Journal of Genetics, Genomics & Plant Breeding 7(1) 12-22 (January, 2023)
ISSN (Online): 2581-3293

RESEARCH ARTICLE

Mutation Breeding: A modern tool for shelf-life extension in Zambian cowpea lines

A.C. Siyunda[1,2], N.M. Mwila[2], M. Mwala[2], K. Munyinda[2], K. Kamfwa[2], G. Chipabika[2]

1. Department of Crop Science, Natural Resources Development College (NRDC), P/Bag CH 99, Chelstone, Lusaka, Zambia

2. Department of Plant Science, School of Agricultural Sciences, University of Zambia (UNZA), P.O. Box 32379, Great East Road Campus, Lusaka, Zambia

Corresponding authors email Id: aaron.siyunda@yahoo.com

Manuscript received: November 9, 2022; Decision on manuscript, December 8, 2022; Accepted: January9, 2023

Abstract

Research studies were conducted to evaluate the potential of mutation breeding as a modern tool in extending the shelf-life of cowpea that suffers heavy losses when attacked by *Callosobruchus maculatus*. The study was done under the no-choice infestation laboratory conditions. Significant variations were observed where mutants outperformed parental genotypes on average across all the parameters under consideration at a 5% level of significance. Mutants recorded on average 50.36 eggs laid/100 seeds compared to 115.86 eggs/100 seeds on parent material. Adult emergence of 48.08% was recorded in mutants compared to 81.31% on parent material. In terms of the developmental period, mutants recorded 25.42 days compared to 19.75 days in parental material. Significant variations were also observed in percent infestation as mutants recorded 32.18% compared to 54.05% in parent material on average. Mutants recorded 12.09% while parent material recorded 26.59% in terms of weight loss percent. On average, a growth index of 4.12 in parent material was also recorded compared to 1.99 in mutant lines. Therefore, a successful extension in the genotype shelf-life through mutation breeding was observed. The researcher recommends the inclusion of BBBT1-11(best performing mutant line across all parameters) in the cowpea breeding programs that have a focus on developing resistant cowpea varieties to address the losses experienced at postharvest in cowpea production, thus extending shelf-life in a more cost-effective, less time consuming and environment-friendly manner.

Key words: *Callosobruchus maculatus*, infestation, mutants, parent material, postharvest

Introduction

According to Chikalipa *et al.*, (2022) and Siyunda *et al.*, (2022a), cowpea [*Vigna unguiculata* (L.) Walp., 2n = 2x = 22] is one of the major leguminous crops of African countries (Chikalipa *et al.*, 2022) having a genome size of 620 million base pairs and cultivated in the semiarid areas across the sub-Saharan region as green manure, folder, and food. Cowpea is considered an important cash crop and it plays a major role in food security and the cropping system as it has contributed immensely to the improvement of agriculture most especially in countries across the African continent (Mwila *et al.*, 2022a). Cowpea has a good nutritional profile, in that it contains approximately 24.1% proteins, 1.9% fat, 62% carbohydrate and it also has a high level of calcium and iron (Khan, 2011).

Nevertheless, cowpea is heavily affected by biotic factors including insects. According to Siyunda *et al.,* (2022b) and Mwila *et al.*, (2022b), huge losses are sustained during postharvest by cowpea producers as a result of the attacks by insects which results in reduced shelf-life. Sarwar (2012), highlights three main cowpea weevil species that are a major contributor to damages sustained by cowpea at postharvest and these are *C. maculatus*, *C. chinensis*, and *C. analis*. *Cowpea weevil*, *C. maculatus*, is an important agricultural insect pest that is found throughout 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 (Waghmare and Bantewad 2020). 90-100% seed loss can potentially be incurred if *C. maculatus* is not controlled (Choudhary, 2015). The step and concept of developing/breeding plant varieties/genotypes that have resistance against insect pests are regarded as a good and feasible idea for sustainable pest management (Kergoat *et al.,* 2007). Therefore, the main objective of the current study was to evaluate the potential that mutation breeding has in breeding cowpea genotypes (mutants) with resistance to *C. maculatus* to improve the shelf-life of cowpea materials.

Materials and methods

Population development of *C. maculatus*

The culturing of *C. maculatus* stock was done in the insectarium laboratory at the University of Zambia in 2021. The protocol used was as described by Sarwar (2012) and Choudhary *et al.,* (2015). The population of *C. maculatus* was maintained at a relative humidity of 70% (±5%) and a temperature of 280C (±20C). The local variety called Namuseba (susceptible to C. maculate) was used as a substrate and check. Before being placed in the six sterilized jars, the substrate was disinfected. Six jars were sterilized by being placed in an oven for 4 hrs while maintaining the temperature at 550C. 150g of seeds were placed in each sterilized jar and 20 weevils in each jar were introduced. Rubber bands were used to cover the mouth of each jar tightly, to prevent adult *C. maculatus* from escaping. The Jars were kept undisturbed in the laboratory and the insect population was advanced to the third generation for mass culturing purposes (Senthilraja and Patel, 2021). Adult insects of *C. maculatus* (1 to 2 days old) were then used for the screening of genotypes.

Germplasm source for cowpea seed

The germplasm used in the current study was provided by the department of plant sciences, school of Agricultural Science at the University of Zambia. The germplasm included parental genotypes; Lutembwe (LT-parent), Bubebe (BB-parent), and Msandile (MS-parent), and also their mutant lines (7 mutants from each parent).

Mutant development

The mutants were developed under the Cowpea Mutation Breeding Program at the University of Zambia, School of Agricultural Sciences, Plant Science Department. The parental material was exposed to radioactive rays (gamma rays). The exposed parental material was then advanced from the first mutant generation (M1) to the seventh mutant generation (M7) to stabilize the mutant material. At the time of the current study, M7 seed was obtained and planted at the University of Zambia field station during the 2020-2021 planting season. M8 seed was then harvested and screened against *C. maculatus.*

Material preparation

Clean and undamaged seeds for each genotype were selected and further examined to ensure that all the seeds did not have any eggs of C. maculatus laid on them before the commencement of the laboratory experiment. The close examination was done using the hand lens. To ensure that the seed was thoroughly cleaned, the seeds of all genotypes were further placed in the deep freezer and kept at -50C for 7 days. Thereafter, the seeds were removed from the deep freezer and placed under laboratory conditions for 24 hrs. (Sarwar, 2012).

No choice test

In the current study, the protocol used for the no-choice test is as described by Senthilraja and Patel (2021). 100 seeds for each genotype were kept separately in 250 ml plastic containers. This was done after weighing the 100 seeds of each genotype. The introduction of two pairs of adult *C. maculatus* (1 or 2 days old) in each 250ml container was done. For sufficient aeration and also to prevent adult *C. maculatus* from escaping, a two-fold muslin cloth was fastened on the mouth of each container using the rubber bands. For maximum insect oviposition, 2 pairs of adult *C. maculatus* infested on the seed in 250mls were released after one week (Choudhary *et al.*, 2015). Completely Randomized Design (CRD) was used with three replications of each genotype. The data generated in the study were subjected to the analysis of variance and CD values were obtained at a 5% level of significance using R-programming software for comparing the mean values of genotypes. Furthermore, the correlation matrix of various parameters of *C. maculatus* on cowpea genotypes was developed.

Number of eggs/100 seeds

The counting of eggs laid on parental and mutant genotypes was done after 7 days of the infestation of *C. maculatus*. To enhance visibility, a hand lens was utilized (Senthilraja and Patel, 2021).

Adult emergence percent

Adult emergence was determined using the formula suggested by Sharma and Thakur (2014) as below. Readings were taken every after 24hrs daily.

Adult emergence (%) = (Number of adults emerged) / (Number of eggs laid) x 100

Development period

The time (number of days) between oviposition and adult emergence was considered a developmental period (Sharma and Thakur, 2014).

Percent infestation

The calculation and classification of percent infestation were done according to the formula and scale suggested by Deshpande *et al.*, (2011).

Percent infestation = (No. of seeds with emergent holes) / (Total number of seeds observed) x 100

Weight loss

Both the initial and final weight of each treatment was taken using the single pan electric balance. Weight loss was further calculated using the formula suggested by Sharma and Thakur (2014).

Weight loss (%) = (Initial weight of seeds (g)- Final weight of seeds (g)) / (Initial weight of seeds (g)) x 100

Growth index

The formula used in the calculation of the growth index was as below, suggested by Senthilraja and Patel, (2021).

Growth index = (Adult emergence (%)/ (Developmental period (days)

Results and discussion

Number of eggs/100seeds

Number of eggs laid by C. maculatus on cowpea seeds of different genotypes differed significantly at 5% level. The highest record was in MS-parent (135.12). MS-parent was statistically at par with Namuseba (123.12), LTBT1-5 (118.21), BBBT1-1 (124.31), MS 1-8-1-4-1 (123.12) and MS1-8-2-6-9-1 (128.67). The lowest number of eggs/100 seeds was recorded in BBBT1-11 (13.01). BBBT1-11 was statistically at par with MS1-8-2-6-6-2 (14.67), BBBT1-7(28.67), BB10-4-2-3-3 (24.33) and BB10-4-2-3-1 (14.47 (Table 1). On average, the parental material (LT, BB, and MS parents) recorded 115.86 eggs/100 seeds compared to the mutant material that recorded an average of 50.36 eggs/ 100 seeds. The mutants derived from the parental genotype Lutembwe, recorded an average of 35.48 eggs/100 seeds while those derived from the parental genotypes Bubebe and Musandire recorded 37.32 eggs/ 100 seeds and 78.29 eggs/ 100 seeds respectively. Significant variations occurred, between the number of eggs recorded on parental material (115.86) and mutant material (50.36). Number of eggs laid on mutants derived from LT-parent (35.48) and BB-parent (37.32), was not significantly different. However, significant differences occurred between mutants derived from MS-parent (78.29) and those derived from LT-parent (35.48) and BB-parent (37.32). Similar results about oviposition preference were recorded by Shaheen *et al.*, (2006). Therefore, the results of the current investigation are similar to the earlier findings. Significant variations between the number of eggs recorded on parental material and mutant material may be due to the genetic variation caused by mutation. This, further indicates that mutation might have successfully manipulated the genome of the genotype (s) Lutembwe, Msandile, and Bubebe and resulted in the formation of the resistant gene(s) found in mutant line (s) (less preferred genotypes for oviposition) and not in parental line (s) (more preferred for oviposition).

Adult emergence percent

Significant variations were observed in the adult emergence among different lines, ranging from 12.72% recorded by BBBT1-11 to 88.11% recorded by LTBT1-5. Mutant line LTBT1-5 was statistically at par with all the parental lines; LT-parent (86.31%), BB-parent (78.21%) and, MS-parent (79.41%) and also some mutant lines BB10-4-2-3-3 (79.72%) and MS 1-8-2-6-9-1 (84.01%) (Table 1). The lowest adult emergence was recorded in the mutant line BBBT1-11 (12.72%) which was statistically at par with mutant lines; LT11-5-2-2-7 (16.71%), LT11-5-2-2-10 (15.34%), and BBBT1-3 (19.71%). On average, the parental genotypes (LT, BB, and MS parents) recorded 81.31% compared to the mutant genotypes which recorded an average of 48.04%. The mutants derived from the parental genotype Lutembwe (LT-parent), recorded an average of 42.46% while those derived from the parental genotypes Bubebe (BB-parent) and Musandire (MS-parent) recorded 43.93% and 57.74% respectively. Significant variations occurred, between the adult emergence recorded on parental material (81.31%) and mutant material (48.04%). Adult emergence of mutants derived from LT-parent (42.46%) and BB-parent (43.93%), was not significantly different. However, significant differences in adult emergence occurred between mutants derived from MS-parent (57.74%) and those derived from LT-parent (42.46%) and BB-parent (43.93%). The lower adult emergence percent exhibited by mutant lines BBBT1-11, LT11-5-2-2-7, LT11-5-2-2-10, and BBBT1-3 is an indication of resistance against *C. maculatus* while the higher adult emergence percent recorded by all parental material including mutant lines; LTBT1-5, BB10-4-2-3-3 and MS 1-8-2-6-9-1 is an indication of susceptibility against *C. maculatus.*

The results of the current study, are in tandem with the results of Senthilraja and Patel, (2021). The resistance against *C. maculatus* exhibited by some mutant lines derived from LT-parent and BB-parent might be due to the successful alteration of the parent material that resulted in the production of high levels of proteins that cannot be digested / metabolized by insects. The presence of undigestible proteins reduced the food supply for the larvae thus interfering in the development of C. maculatus and consequently resulting in low adult emergence in the mutant lines that had the presence of these proteins and high adult emergence in the parental material and some of the mutant lines that did not have the presence of these proteins.

Development period

The development period of *C. maculatus* among different genotypes ranged from 18.90 days recorded by MS-parent to 31.12 days recorded by LT11-5-2-2-4. Significant differences at the 5 % level, were observed among different genotypes. MS-parent (18.90 days) recorded the shortest development period and was statistically at par with BB-parent (20.01), and the mutant lines; MS1-8-2-6-9-1 (22.02 days), MS10-11-1-1-1 (21.32 days), MS1-8-2-6-6-2 (20.02 days), BBBT1-7 (21.67 days), BB10-4-2-3-3 (19.02 days), and LTBT1-5 (20.04 days) (Table 1). The longest development period recorded by LT11-5-2-2-4 (31.12 days) was statistically at par with mutant lines; LT11-5-2-2-2 (30.33 days), BBBT1-3 (30.67 days), BBBT1-11 (30.33 days), BB10-4-2-3-1 (30.33 days) and MS1-8-2-2-5-1 (28.23 days). Parental genotypes (LT, BB, and MS parents) recorded an average development period of 19.75 days compared to the mutant genotypes that recorded an average of 25.42 days. The mutants derived from the parental genotype Lutembwe (LT-parent), recorded an average of 26.35 days while those derived from the parental genotypes Bubebe (BB-parent) and Musandire (MS-parent) recorded 26.05 days and 24.02 days respectively. Significant variations occurred, between the development period recorded on parental material (19.75 days) and mutant material (25.47 days). Non-significant differences were observed among mutants derived from LT-parent (26.35 days), BB-parent (26.05 days), and MS-parent (24.02 days). The genotype ICCV-3137, classified as resistant against C. maculatus in the study by Waghmare and Bantewad (2020) recorded a development period of 31.85 days which correlates with the developmental period of 31.12 days recorded by mutant line LT11-5-2-2-4 in the current study. Thus, the current findings are in accordance with the previous studies. The longer developmental period indicates resistance against *C. maculatus* while the shorter developmental period indicates susceptibility against *C. maculatus*. Further, the longest developmental period exhibited by LT11-5-2-2-4 might be a result of the antibiosis defense mechanism which consequently reduced the development of larvae while in the seed and delayed its emergence. Therefore, the processes of mutation successfully created the variation between some mutant lines (with a longer developmental period) and the parent lines (with a shorter developmental period).

Percent infestation

The percent infestation of *C. maculatus* on various cowpea genotypes varied significantly at a 5% level of significance. Mutant line BBBT1-1 recorded the highest percent infestation of 95.01% followed by LTBT1-5 (93.22%), MS1-8-2-6-9-1 (90.33%), and MS10-11-1-1-1 (89.78%) (Table 1). The lowest percent infestation was recorded by BBBT1-7 (4.02%). BBBT1-7 was statistically at par with mutant lines; BBBT1-11 (10.03), BBBT1-3(12.33), BB10-4-2-3-3 (14.01), BB10-4-2-3-1 (13.01), BB7-9-7-5-3 (11.01), LT11-5-2-2-2 (13.11), LT11-5-2-2-7 (11.67) LT11-5-2-2-10 (13.33), and LTBT1-4 (13.33%).

Table 1: Mean values of parameters measured during the evaluation of Cowpea genotypes against pulse beetle *Callosobruchus maculatus* (Fabricius)

Genotypes	**eggs/100 seeds**	**AEP**	**DP (days)**	**GI**	**PI**	**WLP**
Namuseba(check)	123.12	45.72	21.22	2.15	49.02	23.41
LT-Parent	102.11	86.31	20.33	4.25	43.03	27.77
LT11-5-2-2-2	18.27	27.01	30.33	0.89	13.11	0.94
LT11-5-2-2-4	19.61	54.72	31.12	1.76	16.02	2.68
LT11-5-2-2-7	21.21	16.71	24.67	0.68	11.67	2.36
LT11-5-2-2-10	24.81	15.34	27.33	0.56	13.33	2.69
LT11-5-2-2-20	18.67	32.33	23.64	1.37	8.67	3.66
LTBT1-4	27.61	63.01	27.33	2.31	13.33	5.51
LTBT1-5	118.21	88.11	20.04	4.40	93.22	44.01
BB-Parent	110.34	78.21	20.01	3.91	40.67	15.89
BB7-9-7-5-3	20.07	61.02	26.01	2.35	11.01	2.12
BB10-4-2-3-1	14.67	42.31	30.33	1.39	13.01	1.76
BB10-4-2-3-3	24.33	79.72	19.02	4.19	14.01	0.96
BBBT1-1	124.31	49.72	24.33	2.04	95.01	37.94
BBBT1-3	36.17	19.71	30.67	0.64	12.33	5.63
BBBT1-7	28.67	42.32	21.67	1.95	4.02	6.94
BBBT1-11	13.01	12.72	30.33	0.42	10.03	1.05
MS-Parent	135.12	79.41	18.90	4.20	78.45	36.11
MS1-8-1-4-1	123.12	60.12	27.12	2.22	54.23	21.42
MS1-8-2-2-5-1	43.01	76.74	28.23	2.72	29.31	6.36
MS1-8-2-6-6-1	58.89	38.12	25.31	1.51	22.21	5.42
MS1-8-2-6-6-2	14.67	32.32	20.02	1.61	26.03	3.71
MS1-8-2-6-8-1	77.56	45.45	24.12	1.88	35.11	7.77
MS1-8-2-6-9-1	128.67	84.01	22.02	3.82	90.33	45.86
MS10-11-1-1-1	102.12	67.43	21.32	3.16	89.78	45.12
S. Em ±	9.32	4.81	0.82	0.25	6.12	3.16
CD at 5%	18.16	9.48	3.12	0.47	11.21	7.90
CV%	17.8	9.45	2.57	1.47	9.57	15.78

The parental material recorded a percent infestation of 54.05% which was statistically different from the average percent infestation of mutant genotypes (32.18%). The mutants derived from LT-parent, recorded an average percent infestation of 24.19% while those derived from BB-parent and MS-parent recorded 22.77% and 49.57% respectively. Significant variations occurred, between the percent infestation recorded by parental genotypes (54.05%) and mutant genotypes (32.18%). Percent infestation of mutants derived from LT-parent (24.19%) and BB-parent (22.77%), was not significantly different. However, significant differences in percent infestation occurred between mutants derived from MS-parent (49.57%) and those derived from LT-parent (24.19%) and BB-parent (22.77%).

According to the scale modified from Senthilraja and Patel (2021), mutant materials on average can be classified as moderately resistant compared to the parental materials (LT, BB, and MS) classified as least susceptible. The distinct differences between parental materials and their mutants in terms of percent infestation might be attributed to the successful alteration of the genome of the parental materials as they were exposed to radiation. Mutation might have led to the manipulation of the parental genotype and created resistant gene(s) against *C. maculatus* in some of the mutant lines. However, some mutant lines recorded very high percent infestation compared to parent materials e.g., Mutant line BBBT1-1 (95.01%), LTBT1-5 (93.22%), MS1-8-2-6-9-1 (90.33%) and MS10-11-1-1-1 (89.78%). The high percent infestation in these mutant lines may be attributed to mutation which altered the genome of the parental material and resulted in the creation of high digestible proteins that promoted the growth and development of *C. maculatus*. Thus, resulting in highly susceptible mutant lines.

Weight loss

Significant variations were observed in the weight loss among different lines, ranging from 0.94% recorded by mutant line LT11-5-2-2-2 to 45.86% recorded by mutant line MS1-8-2-6-9-1 (Table 1). The lowest weight loss of LT11-5-2-2-2 (0.94%), was statistically at par with 16 genotypes while the highest weight loss of MS1-8-2-6-9-1 (45.86%) was statistically at par with 3 genotypes. LT, BB, and MS parents recorded an average weight loss of 26.59% compared to the mutant genotypes that recorded an average of 12.09%. Significant variations occurred, between the weight loss recorded on parental material (26.59%) and mutant material (12.09%). However, significant differences in weight loss occurred between mutants derived from MS-parent (19.38%) and those derived from LT-parent (8.84%) and BB-parent (8.06%). Significant variations in weight loss percent indicate varying levels of resistance of cowpea genotypes against *C. maculatus.* Genotypes with high weight loss percent indicate susceptibility while those with low weight loss percent indicate resistance (Deshpande *et al.*, 2011). Adult emergence and percent infestation highly influence the seed weight loss percent caused by *C. maculatus*. On average, the parent material (LT, BB and MS) recorded a significant weight loss percent compared to the mutant material. This can be attributed to the high adult emergence and percent infestation sustained by parental material compared to the mutant material. Further, high adult emergence and percent infestation produce high fresh weight which affected the final weight of the cowpea seed negatively. Thus, genotypic variations were successfully created by mutation, between parental materials and their mutants as observed in their phenotypic variations concerning weight.

Growth index

Significant variations at the 5% level, were observed in the growth index of different cowpea lines. The lowest growth index was recorded on mutant line BBBT1-11 (0.42). BBBT1-11 was statistically at par with mutant lines; BBBT1-3 (0.64), LT11-5-2-2-2 (0.89), LT11-5-2-2-7 (0.68), and LT11-5-2-2-10 (0.56). The highest growth index was recorded by LTBT1-5 (4.40), statistically at par with parental materials LT-parent and MS-parent that recorded 4.25 and 4.20 respectively. LTBT1-5 was also statistically at par with mutant line BB10-4-2-3-3 (4.19). On average, the parental genotypes recorded a growth index of 4.12 which was statistically different from the average growth index of mutant genotypes (1.99). The mutants derived from LT-parent, recorded an average growth index of 1.71 while those derived from BB-parent and MS-parent recorded 1.85 and 2.42 respectively.

Significant variations occurred, between the average growth index recorded on parental genotypes (4.12) and mutant genotypes (1.99). The average growth index of mutants derived from LT-parent (1.71) and BB-parent (1.85), was not significantly different. However, significant differences in growth index occurred between mutants derived from MS-parent (2.42) and those derived from LT-parent (1.71) and BB-parent (1.85). Significant variations in growth index are an indication of significant variations in resistant levels against C. maculatus among different cowpea genotypes under consideration. According to Senthilraja and Patel (2021), a high growth index is an indication of susceptibility while a low growth index is an indication of resistance against C. maculatus. The significantly high growth index in parent materials can be attributed to the short developmental period recorded in parent materials compared to the mutant materials. The short developmental period which led to a high growth index might be attributed to the presence of highly metabolizable proteins in parent materials that created a ready supply of food for larvae growth and development. However, mutant lines on average had a significantly lower growth index. This is an indication of lower levels of the said proteins. Thus, parental material exposure to radiation, successfully created the variation both at the genotypic and phenotypic level, between some mutant lines (with longer developmental period and lower growth index) and the parental lines (with shorter developmental period and higher growth index).

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

Table 2 shows the correlation matrix of various growth parameters of C. maculatus on cowpea lines. The growth index had a significantly strong negative association with the development period (-0.74**), a significant strong positive association with adult emergence (0.83**), a non-significant weak positive association with number of eggs/100 seeds (0.21), significantly moderate positive relationship with percent infestation (0.61**) and significantly positive moderate association with weight loss percent (0.63**). The development period exhibited significant moderately negative relationships with adult emergence (-0.45**), number of eggs/100 seeds (-0.45**), percent infestation (-0.51**), and weight loss percent (-0.55**). A non-significantly weak positive association of 0.28, existed between percent infestation and adult emergence. However, adult emergence had significantly moderate positive associations with number of eggs/100 seeds (0.60**) and weight loss percent (0.61**). Weight loss percent had a non-significant weak positive association with number of eggs/100 seed (0.18) and a significantly strong positive relationship with percent infestation (0.87**).

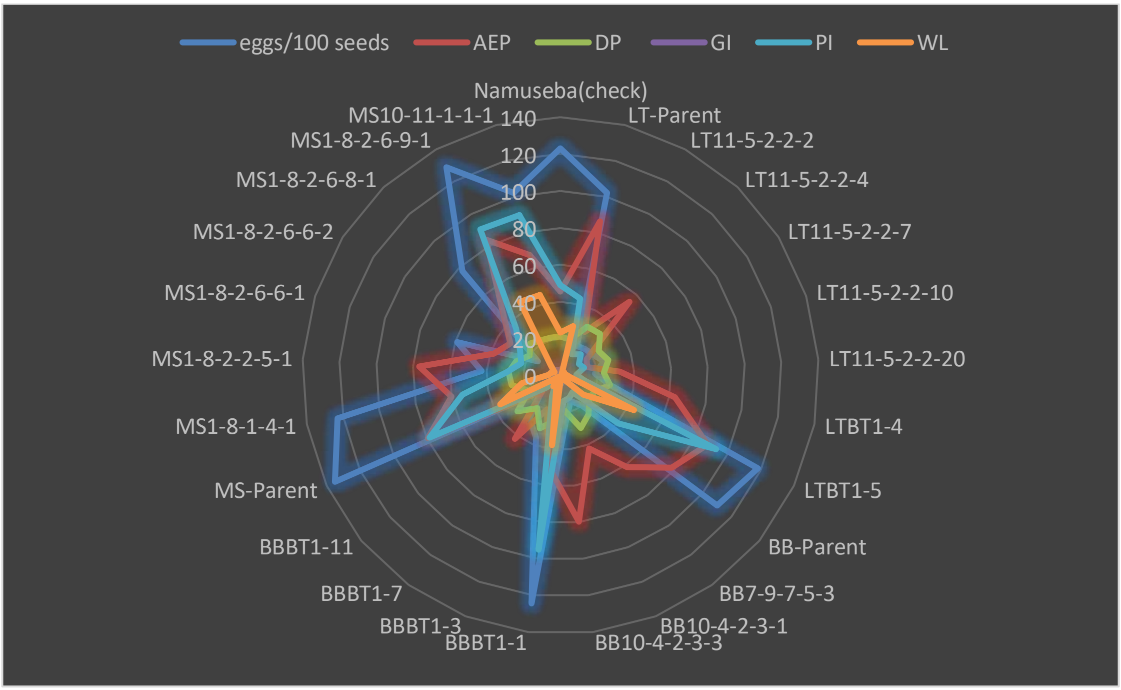
Table2: Correlation matrix for growth parameters of *C. maculatus* on cowpea genotypes

Parameters	**Growth index**	**Development period**	**Adult emergence**	**No. of eggs**	**Percent infestation**	**Weight loss (%)**
Growth index	-	-0.74**	0.83**	0.21^{ns}	0.61**	0.63**
Development period		-	-0.45**	-0.45**	-0.51**	-0.55**
Adult emergence			-	0.60**	0.28^{ns}	0.61**
No. of eggs				-	0.87**	0.18^{ns}
Percent infestation					-	0.87**
Weight loss (%)						-

**Highly significant at 1 percent level

The correlation matrix of various parameters of C. maculatus on cowpea genotype indicates how the parameters interacted (Figure 1). The strong negative association between the development period and growth index indicates the significant variations that exist between susceptible and resistant cowpea genotypes. Unlike the susceptible parental material and some mutant lines, resistant mutant lines were able to delay the growth and development of *C. maculatus*. Thus, lengthening the development period and reducing the growth index. However, adult emergence had a strong positive association with the growth index. This is because susceptible genotypes had an increased number of adult insects that emerged out of the total number of eggs laid on the cowpea seed compared to the resistant lines that had a small number of adult insects that emerged out of the total number of eggs laid on the cowpea seed. The non-significant weak positive association of percent infestation and adult emergence indicates that the number of developed and emerged adults has a non-significant bearing on the number of holes perforated on the cowpea seed surface. Furthermore, this indicates that the number of holes made on a particular cowpea seed has no significant bearing on the total number of *C. maculatus* that develop within a particular seed. The significant strong positive association between number of eggs/100 seeds and percentage infestation, might be an indication that the proteins responsible for deterring egg-laying on the seed surface of cowpea genotypes, may be linked to the non-digestible/metabolizable proteins that can interfere in the development of *C. maculatus* while within the seed.

Fig 1: Interaction matrix of measured parameters during the evaluation of cowpea genotypes against *C. maculatus*



Where, ADP, Adult emergence percent; DP, Development period; GI, Growth index; PI, Percent infestation; WL, Weight loss

In conclusion the study successfully identified the gains made by using mutation breeding as a modern tool for shelf-life extension. This can be seen in the outperformance of some mutants compared to their parents across all the parameters under consideration, especially in the development period and percent infestation. On average, mutation led to the extension of the developmental period of *C. maculatus* from 19.75 days (parental material) to 25.47 days (mutant material) and reduced the percent infestation from 54.05% (parental material classified as least susceptible against *C. maculatus*) to 32.18% (mutant material classified as moderately resistant against C. maculatus). The increase in the developmental period indicates a delay in the growth and development of *C. maculatus* within the cowpea seed while the reduction in percent infestation indicates a reduction in the damage sustained by the cowpea seed from *C. maculatus.* Thus, a successful extension in the genotype shelf-life through mutation breeding. The researcher recommends the inclusion of BBBT1-11(best performing mutant line across all parameters) in the cowpea breeding programs that have a focus on developing resistant cowpea varieties to address the losses experienced at postharvest in cowpea production, thus extending shelf-life in a more cost-effective and environmentally friendly manner.

Acknowledgements

The authors grateful to the University of Zambia (UNZA), for the supply of the germplasm and to the Regional University Forum (RUFORUM) and the Carnegie Corporation for sponsoring this work.

References

Choudhary, M.D., Bharpoda, T.M., Sushma, D., Choudhary, S. 2015. Screening of Cowpea (Vigna unguiculata) varieties against *Callosobruchus chinensis* under storage conditions. J. Food Legumes. 28(2): 55-58.

Deshpande, V.K., Makanur, B., Deshpande, S.K., Adiger, S., Salimath, P.M. 2011. Quantitative and Qualitative losses caused by Callosobruchus maculatus. In: Cowpea during seed storage. Plant Archives. 11(2): 723-731

Chikalipa, E., Mwala, M., Tembo, L. 2022. Evaluation of cowpea parents and hybrids through multivariate analysis under phosphorus limited soils. J. Genet. Genom. Plant Breed. 6(4): 102-110

Kergoat, G.J., Silvain, J.F., Delobel, A., Tuda, M., Anton, K.W. 2007. Defining the limits of taxonomic conservatism in host-plant use for phytophagous insects: Molecular systematics and evolution of host-plant association in the seed-beetle genus *Bruchus Linnaeus* (Coleoptera: Chrysomelidae: Bruchinae). Mol.Phylogeny Evo., 43: 251-269.

Khan, M.A. 2011. Forages and fodder-Indian perspective. Delhi, India: Daya Publishing House. Delhi-110 035.

Mwila, M.N., Munyinda, K., Mwala, M., Kamfwa, K., Kambikambi, T., Siyunda, C.A., Sinyangwe, S., Kanenga, K., Alamu, E.O., Rubaihayo, P. 2022a. Situational analyses on cowpea value chain in Zambia: The case of untapped legume. Cogent food Agri., 8:1.

Mwila, M.N., Mukonze, B., Siyunda, C.A., Munyinda, K. 2022b. Climate smart crop: Evaluation of selected mutant cowpea genotypes for yield, earliness and ground cover in Eastern Zambia. Med Agric. Env Sci. 3(3):33-41.

Sarwar, M. 2012. Assessment of resistance to the attack of bean beetle, *Callosobruchus maculatus* (Fabricius) in chickpea genotypes on the basis of various parameters during storage. Songklanakarin J. Sci. Technol., 34 (3): 287-291.

Senthilraja, N., Patel, P.S. 2021. Screening of cowpea varieties/genotypes against the pulse beetle, *Callosobruchus maculatus* (F.). J. Ent and Zoo Studies. 9(1):680-684.

Shaheen, F.A., Khaliq, A., Aslam, D.M. 2006. Resistance of Chickpea (*Cicer arietinum* L.) cultivars against pulse beetle. Pakistan J. of Botany. 38(4): 1237-1244.

Sharma, S. and Thakur, D.R. 2014. Comparative developmental compatibility of *Callosobruchus maculatus* on cowpea, chickpea, and soybean genotypes. Asian J. Biolog. Sci. 7(6): 270-276.

Siyunda, A.C., Mwila, M.N., Mwala, M., Munyinda, K., Kamfwa, K., Chipabika, G., Nshimbi, D. 2022a. Screening for resistance to cowpea aphids (*Aphis craccivora* Koch.) in mutation derived and cultivated cowpea (*Vigna unguiculata* L. Walp.) genotypes. Int J. Sci and Busi.13(1):15-26.Siyunda, A.C., Mwila, M.N., Mwala, M., Munyinda, K., Kamfwa, K., Chipabika, G., Nshimbi, D. 2022b. Laboratory screening of cowpea (*Vigna unguiculata* L. Walp.) genotypes against pulse beetle (*Callosobruchus maculatus* F.). Int J. Sci. Busi.6(1):85-93.

Waghmare, P.D., Bantewad, S.D. 2020. Screening the seeds of different chickpea genotypes against pulse beetle, *C. chinensis* L. in laboratory conditions. Int. J. Chem. Studies. 8(2): 1442-1450.