

Research Article

Towards Development of Mungbean [*Vigna radiata* (L.) Wilczek] Genotypes with Combined Resistance to Bruchid (*Callosobruchus maculatus*) and Mungbean Yellow Mosaic Virus (MYMV)

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Abstract

Mungbean is an important pulse crop that is grown throughout Asia. Among the biotic constraints, Bruchids and Mungbean Yellow Mosaic Virus are the most destructive in mungbean. Bruchid causes serious damage during storage whereas MYMV disease is a serious threat under field condition. The yield loss is observed up to 100% in both cases with a severe infestation. The seeds derived from eight F₃ families of mungbean were screened against *C. maculatus* in a CRD design under ideal laboratory conditions during *rabt*-2017. Breeding lines from the same crosses were laid out in an RBD design and screened against MYMV resistance in F₄ generation under field conditions in *summer*-2018. The cross derivatives of V-02-802xDGGV-7 and V-02-802xDGGV-2 were resistant to *C. maculatus* with susceptibility index 0.039 and 0.043 respectively. The breeding lines from V-02-802xDGGV-2 were found to be resistant to MYMV, with a percent disease incidence of 7.60. The promising progeny lines selected from the cross V-02-802xDGGV-2 shown resistance to both bruchid and MYMV with yield per plant is 10.0 g (plant code no. 5S-2) and 10.7g (plant code no. 5S-10). These resistant breeding lines were selected separately and it needs special consideration to develop genotypes with combined resistance to *C. maculatus* and MYMV disease.

Keywords: Mungbean; Bruchid; Mungbean yellow mosaic virus; Combined resistance

Introduction

A variety of pulses are cultivated in India as well as in the world and among these pulse crops, mungbean occupies the third position in terms of acreage, production and productivity after chickpea and pigeon pea. India is the largest consumer and producer of mungbean, which alone accounts for 65% of the world acreage and 54% of global production [1]. Mungbean [*Vigna radiata* (L.) Wilczek; Family: Fabaceae] is a diploid species (2n=2x=22) and predominantly self-pollinated. This is an excellent source of high-quality protein with easy digestibility, consumed as whole grains, dal and sprouted in a variety of ways (Tiwari, 2016). The nutritional value of mungbean seeds contains approximately protein (25-28%), carbohydrates (62-65%) and fiber (3.5-4.5%) on a dry weight basis. The concentration of sulfur-containing amino acids like cysteine and methionine are in low quantity but lysine contents are comparatively high, which makes the protein of mungbean is an excellent complement to rice in terms of balanced human nutrition [2]. There are several major constraints in mungbean production like narrow genetic variability, lack of different plant types for different seasons, low harvest index and susceptibility to a number of biotic and abiotic stresses, which limits achieving the goal of higher productivity. Among various biotic stresses; Bruchid (*Callosobruchus spp.*) and Mungbean Yellow Mosaic Virus (MYMV) disease which has been renowned in India for more

than five decades are the most brutal for mungbean production [3].

Bruchid or pulse beetle (*Callosobruchus spp.* F; Coleoptera: Bruchidae) causes serious damage and yield loss in pulse crops including mungbean in field condition as well as during storage [4]. Generally the infestation rate in field condition is low but during storage, the infestation rate is very rapid [5]. There are four developmental stages observed in bruchid *viz.*, egg, larva, pupa and adult. The adult insects lay the egg on the surface of the seeds and from egg to larva developed. The larval stage is the most destructive stage of bruchid for the pulses. From the pupa, adult insects emerged and again lay eggs on the seed surface causing secondary infestation with the rapid multiplication of the pest population during storage and resulting up to 100% grain loss [6]. There are several species of Bruchid that infect the pulse crops, but in the present investigation, we focused on the species *Callosobruchus maculatus*. The wild species of mungbean (*Vigna radiata var. sublobata*) accession TC1966 is completely reported to *C. maculatus* and *C. chinensis* [7], but due to the crossability barrier, a satisfactory level of result is not observed. The cultivated varieties of mungbean V02802 and V02709 were reported highly resistant to both species of bruchid [8,9]. Therefore, in the present study, these two cultivated resistant sources were used to improve the elite breeding lines of mungbean. Other than bruchid, Mungbean Yellow Mosaic Virus (MYMV) is one of the

most devastating and destructive diseases that severely affect the production and productivity of mungbean throughout Asia, including India [10,11]. The yield loss in pulse due to viral diseases accounts for up to 80 percent, while the yield loss due to MYMV in mungbean alone accounts 80-100 percent [12]. The virus belongs to the family geminiviridae and genus Begomovirus with bipartite genome (having two different components, DNA-A and DNA-B) as single-stranded circular DNA of genome size about 2.7kb [13,14,15,16]. The MYMV disease is mainly transmitted by the polyphagous pest whitefly (*Bemisia tabaci*; Hemiptera: Aleyrodidae) in a persistent manner, but not much by the sap, seed or soil. The insect vector and virus survive on different alternate and collateral hosts, including the main crop which serves as the primary source of inoculum to spread the disease all-round the year.

There are several approaches followed to manage bruchid (chemical treatment like methyl bromide, Carbon disulfide, botanicals, etc.) [17] and MYMV (vector control by spraying the insecticides and applications of different plant extracts) [15]. But, the application of chemical pesticides is highly toxic, environmentally undesirable, and poses a threat to food safety whereas on the other side botanicals, plant extracts are slow-release in action and cause a problem in the germination of seed and growth of the plant [18]. Thus, managing these two problems up to 100% is quite difficult. The wide host range, genome size variation of the virus, quantitative nature of inheritance of the disease make the task more challenging to develop mungbean varieties resistant to MYMV [15]. The identification of resistance source is the most reliable, reasonable and environmentally friendly method for the management of bruchid and MYMV in mungbean through innate resistance. Even though some genotypes and germplasms have been identified as a resistance source against bruchid and MYMV separately, but lack of durable resistance has been noticed in most of the cases. Hence, it needs continuous screening in all the seasons to identify the resistance/tolerance lines against bruchid and MYMV. Therefore, considering these constraints into account, the present investigation was aimed at serious attention to screen the advanced breeding lines of mungbean against bruchid (*C. maculatus*) during storage and against the menace of MYMV disease under natural field condition. The yield levels of all the breeding lines were also analyzed to develop superior genotypes with combined resistance/tolerance to bruchid and MYMV disease.

Materials and Methods

Experimental materials

The genetic materials for the present investigation included the F₃ and F₄ families of mungbean breeding lines. There are eight crosses (four direct and four reciprocal) that have been made among four parents *viz.*, DGGV-2, DGGV-7, V-02-802 and V-02-709. The desirable features of experimental materials along with their checks, centers responsible for releasing are mentioned in detail in Table 1. The genotypes DGGV-2 and DGGV-7 are agronomically superior and high yielding but when grow in Northern parts of Karnataka they are susceptible to MYMV under the natural condition and to bruchid during storage. Genotypes V-02-802 and V-02-709 are contributed by AVRDC [19], Taiwan and are resistant to bruchid [*Callosobruchus maculatus* (F.) and *C. chinensis*] but moderately susceptible to MYMV. So, an attempt was made in the present study to identify superior breeding lines with combined resistance to bruchid and MYMV resistance from the direct and reciprocal crosses among these four parents. The experiment was conducted at Main Agricultural Research Station (MARS), AICRP on MULLaRP, University of Agricultural Sciences, Dharwad, Karnataka, India which is geographically located 15° 12' N latitude and 75° 07' E longitudes with an altitude of 678 m above mean sea level.

Methodology for Bruchid Screening

The seeds from eight F₃ families from the above-mentioned crosses (Table 1) were used for screening against *C. maculatus*. The experiment was laid in a Complete Randomized Design (CRD) under ideal laboratory conditions [25±50C and 65±5% RH and photoperiod (16L:8D)] in rabi-2017. The procedure for maintenance of stock culture, bioassay test by free choice test and force choice test (Figure 1a) were practiced according to Ponnusamy et al., [20]; and Soumia et al., [21]. The adult *C. maculatus* species were collected from Pulse storehouse, AICRP on MULLaRP, Dharwad, and reared with very susceptible genotypes of mungbean for mass multiplication. There is an equal number of seeds from each family were taken for free choice tests and further the resistance reaction was screened under the forced-choice test. All the biological parameters like the number of eggs laid on each seed surface, number of adult emergence, weight loss, percent infestation and susceptibility index were taken into consideration from egg-laying to adult emergence up to one complete life cycle. Susceptibility index or growth index which was calculated by using the formula given by Howe (1971), Growth Index=(Log S)/T;

Table 1: Experimental materials and their checks used for screening to the reaction of MYMV and Bruchid(*C. maculatus*).

Sl. No.	Treatments	Sl. No.	Checks	Releasing Centers of the Variety (Check)	Reaction of Checks to MYMV	Reaction of Parents to Bruchid (<i>C. maculatus</i>)
1.	DGGV-2×V-02-709	1.	DGGV-2 (Parent)	University of Agricultural Sciences, Dharwad	Susceptible	Susceptible
2.	DGGV-2×V-02-802	2.	DGGV-7 (Parent)	University of Agricultural Sciences, Dharwad	Susceptible	Susceptible
3.	DGGV-7×V-02-709	3.	V-02-802 (Parent)	Asian Vegetable Research Development Centre, Taiwan	Moderately susceptible	Resistant
4.	DGGV-7×V-02-802	4.	V-02-709 (Parent)	Asian Vegetable Research Development Centre, Taiwan	Moderately susceptible	Resistant
5.	V-02-802×DGGV-2	5.	IPM-409-4	Indian Institute of Pulses Research (IIPR), Kanpur	Moderately resistant	-
6.	V-02-802×DGGV-7	6.	IPM-2-14	Indian Institute of Pulses Research (IIPR), Kanpur	Moderately resistant	-
7.	V-02-709×DGGV-2	7.	LGG-460	Andhra Pradesh Agricultural University (APAU), Lam	Resistant	-
8.	V-02-709×DGGV-7	8.	SML-1815	Punjab Agricultural University (PAU), Ludhiana	Resistant	-

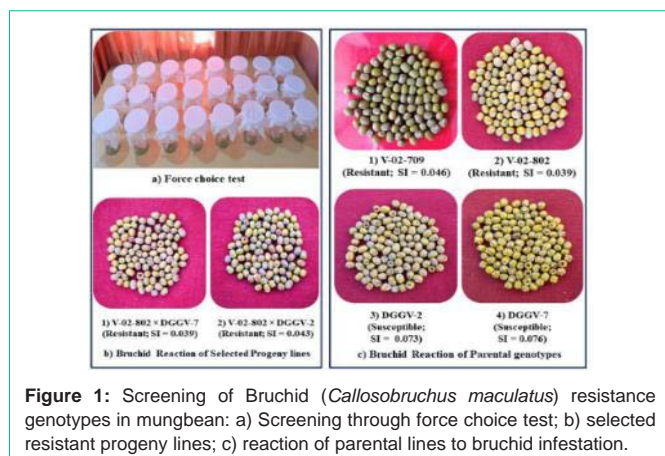


Figure 1: Screening of Bruchid (*Callosobruchus maculatus*) resistance genotypes in mungbean: a) Screening through force choice test; b) selected resistant progeny lines; c) reaction of parental lines to bruchid infestation.

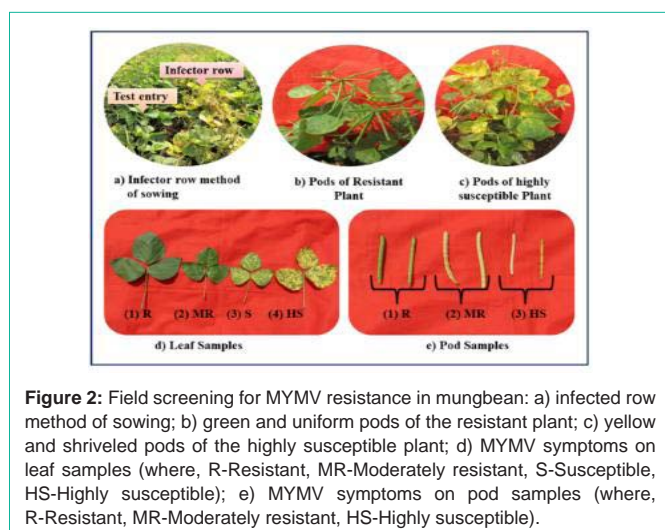


Figure 2: Field screening for MYMV resistance in mungbean: a) infected row method of sowing; b) green and uniform pods of the resistant plant; c) yellow and shriveled pods of the highly susceptible plant; d) MYMV symptoms on leaf samples (where, R-Resistant, MR-Moderately resistant, S-Susceptible, HS-Highly susceptible); e) MYMV symptoms on pod samples (where, R-Resistant, MR-Moderately resistant, HS-Highly susceptible).

Where, S is the percent adult emergence, T is the mean developmental period (days). The lines were classified into different groups based on their Susceptibility Index (SI) value as resistant (<0.05), moderately resistant (0.051-0.060), moderately susceptible (0.061-0.070), susceptible (0.071-0.080) and highly susceptible (>0.081) [22]. The detailed procedure of screening is mentioned and published by Majhi and Mogali, [23]. Therefore, it is suggested that to follow the previously published research article “Characterization and Selection of Bruchid [*Callosobruchus maculatus* (F.)] tolerant Greengram [*Vigna radiata* (L.) Wilczek] Genotypes” by Majhi and Mogali [23]. The statistical analysis for CRD design was accomplished by using WASP software version 1.0. The mean value of each character was compared with the CD values @ $p=0.05$.

Methodology for MYMV disease screening

The same eight F_3 families which were screened for bruchid resistance were again grown in the field to give F_4 population. These eight F_4 families of mungbean were used for screening against MYMV disease resistance under natural field conditions in summer-2018. The experiment was laid out in an RBD design. Each row length was 4m with the row to row spacing 30cm and plant to plant spacing 10cm. Each F_4 family was sown along with their resistant and susceptible checks as well as their parents (Figure 2a). The genotypes DGGV-2 and DGGV-7 were used as infector lines after every five test entries

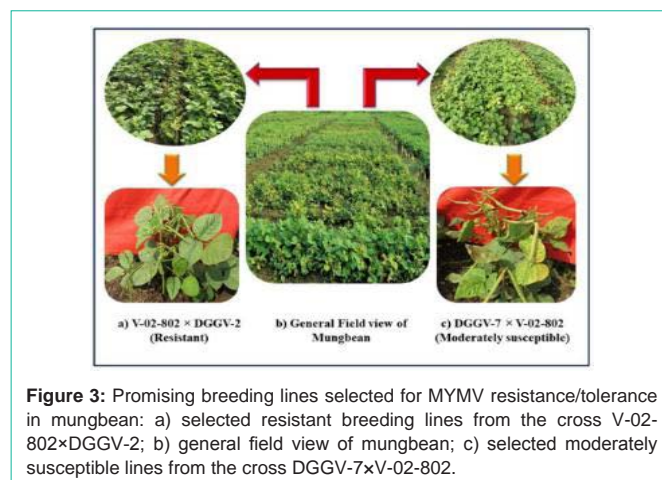


Figure 3: Promising breeding lines selected for MYMV resistance/tolerance in mungbean: a) selected resistant breeding lines from the cross V-02-802×DGGV-2; b) general field view of mungbean; c) selected moderately susceptible lines from the cross DGGV-7×V-02-802.

to study the level of infestation of MYMV under field condition as suggested by Mogali et al. [24]. The observations were recorded at regular intervals. All the agronomical practices followed to raise a good crop. The spraying of chemical insecticide is prevented to control the whitefly population as we need host plant resistance. All the symptoms were noted down with critical observation from a single plant basis. The Percent Disease Incidence (PDI) was calculated by using the formula given by Basir et al., [25] and the breeding lines were categorized into different reaction groups based on their respective PDI values (Table 2).

$$\text{Percent disease incidence (PDI)} = \frac{\text{Total number of plants infected in a row}}{\text{Total number of plants in a row}} \times 100$$

Results and Discussion

Screening for Bruchid resistance

The Susceptibility Index (SI) or growth index was calculated for the eight test entries and was categorized as resistant, moderately resistant, moderately susceptible, susceptible, and highly susceptible based on the estimation of susceptibility index given by Howe [22]. The highest susceptibility index values were recorded for the highly susceptible ones whereas resistant genotypes have shown the lowest susceptibility index. The parents were evaluated as a check to test their resistance/susceptibility reaction to bruchid infestation. The SI value of two susceptible parents was observed and DGGV-2 was more susceptible than DGGV-7 with SI value 0.073 and 0.076 respectively; in contrast among the resistant parents V-02-802 (SI=0.039) and V-02-709 (SI=0.046) respectively (Figure 1c). The cross derivatives of V-02-802×DGGV-7 and V-02-802×DGGV-2 were resistant with susceptibility index 0.039 and 0.043 respectively (Figure 1b). This result may be due to the physical appearance of the seed (shining appearance, color and size) which prevents egg laying or even though eggs laid on the seed coat, which is prevented from hatching by the biochemical substances present in the seed. Moderately resistant reaction was observed in four sets of cross derivatives to bruchid with susceptibility index value *viz.*, V-02-709×DGGV-2 (SI=0.050), DGGV-2×V-02-709 (SI=0.055), DGGV-2×V-02-802 (SI=0.056) and DGGV-7×V-02-709 (SI=0.058); and two moderately susceptible lines *viz.*, DGGV-7×V-02-802 and V-02-709×DGGV-7 with susceptibility index value 0.061 (Table 3). The weight loss was observed highest for the cross V-02-709×DGGV-7 (44.25%) and lowest for the cross V-02-802×DGGV-2 (13.49%); among the segregating lines. The percent

Table 2: Disease scoring scale (0-5) for Mungbean Yellow Mosaic Virus (MYMV) based on the Percentage of Disease Incidence (PDI) under field condition [25].

Disease Scale	Percent infection	Visual symptoms	Category	Reaction group
0	All plants free of virus symptoms	Complete absence of symptoms	Highly resistant	HR
1	1-10 % infection	Small yellowish spots scattered on some leaves	Resistant	R
2	11-20 % infection	Yellowish bright spots common on leaves, easy to observe	Moderately resistant	MR
3	21-30 % infection	Yellowish bright specks on leaves, easy to observe with larger patches of symptoms	Moderately Susceptible	MS
4	30-50 % infection	Bright yellow specks or spots on all leaves, minor stunting of plants and less number of pods	Susceptible	S
5	50% and more infection	Yellowing or chlorosis of all leaves on whole plants, Shortening of internode, severe stunting of plants with no yield or few flowers and deformed pods produced with small, immature and shriveled seeds	Highly Susceptible	HS

Table 3: Performance of mungbean breeding lines (F_3 generation) to *Callosobruchus maculatus* through Force choice test (*rabi-2017*).

Sl. No.	Treatments	No. of eggs/ 100 seeds	Adult emergence (S) (%)	Mean developmental period (days) (T)	Weight loss (%)	Infestation (%)	Susceptibility Index (S.I)	Categorization
1.	DGGV-2×V-02-802	88.66±2.61 ^d	54.89±2.15 ^{cd}	30.97±0.12 ^a	18.95±0.86 ^{cd}	48.00±1.32 ^c	0.056	Moderately Resistant (MR)
2.	DGGV-2×V-02-709	56.00±0.76 ^{ef}	65.81±3.15 ^b	32.93±0.12 ^d	28.07±1.16 ^b	36.66±1.30 ^{de}	0.055	Moderately Resistant (MR)
3.	DGGV-7×V-02-802	135.33±1.3 ^b	67.05±1.11 ^b	29.51±0.09 ^f	26.82±1.40 ^b	90.66±1.16 ^a	0.061	Moderately Susceptible (MS)
4.	DGGV-7×V-02-709	43.00±1.32 ^g	88.38±0.52 ^a	33.55±0.18 ^d	26.12±1.19 ^b	37.66±1.30 ^d	0.058	Moderately Resistant (MR)
5.	V-02-802×DGGV-2	48.00±1.04 ^{fg}	62.79±2.06 ^b	33.53±0.07 ^{cd}	13.49±1.72 ^{de}	30.33±2.33 ^{ef}	0.043	Resistant (R)
6.	V-02-802×DGGV-7	121.66±2.20 ^c	22.79±0.88 ^e	34.76±0.11 ^b	25.18±1.33 ^{bc}	27.66±0.92 ^f	0.039	Resistant (R)
7.	V-02-709×DGGV-2	66.00±0.76 ^e	55.35±1.59 ^{cd}	34.02±0.14 ^{bc}	24.44±1.92 ^{bc}	34.66±1.20 ^{def}	0.051	Moderately Resistant (MR)
8.	V-02-709×DGGV-7	133.33±3.3 ^{bc}	61.31±0.68 ^{bc}	29.02±0.17 ^f	44.25±1.03 ^a	81.66±1.87 ^b	0.061	Moderately Susceptible (MS)
9.	V-02-802 (Check-1)	10.66±0.83 ^h	37.50±1.60 ^d	39.48±0.12 ^a	6.25±0.60 ^f	4.00±0.28 ^g	0.039	Resistant (R)
10.	V-02-709 (Check-2)	8.00±0.57 ^h	66.66±2.42 ^{bcd}	39.05±0.11 ^a	8.84±0.68 ^{ef}	5.33±0.33 ^g	0.046	Resistant (R)
11.	DGGV-2 (Check-3)	181.66± 2.20 ^a	49.57±0.46 ^d	23.15±0.11 ^g	47.70±0.76 ^a	90.00±0.57 ^a	0.073	Susceptible (S)
12.	DGGV-7 (Check-4)	180.33±3.9 ^a	51.24±1.29 ^d	22.34±0.07 ^h	50.87±0.87 ^a	92.00±0.50 ^a	0.076	Susceptible (S)
	F test (5%)	Sig	Sig	Sig	Sig	Sig	-	-
	CD (0.05)	11.96	9.9	0.76	6.99	7.25	-	-

*All values represent mean of three replications, values represent Mean±Standard error.

*Means in a column followed by different letter(s) are significantly different at $P=0.05$.

infestation was highest for the cross DGGV-7×V-02-802 (90) while lowest cross V-02-802×DGGV-7 (27.66), followed by 81.66 percent infestation in the breeding lines of V-02-709×DGGV-7. The highest infestation of these breeding lines was due to the dull color of the seed coat and large seed size which is very suitable for colonization and oviposition of bruchid. More than one adult has emerged from large size seeds. The reports of Somta et al., [9], Duraimurugan et al. [26], Chakraborty [27] and Sekar and Nalini [28] were similar to the present study on oviposition, mean developmental period and weight loss for resistant and susceptible lines. The ovipositional preference of *C. maculatus* has significantly differed among the treatment genotypes. The number of eggs laid by the female beetle was recorded on 100 seeds in each genotype. The number of eggs on 100 seeds ranged between 8.00 to 181.66 on different genotypes. More numbers of eggs laid on the 100 seeds were observed for the cross derivatives DGGV-7×V-02-802 (135.33), V-02-709×DGGV-7 (133.33) and V-02-802×DGGV-7 (121.66). The other five breeding lines showed less than 1 egg per seed. Two susceptible parents viz., DGGV-2 and DGGV-7 showed 181.66 and 180.33 number of eggs per 100 seeds;

while the resistance parents viz., V-02-802 and V-02-709 recorded 10.66 and 8.00 number of eggs respectively.

The biological parameters like oviposition preference, percent adult emergence, Mean Developmental Period (MDP), percent adult emergence, percent infestation, and Susceptibility index were the most consistent indicators for screening resistance reaction of green gram genotypes to the damage of *C. maculatus*. The cross derivatives V-02-802×DGGV-2 and V-02-802×DGGV-7 were found to be resistant with a longer mean developmental period (33.53 and 34.76 days), low infestation (30.33 and 27.66%) and low susceptibility index (0.043 and 0.039) when compared to the susceptible one with a mean developmental period (29.51 days), highest infestation (90.66%) and highest susceptibility index (0.061) among the breeding lines. The results of Divya et al. [29]; Sekar and Nalini [28] were comparable with the present study. When DGGV-2 was used as a female parent in the crosses viz., DGGV-2×V-02-802 and DGGV-2×V-02-709 it was revealed moderately resistant reaction with susceptibility index 0.056 and 0.055 respectively. The cross derivative DGGV-2×V-02-709 (SI of 0.055) and its reciprocal cross V-02-709×DGGV-2 (SI of 0.051)

Table 4: Percent Disease Incidence (PDI) for mungbean breeding lines (F_4 generation) to Mungbean Yellow Mosaic Virus (MYMV) under field condition (summer-2018).

Sl. No.	Treatments	Percent Disease Incidence (PDI %)				Disease Score	Reaction group
		After 7 th week of sowing	After 8 th week of sowing	After 9 th week of sowing	Mean		
	DGGV-2×V-02-709	96.95	99.50	100	99.81	5	HS
	DGGV-2×V-02-802	98.80	99.26	100	99.35	5	HS
	DGGV-7×V-02-709	74.94	85.47	89.94	83.45	5	HS
	DGGV-7×V-02-802	15.68	34.52	35.66	28.62	3	MS
	V-02-802×DGGV-2	6.91	7.66	8.24	7.60	1	R
	V-02-802×DGGV-7	95.23	96.89	100	97.35	5	HS
	V-02-709×DGGV-2	62.62	74.22	82.29	73.04	5	HS
	V-02-709×DGGV-7	95.92	97.20	100	97.70	5	HS

Where, R: Resistant; MR: Moderately Resistant; MS: Moderately Susceptible; S: Susceptible; HS: Highly Susceptible.

Table 5: Mean estimates of yield and attributing traits in advanced breeding lines of Mungbean in F_4 -generation (summer-2018).

Sl. No.	Treatments	Days to 50% flowering (days)	Plant height (cm)	Number of branches per plant	Number of clusters per plant	Number of pods per cluster	Number of pods per plant	Pod length (cm)	Number of seeds per pod	100 seed weight (g)	Seed yield per plant (g)
1.	DGGV-2×V-02-709	40.35	46.54	5.52	6.35	4.22	4.52	8.64	12.3	3.8	8.55
2.	DGGV-2×V-02-802	41.23	47.35	4.66	4.95	4.22	12.35	9.32	14.25	5.1	8.32
3.	DGGV-7×V-02-709	40.52	49.66	4.68	7.54	7.54	15.45	8.45	12.36	3.7	7.56
4.	DGGV-7×V-02-802	40.42	51.22	4.65	7.66	7.66	17.25	9.32	13.25	4.1	8.55
5.	V-02-802×DGGV-2	42.25	49.66	4.98	6.35	4.62	20.32	7.45	11.22	4.2	7.34
6.	V-02-802×DGGV-7	41.32	58.63	3.85	6.55	4.32	16.25	8.28	13.62	4.5	8.62
7.	V-02-709×DGGV-2	41.48	64.32	4.55	5.21	6.35	20.33	8.32	13.55	4.8	9.65
8.	V-02-709×DGGV-7	40.82	63.45	4.88	5.23	4.22	19.32	8.42	13.56	5.8	10.54

were found to be moderately resistant. The above findings were more elaborately mentioned by Majhi and Mogali [23]. Considering all the studied biological parameters, breeding lines of eight crosses were categorized as resistant (2 genotypes), moderately resistant (2 genotypes), moderately susceptible (3 genotypes) and susceptible (1 genotype). The findings from the above study specify that the observed resistance may be due to antixenosis or antixenosis effect of the host. The physical seed traits including seed color, seed shape and size, seed volume and seed coat hardness were varied from genotypes to genotype which shows preferential vitiation by the bruchid. Physical barriers such as thickened plant epidermal layers, waxy deposition on leaves, stems or pods and increased trichomes density prevent the insect to oviposit on the surface, ingest or consume the seed. The resistant breeding lines were attributed to reduced egg viability and egg-to-adult survival rate. Therefore, it is necessary to establish which traits or combinations thereof contributing to the resistance reaction.

Symptomatology and Screening for MYMV resistance

MYMV is the most severe threat in mungbean due to its persistent nature of transmission, the rapid development of the viral recombinant strains and the presence of a wide host range for the vector *Bemisia tabaci* possess a serious constraint to the mungbean production in India. The field was regularly monitored to observe the development symptoms of MYMV under natural conditions. After the first and second weeks of sowing, there was no symptom of MYMV. The symptoms started appearing on the third week onwards in some

susceptible lines as yellowing or chlorotic like appearance. Then it was spread very quickly and subsequently, the leaves became necrotic, shortening of internodes and severe stunting of plants. The infected plants bear few flowers and fewer number pods with deformed shapes containing small, and shriveled seeds (Figure 2b,c,d,e). The difference in the level of resistance was observed in different breeding lines based on the visual symptoms in response to MYMV infection. The present results also in accordance with the observations recorded by Akhtar et al., [30]; Subedi et al., [31]; Darshan et al. [32].

However, the disease incidence was recorded on the 7th, 8th and 9th weeks after sowing to determine the Percent Disease Incidence (PDI) based on the disease scoring scale given by Basir et al., [25]. The disease score varied between the observation taken on the 7th week and 8th week after sowing. But, the disease score did not vary that much between the 8th and 9th week after sowing (Table 4). On the basis of Percent Disease Incidence (PDI), the mungbean breeding lines were classified into six groups based on the classification of Basir et al. [25]. The disease symptoms were observed very early (after 3 weeks of sowing) in most of the breeding lines from viz; DGGV-2×V-02-709, DGGV-2×V-02-802, DGGV-7×V-02-709, V-02-802×DGGV-7, V-02-709×DGGV-2, V-02-709×DGGV-7. The coincidence of temperature, relative humidity and increased whitefly population creates a conducive environment for the rapid spread of the disease. The percent disease incidence varied from 7.6 to 99.1 percent in summer 2018 under field conditions. More than 90 percent of disease

Table 6: Selected promising breeding lines of mungbean and their yield attributes along with combined resistance to Bruchid and MYMV.

Sl. No.	Pedigree	Plant Code No.	Days to 50% flowering (days)	Days to maturity (days)	Number of pods per plant	100 seed weight (g)	Seed yield/Plant (g)	Reaction to Bruchid	Reaction to MYMV
1.	DGGV-7×V-02-802	4S-3	40	75	42	4.1	17.3	MS	MS
		4S-15	40	75	25	4.1	15.2	MS	MS
		4S-16	40	75	36	4.1	16.2	MS	MS
		4S-17	40	75	44	4.1	18.2	MS	MS
2.	V-02-802×DGGV-2	5S-2	42	75	30	4.2	10	R	R
		5S-10	42	75	35	4.2	10.7	R	R
		5S-14	42	75	25	4.2	9.5	R	R
		5S-19	42	75	29	4.2	9.2	R	R
3.	V-02-802×DGGV-7	6S-5	41	75	32	4.5	14	R	HS
		6S-6	41	75	20	4.5	10.2	R	HS
		6S-8	41	75	36	4.5	14.2	R	HS
4.	V-02-709×DGGV-2	7S-4	41	75	25	4.5	13.6	MR	HS
		7S-11	41	75	23	4.5	11.8	MR	HS
		7S-18	41	75	23	4.5	11.6	MR	HS
5.	V-02-709×DGGV-7	8S-2	40	75	38	5.8	20	MS	HS
		8S-12	40	75	33	5.8	16.5	MS	HS
		8S-13	40	75	25	5.8	15.2	MS	HS
		8S-16	40	75	24	5.8	15	MS	HS

incidence was recorded in the breeding lines viz; DGGV-2×V-02-709 (99.81%), DGGV-2×V-02-802 (99.35%) and V-02-802×DGGV-7 (97.35%) and they were classified as a highly susceptible category as per PDI categorization. Moderate susceptibility was observed for only one cross derivative, DGGV-7×V-02-802 with a disease incidence score of 28.62 percent, while the cross derivative of V-02-802×DGGV-2 was observed to be resistant with a disease incidence score 7.60 percent (Figure 3a,b,c). The earlier findings by Mohan et al., [33]; Deepa et al., [34]; Nair et al., [35] and Darshan et al., [32] were also shown the similar conclusions. None of the progeny lines from the present study were found to be highly resistant to MYMV with the available climatic condition. This indicates that the resistance genes were contributed by some modifier genes of the male parent.

Yield potential of advanced breeding lines of mungbean

The mean estimates of the yield and yield attributing traits were recorded to select the high yielding lines. The highest average seed yield per plant (10.54g) was observed in the breeding lines of V-02-709×DGGV-7 and second highest yield (9.65g) in V-02-709×DGGV-2 (Table 5). The seed yield per plant was at par in the cross derivatives of V-02-802×DGGV-7 (8.62g), DGGV-2×V-02-709 (8.55g) and DGGV-7×V-02-802 (8.55g). When the 100 seed weight per plant was compared with other lines, the highest (5.8g) observed in the breeding lines of V-02-709×DGGV-7. Long pod size with a more average number of seeds per pod was observed in the cross derivatives of DGGV-2×V-02-802 (14.25), V-02-802×DGGV-7 (13.62) and V-02-709×DGGV-7 (13.56). The average number of pods per plant was more in the case of V-02-709×DGGV-2 (20.33) and V-02-802×DGGV-2 (20.32). The plant height is ranged between 46.54cm (DGGV-2×V-02-709) to 64.32cm (V-02-709×DGGV-2). The observations recorded earlier by Aparna et al., [36], Das and Barua [37], Vir and Singh [38], Parihar et al., [39], Sandhya and

Saravanan [40]; and Majhi et al., [41] for the traits seed yield per plant, 100 seed weight, number of pods and number of branches per plant are similar as per the present findings. The average number of pods per cluster was recorded 7.66 and 7.54 in breeding lines of DGGV-7×V-02-802 and DGGV-7×V-02-709 respectively. The experimental findings of Majhi et al., [42] were similar to the results recorded in the present studies. Very less difference (40.35 to 42.25 days) in days to 50% percent flowering was observed among the breeding lines. There is a positive correlation was observed among the yield attributing traits which are indirectly contributed to yield. These findings were in accordance with the result of Parihar et al. [39]; Sandhya et al., [40] and Pavan et al., [43]. When the number of branches is more than, the number of clusters and the number of pods per plant was higher. This indicates that selection of the promising lines with more number of branches will give a higher yield as compared to the plants with less number of secondary branches. Therefore, progeny lines with more branches, more pods per cluster, large pod size, more seeds per pod and large bold seed size are the best selection criteria for the development of high yielding lines.

Selection of Promising Breeding lines with combined resistance to MYMV and Bruchid

There are some promising breeding lines were selected from cross derivatives of DGGV-7×V-02-802, V-02-802×DGGV-2, V-02-802×DGGV-7, V-02-709×DGGV-2, and V-02-709×DGGV-7 (Table 6). The individual progenies were selected with a code number based on their overall performance on yield attributing traits like days to 50% flowering, days to maturity, number of pods per plant, 100 seed weight and seed yield per plant along with tolerance to bruchid and MYMV disease. Early days to 50% flowering were observed in progeny lines of DGGV-7×V-02-802 (40 days) and V-02-709×DGGV-7 (40 days), whereas the progeny lines of V-02-802×DGGV-2 has taken

42 days to 50% flowering. But, all the progeny lines from all crosses were matured at the same time i.e. 75 days has taken to maturity. The number of pods per plant ranged from 20 to 44. The highest number of pods per plant was reported in progeny lines of DGGV-7×V-02-802 plant code no. 4S-17 (44 pods). The progeny lines from the cross V-02-802×DGGV-2 were recorded an overall higher number of pods; plant code no. 5S-2 (30 pods), 5S-10 (35 pods), 5S-19 (29 pods). 100 seed weight is recorded highest 5.8g in the progeny lines of the cross V-02-709×DGGV-7, but the lines are highly susceptible to MYMV and moderately susceptible to bruchid. The progeny lines of the cross V-02-802×DGGV-2 shown resistance to both MYMV and bruchid with yield per plant is 10.0g (plant code no. 5S-2) and 10.7g (plant code no. 5S-10). The result indicated that there may be some modifier genes in the male parent which is contributing to combined resistance. There may be some conserved gene blocks present in the rare site of the parental lines for resistance reactions, which are not expressed normally. But, when they are crossed with their favorable counter parents, they express the resistance character. Thus, it needs a few more generations of stringent selection to identify the true resistant advanced breeding lines. Therefore, the breeding lines which are resistant to both bruchid and MYMV need further attention to develop superior genotypes [44-70].

Conclusion

The lack of a sufficient number of highly resistant donor lines from the available germplasm of mungbean increases the interest for extensive research for exploring new sources of resistance/tolerance to bruchid and MYMV. This facilitates to develop tolerant genotypes through interspecific hybridization. The wild relative, *Vigna trilobata* possesses resistance to MYMV, but the success of crossing of this species with *Vigna radiata* is difficult. In the endeavor of the present investigation, the derivative breeding lines of V-02-802×DGGV-2 were found to be resistant to both *C. maculatus* and MYMV disease. The selected progeny lines with plant code no. 5S-2, 5S-10, 5S-14, and 5S-19 were selected for further molecular characterization to confirm the true resistance. Other than combined resistance, some promising lines were selected for high yielding and early maturity from the cross derivative DGGV-7×V-02-802 (plant code no. 4S-3, 4S-15, 4S-16, and 4S-17) with moderate susceptibility to *C. maculatus* and MYMV. The breeding line (plant code no. 7S-4, 7S-11, 7S-18) from the cross V-02-802×DGGV-7 were selected for resistance to bruchid with a reasonable yield level. The advancement in molecular breeding will facilitate the conventional breeding to harness the potential from interspecific *Vigna* gene pools through genomics, transcriptomics, proteomics, and plant innate immunity approaches to identifying and tagging candidate genes for bruchid and MYMV resistance, as well as for determining their precise introgression in mungbean to develop advance breeding materials with high yielding and multiple-resistant to biotic and abiotic stresses. Hence, the above findings need the molecular conformity to develop favorable resistant genotypes to compensate for the yield loss by maintaining good seed quality.

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