

Laboratory screening of cowpea (*Vigna unguiculata*) genotypes against pulse beetle, *Callosobruchus maculatus* (F.)

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Abstract

Research studies were conducted to screen 110 cowpea genotypes against *Callosobruchus maculatus* commonly known as the cowpea weevil, under the no-choice infestation condition. Out of all the genotypes screened, BBT1-11 outperformed all the genotypes across all parameters investigated. BBT1-11 recorded 13.01 no. of eggs/100 seeds compared to MS1-8-2-6-9-1 with 128.67 eggs/100 seeds. The adult emergence on BBT1-11 was at 12.72% compared to the highest LTBT1-5 at 88.11%. The lowest growth index of 0.42 and frass of 0.23g was recorded on BBT1-11. BBT1-11 could be further assessed for the possible presence of biochemical substances, which could explain the observed differences among the genotypes in their reaction to *C. maculatus*. Besides, BBT1-11 can also be incorporated into the breeding program with the target of breeding cowpea varieties that are tolerant to pest infestation.



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1.0 Introduction

Cowpea (*Vigna unguiculata* F.) is one of the major legume crops that is cultivated as green manure crop, fodder, and pulse. Cowpea seeds contain approximately 60% carbohydrates, 1.8% fat, and 23.4% protein (Choudhary, 2015). It is also a rich source of iron and calcium (Khan, 2011). Pulse crops, including cowpea, have contributed to the improvement of agriculture in different countries, especially developing countries (Sarwar et al., 2003; Deeba et al., 2006; Raikar et al., 2011; and Sarwar, 2012). However, heavy losses in cowpea from various insect pests are experienced during storage. According to Semple et al (1992) and Sarwar (2012), three major pulse beetle species responsible for cowpea damage during storage are; *Callosobruchus chinensis*, *Callosobruchus analis*, and *Callosobruchus maculatus*. Cowpea weevil, *Callosobruchus maculatus*, is an important agricultural insect pest that is found throughout the tropical and subtropical regions. The most destructive stage of this beetle is the larval stage which feeds and develops on the seed of legumes (Fabaceae), while, the adults do not feed on the seed but spend their limited time (one to two weeks) mating and egg-laying (Kergoat et al., 2007 and Sarwar, 2012). The beetle *C. maculatus* has the potential to cause 100% seed loss if left uncontrolled. The use of resistant genotypes in insect pest management is considered a good and viable proposition (Waghmare and Bantewad 2020). Therefore, the main objective of the current study was to identify cowpea genotypes with resistance to *C. maculatus*.

2.0 Materials and Methods

2.1 Stock culture

The study was carried out in the Department of Plant Science, University of Zambia, Lusaka, Zambia in 2021. Culturing of the *C. maculatus* population was done at the University of Zambia, in the Insectarium laboratory. The population was maintained at 28°C ($\pm 2^\circ\text{C}$) and Relative Humidity of 70 \pm 5% (Sarwar, 2012). A disinfected susceptible genotype, Namuseba was used as substrate in the culturing of the insect population. Adult insects were placed in twelve glass jars that were sterilized at 55°C for 4hrs in an oven before use. The jars were covered tightly with muslin affixed with rubber bands to prevent the adults from escaping (Choudhary et al., 2015). The seeds (150 g) were placed in each jar and infested with 20 weevils. The jars were kept under laboratory conditions undisturbed and mass cultured up to the third generation (Senthilraja and Patel, 2021). The freshly emerged adults of *C. maculatus* were used for genotype screening (Sarwar, 2012).

2.2 Experimental cowpea seed

The undamaged and clean cowpea seeds for each genotype were examined using a hand lens to make sure that all the seeds were clean and undamaged and that no eggs had been laid on them. The seeds of all 110 test genotypes were then kept at -5 °C for one week in the deep freezer and thereafter left for 24 h under ambient laboratory conditions (Sarwar, 2012).

2.3 No-choice test

Relative resistance and susceptibility of cowpea genotypes against *C. maculatus* were determined under a no-choice test (Giga and Smith, 1981). One-hundred seeds from each genotype were weighed and kept separately in 250ml plastic containers and two pairs of adult beetles (1or 2 days old) were put in each container, separately (Senthilraja and Patel, 2021). Each container was covered on the top with a two-fold muslin cloth fastened with a rubber band to avoid insects from escaping and still provide sufficient aeration (Sarwar, 2012). After infesting the plastic containers with the beetles, the insects were discarded after 7 days, to ensure maximum oviposition (Choudhary et al., 2015). The experimental design used in the study was a Completely Randomized Design (CRD) with 3 replications (Steel and Torrie, 1980).

The data obtained were subject to analysis of variance and LSD values were obtained at a 5% level of significance.

2.3.1 No. of eggs laid/100 seeds

The number of eggs laid on each genotype was counted after 7 days of the release of *C. maculatus*. A hand lens was used in the counting process for easy visibility.

2.3.2 Adult emergence (%)

Adult emergence was recorded every 24h daily. The formula used for calculating adult emergence was the standard formula suggested by Sharma and Thakur (2014).

$$\text{Adult emergence (\%)} = \frac{\text{Number of adults emerged}}{\text{Number of eggs laid}} \times 100$$

2.3.3 Developmental period (days)

The developmental period was considered as the time taken from oviposition to adult emergence (Sharma and Thakur, 2014).

2.3.4 Growth index

The calculation of the growth index was done using the formula suggested by Singh and Pant (1955) as shown below;

$$\text{Growth Index} = \frac{\text{Adult emergence (\%)}}{\text{Developmental period (days)}}$$

2.3.5 Weight loss (%)

The formula suggested by Sharma and Thakur (2014), was used for the calculation of the percent loss in weight as shown below. The final weight for each genotype was taken with a single pan electric balance independently for each treatment.

$$\text{Weight loss (\%)} = \frac{\text{Initial weight of seeds (g)} - \text{Final weight of seeds (g)}}{\text{Initial weight of seeds (g)}} \times 100$$

2.3.6 Frass produced

This was done by separating healthy and damaged grains from dust material by passing each sample (genotype) through a sieve. Thereafter, the frass (dust material) of each treatment was weighed separately using the single pan electric balance (Sarwar, 2012).

2.3.7 Percent infestation

The percent infestation was calculated using the standard formula and categorized according to the suggestion of Deshpande et al (2011) (Table 1).

$$\text{Per cent infestation} = \frac{\text{No. of seeds with emergent holes}}{\text{Total number of seeds observed}} \times 100$$

Table1: Resistance/susceptibility rating based on percent seed infestation.

Class	Percent infestation
Highly resistance	0-20
Moderately resistance	21-40
Least susceptible	41-60
Moderately susceptible	61-80
Highly susceptible	81-100

3.0 Results and discussion

The results obtained in the study revealed that 110 cowpea genotypes screened using the no-choice laboratory condition showed significant differences in expression of resistance to *C. maculatus*. A significant difference was observed among the genotypes for the number of eggs laid per 100 seeds of each genotype, adult emergence, developmental period, growth index, percentage infestation, weight loss, and frass produced.

3.1 No. of eggs laid/100 seeds

Significant variations in the number of eggs laid by two pairs of *C. maculatus* on different cowpea genotypes, occurred significantly at a 5% level of significance, from 13.01 in BBBT1-11 to 128.64 in MS1-8-2-6-9-1 (Table 2). Significantly less oviposition was seen on BBBT1-11(13.01 eggs/100 seeds) which was statistically at par with the mutant genotypes LT11-5-2-2-2(18.27), LT11-5-2-2-4(19.61), LT11-5-2-2-7(21.21), LTBT1-4(27.61), LT11-5-2-2-10(24.81), LT11-5-2-2-20(18.67), BB10-4-2-3-1(14.67), BB10-4-2-3-3(24.33), BB7-9-7-5-3(20.07), MS1-8-2-6-6-2(14.67), and BBBT1-7(28.67). The genotype MS1-8-2-6-9-1 was more preferred by *C. maculatus* in no-choice conditions as it recorded a significantly greater number of eggs laid per 100 seeds, which was statistically at par with 43 genotypes some of which are not recorded in Table 2. Sharma and Thakur (2014), reported that cowpea seeds with smooth surfaces were more preferred for egg-laying by *C. maculatus* than those with a rough seed coat texture. Almost similar results of the relationship between seed size, seed color, and oviposition preference were reported by Shaheen et al., (2006), Patil et al (2009), Raghuwashi et al., (2016) Tripathi et al (2015), and Waghmare and Bantewad (2020). Therefore, the current findings are in accordance with the earlier researchers.

3.2 Adult emergence

The percent adult emergence of *C. maculatus* on different cowpea genotypes varied significantly from 12.72% to 88.11% (Table 2). The lowest adult emergence was recorded on BBBT1-11 (12.72%) which was statistically at par with LT11-5-2-2-7 (16.71%) and LT11-5-2-2-10 (15.34%) indicating resistance. The highest adult emergence was recorded on LTBT1-5 (88.11%) which was statistically at par with MS1-8-2-6-9-1(84.01%) and LT parent (86.31%) indicating susceptibility to *C. maculatus*. The resistance of genotypes such as BBBT1-11, LT11-5-2-2-7, and LT11-5-2-2-10 may be attributed to varying sorts of reserve protein and vicilin which cannot be metabolized by the midgut proteinases of insects, thereby reducing the food supply of the larvae and interfering with *C. maculatus* development (Domingues et al.,2006 and Senthilraja and Patel, 2021).

3.3 Development period

The developmental period varied significantly, from 19.02 to 31.12 days. The shortest developmental period was recorded on BB10-4-2-3-3 which was statistically at par with 83 genotypes out of 110 genotypes screened against *C. maculatus* indicating susceptibility. The longest developmental period was recorded on the mutant genotype LT11-5-2-2-4 which was statistically at par with 9 genotypes out of 110 genotypes screened against *C. maculatus* indicating resistance. The present findings are in accordance with the study by Senthilraja and Patel (2021) who recorded the shortest developmental period of 19.67 days on the genotype GC1710 and also the study by Waghmare and Bantewad (2020), who recorded the longest developmental period of 31.85 days on the genotype ICCV-3137. The results are also in accordance with Tripathi et al., (2015), who reported that the developmental period was significantly shorter on susceptible genotypes and significantly longer on resistant genotypes of cowpea. The longer developmental period of *C. maculatus* in cowpea genotypes may be attributed to the antibiosis resistance, leading to the reduction of adult emergence (Smith and Clement, 2011).

3.4 Weight loss

The results on weight loss caused by *C. maculatus* to the seeds of different cowpea genotypes varied significantly. The lowest weight loss of 0.94%, was recorded on the mutant genotype LT11-5-2-2-2, which was statistically at par with LT11-5-2-2-4(2.68%), LT11-5-2-2-7(2.36%),

LT11-5-2-2-10(2.69%), LT11-5-2-2-20(3.66%), BB10-4-2-3-1(1.76%), BB10-4-2-3-3(0.96%), BB7-9-7-5-3(2.12%), MS1-8-2-6-6-2(3.17%), BBT1-11(1.05%), IT99K241-2(2.03%) and IT99K573-2-1(2.24%) indicating resistance to *C. maculatus*. Significant high weight loss was recorded on LT16-7-2-5-1(58.58%) which was statistically at par with MS1-8-2-6-9-1(55.86%) and other two unlisted genotypes, indicating susceptibility to *C. maculatus*. The results indicated that weight loss was much influenced by the levels of susceptibility in different cowpea genotypes (Waghmare and Bantewad, 2020). The significant susceptibility levels of LT16-7-2-5-1 and MS1-8-2-6-9-1 may be attributed to variable traits that made them more preferred to *C. maculatus* compared to other genotypes (Shaheen et al, 2006). The present findings are also in conformity with Patil et al., (2009), who reported that significantly high weight loss (77.9%) was recorded in Mexican Doler which had a smooth bold seed coat compared to other genotypes with a rough seed coat. The study by Deshpande et al (2011), indicates that variation in seed weight loss by *C. maculatus* occurs mainly due to the variations in adult emergence and percent infestation levels.

Table 2: Mean values of parameters measured during the evaluation of Cowpea genotypes against pulse beetle *Callosobruchus maculatus* (Fabricius) (Results of selected 22 genotypes out of 110 genotypes).

Genotypes	eggs/100 seeds	Adult emergence Percent	Development period (days)	Growth Index	Percent Infestation	Weight loss %	Frass produced (grams)
LT11-5-2-2-2	18.27	27.01	30.33	0.89	13.11	0.94	0.27
LT11-5-2-2-4	19.61	54.72	31.12	1.76	16.02	2.68	0.41
LT11-5-2-2-7	21.21	16.71	24.67	0.68	11.67	2.36	0.37
LTBT1-4	27.61	63.01	27.33	2.31	13.33	5.51	0.69
LT11-5-2-2-10	24.81	15.34	27.33	0.56	13.33	2.69	0.37
LT11-5-2-2-20	18.67	32.33	23.64	1.37	8.67	3.66	0.43
BBBT1-3	36.17	19.71	30.67	0.64	12.33	5.63	0.53
BB10-4-2-3-1	14.67	42.31	30.33	1.39	13.01	1.76	0.23
BB7-9-7-5-3	20.07	61.02	26.01	2.35	11.01	2.12	0.37
BB10-4-2-3-3	24.33	79.72	19.02	4.19	14.01	0.96	0.23
MS1-8-2-6-6-2	14.67	32.32	20.02	1.61	8.03	3.71	0.47
BBBT1-7	28.67	42.32	21.67	1.95	4.02	6.94	0.43
BBBT1-11	13.01	12.72	30.33	0.42	10.03	1.05	0.23
IT99K241-2	67.67	51.71	21.33	2.42	13.04	2.03	0.42
IT99K573-2-1	47.67	79.31	22.33	3.55	17.02	2.24	0.37
LT16-7-2-5-1	123.17	80.31	19.67	4.08	95.42	58.58	6.53
LTBT1-5	118.21	88.11	20.04	4.40	93.22	44.01	5.07
BBBT1-1	124.31	49.72	24.33	2.04	95.01	37.94	4.33
MS1-8-2-6-9-1	128.67	84.01	22.02	3.82	90.33	55.86	5.41
LUNKWAKWA	36.01	66.71	23.33	2.86	23.02	14.87	0.33
LT-Parent	102.11	86.31	20.33	4.25	43.03	27.77	1.73
BB-Parent	110.34	78.21	20.01	3.91	40.67	15.89	1.63
S. Em ±	1.87	1.57	0.40	0.18	0.68	0.49	0.09
CD at 5%	17.57	4.48	5.21	0.37	9.39	2.90	0.42
CV%	3.33	2.44	3.26	1.05	2.11	4.39	2.26

S. Em, Standard Error of the Mean; CD, Critical Difference; CV, Coefficient of Variation.

3.5 Growth index

The data concerning the growth index of *C. maculatus* on different cowpea genotypes varied from 0.42 recorded on BBT1-11 to 4.40 recorded on LTBT1-5. The highest growth index was recorded on LTBT1-5 which was statistically at par with 35 genotypes out of the 110 genotypes screened against *C. maculatus*. The study by Umrao and Verma (2003), reported that the resistant genotypes have low levels of digestible protein content compared to susceptible genotypes that have high levels of digestible protein content.

3.6 Percent Infestation

Percent infestation by *C. maculatus* among different cowpea genotypes, varied from 4.02% recorded on BBT1-7 to 95.42% recorded on LT16-7-2-5-1 (Table 3). Significantly less damage was recorded on the mutant line BBT1-7 at 4.02%, which was statistically at par with LT11-5-2-2-2, LT11-5-2-2-7, LTBT1-4, LT11-5-2-2-10, LT11-5-2-2-20, BBT1-11 and IT99K241-2. Significantly, the highest damage was recorded on the mutant line LT16-7-2-5-1 at an infestation of 95.42%, which was statistically at par with MS1-8-2-6-9-1, LTBT1-5, BBT1-1, and other 22 genotypes not listed in Table 3. Based on percent infestation, 15 genotypes were classified as high resistant, 19 genotypes were classified as moderately resistant, 16 genotypes were classified as least susceptible, moderately susceptible genotypes were 21 and highly susceptible genotypes were 39 (Table 3). The classification of the genotypes is based on the earlier classification reported by Miesho et al (2018) and Senthilraja and Patel (2021). The present study had some genotypes that indicated resistance, this is in conformity with Sarwar (2012), who also reported some genotypes that were resistant to the beetle. The results in the present study are also in conformity with Deshpande et al (2011), Augustine et al (2018), and Senthilraja and Patel (2021), who reported that none of the cowpea genotypes screened in their studies, were completely resistant to the attack of the beetle. This can also be seen in the present study in that, although BBT1-7 was classified as highly resistant but also recorded some degree of damage of 4.02%. The variation in the susceptibility and resistant levels of the cowpea genotypes could be linked to the association between some physiochemical characteristics of the cowpea seeds (Senthilraja and Patel, 2021).

Table 3: Resistance/susceptibility rating based on percent infestations

Class	Percent Infestation	Number of genotypes/Variety
Highly resistant	0-20	15
Moderately resistance	21-40	19
Least susceptible	41-60	16
Moderately susceptible	61-80	21
Highly susceptible	81-100	39

3.7 Frass produced

Significantly low frass of 0.23g, was produced by BBT1-11, BB10-4-2-3-1 and BB10-4-2-3-3, these genotypes were statistically at par with mutant genotypes LT11-5-2-2-2(0.27g), LT11-5-2-2-4(0.41g), LT11-5-2-2-7(0.37g), LT11-5-2-2-10(0.37g), LT11-5-2-2-20(0.43g), BB7-9-7-5-3(0.37g), MS1-8-2-6-6-2(0.47g), BBT1-7(0.43g) and Lunkwakwa(0.33g) and also the pure lines IT99K241-2-1(0.42g) and IT99K573-2-1 (0.37g) indicating resistance. The genotype LT16-7-2-5-1 recorded the highest amount of frass produced (6.53g) which can be attributed to more grain damage caused by *C. maculatus*. The present findings are supported by those of Sarwar (2012) who reported a direct relationship between frass produced and parameters such as percent infestation (grain damage) and adult emergence. In the study by Sarwar (2012), the genotype CH-86/02 which recorded the significantly highest percent infestation of 87% also recorded the significantly highest frass weight of 0.24g, this coincides with the present findings.

4.0 Relationship between Growth Index of *C. maculatus* and various parameters

The relationship between growth index and various growth parameters of *C. maculatus* in different cowpea genotypes (Table 4) showed that growth index had a negative association which was highly significant with the developmental period ($r = -0.83^{**}$), highly significant positive association with adult emergence ($r = 0.85^{**}$), non-significant weak positive association with the number of eggs laid per 100 seeds ($r = 0.18$), non-significant positive relationship with percent infestation ($r = 0.07$), non-significant positive relationship with

weight loss ($r=0.21$) and frass produced ($r=0.41$). The results of the present study are supported by those of Senthilraja and Patel (2021), who reported a significant negative association between growth index with the developmental period ($r= -0.504$) and a significant positive association of growth index with adult emergence ($r= 0.766$). Similar observations were also reported in the studies by Tripathi et al (2015) and Miesho et al (2018). The developmental period had a significant negative association with adult emergence ($r= -0.54^{**}$) and a negative significant association with the number of eggs laid per 100 seeds ($r= -0.65^{**}$). Other parameters also recorded a significant moderate negative association with developmental period viz., percent infestation ($r= -0.49^{**}$), weight loss ($r= -0.49^{**}$) and frass produced ($r= -0.58^{**}$). The significant moderate negative association of developmental period with parameters such as adult emergence, percent infestation, weight loss, and frass produced indicates that the resistant cowpea seeds delayed development of *C. maculatus*, which resulted in increased post-embryonic mortality, reduced percent infestation, and consequently, reducing weight loss and frass produced per resistant cowpea genotype (Appleby and Creland, 2004). The present findings of a negative significant association between developmental period and the number of eggs laid per 100 seeds are in conformity with the report by Miesho et al (2018). This may be a result of the intraspecific competition due to the greater number of eggs laid which reduced the adult emergence, percent infestation, weight loss, and finally, frass produced.

Table 4: Correlation matrix of various growth parameters of *C. maculatus* on cowpea genotypes

Parameters	GI	DP	AE	No. of eggs	PI	WL %	FP
GI	-	-0.83**	0.85**	0.18	0.07	0.21	0.41
DP		-	-0.54**	-0.65**	-0.49**	-0.49**	-0.58**
AE			-	0.23	0.34	0.48**	0.43**
No. of eggs				-	-0.08	0.31	0.06
PI					-	0.69**	0.55**
WL						-	0.89**
FP							-

****Highly significant at 1 percent level; GI=growth index, DP=Developmental Period, AE=Adult Emergence, PI= Percent Infestation, WL= Weight Loss %, FP=Frass Produced**

The significant positive association between adult emergence and weight loss ($r=0.48^{**}$) and frass produced ($r=0.43^{**}$) and also the significant positive association between percent infestation and weight loss ($r=0.69^{**}$) and frass produced ($r=0.55^{**}$) coincides with the results reported by Sarwar (2012) and Senthilraja and Patel (2021). This indicates that, unlike the susceptible genotypes, resistant genotypes were able to delay the development of *C. maculatus*. This may be attributed to varying sorts of reserve protein and vicilin which cannot be metabolized by the midgut proteinases of insects, thereby reducing the food supply of the larvae and interfering with *C. maculatus* development (Domingues et al., 2006 and Senthilraja and Patel, 2021).

5.0 Conclusion

Out of all the genotypes screened, BBT1-11 outperformed all the genotypes across all parameters investigated. BBT1-11 could further be assessed for the possible presence of biochemical and genetic parameters, which could explain the observed differences among the genotypes in their reaction to *Callosobruchus maculatus*. Besides, BBT1-11 can also be incorporated into the breeding program with the target of breeding cowpea varieties that are tolerant to bruchid infestation.

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7.0 Conflict of Interest

The authors did not declare any conflict of interest.

8.0 Data Availability

Data is available upon request, by writing to the corresponding author.

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