Bruchid (Callosobruchus maculates F.) Resistance Studies in F₄ Segregants of Cowpea (Vigna unguiculata (L.) Walp.)

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Abstract

Screening of 230 F₄ segregants of cross DC 615 (Susceptible parent) × Dharwad local (Resistant parent) along with checks (Dharwad local, DC 615, DC 15, DCS 47-1 and C 152) was carried out against bruchids (Callosobruchus maculates) at the Department of Genetics and plant Breeding, University of Agricultural Sciences, Dharwad. Out of 230 segregants, 36 were highly resistant, 43 moderately resistant, 110 least susceptible, 34 moderately susceptible and 7 were found to be highly susceptible. The segregants F₄-191, F₄-199 and F₄-162 were most superior in terms of per cent bruchid infestation, which recorded least values for actual weight loss, apparent weight loss, per cent seed damage, number of bruchids emergence after 45 days of infestation, per cent germination and seedling vigor after bruchid infestation.

Keywords: Bruchids, Cowpea, F₄ Segregants, Resistance, Susceptible

Introduction

About 140 insect species are known to infest cowpea (Vigna unguiculata (L.) Walp.) both in the field as well as during its storage (CAB International, 2007). Of these, pulse beetles (bruchids) causes both qualitative and quantitative losses, making the seeds unfit for planting and for human consumption (Ali et al., 2004). Callosobruchus maculates is the most destructive bruchid beetle species. Under poor threshing, cleaning, drying and storage conditions in humid tropics, 60 to 100% infestation of cowpea seeds by bruchids can occur in less than five months of storage (Lienard and Seck, 1994). As the use of resistant varieties for effective management of this pest is considered as eco-friendly and ecologically viable, present investigation was undertaken to identify resistant genotypes of cowpea against Callosobruchus maculates.

Materials and Methods

In the present study, F₄ population generated from the cross DC 615 (Susceptible parent) × Dharwad local (Resistant parent) along with checks (Dharwad local, DC 615, DC 15, DCS 47-1 and C 152) were screened for resistance to the bruchids Callosobruchus maculates. Initial seed moisture content, germination percentage, seedling length, seedling vigor index and seedling dry weight were recorded as described by ISTA (2013). A sample of 50g
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seeds was placed in plastic bottle of 100 ml capacity. Six pairs of 5 days old weevils were introduced into each bottle, mouth of bottles were covered with muslin cloth and tightly secured by rubber bands.

Bottles were incubated under ambient conditions for 45 days, and thereafter percent bruchid infestation, apparent weight loss, percent seed damage were recorded as described by Girish et al., (1975). The data generated was considered for the calculations of following parameters:

Per cent bruchid infestation = (Number of seeds with emergent holes/ Total number of seeds observed) × 100

Per cent apparent weight loss = [(Initial weight – Final weight)/ (Initial weight)] ×100

Per cent seed damage = [(Initial weight – Weight of undamaged seeds)/ (Initial weight) × 100

Per cent actual weight loss = {[(U (Nd) -D (Nu))/ [U (Nu + Nd)]]} ×100

Where,

Nd - Number of damaged seeds [seeds showing emergent holes];

Nu - Number of undamaged seeds

D - Weight of damaged seeds

U - Weight of undamaged seeds

Nd + Nu - Total number of seeds in 50g.

Based on percent bruchid infestation the segregants were classified as highly resistant (0-20%), moderately resistant (21-40%), least susceptible (41-60 %), moderately susceptible (61-80 %) and highly susceptible (81-100 %) groups against bruchid infestation during storage as suggested by Nagaraj (2006).

Results and Discussion

There were considerable differences among the F₄ segregants in terms of per cent bruchid infestation, apparent weight loss, actual weight loss and seed damage caused by C. maculates in cowpea. Among the 230 segregants, 36 were identified as highly resistant, 43 moderately resistant, 110 least susceptible, 34 susceptible and only 7 were highly susceptible (Table 1).

Minimum seed moisture was reported for F₄-179 (8.02 %) followed by F₄-95 (8.46 %) segregant (Table 3). Seed moisture did not differ much between different classes, however, highly resistant class recorded minimum (9.02 %), while highly susceptible class recorded maximum seed moisture content (10.01%) as given in Table 3. It indicates that, generally seed moisture does not influence susceptibility of cowpea seeds to bruchids. Similar results were obtained by Basavaraj (2010).

The F₄-191 and F₄-199 segregants displayed lowest actual weight loss (0.70 and 0.73 % respectively) and apparent weight loss (1.09 and 1.84 % respectively) indicating that they were highly resistant to C. Maculatus. Among the checks, Dharwad local exhibited minimum actual weight loss (0.6 %) and apparent weight loss (1.54%) while, DCS 47-1, DC 15, DC 615 and C 152 exhibited 4.88, 5.38, 15.12, and 17.26 per cent actual weight loss respectively (Table 2).

The least weight loss in highly resistant segregants might be attributed to low fecundity, adult emergence and minimum seed damage due to infestation by pulse beetle. These results are in agreement with the findings of Divya et al., (2012).
Table.1 Classification of F₄ segregants of the cross DC 615 × Dharwad local in terms of resistance to bruchids based on mean per cent bruchid infestation at 45 days after infestation

| Class                  | Per cent infestation | Bruchid infestation (%) | Number of segregants |
|------------------------|----------------------|--------------------------|----------------------|
| Highly resistant       | 0-20                 | 10.01                    | 36                   |
| Moderately resistant   | 21-40                | 31.69                    | 43                   |
| Least susceptible      | 41-60                | 50.08                    | 110                  |
| Moderately susceptible | 61-80                | 67.16                    | 34                   |
| Highly susceptible     | 81-100               | 84.01                    | 7                    |

Table.2 Seed moisture (%), actual weight loss (%), apparent weight loss (%) percentage seed damage and number of bruchids emerged 45 days after rearing for top twenty resistant F₄ segregants

| Sl. No | F₄-segregants | Seed moisture (%) | Actual weight loss (%) | Apparent weight loss (%) | Percentage seed damage | Number of bruchids emerged 45 days after rearing |
|--------|---------------|-------------------|------------------------|--------------------------|------------------------|-----------------------------------------------|
| 1      | F₄-191        | 9.20              | 0.70                   | 1.09                     | 1.55                   | 5                                             |
| 2      | F₄-199        | 8.90              | 0.73                   | 1.84                     | 1.85                   | 5                                             |
| 3      | F₄-162        | 9.20              | 0.84                   | 2.22                     | 2.55                   | 6                                             |
| 4      | F₄-225        | 9.40              | 0.85                   | 4.09                     | 4.55                   | 7                                             |
| 5      | F₄-247        | 9.24              | 0.98                   | 4.95                     | 5.55                   | 7                                             |
| 6      | F₄-149        | 9.04              | 2.77                   | 8.24                     | 9.55                   | 11                                            |
| 7      | F₄-176        | 8.64              | 0.92                   | 5.39                     | 6.55                   | 9                                             |
| 8      | F₄-180        | 9.46              | 1.23                   | 7.18                     | 8.15                   | 10                                            |
| 9      | F₄-73         | 9.22              | 3.15                   | 14.75                    | 16.45                  | 14                                            |
| 10     | F₄-231        | 8.90              | 1.42                   | 7.15                     | 9.85                   | 12                                            |
| 11     | F₄-206        | 9.40              | 3.02                   | 10.25                    | 12.55                  | 12                                            |
| 12     | F₄-95         | 8.46              | 2.18                   | 8.74                     | 11.55                  | 18                                            |
| 13     | F₄-117        | 10.02             | 1.61                   | 10.87                    | 13                     | 13                                            |
| 14     | F₄-114        | 8.88              | 1.88                   | 6.40                     | 8.80                   | 16                                            |
| 15     | F₄-71         | 8.88              | 2.88                   | 5.98                     | 13.60                  | 16                                            |
| 16     | F₄-100        | 8.84              | 1.66                   | 7.09                     | 16.80                  | 16                                            |
| 17     | F₄-93         | 9.20              | 2.81                   | 7.32                     | 16.00                  | 20                                            |
| 18     | F₄-179        | 8.02              | 0.73                   | 2.10                     | 10.55                  | 6                                             |
| 19     | F₄-181        | 8.82              | 2.95                   | 9.20                     | 11.45                  | 14                                            |
| 20     | F₄-207        | 10.00             | 2.72                   | 6.89                     | 16.95                  | 20                                            |

Checks

| Sl. No | Classes          | Number of segregants in each classes | Seed moisture (%) | Actual weight loss (%) | Apparent weight loss (%) | Percentage seed damage | Number of bruchids emerged 45 days after rearing |
|--------|------------------|--------------------------------------|-------------------|------------------------|--------------------------|------------------------|-----------------------------------------------|
| 01     | Dharwad local    | -                                    | 0.6               | 1.54                   | 1.20                     | 4                      |                                              |
| 02     | DC 615           | -                                    | 15.12             | 16.22                  | 90.60                    | 110                    |                                              |
| 03     | DC 15 (MR)       | -                                    | 5.38              | 10.11                  | 44.8                     | 54                     |                                              |
| 04     | DCS 47-1 (MS)    | -                                    | 4.88              | 7.92                   | 64.8                     | 94                     |                                              |
| 05     | C 152 (HS)       | -                                    | 17.26             | 19.28                  | 83.1                     | 116                    |                                              |

Table.3 Mean seed moisture (%), actual weight loss (%), apparent weight loss (%) seed damage (%) and number of bruchids emerged 45 days after rearing among different classes

| Sl. No | Classes          | Number of segregants in each classes | Seed moisture (%) | Actual weight loss (%) | Apparent weight loss (%) | Percentage seed damage | Number of bruchids emerged 45 days after rearing |
|--------|------------------|--------------------------------------|-------------------|------------------------|--------------------------|------------------------|-----------------------------------------------|
| 1      | Highly resistant | 36                                   | 9.02              | 1.02                   | 5.61                     | 14.17                  | 18.33                                          |
| 2      | Moderately resistant | 43                                | 9.31              | 3.51                   | 7.20                     | 34.33                  | 51.55                                          |
| 3      | Least susceptible | 110                                  | 9.40              | 5.78                   | 10.33                    | 52.49                  | 78.38                                          |
| 4      | Moderately susceptible | 34                               | 9.56              | 8.73                   | 14.16                    | 69.11                  | 97.32                                          |
| 5      | Highly susceptible | 7                                    | 10.01             | 16.00                  | 22.00                    | 85.11                  | 122.71                                         |
Table 4 Effect of *Callosobruchus maculatus* on seed germination (%), seedling length (cm), seedling vigour index and seedling dry weight (g) of top twenty resistant F$_4$ segregants in cowpea

| Sl. No | F$_4$ segregant s | Germination (%) Before screening | Germination (%) After screening | Per cent reduction in germination over initial | Seedling length (cm) Before screening | Seedling length (cm) After screening | Per cent reduction in seedling length | Seedling vigour index Before screening | Seedling vigour index After screening | Per cent reduction in vigour index | Seedling dry weight (mg) Before screening | Seedling dry weight (mg) After screening | Per cent reduction in seedling dry weight |
|-------|------------------|---------------------------------|---------------------------------|-----------------------------------------------|--------------------------------------|--------------------------------------|-----------------------------------|---------------------------------------|--------------------------------------|--------------------------------------|----------------------------------------|----------------------------------------|----------------------------------------|
| 1     | F$_4$-191        | 87.0                            | 84.0                            | 3.4                                           | 42.1                                 | 41.0                                 | 2.6                                | 3662.7                               | 3444.0                               | 6.0                                  | 72.5                                  | 56.6                                  | 21.9                                  |
| 2     | F$_4$-199        | 90.0                            | 88.0                            | 2.2                                           | 36.5                                 | 35.6                                 | 2.5                                | 3285.0                               | 3132.8                               | 4.6                                  | 74.2                                  | 60.5                                  | 18.5                                  |
| 3     | F$_4$-162        | 84.0                            | 79.0                            | 6.0                                           | 38.3                                 | 37.0                                 | 3.4                                | 3217.2                               | 2923.0                               | 9.1                                  | 69.2                                  | 54.4                                  | 21.4                                  |
| 4     | F$_4$-225        | 93.0                            | 90.0                            | 3.2                                           | 32.7                                 | 31.9                                 | 2.4                                | 3041.1                               | 2871.0                               | 5.6                                  | 63.2                                  | 48.5                                  | 23.3                                  |
| 5     | F$_4$-247        | 90.0                            | 86.0                            | 4.4                                           | 31.7                                 | 30.8                                 | 2.8                                | 2853.0                               | 2648.8                               | 7.2                                  | 69.6                                  | 54.6                                  | 21.6                                  |
| 6     | F$_4$-149        | 85.0                            | 82.0                            | 3.5                                           | 36.5                                 | 35.6                                 | 2.5                                | 3102.5                               | 2919.2                               | 5.9                                  | 75.2                                  | 60.5                                  | 19.5                                  |
| 7     | F$_4$-176        | 84.0                            | 80.0                            | 4.8                                           | 39.0                                 | 37.8                                 | 3.1                                | 3276.0                               | 3024.0                               | 7.7                                  | 68.3                                  | 53.6                                  | 21.5                                  |
| 8     | F$_4$-180        | 81.0                            | 78.0                            | 3.7                                           | 40.4                                 | 39.6                                 | 2.0                                | 3272.4                               | 3088.8                               | 5.6                                  | 87.2                                  | 72.2                                  | 17.2                                  |
| 9     | F$_4$-73         | 80.0                            | 77.0                            | 3.8                                           | 33.2                                 | 32.3                                 | 2.7                                | 2656.0                               | 2487.1                               | 6.4                                  | 56.4                                  | 41.6                                  | 26.2                                  |
| 10    | F$_4$-231        | 96.0                            | 90.0                            | 6.3                                           | 41.0                                 | 40.3                                 | 1.7                                | 3936.0                               | 3627.0                               | 7.9                                  | 69.2                                  | 54.2                                  | 21.7                                  |
| 11    | F$_4$-206        | 87.0                            | 81.0                            | 6.9                                           | 38.5                                 | 37.6                                 | 3.3                                | 3349.5                               | 3045.6                               | 9.1                                  | 67.3                                  | 52.3                                  | 22.3                                  |
| 12    | F$_4$-95         | 77.0                            | 75.0                            | 2.6                                           | 33.7                                 | 32.5                                 | 3.6                                | 2594.9                               | 2437.5                               | 6.1                                  | 89.2                                  | 74.2                                  | 16.8                                  |
| 13    | F$_4$-117        | 87.0                            | 82.0                            | 5.7                                           | 33.2                                 | 32.5                                 | 2.1                                | 2888.4                               | 2665.0                               | 7.7                                  | 52.5                                  | 37.4                                  | 28.8                                  |
| 14    | F$_4$-114        | 77.0                            | 74.0                            | 3.9                                           | 39.5                                 | 38.7                                 | 2.0                                | 3041.5                               | 2863.8                               | 5.8                                  | 69.4                                  | 51.2                                  | 26.2                                  |
| 15    | F$_4$-71         | 70.0                            | 68.0                            | 2.9                                           | 35.2                                 | 34.1                                 | 3.1                                | 2464.0                               | 2318.8                               | 5.9                                  | 57.5                                  | 39.5                                  | 31.3                                  |
| 16    | F$_4$-100        | 77.0                            | 71.0                            | 7.8                                           | 33.2                                 | 32.2                                 | 3.0                                | 2556.4                               | 2286.2                               | 10.6                                 | 91.6                                  | 73.4                                  | 19.9                                  |
| 17    | F$_4$-93         | 81.0                            | 77.0                            | 4.9                                           | 35.4                                 | 34.2                                 | 3.4                                | 2867.4                               | 2633.4                               | 8.2                                  | 63.5                                  | 45.5                                  | 28.3                                  |
| 18    | F$_4$-179        | 75.0                            | 71.0                            | 5.3                                           | 39.4                                 | 38.5                                 | 2.3                                | 2955.0                               | 2733.5                               | 7.5                                  | 68.4                                  | 50.6                                  | 26.0                                  |
| 19    | F$_4$-181        | 70.0                            | 66.0                            | 4.7                                           | 36.2                                 | 35.4                                 | 2.2                                | 2534.0                               | 2336.4                               | 7.8                                  | 91.5                                  | 73.5                                  | 19.7                                  |
| 20    | F$_4$-207        | 88.0                            | 79.0                            | 10.2                                          | 36.5                                 | 35.7                                 | 2.2                                | 3212.0                               | 2820.3                               | 12.2                                 | 65.6                                  | 47.6                                  | 27.4                                  |

**Checks**

| Sl. No | Variety            | Check Code | Seed weight (mg) Before screening | Seed weight (mg) After screening | Germination (%) | Seedling length (cm) | Seedling vigour index | Seedling dry weight (mg) |
|-------|--------------------|------------|-----------------------------------|----------------------------------|-----------------|----------------------|----------------------|------------------------|
| 01    | Dharward local     | 94         | 92                                | 2.12                             | 36.50           | 35.50                | 2.46                 | 3431.00                | 3266.00                | 4.8                  | 68.44                             | 51.56                             | 25.21                             |
| 02    | DC 615             | 84         | 72                                | 14.2                             | 35.00           | 32.60                | 6.86                 | 2940.00                | 2347.50                | 20.10                | 67.78                             | 49.78                             | 27.10                             |
| 03    | DC (MR) 15         | 95         | 84                                | 11.58                            | 39.50           | 36.50                | 7.59                 | 3392.00                | 2986.80                | 11.95                | 69.14                             | 50.75                             | 27.07                             |
| 04    | DCS 47-1(MS)       | 78         | 64                                | 17.94                            | 40.10           | 38.10                | 4.99                 | 3145.70                | 2477.10                | 21.25                | 71.57                             | 49.19                             | 31.24                             |
| 05    | C 152 (HS)         | 88         | 64                                | 27.27                            | 35.40           | 32.40                | 8.47                 | 2999.30                | 2219.20                | 26.01                | 63.59                             | 41.00                             | 35.49                             |

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Table 5. Mean seed germination (%), seedling length (cm), seedling vigour index and seedling dry weight (g) among different classes

| Sl. No | Classes            | Germination (%) | Per cent reduction in germination over initial | Seedling length (cm) | Per cent reduction in seedling length | Seedling vigour index | Per cent reduction in vigour index | Seedling dry weight (mg) | Per cent reduction in seedling dry weight |
|--------|--------------------|-----------------|-----------------------------------------------|----------------------|--------------------------------------|-----------------------|-----------------------------------|-------------------------|-------------------------------------------|
| 1      | Highly resistant   | 81.44           | 76.97                                         | 5.54                 | 35.92                                | 34.93                 | 7928.25                           | 2692.07                 | 8.16                                       | 68.44                                   |
| 2      | Moderately resistant | 79.95          | 73.35                                         | 8.25                 | 35.96                                | 34.42                 | 2880.81                           | 2531.56                 | 12.19                                      | 67.78                                   |
| 3      | Least susceptible  | 80.75           | 70.16                                         | 13.17                | 36.28                                | 33.90                 | 2935.08                           | 2384.94                 | 18.85                                      | 69.14                                   |
| 4      | Moderately susceptible | 78.38       | 63.41                                         | 19.16                | 35.61                                | 32.54                 | 2794.76                           | 2066.37                 | 26.11                                      | 71.57                                   |
| 5      | Highly susceptible | 79.14           | 54.86                                         | 30.71                | 37.09                                | 31.91                 | 2942.91                           | 1761.50                 | 40.41                                      | 63.59                                   |

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Minimum seed damage (1.55 and 3.75 %) was observed in F₄-191 and F₄-180 segregants respectively (Table 2). As compared to checks, Dharwad local recorded least seed damage (1.20 %) than DC 15 (44.8 %), DCS 47-1 (64.8 %), C 152 (83.1 %) and DC 615 (90.60 %). Per cent seed damage was highest in case of highly susceptible segregants (85.11 %) whereas, it was lowest for highly resistant segregants (14.17 %) as depicted in Table 3. The extent of damage dependeds upon the number of adults emerged during each generation and the duration of life cycle, therefore seeds supporting rapid and higher rate of adult emergence got damaged. The segregants with highest adult emergence recorded extensive damage. These findings are in agreement with those of Basavaraj (2010).

The F₄-199 segregant reported minimum per cent reduction in germination (2.22 %) followed by F₄-95 (2.60%). Among the checks, Dharwad local reported least reduction in germination (2.12 %) while, DC 15, DC 615, DCS 47-1 and C 152 reported 11.58, 14.2, 17.94 and 27.27 percent reduction in germination respectively as given in Table 4. The reduction in germination percentage in highly resistant and susceptible class of segregants were 5.54 and 30.71 respectively (Table 5) due to C. maculates infestation. The least damaged seeds with C. maculates had better potential for growth and development, whereas, highly damaged seeds had lower germination. Similar reduction in germination due to infestation of the bruchids was reported by Sowmya (2015).

Maximum reduction in seedling length (14.12 %) was observed in highly susceptible segregants, whereas highly resistant segregants reported minimum reduction in seedling length (2.77 %) as given in Table 4. The F₄-230 displayed least reduction in seedling length (1.7 %) followed by F₄-180 (2.0%). Dharwad local recorded minimum reduction in seedling length (2.46 %) while, checks DCS 47-1, DC 615, DC 15, and C 152 noticed 4.99, 6.86, 7.59 and 8.47 per cent reduction in seedling length (Table 5). These results were in agreement with the findings of Nagaraj (2006).

The seedling vigor is an important component that can influence crop plant density and yield (Siddique and Wright, 2004). In the present study, reduction in seedling vigour index was minimum (8.16 %) and maximum (40.41 %) in highly resistant and susceptible segregants respectively (Table 5). The F₄-199 segregant reported minimum reduction in seedling vigour index (4.63 %) followed by the F₄-225 (5.59 %). Least reduction in seedling vigour may due to least seed damage and bruchid infestation. However, the seedling vigor is further dependent on other factors such as thickness of seed coat, color, nutrient status, presence of antixenotic chemicals etc., which vary with genotype. Similar results with respect to seedling index were also reported earlier by Deshpande et al., (2011).

The variation in seed parameters was mainly due to variation in per cent infestation level, adult emergence and reduction in seed weight and also inherent capacity of each genotype response to be bruchids. Similar results with respect to seed parameters were also reported by Bhaduria and Jakhmola (2006) in cowpea. Among highly resistant group, segregants, F₄-191, F₄-199, F₄-162, F₄-225, F₄-247, F₄-149, F₄-176, F₄-180, F₄-73, F₄-231, F₄-206, F₄-95, F₄-117, F₄-114, F₄-71 and F₄-100 exhibited 0-10 per cent infestation. Hence, the identified superior lineshave been recommended to test yield stability across different locations.

Out of the thirty six promising, highly resistant progenies, F₄-191, F₄-199 and F₄-162 were most superior in term of per cent bruchid infestation which also recorded least actual weight loss, apparent weight loss, per cent seed damage, number of bruchids emergence after 45 days of infestation and also exhibited least reduction in germination and seedling vigor after bruchid infestation. These progenies were on par with highly resistant check Dharwad local. Hence, these F₄ segregants can be effectively utilized as a genetic source of resistance to bruchids in future breeding programmes to developing high
yielding cowpea varieties resistant to bruchid infestation.

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