

## Original Research Article

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## Bruchid Resistance Studies in Advanced Stabilized F<sub>6</sub> Lines Derived From Interspecific Hybridization between Cowpea and Rice Bean

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### ABSTRACT

#### Keywords

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Cowpea [*Vigna unguiculata* (L.) Walp.] is an important tropical legume crop, its grain contains about 25% protein and 64% carbohydrate, therefore, it is also called as a vegetable meat. In storage, *Callosobruchus maculatus*, also called cowpea beetle, cowpea weevil or bruchid, is regarded as the most important and common pest of cowpea both in Africa and Asia. All vigna crops except rice bean are susceptible to bruchids. So in this context in the present study the 100 stabilized families in F<sub>6</sub> generation of cross between cowpea and rice bean were evaluated. The segregants F<sub>6</sub> -229 and F<sub>6</sub> -239 were identified as a resistant to bruchids since these have a less bruchid infestation (%) with values of 8.5% and 12.5% respectively.

### Introduction

Cowpea [*Vigna unguiculata* (L.) Walp.] is an important tropical legume crop, its grain contains about 25% protein and 64% carbohydrate, therefore, has a tremendous potential to contribute to the alleviation of malnutrition among resource-poor farmers, so it provides nutritious grain and an inexpensive source of protein for both rural poor and urban consumers. Meanwhile, it is more important as the source of green as well as dry fodder.

Cowpea has the ability to do well even in the drought conditions. However, cowpea production is faced with a wide range of biotic constraints like virus (Cowpea Aphid-Borne Mosaic Virus, CABMV), Pest like bruchids, aphids, pod borer etc. (Singh, 2005).

In storage, *Callosobruchus maculatus*, also called cowpea beetle, cowpea weevil or bruchid, is regarded as the most important and common pest of cowpea both in Africa and Asia (Jackai and Daoust, 1986; Deshpande *et al.*, 2011). This weevil has caused losses both in quality and quantity of the stored seeds. An estimate of storage losses of 90-100% is evident (Umeozor, 2005). Due to perforations by this weevil, thus reducing the degree of usefulness and making the seeds unfit either for planting or human consumption (Ali *et al.*, 2004). The control of this storage grain pest carried out by chemical methods is not viable since it cause harmful effect to human as well as to the environment. In this regard alternative methods could be the development of the resistance varieties which are actually economically viable, environmentally safe,

ecologically stable and long lasting (Lale *et al.*, 1996). Legume researchers have been seeking for sources of bruchid resistance in both cultivated and wild vigna species. The sources of resistance in the primary gene pool of cultivated cowpea is very limited. All vigna crops except rice bean are susceptible to bruchids. Rice bean is one crop under vigna genus which is completely resistant to bruchids. Hence, earlier Dr. S. K. Deshpande, Cowpea breeder, University of Agricultural sciences, Dharwad during the year 2014 had attempted interspecific crosses between Cowpea and Rice bean and advanced the generations till F<sub>5</sub>. So in the present study an attempt was made to evaluate 498 F<sub>5</sub> families under field condition for yield traits and based on yield *per se* 100 best F<sub>6</sub> families were screened for bruchid resistance under artificial infestation in laboratory.

### Materials and Methods

The experiment was conducted during *kharif* 2017 at Botanical garden, Department of Genetics and Plant Breeding, University of Agricultural Sciences, Dharwad. The F<sub>5</sub> population generated earlier between cross DC 615 (Susceptible to bruchids) × Rice bean (Resistant to bruchids) was used for the present study. About 498 F<sub>5</sub> families were selfed and advanced to F<sub>6</sub> generation. Based on yield *per se* top 100 F<sub>6</sub> progenies were selected and used in the present study. Two checks of cowpea such as DC 15 (Moderately resistant check) and DCS 47-1 (susceptible check) and three rice bean checks (Dharwad local and ricebean germplasms IC-524075 and IC-341983) were also used.

Initial culture of *Callosobruchus maculatus* was obtained from the infected grains of cowpea from the farm stores. They were reared and maintained on cowpea by releasing ten pairs of adults (males and females) in stem jars. The mouth of the jar was covered with

muslin cloth secured by rubber band. The artificial screening of the cowpea seeds against bruchids was undertaken according to the procedure followed by Basavaraj (2010). Seeds of each progeny weighing 20 g were kept in a plastic glass of 100 ml capacity, six pairs of adults (males and females) were introduced in each plastic glass and tops were kept covered with muslin cloth and tightly concealed by rubber bands. After seven days adults were removed and were kept under normal room temperature and humidity for a period of 45 days. After the specified period of 45 days each bottle was examined for loss of weight in terms of actual weight loss and apparent weight loss was determined.

Actual weight loss was calculated by using the following formula (Adams and Schulten, 1978).

$$\text{Actual weight loss (\%)} = \frac{U (N_d) - D (N_u)}{U (N_u + N_d)} \times 100$$

Where;

(N<sub>d</sub>) = Number of damaged seeds [seeds showing emergent holes].

(N<sub>u</sub>) = Number of undamaged seeds.

(D) = Weight of damaged seeds.

(U) = Weight of undamaged seeds.

(N<sub>d</sub> + N<sub>u</sub>) = Total number of seeds in 20 g.

Apparent weight loss was worked out using the following formula (Girish *et al.*, 1975).

$$\text{Apparent weight loss (\%)} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100$$

In general,

$$\text{Apparent weight loss (\%)} = \frac{W_i - W_f}{W_i} \times 100$$

Where,

$W_i$  = Initial weight of seeds at the beginning experiment

$W_n$  = Weight of seeds after 'n' days (45 days)

The moisture content of seeds was determined by the oven dry ( $103 \pm 1$  °C for  $17 \pm 1$  hours) method as per ISTA rules. The moisture content on wet basis was determined and expressed in percentage using the following formula.

$$\text{Moisture content (\%)} = \frac{M_2 - M_3}{M_2 - M_1} \times 100$$

Where,

$M_1$  – Weight of the empty metal box (g)

$M_2$  – Weight of metal box with seed sample before drying (g)

$M_3$  – Weight of metal box with seed sample after drying (g)

The percentage infestation was calculated by using following formula

$$\text{Infestation (\%)} = \frac{\text{Number of seeds with emergent holes}}{\text{Total number of seeds observed}} \times 100$$

Based on per cent bruchid infestation, cowpea genotypes were classified according to Nagaraj (2006). The classification is as follows.

Similarly germination percentage was also worked out before and after infestation according to paper towel germination test and reduction in germination was calculated and genotypes were compared based on these results.

$$\text{Germination (\%)} = \frac{\text{Number of normal seedlings obtained}}{\text{Total number of seeds put for germination}} \times 100$$

From the germination test, ten random seedlings from each of the genotype were carefully used for measuring seedling length. The seedling length from tip of shoot to tip of root was measured and the average length of the seedling was noted down. From the germination percentage and seedling length, Seedling vigor index was calculated by following formula as per Abdulkaki and Anderson (1973).

$$\text{Seedling vigor index} = \text{Germination (\%)} \times \text{Seedling length (cm)}$$

## Results and Discussion

Based on per cent bruchid infestation the  $F_6$  segregants were classified as highly resistant, moderately resistant, least susceptible, moderately susceptible and highly susceptible, as presented in the Table 1. As shown in Table 1, the per cent bruchid infestation varied much among the different classes, the highly resistant (HR) class recorded 12.50 per cent bruchid infestation and moderately resistant (MR), least susceptible (LS), moderately susceptible (MS), highly susceptible (HS) classes recorded 30.71 per cent, 50.45 per cent, 68.40 per cent and 85.50 per cent bruchid infestation respectively. Accordingly, top 10 resistant  $F_6$  segregants are given in the Table 2. The results revealed that,  $F_6$ -229 segregant recorded least per cent bruchid infestation (8.5 %) followed by  $F_6$ -239 (12.5 %). Among the rice bean resistant checks, Dharwad local, germplasm lines IC-524075 and IC-341983 recorded 0 per cent infestation, the variety DC 15 exhibited infestation of 23.50 per cent and considered as moderately resistant and DCS 47-1 reported 65.5 per cent

infestation, regarded as moderately susceptible. Since highly resistant have considerable amount of protein and nutritional factors like saponins, phenols, Trypsin inhibitors, chymotrypsin inhibitors, lectins and antifungal peptides or those are not favorable for insect oviposition and egg laying. These factors contribute to the less bruchid infestation in resistant genotypes. The seed moisture content of different classes of F<sub>6</sub> progenies are presented in Table 3. The highly resistant (HR) class reported 6.53 per cent moisture content. While, moderately resistant (MR), least susceptible (LS), moderately susceptible (MS) and highly susceptible (HS) classes reported 7.54 per cent, 8.99 per cent, 13.75 per cent and 15.96 per cent moisture content respectively, indicates the presence of considerable variability among different classes for seed moisture content. Similarly, seed moisture (%) of top 10 resistant F<sub>5</sub> progenies is presented in Table 4. The progeny, F<sub>6</sub>-229 contained minimum moisture (6.48 %) followed by F<sub>6</sub>-239 (6.55 %). Since there is difference among different classes, this clearly indicates that seed moisture content of genotypes influence the resistance or susceptibility against bruchids attack. While, the moderately resistant check DC 15(MR) recorded 8.65 per cent moisture, moderately susceptible check DCS 47-1 (MS) recorded 10.08 per cent moisture. Highly resistant rice bean checks Dharwad local, IC-524075 and IC-341983 have 12.35, 14.25 and 11.44 per cent moisture respectively, so mostly in these rice bean checks compared to moisture content, seed size, hard seed coat and other biochemical factors play an important role in resistance. So less moisture is associated with the resistant nature of the cowpea seeds.

The results pertaining to the per cent actual weight loss in different groups of F<sub>6</sub> lines after infestation are presented in Table 5. There was a variability existed among the different F<sub>6</sub>

lines. The highly resistant (HR) group showed 3.58 per cent actual weight loss, while moderately resistant (MR), least susceptible (LS), moderately susceptible (MS) and highly susceptible (HS) groups showed 7.26 per cent, 11.97 per cent, 16.93 per cent and 22.35 per cent actual weight loss respectively. The actual weight loss (%) of top 10 resistant lines out of 100 F<sub>6</sub> lines is given in Table 6. The results showed that the progenies F<sub>6</sub>-229 and F<sub>6</sub>-239 recorded least actual weight loss of 3.25 and 3.20 per cent respectively. Among the checks IC-341983 exhibited minimum weight loss (0.11 %), while IC-524075, Dharwad local, DCS 47-1 and DC 15 exhibited 0.744 per cent, 0.725 per cent, 6.54 per cent, and 5.36 per cent actual weight loss respectively. The minimum actual weight loss is attributed to minimum adult emergence and less seed damage. Lambrides and Imrie (2000) reported that the tolerant varieties showed the least weight loss against bruchids, which could be attributed to the small size, seed coat thickness, seed coat width and presence of well-formed texture layer on the seed. In Table 5 results indicates the apparent weight loss (%) of different classes of F<sub>6</sub> families due to infestation at 45 days after release of bruchids was minimum in the highly resistant (HR) class with 5.43 per cent apparent weight loss, while moderately resistant (MR), least susceptible (LS), moderately susceptible (MS) and highly susceptible (HS) classes reported 8.86 per cent, 13.18 per cent, 18.36 per cent and 23.82 per cent apparent weight loss respectively. Among top 10 resistant lines mentioned in Table 6 the segregant F<sub>6</sub>- 229 have less apparent weight loss with 4.9 per cent where it has values 6.4, and 7.3, in moderately resistant and susceptible checks such as DC15, DC S47-1, and 2.75, 2.3 and 2.2 percent in resistant checks of ricebean namely IC-524075, IC-341983 and Dharwad local respectively. These results are in agreement with findings of Divya *et al.*, (2012) who stated that entries which were

least favored by bruchids for oviposition, adult emergence and insect recorded less percent weight loss are resistant to bruchids.

The results pertaining to number of adults emerged out of different classes of stabilized F<sub>6</sub> lines are depicted in Table 5. High degree of variation was observed among the different stabilized F<sub>5</sub> lines. The highly resistant (HR) class reported 12.16 number of bruchids emerged, while moderately resistant (MR), least susceptible (LS), moderately susceptible (MS) and highly susceptible (HS) classes reported 31.81, 52.93, 73.82 and 95 number of bruchids emerged respectively. Considerably least number of bruchids were emerged in F<sub>6</sub> lines namely, F<sub>6</sub>-229 and F<sub>6</sub>-239 exhibited number of bruchids with 11 and 12 adults respectively (Table 6). As far as checks are concerned, checks Dharwad local, IC-524075 and IC-341983 have 0 number of bruchids adult emergence whereas, DC 15 and DCS 47-1 showed 35 and 105 adults respectively after

45 days of infestation. The reason for resistance may be due to oviposition and antibiosis to egg and larva. Adult recovery is hampered by unpalatable physicochemical characteristics of grains. The seed germination (%) at initial and 45 days after release of bruchids was recorded (Table 7). The results shows that the minimum reduction in germination percentage was seen in highly resistant class with 2.46% and it was followed by moderately resistant (MR), least susceptible (LS), moderately susceptible (MS) and highly susceptible (HS) classes reported 4.35, 6.33, 9.05 and 13.19 per cent reduction respectively. While among top 10 resistant lines (Table 8), F<sub>6</sub>- 229 have least reduction in germination per centage with value of 2.35 and among checks lowest reduction was observed for check IC-341983 (1.09%) and it was followed by IC-524075, Dharwad local, DC 15 and DCS 47-1 with values 1.10, 2.2, 6.59 and 16.67 respectively.

**Based on per cent bruchid infestation, cowpea genotypes were classified according to Nagaraj (2006)**

Class	Bruchid infestation (%)
Highly resistant	00-20
Moderately resistant	21-40
Least susceptible	41-60
Moderately susceptible	61-80
Highly susceptible	81-100

**Table.1** Classification of F<sub>6</sub> Segregants in terms of resistance to bruchid based on mean percent bruchid infestation at 45 days after infestation

Class	Range	Average infestation	No of segregants
Highly resistant	0-20	12.50	3
Moderately resistant	21-40	30.71	36
Least susceptible	41-60	50.45	37
Moderately Susceptible	61-80	68.40	20
Highly Susceptible	81-100	85.50	4

**Table.2** Bruchid infestation among the top 10 resistant and stabilized F6 lines derived from Cowpea x Rice bean crosses

Sl. No.	Segregants	% bruchid infestation
1	F <sub>6</sub> -229	8.5
2	F <sub>6</sub> -239	12.5
3	F <sub>6</sub> -127	16.5
4	F <sub>6</sub> -226	20.5
5	F <sub>6</sub> -224	21
6	F <sub>6</sub> -85	23
7	F <sub>6</sub> -44	23.5
8	F <sub>6</sub> -222	25
9	F <sub>6</sub> -193	25.5
10	F <sub>6</sub> -226	25.5
Checks		
1	DC15 (Cowpea variety)	23.5
2	DCS 47-1 (Cowpea variety)	65.5
3	IC-524075 (Rice bean germplasm accession)	0
4	IC-341983 (Rice bean germplasm accession)	0
5	Dharwad local (Local Rice bean genotype)	0

DC 15– Cowpea variety moderately resistant check

DC47-1 – Susceptible check

IC-524075- Ricebean germplasm, resistant check

IC- 341983- Ricebean germplasm, resistant check

Dharwad local- Local Rice bean genotype, resistant check

**Table.3** Mean seed moisture (%) among the different bruchid resistant classes

Class	No of segregants	Seed moisture (%)
Highly resistant	3	6.53
Moderately resistant	36	7.54
Least susceptible	37	8.99
Moderately Susceptible	20	13.75
Highly Susceptible	4	15.96

**Table.4** Seed moisture content among top 10 resistant F<sub>6</sub> segregants

Sl. No.	Segregants	Seed moisture (%)
1	F <sub>6</sub> -229	6.48
2	F <sub>6</sub> -239	6.55
3	F <sub>6</sub> -127	6.59
4	F <sub>6</sub> -226	6.66
5	F <sub>6</sub> -224	6.81
6	F <sub>6</sub> -85	6.84
7	F <sub>6</sub> -44	6.88
8	F <sub>6</sub> -222	6.99
9	F <sub>6</sub> -193	6.99
10	F <sub>6</sub> -226	7.10
<b>Checks</b>		
1	DC15 (Cowpea variety)	8.65
2	DCS 47-1(Cowpea variety)	10.08
3	IC-524075 (Rice bean germplasm accession)	14.25
4	IC-341983 Rice bean germplasm accession)	11.44
5	Dharwad local (Local Rice bean genotype)	12.35

**Table.5** Mean actual weight loss, apparent weight loss, number of bruchids emerged among bruchid resistance classes F<sub>6</sub> segregants

Class	No of segregants	Actual loss (%)	Apparent loss (%)	No of bruchid emrged
Highly resistant	3	3.58	5.43	12.16
Moderately resistant	36	7.26	8.86	31.81
Least susceptible	37	11.97	13.18	52.93
Moderately Susceptible	20	16.93	18.36	73.82
Highly Susceptible	4	22.35	23.82	95.5

**Table.6** Actual weight loss, apparent weight loss, number of bruchids emerged among top 10 bruchid resistance classes of F<sub>6</sub> segregants

Sl. No.	Segregants	Actual loss (%)	Apparent loss (%)	No of bruchid emrged
1	F <sub>6</sub> -229	3.25	4.9	11
2	F <sub>6</sub> -239	3.25	5.1	12
3	F <sub>6</sub> -127	4.25	6.3	13.5
4	F <sub>6</sub> -226	4.56	6.7	18
5	F <sub>6</sub> -224	5.21	6.75	20
6	F <sub>6</sub> -85	5.25	6.75	22
7	F <sub>6</sub> -44	5.25	6.75	22
8	F <sub>6</sub> -222	5.58	7.2	26.5
9	F <sub>6</sub> -193	5.58	7.3	27
10	F <sub>6</sub> -226	5.58	7.3	27
<b>Checks</b>				
1	DC15 (Cowpea variety)	5.36	6.4	35
2	DCS 47-1(Cowpea variety)	6.54	7.3	105
3	IC-524075 (Rice bean germplasm accession)	0.744	2.75	0
4	IC-341983 Rice bean germplasm accession)	0.11	2.3	0
5	Dharwad local (Local Rice bean genotype)	0.725	2.2	0

This reduction in the seed germination is due to reduction in seed viability due to increased seed damage and bruchid infestation. (Tomaz *et al.*, 2007) found in some cases larval feeding effectively kills the embryo or feeds on the endosperm so that that the seed cannot be germinated. Sometimes larval feeding create opening for pathogenic bacteria and fungi (Chang *et al.*, 2011).

The results on percent reduction in seedling length (Table 7) revealed that reduction is maximum in highly susceptible class with 17.68 percent and it is followed with moderately susceptible, least susceptible, moderately resistant and highly resistant class with 12.03, 9.17, 6.62, and 3.79 per cent respectively. Among top 10 resistant lines (Table 8), the lowest percent reduction was for the segregant F<sub>6</sub>-229 followed by F<sub>6</sub>-239.

Among checks, Dharwad local, recorded minimum reduction in seedling length (3.15 %) while checks, IC-341983, IC-524075, DC 15 and DCS 47-1 shows 3.48, 4.72, 6.82, and 8.03 per cent reduction in seedling length respectively. The reduction in seedling length in highly susceptible plants is may be attributed to the depletion of cotyledon reserves due to the larval feeding of bruchid which later leads to the slower and reduced growth of the plants.

The results pertaining to seedling vigor index is shown in table 7. In case of highly resistant varieties the reduction in seedling vigor index was less with value of 6.16 per cent compared to moderately resistant, least susceptible, moderately susceptible and highly susceptible types with values of 10.67, 14.92, 19.98 and 28.52 percent reduction respectively. Among

top 10 resistant F<sub>6</sub> stabilized lines (Table 8), F<sub>6</sub>- 229 registered less reduction in seedling vigor index with the value of 5.63% and it was followed by F<sub>6</sub>- 239 with 6.17%. Among the checks, the seedling vigor index was more reduced in check DCS 47-1 with 21.69 % and it was followed by DC15, IC524075, IC341983 and Dharwad local with 12.97, 6.78, 4.50 and 6.3 percents reduction respectively. The reduction in seedling vigor index is mainly attributed to lowest value in seedling germination % and less seedling length in susceptible varieties since vigor is calculated by using both parameters.

So from this study we have identified the some of the stabilized F<sub>6</sub> lines such as F<sub>6</sub>- 229, F<sub>6</sub> - 239 of interspecific cross between cowpea × ricebean to exhibit enhanced level of resistance to bruchids along with desirable seed characters. These stabilized F<sub>6</sub> segregants further will be evaluated for biochemical and artificial bruchid infestation studies and desirable transgressive segregants for bruchid resistance and favorable seed characteristics will be identified in future studies. Such new stabilized lines hold promise will be used to develop new bruchid resistant high yielding cowpea varieties or as parents in cowpea hybridization programs.

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