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RESEARCH ARTICLE

# Resistance gene analogue assisted screening for mung bean yellow mosaic virus in mung bean (*Vigna radiata* L. Wilczek) genotypes

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## Abstract

Mung bean production is severely constrained by mung bean yellow mosaic virus (MYMV) disease, making host resistance a priority for sustainable crop improvement. Resistance gene analogue (RGA) primers serve as effective molecular tools for identifying MYMV-resistant genotypes. The present study was carried out to screen 302 mung bean genotypes using RGA primers to identify resistant genotypes and validate their field performance under hotspot conditions. Among the four tested RGA primers, only two primers *viz., CYR-1* (1236 bp) and *VMR-1* (445bp) were successful in amplifying MYMV resistance related sequences. Among 25 genotypes, 13 were amplified by *CYR-1*and 24 genotypes were amplified by *VMR-1*. Further, thirteen genotypes showed amplification for both the primers in common, indicating the presence of resistance genes for both MYMV and mung bean yellow mosaic India virus (MYMIV). These results were confirmed by t test indicating significant ($p = 0.001$) association of these two markers with MYMV disease in mung bean. The findings highlight the efficiency of *CYR-1* and *VMR-1* primers as reliable markers for screening MYMV resistance. Their integration into marker-assisted selection (MAS) can accelerate breeding programs aimed at developing resistant mung bean varieties.

**Keywords**: Mungbean, MYMV, Resistance gene analogue, Molecular characterization

## Introduction

Mungbean is one of the important pulse crops of India and contributing 10 per cent to the total pulse production. However, a number of biotic and abiotic stresses impede the production. Mungbean yellow mosaic virus (MYMV) is one of the most damaging biotic agents; its intensity and crop growth stage can cause production reductions of up to 85 per cent. Prominent sign of MYMV on the foliage is little yellow-coloured spots dispersed on the young leaves' and along the veinlet's spreads throughout the lamina; subsequently, those spots progressively expand in size and the entire leaf becomes chlorotic (Akhtar et al. 2011). Resistance gene analogues (RGA) are emerging marker system, and their uses are encouraging the development of resistance breeding. While RGA itself is not a resistance gene analogue and RGA in plants are related to the class of disease resistance genes known as nucleotide binding site and leucine rich repeat (NBS-LRR), which are distinguished by NBS-LRR regions. The usage of RGA from distantly related plants is made easier by the presence of numerous distinct sequence motifs in the NBS that are substantially conserved among disease resistance genes (Sekhwal et al. 2015; Tantasawat et al. 2021). As a result, the use of RGA markers is relatively new and can be created using the diagnostic patterns of genes that are known to resist certain diseases (Yan et al. 2003). Resistance gene analogue (RGA) primers serve as effective molecular tools for identifying MYMV resistant genotypes. The quick selection of genotypes bearing resistance genes will be aided by the identification of resistant lines through field screening

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and indirect selection utilizing RGA markers. The present study was, therefore, conducted to screen a large number of mung bean (*Vigna radiata* L. Wilczek) genotypes using RGA primers to identify resistant genotypes and validate their field performance under hotspot locations.

## Materials and methods

The field experiment was carried out at the research plot of the Department of Genetics and Plant Breeding, University of Agricultural Sciences, Bangalore. The material used in this investigation includes 250 advanced breeding lines (ABLs) obtained from UAS Dharwad, 50 germplasms obtained from IIPR, Kanpur, and two susceptible checks, LM 1668 and China mung. Augmented design was followed to lay out the experiment, and all agronomic procedures were followed to raise the crop except prophylactic spray for MYMV.

### *Screening for MYMV resistance under natural conditions*

In summer 2020, 302 genotypes including two susceptible checks namely, Chinamung and LM 1668 were screened for responses to MYMV disease. The infector row method with spreader rows of susceptible variety (China mung and LM 1668) was adopted. In addition to this, two rows of susceptible genotypes were planted all around the experimental plot to increase the disease incidence. The genotypes were scored for the response to MYMV after 80% of the plants in spreader rows infected with MYMV. The per cent disease index (PDI) of each genotype were scored at 30, 45 and 55 days after sowing (DAS) using 1–6 scale (AVRDC scale) developed by world vegetable centre (WVC) and modified by Akhtar et al. (2009). Disease incidence (DI) was determined based on the percentage of plants infected. According to Campbell and Madden (1990), the trapezoidal integration of PDI estimated at 30, 45, and 55 DAS was used to determine the area under disease progress curve (AUDPC) for each genotype.

DI, PDI, and AUDPC were calculated as follows:

$$\textit{Per cent disease index} = \frac{\textit{Sum of individual disease ratings}}{\textit{Total no.of sample leaves assessed} \times \textit{maximum grade}} \times 100$$

$$\textit{Disease incidence} = \frac{\textit{No. of plants infected in a row}}{\textit{Total no. of plants in a row}} \times 100$$

$$AUDPC = \sum_{i=1}^{n-1} \frac{Yi + Yi + 1}{2} \times (ti + 1 - ti)$$

Where,

n- number of assessments

Y-per cent disease index

(ti+1-ti)- duration between two consecutive assessments

Field evaluation under hotspot conditions, identification and characterization of analogues (R genes) controlling resistance to mungbean yellow mosaic virus

To identify potential sources of resistance against MYMV, experiment was conducted at the experimental plot at main research station, Hebbal in summer, 2021. Based on results obtained from the screening under field condition and wet lab experiment by using RGA primers, 24 genotypes were selected from among 302 genotypes. These selected genotypes consisted of 15 ABLs and nine germplasms. The infector row strategy involving sowing of test entries coupled with susceptible checks alternatively was adopted. The PDI were scored at 30, 45 and 55 DAS for each genotype using the AVRDC scale. DI was recorded by identifying the number of planted infected in a row out of total number of plants in a row. The Area Under the Disease Progress Curve (AUDPC) was observed for each genotype using trapezoidal integration of PDI estimated at 30, 45 and 55 DAS (Campbell and Madden 1990).

The total genomic DNA was isolated from mung bean leaves of 302 genotypes using CTAB method. The molecular identification of resistance genes in 302 mung bean genotypes for MYMV was tested out using RGA primers tabulated in Table 1. PCR for the RGA marker analysis was done in 10 µl reaction volume.

## Results and discussion

### *Field evaluation of mung bean genotypes for MYMV under natural condition*

A collection of 302 mung bean genotypes along with checks were screened under natural condition for MYMV disease resistance during summer, 2020. DI, PDI, and AUDPC for all the 302 mung bean genotypes were recorded. All the genotypes evaluated under field condition showed variable responses to severity of the disease (Fig. 1). PDI of mung beangenotypes varied from 19.92 to 78.02 per cent. Disease incidence of mung bean genotypes under this study varied between 30 *per cent*and80*percent*. Further, AUDPC of mung bean genotypes ranged from 701.35 to 2892.55. Both susceptible checks were recorded higher AUDPC (Table 2). This experiment revealed that 25 genotypes (about 8%) including 16 ABLs and nine germplasms have recorded lowest PDI and possess resistance to MYMV under field conditions (Table3). Present findings are in accordance with

**Table 1.** RGA primers used in the present study

| Markers | Primer sequences | Reference |
|---|---|---|
| *CYR - 1* (Mungbean) | F: GGGTGGTTTGGGTAAGACCC<br>R:TTCGCGGTGTGAAAAGTCT | Kabi et al. 2017 |
| *CYR - 1*Degenerate (Mungbean) | F: GGGTGGNTTGGGTAAGACCAC<br>R:NTCGCGGTGNGTGAAAAGNCT | Maiti et al. 2011 |
| VMYR - 1 (Mungbean) | F: AGTTTATAATTCGATTGCT<br>R:ACTACGATTCAAGACGTCCT | Basak et al. 2005 |
| YR-4 (Mungbean) | F:GGNAAGACGACACTCGCNTTA<br>R: GACGTCCTNGTAACNTTGATCA | Maiti et al. 2011 |

**Table 2.** Range of disease incidence, per cent disease index and AUDPC for resistant, moderately resistant and moderately susceptible genotypes

| S. No. | Category | Per cent disease index | Disease incidence | AUDPC |
|---|---|---|---|---|
| 1 | Resistant | 19.92 – 78.02 | 30 - 80 | 701.95 – 2892.55 |
| 2 | Moderately Resistant | 19.92 -56.44 | 30 - 80 | 701.35 – 2058.40 |
| 3 | Moderately susceptible | 19.92 – 59.76 | 30 - 80 | 709.65 – 2211.95 |

**Table 3.** Disease incidence, per cent disease index and AUDPC of selected 25 resistant genotypes

| S.No. | Name | DI | PDI | AUDPC | Disease Score | Disease Reaction |
|---|---|---|---|---|---|---|
| 1 | GG-ABL-431 | 30 | 21.58 | 747 | 2 | R |
| 2 | GG-ABL-1 | 40 | 21.58 | 767.75 | 2 | R |
| 3 | GG-ABL-419 | 30 | 23.24 | 796.8 | 2 | R |
| 4 | GG-ABL-241 | 30 | 24.9 | 879.8 | 2 | R |
| 5 | GG-ABL-244 | 30 | 19.92 | 701.35 | 2 | R |
| 6 | GG-ABL-357 | 40 | 19.92 | 701.35 | 2 | R |
| 7 | GG-ABL-265 | 30 | 21.58 | 730.4 | 2 | R |
| 8 | GG-ABL-407 | 40 | 21.58 | 805.1 | 2 | R |
| 9 | GG-ABL-457 | 30 | 19.92 | 738.7 | 2 | R |
| 10 | GG-ABL-349 | 40 | 23.24 | 834.15 | 2 | R |
| 11 | GG-ABL-334 | 50 | 19.92 | 738.7 | 2 | R |
| 12 | GG-ABL-480 | 40 | 23.24 | 796.8 | 2 | R |
| 13 | GG-ABL-526 | 30 | 21.58 | 730.4 | 2 | R |
| 14 | GG-ABL-516 | 30 | 23.24 | 834.15 | 2 | R |
| 15 | GG-ABL-511 | 40 | 24.9 | 937.9 | 2 | R |
| 16 | GG-ABL-499 | 30 | 24.9 | 900.55 | 2 | R |
| 17 | HUM12 | 50 | 19.92 | 759.45 | 2 | R |
| 18 | AKP/NP/8/9 | 30 | 21.58 | 788.5 | 2 | R |
| 19 | EC 496839 | 30 | 23.24 | 834.15 | 2 | R |
| 20 | OMG-1030 | 50 | 21.58 | 730.4 | 2 | R |
| 21 | PS-16 | 40 | 21.58 | 709.65 | 2 | R |
| 22 | EC- 550831 | 30 | 21.58 | 767.75 | 2 | R |
| 23 | TJM- 3 | 30 | 23.24 | 871.5 | 2 | R |
| 24 | EC- 398131 | 40 | 23.24 | 776.05 | 2 | R |
| 25 | RMG-353 | 30 | 21.58 | 788.5 | 2 | R |

the earlier reported results of Meghashree et al. (2017) and Win et al. (2020), who evaluated nine mung bean cultivars and found that IPM-2-3 and PDM-139 were most potential MYMV resistant cultivars with good yield potential and did not find any entry to be highly resistant. Similarly, Munawwar et al. (2014) screened 64 genotypes of mung bean against MYMVunder field condition and identified six genotypes *viz.*, AZRI-1, NCM-15-11, NCM-21,NCM-11-8, 14063 and AZRI-06 with resistant reaction.

***Molecular detection of analogs candidate R genes controlling resistance against MYMV in mungbean genotypes using resistant gene analogs***

To confirm the presence of MYMV, 302 genotypes of mung bean were subjected to molecular analysis using RGA primers. Based on the specificity, four RGA primers were selected however, only two primers were amplified *viz., CYR-1* (1236 bp) and *VMR-1*(445 bp). A total of 13 genotypes amplified *CYR-1* primers *viz.,* GG-ABL-1, GG-ABL-


**Fig. 1.** Progressive increase of MYMV symptoms in mungbean genotypes during summer, 2020

419, GG-ABL-241, GG-ABL-244, GG-ABL-357, GG-ABL-265, GG-ABL-349, GG-ABL-526, GG-ABL-511, OMG-1030, PS-16, TJM-3 and RMG-353 (Fig. 2). On the other hand, *VMR-1* primer was amplified in 24 genotypes *viz.,* GG-ABL-431, GG-ABL-1, GG-ABL-419, GG-ABL-241, GG-ABL-244, GG-ABL-357, GG-ABL-265, GG-ABL-407, GG-ABL-457, GG-ABL-349, GG-ABL-334, GG-ABL-480, GG-ABL-526, GG-ABL-516, GG-ABL-511, HUM12, AKP/NP/8/9, EC-496839, OMG-1030, PS-16, EC 550831, TJM-3, EC-398131 and RMG-353 (Fig. 3) and confirmed the presence of resistance gene against MYMV. Furthermore, it was determined that both markers were not amplified in susceptible check samples, China mung and LM 1668, proving the existence of an RGA primer against MYMV. Thirteen mung bean genotypes out of 24 showed amplification for both primers (*CYR-1* and *VMR-1*), indicating the genotypes were MYMV-resistant. These results are confirmed by t test indicating significant associations with MYMV resistance (Table 4).The list of the genotypes amplified using the *CYR-1* and *VMR-1* primers are tabulated in Table 5. These findings bear resemblance to Panigrahi et al. (2016), who demonstrated a strong correlation between this marker and YMV resistance by amplifying *CYR-1* primer (1236 bp) in segregating $F_2$ populations and $F_3$ families. Maiti et al. (2011) conducted related research using the *CYR-1* primer, which demonstrated linkage with MYMIV-resistant germplasms and co-segregating with MYMV-resistant $F_{2s}$, and $F_3$ urdbean progenies. Using *CYR-1* markers, Kabi et al. (2017) determined that seven genotypes of mung bean (OBGG-2013-8, OBGG-2013-12, OBGG-2013-11, OBGG-2013-16, OBGG2013-20, OBGG-2013-21, and OBGG-2013-39) have a gene exhibiting resistance to yellow mosaic virus. The marker can be effectively utilized for selection of YMV resistant lines.

**Table 4.** t test of markers linked to MYMV disease in mungbean genotypes

| S. No. | Marker | Degrees of freedom | t - statistic | p-value |
|---|---|---|---|---|
| 1. | *CYR-1* | 299 | 8.681*** | $2.5 \times 10^{-16}$ |
| 2. | *VMR-1* | 299 | 13.723*** | $2.2 \times 10^{-16}$ |

***Significantat ($p = 0.001$)levelofsignificance

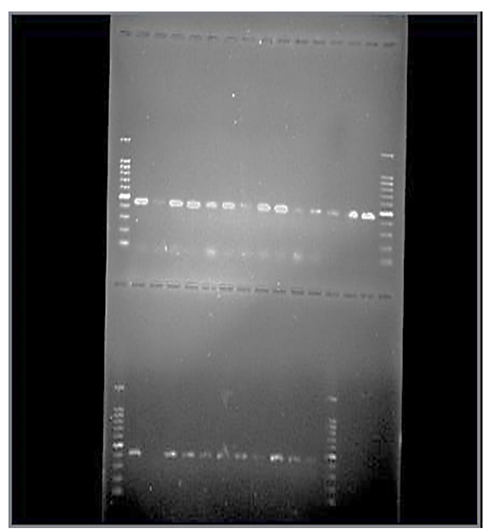

**Fig. 2.** PCR amplification of CYR-1 primer in resistant genotypes

### *Molecular characterization of RGA against MYMV*

Sequencing verified the PCR result of mung bean samples with RGA against MYMV. The NCBI BLASTn search was used to do the sequence analysis. Research has shown that the RGAs of *CYR1* and *VMR1* were substantially similar to the RGAs of the sequences that were deposited in the NCBI

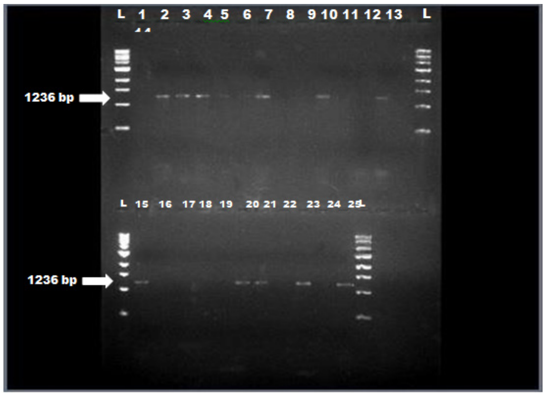


**Fig. 3.** PCR amplification of VMR-1 primer in resistant genotypes

database. The present *RGA-CYR1* isolate exhibits 98.02% identity with the complete sequence of the *Vigna mungo* disease resistance protein (*CYR-1*) mRNA (HQ704837.1), 97.94% identity with the partial sequence of the *Vigna mungo* disease resistance protein (*CYR - 1*) (HQ704838.1) and 97.40 *percent* identity with the complete sequence of *Vigna mungo* disease resistance protein (*CYR-1*) gene, *cyr1* allele (KR350634.1). Table 6 compiles a list of published sequences utilized to compare the mung bean*CYR1* sequence. RGA-VMR - 1 isolate has 92.47% similarity with *Vigna mungo* resistance protein (*VMR-2*) gene partial sequence (AY301991.1), 89.21% similarity with *Vigna mungo* disease resistance protein (*VMYR-1*) gene partial sequence (AY297425.2) and also 91.45 *per cent* similarity with *Vigna mungo* disease resistance

**Table 5.** Reaction of resistant mung bean genotypes to *VMR-1* and *CYR-1* primers

| S. No. | Genotypesname | VMR-1 | CYR-1 | Common | Disease score | Diseasereaction |
|---|---|---|---|---|---|---|
| 1 | GG-ABL-431 | | | | 2 | R |
| 2 | GG-ABL-1 | | | | 2 | R |
| 3 | GG-ABL-419 | | | | 2 | R |
| 4 | GG-ABL-241 | | | | 2 | R |
| 5 | GG-ABL-244 | | | | 2 | R |
| 6 | GG-ABL-357 | | | | 2 | R |
| 7 | GG-ABL-265 | | | | 2 | R |
| 8 | GG-ABL-407 | | | | 2 | R |
| 9 | GG-ABL-457 | | | | 2 | R |
| 10 | GG-ABL-349 | | | | 2 | R |
| 11 | GG-ABL-334 | | | | 2 | R |
| 12 | GG-ABL-480 | | | | 2 | R |
| 13 | GG-ABL-526 | | | | 2 | R |
| 14 | GG-ABL-516 | | | | 2 | R |
| 15 | GG-ABL-511 | | | | 2 | R |
| 16 | GG-ABL-499 | | | | 2 | R |
| 17 | HUM12 | | | | 2 | R |
| 18 | AKP/NP/8/9 | | | | 2 | R |
| 19 | EC496839 | | | | 2 | R |
| 20 | OMG-1030 | | | | 2 | R |
| 21 | PS-16 | | | | 2 | R |
| 22 | EC-550831 | | | | 2 | R |
| 23 | TJM- 3 | | | | 2 | R |
| 24 | EC-398131 | | | | 2 | R |
| 25 | RMG-353 | | | | 2 | R |
| | Presence of *VMR – 1* | | | | | |
| | Presence of *CYR – 1* | | | | | |
| | Presence of both *VMR – 1* and *CYR – 1* | | | | | |

protein (YR-3) mRNA partial sequence (EF446378.1). List of reported sequences used for comparison of *VMR1* sequence of mung bean is documented in Table 7. NBS-LRR are the most common functional domains found in the majority of disease resistance genes (R-genes) found in plants. *CYR-1* and *VMR-1* marker have these domains which were confirmed by NCBI blast analysis (Sekhwal et al. 2015, Panigrahi et al. 2016). Molecular characterization of RGA of genotypes used in the present study suggested that, per cent similarity of RGA sequence with reported RGA sequences in NCBI database provides information about the domains evolutionarily responsible for their resistance.

### *Screening of mungbean genotypes under hotspot conditions for MYMV resistance*

In summer, 2021, 24 genotypes of mung bean, along with checks, were evaluated for MYMV in disease pressure point areas. Based on MYMV incidence, severity, and disease parameters, scoring was done for DI, PDI and AUDPC (Table 8) and the readings were recorded similar to the summer, 2020 screening investigation. The outcome demonstrated wildly disparate reactions to the disease severity. Out of 24 resistant genotypes, 15 were ABL and 9 were germplasms. The PDI of twenty-four resistant mung bean genotypes ranged from twenty-one to twenty-nine per cent. When the

**Table 6.** List of reported sequences used for comparison of CYR-1 sequence of mung bean

| S. No. | Resistant gene analog | Per centidentity(%) | Accession number |
|---|---|---|---|
| 1. | *Vigna mungo* disease resistance protein(*CYR1*)mRNA,Completesequence | 98.02 | HQ704837.1 |
| 2. | *Vigna mungo* disease resistance protein(*CYR1*)gene,Partialsequence | 97.94 | HQ704838.1 |
| 3. | *Vigna mungo* disease resistance protein(*CYR1*) gene, *cyr1* allele, Completesequence | 97.40 | KR350634.1 |
| 4. | *Vigna mungo* cultivar WBU-108 diseaseresistance protein *CYR1* (*YR2*) gene, Partialsequence | 99.14 | EU258701.1 |
| 5. | *Vigna angularis* putative disease resistanceRPP-13-like protein 1 (LOC108345928),mRNA | 96.13 | XM017584784.1 |
| 6. | *Vigna radiata* var. *radiata* putative diseaseresistance RPP13-like protein 1(LOC106763260),mRNA | 98.97 | XM014647468.2 |
| 7. | *Vigna radiata* var. *radiata* putative diseaseresistance RPP13-like protein 1(LOC111241710),mRNA | 85.02 | XM022781474.1 |
| 8. | *Vigna unguiculata*putative disease resistanceproteinAt3g14460(LOC114175366),mRNA | 85.15 | XM028060141.1 |
| 9. | *Phaseolus vulgaris* NBS-LRR type putativedisease resistance protein CNL-B18 gene,completecds | 85.34 | EU856774.1 |
| 10. | *Phaseolus vulgaris* NBS-LRR resistance likeproteinJ71(J71)gene,Completesequence | 85.39 | AF306505.1 |

**Table 7.** List of reported sequences used for comparison of *VMR-1* sequence of mung bean

| S. No. | Resistant gene analog | | Accession number |
|---|---|---|---|
| 1. | *Vigna mungo* resistance protein (*VMR-2*) gene, Partialsequence | 92.47 | AY301991.1 |
| 2. | *Vigna mungo* disease resistance protein (*YR-3*)mRNA,Partialsequence | 91.45 | EF446378.1 |
| 3. | *Vigna radiata* var. radiata TMV resistance proteinN-like(LOC106763203),transcript variant | 93.16 | XM022784106.1 |
| 4. | *Vignaradiata*viralresistancecandidate(*MYR-1*)gene,Partialsequence | 90.55 | AY301990.1 |
| 5. | *Vignamungo*diseaseresistanceprotein(*VMYR-1*)gene,Partialsequence | 89.21 | AY297425.2 |
| 6. | *Vigna radiata* var. radiata TMV resistance proteinN-like(LOC111241413), mRNA | 87.89 | XM022779288.1 |
| 7. | *Vigna angularis* var. radiata TMV resistanceprotein N-like (LOC108343899), transcript variantX2,mRNA | 87.11 | XM017582331.1 |
| 8. | *Vigna angularis* var. radiata TMV resistanceprotein N-like (LOC108343899), transcript variantX1,mRNA | 87.11 | XM017582330.1 |
| 9. | *Phaseolusvulgaris*hypotheticalprotein(PHAVU004G140800g),mRNA,Completesequence | 86.58 | XM007152503.1 |
| 10. | *Phaseolusvulgaris*hypotheticalprotein(PHAVU004G140800g),mRNA,Completesequence | 86.58 | XM007152504.1 |

**Table 8.** Disease reaction of 24 resistant lines for MYMV disease summer, 2021

| S. No | Resistant lines | DI | PDI | AUDPC |
|---|---|---|---|---|
| 1 | GG-ABL-431 | 30 | 21.58 | 688.9 |
| 2 | GG-ABL-1 | 40 | 23.24 | 759.45 |
| 3 | GG-ABL-419 | 40 | 23.24 | 871.5 |
| 4 | GG-ABL-241 | 30 | 24.9 | 900.55 |
| 5 | GG-ABL-244 | 40 | 23.24 | 814.2 |
| 6 | GG-ABL-357 | 30 | 23.24 | 854.9 |
| 7 | GG-ABL-265 | 40 | 21.58 | 788.5 |
| 8 | GG-ABL-407 | 30 | 21.58 | 767.75 |
| 9 | GG-ABL-457 | 30 | 21.58 | 767.75 |
| 10 | GG-ABL-349 | 30 | 23.24 | 813.4 |
| 11 | GG-ABL-334 | 30 | 21.58 | 730.4 |
| 12 | GG-ABL-480 | 30 | 23.24 | 834.15 |
| 13 | GG-ABL-526 | 30 | 21.58 | 805.1 |
| 14 | GG-ABL-516 | 40 | 23.24 | 854.9 |
| 15 | GG-ABL-511 | 30 | 24.9 | 842.45 |
| 16 | HUM 12 | 30 | 21.58 | 747 |
| 17 | AKP/ NP/8/9 | 40 | 21.58 | 730.4 |
| 18 | EC 496839 | 30 | 23.24 | 813.4 |
| 19 | OMG- 1030 | 40 | 21.58 | 709.65 |
| 20 | PS- 16 | 40 | 23.24 | 813.4 |
| 21 | EC- 550831 | 30 | 21.58 | 805.1 |
| 22 | TJM- 3 | 30 | 23.24 | 776.05 |
| 23 | EC- 398131 | 40 | 24.9 | 842.45 |
| 24 | RMG- 353 | 30 | 21.58 | 709.65 |

PDI of summer, 2020 and 2021 were compared, it was found that range of summer, 2021 was greater than summer, 2020. Disease incidence of the 24 selected resistant mung bean genotypes ranged between 30 and 40%. On comparing the range of DI of both the seasons (summer, 2020 and 2021), range of DI in summer 2021 was lower than summer, 2020. AUDPC of selected 24 resistant genotypes of mungbean ranged from 709.65 to 900.55 in which both minimum and maximum AUDPC were observed from ABLs. On comparing the range of AUDPC of both the seasons (summer ,2020 and 2021), range of AUDPC in summer 2021 was lower compared to summer, 2020.

Thepresent work, which included molecular screening of 24 distinct mung bean genotypes using RGA primers and field screening at two distinct locations, revealed that these genotypes were resistant to MYMV and can be employed as resistant gene donors in hybridization programs. Thirteen of the twenty-four genotypes of mung beans showed amplification for both primers, *CYR -1* and *VMR -1*, indicating the presence of a MYMV resistance gene. Results validate the use of *CYR - 1* and *VMR - 1* primers in marker assisted selection (MAS) and as a fundamental screening technique for identifying susceptible and resistant genotypes across various genotypes. Additionally, genotypes that exhibit resistance that were discovered through field screening and RGA primers may be sent for confirmation of their resistance to rolling circle amplification-PCR.

## Authors' contribution

Conceptualization of research (RN, SBM); Designing of the experiments (RN, SBM, AR); Contribution of experimental materials (RN, HHS, A); Execution of field/lab experiments and data collection (SBM); Analysis of data and interpretation (RN, AR, A, HHS); Preparation of the manuscript (RN, SBM, HHS).

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