

## Research Article

### Development of *Mungbean yellow mosaic virus* Resistant Genotypes in Mungbean through Interspecific Crosses of Wild *Vigna* Species

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#### Abstract

Mungbean Yellow mosaic disease is a main destructing viral disease in mungbean caused by *Mungbean yellow mosaic virus* (MYMV) which leads to severe sometime 100 percent yield reduction and it necessitates for developing MYMV resistant lines. The present investigation was carried out with an objective of evolution of MYMV resistant progenies through incorporating wide genes of same genera. An attempt was made between *V. radiata* x *V. umbellata* crosses. This agroinoculation technique was employed to examine the F<sub>2</sub> individuals, which were derived from a cross between VMGG012-005 (Moderately susceptible) x VGGru1 (resistant homozygous advanced line from *Vigna radiata* x *Vigna umbellata* derivative used as MYMV donor) to screen for the MYMV resistant progenies. In the field condition, MYMV infection can be evaluated by MYMV disease rating scale (1-9). Out of the 225, F<sub>2</sub> individuals, 48 individuals were identified as resistant to MYMV and subjected to agroinoculation. The two tandem viral constructs of MYMV, VA 221 (KA30 DNA A + KA22 DNA B) and VA 239 (RA30 DNA A + KA27 DNA B) mobilized in *Agrobacterium tumefaciens* strains Ach 5 and C 58 were used for

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Agroinoculation. The results shows that among the 48 individuals, only ten individuals namely Resplant3, Resplant6, Resplant7, Resplant9, Resplant13, Resplant19, Resplant25, Resplant37, Resplant40 and Resplant43 showed resistance to VA 221 and the same entries found to be susceptible to VA239 strain. The all 48 entries again raised as F<sub>3</sub> generation in next season in the field it showed resistant to MYMV. Other 177 individuals are susceptible to both strains, VA 221 and VA 239. All these field resistant individuals are forwarded to further generations and confirmed for resistance in field condition. In F<sub>3</sub> generation only ten genotypes which was showed resistance for VA221.

**Keywords:** Agroinoculation; Drought tolerant; Mungbean; *Mungbean yellow mosaic virus* resistant lines; Short duration; Summer season; VMG012-005

#### Introduction

Mungbean (*Vigna radiata* L.) is an important pulse crop in developing countries of Asia especially India, China, Japan, Myanmar and other countries and Latin America where it is consumed as dry seeds, fresh green pods [1]. Mungbean serves as vital source of vegetable protein (19.1-28.3%), mineral (0.18-0.21%) and vitamins. It is a native of India -Burma and is cultivated extensively in Asia [2]. India is the leading mungbean cultivating country, for mungbean improvement research has elaborately taken by mungbean breeders in India with covers up to 55% of the total area [3]. The World acreage and 45% of total production [4]. Among the biotic sources plant viruses are responsible for a significant proportion of crop disease [5]. It causes serious economic losses in many major crops by reducing economic part like seed yield and quality [6]. *Mungbean yellow mosaic virus* Disease (MYMV) is reported to be the most destructive viral disease among the various viral diseases, caused by *Mungbean yellow mosaic virus*. It causes severe yield reduction in all mungbean growing countries in Asia including India [7]. MYMV belongs to the family Geminiviridae [8]. The family Geminiviridae is divided into four genera (Mastrevirus, Curtovirus, Topocovirus and Begomovirus) based on genome structure, type of insect vector and host range [9]. Begomovirus is the largest genus of the family Geminiviridae [10], which is characterized by a bipartite genome (DNA-A and DNA-B) or monopartite genomes that were transmitted in a circulative persistent manner by white fly *Bemisia* [11]. Conventional breeding methods are unsuccessful in developing Yellow Mosaic Virus (YMV) resistant mungbean lines due to rapid explosion of new isolates and also the complexity of mechanism in controlling the resistance to MYMV [12]. The major problem encountered by scientists to develop the MYMV resistant variety is the identification of MYMV resistant lines in segregating population. Identifying the resistant lines is very complicated task due to the lack of reliable screening protocol for assessing the resistance/susceptibility against MYMV. Hence the scientists are in need of any biological/molecular tool that can lead to screening of the resistant or susceptible lines for MYMV. Developing host resistance to the disease or the vector has therefore been considered as the only solution to control this disease [6]. Plant genetic

transformation is of particularly benefit to molecular genetic studies and crop improvement programmes [13]. The entry VMGG012-005 is a extra early duration genotype, tolerant to high temperature with drought. If improve this culture for high level of MYMV resistant which can be used directly as variety and also used as donor for short duration, drought tolerant and high temperature resistant along with MYMV donar.

The emerging field or genetic engineering endows with a new technique agroinoculation was successful for screening virus resistant plants [14,15]. Jacob SS et al., [16] demonstrated the feasibility of using an *in vitro* molecular protocol to screen for resistance/susceptibility against MYMV and proved that agroinoculation can be successfully adopted for screening MYMV resistant mungbean genotypes. Exploitation of this reproducible and less expensive technique may lead to the development of a MYMV resistance genotype. With this background knowledge this present investigation was carried out with the aim of identifying the MYMV resistant progenies through agroinoculation.

## Materials and Methods

### Plant materials

The experimental material for the present investigation consisted of 225 F<sub>2</sub> individuals, 48 F<sub>3</sub> progenies and parents VMGG012-005 (short duration, high temperature drought tolerant genotype and moderately susceptible to MYMV), high yield under high temperature with drought condition. The entry VMGG 012-005 is derived from *Vigna radiata* (VRM (Gg)1) x *Vigna radiata* (*Pusa bold*), developed for the purpose of Moderately resistance / tolerance to Mungbean yellow mosaic virus. It was developed through intraspecific hybridization followed by pedigree selection and VGG ru1 ( high level MYMV resistant derivative of *Vigna radiata* x *Vigna umbellata*), which were raised at Agricultural Research Station, Virinjipuram, Tamil Nadu Agricultural University, Vellore, and VMGG012-005 developed at Virinjipuram is an agronomically superior high yielding with short duration variety but Moderately susceptible to MYMV, tolerant to drought and also high temperature tolerant with shortest duration. VGGru 1 is a field resistant genotype to MYMV. The field experiment was conducted in five consecutive seasons, namely Kharif 2013, Rabi 2013-14, Kharif 2014 Rabi 2014-15 and summer 2015 at Agricultural Research Station, Virinjipuram.

### Phenotyping of mapping population

In the field condition, the MYMV infection can be evaluated by infector row method as described [12]. The variety Co5 greengram is used as susceptible check at augmented design. The test materials were scored after 80% of plants showed MYMV incidence. The 225 individuals and progenies in the F<sub>2</sub> and 48 F<sub>3</sub> generation plants were scored for MYMV infection using 1-9 rating scale [17] is adopted.

### Agroinoculation

The study on agroinoculation was conducted in the Centre for Plant Molecular Biology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India. The tandem viral constructs of MYMV, VA 221 (KA30 DNA A + KA22 DNA B) and VA 239 (KA30 DNA A + KA27 DNA B) mobilized in *Agrobacterium tumefaciens* strains Ach 5 and C 58 were collected from Madurai Kamaraj university, Madurai and used for further studies. Agroinoculation was done on surface

sterilized overnight sprouted seeds of the parents (VMGG012-005 and VGGru 1) and F<sub>2</sub> individuals. *Agrobacterium tumefaciens* strains harbouring the appropriate partial tandem repeat clone were grown to 1 Optical Density at 600 nm in 2 mL AB minimal medium pH 7.0 containing the antibiotics like streptomycin (150 mg L<sup>-1</sup>), spectinomycin (50 mg L<sup>-1</sup>) and tetracycline (5 mg L<sup>-1</sup>) at 28°C at 220 rpm. From this, 1 mL of the culture was taken to inoculate, another 50 mL of AB minimal medium (pH - 7.0) containing the above mentioned antibiotics and grown to 1 OD L 600 nm at 28°C at 220 rpm. The culture was spinned at 4000 rpm for 10 min at 25°C. Cells obtained were re-suspended in 50 mL of AB minimal medium (pH - 5.6) with 100 in acetosyringone (100 µm). Seed coat of the sprouted seeds was removed by using forceps and pricked around the hypocotyl region and were immediately immersed in the appropriate culture of *A. tumefaciens*. After the overnight incubation, seeds were washed with distilled water and sown in pots containing autoclaved sand and vermiculite in the ratio of 1:1. Agroinoculated plants were maintained in a growth chamber at 25°C, 60-70% relative humidity and a photoperiod of 16/18h. The Hoagland's solution was applied twice in a week for proper growth of the plants and transferred to green house after 15 days for symptom observation [6] and used the same protocol for agroinoculation test [6]. For this study also followed the same inoculation procedure.

## Results and Discussion

Most of the ruling mungbean varieties are susceptible to MYMV accepted by the farmers. It is necessitates for developing MYMV resistant varieties because mungbean is important pulse food crop in Asia. Most of previous screening studies for MYMV resistance in Asia were conducted in field condition under high MYMV epidemic pressure. Evolution and identification of MYMV resistant lines through conventional breeding method relies on field screening. Even though it is time consuming one and requires evaluation at hot spot area [12]. Sometimes the screening based on natural occurrence in the hot spot areas also does not give consistent results due to the stain presence and environmental condition. For identifying the real resistant source and combination with plant breeding approaches will likely to be needed for the improvement of crops [18,19]. Interspecific hybridization in *Vigna radiata* x *Vigna trilobata* for MYMV donar development study revealed same result for many characters reported by [20]. Diversity analysis also made among the Wild *Vigna* species for many characters same results were reported [21].

Pathogen Derived Resistance (PDR) is a very effective genetic engineering approach to control plant viruses [22]. Advancements in the field of genetic engineering provide a new technique called agroinoculation. It is an effective method by which infectious viral clones can be introduced into plants using *A. tumefaciens* [14,23]. reported agroinoculation is all efficient method to employ the virus resistant plants. In earlier report successful agro-inoculation of mungbean with MYMV was recorded by [16,24] proved for MYMV studies in mungbean. Genetic transformation in combination with conventional breeding increases the efficiency of the breeding programme especially the incorporation of the disease resistance into the varieties and is the most predominant and a powerful tool to achieve the goal. The parents VMGG012-005 special features (Table 1) and VGGru 1 initially subjected to agroinoculation, revealed that VGGru1 did not develop any mosaic or leaf curling symptoms upon inoculation with VA 221 strain but at the same time they exhibited susceptibility against VA

239 strain. VMGG012-005 is the moderately susceptible mungbean, developed typical yellow mosaic and leaf curling symptoms in the trifoliolate leaves upon agroinoculation with both the *Agrobacterium* strains (VA 221 and VA 239). Similar to the present finding reported [24] and also observed the leaf curling and mild yellow mosaic symptoms from agroinoculation in *Vigna* sp. using two different MYMV isolates. The 225 F<sub>2</sub> individuals with MYMV infection, evaluated under field condition indicated that 48 individuals were identified as resistant and their seeds were harvested and subjected to agroinoculation which aims to identify the MYMV resistant mungbean progenies in the segregating population.

SL.No	Name of the variety	VMGG 012-005
1	Pedigree	Vigna radiata (VRM(Gg)1 ) x Vigna radiata Pusa bold
2	Origin (Name of the Institute)	Agricultural Research Station, Virinjipuram, Vamban - 632 104, TNAU, India
3	Plant growth habit	Erect upright
4	Plant habit	Determinate
5	Stem colour	Green
6	Stem pubescence	Light Present
7	Shape of leaf pinnae	Basal leaf trifoliolate, upper leaf trifoliolate
8	Colour of the leaf	Green
9	Leaf pubescence	Present
10	Petiole colour	Green
11	Pod colour: intensity of colour of premature pods	Uniform green
12	Pod pubescence	No
13	Pod colour at maturity	Brownish black
14	Seed colour	Green
15	Seed lusture	Non shiny
16	Seed shape	Globose
17	Days to 50% flowering	25 days
18	Days to maturity (days)	50 days
19	Plant height (cm)	20 cm
20	Seeds per pod	6-9
21	100 seed weight (g)	Medium (2.5-3.5 )
22	Disease reaction	Moderately Resistant against MYMV

**Table 1:** Morpho agronomic Characters of VMGG012-005.

The list of the MYMV field resistant genotypes subjected to agroinoculation screening is presented in Table 2.

The results of the agroinoculation showed that, among the 48 individuals only ten individuals showed to be resistant. The ten individuals namely Resplant3, Resplant6, Resplant7, Resplant9, Resplant13, Resplant19, Resplant25, Resplant37, Resplant40 and Resplant43 did not develop any mosaic or leaf curling symptoms upon inoculation with VA 221 strain. But at the same time they exhibited susceptibility reaction against the strain VA 239. The remaining thirty eight individuals developed typical yellow mosaic symptom for both the strains (VA 221 and VA 239). The ten resistant, individuals were found to have behaved the same way as that of their resistant, parent VGGru1. The same result revealed in mungbean [25-28].

The agroinoculated mungbean plants started developing yellow mosaic symptoms from the 17<sup>th</sup> day to 23<sup>th</sup> day and there were no symptoms in the control plants.

S.No.	Mungbean parents and MYMV resistant F <sub>2</sub> Individuals	MYMV score
1	VGGru1	1.0-2.0
2	VMGG012-005	1.0-2.0
3	RESPLANT 01	1.0-2.0
4	RESPLANT 02	1.0-2.0
5	<b>RESPLANT 03</b>	<b>1.0-2.0</b>
6	RESPLANT 04	1.0-2.0
7	RESPLANT 05	1.0-2.0
8	<b>RESPLANT 06</b>	<b>1.0-2.0</b>
9	<b>RESPLANT 07</b>	<b>1.0-2.0</b>
10	RESPLANT 08	1.0-2.0
11	<b>RESPLANT 09</b>	1.0-2.0
12	RESPLANT 10	1.0-2.0
13	RESPLANT 11	1.0-2.0
14	RESPLANT 12	1.0-2.0
15	<b>RESPLANT 13</b>	<b>1.0-2.0</b>
16	RESPLANT 14	1.0-2.0
17	RESPLANT 15	1.0-2.0
18	RESPLANT 16	1.0-2.0
19	RESPLANT 17	1.0-2.0
20	RESPLANT 18	1.0-2.0
21	<b>RESPLANT 19</b>	<b>1.0-2.0</b>
22	RESPLANT 20	1.0-2.0
23	RESPLANT 21	1.0-2.0
24	RESPLANT 22	1.0-2.0
25	RESPLANT 23	1.0-2.0
26	RESPLANT 24	1.0-2.0
27	<b>RESPLANT 25</b>	<b>1.0-2.0</b>
28	RESPLANT 26	1.0-2.0
29	RESPLANT 27	1.0-2.0
30	RESPLANT 28	1.0-2.0
31	RESPLANT 29	1.0-2.0
32	RESPLANT 30	1.0-2.0
33	RESPLANT 31	1.0-2.0
34	RESPLANT 32	1.0-2.0
35	RESPLANT 33	1.0-2.0
36	RESPLANT 34	1.0-2.0
37	RESPLANT 35	1.0-2.0
38	RESPLANT 36	1.0-2.0
39	<b>RESPLANT 37</b>	<b>1.0-2.0</b>
40	RESPLANT 38	1.0-2.0
41	RESPLANT 39	1.0-2.0
42	<b>RESPLANT 40</b>	<b>1.0-2.0</b>
43	RESPLANT 41	1.0-2.0
44	RESPLANT 42	1.0-2.0
45	<b>RESPLANT 43</b>	<b>1.0-2.0</b>
46	RESPLANT 44	1.0-2.0
47	RESPLANT 45	1.0-2.0
48	RESPLANT 46	1.0-2.0
49	RESPLANT 47	1.0-2.0
50	RESPLANT 48	1.0-2.0

**Table 2:** List of MYMV field resistant F<sub>2</sub> individuals.

At the 25<sup>th</sup> day, the yellow mosaic symptoms were clearly seen on the leaves. The average infectivity of MYMV strains through agroinoculation in mungbean ranged from 0 to 100%. Average per cent infection of resistant VGGru in the strain VA 221 is 0.00 and VA 239 strain is 42.33. The moderately susceptible parent VMGG012-005 recorded an average infection of 97.00 and 100.00% in VA 221 and VA 239, respectively. In blackgram, high yield with MYMV disease resistance were achieved through interspecific cross combination, this study as similar results reported in mungbean [25,28] and in blackgram [29].

No infection was recorded on Resplant3, Resplant6, Resplant7, Resplant9, Resplant13, Resplant19, Resplant25, Resplant37, Resplant40 and Resplant43 in VA-221 strain; however infection was recorded in these ten individuals ranging from 49.0 to 85. % in VA 239 strain. Among the individuals the highest average infection was recorded in Resplant28 and Resplant24 24 (92.5.00%) followed by Resplant 27 (92.00%), Resplant11 (89.58.00%) and Resplant (87.00) for VA 239 and Resplant 27 (93.66), Resplant 24 and 28 (91.00%) followed by Resplant 20 (87.5 %) and Resplant 29 (29.87.33 %) in VA 221strains. This results same as the cytological irregularities occur when MYMV resistant development process started by crossing of non resistant with resistance genotypes this result agree [30].

*V. umbellata* is single resistant parent is having both MYMV and Bruchids resistant which is used for development MYMV resistance in mungbean this result agree [31,32]. The six wild *Vigna* species were taken for development of MYMV resistance in mungbean the same result exhibited [33]. In intraspecific crosses were made among *Vigna radiata* x *Vigna radiata* for MYMV resistance agree with results [19,34]. Various wild *Vigna* species cross combinations showed various level of pollen fertility percentage agree [35]. The broadening of genetic base derived from the wild crosses followed by mutation same result agree with [31]. Thirteen wild *Vigna* species crossed with *Vigna radiata* for MYMV donar development and all the characters studied now is revealed same result for many characters reported [3]. Thirteen wild *Vigna* species crossed with *Vigna radiata* for MYMV donar development and genetic diversity for variation studied completed, this study revealed same result for many characters [27]. In blackgram, high yield with MYMV disease resistance were achieved through interspecific cross combination, this study results was similar to that [29] in blackgram. The individuals Resplant 9 (22.05 %) and Resplant6 (52.33) recorded the lowest average infection in the strains VA 239 and Resplant4 (60.88 %) and Resplant 26 (63.0 %) in VA 221, (Table 3). These findings are nearly in close conformity with the reports [14] in agro inoculating mungbean with 71 to 95% of MYMV [19, 21,25,28].

SL.No.	Mungbean parents and MYMV resistant F <sub>2</sub> Individuals	<i>Agrobacterium tumifaciens</i> strains	No. of days taken for symptom development	Infectivity (%) (Replication 1)	Infectivity (%) (Replication 2)	Average Infectivity (%)
1	VGGru1	VA 221	-	0.00	0.00	0.00
		VA 239	21	40.00	45.00	42.50
2	VMGG012-005	VA 221	21	100.00	96.00	95.00
		VA 239	21	100.00	100.00	100.00
3	RESPLANT 01	VA 221	22	75.00	72.50	68.75
		VA 239	17	60.00	75.00	77.50
4	RESPLANT 02	VA 221	21	88.00	85.00	71.50
		VA 239	20	78.00	75.60	86.80
5	RESPLANT 03	VA 221	-	0.00	00.00	00.00
		VA 239	21	80.00	85.00	90.00
6	RESPLANT 04	VA 221	21	55.00	60.00	67.50
		VA 239	21	52.00	65.00	58.50
7	RESPLANT 05	VA 221	21	77.00	60.00	63.50
		VA 239	18	70.00	78.00	79.00
8	RESPLANT 06	VA 221	-	0.00	0.00	0.00
		VA 239	21	45.00	53.00	59.00
9	RESPLANT 07	VA 221	-	0.00	0.00	0.00
		VA 239	20	68.00	90.00	89.00
10	RESPLANT 08	VA 221	17	85.00	75.00	75.00
		VA 239	21	70.00	68.50	69.25
11	RESPLANT 09	VA 221	-	0.00	0.00	0.00
		VA 239	21	0.49	0.60	65.00
12	RESPLANT 10	VA 221	23	75.00	70.00	72.50
		VA 239	21	75.60	78.00	81.80
13	RESPLANT 11	VA 221	17	74.00	70.00	82.00
		VA 239	19	92.50	100.00	76.25
14	RESPLANT 12	VA 221	18	85.00	85.00	75.00
		VA 239	23	68.00	58.00	69.00
15	RESPLANT 13	VA 221	-	0.00	0.00	0.00
		VA 239	20	75.00	72.50	78.75

16	RESPLANT 14	VA 221	23	70.00	75.00	87.50
		VA 239	17	68.00	85.00	71.50
17	RESPLANT 15	VA 221	23	88.00	75.60	86.80
		VA 239	23	84.00	75.00	79.50
18	RESPLANT 16	VA 221	23	80.00	80.00	80.00
		VA 239	17	95.00	90.00	67.50
19	RESPLANT 17	VA 221	23	0.00	0.00	0.00
		VA 239	23	87.00	60.00	73.50
20	RESPLANT 18	VA 221	23	80.00	78.00	79.00
		VA 239	23	75.00	74.00	74.50
21	RESPLANT 19	VA 221	-	0.00	0.00	0.00
		VA 239	23	70.00	73.50	66.75
22	RESPLANT 20	VA 221	23	85.00	93.50	84.25
		VA 239	23	85.00	84.00	84.50
23	RESPLANT 21	VA 221	23	78.00	69.00	73.50
		VA 239	17	90.00	88.00	84.00
24	RESPLANT 22	VA 221	18	56.00	60.00	63.00
		VA 239	18	75.00	85.00	75.00
25	RESPLANT 23	VA 221	0.0	0.00	0.00	0.00
		VA 239	19	94.00	90.00	92.00
26	RESPLANT 24	VA 221	23	90.00	92.00	91.00
		VA 239	23	95.00	90.00	92.50
27	RESPLANT 25	VA 221	-	0.00	0.00	0.00
		VA 239	23	90.00	78.00	84.00
28	RESPLANT 26	VA 221	23	56.00	60.00	73.00
		VA 239	23	85.00	85.00	75.00
29	RESPLANT 27	VA 221	23	98.00	96.00	87.00
		VA 239	23	94.00	90.00	92.00
30	RESPLANT 28	VA 221	20	90.00	92.00	91.00
		VA 239	19	95.00	90.00	92.50
31	RESPLANT 29	VA 221	21	90.00	88.00	84.00
		VA 239	19	86.00	90.00	73.00
32	RESPLANT 30	VA 221	20	89.00	87.00	83.00
		VA 239	21	86.00	92.00	79.00
33	RESPLANT 31	VA 221	20	62.50	65.00	58.75
		VA 239	20	75.00	72.50	68.75
34	RESPLANT 32	VA 221	23	70.00	75.00	77.50
		VA 239	19	68.00	85.00	71.50
35	RESPLANT 33	VA 221	23	78.00	75.60	86.80
		VA 239	21	84.00	85.00	79.50
36	RESPLANT 34	VA 221	23	90.00	90.00	90.00
		VA 239	17	75.00	90.00	67.50
37	RESPLANT 35	VA 221	22	62.00	65.00	58.50
		VA 239	23	77.00	60.00	73.50
38	RESPLANT 36	VA 221	22	80.00	78.00	79.00
		VA 239	23	75.00	74.00	74.50
39	RESPLANT 37	VA 221	-	0.00	0.00	0.00
		VA 239	23	70.00	63.50	66.75
40	RESPLANT 38	VA 221	23	65.00	83.50	84.25
		VA 239	13	85.00	84.00	84.50
41	RESPLANT 39	VA 221	19	68.00	69.00	73.50
		VA 239	19	70.00	78.00	84.00
42	RESPLANT 40	VA 221	-	0.00	0.00	0.00
		VA 239	20	75.00	85.00	75.00
43	RESPLANT 41	VA 221	0.00	0.00	0.00	0.00



		VA 239	20	84.00	80.00	82.00
44	RESPLANT 42	VA 221	23	90.00	92.00	91.00
		VA 239	23	95.00	90.00	92.50
45	RESPLANT 43	VA 221	-	0.00	0.00	0.00
		VA 239	23	56.00	49.50	52.75
46	RESPLANT 44	VA 221	23	85.00	85.00	80.00
		VA 239	22	80.00	78.50	79.25
47	RESPLANT 45	VA 221	20	65.00	70.00	67.50
		VA 239	20	80.00	85.00	77.50
48	RESPLANT 46	VA 221	20	88.00	85.00	81.50
		VA 239	19	80.00	94.00	82.00
49	RESPLANT 47	VA 221	23	85.00	90.00	87.50
		VA 239	18	94.00	92.00	93.00
50	RESPLANT 48	VA 221	22	79.00	77.00	73.00

**Table 3:** Average infectivity of Mungbean parents and 48 MYMV resistant F<sub>2</sub> individuals Agroinoculation for MYMV.

To verify the non presence of viral DNA inside the host genome phenotypic showed in the field condition at consecutive sown. The same 10 genotypes which was resistant for VA 221 raised in the field condition showed resistance again. These results are in accordance with the reports [14,19,35].The indicating the presence of viral DNA in agroinoculated symptomatic plants and their absence in asymptomatic plants with coat protein specific primers for the DNA A and B components [19,25,36,37]. The identified resistant individuals namely, Resplant3, Resplant6, Resplant7, Resplant9, Resplant13, Resplant19, Resplant25, Resplant37, Resplant40 and Resplant43 were similar to their resistant parent, exhibiting the presence of viral coat protein gene for both the strains. This clearly depicts their pure inheritance pattern of the resistance for MYMV. For further confirmation, the individuals which showed resistance in field (F<sub>2</sub> generation) and agro inoculation, were alone forwarded to next generation (F<sub>3</sub>) and as per this, the ten individuals were forwarded to next generation along with five susceptibles. In F<sub>3</sub> generation MYMV infection can be evaluated by MYMV disease rating scale. Interestingly, the ten individuals which showed resistance in field (F<sub>2</sub> generation) and agroinoculation, are found to be resistant in F<sub>3</sub> generation and F<sub>4</sub> generations under field conditions also.

## Conclusion

The results of this study show that the ten progenies namely Resplant5, Resplant22, Resplant28, Resplant35, Resplant88, Resplant92, Resplant123, Resplant156, Resplant157 and Resplant5168 are confirmed for resistance in both agroinoculation and field screening. From these five progenies, a MYMV resistant mungbean genotype can probably be developed by adopting future breeding programmes.

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