportunities for severe disease development in the material under study.

It is obvious from the results that out of 165 RILs, 78 RILs appeared as resistant, 7 RILs moderately resistant, 6 RILs moderately susceptible, 37 RILs susceptible, 37 RILs highly susceptible and none of the RILs showed immune reaction. It is observed that the genotypes screened against MYMV disease at these locations exhibited differential response to resistance. The differential response of MYMV disease severity at different locations may be the possibility of existence of different strains of the virus. The other possible causes may be vector load (Whitefly), climatic condition and genetic background as well as lineage of varieties.

Use of virus resistant genotypes has become an imperative measure for an effective disease control but there is little success achieved in this respect. Similar kind of screening technique was utilized by Kooner and Harpreet (2005) [1]. They screened fifty seven mungbean genotypes for MYMV resistant in rainy season for four years. Selvi *et al.* (2006) [14] screened MYMV resistant mungbean line ML 267 in field at Coimbatore utilizing the similar procedure. The test materials were scored in field with spreader rows of highly susceptible genotype CO5 mungbean. Iqbal *et al*. (2011) [8] screened one hundred genotypes of mungbean germplasm against MYMV during summer season under natural field conditions at Islamabad. The genotype grouped under resistant category could be utilized as donors to develop MYMV resistant lines. For additional corroboration, these genotypes may be screened through artificial screening methods like forced feeding method and agroinoculation method to confirm resistance against MYMV. The previous studied indicated monogenic dominant nature of MYMV resistance in lined with lineage of this resistance from black gram in popular variety Meha.

Conclusion

The yellow mosaic disease caused by MYMV is limiting the production of green gram across the world. The varied incidence of this virus is reported from various parts of green gram cultivating countries including India. Heterozygous Inbred Lines identified from the progenies of resistant F5 RILs may be utilized for molecular mapping and dissection of MYMV resistance in mungbean utilizing genomics tools like transcriptome sequencing coupled with quantitative gene expression studies using qRT-PCR (quantitative real time PCR). The whole genome mungbean sequence available may aid and hasten this process.

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