Genetic Studies on Differential Expression of Mungbean Yellow Mosaic Virus Resistance Related to Trichome Density in Urd bean (*Vigna mungo* (L.) Hepper)

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In blackgram, five cross combinations were taken for generation mean analysis to study the inheritance pattern of *Mungbean yellow mosaic virus* (MYMV) disease which revealed different gene actions. It is found that the crosses Co5 x VBN (Bg) 4 and Co5 x VBG 66, the segregation was governed by digenic complementary interaction. However, in Co5 x VBG 73 the YMV incidence was governed by digenic duplicate interaction. In contrast, in the crosses KBG 98005 x VBN (Bg) 4 and KBG 98005 x VBG 73, the incidence of YMV was governed by trigenic inhibitory interaction. The putative gene symbols assigned for the five genotypes *viz.,* Co5, KBG 98005, VBN (Bg) 4, VBG 66 and VBG 73 are $r_1r_1r_2r_2r_3r_3r_4r_4$, $r_1r_1r_2r_2r_3r_3r_4r_4II$, $R_1R_1R_2R_2ii$, $R_1R_1R_2R_2$ and $R_3R_3R_4R_4ii$, respectively. Besides this, the presence of trichomes in leaf of urdbean genotypes reveals that trichomes do not hinder the activities of whitefly and the resistance to MYMV may be due to some other factors like chemical, physiological and morphological factors.

Key Words: Blackgram, Genetic studies, Inheritance, Mungbean yellow mosaic virus, Trichome density

Introduction

Mungbean yellow mosaic virus (MYMV) is the one of the most destructive diseases and is prevalent on mungbean, blackgram and soybean throughout India mainly in the *Kharif* season and rice fallow situation. The virus is transmitted by whitefly, *Bemisia tabaci*. The yield losses upto 100 % have been reported by Nair (1971) in blackgram. Khattak *et al*. (2000) reported 32.2 to 78.6% decrease in grain yield under field conditions in mungbean. Hence, the present investigation was taken up to know the inheritance pattern of MYMV, Which is the prerequisite to evolve high yielding blackgram varieties combined with resistance to MYMV. Further, the presence of trichomes on leaf was also studied to know the relationship between MYMV and trichome density.

Materials and Methods

To study the inheritance pattern of MYMV, the five cross combinations *viz*., Co5 x VBN (Bg) 4, Co5 x VBG 73, Co5 x VBG 66, KBG 98005 x VBN (Bg) 4 and KBG 98005 x VBG 73 were chosen for the study. The 'lines' (Co5 and KBG 98005) are highly susceptible and three 'testers' (VBN (Bg) 4, VBG 73 and VBG 66) are highly resistant for MYMV. Six generations *viz.,* P_1, P_2, F_1, F_2, B_1 and B_2 for each of the five crosses were generated to understand the inheritance pattern of MYMV. The six generations of the five selected cross combinations were raised at National Pulses Research Centre, Vamban, which is a hot spot area for MYMV. The above said materials were raised in ridges of two meter length spaced at 30 cm between ridges and 10 cm between plants in two replications. For every 10^{th} row and as border rows of the experimental plot the check Co5 (highly susceptible to MYMV) was raised as infector row so as to effectively spread the inoculum.

For trichome density studies, fifth leaf from the top of the selected plants in parents alone was taken at the time of initiation of flowering. The number of trichomes per cm^2 was estimated after removing chlorophyll from the leaf samples as per the method suggested by Maite *et al.* (1980).

The MYMV incidence was recorded on all the plants based on the visual scores on 50^{th} day while the susceptible check Co5 recorded scale 9. The classification was made into scale 1-9 as follows based on the scale adopted by Singh *et al.,* (1988) and is given below:

The plants in the F_2 and back cross generations were classified as resistant (1-3) and susceptible (5-9) following Reddy and Singh (1993).

The goodness of fit to Mendelian segregation ratio for MYMV (resistance: susceptible) in the segregating population was tested through Chi-square test.

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Scales	Percentage of plant foliage affected	Reaction
1	Mottling of leaves covering 0.1 to 5.0% of the leaf area	Resistant
3	Mottling of leaves covering 5.1 to 10.0% of the leaf area	Moderately resistant
5	Mottling and yellow discoloration of 10.1to 25.0% of the leaf area	Moderately susceptible
7	Mottling and yellow discoloration of 25.1to 50.0% of the leaf area	Susceptible
9	Severe yellow mottling on more than 50.0% and up to 100% of the leaf area	Highly susceptible

The mean disease scale of parents and $F_1$was calculated following Singh (1980).
Mean scale = $\sum$ (Infection rate x Frequency)/Total number of plants scored.

Results and Discussion

For the study of inheritance of MYMV resistance, the Chi-square test for the deviation from the expected genetic ratios of the segregating generations *viz*., F_2, B_1 and B_2 of five crosses was made and the results are presented in Table 1.

The F_1 of the crosses *viz*., Co5 x VBN (Bg) 4), (Co5 x VBG 73) and (Co5 x VBG 66) were resistant to MYMV, while it was susceptible in the crosses KBG 98005 x VBN (Bg) 4 and KBG 98005 x VBG 73. Regarding the segregating generations, in crosses Co5 x VBN (Bg) 4 and Co5 x VBG 66 the chi-square test for the expected ratio of 9:7 (resistant: susceptible) in F_2 and 1:3 (resistant: susceptible) in B_1 was not significant. In B_2 generation, all plants were resistant. In the case of Co5 x VBG 73 cross, the chi-square test revealed that the F_2 generation showed a expected ratio of 15:1 for resistance: susceptible and B_1 showed a ratio of 3:1 (resistant: susceptible). In B_2, all plants were resistant. In the crosses KBG 98005 x VBN (Bg) 4 and KBG 98005 x VBG 73, the chi-square test was non-significant showing fitness of the expected ratios of 15:49 (resistant: susceptible) and 1:1 (resistant: susceptible) in F2 and B_2 generations respectively. All the plants in B_1 were susceptible to MYMV.

Among these five crosses, three crosses *viz*., (Co5 x VBN (Bg) 4), (Co5 x VBG 73) and (Co5 x VBG 66) were found to be resistant to MYMV in $F_{1,}$ where the female parent was Co5. Therefore, resistance is dominant over susceptibility. In black gram, Dahiya *et al*. (1977), Verma and Singh (1980) and Kaushal and Singh (1988) and in green gram Reddy and Singh (1993) Selvi (2002) and Gupta *et al*. (2005) and in mungbean, Patel *et al*. (2009) reported that the resistance was dominant over susceptibility. The segregation of YMV resistance in the present study was

Table 1. Chi-square test for inheritance of MYMV resistance in blackgram

Generation	Observed values		Expected ratio	c^2 values	Probability between
	Resistant	Susceptible			
Co5 X VBN(Bg) 4					
F_1	Resistant	–			
F_2	151	111	9:7	0.21	0.70-0.50
B_1	31	71	1:3	1.59	0.30-0.20
B_2	121	–			
Co5 X VBG 73					
F_1	Resistant	–			
F_2	254	20	15:1	0.52	0.50-0.30
B_1	80	30	3:1	0.31	0.70 – 0.50
B_2	120	–			
Co5 X VBG 66					
F_1	Resistant	–			
F_2	172	146	9:7	0.6	0.50-0.30
B_1	35	80	1:3	1.8	0.20-0.10
B_2	115	–			
KBG 98005 X VBN(Bg) 4					
F_1	–	Susceptible			
F_2	54	212	15:49	1.44	0.20-0.30
B_1	–	112			
B_2	52	56	1:1	0.15	0.70
KBG 98005 X VBG 73					
F_1	–	Susceptible			
F_2	57	218	15:49	1.13	0.2- 0.30
B_1	–	115			
B_2	57	50	1:1	0.45	0.50

Table 2. Details of the parents

Genotypes	Parentage	Reaction to MYMV	Place of origin	Special features
CO 5	Selection from Musiri local	Highly Susceptible	Coimbatore	High yielding suitable for rabi seasons
KBG 98005	Selection from Kalugumalai local.	Highly Susceptible	Kovilpatti	Suitable for rainfed situation
VBN (Bg) 4	Co 4 x PDU 102	Highly Resistant	Vamban	High yielding, suitable for all seasons
VBG 73	Vamban 1 x UK 17	Highly Resistant	Vamban	Short duration (60 days)
VBG 66	Pure line selectionfrom IPU 94-2	Highly Resistant	Vamban	Suitable for rainfed situation

appeared to be governed by digenic complementary interaction as seen from the ratio of 9:7 (resistant: susceptible) in F_2 of Co5 x VBN (Bg) 4 and Co5 x VBG 66. The pattern of segregation in B_1 (1 resistant: 3 susceptible) and B_2 (all resistant) of these two crosses (Co5 x VBN (Bg) 4 and Co5 x VBG 66) also confirmed the result of complementary interaction (duplicate recessive genes) (Table 1). A similar type of duplicate recessive genes for MYMV resistance in black gram was reported by Verma and Singh (1980), Sandhu *et al.* (1985) and Shukla and Pandiya (1985) and in green gram by Selvi (2002).

However, in the cross (Co5 x VBG 73), the segregation of F_2 showed a ratio of 15:1 (resistant: susceptible). The segregation ratio in B_1 (3 resistance: 1 susceptible) and B_2 (all resistant) also confirmed the ratio observed in F_2. This revealed that, in the cross Co5 x VBG 73 the YMV was governed by interaction of two duplicate genes (duplicate dominant genes). Shukla *et al.* (1978) and Singh (1980) reported the presence of duplicate dominant genes for YMV in black gram. From the above discussion it was found that, though the three crosses had same female parent (Co5), two different types of gene action of complimentary interaction in (Co5 x VBN (Bg) 4 and Co5 x VBG 66) and duplicate interaction in Co5 x VBG 73 were noticed. The reason for the difference in gene action may be attributed to the presence of two different sets of genes in male parents of the crosses. The male parents of the crosses (Co5 x VBN (Bg) 4 and Co5 x VBG 66) namely VBN(Bg) 4 and VBG 66, may have same alleles ($R_1R_1R_2R_2$), which act in complementation. However, the male parent of the cross Co5 x VBG 73 namely VBG 73, may have another set of non-allelic genes ($R_3R_3R_4R_4$), which act in a duplicate manner. The common female parent of all these three crosses, namely Co5, may have recessive alleles for all these four loci.

In the crosses KBG 98005 x VBN (Bg) 4 and KBG 98005 x VBG 73, the F_1 was found to be susceptible, where the female parent was KBG 98005. The segregation of F_2 was observed to be 15:49 (resistant: susceptible). It showed that the reaction to MYMV was governed by trigenic inhibitory interaction. The segregation in B_1 (all susceptible) and B_2 (1 resistant : 1 susceptible) also confirmed the trigenic inhibitory interaction. In blackgram, inhibitory gene action was reported by several workers in blackgram (Solanki *et al.*, 1982; Verma,1985; Verma and Singh, 1986; Reddy and Singh 1990); in greengram (Mishra and Asthana, 1996; Khattak *et al.*, 2000). From the above results, it could be concluded that the male parents of these two crosses namely VBN (Bg) 4 and VBG 73 respectively, may have recessive alleles for an inhibitory gene (ii) apart from two sets of alleles already indicated. The female parent KBG 98005 of these two crosses may have a set of dominant inhibitory alleles (II) at a locus apart from recessive alleles for susceptibility to MYMV at four loci.

Therefore, the following putative gene symbols are proposed for the parents involved in the five crosses:

Parent	Reaction to MYMV	Gene symbol for MYMV resistance
CO5	Susceptible	$r_1r_1r_2r_2r_3r_3r_4r_4$
KBG 98005	Susceptible	$r_1r_1r_2r_2r_3r_3r_4r_4II$
VBN(Bg) 4	Resistant	$R_1R_1R_2R_2ii$
VBG 66	Resistant	$R_1R_1R_2R_2$
VBG 73	Resistant	$R_3R_3R_4R_4ii$

However, the gene symbols are allotted subject to confirmation by allelic tests. The allelic tests may be conducted by intercrossing all the three male parents and studying the resistant pattern for MYMV resistance.

From the present investigation it was confirmed that the MYMV was controlled by two and three genes with various types of interaction. Hence, recombination breeding with two or three cycles of recurrent selection is required to obtain desirable segregants of high yielding ability coupled with MYMV resistance.

MYMV Resistance and Trichome density on leaves

Limited studies have been conducted to reveal the relationship between white fly and MYMV incidence. Earlier results reported that there was an association between trichomes on leaves and whitefly resistance (Chand and Verma, 1980). Hence, the present study was undertaken utilizing the MYMV scores and the number of

Table 3. Correlation between number of trichomes and MYMV scores in parents

Code No.	Genotypes	No. of trichomes/cm^2	Mean MYMV score	Type of trichome
L_1	Co 5	10 (3.16)	9.0	Non septate - Long
L_2	KBG 98005	2 (1.41)	9.0	Non septate - Medium
L_3	Vamban 1	10 (3.16)	2.3	Septate - Medium
L_4	Vamban 2	6 (2.45)	1.3	Non septate - Very long
L_5	Vamban 3	10 (3.16)	1.3	Septate - Small
L_6	ADT 3	16 (4.00)	7.0	Medium - Septate
L_7	ADT 5	28 (5.29)	1.0	Small - Septate (distinct septate)
L_8	T 9	12 (3.46)	1.6	Non septate - Very short
L_9	TMV 1	10 (3.16)	7.9	Non septate - Very long - Sharp
L_{10}	VBG 81	1 (1.00)	1.9	Septate - Small
L_{11}	ADB 2003	14 (3.74)	4.6	Non septate - Medium
T_1	VBN (Bg) 4	10 (3.16)	1.0	Septate - Long
T_2	VBG 73	6 (2.45)	1.0	Non septate - Medium
T_3	VBG 66	12 (3.46)	1.0	Non septate - Long - Glandular

Square root transformed values are in paranthesis
Correlation coefficient (r) = 0.006 (ns)

trichomes observed in 14 parents and results obtained are discussed. The results obtained from the present study showed that there was no correlation between number of trichomes on leaves and MYMV resistance (Table 3). There was no distinct pattern of variation between the resistant and susceptible varieties for number of trichomes. Hence it is concluded from the present study that trichomes in leaf does not hinder the activities of whitefly and the resistance to MYMV may be also due to some other factors like cuticle thickness, chemical components, wax coating, compact vascular bundles, lignification of cell walls *etc.* in leaves.

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