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# Evaluation of Screening Methods for Bruchid Beetle (*Callosobruchus chinensis*) resistance in Greengram (*Vigna radiata*) and Blackgram (*Vigna mungo*) genotypes and influence of seed physical characteristics on its infestation

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**Abstract** Bruchid beetle, *Callosobruchus* spp. (Coleoptera: Bruchidae) cause substantial reduction in quantity and quality of legumes during storage in tropical and sub-tropical regions of the world. *C. chinensis* is the most important bruchid in Asia and causes considerable damage to *Vigna* seeds. Four hundred seventy five accessions of two *Vigna* species, [335 of *Vigna radiata* (greengram) and 140 of *Vigna mungo* (blackgram)] were initially evaluated for resistance to bruchid beetle through 'free choice' test. Eight greengram and nine blackgram resistant accessions were further evaluated under lab conditions using 'no choice' test. Four greengram accessions (LM 131, V 1123, LM 371 and STY 2633) were found moderately resistant with less percentage survival (38.9 to 51.6%) and prolonged developmental period (30.5 to 31.5 days) as compared to susceptible check (90.8% survival and 26.1 days of developmental period). Similarly in blackgram, three accessions (UH 82-5, IC 8219 and SPS 143) were moderately resistant with less percentage survival (33.7 to 42.0%) and prolonged developmental period (26.8 to 28.1 days) as compared to susceptible check (83.2% survival and developmental period of 24.2 days). Physical characteristics of the seeds were related with the ovipositional preference of the bruchid. In greengram, lesser number of eggs were recorded in small and shiny seeds as compared to large and dull seeds while in blackgram, small and black seeds recorded lower number of eggs as compared to large and green seeds. In this study, 'no choice' test seemed to be most reliable and potentially useful for screening of bruchid resistance. Since, no cultivated germplasm exhibited complete resistance, the moderately resistant genotypes identified in the present study hold promise in breeding programme of greengram and blackgram.

**Keywords:** Coleoptera, Bruchidae, Legumes, Mungbean, Urdbean, Germplasm, Host plant resistance

## Introduction

Greengram [*Vigna radiata* (L.) Wilczek] and blackgram [*Vigna mungo* (L.) Hepper] belonging to genus *Vigna,* subgenus *Ceratotropis* are important grain legumes grown widely in South and Southeast Asia. These crops are grown in India, Pakistan, China, Myanmar, Bangladesh, Thailand, Philippines, Malaysia, Kenya, Malawi and United States. These low input, short duration crops are prized for their seeds, which are high in protein, easily digested, and consumed as food in various culinary preparations. Both these crops are important source of dietary protein, especially in the Indian Sub-continent dominated by vegetarian diet. In India, these crops together are grown on 6.62 million ha area with a production of 3.40 million tonnes of grains in 2011-12 (DAC 2013).

Bruchid beetle, *Callosobruchus* spp. (Coleoptera: Bruchidae) cause substantial reduction in quantity and quality of legumes during storage in tropical and sub-tropical regions of the world (Duraimurugan *et al.* 2011). *C. chinensis* is the most important bruchid in Asia and causes considerable damage to *Vigna* seeds. Infestation starts in the field and carried to store, where sometimes it causes total destruction of the seeds within six months (Srinivasan *et al.* 2010). The dam-

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age is caused by the grubs, which feed on the entire grain contents leaving only the shell behind. The seed thus loses its viability and the grain is rendered unfit for human consumption. The available pest management methods do not provide the expected levels of control, beside the high cost and pesticide residue issues. Developing host resistance to bruchids in improved cultivars of greengram (mungbean) and blackgram (urdbean) remain an economically viable option to reduce heavy post-harvest losses. To date, no resistant varieties in these crops have been developed because of lack of reliable resistance sources against bruchids and a reliable rapid method of screening.

A very limited number of bruchid resistant genotypes have been identified in greengram, but none in blackgram. Talekar and Lin (1992) reported moderate to high resistance to *C. chinensis* in cultivated mungbean germplasm lines, V2709 and V2802. Similarly Somta *et al*. (2008) also identified two such accessions, V1128 and V2817. An accession, TC 1966, of wild mungbean (*Vigna radiata* var. *sublobata*) was first reported as resistant against bruchids (Fujii and Miyazaki 1987), while two other accessions of this species, ACC 23 and ACC 41 were also reported from Australia (Lambridges and Imrie 2000). To accelerate breeding efforts for developing bruchid resistant cultivars, new sources of resistance need to be identified in cultivated *Vigna* species.

Seed characteristics like size, colour, lustre, etc. are known to affect resistance or preference of bruchids. Bruchid species predation is considered a selection criterion for smaller seeds in wild legumes (Janzen 1969, Winn 1988). The explanation for such information is useful in preparing selection indices for developing bruchid resistant cultivars. One objective of the present study was to develop efficient method of screening genotypes for bruchid resistance which would work well in eliminating susceptible genotypes in large numbers of germplasm or early-generation lines. Thus, the experiments were conducted for identification of new sources of resistance in 475 accessions of two *Vigna* species against *C. chinensis* using 'free choice' and 'no-choice' techniques.

## Materials and Methods

### Source of seeds

Three hundred thirty five accessions of greengram and one hundred forty accessions of blackgram comprising germplasm lines, released varieties and breeding material obtained from Gene Bank of Indian Institute of Pulses Research, Kanpur were used in the study. Seeds of these accessions were grown on main research farm of the Institute from August to October 2010. Plants were grown using normal recommended cultivation practices with an exception that no insecticides were used throughout the growing period. Seeds produced from these plants were used for evaluation of bruchid resistance.

### Insect culture

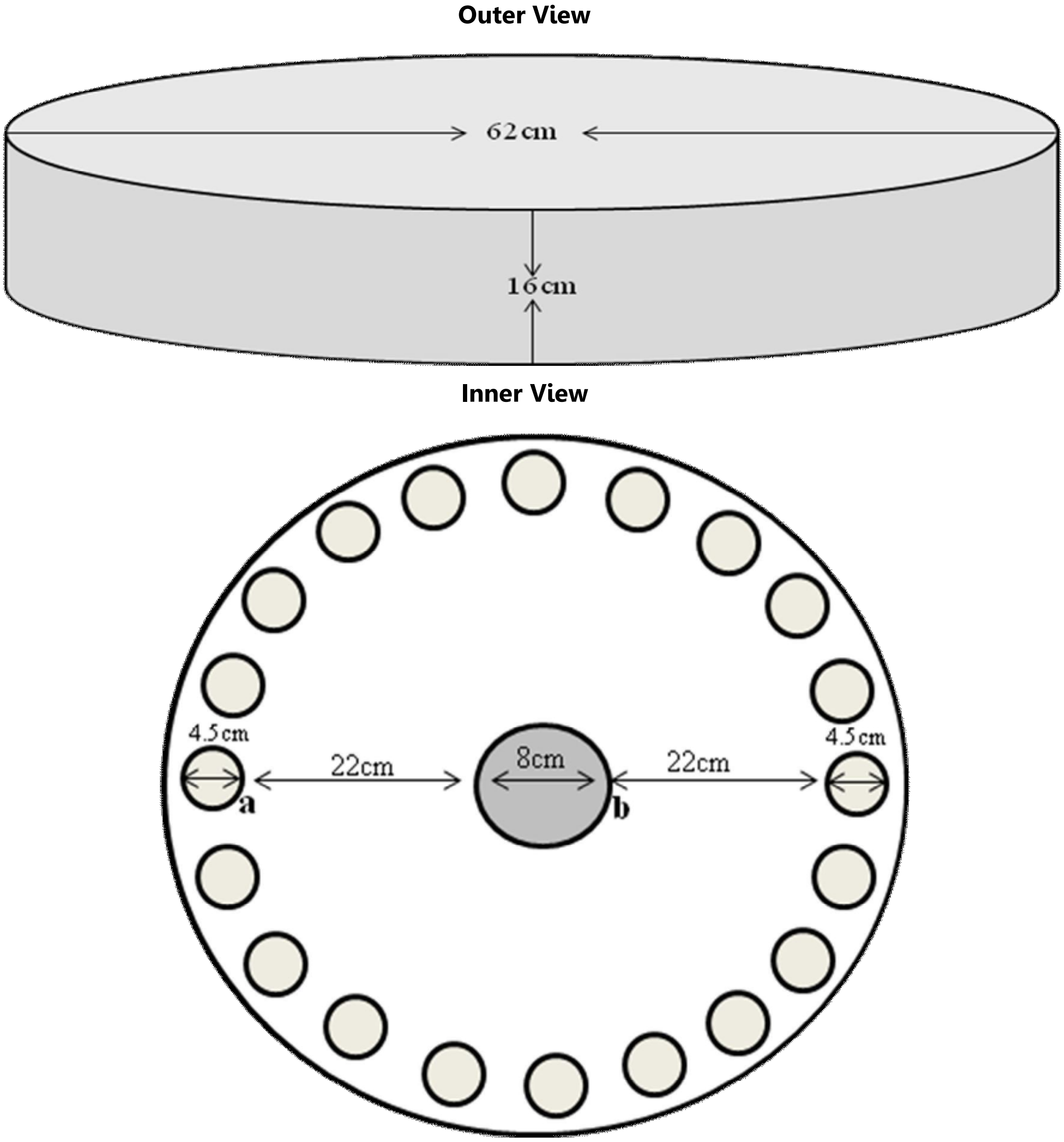
Bruchid population generally comprises of two species *viz., C. chinensis* and *C. maculatus.* Pure culture of *C. chinensis* was obtained from Crop Protection Division of Indian Institute of Pulses Research, Kanpur and mass reared on fresh greengram seeds at 30±2$^{o}$C and 70±5% relative humidity. Subculturing of this beetle was done at regular intervals so as to maintain a continuous supply of insects for experiments throughout the period of study.

### Bioassay for bruchid resistance

**(i) 'Free choice' test**:

Three hundred thirty five greengram and one hundred forty blackgram accessions were screened by 'free choice' test for their comparative resistance to *C. chinensis* under laboratory conditions (30±2ºC and 70±5% RH). The 'free choice' test was conducted by putting 50 seeds of each accession in 20 separate circular disk-like craters (4.5cm diameter and 1cm depth) carved out in the outer periphery of the wooden bottom of a specially designed cylindrical cage (62cm diameter and 16cm height) (Fig. 1). After arranging the seeds onto the circular craters inside the cage, 100 pairs of freshly emerged adult were released at the centre of the cage and covered with muslin cloth. The insects were allowed to remain there for the purpose of oviposition for three days. Three days after the release of adult beetles, seeds with eggs were recorded and transferred to a plastic container (6cm x 4cm, height x diameter). As this was a preliminary screening, the experiment was done unreplicated. The data on per cent survival of adults, mean developmental period and the susceptibility index were worked out as described by Howe (1971). The accessions were categorized based on this index as resistant (< 0.05), mod-

Fig 1. Circular wooden cage fabricated for conducting 'free choice' test (a - Arena for keeping seed, 20 accessions can be kept for screening at a time; b - Arena for releasing insect)




erately resistant (0.051 – 0.060), moderately susceptible (0.061 – 0.070), susceptible (0.071 – 0.080) and highly susceptible (> 0.081) as per Howe (1971) and Sulehrie *et al.* (2003).

**(ii) 'No choice' test**:

Eight greengram and nine blackgram accessions having low susceptibility index (resistance) in the preliminary screening under 'free choice' test were selected for further confirmation for resistance under 'no choice' test in a completely randomized design (CRD) with three replications. In the 'no-choice' test, twenty five seeds of each entry were kept separately in a plastic container (6cm x 4cm, height x diameter) and air circulation was provided through a 1cm diameter hole in the lid, that is covered with nylon mesh. Male and female bruchids were sexed morphologically as described by Raina (1970). Three pairs of well characterised and freshly emerged male and female adults of bruchid (*C. chinensis*) were released in the plastic container and allowed for oviposition. The insects were allowed to remain there for the purpose of oviposition for three days, and were subsequently removed. Three days after the release of adult beetles, observations were recorded on the number of eggs laid. Infested seeds were kept under laboratory conditions at 30±2ºC and 70±5% RH. The number of emerged adults and damaged seeds were counted daily. The observations were continued for next 40 days. Seeds that showed no surface damage at the end of evaluation were further examined by

Table 1. Reaction of greengram and blackgram accessions to bruchid (*C. chinensis*) on the basis of 'free choice' test

| Classification | Susceptibility index | Number of greengram accessions | Number of blackgram accessions |
|---|---|---|---|
| Resistant | < 0.05 | 3 | 4 |
| Moderately resistant | 0.051-0.060 | 5 | 5 |
| Moderately susceptible | 0.061-0.070 | 47 | 29 |
| Susceptible | 0.071-0.080 | 155 | 77 |
| Highly susceptible | >0.081 | 125 | 25 |
| Total | | 335 | 140 |

soaking them in water and then cut open for examination under stereo-microscope. The data on number of eggs laid, per cent survival of adults, mean developmental period and the susceptibility index (Howe, 1971) were worked out as described already in the sub-section 'free choice' test.

**Comparative assessment of bruchid infestation on the basis of seed traits**

To know the effect of certain physical characteristics of the seeds on bruchid infestation, greengram and blackgram accessions having variable seed size, seed lustre and seed colour were evaluated using 'free choice' test under laboratory conditions (30±2° C and 70±5% RH). Accessions for small, medium and large seed size category were assessed for bruchid resistance. Beside seed size, seed lustre was also taken into consideration. In greengram, five accessions each with shiny and dull seeds were selected and evaluated for bruchid resistance while in blackgram, five accessions each of black and green seed coat colour types were evaluated by keeping the seeds inside a wooden cage as described earlier. Fifty seeds of each accession were kept on separate craters. Fifty pairs of freshly emerged adults were released at the centre of the cage and allowed for oviposition. All observations were made as described earlier under free choice test.

**Statistical analysis**

Data collected for 'no choice' test were analyzed as per Steel and Torrie (1980). Upon significance of F-test for treatment difference, a mean separation was performed using Duncan's Multiple Range Test at P=0.05. Correlations between biological parameters of bruchid on all accessions of greengram and blackgram were determined using Pearson's Correlation Coefficients.

## Results and Discussion

### Screening of greengram and blackgram accessions for resistance to bruchid

**'Free choice' test :** A total of 335 accessions of green gram and 140 of blackgram were subjected to 'free choice' test. These accessions were categorised and grouped on the basis of their susceptibility index (Table 1). Since susceptibility index of each accession was based on reaction of bruchids in respect of their biological parameters like oviposition, developmental period and adult emergence, it provided the preliminary evaluation data of each accession. The susceptible check ML 552 of green gram and PLU 158 of blackgram recorded susceptibility index of more than 0.075. In case of green gram, 97.61% accessions were placed in susceptible category while in blackgram 93.57% accessions exhibited susceptible reaction. This indicates that greengram is more susceptible to bruchids in comparison to blackgram. Comparative resistance to bruchids among several species of *Vigna* subgenus *Ceratotropis* were reported by Tomooka *et al.* (2000). Only eight accessions of green gram and nine of black gram exhibited resistant reactions with susceptibility index of less than 0.060. These accessions were subjected to detailed screening using 'no choice' test for the confirmation of their reactions against bruchid.

**'No choice' test :** Eight greengram accessions found promising among the 335 accessions through the 'free choice' test were screened further by 'no-choice' test to assess their reaction to bruchid (*C. chinensis*). The oviposition, developmental period and percent survival of bruchids significantly differed between different greengram accessions (Table 2). The accessions STY 2633, LM 131 and LM 371 recorded significantly less number of eggs (11.7, 14.0 and 16.0 per 25 seeds) while WAGHOLT and PLM 156 recorded the highest number of eggs (32.3 and 30.3 per 25 seeds). The susceptible check genotype ML 552 registered an egg laying of 27.3 per 25 seeds. The accession LM 131 followed by LM 371, STY 2633 and V 1123 exhibited longer mean developmental period (31.5, 30.9, 30.7 and 30.5 days, respectively) as against the least developmental period of 26.1 days in the sus-

Table 2. Assessment of bruchid (*C. chinensis*) resistance in selected greengram (*Vigna radiata*) and blackgram (*V. mungo*) accessions using 'no choice' test

| Accession | No. of eggs / 25 seeds | Developmental period (days) | No. of adults emerged | % Survival | Susceptibility Index | Category |
|---|---|---|---|---|---|---|
| Greengram (*Vigna radiata*) | | | | | | |
| LM 131 | 14.0 ± 4.50[a] | 31.5 ± 0.52[a] | 6.3 ± 2.84[a] | 41.8 ± 5.51[a] | 0.051 | Moderately resistant |
| V 1123 | 27.0 ± 7.00[bc] | 30.5 ± 0.18[ab] | 10.0 ± 1.73[a] | 38.9 ± 3.30[a] | 0.052 | Moderately resistant |
| LM 371 | 16.0 ± 7.50[a] | 30.9 ± 0.36[ab] | 6.0 ± 2.51[a] | 39.1 ± 2.71[a] | 0.052 | Moderately resistant |
| STY 2633 | 11.7 ± 2.72[a] | 30.7 ± 0.23[ab] | 6.3 ± 2.33[a] | 51.6 ± 7.17[ab] | 0.055 | Moderately resistant |
| V 3651 | 19.0 ± 7.21[ab] | 28.9 ± 0.54[c] | 11.3 ± 4.25[ab] | 59.8 ± 0.65[abc] | 0.061 | Moderately susceptible |
| WAGHOLT | 32.3 ± 6.56[c] | 29.4 ± 0.47[c] | 20.3 ± 3.48[bc] | 65.7 ± 13.04[bc] | 0.062 | Moderately susceptible |
| LM 1021 | 19.0 ± 1.52[ab] | 29.0 ± 0.11[c] | 12.0 ± 3.05[ab] | 61.4± 10.46[abc] | 0.062 | Moderately susceptible |
| PLM 156 | 30.3 ± 4.25[c] | 29.7 ± 0.25[bc] | 25.0 ± 6.65[c] | 78.9 ± 12.33[cd] | 0.063 | Moderately susceptible |
| ML 552 (C) | 27.3 ± 3.33[bc] | 26.1 ± 0.11[d] | 24.3 ± 0.88[c] | 90.8 ± 7.24[d] | 0.075 | Susceptible |
| SEd | 4.45 | 0.92 | 4.88 | 11.4 | - | - |
| CD (*P* = 0.05) | 9.34 | 1.04 | 10.27 | 23.9 | - | - |
| Blackgram (*Vigna mungo*) | | | | | | |
| IC 8219 | 9.0 ± 1.52[a] | 26.8 ± 0.44[bc] | 3.0 ± 0.57[a] | 33.7 ± 5.15[a] | 0.057 | Moderately resistant |
| UH 82-5 | 20.0 ± 1.15[bc] | 28.1 ± 0.19[a] | 7.0 ± 0.57[ab] | 35.4 ± 4.54[a] | 0.055 | Moderately resistant |
| SPS 143 | 32.0 ± 1.52[e] | 27.7 ± 0.34[ab] | 13.3 ± 1.33[cd] | 42.0 ± 5.09[a] | 0.058 | Moderately resistant |
| UH 84-1 | 12.0 ± 2.30[a] | 25.9 ± 0.71[cd] | 5.0 ± 0.57[a] | 43.1 ± 3.67[a] | 0.063 | Moderately susceptible |
| SPS 35 | 14.7 ± 4.63[ab] | 24.5 ± 0.06[ef] | 10.0 ± 3.21[bc] | 67.0 ± 6.24[b] | 0.075 | Susceptible |
| PLU 25 | 27.3 ± 3.52[cde] | 24.3 ± 0.10[ef] | 21.0 ± 1.52[fg] | 78.3 ± 7.07[bc] | 0.078 | Susceptible |
| PLU 99-52 | 30.3 ± 1.45[de] | 25.2 ± 0.06[de] | 21.3 ± 0.88[fg] | 70.6 ± 3.78[bc] | 0.073 | Susceptible |
| PLU 96-6 | 23.7 ± 1.45[cd] | 25.1 ± 0.44[def] | 20.0 ± 1.15[ef] | 84.6 ± 0.68[c] | 0.077 | Susceptible |
| L 23 | 20.7 ± 2.02[bc] | 24.3 ± 0.12[ef] | 16.7 ± 1.76[de] | 81.4 ± 8.24[bc] | 0.079 | Susceptible |
| PLU 158 (C) | 30.7 ± 2.96[de] | 24.2 ± 0.03[f] | 25.0 ± 0.57[g] | 83.2 ± 9.14[bc] | 0.080 | Susceptible |
| SEd | 3.55 | 0.47 | 2.04 | 8.31 | - | - |
| CD (*P*=0.05) | 7.42 | 0.98 | 4.27 | 17.34 | - | - |

All values represent mean of three replications, Values represent Mean ± Standard Error; C- Check
Means in a column followed by different letter(s) are significantly different at *P*=0.05

Table 3. Correlation coefficients (r) between different biological parameters of bruchid and susceptibility index in greengram (*Vigna radiata*) and blackgram (*V. mungo*) accessions[#]

| Biological parameters of bruchid and Susceptibility index | Egg laying | Developmental period | Percentage survival | Susceptibility Index |
|---|---|---|---|---|
| Egg laying | $_{bg}r^{gg}$ | $-0.47^{NS}$ | $0.57^{NS}$ | $0.53^{NS}$ |
| Developmental period | $-0.15^{NS}$ | $_{bg}r^{gg}$ | -0.85** | -0.96** |
| Percentage survival | $0.47^{NS}$ | -0.88** | $_{bg}r^{gg}$ | 0.96** |
| Susceptibility Index | $0.36^{NS}$ | -0.95** | 0.98** | $_{bg}r^{gg}$ |

NS-Non-significant * - Significant at 5% level ** - Significant at 1% level

[#]Correlation values for greengram (gg: upper diagonal) and blackgram (bg: lower diagonal)

ceptible check. Similarly, the percentage survival exhibited noticeable differences in V 1123, LM 371 and LM 131 registering lesser survival (38.9, 39.1 and 41.8%, respectively) as compared to susceptible check (ML-552) which showed a survival of 90.8%. Based on the observations on oviposition, percentage survival and developmental period and susceptibility index indicated that none of the accessions was completely resistant to bruchids. Nevertheless, four accessions *viz*., LM 131, V 1123, LM 371 and STY 2633 were categorized as moderately resistant (susceptibility index of 0.051, 0.052, 0.052 and 0.055, respectively) while the remaining four accessions (V 3651, WAGHOLT, LM 1021, PLM 156) were categorised as moderately susceptible.

Nine black gram accessions that were found promising among 140 accessions in the 'free choice' test were also screened for 'no choice' test along with the susceptible accession (PLU-158). Significantly less egg laying was noticed in IC 8219 (9.0 per 25 seeds) and UH 84-1 (12.0 per 25 seeds) while maximum egg laying was observed in SPS 143 (32.0 per 25 seeds). Accession UH 82-5 followed by SPS 143 registered significantly longer developmental periods (28.1 and 27.7 days, respectively) as compared to PLU 158 (24.2 days). Interestingly, the percent survival showed a different trend with IC 8219, UH 82-5, SPS 143 and UH 84-1 recording the least survival percentage (33.7 to 43.1%)

Table 4. Influence of physical characteristics of the seed on the infestation of bruchid in greengram and blackgram.

| Classification | No. of eggs / 50 seeds | Developmental Period (days) | Adult emergence (%) |
|---|---|---|---|
| Influence of seed size on bruchid infestation in greengram | | | |
| Small | 62.6 ± 3.8 | 23.8 ± 0.2 | 78.4 ± 3.2 |
| Medium | 68.5 ± 5.3 | 23.9 ± 0.1 | 82.9 ± 3.4 |
| Large | 85.0 ± 2.9 | 24.4 ± 0.1 | 90.4 ± 3.5 |
| Influence of seed lusture on bruchid infestation in greengram | | | |
| Shining | 69.9 ± 3.9 | 23.9 ± 0.1 | 74.3 ± 2.7 |
| Dull | 75.3 ± 2.3 | 23.7 ± 0.1 | 80.7 ± 1.0 |
| Influence of seed size on bruchid infestation in blackgram | | | |
| Small | 22.0 ± 1.6 | 24.9 ± 0.4 | 72.5 ± 7.9 |
| Medium | 49.0 ± 4.9 | 25.1 ± 0.3 | 72.2 ± 5.8 |
| Large | 73.8 ± 1.4 | 25.3 ± 0.3 | 74.6 ± 1.2 |
| Influence of seed colour on bruchid infestation in blackgram | | | |
| Black | 40.5 ± 5.4 | 24.9 ± 0.1 | 68.2 ± 5.8 |
| Green | 85.8 ± 3.3 | 25.6 ± 0.4 | 74.1 ± 5.3 |

Values represent Mean ± Standard Error

while the check (PLU 158) registered the maximum percentage survival (83.2%). Based on the index of susceptibility, three accessions *viz.,* UH 82-5, IC 8219 and SPS 143 (susceptibility index of 0.055, 0.057 and 0.058, respectively) were found to be moderately resistant. The accession UH 84-1 (susceptibility index of 0.063) was found to be moderately susceptible while the remaining five accessions (SPS 35, PLU 25, PLU 99-52, PLU 96-6 and L 23) were found susceptible.

**Correlations between biological parameters of bruchid and susceptibility index**

In order to assess the important biological parameters affecting bruchid resistance, correlation coefficients were worked out among different biological parameters *viz*., egg laying, developmental period, adult emergence, percent survival and susceptibility index (Table 3). The egg laying did not show significant correlation with any other parameters in both green gram and black gram. This suggests that egg laying alone can not be considered for determining the criteria for selecting the genotypes for bruchid resistance. Dick and Credland (1984) observed that oviposition preference is influenced by host availability to a greater extent and has nothing to do with the actual resistance nature of an accession. The reduction in per cent adult emergence is an indication of the presence of unfavourable chemical constituents inside the cotyledons. Further, such unfavourable chemicals present inside a seed directly affect the development of a growing grub resulting in prolongation of developmental period (Dongre *et al*. 1996). Sulehrie *et al*. (2003) also observed that a delay in developmental period may lead to a considerable reduction in seed loss during storage. This is in conformity with the present observations of significant negative correlation of susceptibility index with developmental period and significant positive correlation with per cent survival in resistant greengram and blackgram accessions. Greengram accessions PLM 262 and PLM 89 were reported as resistant to *C. maculatus* based on susceptibility index (Srinivasan and Durairaj 2007). Four greengram (LM 131, V 1123, LM 371, STY 2633) and three blackgram (IC 8219, UH 82-5, SPS 143) accessions identified in the present study have potential for use in conventional breeding programs as well as hybridization on account of their favourable traits *viz*., lesser percent survival and prolonged developmental period.

**Effect of physical characteristics of the seeds on bruchid infestation**

The effect of physical characteristics of the seeds (size, colour and lustre) were studied on oviposition, developmental period and per cent emergence of bruchids (*C. chinensis*) (Table 4). In greengram, less numbers of eggs and lower percentage of emergence was recorded in small (62.6 per 50 seeds and 78.4% emergence) and shiny seeds (69.9 per 50 seeds and 74.3% emergence) as compared to the large (85.0 eggs and 90.4% emergence) and dull (75.3 eggs and 80.7% emergence) seeds. Preference of female for egg laying in large and dull seeds could be possibly due to the ease for settling of adults for ovipoistion. Similarly in blackgram, small sized (22.0 eggs per 50 seeds) and black colour (40.5 eggs per 50 seeds) seeds recorded lower number of eggs as compared to 73.8 eggs in large seeds and 85.8 eggs in green coloured seeds. However, it did not show much variation in developmental period and percent adult emergence. Jason and Charles (2003) reported that bruchid females are better at judging seed mass and distribute their eggs in a manner that maximize the amount of resources per offspring. Rathore and Chaturvedi (1997) also found that larger seeds of chickpea were ovipoisited with more number of eggs by *C. chinensis* as compared to smaller seeds. The results of the current study corroborate the above findings. Chavan *et al*. (1997) observed that dark coloured seeds (black, greyish mosaic, red and brown) were more preferred for oviposition than the white coloured lines in cowpea. In contrast, ovipositional preference for green coloured seeds compared to black coloured seeds of blackgram accessions observed in the present study may be due to the relatively better camouflaging for egg protection on green coloured seed compared to black coloured seed. It is evident from above information that significant variability exists in greengram and blackgram germplam against bruchid resistance and the less susceptible genotypes may be utilized in crop improvement programme.

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