

Laboratory Screening of Cowpea (*Vigna unguiculata*) Genotypes against Pulse Beetle, *Callosobruchus maculatus* (F.)

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Abstract

Research studies aimed at screening cowpea genotypes against *Callosobruchus maculatus* commonly known as a bruchid, under the no-choice infestation condition, were conducted at the Department of Plant Science, University of Zambia in Lusaka, Zambia during 2021 on 110 cowpea genotypes. The cowpea genotypes evaluated included 3 parental lines, 97 mutant lines, and 10 pure lines in order to discern the tolerant cowpea genotype against *Callosobruchus maculatus* that would aid the reduction of storage losses encountered in cowpea production. Parameters measured included, number of eggs laid per 100 seeds, adult emergence per cent, developmental period, growth index, per cent infestation, weight loss per cent, and frass produced. Significant differences were observed among the genotypes for all the parameters considered in the investigation. The most tolerant genotype BBBT1-11 across all parameters investigated, recorded 13.01 no. of eggs/100 seed compared to MS1-8-2-6-9-1 with 128.67 eggs/100 seeds. The adult emergence of BBBT1-11 was at 12.72% compared to the highest LTBT1-5 at 88.11%. BBBT1-11 also recorded the lowest growth index of 0.42 and frass produced of 0.23g compared to the highest growth index record of 4.19 in BB10-4-2-3-3 and the highest frass produced of 5.41g recorded in MS1-8-2-6-9-1. BBBT1-11 could be further assessed for the possible presence of bio chemical and genetic parameters, that could explain the basis of the observed differences among the genotypes in their reaction to *Callosobruchus maculatus*. Besides, BBBT1-11 can also be incorporated in the breeding program with the target of breeding cowpea varieties that are tolerant to bruchid infestation. Furthermore, BBBT1-11 may be released so as to address the storage losses experienced at postharvest in cowpea production.

Keywords: *Callosobruchus maculatus*; bruchid; tolerance

Abbreviations

IAEA: International Atomic Energy Agency, ZARI: Zambia Agricultural Research Institute, RUFORUM: Regional University Forum, NRDC: Natural Resources Development College, UNZA: University of Zambia, CD: Critical Difference, CV: Coefficient of Variation, S. Em: Standard Error of mean.

Introduction

Cowpea (*Vigna unguiculata* F.) is one of the major pulse crops that belong to the family Leguminosae. It is cultivated as green manure crop, fodder and pulse. Cowpea seeds contain approximately 60.3 per cent carbohydrates, 1.8 per cent fat and 23.4 per cent protein [3]. It is also a rich source of iron and calcium [7, 10]. Pulse crops, cowpea inclusive, have contributed to the improvement of agriculture of different countries, especially developing countries [16, 4, 14, 15].

Cowpea is, however, heavily damaged during storage from various insect pests. According to Semple et al (1992) and Sarwar (2012), three major pulse beetle species responsible for cowpea damage during storage are; *C. chinensis*, *C. analis* and *C. maculatus*. Bean beetle, *C. maculatus* (Fabricius), 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 [9, 12]. The beetle *C. maculatus*, has the potential to cause 100 per cent seed loss if left uncontrolled.

Management options that would be required, would be those that are cheap and effective. Efforts are being made to replace synthetic pesticides with botanical pesticides which are safe, cheap, less-persistent, eco-friendly and more specific. The use of resistant genotypes in insect pest management is considered a good and viable proposition [26].

Materials and Methods

Stock culture of insect pest

The study was carried out in the Department of Plant Science, University of Zambia, Lusaka, Zambia during 2021. Culturing of the Bruchid population was done at the University of Zambia, in the Insectarium laboratory. The population was maintained at the temperature of 28°C ($\pm 2^\circ\text{C}$) and Relative Humidity of 70 \pm 5% [15]. A random sample of three disinfected susceptible genotypes, Namuseba and Musandile and also one mutant for Musandile (MS1-8-1-4) were 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 4 hours in an oven before use. The jars were covered tightly with muslin affixed with rubber bands in order to prevent the adults from escaping [3]. Substrate (seed) weighing 150 grams was placed in each jar which were then infested with 20 unisex bruchids. The jars were kept under laboratory conditions undisturbed and mass cultured up to the third generation [18]. The freshly emerged adults of *C. maculatus* obtained, were further used for genotype screening [15].

Experimental cowpea seed

110 cowpea genotypes consisting of 3 parental materials provided by Zambia Agricultural Research Institute (ZARI), 97 mutants provided by the department of Plant Science of the University of Zambia, School of Agricultural Sciences, from the cowpea mutation development program carried out in collaboration with International Atomic Energy Agency (IAEA) and ZARI and 10 purelines which were provided by International Institute of Tropical Agriculture (IITA) were screened. The undamaged and clean cowpea seeds for each genotype used, 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 [15].

No-Choice test

Relative resistance and susceptibility of cowpea genotypes against *Callosobruchus maculatus* was done under no-choice test [8]. 100 seeds for each genotype, were weighed and kept separately in 250mls plastic containers and two pairs of adult pulse beetle (2 females and 2 males, 1-2 days old) were put in each container, separately [18]. Each container was covered on the top with two-fold muslin cloth fastened with a rubber band to avoid insects from escaping and yet provide sufficient aeration [15]. After infesting the plastic containers with the pulse beetles, the insects were discarded after 7 days from each plastic container, to ensure maximum oviposition [3]. The experimental design used in the study, was Completely Randomized Design (CRD) with 3 replications [23]. The data obtained was subject to analysis of variance and LSD values were obtained at 5% level of significance.

No. of eggs laid/100 seeds

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

Adult emergence (%)

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

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

Developmental period (days) of pulse beetle on each genotype

Developmental period was considered as time taken from oviposition to adult emergence [20].

Growth index of pulse beetle on different genotypes

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

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

Weight Loss (%)

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

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

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 [15].

Per cent infestation

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

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

S. No	Class	Per cent infestation
1	Highly resistance	0-20
2	Moderately resistance	21-40
3	Least susceptible	41-60
4	Moderately susceptible	61-80
5	Highly susceptible	81-100

Table1: Resistance/susceptibility rating based on Per cent seed infestation.

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 pulse beetle, *Callosobruchus maculatus*. Significant difference was observed among the genotypes for number of eggs laid per 100 seed of each genotype, adult emergence, developmental period, growth index, percentage infestation, weight loss and frass produced.

No. of eggs laid/100 seeds

It is clearly indicated from Table 2, that the number of eggs laid in no-choice condition by two pairs of *C. maculatus* on different cowpea genotypes varied significantly at 5% level of significance, from 13.01 in BBBT1-11 to 128.64 in MS1-8-2-6-9-1. Significantly less oviposition at 5% level of significance was seen in 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 condition as it recorded significantly greater number of eggs laid per 100 seeds at 5% level of significance, which was statistically at par with 43 genotypes some of which are not recorded in Table 2. Sharma and Thakur (2014), also reported that seeds with smooth surfaces were more preferred for laying eggs by *C. maculatus* as compared to cowpea seed that has a rough seed coat texture. Almost similar results of the relationship between seed size, seed colour and ovipositional preference was reported by Shaheen et al., (2006), Patil et al (2009), Tripathi et al (2015) and Raghuvashi et al., (2016) and Waghmare and Bantewad (2020). Therefore, the current findings are in tandem with the earlier researchers.

Adult emergence

The per cent adult emergence of *C. maculatus* on different cowpea genotypes varied significantly from 12.72% to 88.11% (Table 2). The lowest adult emergence per cent was recorded in 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 per cent was recorded in 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 [6, 18].

Development period

Developmental period of *C. maculatus* can be considered as the number of days taken by the adult *C. maculatus* to emergence since the oviposition period (2020). Developmental period varied significantly at 5% level of significance, from 19.02 days to 31.12 days. The lowest developmental period of 19.02 days was recorded in BB10-4-2-3-3 which was statistically at par with 83 genotypes out of 110 genotypes screened against *C. maculatus* indicating susceptibility. The highest developmental period of 31.12 days was recorded in 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 lowest developmental period of 19.67 days in the genotype GC1710 and also the study by Waghmare and Bantewad (2020), who recorded the highest developmental period of 31.85 days in the genotype ICCV-3137. The results are also in accordance with Tripathi et al., (2015), who reported that the developmental period was significantly shorter in susceptible genotypes and significantly longer in resistant genotypes of cowpea. The longer developmental period of *C. maculatus* in cowpea genotypes may be attributed to the antibiosis type of resistance characterized by an increased length of time between the egg and adult phases which results into the reduction in adult emergence (2012).

Growth index

The data concerning growth index of *C. maculatus* on different cowpea genotypes varied significantly at 5% level of significance, ranging from 0.42 recorded in BBBT1-11 to 4.40 recorded in LTBT1-5. BBBT1-11 with the growth index of 0.42 was statistically at par with LT11-5-2-2-7(0.68), LT11-5-2-2-10(0.56) and BBBT1-3(0.64). The highest growth index was recorded in 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.

S. No	Genotypes	No. of eggs/ 100 seed	Adult emergence %	Development period (days)	Growth Index	Percent Infestation	Weight loss %	Frass produced (grams)
1	LT11-5-2-2-2	18.27	27.01	30.33	0.89	13.11	0.94	0.27
2	LT11-5-2-2-4	19.61	54.72	31.12	1.76	16.02	2.68	0.41
3	LT11-5-2-2-7	21.21	16.71	24.67	0.68	11.67	2.36	0.37
4	LTBT1-4	27.61	63.01	27.33	2.31	13.33	5.51	0.69
5	LT11-5-2-2-10	24.81	15.34	27.33	0.56	13.33	2.69	0.37
6	LT11-5-2-2-20	18.67	32.33	23.64	1.37	8.67	3.66	0.43
7	BBBT1-3	36.17	19.71	30.67	0.64	12.33	5.63	0.53
8	BB10-4-2-3-1	14.67	42.31	30.33	1.39	13.01	1.76	0.23
9	BB7-9-7-5-3	20.07	61.02	26.01	2.35	11.01	2.12	0.37
10	BB10-4-2-3-3	24.33	79.72	19.02	4.19	14.01	0.96	0.23
11	MS1-8-2-6-6-2	14.67	32.32	20.02	1.61	8.03	3.71	0.47
12	BBBT1-7	28.67	42.32	21.67	1.95	4.02	6.94	0.43
13	BBBT1-11	13.01	12.72	30.33	0.42	10.03	1.05	0.23
14	IT99K241-2	67.67	51.71	21.33	2.42	13.04	2.03	0.42
15	IT99K573-2-1	47.67	79.31	22.33	3.55	17.02	2.24	0.37
16	LT16-7-2-5-1	123.17	80.31	19.67	4.08	95.42	58.58	6.53
17	LTBT1-5	118.21	88.11	20.04	4.4	93.22	44.01	5.07
18	BBBT1-1	124.31	49.72	24.33	2.04	95.01	37.94	4.33
19	MS1-8-2-6-9-1	128.67	84.01	22.02	3.82	90.33	55.86	5.41
20	LUNKWAKWA	36.01	66.71	23.33	2.86	23.02	14.87	0.33
21	LT-Parent	102.11	86.31	20.33	4.25	43.03	27.77	1.73
22	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	2.9	0.42
CV %		3.33	2.44	3.26	1.05	2.11	4.39	2.26

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

Per cent Infestation

Per cent infestation by *C. maculatus* among different cowpea genotypes, varied from 4.02 per cent recorded by BBBT1-7 to 95.42 per cent recorded by LT16-7-2-5-1 (Table 3). Significantly less damage was recorded in the mutant line BBBT1-7 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, BBBT1-11 and IT99K241-2. Significantly, the highest damage was recorded in the mutant line LT16-7-2-5-1 at infestation of 95.42%, which was statistically at par with MS1-8-2-6-9-1, LTBT1-5, BBBT1-1 and other 22 genotypes not listed in Table 3. Based on per cent infestation, 15 genotypes were classified as highly resistance, 19 genotypes were classified as moderately resistance, 16 genotypes were classified as least susceptible, 21 genotypes were classified as moderately susceptible and 39 genotypes were classified as highly susceptible (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 BBBT1-7 was classified as highly resistant but also recorded some degree of damage of 4.02 percent. 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).

S. No	Class	Per cent Infestation	Number of genotypes/Variety
1	Highly resistant	0-20	15
2	Moderately resistance	21-40	19
3	Least susceptible	41-60	16
4	Moderately susceptible	61-80	21
5	Highly susceptible	81-100	39

Table 3: Resistance/susceptibility rating based on percent infestations.

Weight loss

The results on per cent weight loss caused by *C. maculatus* to the seeds of different cowpea genotypes varied significantly. The lowest per cent weight loss of 0.94 percent, was recorded in 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%), BBBT1-11(1.05%), IT99K241-2(2.03%) and IT99K573-2-1(2.24%) indicating resistance to *C. maculatus*. Significantly high weight loss at 5% level of significance, was recorded in 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, per cent weight loss was much influenced by the levels of susceptibility in different cowpea genotypes [26]. The significant susceptibility levels of LT16-7-2-5-1 and MS1-8-2-6-9-1 at 5% level of significance, may be attributed to variable traits that made them to be more preferred to *C. maculatus* compared to other genotypes [19]. The present findings are also in conformity with Patil et al., (2009), who reported that significantly high per cent weight loss (77.9%) was recorded in Mexican Doller which had smooth bold seed coat compared to other genotypes with 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 per cent infestation levels.

Frass produced

Significantly low frass of 0.23g at 5% level of significance, was produced by BBBT1-11, 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), BBBT1-7(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 per cent infestation (grain damage) and adult emergence. In the study by Sarwar (2012), the genotype CH-86/02 which recorded the significantly highest per cent infestation of 87% also recorded the significant highest frass weight of 0.24g, this coincides with the present findings.

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 developmental period ($r = -0.83^{**}$), highly significant positive association with adult emergence ($r = 0.85^{**}$), non-significant weak positive association with number of eggs laid per 100 seeds ($r = 0.18$), non-significant positive relationship with per cent 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 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).

Developmental period had a significant negative association with adult emergence ($r = -0.54^{**}$) and a negative significant association with 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 indicate 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 [1]. The present findings of a negative significant association between developmental period and number of eggs laid per 100 seeds is in conformity with the report by Miesho et al (2018). This may be as 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.

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 [6, 18].

Parameters	GI	Developmental Period	Adult Emergence	No. of eggs	Percent Infestation	Weight loss %	Weight loss %
GI	-	-0.83 ^{**}	0.85 ^{**}	0.18	0.07	0.21	0.41
Developmental Period		-	-0.54 ^{**}	-0.65 ^{**}	-0.49 ^{**}	-0.49 ^{**}	-0.58 ^{**}
Adult Emergence			-	0.23	0.34	0.48 ^{**}	0.43 ^{**}
No. of eggs				-	-0.08	0.31	0.06
Percent Infestation					-	0.69 ^{**}	0.55 ^{**}
Weight loss						-	0.89 ^{**}
Frass Produced							-

^{**}Highly significant at 1 percent level; GI=growth index

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

Conclusion

The most tolerant genotype BBBT1-11 across all parameters investigated, recorded 13.01 no. of eggs/100 seed compared to MS1-8-2-6-9-1 with 128.67 eggs/100 seeds. The adult emergence of BBBT1-11 was at 12.72% compared to the highest LTBT1-5 at 88.11%. BBBT1-11 also recorded the lowest growth index of 0.42 and frass produced of 0.23g compared to the highest growth index record of 4.19 in BB10-4-2-3-3 and the highest frass produced of 5.41g recorded in MS1-8-2-6-9-1. BBBT1-11 could further be assessed for the possible presence of bio chemical and genetic parameters, that could explain the basis of the observed differences among the genotypes in their reaction to *Callosobruchus maculatus*. Besides, BBBT1-11 can also be incorporated in the breeding program with the target of breeding cowpea varieties that are tolerant to bruchid infestation. Furthermore, BBBT1-11 may be released so as to address the storage losses experienced at postharvest in cowpea production.

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Conflict of Interest

The authors have not declared any conflict of interests.

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