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Bruchid (*Callosobruchus maculates* F.) Resistance Studies in F_4 Segregants of Cowpea (*Vigna unguiculata* (L.) Walp.)

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A B S T R A C T

Screening of 230 F_4 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 maculatus*) 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 F_4-191, F_4-199 and F_4-162 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.

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 maculatus*.

Materials and Methods

In the present study, F_4 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

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 F_4 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 F_4-179 (8.02 %) followed by F_4-95 (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 F_4-191 and F_4-199 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 F_4 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 bruchid 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 F_4 segregants

Sl. No	F_4-segregants	Seed moisture (%)	Actual weight loss (%)	Apparent weight loss (%)	Percentage seed damage	Number of bruchids emerged 45 days after rearing
1	F_4-191	9.20	0.70	1.09	1.55	5
2	F_4-199	8.90	0.73	1.84	5.95	5
3	F_4-162	9.20	0.84	2.22	7.05	6
4	F_4-225	9.40	0.85	4.09	8.20	7
5	F_4-247	9.24	0.98	4.95	9.12	11
6	F_4-149	9.04	2.77	8.24	11.80	21
7	F_4-176	8.64	0.92	5.39	8.70	9
8	F_4-180	9.46	1.23	7.18	3.75	10
9	F_4-73	9.22	3.15	14.10	8.46	14
10	F_4-231	8.90	1.42	7.15	13.75	12
11	F_4-206	9.40	3.02	10.25	10.90	12
12	F_4-95	8.46	2.18	8.74	16.80	18
13	F_4-117	10.02	1.61	10.87	8.50	13
14	F_4-114	8.88	1.88	6.40	8.80	16
15	F_4-71	8.88	2.88	5.98	13.60	16
16	F_4-100	8.84	1.66	7.09	16.80	16
17	F_4-93	9.20	2.81	7.32	16.00	20
18	F_4-179	8.02	0.73	2.10	10.55	6
19	F_4-181	8.82	2.95	9.20	11.45	14
20	F_4-207	10.00	2.72	6.89	16.95	20
Checks						
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-segregants	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
		Before screening	After screening		Before screening	After screening		Before screening	After screening		Before screening	After screening	
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	2.3	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	5.7	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													
01	Dharwad 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 15 (MR)	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	35.4	32.4	8.47	2999.3	2219.20	26.01	63.59	41.00	35.49

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
		Before screening	After screening		Before screening	After screening		Before screening	After screening		Before screening	After screening	
1	Highly resistant	81.44	76.97	5.54	35.92	34.93	2.77	2928.25	2692.07	8.16	68.44	51.56	25.21
2	Moderately resistant	79.95	73.35	8.25	35.96	34.42	4.30	2880.81	2531.56	12.19	67.78	49.78	27.10
3	Least susceptible	80.75	70.16	13.17	36.28	33.90	6.54	2935.08	2384.94	18.85	69.14	50.75	27.07
4	Moderately susceptible	78.38	63.41	19.16	35.61	32.54	8.60	2794.76	2066.37	26.11	71.57	49.19	31.24
5	Highly susceptible	79.14	54.86	30.71	37.09	31.91	14.12	2942.91	1761.50	40.41	63.59	41.00	35.49

Minimum seed damage (1.55 and 3.75 %) was observed in F_4-191 and F_4-180 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 highestin 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 F_4-199 segregant reported minimum per cent reduction in germination (2.22 %) followed by F_4-95 (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 inhighly resistant and susceptible class of segregants were5.54and 30.71 respectively (Table 5) due to *C. maculates* infestation. The least damaged seeds with *C. maculatus* 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 F_4-230 displayed least reduction in seedling length (1.7 %) followed by F_4 -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 F_4-199 segregant reported minimum reduction in seedling vigour index (4.63 %) followed by the F_4-225 (5.59 %). Least reduction in seedling vigor 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, F_4-191, F_4-199, F_4-162, F_4-225, F_4-247, F_4-149, F_4-176, F_4-180, F_4-73, F_4-231, F_4-206, F_4-95, F_4-117, F_4-114, F_4-71 and F_4-100 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, F_4-191, F_4-199 and F_4-162 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 F_4 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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