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The Hunt for Mungbean (*Vigna radiata* (L.) Wilczek) Genotypes and Breeding Lines Resistance to South Indian Bruchid Strain

Santhi Madhavan Samyuktha [1], Devarajan Malarvizhi [2], Irulappan Mariyammal [1], Adhimoolam Karthikeyan [3], Devina Seram [4], Manickam Dhasarathan [5], Sundarrajan Juliet Hepziba [1], Venugopal Sheela [6], Arumugam Thanga Hemavathy [7], Duraisamy Kavithamani [8], Shanmugam Kavitha [9] and Natesan Senthil [10,*]

1 Department of Plant Breeding and Genetics, Agricultural College and Research Institute, Tamil Nadu Agricultural University, Madurai 625104, India; samyukthaliea@gmail.com (S.M.S.); mari.tnau@gmail.com (I.M.); juliethepziba.s@tnau.ac.in (S.J.H.)
2 Agricultural Research Station, Tamil Nadu Agricultural University, Bhavanisagar 638451, India; dmalarvizhi@tnau.ac.in
3 Department of Biotechnology, Centre of Innovation, Agricultural College and Research Institute, Tamil Nadu Agricultural University, Madurai 625104, India; karthik2373@gmail.com
4 Department of Entomology, School of Agriculture, Lovely Professional University, Phagwara 144001, India; devnah@gmail.com
5 Agro Climate Research Centre, Directorate of Crop Management, Tamil Nadu Agricultural University, Coimbatore 641003, India; plantdr.dhasarathan@gmail.com
6 Department of Rice, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore 641003, India; sheelavenugopal@tnau.ac.in
7 Department of Pulses, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore 641003, India; thangahemavathy@tnau.ac.in
8 Department of Millets, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore 641003, India; dkavithamani@tnau.ac.in
9 Department of Plant Genetic Resources, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore 641003, India; skavitha@tnau.ac.in
10 Department of Plant Molecular Biology and Bioinformatics, Centre for Plant Molecular Biology and Biotechnology, Tamil Nadu Agricultural University, Coimbatore 641003, India
* Correspondence: senthil_natesan@tnau.ac.in

Abstract: Mungbean (*Vigna radiata*) is an important short-season legume widely cultivated in Asia, particularly India. It is highly susceptible to bruchids and developing bruchid resistance is an important goal in mungbean breeding programs. In the present study, 52 mungbean genotypes were evaluated for bruchid resistance based on the "no-choice test" and identified two highly resistant genotypes (V2802BG and V2709) with no adult emergence and seed damage. Further, these two resistant genotypes were crossed with six high-yielding bruchid susceptible cultivars (CO 5, CO 6, CO 7, CO 8, VBN 2, and VBN 3), and 12 independent F_1 populations were generated. Of these, one population derived from CO 6 × V2802BG was selected (based on the good combining ability of the parents) and forwarded to later generations to trace the bruchid-resistant lines. A total of 159 $F_{2:3}$ families were screened for bruchid resistance, and the results showed that seven families were highly resistant, whereas the remainder were resistant to highly susceptible. Further, those seven families were evaluated in F_4 and F_5 generations. As a result, five highly resistant lines (BSR-GG-1-49-3-1, BSR-GG-1-56-2-2, BSR-GG-1-160-5-3, BSR-GG-1-170-2-4, and BSR-GG-1-198-1-4) with good agronomic performances were identified. The newly developed lines could be tested in multi-location trials and then be utilized as a potential source of genetic material for improving the bruchid resistance in mungbean breeding programs.

Keywords: bruchid resistance; *Callosobruchus* spp.; introgression breeding; mungbean

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1. Introduction

Mungbean (*Vigna radiata*) is a major grain legume and versatile crop cultivated throughout Asia. It is an excellent source of protein, carbohydrates, minerals, and vitamins for people [1,2]. Mungbean has the ability to fix nitrogen even in poor soils. Also, it is compatible with growing as an intercrop with many cereals and root crops, as well as with cotton, sugarcane, and several plantation crops [1,3–6]. With these characteristics, the mung bean is highly adaptable for sustainable agriculture in marginal lands and drier tropical regions. Asia alone accounts for 90% of mungbean production, and some mung bean is also produced in Africa, Australia, and the United States [2]. The important mungbean growing countries are India, Pakistan, Bangladesh, China, Myanmar, and Nepal. India is the largest producer in the world and produces a significant amount of mung bean. The total mungbean area in India was 4.07 million ha, with a total production of 1.9 million tonnes [7]. However, the standard yield of mungbean is low, and its production has not been significantly increased yet.

Insect pests are a formidable threat to flourishing mungbean production in Asia, including India. The outbreak of insect pests seriously reduces the yield and quality of mungbean. Among the insect pests, bruchids (*Callosobruchus* spp. Coleoptera, Bruchidae) are major insects causing severe damage to seeds in storage conditions [8–10]. The two most common species infecting mungbean seeds are *Callosobruchus chinensis* (L.) and *Callosobruchus maculatus* (F.) [11]. Bruchid infection in field conditions have no severe impacts because of oviposition on the surface of green pods. However, infection in storage conditions leads to severe damage to seeds [12,13]. A single insect-infested seed is a potential source for bruchid population development under storage conditions. Bruchid damage significantly reduces the grain's commercial and nutritional values [14–16]. Therefore, the seeds are not suitable for human consumption and agricultural use.

Breeding resistant cultivars is a cost-effective and environment-friendly method for managing bruchids in mungbean production [10,17–20]. However, over the past decade, limited progress has been made in mungbean breeding to identify resistance sources and develop resistant varieties. So far, several genotypes with resistance to bruchids have been identified by screening a set of mungbean germplasm [21–25]. However, the resistance breakdown occurs with the emergence of a new bruchid population. Moreover, resistant genotypes are not well adapted to different agro-climatic zones, and their agronomic performance is low. Therefore, it is essential to develop bruchid-resistant high-yielding genotypes adapted to different agro-climatic zones. With this backdrop, the objectives of the present study were to: (i) screen mungbean genotypes' resistance to South Indian bruchid strain based on "no-choice" testing; (ii) develop the breeding lines with bruchid resistance, (iii) assess the agronomic performance of the resistant lines.

2. Materials and Methods

2.1. Plant Genetic Materials

Fifty-two mungbean genotypes from different parts of Asian and African countries were used to evaluate resistance to bruchids (*C. maculatus*). The mungbean seeds were obtained from the Department of Plant Genetic Resources, Tamil Nadu Agricultural University (TNAU), Coimbatore, India; National Bureau of Plant Genetic Resources (NBPGR), New Delhi, India; and Asian Vegetable Research and Development Center (AVRDC), Taiwan.

2.2. Source and Identification of Callosobruchus maculatus

The culture of the test insect was maintained in the plastic containers (20 × 10 cm) on a BOD-incubator (Bio-Oxygen Demand) under the temperature of 27 ± 1 °C with 65 ± 5% of relative humidity (RH) at the storage laboratory, Agricultural Research Station (ARS), TNAU, Bhavanisagar, Tamil Nadu, India. To raise the culture in the laboratory, bruchid adults were collected from the local grain market, and *C. maculatus* was carefully separated based on the morphological characters with the support of a stereo zoom microscope (Olympus SZ61, Tokyo, Japan). Adult males and females of *C. maculatus* were differentiated

through readily observable morphological characters (i.e., the abdomen's size and shape) (Figure 1). Males had a shorter abdomen than females, with the dorsal side of terminal segments bent strongly downwards. Females have dark stripes on each side of their posterior dorsal belly, but males do not have dark stripes [26].

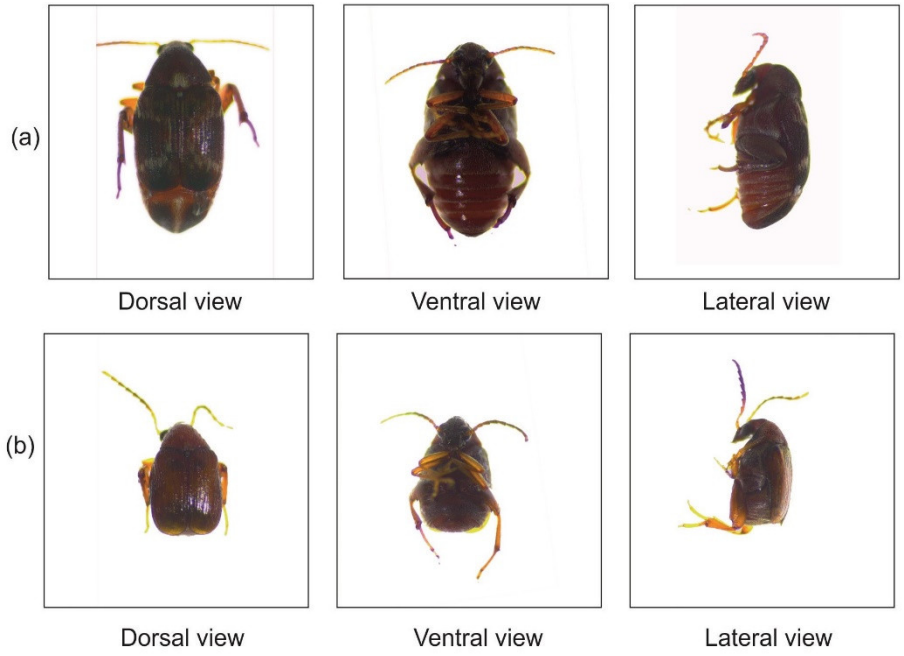

Figure 1. Female (**a**) and male (**b**) bruchid beetles (*Callosobruchus maculatus*).

2.3. Mass Culturing and Maintenance of Callosobruchus maculatus

The infested mungbean seeds collected from the local grain markets of Bhavanisagar, Tamil Nadu, India, were kept for one generation as a source of initial bruchid cultures (*Callosobruchus maculatus*). The pests obtained from initial cultures were reared on fresh CO 6 mungbean seeds susceptible to bruchids following the procedure adopted by Seram et al. [27] with some modifications. Around 50 pairs of *C. maculatus* adults were placed into 600 mL plastic containers containing 200 g of mungbean seeds (CO 6). The containers were covered using a muslin cloth, which allowed adequate ventilation but prevented insects from escaping and placed inside the incubator at 27 ± 1 °C with relative humidity (RH) of 65 ± 5% to facilitate maximum oviposition. The insects were allowed to oviposit for 10 days before being discarded. The dead adults were discarded from each container daily by visual screening using a microscope. Freshly emerging progenies were used to generate the subsequent generations of the laboratory population after 25–30 days. A specific number of females were collected from stock culture, transferred individually to different containers, and maintained as subcultures adopting the above-mentioned procedure. Infested seeds were regularly replaced with fresh ones at monthly intervals.

2.4. Assay Methodology for Screening Mungbean Genotypes and Resistance Evaluation

Bruchid resistance evaluation was made in 52 mungbean genotypes using a "no-choice" test according to the method described by Venkataramana et al. [28] with minor modifications. The stepwise procedures for conducting a "no-choice" test are detailed. Briefly, the sample containers were first labeled (i.e., Genotype name, replication number, and date of infestation), and then each sample was kept in respective containers in three replications containing 20 seeds each replication. Five pairs of freshly emerged adults were introduced per container, covered with muslin cloth on the top and tied with rubber bands. For oviposition, the containers were left undisturbed for three days. Containers were kept inside an incubator at 27 ± 1 °C with RH of 65 ± 5% to facilitate proper oviposition. After three days, containers with egg-laden seeds were carefully taken out, and the number of

eggs oviposited on each seed sample was counted. The presence of frass was checked, and the number of eggs hatched on the 4th and 5th day (After oviposition or release of insects) was recorded. Containers (with egg-laden seeds) were undisturbed until the first adult emergence of a new insect generation. Then, the observations were taken up to the cessation of adult emergence. The observations were recorded based on the following biological and damage assessment parameters:

I. The number of eggs laid: The total number of eggs laid was counted in each genotype for seven days after adult release;
II. The number of adults emerged: The total number of adults emerged was counted daily to determine the mean developmental period (days) and continued till the cessation of emergence;
III. Adult emergence percentage: (Number of adults emerged/number of eggs laid) × 100;
IV. Female to male ratio;
V. Mean developmental period (MDP) recorded in days.

The mean developmental period is the time required for the emergence of 50% of adults. It was calculated by the formula given by Howe [29]

$$MDP = D_1A_1 + D_2A_2 + D_3A_3 + \ldots + D_nA_{n/}\text{Total number of adults emerged}$$

where D_1 = day at which the first adult started emerging (1st day), A_1 = total number of adults emerged on the D_1th day

a. Susceptibility Index: log (per cent adult emergence)/mean developmental period.
b. Percentage of (%) seed damage: Number of seeds damaged/Number of seeds taken × 100. Based on seed damage percent, the genotypes were categorized as highly resistant (0–10%), resistant (10.1–20%), moderately resistant (20.1–40%), susceptible (40.1–80%), and highly susceptible (80.1–100%) [10].

2.5. Generation of Breeding Population Using Resistant and Susceptible Genotypes

The two resistant genotypes (V2802BG and V2709) and six high-yielding susceptible cultivars (CO 5, CO 6, CO 7, CO 8, VBN 2, and VBN 3) were raised in a crossing block during June to September 2018 at ARS, Bhavanisagar. The genotypes were raised on 4-m length ridges at 30 cm spacing between ridges and 10 cm between plants. Two-staggered sowing of parents was used to synchronize flowering and continuous supply of pollen for the crossing program. The crossed pods from the following cross combinations *viz.*, CO 5 × V2802BG, CO 5 × V2709, CO 6 × V2802BG, CO 6 × V2709, CO 7 × V2802BG, CO 7 × V2709, CO 8 × V2802BG, CO 8 × V2709, VBN 2 × V2802BG, VBN 2 × V2709, VBN 3 × V2802BG and VBN 3 × V2709 were collected separately, threshed, and used to raise the F_1 generation at ARS, Bhavanisagar during November 2018 to February 2019. Then, F_2 generation was raised during March to June 2019, and the seeds of each plant were collected individually, and the selected progenies were forwarded to F_3–F_5. The F_3, F_4, and F_5 generations were raised during July to October 2019, December 2019 to March 2020, and April to July 2020. All the generations were raised at ARS, Bhavanisagar.

2.6. Assessment of Grub and Morphological Traits

Grub development and morphological traits were examined in five resistant genotypes (BSR-GG-1-49-3-1, BSR-GG-1-56-2-2, BSR-GG-1-160-5-3, BSR-GG-1-170-2-4, and BSR-GG-1-198-1-4) in the F_5 generation and parents of the cross CO 6 × V2802BG. The morphometric measurement (length and breadth) of the grub of *Callosobruchus maculatus* was measured with the help of a stereo zoom microscope with ten replications. At about 20 days after insect infestation (DAI), seeds with developing grubs inside were carefully cut open, and the measurements were taken. The morphological traits were evaluated in five stable resistant lines from the F_5 generation raised in three replications to determine the agronomic performance. The morphological observations were recorded based on the standard descriptors of mungbean [30]. The traits recorded were plant height (cm), days to fifty

percent flowering, number of pods per plant, pod length (cm), number of seeds per pod, hundred seed weight (g), and single plant yield (g).

2.7. Statistical Analysis

The data on the biological and damage assessment parameters of *C. maculatus* in different genotypes and breeding populations were subjected to square root transformation in case of number values and angular transformation in case of percent values and analyzed using a Completely Randomized Design suggested by Panse and Sukhatme [31]. Analysis of variance (ANOVA) was carried out using SPSS 16.0 version. The general combining ability effects of the parents was worked out as suggested by Kempthorne [32]. Combining ability analysis was carried out using the TNAUSTAT software package [33].

3. Results

3.1. Bruchid Resistance Determination on 52 Mungbean Genotypes

Seed characteristics showed only slight variation among the 52 mungbean genotypes examined. The majority of the genotypes had light green color seeds with shiny lustre. Greenish-yellow or dark green seeds with a dull lustre were seen in a few genotypes. Both oval (24 accessions) and drum (28 accessions) types of seeds were common among the genotypes. The total number of eggs in each genotype ranged from 22 (V2802BG) to 69 (EC 396121), which was significantly higher and lower than the overall mean (Table 1; Figure 2).

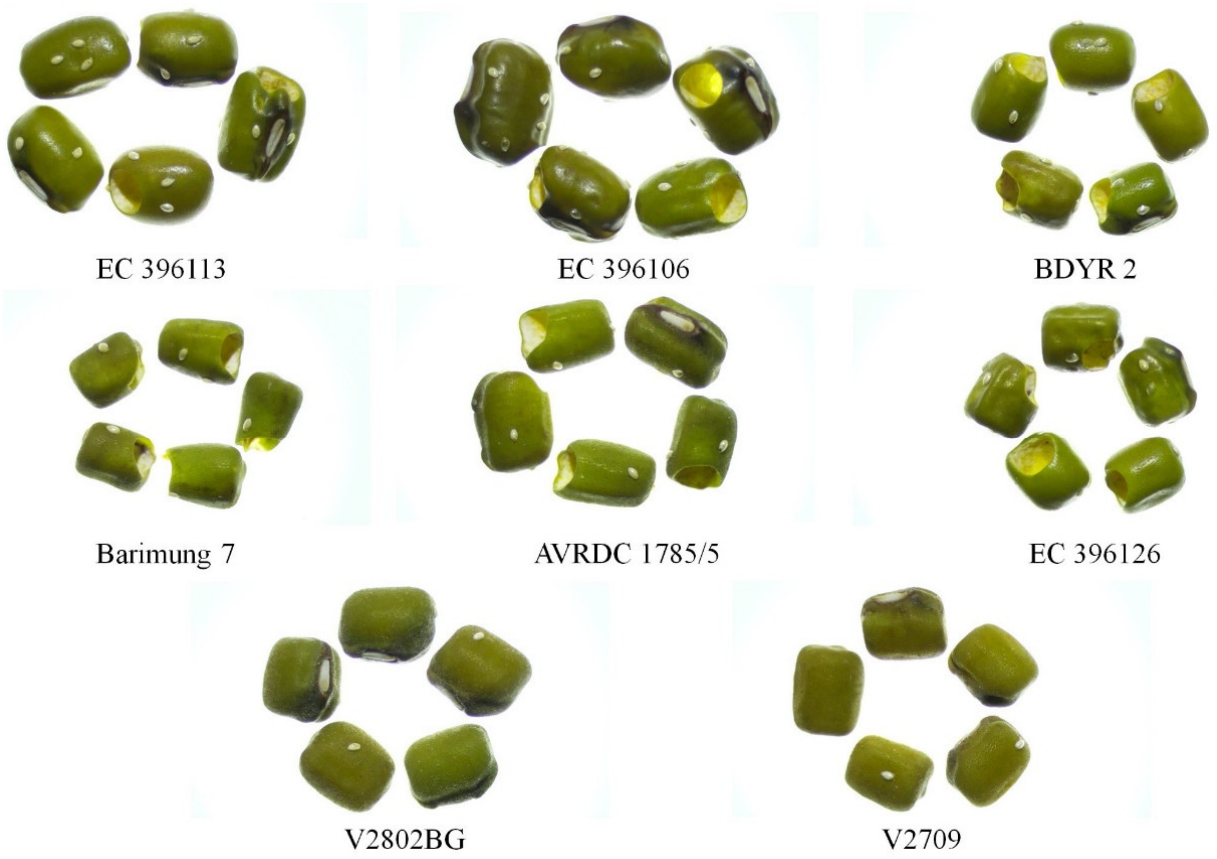

Figure 2. Mungbean genotypes response to bruchid beetles (*Callosobruchus maculatus)* Note: V2802BG and V2709 (Highly resistant); EC 396113, EC 396106, BDYR2, Barimung 7, AVRDC 1785/5, and EC 396126 (Highly susceptible).

Table 1. Screening of 52 mungbean genotypes for bruchid resistance.

S.No.	Genotypes	No.of Eggs Laid	No. of Adults Emerged	Adult Emergence Percentage	Mean Developmental Period	No. of Males Emerged	No. of Females Emerged	Female to Male Ratio	Susceptibility Index	Damage Percentage	Category
1	NM94	52 ± 3.28 ^{h}	20	38.46 ^{pq}	25.75 ± 0.65 ^{qrstuv}	14 ± 0.88 ^{b}	6 ± 0.88 ^{j}	0.43 ^{j}	0.062 ^{defghijklmnopq}	100	HS
2	Binamung 2	30 ± 3.76 ^{x}	20	66.67 ^{a}	23.35 ± 0.63 ^{y}	12 ± 1.15 ^{d}	8 ± 1.15 ^{h}	0.67 ^{h}	0.078 ^{a}	100	HS
3	Barimung 7	50 ± 5.29 ^{i}	20	40.00 ^{op}	25.75 ± 0.88 ^{qrstuv}	11 ± 1.45 ^{e}	9 ± 1.45 ^{g}	0.82 ^{g}	0.062 ^{cdefghijklmnop}	100	HS
4	Barimung 4	63 ± 2.96 ^{c}	20	31.75 ^{t}	24.85 ± 0.49 ^{vwx}	11 ± 0.88 ^{e}	9 ± 0.88 ^{g}	0.82 ^{g}	0.060 ^{ghijklmnopq}	100	HS
5	Binamung 7	36 ± 4.33 ^{u}	20	55.56 ^{d}	25.80 ± 0.28 ^{pqrstu}	6 ± 0.33 ^{j}	14 ± 0.33 ^{b}	2.33 ^{b}	0.068 ^{bcdefg}	100	HS
6	Barimung 5	48 ± 2.73 ^{j}	20	41.67 ^{no}	28.95 ± 0.58 ^{cdefg}	10 ± 0.58 ^{f}	10 ± 0.58 ^{f}	1.00 ^{f}	0.056 ^{mnopqrs}	100	HS
7	Nigerian variety	40 ± 2.19 ^{qr}	20	50.00 ^{gh}	26.80 ± 0.65 ^{jklmnopq}	13 ± 0.88 ^{c}	7 ± 0.88 ^{i}	0.54 ^{i}	0.063 ^{bcdefjklm}	100	HS
8	DM 2	58 ± 2.91 ^{d}	20	34.48 ^{s}	26.60 ± 0.62 ^{y}	7 ± 0.58 ^{i}	13 ± 0.58 ^{c}	1.86 ^{c}	0.058 ^{bcdefghi}	100	HS
9	Ilangai 2	36 ± 3.18 ^{u}	20	55.56 ^{d}	25.15 ± 0.82 ^{tuvw}	13 ± 0.88 ^{c}	7 ± 0.88 ^{i}	0.54 ^{i}	0.069 ^{bc}	100	HS
10	Ilangai 1	32 ± 3.18 ^{w}	20	62.50 ^{b}	25.45 ± 0.85 ^{rstuvw}	10 ± 1.33 ^{f}	10 ± 1.33 ^{f}	1.00 ^{f}	0.071 ^{b}	100	HS
11	EC 396097	42 ± 6.08 ^{op}	20	47.62 ^{ij}	27.95 ± 0.69 ^{fghij}	13 ± 1.53 ^{c}	7 ± 1.53 ^{i}	0.54 ^{i}	0.060 ^{hijklmnopq}	100	HS
12	HUM 2	41 ± 3.79 ^{pq}	20	48.78 ^{hi}	26.20 ± 0.70 ^{nopqrst}	7 ± 0.88 ^{i}	13 ± 0.88 ^{c}	1.86 ^{c}	0.064 ^{bcdefghijk}	100	HS
13	EC 396099	41 ± 2.91 ^{pq}	20	48.78 ^{hi}	29.05 ± 0.66 ^{cdef}	12 ± 1.86 ^{d}	8 ± 1.86 ^{h}	0.67 ^{h}	0.058 ^{jklmnopqr}	100	HS
14	EC 396103	38 ± 3.84 st	20	52.63 ^{ef}	28.15 ± 0.17 ^{efghi}	8 ± 0.88 ^{h}	12 ± 0.88 ^{d}	1.50 ^{d}	0.061 ^{efghijklmnopq}	100	HS
15	EC 396107	32 ± 5.04 ^{w}	20	62.50 ^{b}	27.75 ± 0.88 ^{ghijk}	12 ± 1.53 ^{d}	8 ± 1.53 ^{h}	0.67 ^{h}	0.065 ^{bcdefghijk}	100	HS
16	EC 396104	48 ± 4.33 ^{j}	20	41.67 ^{no}	29.25 ± 0.69 ^{bcde}	11 ± 0.58 ^{e}	9 ± 0.58 ^{g}	0.82 ^{g}	0.055 ^{opqrs}	100	HS
17	EC 396114	51 ± 3.18 ^{hi}	20	39.22 ^{p}	26.15 ± 0.89 ^{nopqrst}	8 ± 0.88 ^{h}	12 ± 0.88 ^{d}	1.50 ^{d}	0.061 ^{fghijklmnopq}	100	HS
18	EC 396115	48 ± 2.73 ^{j}	20	41.67 ^{no}	29.05 ± 0.81 ^{cdef}	12 ± 1.45 ^{d}	8 ± 1.45 ^{h}	0.67 ^{h}	0.056 ^{nopqrs}	100	HS
19	EC 396126	39 ± 2.85 ^{rs}	20	51.28 ^{fg}	27.65 ± 0.69 ^{hijkl}	7 ± 0.33 ^{i}	13 ± 0.33 ^{c}	1.86 ^{c}	0.062 ^{defghijklmnopq}	100	HS
20	EC 396100	41 ± 2.65 ^{pq}	20	48.78 ^{hi}	26.40 ± 0.32 ^{mnopqrs}	11 ± 1.45 ^{e}	9 ± 1.45 ^{g}	0.82 ^{g}	0.064 ^{bcdefghijk}	100	HS
21	EC 396121	69 ± 1.73 ^{a}	20	28.99 ^{u}	25.05 ± 0.92 ^{tuvw}	15 ± 0.58 ^{a}	5 ± 0.58 ^{k}	0.33 ^{k}	0.058 ^{ijklmnopqr}	100	HS
22	BDYR 3	34 ± 1.53 ^{v}	20	58.82 ^{c}	26.05 ± 0.45 ^{opqrstu}	9 ± 1.53 ^{g}	11 ± 1.53 ^{e}	1.22 ^{e}	0.068 ^{bcdef}	100	HS
23	EC 396106	62 ± 0.88 ^{c}	20	32.26 ^{t}	27.75 ± 0.37 ^{ghijk}	12 ± 1.20 ^{d}	8 ± 1.20 ^{h}	0.67 ^{h}	0.054 ^{qrs}	100	HS
24	EC 396110	46 ± 3.76 ^{kl}	20	43.48 ^{mn}	28.45 ± 0.91 ^{defgh}	13 ± 1.33 ^{c}	7 ± 1.33 ^{i}	0.54 ^{i}	0.058 ^{klmnopqr}	100	HS
25	EC 396108	41 ± 1.15 ^{pq}	20	48.78 ^{hi}	28.05 ± 0.50 ^{efghi}	8 ± 1.45 ^{h}	12 ± 1.45 ^{d}	1.50 ^{d}	0.060 ^{ghijklmnopq}	100	HS
26	EC 396105	56 ± 4.62 ^{ef}	20	35.71 ^{rs}	27.00 ± 0.18 ^{ijklmnop}	11 ± 1.20 ^{e}	9 ± 1.20 ^{g}	0.82 ^{g}	0.058 ^{klmnopqr}	100	HS
27	EC 396118	41 ± 1.76 ^{p}	20	48.78 ^{hi}	30.30 ± 0.23 ^{ab}	10 ± 1.45 ^{f}	10 ± 1.45 ^{f}	1.00 ^{f}	0.056 ^{t}	100	HS
28	EC 396120	57 ± 2.96 ^{de}	20	35.09 ^{gs}	26.45 ± 0.42 ^{lmnopqrs}	8 ± 0.88 ^{h}	12 ± 0.88 ^{d}	1.50 ^{d}	0.058 ^{ikmnopqr}	100	HS
29	EC 118889	39 ± 3.76 ^{rs}	20	51.28 ^{f}	26.10 ± 0.56 ^{opqrst}	14 ± 0.33 ^{b}	6 ± 0.33 ^{j}	0.43 ^{j}	0.066 ^{bcdefghij}	100	HS
30	AVRDC 1785/5	38 ± 1.15 st	20	52.63 ^{ef}	24.40 ± 0.49 ^{wxy}	5 ± 0.33 ^{k}	15 ± 0.33 ^{a}	3.00 ^{a}	0.071 ^{b}	100	HS
31	BDYR 2	45 ± 1.76 ^{lm}	20	44.44 ^{lm}	25.05 ± 0.42 ^{tuvw}	12 ± 0.88 ^{d}	8 ± 0.88 ^{h}	0.67 ^{h}	0.066 ^{bcdefghi}	100	HS
32	EC 396101	54 ± 3.18 ^{g}	20	37.04 ^{q}	26.30 ± 0.56 ^{vwx}	13 ± 0.88 ^{c}	7 ± 0.88 ^{i}	0.54 ^{i}	0.060 ^{bcdefghijklmn}	100	HS
33	EC 396102	44 ± 1.73 ^{mn}	20	45.45 ^{kl}	29.45 ± 0.75 ^{bcd}	6 ± 0.67 ^{j}	14 ± 0.67 ^{b}	2.33 ^{b}	0.056 ^{lmnopqrs}	100	HS
34	EC 396111	65 ± 2.03 ^{b}	20	30.77 ^{tu}	30.00 ± 0.22 ^{bc}	14 ± 0.33 ^{b}	6 ± 0.33 ^{j}	0.43 ^{j}	0.050 ^{s}	100	HS
35	EC 396116	38 ± 1.45 st	20	52.63 ^{ef}	27.45 ± 0.86 ^{gijklm}	13 ± 1.15 ^{c}	7 ± 1.15 ^{i}	0.54 ^{i}	0.063 ^{cdefijklmno}	100	HS
36	EC 396117	37 ± 1.45 ^{tu}	20	54.05 ^{de}	31.50 ± 0.71 ^{a}	12 ± 1.15 ^{d}	8 ± 1.15 ^{h}	0.67 ^{h}	0.055 ^{pqrs}	100	HS
37	EC 396125	33 ± 2.03 ^{vw}	20	60.61 ^{bc}	27.10 ± 0.26 ^{ijklmno}	11 ± 0.88 ^{e}	9 ± 0.88 ^{g}	0.82 ^{g}	0.066 ^{bcdefghi}	100	HS
38	EC 396113	63 ± 2.03 ^{c}	20	31.75 ^{t}	25.75 ± 0.71 ^{qrstuv}	7 ± 0.88 ^{i}	13 ± 0.88 ^{c}	1.86 ^{c}	0.058 ^{ijklpqr}	100	HS

Table 1. *Cont.*

S.No.	Genotypes	No.of Eggs Laid	No. of Adults Emerged	Adult Emergence Percentage	Mean Developmental Period	No. of Males Emerged	No. of Females Emerged	Female to Male Ratio	Susceptibility Index	Damage Percentage	Category
39	EC 396123	47 ± 1.45^{jk}	20	42.55^{mn}	23.75 ± 0.20^{xy}	10 ± 1.20^{f}	10 ± 1.20^{f}	1.00^{f}	0.069^{bcde}	100	HS
40	EC 396122	42 ± 3.48^{op}	20	47.62^{ij}	25.50 ± 0.45^{rstuvw}	13 ± 0.33^{c}	7 ± 0.33^{i}	0.54^{i}	$0.066^{bcdefghi}$	100	HS
41	BDYR 1	55 ± 2.96^{fg}	20	36.36^{rs}	30.15 ± 0.76^{bc}	14 ± 0.88^{b}	6 ± 0.88^{j}	0.43^{j}	0.052^{rs}	100	HS
42	V2709	25 ± 2.40^{y}	0	0.00^{v}	0.00 ± 0.00^{z}	0 ± 0.00^{l}	0 ± 0.00^{l}	0.00^{l}	0.000^{u}	0	HR
43	HG 22	32 ± 4.36^{w}	20	62.50^{b}	26.10 ± 0.45^{opqrst}	12 ± 0.58^{d}	8 ± 0.58^{h}	0.67^{h}	0.069^{bcd}	100	HS
44	ML 818	42 ± 6.06^{op}	20	47.62^{ij}	27.35 ± 0.75^{gijklmn}	13 ± 1.45^{c}	7 ± 1.45^{i}	0.54^{i}	$0.061^{defghijklmnopq}$	100	HS
45	VGGRU 1	58 ± 3.06^{d}	20	34.48^{s}	25.60 ± 0.19^{qrstuvw}	9 ± 1.45^{g}	11 ± 1.45^{e}	1.22^{e}	$0.060^{hijklmnopq}$	100	HS
46	ML 1108	62 ± 5.13^{c}	20	32.26^{t}	26.15 ± 0.51^{nopqrst}	12 ± 0.88^{d}	8 ± 0.88^{h}	0.67^{h}	$0.058^{klmnopqr}$	100	HS
47	Basanti	34 ± 3.21^{v}	20	58.82^{c}	$26.55 \pm 0.42^{klmnopqr}$	5 ± 0.58^{k}	15 ± 0.58^{a}	3.00^{a}	$0.067^{bcdefgh}$	100	HS
48	KMG 189	39 ± 6.06^{rs}	20	51.28^{fg}	25.30 ± 0.92^{stuvw}	12 ± 1.15^{d}	8 ± 1.15^{h}	0.67^{h}	0.068^{bcdef}	100	HS
49	EC 396098	40 ± 6.11^{qr}	20	50.00^{gh}	26.05 ± 1.08^{opqrstu}	6 ± 0.33^{j}	14 ± 0.33^{b}	2.33^{b}	$0.065^{bcdefghij}$	100	HS
50	LM 469	43 ± 5.49^{no}	20	46.51^{hjk}	26.20 ± 0.78^{nopqrst}	13 ± 1.20^{c}	7 ± 1.20^{i}	0.54^{i}	$0.064^{bcdefghijkl}$	100	HS
51	T 1	41 ± 6.17^{pq}	20	48.78^{i}	25.75 ± 1.22^{qrstuv}	9 ± 0.58^{g}	11 ± 0.58^{e}	1.22^{e}	$0.066^{bcdefghij}$	100	HS
52	V2802 BG	22 ± 3.46^{z}	0	0.00^{v}	0.00 ± 0.00^{z}	0 ± 0.00^{l}	0 ± 0.00^{l}	0.00^{l}	0.000^{u}	0	HR
Mean		44.42	19.23	44.44	25.73	10.17	9.06	1.01	0.059	96.15	-
SEd		0.91	-	0.96	0.61	0.24	0.19	0.02	0.004	-	-
CD ($p = 0.05$)		1.81	-	1.91	1.21	0.48	0.38	0.04	0.008	-	-

Values are mean ± SE of three replicates; Mean values followed by different letters in the same column are significantly different at the 5% level by LSD.

Two mungbean genotypes, V2709 and V2802BG, exhibited no adult emergence, whereas a maximum of 20 adults emerged from the remaining 50 genotypes. The adult emergence percentage was significantly higher in Binamung 2 (66.67%), followed by 62.50% in Ilangai 1, EC 396107, and HG 22. EC 396117 had a considerably higher mean developmental period (31.50 days), comparable to EC 396118 (30.30 days). The mean developmental period cannot be calculated since there was no adult emergence in V2709 and V2802BG. Other than these two genotypes (V2709 and V2802BG), Binamung 2 exhibited a significantly minimum mean developmental period of 23.35 days that was found to be on par with the following genotypes, EC 396123 (23.75 days), AVRDC 1785/5 (24.40 days), and Barimung 4 (24.85 days). Apart from V2709 and V2802BG, a significant minimum female to male ratio of 0.33 was observed from EC 396121. The significantly highest female to male ratio of 3.00 was shown by AVRDC 1785/5 and Basanti, followed by 2.33 in Binamung 7, EC 396102, and EC 396098. The susceptibility index was zero for V2709 and V2802BG, whereas a significantly higher susceptibility index of 0.078 was observed in Binamung 2, followed by 0.071 (Ilangai 1, AVRDC 1785/5) and 0.069 (Ilangai 2, HG 22, EC 396123). V2709 and V2802BG were categorized as highly resistant (HR) with 0% seed damage, and all other genotypes were classified as highly susceptible (HS) with 100% seed damage.

3.2. Development of Breeding Lines with Bruchid Resistance

Two resistant genotypes (V2709 and V2802BG) were crossed with six high-yielding cultivars (CO 5, CO 6, CO 7, CO 8, VBN 2, and VBN 3), and 12 independent populations of F_1 were generated. Of these, one population derived from CO 6 × V2802BG was selected based on good combining ability (Table 2; Figure 3) and forwarded to later generations.

Table 2. General combining ability effects for different morphological traits.

Parents	Plant Height	Days to 50% Flowering	No. of Pods/Plant	Pod Length	No. of Seeds/Pod	Hundred Seed Weight	Single Plant Yield
Lines							
CO 5	6.28 **	0.31	3.44	0.15	0.03	−0.14 **	0.82
CO 6	10.83 **	−0.03	7.11 **	0.95 **	0.86 **	0.09 **	5.63 **
CO 7	2.31 **	−0.53 *	−3.56	0.32 **	0.86 **	0.30 **	2.24
CO 8	−4.75 **	−0.19	−4.89 *	−0.85 **	0.19	−0.21 **	−3.50 **
VBN 2	−8.72 **	−1.53 **	6.11 *	−0.12	−0.47	0.09 **	1.70
VBN 3	−5.95 **	1.97 **	−8.22 **	−0.44 **	−1.47	−0.13 **	−6.89 **
Testers							
V2802BG	0.03	−0.36 **	0.83	0.12 *	−0.25	0.01	0.05
V2709	−0.03	0.36 **	−0.83	−0.12 *	0.25	−0.01	−0.05
SE (Lines)	1.06	0.21	2.24	0.08	0.28	0.02	1.17
SE (Testers)	0.61	0.11	1.29	0.05	0.16	0.02	0.67

* Significant at 5% level, ** Significant at 1% level.

CO 6 × V2802BG population comprising 159 $F_{2:3}$ families were screened for bruchid resistance, and results showed that seven $F_{2:3}$ families exhibited high resistance with 0% seed damage, whereas the remainder were resistant to highly susceptible. Further, these seven $F_{2:3}$ families were (BSR-GG-1-42, BSR-GG-1-49, BSR-GG-1-56, BSR-GG-1-97, BSR-GG-1-160, BSR-GG-1-170, and BSR-GG-1-198) evaluated for bruchid resistance in the F_4 generation. Of these, five families *viz.*, BSR-GG-1-49, BSR-GG-1-56, BSR-GG-1-160, BSR-GG-1-170, and BSR-GG-1-198 exhibited high resistance with 0% seed damage. Two families, namely BSR-GG-1-42 and BSR-GG-1-97, showed segregation with 0–35% seed damage (Table 3). Further, we have evaluated the five resistant lines in the F_5 generation and confirmed the resistance.

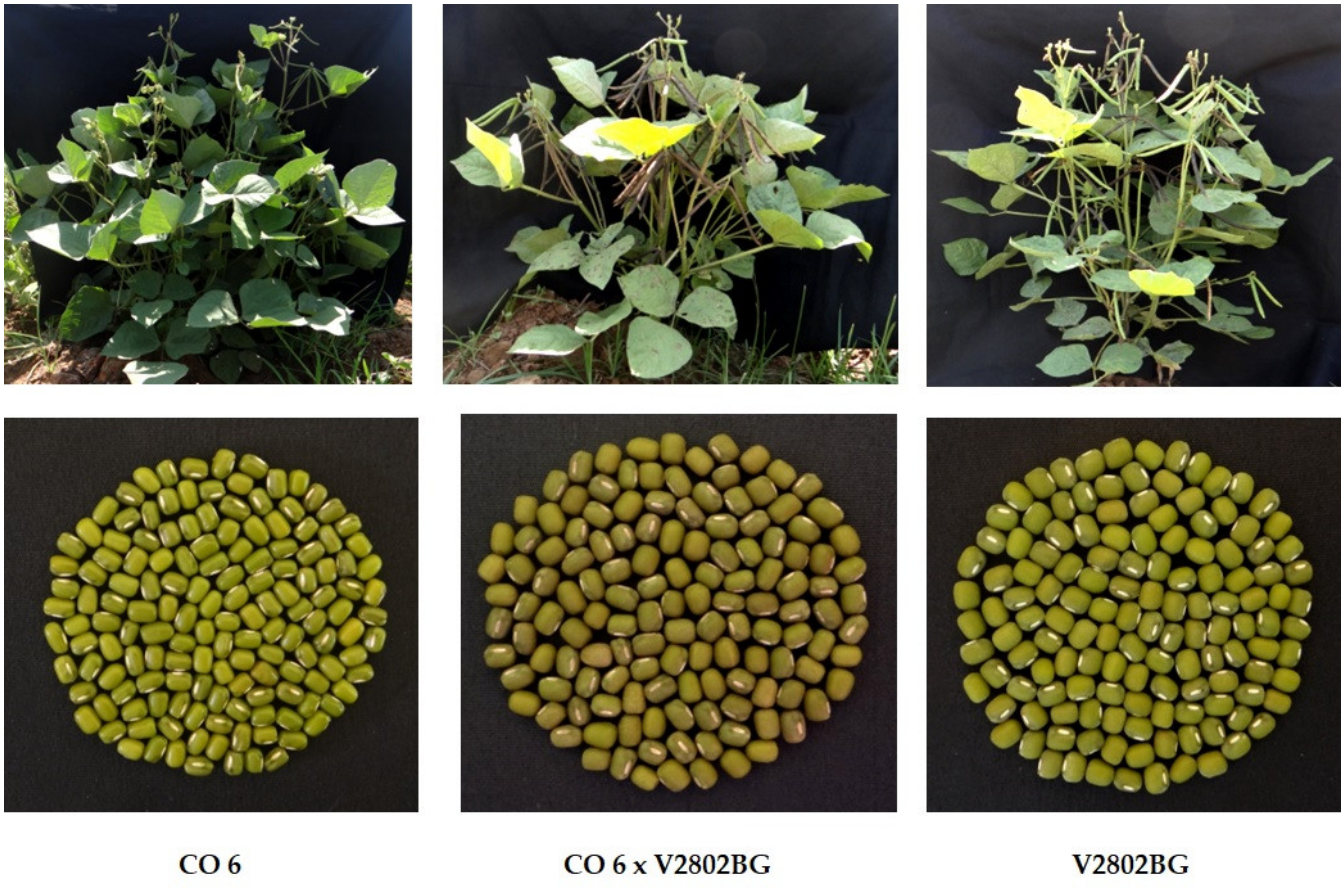

Figure 3. Parents, F_2 plants, and F_3 seeds of the cross CO 6 × V2802BG.

3.3. Grub Development in the Resistant Lines

The development of grub in five resistant lines in the F_5 generation was examined along with the parents (Table 4, Figure 4). Grub length was observed as follows in the resistant lines *viz.*, BSR-GG-1-49-3-1 (0.19 mm), BSR-GG-1-56-2-2 (0.20 mm), BSR-GG-1-160-5-3 (0.20 mm), BSR-GG-1-170-2-4 (0.22 mm) and BSR-GG-1-198-1-4 (0.21 mm), which was found to be on par with the resistant parent V2802BG (0.21 mm). However, grub length was significantly higher in CO 6 (4.05 mm) than in resistant lines. On the other hand, the grub breadth of resistant lines varied from 0.11 to 0.14 mm and was on par with the resistant parent V2802BG (0.13 mm), while in CO 6, grub breadth (2.65 mm) was significantly higher than the resistant lines.

Table 3. Screening of bruchid resistance in seven F_4 families derived from the cross of CO 6 × V2802BG.

S.No.	Genotypes	No. of Eggs Laid	Adult Emergence	Adult Emergence Percentage	Mean Developmental Period	No. of Males Emerged	No. of Females Emerged	Female to Male Ratio	Susceptibility Index	Damage Percentage	Score
1	BSR-GG-1-42-1	23 ^{q}	0 ^{f}	0.00 ^{h}	0.00 ^{e}	0 ^{f}	0 ^{e}	0.00 ^{e}	0.000 ^{e}	0.00 ^{f}	HR
2	BSR-GG-1-42-2	28 ^{m}	1 ^{f}	3.57 ^{d}	31.00 ^{a}	1 ^{e}	0 ^{e}	0.00 ^{e}	0.018 ^{d}	5.00 ^{e}	HR
3	BSR-GG-1-42-3	78 ^{a}	3 ^{d}	3.85 ^{g}	31.67 ^{a}	3 ^{c}	0 ^{c}	0.00 ^{e}	0.019 ^{d}	15.00 ^{d}	R
4	BSR-GG-1-42-4	50 ^{f}	7 ^{b}	14.00 ^{f}	31.71 ^{a}	4 ^{b}	3 ^{c}	0.75 ^{c}	0.036 ^{c}	35.00 ^{b}	MR
5	BSR-GG-1-42-5	48 ^{g}	0 ^{f}	0.00 ^{h}	0.00 ^{e}	0 ^{f}	0 ^{e}	0.00 ^{e}	0.000 ^{e}	0.00 ^{f}	HR
6	BSR-GG-1-49-1	21 ^{rs}	0 ^{f}	0.00 ^{h}	0.00 ^{e}	0 ^{f}	0 ^{e}	0.00 ^{e}	0.000 ^{e}	0.00 ^{f}	HR
7	BSR-GG-1-49-2	26 ^{op}	0 ^{f}	0.00 ^{h}	0.00 ^{e}	0 ^{f}	0 ^{e}	0.00 ^{e}	0.000 ^{e}	0.00 ^{f}	HR
8	BSR-GG-1-49-3	51 ^{f}	0 ^{f}	0.00 ^{h}	0.00 ^{e}	0 ^{f}	0 ^{e}	0.00 ^{e}	0.000 ^{e}	0.00 ^{f}	HR
9	BSR-GG-1-49-4	22 ^{qr}	0 ^{f}	0.00 ^{h}	0.00 ^{e}	0 ^{f}	0 ^{e}	0.00 ^{e}	0.000 ^{e}	0.00 ^{f}	HR
10	BSR-GG-1-49-5	23 ^{q}	0 ^{f}	0.00 ^{h}	0.00 ^{e}	0 ^{f}	0 ^{e}	0.00 ^{e}	0.000 ^{e}	0.00 ^{f}	HR
11	BSR-GG-1-56-1	32 ^{j}	0 ^{f}	0.00 ^{h}	0.00 ^{e}	0 ^{f}	0 ^{e}	0.00 ^{e}	0.000 ^{e}	0.00 ^{f}	HR
12	BSR-GG-1-56-2	28 ^{m}	0 ^{f}	0.00 ^{h}	0.00 ^{e}	0 ^{f}	0 ^{e}	0.00 ^{e}	0.000 ^{e}	0.00 ^{f}	HR
13	BSR-GG-1-56-3	36 ^{i}	0 ^{f}	0.00 ^{h}	0.00 ^{e}	0 ^{f}	0 ^{e}	0.00 ^{e}	0.000 ^{e}	0.00 ^{f}	HR
14	BSR-GG-1-56-4	67 ^{b}	0 ^{f}	0.00 ^{h}	0.00 ^{e}	0 ^{f}	0 ^{e}	0.00 ^{e}	0.000 ^{e}	0.00 ^{f}	HR
15	BSR-GG-1-56-5	60 ^{c}	0 ^{f}	0.00 ^{h}	0.00 ^{e}	0 ^{f}	0 ^{e}	0.00 ^{e}	0.000 ^{e}	0.00 ^{f}	HR
16	BSR-GG-1-97-1	27 ^{o}	0 ^{f}	0.00 ^{h}	0.00 ^{e}	0 ^{f}	0 ^{e}	0.00 ^{e}	0.000 ^{e}	0.00 ^{f}	HR
17	BSR-GG-1-97-2	27 ^{o}	5 ^{c}	18.52 ^{b}	26.20 ^{c}	3 ^{c}	1 ^{d}	0.33 ^{d}	0.048 ^{b}	25.00 ^{c}	MR
18	BSR-GG-1-97-3	38 ^{h}	1 ^{e}	2.63 ^{e}	25.00 ^{d}	1 ^{e}	0 ^{e}	0.00 ^{e}	0.017 ^{d}	5.00 ^{e}	HR
19	BSR-GG-1-97-4	58 ^{d}	7 ^{b}	12.07 ^{c}	29.28 ^{b}	2 ^{d}	5 ^{b}	2.50 ^{a}	0.037 ^{c}	35.00 ^{b}	MR
20	BSR-GG-1-97-5	30 ^{kl}	0 ^{f}	0.00 ^{h}	0.00 ^{e}	0 ^{f}	0 ^{e}	0.00 ^{e}	0.000 ^{e}	0.00 ^{f}	HR
21	BSR-GG-1-160-1	25 ^{p}	0 ^{f}	0.00 ^{h}	0.00 ^{e}	0 ^{f}	0 ^{e}	0.00 ^{e}	0.000 ^{e}	0.00 ^{f}	HR
22	BSR-GG-1-160-2	20 ^{s}	0 ^{f}	0.00 ^{h}	0.00 ^{e}	0 ^{f}	0 ^{e}	0.00 ^{e}	0.000 ^{e}	0.00 ^{f}	HR
23	BSR-GG-1-160-3	31 ^{jk}	0 ^{f}	0.00 ^{h}	0.00 ^{e}	0 ^{f}	0 ^{e}	0.00 ^{e}	0.000 ^{e}	0.00 ^{f}	HR
24	BSR-GG-1-160-4	55 ^{e}	0 ^{f}	0.00 ^{h}	0.00 ^{e}	0 ^{f}	0 ^{e}	0.00 ^{e}	0.000 ^{e}	0.00 ^{f}	HR
25	BSR-GG-1-160-5	48 ^{g}	0 ^{f}	0.00 ^{h}	0.00 ^{e}	0 ^{f}	0 ^{e}	0.00 ^{e}	0.000 ^{e}	0.00 ^{f}	HR
26	BSR-GG-1-170-1	23 ^{q}	0 ^{f}	0.00 ^{h}	0.00 ^{e}	0 ^{f}	0 ^{e}	0.00 ^{e}	0.000 ^{e}	0.00 ^{f}	HR
27	BSR-GG-1-170-2	25 ^{p}	0 ^{f}	0.00 ^{h}	0.00 ^{e}	0 ^{f}	0 ^{e}	0.00 ^{e}	0.000 ^{e}	0.00 ^{f}	HR
28	BSR-GG-1-170-3	26 ^{op}	0 ^{f}	0.00 ^{h}	0.00 ^{e}	0 ^{f}	0 ^{e}	0.00 ^{e}	0.000 ^{e}	0.00 ^{f}	HR
29	BSR-GG-1-170-4	32 ^{j}	0 ^{f}	0.00 ^{h}	0.00 ^{e}	0 ^{f}	0 ^{e}	0.00 ^{e}	0.000 ^{e}	0.00 ^{f}	HR
30	BSR-GG-1-170-5	30 ^{kl}	0 ^{f}	0.00 ^{h}	0.00 ^{e}	0 ^{f}	0 ^{e}	0.00 ^{e}	0.000 ^{e}	0.00 ^{f}	HR

Table 3. *Cont.*

S.No.	Genotypes	No. of Eggs Laid	Adult Emergence	Adult Emergence Percentage	Mean Developmental Period	No. of Males Emerged	No. of Females Emerged	Female to Male Ratio	Susceptibility Index	Damage Percentage	Score
31	BSR-GG-1-198-1	39 ^{h}	0 ^{f}	0.00 ^{h}	0.00 ^{e}	0 ^{f}	0 ^{e}	0.00 ^{e}	0.000 ^{e}	0.00 ^{f}	HR
32	BSR-GG-1-198-2	29 ^{lm}	0 ^{f}	0.00 ^{h}	0.00 ^{e}	0 ^{f}	0 ^{e}	0.00 ^{e}	0.000 ^{e}	0.00 ^{f}	HR
33	BSR-GG-1-198-3	27 ^{o}	0 ^{f}	0.00 ^{h}	0.00 ^{e}	0 ^{f}	0 ^{e}	0.00 ^{e}	0.000 ^{e}	0.00 ^{f}	HR
34	BSR-GG-1-198-4	60 ^{c}	0 ^{f}	0.00 ^{h}	0.00 ^{e}	0 ^{f}	0 ^{e}	0.00 ^{e}	0.000 ^{e}	0.00 ^{f}	HR
35	BSR-GG-1-198-5	32 ^{j}	0 ^{f}	0.00 ^{h}	0.00 ^{e}	0 ^{f}	0 ^{e}	0.00 ^{e}	0.000 ^{e}	0.00 ^{f}	HR
					Parents						
36	CO6	48 ^{g}	20 ^{a}	41.67 ^{a}	26.10 ^{c}	9 ^{a}	11 ^{a}	1.22 ^{b}	0.062 ^{a}	100.00 ^{a}	HS
37	V2802 BG	16 ^{t}	0 ^{f}	0.00 ^{h}	0.00 ^{e}	0 ^{f}	0 ^{e}	0.00 ^{e}	0.000 ^{e}	0.00 ^{f}	HR
	Mean	36.33	1.00	2.21	5.72	0.49	0.46	0.11	0.01	4.86	-
	SEd	0.91	0.11	0.07	0.22	0.02	0.03	0.01	0.00	0.57	-
	CD (p = 0.05)	1.82	0.22	0.13	0.44	0.05	0.05	0.02	0.00	1.13	-

Values are the mean of three replicates; Mean values followed by different letters in the same column are significantly different at 5% level by LSD. HR, Highly resistant; R, Resistant; MR, Moderately resistant; HS, Highly susceptible.

Table 4. Grub dimensions of *Callosobruchus maculatus* in five stable resistant lines from F_5 generation derived from the cross of CO 6 × V2802BG.

S.No.	Parents and Resistant Lines	Length (mm)	Breadth (mm)
1.	BSR-GG-1-49-3-1	0.19 ^{b}	0.12 ^{b}
2.	BSR-GG-1-56-2-2	0.20 ^{b}	0.14 ^{b}
3.	BSR-GG-1-160-5-3	0.20 ^{b}	0.11 ^{b}
4.	BSR-GG-1-170-2-4	0.22 ^{b}	0.13 ^{b}
5.	BSR-GG-1-198-1-4	0.21 ^{b}	0.13 ^{b}
6.	CO 6	4.05 ^{a}	2.65 ^{a}
7.	V2802 BG	0.21 ^{b}	0.13 ^{b}
	Mean	0.75	0.49
	SEd	0.04	0.01
	CV%	6.41	3.56

Values are the mean of three replicates; Mean values followed by different letters in the same column are significantly different at the 5% level by LSD.

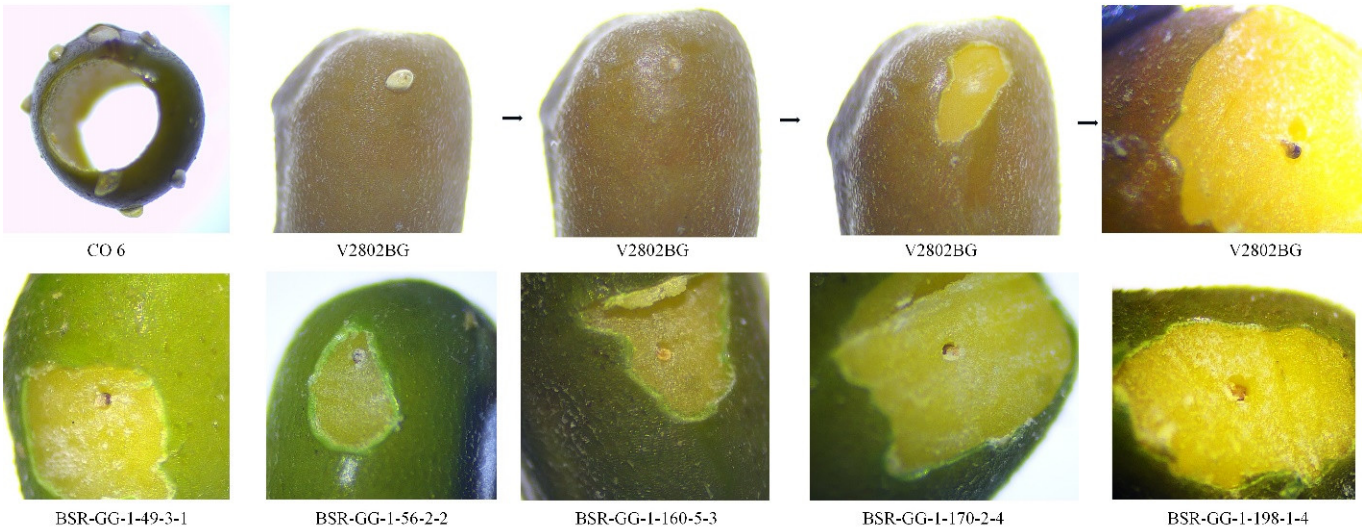

Figure 4. Grub development in five highly resistant lines from the cross of CO6 × V2802BG. CO 6 (Highly susceptible) and V2802BG (Highly resistant). BSR-GG-1-49-3-1, BSR-GG-1-56-2-2, BSR-GG-1-160-5-3, BSR-GG-1-170-2-4, and BSR-GG-1-198-1-4 (Highly resistant lines).

3.4. Agronomic Performance of the Resistant Lines

The agronomic performance of five resistant lines in the F_5 generation was evaluated along with CO 6 (Table 5). Apart from BSR-GG-1-56-2-2 (60.17 cm), all the four resistant lines had increased mean plant height compared to CO 6 (60.62 cm). Two resistant lines, BSR-GG-1-160-5-3, and BSR-GG-1-49-3-1 attained 50 percent flowering at 35 days after sowing was earlier than CO 6 (36 days). Excluding BSR-GG-1-49-3-1 (41.33), all the resistant lines produced more pods per plant than CO 6. The maximum number of pods per plant was exhibited by BSR-GG-1-198-1-4 (48.33). All the resistant lines expressed increased pod length. The mean value for the number of seeds per pod of BSR-GG-1-170-2-4 (12.33) was found to be on par with CO 6, whereas other resistant lines exhibited a higher number of seeds per pod. All the resistant lines expressed a higher hundred seed weight and single plant yield than CO 6.

Table 5. Agronomic performance of five stable resistant lines from F_5 generation derived from the cross of CO 6 × V2802BG.

S.No.	Genotypes	PH	DFPF	PPP	PL	SPP	HSW	SPY
1	BSR-GG-1-49-3-1	64.23 ± 6.31	35.33 ± 0.33	41.33 ± 1.76	8.27 ± 0.15	13.00 ± 0.58	3.95 ± 0.02	16.71 ± 0.66
2	BSR-GG-1-56-2-2	60.17 ± 4.13	35.67 ± 0.33	43.67 ± 3.38	7.83 ± 0.12	12.67 ± 0.33	4.04 ± 0.05	18.18 ± 0.83
3	BSR-GG-1-160-5-3	62.40 ± 2.15	34.67 ± 0.33	45.67 ± 2.73	8.53 ± 0.06 *	13.00 ± 0.58	4.02 ± 0.06	19.70 ± 1.41
4	BSR-GG-1-170-2-4	61.97 ± 2.44	35.67 ± 0.33	44.00 ± 2.52	7.73 ± 0.15	12.33 ± 0.33	3.98 ± 0.03	16.31 ± 0.50
5	BSR-GG-1-198-1-4	62.73 ± 3.46	36.33 ± 0.33	48.33 ± 2.60	8.23 ± 0.15	12.67 ± 0.33	3.90 ± 0.04	18.72 ± 1.00
6	CO 6	60.62 ± 2.66	35.67 ± 0.33	42.33 ±2.03	7.33 ± 0.06	12.33 ± 0.33	3.79 ± 0.04	15.99 ± 0.34
	Mean	62.02	35.56	44.22	7.99	12.67	3.95	17.60
	SEd	5.45	0.41	3.56	0.11	0.62	0.06	1.31
	CD (p = 0.05)	12.15	0.92	7.93	0.24	1.37	0.14	2.91

Values are mean ± SE of three replicates; * Significance at 5% level; PH, Plant height (cm); DFPF, Days to fifty percent flowering; PPP, Number of pods per plant; PL, Pod length (cm); SPP, Number of seeds per pod; HSW, Hundred seed weight (g); SPY, Single plant yield (g).

4. Discussion

4.1. Search for Mungbean Genotypes Resistance to South Indian Bruchid Strain

The initial screening for identifying bruchid-resistant genotypes was made in a set of 52 mungbean genotypes. The genotypes comprised the high frequency of light green seeds with shiny lustre, whereas few genotypes possessed greenish-yellow or dark green seeds with dull lustre. The proportion of oval and drum-shaped seeds was almost equal among the mungbean genotypes. First, we observed the egg deposition in all the mungbean genotypes used in the study. There was no significant difference in egg deposition among the mungbean genotypes of various sizes and shapes. The egg distribution also revealed no difference among seeds with dull and shiny lustres. It showed that the seed texture had no role in preventing the pest from laying eggs. AVRDC [34] reported that the texture layer could not prevent mungbean seeds from the damage caused by *C. maculatus* and *C. chinensis*. Singh and Singh [35] also reported that the seed coat texture of cowpea could not be considered a reliable trait in breeding against *C. maculatus*. Edde and Amatobi [36] reported that the type of seed coat (Wrinkled or smooth) had no effect on the ovipositional intensity of *C. maculatus* on cowpea. Hence, it is concluded that none of the seed traits, *viz.*, seed colour, seed shape, and seed lustre prevented the bruchid infestation in mungbean.

Further, the seed damage is measured by observing the following traits *viz.*, the number of eggs laid, adult emergence percentage, mean developmental period, female to male ratio, susceptibility index, and seed damage percentage (%). Results showed that no adult emergence and seed damage were reported in two genotypes (V2709 and V2802BG). In contrast, the maximum number of adult emergence, with 100% seed damage, was found in the remaining 50 mungbean genotypes. The adult emergence percentage, mean developmental period, female to male ratio, and susceptibility index of 50 highly susceptible genotypes ranged from 28.99 to 66.67%, 23.75 to 31.50 days, 0.33–3.00, and 0.050–0.078, respectively. Similar findings for adult emergence percentage, mean developmental period, and susceptibility index were reported by Soumia et al. [24] in mungbean infested with *C. maculatus*. A lower number of females than males of *C. maculatus* in mungbean was reported by Bashir et al. [37] and Sharma et al. [38]. The egg-laying and hatching were observed in the resistant (V2802BG and V2709) and all remaining susceptible genotypes. This indicates that the antixenosis mechanism exhibited by seed traits *viz.*, seed colour, seed lustre, and seed shape had no role in imparting resistance against bruchids and coupled with the results of Seram et al. [27].

4.2. Breeding Resistant Lines with Better Agronomic Performances

To develop the widely adopted resistant lines, two resistant genotypes (V2709 and V2802BG) were crossed with six high-yielding cultivars (CO 5, CO 6, CO 7, CO 8, VBN 2, and VBN 3), and 12 independent populations of F_1 were generated. Of these, one population derived from CO 6 × V2802BG was selected based on the good combining ability of the parents (CO 6 and V2802BG) for most of the promising traits and forwarded to later

generations. Good combiners will yield better recombinant progenies in later generations. Furthermore, CO 6 is the high-yielding and ruling variety in Tamil Nadu. A total of 159 $F_{2:3}$ families were examined for bruchid resistance. The results revealed that seven F2:3 families were highly resistant with 0% seed damage, and the rest were resistant to highly susceptible. The percentage of seed damage varied from 0 to 100%, with a mean of 44.4%. Similarly, the F_2 population derived from Kamphaeng Saen 2 (Susceptible) × ACC41 (Resistant) exhibited 0 to 100% seed damage (*C. maculatus*) with a mean of 46.30% [39]. The $BC_{11}F_2$ population derived two crosses, KPS1 × V2802 [40] and KPS 1 and V2709 [41], also recorded 0 to 100% seed damage (*C. maculatus*) with a mean of 48.58% and 44.60%, respectively. Chen et al. [42] also recorded 0 to 100% seed damage in F_{12} RILs derived from the cross NM92 × TC1966. The F_{10} population of the cross Berken × ACC41 also recorded 0 to 100% seed damage with a mean of 46.5% [43]. Further, we evaluated the seven $F_{2:3}$ families in the advanced generations (F_4 and F_5) and found five highly resistant lines (BSR-GG-1-49-3-1, BSR-GG-1-56-2-2, BSR-GG-1-160-5-3, BSR-GG-1-170-2-4, and BSR-GG-1-198-1-4) in the F_5 generation.

4.3. Development of Grub and Agronomic Performance of the Resistant Lines

When we compare the development of grub in resistant lines with the parental lines, it showed that the underdevelopment of grub in all the five stable resistant lines was the same as that of the resistant parent, V2802BG, at the early instar level and confirmed the transfer of resistance from V2802BG to the population of CO 6 × V2802BG. These results are consistent with the reports of Somta et al. [44], who described the death (62.9%) of bruchids (*C. chinensis* and *C. maculatus*) at the first instar larval stage in undamaged seeds. The present study recorded normal growth of grub from the susceptible genotypes. In contrast, in resistant genotypes, the underdevelopment of grub and death of grub was observed at a lower instar level in the undamaged seeds. It was already discussed that there was no role of antixenosis factors in imparting resistance against *C. maculatus.* Hence, the resistance was due to the compounds in the seed's cotyledon. Antibiosis resistance resulted in grub mortality, disturbance in the life cycle, reduction in fecundity, and insect fertility [45,46]. Plant morphological traits and some chemical factors are responsible for the antibiosis mechanism of host plants against insects [46,47]. Edwards and Singh [48] and Eduardo et al. [49] reported antibiosis as an effective defense strategy exhibited by the legumes against stored seed insect pests. The *C. maculatus* grub with morphometric measurements *viz.*, length (3.64 mm) and breadth (2.00 mm) reared on mungbean was reported by Devi and Devi [50]. The fate of *C. maculatus* during development is determined by the biochemical factors operating after hatching and commencement of feeding by the developing grub [51–53]. The antibiosis mechanism of resistance due to the presence of toxic secondary metabolites in mungbean was reported by AVRDC [54] and Talekar and Lin [55]. The antibiosis mechanism of resistance against bruchids in various legume crops was reported by several researchers Seram et al. [27], Souframanien and Gopalakrishna [56], Castro et al. [57], Kaur et al. [58], Miesho et al. [59], Grazziotin et al. [60], Jaba et al. [61] and Caroline et al. [62]. Furthermore, the stable resistant lines recorded a comparable yield to the CO6. The five resistant lines in the F_5 generation showed good agronomic performance like high-yielding parent CO 6. An agronomic performance similar to parents in the F_5 generation was reported by Krisnawati et al. [63].

5. Conclusions

In summary, we have successfully developed the bruchid resistance lines with good agronomic performance. Furthermore, in the present study, the mechanism of bruchid resistance is described as antibiosis. The resistant lines developed in this study could be evaluated in multi-location trials and then exploited as a budding source of genetic material for improving bruchid resistance in mungbean breeding programs.

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