Journal of Advances in Biology & Biotechnology

Volume 27, Issue 6, Page 847-854, 2024; Article no.JABB.117632
ISSN: 2394-1081

Identification of Resistance Gene for Mungbean Yellow Mosaic Virus (MYMV) in Resistant Mungbean Genotypes through RGA Markers

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.9734/jabb/2024/v27i6948

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/117632

Original Research Article

Received: 20/03/2024
Accepted: 25/05/2024
Published: 30/05/2024

ABSTRACT

Mungbean is one among the important and major pulse crop for supplementation of protein in subtropical zones of the world. The yield of mungbean is affected by both abiotic and biotic factors. Among the biotic factors, Mungbean yellow mosaic virus (MYMV) is the major bottleneck for pulse producers. MYMV cause a Yellow mosaic disease and its transmitted by Aleyrodidae insects. The virus belong to the family Geminiviridae and the genus Begomovirus. Fourteen mungbean genotypes procured from Asian Vegetable Research and Development Centre (AVRDC), Taiwan

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Cite as: *Shilpa , M., Manjunath , B., Sunil, C., Sudarshan, G.K., & Nagaraju, N. (2024). Identification of Resistance Gene for Mungbean Yellow Mosaic Virus (MYMV) in Resistant Mungbean Genotypes through RGA Markers. Journal of Advances in Biology & Biotechnology, 27(6), 847–854. https://doi.org/10.9734/jabb/2024/v27i6948*

were screened for Mung bean Yellow Mosaic Virus resistance in field and laboratory conditions during Kharif 2022 and summer 2023. Genotypes MYB-6, 7, 8, 9 and 12 were found to be resistant under field conditions by less PDI and AUDPC values. The molecular confirmation of MYMV resistance was tested using Resistant Gene Analog (RGA) in all 14 genotypes. The RGA was amplified at 90bp in resistant genotypes viz MYB-6, 7, 8 and 9. Since MYMV resistant lines MYB-6, 7, 8 and 9 showed resistance in both field and molecular studies, hence these lines can be used as a YMV donor in breeding process and also can be released as a variety after conducting a multi-location trails in MYMV hotspot conditions.

Keywords: Mungbean; genotypes; resistance; markers; yellow mosaic virus.

1. INTRODUCTION

"Being a short duration catch crop mungbean is widely grown between two principal or main crops in Indian subcontinent. It contains carbohydrates (51%), proteins (24-26%), minerals (4%), and vitamins (3%). Apart from providing protein in the diet, it also has the appreciable quality of helping the symbiotic root rhizobia by contributing to fixing atmospheric nitrogen enriches the fertility of soils" [1].

The yield of mungbean is affected by both abiotic and biotic factors. Among the biotic factors, Mungbean yellow mosaic virus (MYMV), Mungbean leaf curl virus (MLCV), Mosaic mottle virus (BCMV), Urdbean Leaf crinkle virus (ULCV) are of prime importance in reducing crop yield [2]. "Mungbean yellow mosaic virus is transmitted by white fly (*Bemisia tabaci*) belongs to group begomoviruses with bipartite genomes" [3]. "This disease causes 10–100 percent yield losses depending on the crop stage at which the plants being infected" [4,5], (Pandey et al., 2009).

"Plant resistance gene analogs (RGAs), as resistance (R) gene candidates, have conserved domains and motifs that play specific roles in pathogens' resistance. Development of markers to identify YMV resistance in greengram and deploying them through marker-aided selection in breeding programme would fasten the process of developing resistant lines" [6].

Breeding of resistant varieties is considered to be the most important and environmentally friendly approach to come back from several drawbacks which are presently witnessing in the mungbean most of the efficient cultivars are prone to susceptibility over a period of time due to several factors. Thereby screening of resistant genotypes based on biochemical and molecular approaches are gaining importance. Hence the present study was carried out to identify the resistance gene through RGA markers in resistant mungbean genotypes.

2. MATERIALS AND METHODS

2.1 Evaluation of MYMV under Field Conditions

The field experiments were conducted at the experimental plot, Main Research Station ,Hebbal, Karnataka, India to identify the potential resistance genotypes against MYMV. Fourteen Mungbean genotypes including one susceptible control known as MYB-11 and KKM-3, a (UASB released variety) were screened during kharif 2022 and summer 2023. These 13 genotypes were obtained from Asian Vegetable Research and Development Center (AVRDC) Taiwan formerly which is known as World Vegetable Centre.

The Mungbean genotypes were sown with spacing of 30 × 10 cm, with a row length of 2.5 m in Randomized Complete Block Design (RCBD) with two replications. The recommended package of practices was followed for raising the crop. Plants were allowed for natural infection of MYMV. The observations were recorded on symptom expression, disease incidence, severity and yield during the experiment.

2.2 Mungbean Yellow Mosaic Disease Incidence

The MYMV disease incidence was scored by counting the total number of plants infected in each row and per cent disease incidence was calculated by using the following formula,

$$\text{Disease incidence (DI \%)} = \frac{\text{No. of infected plants in a row}}{\text{Total No. of plants in a row}} \text{ X } 100$$

2.3 Mungbean Yellow Mosaic Disease Severity (Per Cent Disease Index)

Mungbean yellow mosaic disease severity was recorded at 30, 45 and 55 Days After Sowing (DAS) from all the plants from each row. Severity

of disease was scored according to phenotypic disease severity scale developed from AVRDC. The mungbean genotypes were categorized based on modified phenotypic disease severity scale of AVRDC. The per cent disease index (PDI) was computed by using the following formula [7].

$$\text{Per cent disease index (\%)} = \frac{\text{Sum of numerical observation}}{\text{Maximum disease scale x No.of observations}} \text{ x } 100$$

2.4 RGA Detection

The leaf samples were collected from the 14 mungbean genotypes at 30 DAS from the experimental plot in all the season i.e. kharif 2019 and summer 2020 for identification of the presence of resistance gene analogs in all 14 genotypes by using specific primers.

2.5 DNA Extraction from the Collected Leaf Samples

The total genomic DNA was extracted from mungbean leaves. The leaf samples were collected from 15-day old seedlings and utilized for genomic DNA extraction. Isolated genomic DNA was checked for its purity and intactness and then quantified. The crude genomic DNA was resolved by agarose gel electrophoresis (0.8 % agarose gel) stained with ethidium bromide and was visualized in a gel documentation system (Alpha ImagerTM1200, Alpha Innotech Corp., CA, USA). DNA was quantified by using Spectrophotometer by reading the absorbance at 260 nm. Based on the quantitative data; DNA dilutions were made in 1X TE buffer to a final concentration of 50ng/µl and stored in -20°C for further use.

2.6 Amplification of MYMV DNA by PCR

The molecular identification of resistance genes in thirteen greengram genotypes for YMV was tested out using a resistant gene analogous (RGA) marker named CYR1 [8]. The genomic DNA was isolated from 5 – 20 days old seedlings of these genotypes by using standard CTAB (Cetyl Trimethyl Ammonium Bromide) method (Doyle and Doyle, l990). The extracted DNA samples from 14 greengram genotypes was correlated with the above mentioned RGA markers for the detection of resistance gene [9].

2.7 Analysis

The analysis of the obtained data was done using the software R version 3.6.2 (2019-12-12).

List 1. Phenotypic disease severity scale for MYMV by world vegetable centre, Taiwan

Score	Description	Reaction
1	No visible symptoms on leaves	Highly resistant (HR)
2	Small yellow specks with restricted spread covering upto 5 per cent leaf area	Resistant (R)
3	Yellow mottling covering 5.1 to 15 per cent leaf area	Moderately Resistant (MR)
4	Yellow mottling and discolouration of 15.1 to 30 per cent leaf area	Moderately susceptible (MS)
5	Pronounced yellow mottling and discoloration of leaves (covering 30.1 to 75 per cent of area) and pods, reduction in leaf size and stunting of plants	Susceptible (S)
6	Severe yellow discoloration covering >75 per cent of foliage, stunting of plants and reduction in pod size.	Highly susceptible (HS)

List 2. Primers used for the experiment

Primers	Primer sequences (5′-3′)	% GC	Annealing temp.	Expected product	Reference
Rga22f2	Gggtggtttgggtaagaccac	57.1	60	90	Maiti et al., [9]
Rga24r2	Ttcgcggtgtgtgaaaagtct	47.6	58	90	Maiti et al., [9]

3. RESULTS AND DISCUSSION

The appearance of first symptom on the mungbean lines and tested susceptible line (MYB-11), and number of days taken to infect 50 per cent leaf area on susceptible line (MYB-11) were recorded by observing the plants every day after sowing to categorize the test lines for different degree of resistance.

The different types of symptoms were identified and recorded in two different seasons i.e. during Kharif 2022 and summer 2023. The initial symptoms appear in the susceptible control and days taken on 50 percent leaf area affected in susceptible control were recorded. The appearance of initial symptom ranged between 20 DAS to 30 DAS in susceptible and resistant genotypes respectively. The disease has occurred two days earlier during the summer as compared to Kharif season (Table 1).

The maximum PDI was noticed in susceptible control (MYB-11) during both Kharif (56.35%) and summer (72.65%). Whereas MYB-7 is having least PDI in Kharif (11.05%) and in summer MYB-9(20.7%) has recorded least PDI. However MYB-6 (19.47%), MYB- 7 (11.05%), MYB-8 (17.04%), MYB-9 (15.03%) and MYB-12 (17.20%) recorded lesser PDI, the PDI of other genotypes are shown in Tables 2 and 3.

Table 1. Expression of yellow mosaic virus disease symptom in mungbean genotypes

Sl. No.	Genotype	*Kharif* 2022 (DAS)	Summer 2023 (DAS)
1	MYB-1	22	21
2	MYB-2	25	27
3	MYB-3	24	24
4	MYB-4	29	27
5	MYB-5	23	25
6	MYB-6	29	31
7	MYB-7	34	29
8	MYB-8	33	28
9	MYB-9	30	29
10	MYB-10	26	28
11	MYB-11*	20	18
12	MYB-12	26	22
13	MYB13	25	24
14	KKM-3	28	27

*DAS= Days After Sowing, * Susceptible control, MYB= Mungbean Yellow Mosaic Bengaluru*

Table 2. Disease incidence of MYMV in mungbean genotypes during Kharif 2022

Genotypes	Per cent disease incidence (%)			Mean
	30 DAS	45 DAS	55 DAS	
MYB-1	38.98	46.32	53.32	46.20
MYB-2	42.20	33.22	40.50	38.64
MYB-3	41.42	43.00	45.17	43.19
MYB-4	38.69	41.89	48.39	42.99
MYB-5	24.28	30.21	35.10	29.86
MYB-6	17.16	19.99	21.27	19.47
MYB-7	10.08	11.00	12.08	11.05
MYB-8	14.18	16.87	20.08	17.04
MYB-9	11.11	16.00	18.00	15.03
MYB-10	24.72	37.33	42.10	34.71
MYB-11*	49.83	54.12	65.10	56.35
MYB-12	11.76	18.76	21.10	17.20
MYB-13	30.50	34.20	43.20	67.23
KKM-3	35.29	39.11	40.90	38.43

DAS= Days After Sowing, Susceptible control, MYB= Mungbean Yellow Mosaic Bengaluru*

Table 3. Disease incidence of MYMV in mungbean genotypes during Summer 2023

Genotypes	Per cent disease incidence (%)			MEAN
	30 DAS	45 DAS	55 DAS	
MYB-1	33.54	43.33	48.98	41.95
MYB-2	30.76	42.11	47.71	40.19
MYB-3	34.11	44.80	48.54	42.48
MYB-4	37.12	39.12	52.43	42.89
MYB-5	32.17	40.20	52.66	41.67
MYB-6	20,12	22.67	28.55	23.78
MYB-7	18.22	23.89	29.65	23.92
MYB-8	19.44	25.77	27.13	24.11
MYB-9	16.15	21.98	24.22	20.70
MYB-10	39.33	45.67	49.23	44.73
MYB-11*	64.33	74.66	78.98	72.65
MYB-12	27.22	39.20	43.66	36.69
MYB-13	32.29	40.22	45.33	39.28
KKM-3	41.23	53.66	56,68	50.52

Table 4. Area under disease progress curve (AUDPC) of MYMV in mungbean genotypes over the time period in different seasons based on PDI values

Genotypes	PDI of *Kharif* 2022	AUDPC	PDI of *Summer* 2023	AUDPC
MYB-1	40	300.00	34.96	262.60
MYB-2	40.29	602.18	45.09	600.38
MYB-3	48.2	663.68	42.5	656.93
MYB-4	41.76	674.70	41.2	627.75
MYB-5	32.47	556.73	34.1	564.75
MYB-6	26.22	440.18	19.6	402.75
MYB-7	15.96	316.35	18.9	288.75
MYB-8	13.36	219.90	19.8	290.25
MYB-9	19.65	247.58	19.5	294.75
MYB-10	26.47	345.90	40.27	448.28
MYB-11*	59.91	647.85	58.87	743.55
MYB-12	19.75	597.45	20.08	592.13
MYB-13	32.25	390.00	45.58	492.45
KKM-3	26.86	443.33	34.27	598.88

The cumulative disease progress was calculated based on the per cent disease index values which are recorded at 15, 30 and 45 DAS for two seasons. The genotypes showed a varying disease progress in the seasons ranging from AUDPC value 219.9 during kharif and 288.75 in summer respectively in resistant genotype (MYB - 8 and 7) as compared to 647.85, 745.55 respectively during Kharif and summer, in susceptible control (MYB-11). The genotype KKM-3 which is a university released variety with moderate resistance reaction recorded AUDPC of 443.33 in kharif and 598.88 during summer (Table 4).

The used primers CYR-1 are markers linked to the YMV resistance [8] and from the gel documentation picture the bands are obtained in the wells where the MYB-6, 7, 8, and 9th samples were run. The molecular marker which is linked to MYMV resistance CYR-1 showed amplification in four mungbean genotypes, i. e. MYB-6, 7, 8 and 9. But it does not showed amplification in the susceptible check MYB-11. RGA22F2/RGA24R2 (CYR-1) markers produced an allele size of approximately of 90 bp .So, these were considered as resistant lines. The YMV resistance lines MYB-6, 7, 8, and 9 were confirmed by both field and laboratory studies so they will be useful for YMV donor in the future breeding process or can be released as a variety after further multi-location evaluations.

In conclusion the CYR-1 marker is validated in this present study endowed with features of resistance gene candidates may be useful for generating superior genotypes in short duration of time with durable YMV resistance, the marker will be in use of Marker assisted selection.

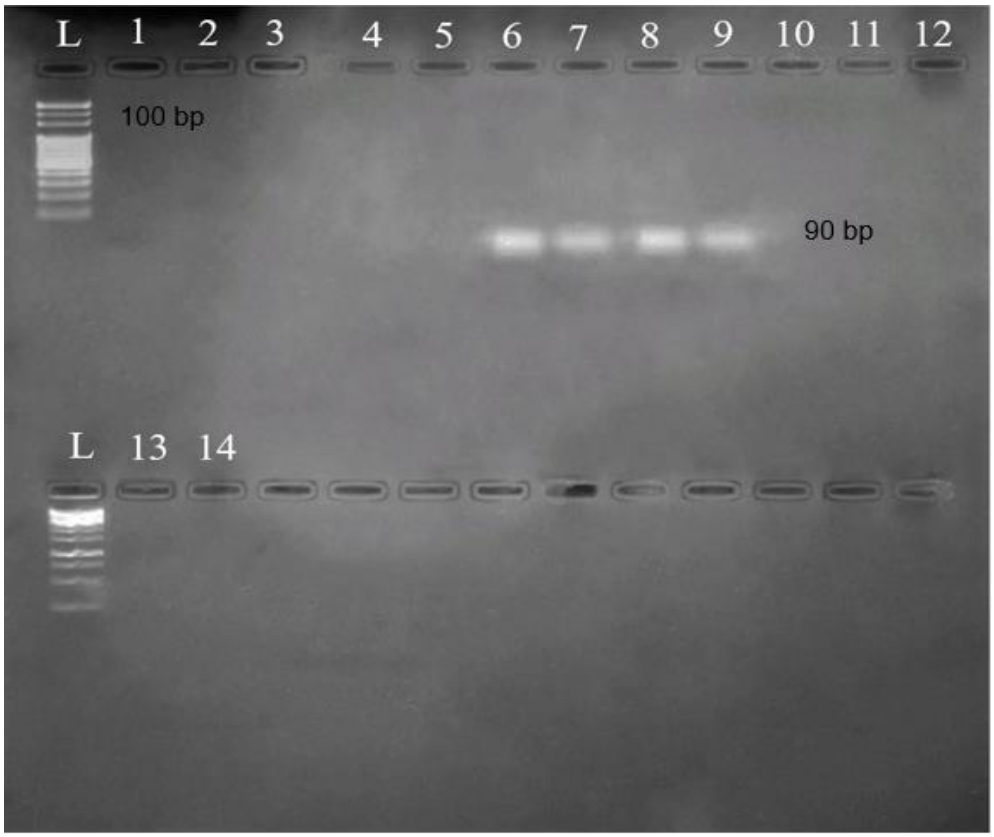

Plate 1. PCR amplification of CYR-1 marker in resistant genotypes

L= Ladder (100bp), 1=MYB-1, 2=MYB-2, 3=MYB-3, 4=MYB-4, 5=MYB-5, 6=MYB-6, 7=MYB=7, 8=MYB-8, 9=MYB-9, 10=MYB-10, 11=MYB-11 (susceptible control), 12=YB-12, 13=MYB-13, 14=KKM-3

The results were supported by Kabi et al. [8] where "screening was done against MYMV. The molecular analysis of twenty six different mungbean genotypes for YMV using a resistant gene analogous (RGA) marker named CYR1 which produced amplicon at 90 bp in seven genotypes (OBGG-2013-8, OBGG2013-21, OBGG-2013-16, OBGG-2013- 11, OBGG-2013-20, OBGG-2013-39 and OBGG-2013-12) concluded that these seven genotypes have yellow mosaic virus resistance gene and this marker is an efficient and ubiquitous for genotyping of YMV reaction. OBGG 2013-20 was an YMV resistance and high yielding line which can be used as YMV donor or can be released as a variety. OBGG 2013-34 had 23.88 per cent higher yield potential than best control but moderately susceptible to YMD thus needs further improvement by hybridization with a suitable YMV resistant varieties as reported" [10]. Both CYR1 and YMV1 marker showed consistent polymorphism with respect to disease reaction in seven resistant genotypes. CYR1 produced an allele size of approximately 90 bp. which concluded that seven genotypes (OBGG-2013-8, OBGG-2013-12, OBGG-2013-11, OBGG-2013-16, OBGG-2013-20, OBGG-2013-21 and OBGG-2013-39) had yellow mosaic virus resistance gene and both the markers were efficient and ubiquitous for genotyping of YMV reaction. OBGG 2013-20 was an YMV resistance and high yielding line.

Tian et al. [11] conducted "an experiment through degenerate primers identification and characterization of resistance genes showing varied level of resistance and tolerance to MYMV was carried out for 25 mungbean accessions. Based on multiple sequence alignment, 8 and 69 were polymorphic sites identified for RGA1 & RGA8 respectively. Out of 15 tolerant accessions, 5 accessions produced amplicons for RGA1 primer shows that resistance gene linked with tolerant accessions". Variability in MYMV resistance for RGA1 and RGA8 sequences at genome level were calculated.

"The isolation of R genes was achieved with the development of technologies for cloning plant genes of unknown structure or molecular

function. Several methods can be use for identifying and cloning genes that are differentially regulated including cDNA –RFLP" (19, Durrant et al., [12]), differential display PCR, and microarray and gene chip technologies [13,14].

Rishee et al. [15] evaluated 100 genotypes of mungbean for resistance to yellow mosaic disease in the field using the disease grading system during Rabi 2021–2022. Out of the 100 mungbean genotypes, 49 were resistant to the yellow mosaic disease,22 as Moderately resistant, 11 as Moderately susceptible, 3 as susceptible, and 15 were highly susceptible. Five pairs of RGA primers were successfully amplified in all resistant genotypes as a consequence, but not in all highly susceptible genotypes. Moreover, identical results were published, except in the resistant genotype, just one primer RGA pair 1F-CG/RGA 1R amplified a single 445 bp band while being lacking in the susceptible genotype [16,17,18,19].

4. CONCLUSION

Fourteen mungbean lines were tested for MYMV resistance during Kharif 2019 and summer 2020 under field conditions. Five genotypes viz., MYB-6, 7, 8, 9 and 12 were found resistant to the disease, low severity of disease and low cumulative disease development over the time period. However, KKM-3 (UASB released variety) was found moderately resistant across the seasons and none of the mungbean genotypes found highly resistant or highly susceptible to MYMV.

The molecular marker which is linked to MYMV resistance CYR-1 showed amplification in four mungbean genotypes, i. e. MYB-6, 7, 8 and 9. But it does not showed amplification in the susceptible check MYB-11. RGA22F2/RGA24R2 (CYR-1) markers produced an allele size of approximately of 90 bp.

The YMV resistance lines MYB-6, 7, 8, and 9 were of more promising in both in field and laboratory studies so that they can be used for YMV resistance donor in the future breeding process or can be released as a variety after further multi-location evaluations.

ACKNOWLEDGEMENT

The authors are highly grateful to Department of Plant Pathology for encouragement and financial support to the research work.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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