Molecular marker characterization of mungbean yellow mosaic disease resistance in blackgram [*Vigna mungo* (L.) hepper]

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Abstract
Molecular marker analysis was conducted to characterize mungbean yellow mosaic virus (MYMV) resistance in blackgram. Crosses have been made between varieties with high yielding ability but with MYMV susceptible line (ADT 3, MDU 1, CO 6, LBG 752, ADT 5 and KUG 688) and five promising MYMV resistant testers (VBN (BG) 4, VBN (BG) 6, Mash – 114, Uttara and PU 31). Promising MYMV resistant genotype from the segregating generation was selected based on the earliness, high yielding and molecular markers. Marker analysis revealed the dominant marker MYMVR-583 (SCAR marker) exhibiting the polymorphism between parents as well as in F_2s in the presence of single band at 583 bp. Similarly two MYMV resistant gene analouge markers YR4 and CYR1 had the polymorphism at 456 bp and 1236 bp respectively among parents and their segregants confirmed the MYMV resistance. These marker could be used in MAS for MYMV resistance in blackgram.

While one SSR marker CEDG-180 exhibited a monomorphic banding profile across the parents and F_2 population in the presence of single band at 136 bp, field test showed that F_1s appeared with devoid of symptom confirming that the MYMV resistance was controlled by dominant gene action. MYMV inheritance study was carried out in field with Chi-square ($\chi 2$) test working out in the F_2 segregants of eight crosses and confirmed the presence of digenic dominant gene action acting in duplicative dominant or complementary interaction manner.

Keywords: Chi-square ($\chi 2$) test, inheritance, MYMV, dominant, duplicate and complementary gene.

Introduction
Blackgram is one of the important legume crops predominantly cultivated in India for dhal as well as fodder. Mostly it has been cultivated as intercrop in marginal land under rainfed in the various agro climatic conditions [25]. Though it is highly an adapted crop, its production is far behind the actual requirement[14]. Mungbean yellow mosaic disease caused by mungbean yellow mosaic virus (MYMV) is one of the most detrimental yield limiting factors for blackgram cultivation [26]. MYMV belongs to the genus begomovirus and is transmitted persistently by white fly (*Bemisia tabaci*) through the phloem cells of the host plant. Consequently, the virus causes irregular green and yellow patches on the leaf lamina. Symptom is characterized by complete yellowing of younger leaves and intermittent green and yellow patches on the older leaves.

A severely infected plant has stunted growth, delayed maturity, reduced number of flowers and pods (symptom spreads to flower and pods make plant nonproductive). This disease has been cause of a major destruction to legume crops cultivation in countries like Pakistan, Srilanka, India[3,7,12,17]. Depending upon the severity of infection yield, reduction may goes up to 70 – 100%. Management of mungbean yellow mosaic disease by controlling vector using chemical measures is often ineffective and causes environmental pollution [5,6].

Hence, durable resistance could be the permanent long lasting solution for controlling the MYMV. Selection of genotypes with durable resistance is based on exploiting the host plant resistance principles through introgression breeding. Studying the inheritance pattern of MYMV resistance has clearly shown the nature of gene action involved in controlling MYMV resistance[1,11,20]. The nature of gene controlling MYMV reaction is changing with genotypes as well as environment. MYMV virus consists of bipartite genome which would enable the virus to express itself with varied environments[16,24].

Identification of MYMV resistance genotypes by conventional approaches has been time consuming and technologically not sound[22,23]. Hence conventional approaches assisted with marker assisted selection (MAS) may be rapid in the selection process. In the light of above fact, the present investigation was conducted in blackgram to delineate the nature of gene action involved in MYMV resistance and characterization of MYMV resistance in the segregating population using molecular markers.

Material and Methods
Selection of genotypes: The experimental materials used for this study consisted of six lines (high yielding ability but MYMV susceptible *viz.* ADT 3, MDU 1, CO 6, LBG 752, ADT 5 and KUG 688) and five testers (promising genotypes resistant to MYMV *viz.* VBN (BG) 4, VBN (BG) 6, Mash – 114, Uttara and PU 31 (Pant urd- 31). Parental material for crossing programme was collected from Nationa Pulses Research Centre (NPRC), Vamban, Department of Pulses,

Tamil Nadu Agricultural University (TNAU), Coimbatore and Agriculture College and Research Institute, Madurai.

Crossing programme: Seeds of the parental materials raised in field and crosses were made in Line x Tester matting design. Hand emasculation and pollination were done at early morning (6.30 am). Totally 30 crosses were obtained and were analysed to test for combining ability. Out of 30 crosses, eight (ADT 3 x PU 31, MDU 1 x Mash-114, MDU 1 x VBN (Bg) 6, MDU 1 x Uttara, LBG- 752 x Mash-114, LBG – 752 x VBN (Bg) 6, MDU 1 x PU 31 and CO 6 x VBN (Bg) 6) were selected based upon the combining ability of the parents for earliness and yield. Six generations (P_1, P_2, F_1, F_2, BC_1 and BC_2) have been raised in these eight crosses to screen for MYMV resistance.

Seeds of these six generations from eight crosses were raised in eight row 4 metre plot at a spacing of 30 x 10 cm in the infector row method at NPRC, Vamban during Kharif, 2015, which is the hot spot for MYMV disease. CO 5 (susceptible cultivar) blackgram variety had been used as an infector sown at every five rows of test materials as well as in border rows around the experimental plot under unprotected condition.

Selection of MYMV resistance genotype: Data from test materials recoded after the symptom on susceptible check reached up to 80% and infector row was pulled out. Disease score had recorded on five randomly selected plants in parents (P_1 and P_2) and every plant in F_1, F_2, BC_1 and BC_2 generations. The mean MYMV score was worked out for each generation. Genotypes were later classified into different classes based on standard arbitrary scale 1 to 9 reported by Alice and Nadarajan[2] (Table 1).

Based upon the mean MYMV score, plants were classified into five categories like resistant (R), moderately resistant (MR), moderately susceptible (MS), susceptible (S) and highly susceptible (HS). Later on these groups were shorted into two category as the plants that are moderately susceptible (MS), susceptible (S) and highly susceptible (HS) included in susceptible (S) group, while the resistant (R) and moderately resistant (MR) plants were included in resistant (R) group.

Molecular marker analysis

DNA extraction: The details of molecular markers used for this study are presented in table 2. Marker analysis was carried out in young plants. DNA was isolated from young leaves at 6-14 days old plants which were collected from parents as well as F_2 generations of four selected crosses (MDU 1 x VBN (Bg) 6, MDU 1 x Mash-114, ADT 3 x PU 31 and CO 6 x VBN (Bg) 6) in the MYMV inheritance study field raised at NPRC, Vamban during Kharif, 2015. Genomic DNA was isolated by the method described by Basak et al[4] and quantified using Nano Drop™1000 Spectrophotometer.

Marker analysis: One SSR (CEDG-180), two resistance gene analog primers (YR4 and CYR1) and one SCAR marker ($MYMVR_{583}$) were used for marker study. Markers have been selected based on the previous report by Souframanien and Gopalakrishna[22], Chatieng et al[8], Gupta et al[11], Dhole and Reddy[9] and John et al[12] in greengram, blackgram and cowpea. Primers were purchased from Eurofins Genomics India Pvt. Ltd. 15µL PCR reaction mixture consists of 50 ng template DNA, 0.2 µM each forward and reverse primer, 0.25 mM each dNTPs (ATP, GTP, CTP and TTP), 0.3 mM $MgCl_2$, 0.20 µl *Taq* polymerase (Bangalore Genei Pvt. Ltd., Bangalore), 1x reaction buffer (10mM Tris HCl, pH 9.0, 15 mM KCl (pH 8.3) and 1.5 mM $MgCl_2$).

Table 1
Grouping of genotypes into different categories based on 1 to 9 arbitrary scales given by Alice and Nadarajan[2]

Scale	Disease incidence	Rating	Reaction
1.	No visible symptoms on leaves or very minute yellow specks on leaves.	1.0 to 2.0	Resistant (R)
2.	Small yellow specks with restricted spread covering 0.1 to 5 % leaf area.		
3.	Yellow mottling of leaves covering 5.1 to 10 % leaf area.	2.1 to 4. 0	Moderately resistant (MR)
4.	Yellow mottling of leaves covering 10.1 to 15 % leaf area.		
5.	Yellow mottling and discoloration of 15.1 to 30 % leaf area.	4.1 to 5.0	Moderately susceptible (MS)
6.	Yellow discoloration of 30.1 to 50 % leaf area.	5.1 to 7.0	Susceptible (S)
7.	Pronounced yellow mottling and discoloration of leaves and pods, reduction in leaf size and stunting of plants covering 50.1 to 75 % foliage.		
8.	Severe yellow discoloration of leaves covering 75.1 to 90 % of foliage, stunting of plants and reduction in pod size.	7.1 to 9.0	Highly susceptible (HS)
9.	Severe yellow discoloration of entire leaves covering above 90.1 % of foliage, stunting of plants and no pod formation.		

Table 2
Markers used for validation of MYMV resistance

S. N.	Marker	Sequence		
1.	MYMVR-583[9]	F (5'–3')	GTGATGCACACGGTTACGGT	SCAR
		R (5'–3')	GGTGACGCAGTCCATACAAATTT	
2.	CEDG180[11]	F (5'–3')	GGTATGGAGCAAAACAATC	SSR $(AG)_{11}$
		R (5'–3')	GTGCGTGAAGTTGTCTTATC	
3.	YR 4[14]	F (5'–3')	GGNAAGACGACACTCGCNTTA	RGA
		R (5'–3')	GACGTCCTNGTAACNTTGATCA	
4.	CYR 1[14]	F (5'–3')	GGGTGGNTTGGGTAAGACCAC	RGA
		R (5'–3')	NTCGCGGTGNGTGAAAAGNCT	

The PCR profile was programmed with an initial denaturation at 94 °C for 7 min followed by 35 cycles at 94 °C for1 min, annealing at 72 °C for 2 min and final extension at 72 °C for 7 min. Annealing temperature was optimized for the primers using gradient PCR. After adding 2 µL of loading buffer [98% formaamide, 10 mM EDTA (pH 8.0), 0.005% bromophenol blue and 0.005% Xylene cynaol], 10 µl of each product loaded in 3% Agarose gel was subjected to electrophoresis in 1x TBE buffer (0.9 M Tris-HCl, 0.025 M EDTA Na^{2+}, 0.9 M Boric acid) at 90V. A 100 bp DNA ladder was used as reference DNA to quantify sample DNA. The gel was documented using Syngene G:Box™ – Documentation and analysis system (Syngene, USA).

Statistical analysis

MYMV inheritance study: Segregating pattern of MYMV was cheked by Chi square test as the test for deviation between observed and expected ratio in the F_2, BC_1 and BC_2 generation.

Results and Discussion

MYMV inheritance study: Chi-square test was worked out to reveal the inheritance pattern of MYMV in the F_2, BC_1 and BC_2 generation of eight crosses (Table 3). Information on MYMV inheritance confirmed the F_1s of all the crosses *viz.* ADT 3 x PU 31, MDU 1 x Mash-114, MDU1 x VBN (Bg) 6, MDU x PU 31, MDU 1 x Uttara, LBG- 752 x Mash-114, LBG – 752 x VBN (Bg) 6 and CO 6 x VBN (Bg) 6 devoid of symptom. Absence of symptom in F_1 clearly showed that resistance was controlled by dominant gene action. With respect to observed: expected F_2 segregation ratio for resistance: susceptible plants, out of eighht crosses, seven crosses *viz.* ADT 3 x PU 31, MDU 1 x Mash-114, MDU 1 x VBN (Bg) 6, MDU 1 x Uttara, LBG- 752 x Mash-114, LBG – 752 x VBN (Bg) 6 and CO 6 x VBN (Bg) 6 were recorded with non-significant chi-square value. The observed ratio (15(resistant): 1 (susceptible)) in F_2 perfectly fits with expected ratio. Hence, null hypothesis is accepted.

The observed deviation is by chance and not real. Segregating pattern of BC_1F_1 was 1:1 (resistant: susceptible) whereas in BC_2 F_1, all the plants were resistant. Inheritance of MYMV resistance in seven crosses appeared to be under the control of digenic dominant gene acting in duplicate dominant type of non allelic interaction. These results were in agreement with the previous report by Murugan and Nadarajan[18].

However the cross MDU 1 x PU 31 exhibited that the observed ratio fitted well with 9:7 expected ratio in F_2 and 1:3 in BC_1, and all the plants in BC_2 were resistant. This in accordance with the dominant and recessive gene acts in complementary type of non alellelic interaction. This result was in agreement with reports of Durga Prasad et al[10].

Shukla et al[19] and Singh[21] also reported the presence of duplicate dominant genes for MYMV resistance in blackgram. It is clear that in all the eight crosses, the F_1 was found to be resistant whereas the female parents ADT 3, MDU 1, LBG-752 and CO 6 were susceptible and male parents were observed with free of symptom designated as resistant. Based on the segeregating pattern of MYMV in segregating generation, the putative gene symbol (Table 4) assigned for female parents is $r_1r_1r_2r_2$, for male parents (Uttara, VBN (Bg) 6 Mash-114 and PU 31) is $R_1R_1R_2R_2$. Since the number of plants segregated in the F_2s typically follows dihybrid ratio, gene symbols were assigned with assumption of two gene.

Molecular marker analysis: Molecualar marker analysis was carried out in selected four crosses using four different DNA markers linked with the MYMV resistance reported in mungbean[9] and blackgram[22]. Nearly 50 F_2 individuals from each of the selected four crosses were analyzed for validating MYMV resistance at young leaf stage collected from MYMV inheritance study field. Four primers [one SCAR marker $MYMVR_{583}$, two RGA (Resistant Gene Analog) CRY1 and YR4 and one SSR marker CEDG -180] were used to validate the MYMV resistance. Banding profile of the markers in parents and F_2s was depicted in fig. 1 and 2.

Each of the primers had the band at the respective bp as CEDG-180 at136 bp, $MYMVR_{583}$ amplified at 583 bp, YR4 and CYR1 at 456 bp and 1236 bp respectively. Among the four primers used, three primers were found to be polymorphic across the parents for resistance and one marker, CEDG -180 exhibited monomorphic banding pattern across the population.

Table 3
Chi-square test for inheritance of MYMV resistance in urdbean

Generation	Observed values		Expected ratio	χ^2 values	Probability
	Resistant	Susceptible			
MDU1 x Uttara					
F_1	15	-	--		
F_2	268	15	15:1	0.44	0.50
BC_1	64	22	3:1	0.016	0.90
BC_2	26	----			
MDU1 x MASH-114					
F_1	17	-	--		
F_2	272	15	15:1	0.50	0.50 - 0.40
BC_1	62	19	3:1	0.11	0.70 – 0.60
BC_2	31	----			
MDU1 x VBN (BG)6					
F_1	16	-	--		
F_2	269	14	15:1	0.83	0.40 - 0.30
BC_1	89	27	3:1	0.19	0.70 – 0.60
BC_2	28	----			
MDU1 x PU 31					
F_1	17	--	--		
F_2	292	134	9:7	0.06	0.80 - 0.70
BC_1	21	73	1:3	0.85	0.40 – 0.30
BC_2	33	----			
LBG-752 x VBN(Bg) 6					
F_1	15	-	--		
F_2	272	14	15:1	0.56	0.50 - 0.40
BC_1	58	14	3:1	1.2	0.30 – 0.20
BC_2	23	----			
LBG-752 x MASH-114					
F_1	18	-	--		
F_2	200	16	15:1	0.51	0.50 - 0.50
BC_1	76	22	3:1	0.34	0.60 – 0.50
BC_2	38	----			
CO6 x VBN (Bg) 6					
F_1	14	-	--		
F_2	255	13	15:1	0.90	0.40 - 0.30
BC_1	60	16	3:1	0.63	0.50 – 0.40
BC_2	22	----			
ADT3 x PU 31					
F_1	15	--	--		
F_2	292	17	15:1	0.69	0.80 - 0.70
BC_1	71	23	3:1	0.65	0.40 – 0.30
BC_2	31	----			

Table 4
Mean MYMV score, disease reaction category and putative gene symbols for parents

Genotypes	Mean MYMV score (%)	MYMV Reaction	Putative gene symbol
VBN (Bg) 6	0.95	Resistant	$R_1R_1\ R_2R_2$
PU 31	1.0	Resistant	$R_1R_1\ R_2R_2$
Uttara	0.6	Resistant	$R_1R_1\ R_2R_2$
Mash – 114	0.7	Resistant	$R_1R\ R_2R_2$
ADT 3	34	Susceptible	$r_1r_1r_2r_2$
CO 6	35	Susceptible	$r_1r_1r_2r_2$
MDU 1	33	Susceptible	$r_1r_1r_2r_2$
LBG-752	32	Susceptible	$r_1r_1r_2r_2$

Presence of MYMV resistance was confirmed in both parents and F_2s using YR4 while only with parents using CRY1. Both the primers were identified to be polymorphic across the population for MYMV resistance. Based on the marker profile, it has been proposed that the resistant parents namely PU 31, Mash-114 and VBN6 were identified with single band for both the primers while no band was observed in the parents *viz*. ADT 3, MDU 1 and CO 6.

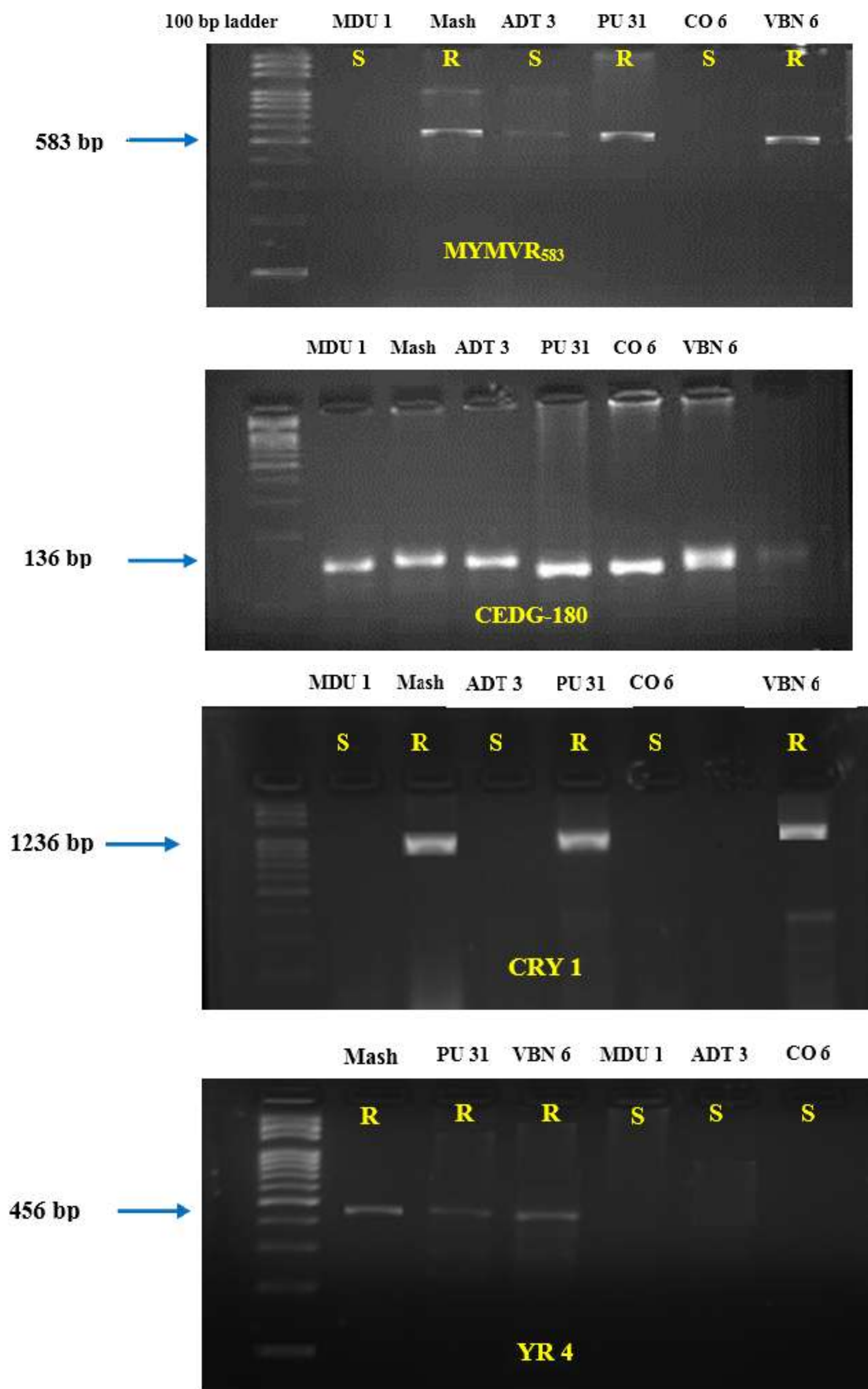
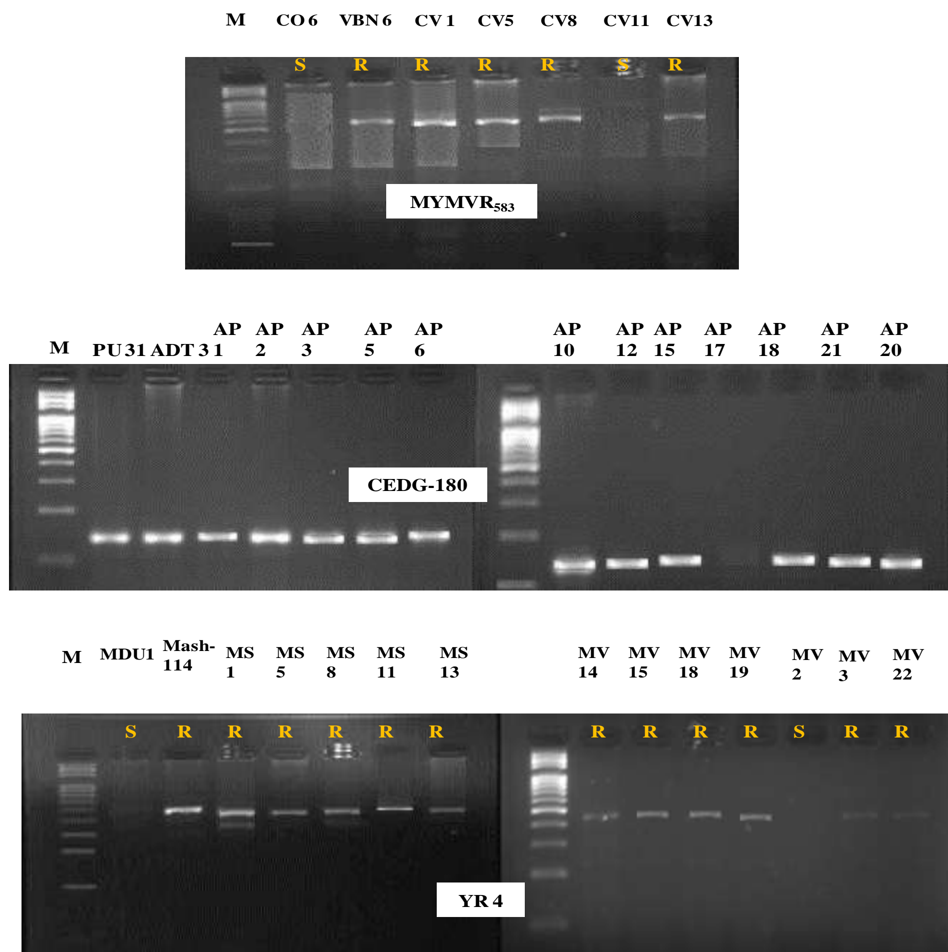


Figure 1: Marker analysis in parents for MYMV resistance



Lane M - 100 bp ladder; R- Resistant; S- Susceptible, CV- CO 6 x VBN 6 F_2;
MS- MDU 1 x Mash-114 F_2; MV- MDU 1 x VBN6 F_2; AP- ADT 3 x PU 31 F_2

Figure 2: Marker analysis in F_2s for MYMV resistance

A similar result was reported by Kundu and Pal[14] in blackgram on MYMV introgression in RILs through genotyping based on MYMV resistance tagged molecular markers YR4 and CYR1. One SCAR marker (MYMVR-583) has the band at 583 bp in 46 F_2s out of 50 classified as resistant to moderately resistant while no band was observed in 4 F_2 individuals coming under susceptible category. Therefore, the marker $MYMV_{583}$ was considered to be a dominant marker for the allele conferring to MYMVD resistance. The present report was in agreement with previous studies of Dhole and Reddy[9] and Binyamin et al[6] in mungbean using SCAR marker (MYMVR-583).

Gupta et al[11] reported that marker CEDG 180 was linked with resistance gene in the F_2 population of blackgram derived from a cross DPU 88-31(resistant) x AKU 9904 (susceptible). However this is contrary to our result that the marker CEDG180 did not show any polymorphism between parents and F_2 individuals of all the four crosses. CEDG180 produced a monomorphic banding pattern across the population at 136 bp. Because of their monomorphic banding pattern this marker might not be used for marker assisted selection forMYMV introgression breeding. Dhole and Reddy[19], Kitsanachandee et al[13] and Mahbubul Alam et al[15] reported similar result in mungbean, using CEDG180 showing monomorphism between resistant and susceptible parents.

Conclusion

It has been concluded that the complicate nature of digenic dominant gene interaction restricts the understanding on nature of gene action involved in the MYMV inheritance. Therefore the complex nature of inheritance makes the ealry generation selection ineffective and may be postponed to later generation accompanied with recurrent selection get desirable recombinant.

References

1. Akhtar K.P., Kitsanachandee R., Srinives P., Abbas G., Asghar M.J., Shah T.M., Atta B.M., Chatchawankanphanich O., Sarwar G., Ahmad M. and Sarwar N., Field evaluation of mungbean recombinant inbred lines against mungbean yellow mosaic disease using new disease scale in Thailand, *Plant Path. J.*, **25**, 422–428 **(2009)**

2. Alice D. and Nadarajan N., Screening techniques and assessment methods for disease resistance, Department of Pulses, TNAU **(2007)**

3. Bakar A.K., Pest and disease problems of mung bean in west Malaysia, *Malaysian J. of Agri.*, **53**, 29-33 **(1981)**

4. Basak J., Kundagramy S., Ghose T.K. and Pal A., Development of yellow mosaic virus (YMV) resistance linked DNA-marker in *Vigna mungo* from population segregating for YMV reaction, *Mole. Bree.*, **14**, 375–383 **(2005)**

5. Binyamin R., Khan M.A., Khan A.I. and Khan M.A., Molecular characterization of urdbean (*Vigna mungo*) germplasm related to resistance against urdbean leaf crinkle virus, *Gene and Mole Res.*, **10**, 1681-1688 **(2011)**

6. Binyamin R., Khan M.A., Khan N.A. and Khan A.I., Application of SCAR markers linked with mungbean yellow mosaic virus disease resistance gene in Pakistan mungbean germplasm, *Gene and Mole Res.*, **14(1)**, 2825-2830 **(2015)**

7. Biswass K.K., Malathi V.G. and Varma A., Diagnosis of symptomless yellow mosaic begomovirus infection in pigeon pea by using cloned mungbean yellow mosaic India virus a probe, *J. Pl. Biochemistry and Biotechnology*, **17**, 9-14 **(2008)**

8. Chatieng B., Kaga A., Tomooka N., Isemura J., Kurocla Y. and Vaughan D.A., Development of a blackgram (*Vigna mungo* (L.) Hepper) linkage map and its comparison with an azukibean (*Vigna angularis* (Willd.) Ohwi & Ohashi), *Theor and Appl Genet.*, **113**, 1261–1269 **(2006)**

9. Dhole V.J. and Reddy K.S., Development of a SCAR marker linked with a MYMV resistance gene in mungbean (*Vigna radiata* L. Wilczek), *Plant Bree.*, **132**, 127-132 **(2013)**

10. Durga Prasad A.V.S., Murugan E. and Vanniarajan C., Inheritance of resistance of mungbean yellow mosaic virus in Urdbean (*Vigna mungo* (L.) Hepper), *Curre Bioti.*, **8(4)**, 413-417 **(2015)**

11. Gupta S., Gupta D.S., Anjum K.T., Pratap A. and Kumar J., Inheritance and molecular tagging of MYMIV resistance gene in blackgram (*Vigna mungo* L. Hepper), *Euphytica*, **193**, 27–37 **(2013)**

12. John P., Sivalingam P.N., Haq Q.M.I. and Kumar N., Cowpea golden mosaic disease in Gujarat is caused by a mungbean yellow mosaic India virus isolate with a DNA B variant, *Arch. Viroloy*, **153**, 1359-1365 **(2008)**

13. Kitsanachandee R., Somta P., Chatchawankanphanich O., Akhtar K.P., Shah T.M., Nair R.M., Bains T.S., Sirari A., Kaur L. and Srinives P., Detection of quantitative trait loci for mungbean yellow mosaic India virus (MYMIV) resistance in mungbean (*Vigna radiata* (L.) Wilczek) in India and Pakistan, *Bree Sci*, **63**, 367–373 **(2013)**

14. Kundu A. and Pal A., Identification and characterization of elite inbred lines with MYMIV-resistance in *Vigna mungo*, *Field Crop Res*, **135**, 116–125 **(2012)**

15. Mahbubul Alam, Prakit Somta and Srinives P., Generation mean and path analyses of reaction to mungbean yellow mosaic virus (MYMV) and yield related traits in mungbean (*Vigna radiata* (l.) wilczek), *SABRAO J. Bree. and Genet*, **46(1)**, 150-159 **(2014)**

16. Malathi V.G. and John P., Gemini viruses infecting legumes, In Rao G.P., Lava Kumar P. and Holguin-Pena R.J., eds., Characterization, diagnosis and management of plant viruses, Studium Press, Houston, 97–123 **(2008)**

17. Malik J.A., Breeding for Resistance to MYMV and its Vector in Pakistan, Mungbean YMDs, Proceedings of an International Workshop, Green S.K. and Kim D., eds., Bangkok, Thailand, AVRDC, 41-53 **(1991)**

18. Murugan E. and Nadarajan N., Genetic studies on differential expression of mungbean yellow mosaic virus resistance related to trichome density in urdbean (*Vigna mungo* (L.). Hepper), *Ind J .Pl Genet Resour*, **25(2)**, 135-138 **(2012)**

19. Shukla G.P., Pandya B.P. and Singh D.P., Inheritance of resistance to yellow mosaic in mungbean, *Int J. Pl Bree and Genet*, **38**, 357-360 **(1978)**

20. Sibiya J., Tongoona P., Derera J., van Rij N. and Makanda I., Combining ability analysis for Phaeosphaeria leaf spot resistance and grain yield in tropical advanced maize inbred lines, *Field Crop Res*, **120**, 86–93 **(2011)**

21. Singh D.P., Inheritance of resistance to mungbean yellow mosaic disease in blackgram, *Theoritical and Applied Genetics*, **57** 233-235 **(1980)**

22. Souframanien J. and Gopalakrishna T., ISSR and SCAR marker linked to the mungbean yellow mosaic virus (MYMV) resistance gene in blackgram (*Vigna mungo* L. Hepper), *Plant Breeding*, **125**, 619-622 **(2006)**

23. Tanksley S.D., Young N.D., Paterson A.H. and Bonierable M.D., RFLP mapping in plant breeding: new tools for old science, *Bio Tech*, **7**, 257-264 **(1989)**

24. Varma A. and Malathi G., Emerging geminivirus problems: a serious threat to crop production, *Ann. Applied Biol.,* **142**, 145–164 **(2003)**

25. Vishalakshi B., Umakanth B., Shanbhag A.P., Ghatak A., Nitish Sathyanarayanan, Madhav M.S., Gopala Krishna G. and Hari Yadla, RAPD assisted selection of black gram (*Vigna mungo* L. Hepper) towards the development of multiple disease resistant germplasm, *Biotechnology*, **7**, 1 **(2017)**

26. Vyas D., Joshi A., Rajamani G., Jain D. and Kaur G., Genetic diversity analysis in different genotypes of black gram (*Vigna mungo* (L.) Hepper) using RAPD marker, *Leg Res*., *International Journal*, doi:10.18805/lr.v0iof.9430 (2016).

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