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**RESEARCH ARTICLE**

# Screening of $M_3$ mutants for yellow vein mosaic virus resistance in greengram [*Vigna radiata* (L.) wilczek]

■ **N. VAIRAM, S. ANANDHI LAVANYA, M. MUTHAMILAN AND C. VANNIARAJAN**

## SUMMARY

Greengram [*Vigna radiata* (L.) Wilczek] is a cheap source of dietary protein for the poor, with high levels of folate and iron compared with many other legumes. Mungbean yellow mosaic virus is also one of the destructive viral disease affecting yield potential of greengram both quantitatively and qualitatively. Induced mutations, have offered a single and short alternative to conventional breeding including isolation, screening, selection and testing generation after generation. An investigation was carried out in two mungbean genotypes *viz.*, CO (Gg) 7 and NM 65 treated by two mutagens *viz.*, gamma rays at the doses of 300, 400 and 500 Gy and EMS treatments of 10, 20 and 30 mM. The trial was conducted in the research farm of Agricultural College and Research Institute, TNAU, Madurai during *Kharif* season 2013. The $M_2$ generation was raised as individual $M_1$ plant basis. The treated and control populations of $M_2$ generation were carefully screened for pod shattering resistance. The yellow vein mosaic virus disease (YMV) incidence was recorded for all the plants in $M_3$ generation for the selected 22 mutants. Based on field scoring, the mutants *viz.*, $M_5$, $M_{18}$, $M_{26}$, $M_{46}$, $M_{54}$, $M_{58}$, $M_{70}$, $M_{71}$, $M_{92}$ and $M_{98}$ were identified as yellow vein mosaic virus resistant mutants. The mutants which showed field resistance were checked for YMV resistant gene using the gene specific primer ISSR811. Five mutants confirmed with marker studies. These resistant mutants have been forwarded to next generation for further yield performance.

**Key Words :** Mungbean, Induced mutation, Yellow vein mosaic virus, Scoring, Mutants, Marker

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Mungbean [*Vigna radiata* (L.) Wilczek] is grown mainly in India, Pakistan, Thailand, Philippines, Sri Lanka, Myanmar and Bangladesh and about 70 per cent of the total world mungbean production occurs in India (Raturi *et al.*, 2012). Among the diseases, yellow mosaic virus disease (YMV) is a major one in greengram. Disease symptoms vary from a few small yellow specks or spots on a few leaves, to yellowing or chlorosis of all leaves of the whole plant followed by necrosis. In highly susceptible plants,

symptoms includes shortening of internodes, severe stunting of plants with no yield or few flowers and deformed pods producing small, immature and shriveled seeds (Kitsanachandee *et al.,* 2013). The YMV disease incidence varies from 5 to 90 per cent depending upon the susceptibility of cultivars and population of whitefly.

Mutation breeding provides the means for enhancing the genetic variation and genetic variability may also be created by modulating the effects of mutagens. Adaptation of mutation breeding in such a situation is promising not only for creating and utilizing the new variability but also an indispensable approach through which yield might be improved without altering the existing desirable characters. The use of resistant varieties is the most desirable strategy to manage the disease in an economical and environmentally-friendly way (Sudha *et al.,* 2013). Development of varieties with resistance to major diseases such as mungbean yellow mosaic disease continues to be a breeding priority. Hence, screening of elite mutants for identifying resistance to yellow vein mosaic virus with high yielding potential will help to increase the production to a greater extent.

## MATERIAL AND METHODS

Two greengram genotypes *viz.,* CO (Gg) 7 and NM 65 obtained from the Department of Pulses, Centre for Plant Breeding and Genetics, TNAU, Coimbatore. The genotypes were subjected to gamma irradiation at the doses of 300, 400 and 500 Gy and EMS treatments of 10, 20 and 30 mM. The trial was conducted in the research farm of Agricultural College and Research Institute, TNAU, Madurai during *Kharif* season 2013. The $M_2$ generation was raised as individual $M_1$ plant basis. A total of 100 mutants were identified in $M_2$ generation. Twenty two mutants showed tolerance to pod shattering in $M_2$ generation. The yellow mosaic virus disease (YMV) incidence was recorded for all the plants in $M_3$ generation for 22 mutants based on the visual scores on 50$^{th}$ day when the susceptible check Co 5 Blackgram recorded scale 9 (Table A).

### Molecular analysis :

The experiment was conducted in the Department of Plant Molecular Biology and Biotechnology, Centre for Plant Molecular Biology, Tamil Nadu Agricultural University, Coimbatore. For this purpose, 10 $M_3$ mutants which showed resistance under field condition were raised in paper cups for isolation of genomic DNA. A total of five markers were surveyed to study the presence of resistance existing among the mutants and the parents. The details of markers are presented in Table B.

### Isolation of DNA :

The young leaves of greengram at three leaf stage (18 DAS) were collected and the DNA was extracted

**Table A : Screening for YMV based on scales given by Singh *et al.* (1988)**

| Scales | Percentage of plant foliage affected | Reaction |
|---|---|---|
| 1 | Mottling of leaves covering 0.1 to 5.0 per cent of leaf area | Resistant |
| 3 | Mottling of leaves covering 5.1 to 10 per cent of leaf area | Moderately resistant |
| 5 | Mottling and yellow discoloration of 10.1 to 25.0 per cent of leaf area | Moderately susceptible |
| 7 | Mottling and yellow discoloration of 25.0 to 50.0 per cent of leaf area | Susceptible |
| 9 | Severe yellow mottling on more than 50.0 per cent and up to 100 per cent of the leaf area | Highly susceptible |

**Table B : List of markers used for screening of MYMV**

| Sr. No. | Marker | Sequence | | |
|---|---|---|---|---|
| 1. | RGA-1-F-CG | (5'-3') | AGTTTATAATTCGATTGCT | RGA |
| 2. | $YMV_1$ | F(5'-3') | GAGAGAGAGAGAGAGACAAAG | SCAR |
| | | R(5'-3') | GAGAGAGAGAGAGAGACAGGA | |
| | ISSR811 | | $(GA_8)C$ | ISSR |
| 3. | RGA22F2 | (5'-3') | GGGTGGNTTGGGTAAGACCAC | RGA |
| | RGA24R2 | (5'-3') | NTCGCGGTGNGTGAAAAGNCT | |
| 4. | $CEDG_{180}$ | F(5'-3') | GGTATGGAGCAAAACAATC | SSR |
| | | R(5'-3') | GTGCGTGAAGTTGTCTTATC | |
| 5. | MYMVR-583 | F(5'-3') | GTGATGCACACGGTTACGGT | SCAR |
| | | R(5'-3') | GGTGACGCAGTCCATACAAATT | |

by CTAB (mini-prep) method. The quantity and quality of the DNA was checked for polymerase chain reaction (PCR). The isolated genomic DNA was quantified using the NanoDrop™1000 spectrophotometer. The quality of DNA was also checked using 0.8 per cent agarose gel electrophoresis.

**Analysis using SSR markers :**

The SSR markers were obtained from Grameni (*http://www.grameni.org.in*) and they were commercially purchased from Sigma Aldrich Corporation, Bangalore. PCR amplifications were performed in 200 µl microcentrifuge tubes containing 15 µl of reaction mixture consisting of 5ng of template DNA, 0.2 µM of each forward and reverse primer, 0.25 mM each dNTPs (ATP, GTP, CTP, TTP), 0.3 mM of $MgCl_2$, 0.75 µl of *Taq* polymerase (Bangalore Genei Pvt. Ltd. Bangalore) and 1x reaction buffer (10 mM Tris HCl, pH 9.0, 15 mM KCl (pH 8.3) and 1.5 mM $MgCl_2$).

**PCR amplification :**

Amplification reaction was in a volume of 15 µl containing 5 ng of genomic DNA and amplification was performed in Mastercycler gradient (EPPENDORF) with the following sequential thermal cycling: initial denaturation step at 94°C for 5 min, followed by 35 cycles at 94°C for 1 min, an annealing step at 56°-60°C for 1 min and an extension at 72°C for 1 min with the final extension step at 72°C for 7 min. The final products were held at 4°C. Gel electrophoresis was performed to separate amplification products. PCR amplified products were resolved on 3 per cent metaphor agarose gel. 10 µl each of the product were loaded and subjected to gel electrophoresis in 1X TBE buffer at 90 V for 3 hours. The bands were visualized after staining with ethidium bromide and UV trans illumination. Fragment length was determined visually by comparing with DNA ladder. The gels were documented using Alpha Imager™ 1200 documentation and analysis system (Alpha Innotech Corporation, USA).

## RESULTS AND DISCUSSION

Mungbean yellow mosaic virus is also one of the destructive viral disease affecting yield potential of greengram both quantitatively and qualitatively. MYMV incidence was first reported from the fields of IARI, New Delhi by Nariani (1960). This disease is well spread throughout the Indian sub-continent. Use of chemicals to control the disease is an age old practice in the plant protection. But managing the disease by means of resistant genotype is an effective, cheapest and safest means of disease control. Improved resistance to YMD is now the major goal in mungbean breeding programs in several mungbean production countries.

In this study, the field screening for MYMV resistance in $M_3$ generation were carried out. A total of 22 mutants along with the control were screened for reaction against yellow mosaic virus and the results are presented in Table 1 and Fig. 1. The results showed 10 mutants *viz.*, $M_5$, $M_{18}$, $M_{26}$, $M_{46}$, $M_{54}$, $M_{58}$, $M_{70}$, $M_{71}$,

**Table 1 : Field screening for yellow vein mosaic virus in $M_3$ mutants of green gram**

| Mutants | Treatments | Scoring % | Grade |
|---|---|---|---|
| $M_5$ | CO (Gg) 7- 300 Gy | 2.70 | Resistant |
| $M_9$ | | 13.50 | Moderately susceptible |
| $M_{18}$ | CO (Gg) 7-400 Gy | 4.50 | Resistant |
| $M_{26}$ | | 4.80 | Resistant |
| $M_{36}$ | CO (Gg) 7-500 Gy | 6.40 | Moderately resistant |
| $M_{38}$ | | 22.30 | Moderately susceptible |
| $M_{42}$ | | 5.80 | Moderately resistant |
| $M_{44}$ | | 15.50 | Moderately susceptible |
| $M_{46}$ | NM 65-300 Gy | 3.50 | Resistant |
| $M_{47}$ | | 13.40 | Moderately susceptible |
| $M_{54}$ | | 3.70 | Resistant |
| $M_{55}$ | | 44.20 | Susceptible |
| $M_{58}$ | NM 65-400 Gy | 5.00 | Resistant |
| $M_{66}$ | CO (Gg) 7-10 mM | 94.50 | Highly susceptible |
| $M_{70}$ | | 4.50 | Resistant |
| $M_{71}$ | CO (Gg) 7-20 mM | 3.90 | Resistant |
| $M_{77}$ | CO (Gg) 7-30 mM | 5.60 | Moderately resistant |
| $M_{84}$ | NM 65-10 mM | 22.90 | Moderately susceptible |
| $M_{89}$ | NM 65-20 mM | 65.80 | Highly susceptible |
| $M_{91}$ | | 56.00 | Highly susceptible |
| $M_{92}$ | | 3.50 | Resistant |
| $M_{98}$ | NM 65-30 mM | 1.70 | Resistant |
| $V_1$ Control | CO (Gg) 7 | 7.80 | Moderately resistant |
| $V_2$ Control | NM 65 | 18.40 | Moderately susceptible |

Scoring:
0.1-5 % - Resistant
5.1 -10 % -Moderately resistant
10.1-25 % - Moderately susceptible
25-50 % - Susceptible
50- 100 % - Highly susceptible.

$M_{92}$ and $M_{98}$ as MYMV resistant, three mutants *viz.*, $M_{36}$, $M_{42}$ and $M_{77}$ as moderately MYMV resistant individuals, five mutants *viz.*, $M_9$, $M_{38}$, $M_{44}$, $M_{47}$ and $M_{84}$ as MYMV moderately susceptible individuals, one mutant $M_{55}$ as susceptible and three mutants *viz.*, $M_{66}$, $M_{89}$ and $M_{91}$ as highly susceptible individuals. The control, CO (Gg) 7 scored under moderately resistant and NM 65 fell under moderately susceptible categories.

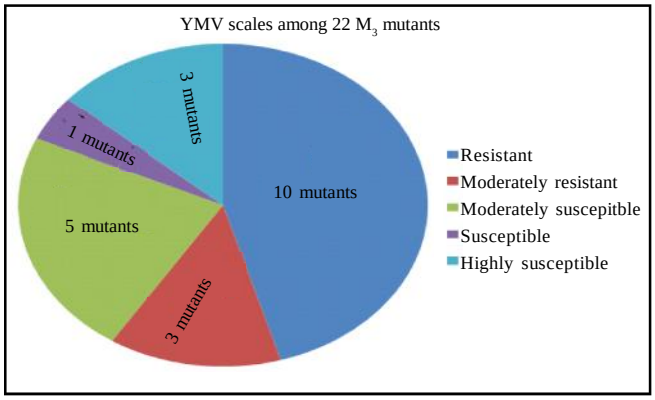


**Fig. 1: Yellow mosaic virus among $M_3$ mutants in greengram**

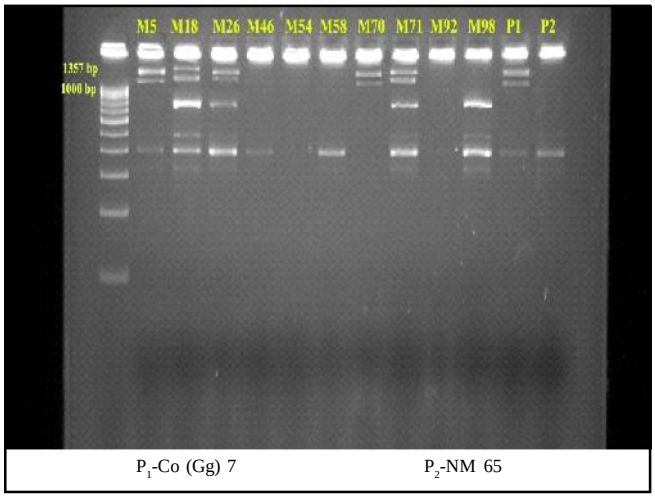


**Fig. 2 : Molecular confirmation for YMV using $ISSR_{811}$ primer**

The results are in agreement with the findings of Karthikeyan *et al.* (2012) and Chandra Paul *et al.*, 2013). A major difficulty in breeding YMD-resistant mungbean is field screening for the disease, which is hampered by non-uniform development of the disease due to fluctuation of the whitefly population in different locations and different seasons. The summer season was highly favourable for MYMV incidence.

Marker validation is a process of examining the behaviour of markers and the associated polymorphism in different genetic backgrounds (Gupta *et al.*, 1999). Identification of flanking DNA markers located within 5–10 cM from a gene of interest has yielded high levels of selection accuracy for resistance (Hittalmani *et al.*, 1995). Viral resistance genes have been tagged with markers in several other crops like soybean (Jeong and Saghai Maroof, 2004), Phaseolus (Urrea *et al.*, 1996) and pea (Gao *et al.*, 2004). Marker assisted indirect selection of resistant genotypes using linked markers has been reported as an effective breeding approach for developing YMD resistant cultivars in blackgram (Sowmini and Jayamani, 2014).

In this present investigation, the best identified ten mutants *viz.*, $M_5$, $M_{18}$, $M_{26}$, $M_{46}$, $M_{54}$, $M_{58}$, $M_{70}$, $M_{71}$, $M_{92}$ and $M_{98}$ that showed field resistance along with two parents were validated using five primers *viz.*, RGA22F2, RGA24R2, $ISSR_{811}$, RGA-1-F-CG (RGA), $CEDG_{180}$ (SSR), MYMVR-583. SSR markers will also provide a platform to expedite the molecular breeding effort in other *Vigna* species, particularly blackgram, azuki bean, rice bean, and moth bean, as sufficient genomic resources are not available for these *Vigna* species (Gupta *et al.*, 2014). The SCAR marker (MYMVR-583) linked with a recessive MYMV resistance gene would hasten the introgression of the resistance gene and may be useful in mapping resistance gene and development of high-yielding YMD-resistant genotypes through MAS without the need for artificial screening (Dhole and Reddy, 2013).

Among the five primers only $ISSR_{811}$ primer got amplified and produced respective allele in resistance mutants and absent in susceptible mutants. Five mutants *viz.*, $M_5$, $M_{18}$, $M_{26}$, $M_{70}$ and $M_{71}$ and the parent CO (Gg) 7 produced respective allele at 1357bp for MYMV resistance. Five mutants *viz.*, $M_{46}$, $M_{54}$, $M_{58}$, $M_{92}$ and $M_{98}$ and the parent NM 65 did not produce allele, hence, they are susceptible for MYMV resistance. To conclude, among the five markers validated, four markers was found to be not linked with YMV resistance genes whereas the marker $ISSR_{811}$ were found to be partially linked with Yellow mosaic disease resistance. While comparing the marker data with phenotypic evaluation, few deviations were noticed for this marker. It is in accordance by the findings of Souframanien and Gopala Krishnan (2006) and Sowmini and Jayamani (2014) in blackgram.

## Conclusion :

In this study, $ISSR_{811}$ primer was used to confirm

the presence of resistant gene in YMV resistant mutants in $M_3$ generation along with control. Five mutants *viz.*, $M_5$, $M_{18}$, $M_{26}$, $M_{70}$, $M_{71}$ and the parent CO (Gg) 7 produced respective allele at 1357bp for MYMV resistance. It showed that $ISSR_{811}$ was partially linked to the YMV resistance gene. These resistant mutants have been forwarded to next generation for further yield performance.

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