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Epistatic Basis of Mosaic Resistance in Mungbean [*Vigna radiata* (L.) Wilczek]

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Hybridization was carried out between highly resistant genotype Meha and four susceptible genotypes *viz*., Pusa Vishal, GJM-1006, GM-4 and GJM-1008 to deduce genetics of Mungbean Yellow Mosaic Virus (MYMV) resistance in mungbean. Parents, F_1s, F_2s and F_3 progenies were evaluated for their response to MYMV infection utilizing highly susceptible variety GM-4 as an infector row keeping one row of GM-4 after every two rows of parents, F_1s, F_2s and F_3 progenies. No insecticides were sprayed to build up white fly vector population. Segregation pattern of resistance and susceptibility in F_2 generation indicated duplicate epistasis (15: 1) which was confirmed by non-significant χ^2 test. The proposed genotypes for Meha, susceptible male parents and hybrids are $R_1R_1R_2R_2$, $r_1r_1r_2r_2$ and $R_1r_1R_2r_2$, respectively. Ten resistant F_2 plants were selfed to further confirm digenic duplicate epistatic nature of mosaic resistance. Out of ten F_3 progenies, seven progenies exhibited 15: 1, while three manifested 3: 1 segregation pattern of resistant and susceptible plants in support to duplicate epistasis. The proposed genotype of F_2 individuals showing 15: 1 segregation pattern in F_3 progenies is $R_1r_1R_2r_2$. While, F_3 progenies indicating 3: 1 ratio predicted F_2 individual's genotype either as $R_1r_1r_2r_2$ or $r_1r_1R_2r_2$ from which they are derived. The results are also in accordance with the lineage of mosaic resistance in Meha from blackgram. The role of duplicate epistasis observed in present study will pave the ways to transfer mosaic resistance in high yielding susceptible cultivars as well as for molecular breeding of mungbean against MYMV infection.

Introduction

Mungbean [*Vigna radiata* (L.) Wilczek], belongs to Fabacease family, is well known self-fertilising diploid pulse crop of Asian countries with 2n = 2x = 22 chromosomes and a genome size of 579 Mb (Parida *et al.*, 1990). It is one of the thirteen food legumes grown in India and third most important pulse crop of India after chickpea and pigeonpea. It is known by various names in India as mung, moong, but the name greengram is more common (Chatterjee and Randhawa, 1952).

Mungbean is an excellent source of high quality protein in the form of split pulse and fresh sprouts. Mungbean seeds are rich in protein, easily digestible and lack flatulence which makes it important component of balance diet. Seeds are also very good source

of minerals (calcium, iron, zinc, potassium and phosphorus), vitamins (folate and vitamin K) and dietary fibres (Keatinge *et al.,* 2011). Ascorbic acid is synthesized in sprouted seeds of mungbean with increment in riboflavin and thiamine. The protein is rich in lysine, an amino acid that is deficient in cereals.

Mungbean is grown in all seasons in India, however, summer cultivation during February to June is the most suitable growing period when there is a plenty of sunshine, high temperature and low humidity that keep insects and disease infestations at their lowest. Being a leguminous crop, it has the capacity to fix atmospheric nitrogen through symbiotic nitrogen fixation and is also used as a green manure crop. It is a short duration crop and therefore widely used as mixed intercrop or in crop rotation to improve nitrogen status of soil to break disease/ pest cycles.

Among various biotic stresses, mosaic disease caused by Mungbean Yellow Mosaic Virus (MYMV) drastically reduces yield up to cent per cent. Yellow Mosaic Disease (YMD) is caused by different species of whitefly transmitted geminivirus which belongs to genus begomovirus and family geminiviridae. Reports on losses due to disease revealed reduction of 9.6 to 38.2 % in height, 7 to 28.5 % in fresh weight of shoot and 4.3 to 22.1 % in dry weight, 25.7 % in 1000 seed weight of susceptible cultivar (Premchand and Varma, 1983) and yield loss of 83.9 % and a maximum growth reduction of 62.94% in MYMV infected cultivar (Quaiser Ahmed, 1991). MYMV disease on greengram was first ever reported from fields of IARI, New Delhi in 1960 and is transmitted principally by whitefly, *Bemisia tabaci* (Genn.) and grafting but not by sap, seed or soil (Nariani, 1960). MYMV causes irregular green and yellow patches in older leaves and complete yellowing of younger leaves. Yellow mosaic disease in northern and central India is caused by MYMIV, whereas the disease in southern and western India is caused by MYMV (Usharani *et al.,* 2004). Affected plants produce fewer flowers and pods, pods often develop mottling, remain small and contain fewer and smaller seeds thus affecting yields qualitatively and quantitatively. Reduction in number of pods per plant, seeds per pod and seed weight are the main contributing factors for yield reduction upon mosaic infection (Nene, 1973; Dhingra and Chenulu, 1985). MYMV disease can be controlled by chemical, cultural and genetic methods. Nevertheless, host plant resistance is preferred over other methods because it is economical and eco-friendly approach. However, screening at hot spot with infector row is more reliable. Most efficient method of screening for MYMV resistance is force inoculation.

Knowledge on inheritance patterns is pre-requisite for resistance breeding against mosaic disease in mungbean. Reports on genetics of mosaic resistance in mungbean varies from monogenic recessive to quantitative in nature (Khan *et al.,* 2007; Dhole and Reddy, 2013; Jain *et al.,* 2013; Kitsanachandee *et al.,* 2013; Mahbubul Alam *et al.,* 2014). In the present study, an effort was made to study genetics of mosaic resistance in mungbean utilizing five diverse parents.

Materials and Methods

To deduce the inheritance pattern of mosaic resistance, resistant genotype Meha was utilized as common parent and was crossed as female parent with susceptible male parents Pusa Vishal, GJM-1006, GM-4 and GJM-1008. Pusa Vishal is tolerant genotype, however, symptoms do appear similar to susceptible genotypes at Navsari. Five parents, their resulting F_1s, F_2s and F_3 progenies were evaluated for their reaction to mosaic in summer seasons during 2014 and subsequent

years keeping highly susceptible variety GM-4 as an infector row (Fig. 1). Inter and intra row spacing was kept as 60 cm and 15 cm, respectively. To build up whitefly vector population, the material was kept free from pesticides during all these evaluation trials. The reaction to disease was evaluated every 10 days after sowing and genotypes were characterized as resistant and susceptible according to Singh *et al.,* (1988). Few resistant and susceptible F_2 and F_3 individuals were selfed to develop subsequent progenies to trace the inheritance pattern in subsequent segregating generations.

(a) Infected GM-4 plant

(b) Status of GM-4 infector row

(c) Resistant F_3 progenies and heavily infected GM-4 infector rows

Fig.1 Status of infector row and F_3 progenies

Table.1 Observed and expected number of resistant and susceptible plants in different generations of four crosses in mungbean

Parents/ Cross	Proposed genotypes	Phenotypic classes	Observed number		Expected number		Expected ratio	χ^2 value	Table χ^2 (1 df, 0.05)
			R	S	R	S			
Parents									
Meha	$R_1R_1R_2R_2$	R	20	00	20	00	1 : 0	NA	NA
Pusa Vishal*	$r_1r_1r_2r_2$	S	00	20	00	20	0 : 1	NA	NA
GJM-1006	$r_1r_1r_2r_2$	S	00	20	00	20	0 : 1	NA	NA
GM-4	$r_1r_1r_2r_2$	S	00	20	00	20	0 : 1	NA	NA
GJM-1008	$r_1r_1r_2r_2$	S	00	20	00	20	0 : 1	NA	NA
Hybrids									
(A) Meha x Pusa Vishal	$R_1r_1R_2r_2$	R	20	00	20	00	1 : 0	NA	NA
(B) Meha x GJM-1006	$R_1r_1R_2r_2$	R	20	00	20	00	1 : 0	NA	NA
(C) Meha x GM-4	$R_1r_1R_2r_2$	R	20	00	20	00	1 : 0	NA	NA
(D) Meha x GJM-1008	$R_1r_1R_2r_2$	R	20	00	20	00	1 : 0	NA	NA
F_2s									
(A) Meha x Pusa Vishal	$R_1R_1R_2R_2$	R/S	93	7	93.75	6.25	15 : 1	0.096	3.84
(B) Meha x GJM-1006	$R_1r_1R_2r_2$	R/S	95	5	93.75	6.25	15 : 1	0.266	3.84
(C) Meha x GM-4	$R_1r_1r_2r_2$	R/S	93	8	93.75	6.25	15 : 1	0.096	3.84
(D) Meha x GJM-1008	$r_1r_1R_2r_2$ $r_1r_1r_2r_2$	R/S	96	4	93.75	6.25	15 : 1	0.864	3.84
F_3 progenies (Genotypes of susceptible individuals is $r_1r_1r_2r_2$)									
11A	$R_1r_1R_2r_{2/}$	R/S	56	4	56.25	3.75	15 : 1	0.017	3.84
40A	$R_1r_1R_2r_2$	R/S	58	2	56.25	3.75	15 : 1	0.871	3.84
15C	$R_1r_1R_2r_2$	R/S	58	2	56.25	3.75	15 : 1	0.871	3.84
44C	$R_1r_1R_2r_2$	R/S	57	3	56.25	3.75	15 : 1	0.160	3.84
17A	$R_1r_1R_2r_2$	R/S	53	7	56.25	3.75	15 : 1	3.004	3.84
56C	$R_1r_1R_2r_2$	R/S	53	7	56.25	3.75	15 : 1	3.004	3.84
65C	$R_1r_1R_2r_2$	R/S	53	7	56.25	3.75	15 : 1	3.004	3.84
13A	$R_1r_1r_2r_2 / r_1r_1R_2r_2$	R/S	40	20	45	15	3 : 1	2.222	3.84
60C	$R_1r_1r_2r_2 / r_1r_1R_2r_2$	R/S	41	19	45	15	3 : 1	1.422	3.84
48A	$R_1r_1r_2r_2 / r_1r_1R_2r_2$	R/S	47	13	45	15	3 : 1	0.355	3.84

* - Pusa Vishal is tolerant but symptoms are similar to susceptible genotypes, R – Resistant, S – Susceptible, NA- Not applicable

Results and Discussion

With a view to study heredity pattern of mosaic resistance in mungbean caused by MYMV which is very important economic pest, crossing was done between mosaic resistant genotype Meha with susceptible genotypes Pusa Vishal, GJM-1006, GM-4 and GJM-1008. The parents, their hybrids, subsequent generation and progenies were screened for their response to MYMV infection keeping highly susceptible variety GM-4 as an infector row. Evaluation was carried out during summer seasons as severe MYMV infection is observed during this season at Navsari. The observed and expected numbers of individuals along with chi-square test are presented in Table 1. All the plants of Meha and F_1 hybrids showed resistant reactions while all the plants of Pusa Vishal, GJM-1006, GM-4 and GJM-1008 exhibited susceptible reactions. Observed and expected number of individuals in four F_2 populations along with non-significant χ^2 value indicated duplicate epistasis as the data were perfectly in agreement with 15 : 1 segregation ratio of resistant and susceptible individuals. Confirming digenic duplicate epistasis, proposed genotypes of Meha, Pusa Vishal, GJM-1006, GM-4 and GJM-1008 are $R_1R_1R_2R_2$, $r_1r_1r_2r_2$, $r_1r_1r_2r_2$, $r_1r_1r_2r_2$ and $r_1r_1r_2r_2$, respectively. Similarly proposed genotype of F_1 individuals is $R_1r_1R_2r_2$. To further confirm the result and trace the inheritance pattern of mosaic resistance, ten resistant plants *viz*., 11A, 40A, 15C, 44C, 17A, 56C, 65C, 13A, 60C and 48A from F_2 generation were selfed to obtain F_3 progenies. Ten F_3 progeny families each comprising of 60 individual plants were evaluated for their reaction to MYMV infection keeping GM-4 as an infector row. The segregation patterns of these progenies are depicted in Table 1. Of these, seven F_3 progeny families obtained from F_2 individuals *viz*., 11A, 40A, 15C, 44C, 17A, 56C and 65C segregated in accordance with 15: 1 ratio of duplicate epistasis. The proposed genotype of these F_2 plants is $R_1r_1R_2r_2$. While, three F_3 progeny populations obtained from 13A, 60C and 48A segregated in agreement with 3: 1 ratio. Proposed genotype of relevant F_2 individuals for this kind of inheritance pattern is either $R_1r_1r_2r_2$ or $r_1r_1R_2r_2$. Besides, all the susceptible plants produced susceptible progenies (data not shown). In most of the previously reported results, monogenic recessive nature of mosaic resistance was observed by Malik *et al.,* (1986 & 1988), Thakur *et al.,* (1997), Pal *et al.*, (1991), Reddy and Singh (1995) or two recessive genes (Dhole and Reddy, 2012) or complementary recessive genes (Shukla and Pandya, 1985). Surprisingly, in our study, digenic duplicate epistasis was observed where two dominant genes together or alone are responsible for mosaic resistance. Meha is a very popular variety of mungbean and has been developed from the interspecific cross of *Vigna radiata* (Pant moong-2) and *Vigna mungo* (AMP-46) (Nadarajan and Gupta, 2010). In blackgram, monogenic dominant nature of resistance was reported by Dahiya *et al.*, (1977), Kaushal and Singh (1988), Gupta *et al.*, (2005) and Gupta *et al.*, (2013), while it was reported to be digenic recessive by Singh (1980), Dwivedi and Singh (1985) and Verma and Singh (1986). Duplicate epistatic nature of mosaic resistance observed in present study is in agreement with previously reported results of Murugan and Nadarajan (2012), Durga Prasad *et al.,* (2015) and Thamodran *et al.,* (2016) in black gram. However, the results obtained are dependent upon genetic background, environment under which mosaic screening is performed as well as prevailing pathotypes in that area. The present findings will contribute to future breeding strategies aimed to incorporate mosaic resistance in mungbean. This information will also be useful for identification of molecular markers linked to mosaic resistance and molecular dissection of mosaic disease resistance.

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