Genotypic Analysis for Retrieval of MYMV Resistant Progenies from Certain Crosses of Mungbean [Vigna radiata (L.) Wilczek]

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ABSTRACT
The present investigation was conducted to study the nature of inheritance of MYMV among intra-specific crosses among three MYMV susceptible and five MYMV resistant genotypes of green gram. The molecular screening and hotspot evaluation is carried out in fifteen F₁ progenies and 8 F₂ families, respectively. The yellow mosaic-affected leaf samples gave positive results with MYMV specific primer pairs YFP1/YRP1 and YFP2/YRP2 amplified fragments having size of 704 bp and 648 bp, respectively. Coat protein gene sequence analysis revealed that the identified virus is homologous to MYMV. The inheritance study based on F₂ data indicated the ratio of MYMV resistant plants and susceptible plants showed a good fit to expected 3:1 (Susceptible: Resistant) suggesting a typical monogenic recessive gene governing resistant reaction to MYMV besides resting the susceptibility from the susceptible parents.

Key words: Chi square test, First and Second filial selected progenies, Greengram, Intra specific crosses, MYMV Disease.

INTRODUCTION
Green gram is grown primarily in India, Pakistan, Bangladesh, Sri Lanka, Philippines, Taiwan and Nepal etc sustaining the protein requirement of human population. In India, it is the third important pulse crop after chickpea and pigeonpea with a very low yield potential of 384 kg/ha (Singh, 2011, Khajudparn et al., 2019 and Raturi et al., 2015) and limited success has been achieved so far in augmenting the yield. The production of mungbean is adversely affected by many biotic and abiotic stresses. Among the biotic stresses, fungi, fowler diseases, powdery mildew and Yellow mosaic viral disease are found to appear in an epiphytotic form, thereby causing immense loss to farmers (Nene, 1988, Bhattacharya and Vijaya lakshmi, 2005). Yellow mosaic disease, a serious disease was first reported in lima bean and later in Dolichos accounting enormous losses in the mungbean production in India (Narasimhulu et al., 2016, Maheswari et al, 2014 and Karthikeyan et al, 2014). The annual production of pulses is greatly challenged by Yellow Mosaic Virus disease resulting in an economic loss of 85% which is spreading faster towards newer areas (Karthikeyan et al, 2014), The yield losses in mungbean vary from 5 to 100 per cent depending upon disease severity, susceptibility of cultivars and population of whitefly (Nene, 1972; Singh, 1980 and Rathli, 2002). The genome of such viruses transmitted whiteflies (Bemisia tabaci) possess bipartite, single stranded, circular DNA genome referred as DNA A and DNA B (Lazarowitz and Shepherd, 1992). Detection of a virus in a plant does not necessarily adjudge for expression of the disease. Disease diagnosis based on symptom is unreliable for the reason that different viruses may cause similar symptoms and that different symptoms may be induced by one virus (Singh and Mishra, 2016 and Ozi et al., 2007). Many abiotic stresses and other pathogens such as phytoplasma like organism may cause symptoms characteristic of virus infection. Even after one become familiar with the symptoms typically caused by a virus in a particular plant, it is essential to confirm the diagnosis with reliable methods. Enzyme-linked immune sorbent assays (ELISA) and polymerase chain reactions (PCR) are the most widely used virus detection methods because of their rapidness and sensitivity (Kumar et al., 2007). PCR is a scientific technique in molecular biology to amplify a piece of DNA to generate a millions of copies (Joshi et al., 2010). In the field of virology, this technique is routinely used to detect the presence of virus and to amplify their genome for further characterization. Breeding for cultivars with resistance is a commonly accepted and effective strategy for controlling the MYMV disease and also prevent the multiplication of virus. The knowledge of inheritance of resistance genes and role of each gene in the development of resistance or susceptibility will be very useful for the mung bean breeders to breed MYMV resistant varieties (Sudha et al, 2013). MYMV infection can be evaluated by MYMV disease rating scale suggested by Singh et al., (1988). The main objective of this present study is to decipher gene
action involved in the inheritance of MYMV resistance in intra-specific crosses of green gram.

**MATERIALS AND METHODS**

Materials for the present investigation comprised of five MYMV resistant mungbean lines viz., MH 565, CO GG 930, IPM 99 125, IPM 0214, Pusa Vishal and three susceptible lines viz., PANT M 103, AGG 10092, EC 396120, F$_1$ and F$_2$ generation plants (derived from crossing between resistant and susceptible parents). Eight parents and fifteen F$_1$ progenies were raised at Agricultural College and Research Institute, Kilikulam, Genetics and Plant Breeding Research Farm besides eight F$_2$ families were raised at Panpoozhi village, Tirunelveli district during the year of 2016-17. The parents along with their F$_1$ progenies and F$_2$ progenies were planted in the single row of 2 m length with a spacing of 40 × 20 cm and 60 × 20 cm, respectively for evaluation. The plant populations of F$_2$ progenies were composed of 50 to 70 individual plants. No pesticide and fungicide spray was given in order to maintain the white fly population in the experimental field.

**Screening for MYMV disease**

Molecular screening method is followed to screen the MYMV incidence in F$_1$ population using two coat protein gene specific primers YFP 1/ YRP 1, YFP 2/ YRP 2 and the F$_2$ families were subjected to hotspot screening in Panpoozhi village near Tenkasi taluk of Tirunelveli district for MYMV incidence. On individual plant basis, the percent infection by MYMV in parents, F$_1$ and F$_2$ generations were recorded on 50 days after planting. The mean disease score for parents, F$_1$ and F$_2$ generations were calculated as (Infection rate x frequency/ total number of plants). The disease incidences were categorized on the basis of the rating scale given in order to maintain the white fly population in the experimental field.

**RESULTS AND DISCUSSION**

Establishment of efficient breeding strategies construe requisite knowledge on inheritance of resistance genes and gene action for the breeders. The objective of this study was to determine the inheritance of MYMV resistance among intra specific crosses of mungbean resistant and susceptible lines. The presence of MYMV among fifteen hybrids was diagnosed by PCR. The PCR was carried out to amplify the CP gene of YMV using two gene specific primers namely YMV forward primer 1 (YFP1), YMV reverse primer 1 (YRP1) and YMV forward primer 2 (YFP2), YMV reverse primer 2 (YRP2). Fifteen hybrids were subjected to detect the viral coat protein gene. The expected amplification was obtained for all the hybrids with both the primers. The MYMV CP gene specific primer pairs, YFP1/YRP1 and YFP2/YRP2 resulted in amplified bands of expected size.
in the amplification of the YMV CP gene of size 704 and 648 bp, respectively. CP gene sequence analysis revealed that the identified virus is homologous to MYMV (Plate 1). The present findings agreed to those of Maheswari et al. (2014) and Balakrishna et al. (2019) for the same primers, whereas Gautam et al. (2014) for the primers of NMI/NM2 and MYMIV-M)PF/MYMIVMPR besides Manjunatha et al. (2015) also for universal primer with amplification of 520 bp and Marabi et al., (2017) for two molecular marker DNA-A (cp) and DNA-B at the base pair of 750 and 541, respectively in soybean. The degree of resistance was assessed based on the mean YMV score value in F$_2$ families. The plants which show moderately susceptible (MS), susceptible (S) and highly susceptible (HS) were included in susceptible group while resistant (R) and moderately resistant (MR) plants were included under resistant group. The susceptible parents and F$_1$ plants of all the fifteen crosses showed susceptible reaction (S), that is, symptoms observed on both leaves and pods. The mean disease score, disease reaction and distribution of plants of eight F$_2$ families of mungbean genotypes are presented in Table 3. The mean disease score of MYMV ranged from 4.94 in AGG 10092 x IPM 0214 to 5.4 in EC 396120 x IPM 0214. The non-significant value from chi square test showed that the segregation ratio of 3 (MS-HS): 1 (MR-R) was observed in progenies of all F$_2$ populations of eight different combinations. The observed ratio was highly fitted to the expected ratio by the probability of maximum value viz., 0.95 - 0.80 for EC 396120 x IPM 0214 and Pant M 103 x CO GG 930 while the probability of minimum value, 0.10 - 0.05 was observed in EC 396120 x PUSA VISHAL (Table 4). Breeding for cultivars with resistance is a commonly accepted and effective strategy for controlling the MYMV disease and also prevent the multiplication of virus. MYMV infection can be evaluated by MYMV disease rating scale suggested by Subhash et al., (2019). Crossing is made with susceptible and resistance

**Table 3:** Distribution of Mungbean F$_2$ plants of different combination in term of MYMV disease rating.

| Cross combination | Total plants | Number of F$_2$ plants according to disease score | Mean disease score |
|-------------------|--------------|-----------------------------------------------|-------------------|
| PANT M 103 x MH 565 | 52 | | |
| PANT M 103 x CO GG 930 | 55 | | |
| AGG 10092 x IPM 99 125 | 58 | | |
| AGG 10092 x CO GG 930 | 50 | | |
| AGG 10092 x IPM 0214 | 54 | | |
| EC 396120 x IPM 99 125 | 67 | | |
| EC 396120 x Pusa Vishal | 48 | | |
| EC 396120 x IPM 0214 | 55 | | |

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| EC 396120 x Pusa Vishal | 48 | | |
| EC 396120 x IPM 0214 | 55 | | |

**Table 4:** Chi-square test for segregation of susceptibility and resistance in F$_2$.

| F$_2$ Generation | Total plants | Yellow mosaic virus | Expected ratio S:R | $\chi^2$ | P value |
|------------------|--------------|---------------------|-------------------|--------|--------|
| PANT M 103 x MH 565 | 52 | 36 16  39 13 | 3 : 1 | 0.64 | 0.50 - 0.30 |
| PANT M 103 x CO GG 930 | 55 | 41 14  41.25 13.75 | 3 : 1 | 0.01 | 0.95 - 0.80 |
| AGG 10092 x IPM 99 125 | 58 | 46 12  43.5 14.5 | 3 : 1 | 0.37 | 0.60 - 0.50 |
| AGG 10092 x CO GG 930 | 50 | 34 16  37.5 12.5 | 3 : 1 | 0.96 | 0.40 - 0.30 |
| AGG 10092 x IPM 0214 | 54 | 36 18  40.5 13.5 | 3 : 1 | 1.58 | 0.30 - 0.20 |
| EC 396120 x IPM 99 125 | 67 | 52 15  50.25 16.75 | 3 : 1 | 0.12 | 0.80 - 0.70 |
| EC 396120 x Pusa Vishal | 48 | 31 17  36 12 | 3 : 1 | 2.25 | 0.10 - 0.05 |
| EC 396120 x IPM 0214 | 55 | 40 15  41.25 13.75 | 3 : 1 | 3.01 | 0.95 - 0.80 |
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Plate 2: Segregation of YMV disease in Second Filial Generation.

parents to study the inheritance of MYMV resistance. In first filial generation, the plants of all the crosses showed susceptible symptom indicating that susceptibility was dominant over resistance (Narasimhulu et al., 2016, Webster et al., 2000). Similar results on the dominant behavior of MYMV susceptibility in the F₁ were also reported in the mungbean by Khan et al. (2007) and Rani et al. (2017). To deduce the inheritance pattern of MYMV resistance, eight first filial crosses were forwarded to second filial generation for the study of segregating pattern. Chi square test was also carried out to confirm the expected deviation from the Mendelian segregation ratio of segregating generation. The inheritance study based on F₂ data indicated the ratio of resistant plants and susceptible plants showed a good fit to expected 3:1 (Susceptible : Resistant) suggesting a typical monogenic recessive gene governing resistant reaction against MYMV resistance and the susceptibility was from the susceptible parents (Plate 2). Such monogenic recessive inheritance of MYMV resistance in mungbean has also been reported by previous researchers like Khattak et al. (2000), Khan et al. (2007), Sudha et al. (2013) and Vinod et al. (2013).

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