# Evaluation of Four Cowpea Lines for Bruchid (*Callosobruchus maculatus*) Tolerance

Oluwafemi D. Amusa<sup>1\*</sup>, Adebayo L. Ogunkanmi<sup>1</sup>, Kehinde Bolarinwa<sup>1</sup>, Omoche Ojobo<sup>2</sup>
1. Department of Cell Biology and Genetics, University of Lagos, Akoka, Nigeria
2. Department of Botany, University of Ibadan, Ibadan, Nigeria
\*Email of corresponding author: femisworld@yahoo.com

#### Abstract

Cowpea, an important legume in many developing countries, is face by varieties of biotic stresses. Among them is the infestation of stored grains by *Callosobruchus maculatus*, an insect pest capable of causing high grain loss both in quantity and quality. However, resistant genotypes were developed to reduce the damages on stored seed grains by this insect pest. The study evaluated the level of resistance in four cowpea genotypes collected from the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. These cultivars were infested with bruchids for three days in four replicates. Comparative data were then collected from the samples study for analysis. This study showed that there was a positive perfect correlation between percentage pest tolerance and the number of undamaged seeds. The study also showed that most of the resistant genotypes were susceptible with TVx 3236 being the most susceptible. IT99K-494-6 and IT84S-2246-4 suggested in earlier literatures to be resistant to bruchid attack were also susceptible. However, IT81D-994 showed a moderate tolerance to the infestation with percentage pest tolerance of 60. It is therefore imperative that bruchid tolerance evaluation be done periodically on released resistant genotypes to ascertain the stability and durability of their resistance against bruchid infestation.

Key words: Cowpea, Callosobruchus maculatus, Pest tolerance, Pest infestation, Susceptibility

### 1. Introduction

Cowpea (*Vigna unguiculata* (L.) Walpers) is an important legume in many developing countries (Adam and Baidoo, 2008), grown mainly for its grains (Fatokun, 2002). It is one of the cheapest sources of plant protein in the diet of people that cannot afford protein foods such as meat and fish (Traver *et al.*, 2005; Olakojo *et al.*, 2007; Laphale *et al.*, 2012). Cowpea is favoured by farmers because of its ability to maintain soil fertility (Blade *et al.*, 1997), income (Singh, 2002; Timko *et al.*, 2007), its use as animal fodder (Deshpande *et al.*, 2011) and comparably high yields in harsh environment where other food legumes do not thrive (Shimingani and Shimelis, 2011). Cowpea feeds millions of people in developing worlds with an annual world-wide production estimated around 4.5 metric tons on 12-14 million ha (Diouf, 2011).

However, cowpea production is faced with a wide range of biotic constraints like virus (Cowpea Aphid-Borne Mosaic Virus, CABMV), bacteria (*Xanthomonas campestris* pv *vignicola*), fungi (*Choanephora spp*), insects (*Aphis carccivora, Megalurothrips sjostedti, Callosobruchus maculatus* etc) and plants (*Striga gesnenoides* and *Alectra vogeli*) (Singh, 2005). In storage, *Callosobruchus maculatus*, also called cowpea beetle, cowpea weevil or bruchid, is regarded as the most important and common pest of cowpea both in Africa and Asia (Jackai and Daoust, 1986; Deshpande *et al.*, 2011). This weevil has caused losses both in quality and quantity of the stored seeds. Estimates of storage losses are highly variable ranging widely from 4 - 90% (IITA, 1989; Umeozor, 2005) due to perforations by this weevil, thus reducing the degree of usefulness and making the seeds unfit either for planting or human consumption (IITA, 1989; Ali *et al.*, 2004). Similar to other stored product insect pests, oviposition, development and survival of these pests are influenced to a large extent by temperature and humidity (Lale *et al.*, 1996).

Sub-Saharan Africa has been known to provide a favourable environment for the pest. Several attempt to preserve the seeds majorly through chemical means apart from being expensive sometimes result in the poisoning of cowpea and environmental toxicity (Olakojo *et al.*, 2007). This suggests the need for alternative management method that would protect the crop and also the environment.

The importance of cowpea in sub-Saharan Africa with respect to its production capacity and utilization potential therefore informed the need to breed insect pest resistance and high yielding genotypes for use as part of the farming system of the people. International Institute of Tropical Agriculture (IITA) in 1990 recognized this when she requested Gatsby to fund collaborative research project with UK's John Innes Centre to modify cowpea genetically for insect pests' resistance (IITA, 1990). The main objective of this study was therefore, to screen four cowpea genotypes against *C. maculatus* based on their tolerance to the insect infestation with the hope to ascertain their resistance status.

#### 2. Materials and Methods

Four cowpea genotypes (IT99K-4944-6, IT84S-2246-4, TVx 3236 and IT81D-994) were collected from International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria for screening at Central Laboratory, University of Lagos, Akoka, Lagos, Nigeria (6.514°N 3.397°E). Screening was done according to Lephale *et al.*, (2012) with modifications. Thus, seeds collected were oven dried at 40°C for 24hours to kill off any bruchid eggs or larvae that might be in the seeds and also to keep moisture level of the seeds the same.

Ten seeds from each genotype were weighed and put into petri dish (90x15mm) and 2 pairs of newly emerged adult bruchid (2 males and 2 females) were introduced into each petri dish containing the cowpea seeds. The insects were left in the dishes for 3 days to allow for mating and oviposition. The experiment was laid in random complete block design with four replicates. A control was setup for each cowpea genotype with no weevil introduction. Data were collected from the infested replicates of each genotype for the duration of 37 days for the following parameters: initial seed weight (g), number of seed damaged (number of seeds perforated by beetles), numbers of undamaged seed (number of seeds not perforated by beetles), residual seed weight (g), (weight of seed after the experiment), seed weight loss (g) (initial seed weight-final seed weight), % weight loss (initial seed weight x100), emerged insect population (number of insect that emerged out of the eggs used) and % pest tolerance (total number of initial seeds - number of damaged seeds/total number of initial seeds/100).

Data from the four replicates were pooled together and statistically analyzed using SPSS v. 15.0 (2006). Analysis of variance was used to compare F-values for the different genotypes. Correlation coefficients (r) for the cowpea seeds were also compared.

#### 3. Results

Analysis of mean number of cowpea weevils' emergence in the four genotypes examined showed that there was no emergence in all the genotypes at 19 days after infestation (DAI). At 22 DAI, two genotypes (IT84S-2246-4 and TVx 3236) recorded some bruchids emergence. At 25 DAI, all the genotypes had been infested with bruchids. TVx 3236 recorded the highest mean number of bruchids (24.33), both IT99K-494-6 and IT84S-2246-4 recorded a mean number of 17.00 and 18.67 bruchids respectively while IT81D-994 had the lowest mean number of bruchids (8.50) at 37 DAI (Table 1)

Table 2 shows significant variation among the different parameters on the genotypes sampled. However, significant variation was observed in percentage weight loss, number of damaged seeds, number of undamaged seeds and percentage pest tolerance. It was also observed that the genotype with lowest percentage weight loss had the highest number of undamaged seeds and consequently the highest percentage seed tolerance (IT81D-994). While the genotype with the highest percentage weight loss had the highest number of damaged seeds and consequently the least percentage pest tolerance recorded (TVx 3236).

Correlation coefficient (r) of the cowpea parameters taken under the infestation of bruchids presented in Table 3 indicated that a perfect positive correlation (r = 1.0) exist between the number of undamaged seeds and percentage pest tolerance which was significant at p < 0.01. Also, the number of damaged seeds was positive and significantly correlated with the percentage weight loss at p < 0.05. Similar trend was observed with initial seed weight, it was positive and significantly correlated with residual weight at p < 0.01. Residual seed weight however showed a negative and significant correlation with percentage weight loss at p < 0.05.

#### 4. Discussion

Variability in grain characteristics have been found useful in the selection of genotypes for insect resistance. The varied parameters indicated that TVx 3236 showed the least tolerance and IT81D-994 showed the most tolerance to the bruchid infestation confirms that the different genotypes possessed different characteristics and therefore response to the infestation was different. Laphale *et al.* (2012) in a similar study reported a similar result and went further to indicate that some characteristics which include seed size, testa thickness and hardness in the genotypes may influence cowpea seed response to bruchid attacks.

Some studies have attributed grain resistance to differences in grain size (mass) and asserted that the larger grains supply more food and space for insect growth and that the smaller grains or grains with less mass offer more resistance to pests attack than the larger grains (Singh *et al.*, 1974). However, this is true to some extent in some genotypes, in this study however, two genotypes (IT99K-494-6 and IT81D-994) showed no significant difference in the grain size, yet showed different level of tolerance to the bruchid infestation. This indicating that grain size did not affect the genotype's resistance to the bruchid attack.

The rate of insect population is known to be affected by the resistance of a particular genotype or variety offers, by causing a reduction in the rate of oviposition through physical or mechanical barrier (Semple, 1992). The barrier may either limit access into the grain or make it unsuitable for oviposition. The barrier may make it difficult for eggs to adhere to the seed or prevent the larva's penetration into the seed when they are hatched

#### (Laphale et al., 2012).

The physical characteristics of seeds can determine the acceptability for oviposition but may not be related to the antibiotic nature of the seed (Messina and Renwick, 1985). Nwanze *et al.* (1975) showed that rough seeds were less acceptable to *C. maculatus* than smooth ones. On the other hand, Murdock *et al.* (1997) indicated that varieties with smooth and glossy seed coat constantly are more resistance than rough seeded varieties suggesting that other factor besides seed coat appearance affect cowpea's resistance to bruchid infestation.

IT99K-494-6 was considered a bruchid resistant genotype (IITA Annual Report, 2004); IT84S-2246-4 was considered bruchid resistant (Jackai and Singh, 1988; Norris, 1996) and moderately resistant to bruchid infestation (Dugje et al., 2009); TVx 3236 bruchid susceptible (Maina and Lale, 2004; Maina et al., 2006); IT81D-994 bruchid resistant (ICRISAT, 1987; Norris 1996) respectively. The result from this present study supports the works of Maina and Lale (2004), and Maina et al. (2006) that TVx 3236 was a bruchid susceptible cowpea genotype which showed the least tolerance to the bruchid attack, highest damaged from the infestation in terms of weight loss, number of adult emergence and number of seeds damaged. However, IT99K-494-6 and IT84S-2246-4 showed a low percentage pest tolerance level of 20.00 and 24.44 respectively. This indicating that the two genotypes were susceptible to the bruchid attack which is in contrast to IITA Annual Report (2004), Jackai and Singh (1988); Norris (1996) and Dugje et al. (2009). This study also shows IT81D-994 with percentage pest tolerance of 60.00, indicating a moderately resistant genotype to the bruchid attack. This also is in contrast to the works of Norris (1996) which suggested the genotype to be bruchid resistant. The breakdown in resistance of these genotypes to C. maculatus attacks may result from the degrading of the resistant characteristic barriers of the seeds or the virulent nature of the insect pest. Shade et al. (1996, 1999) had earlier reported a virulent strain of bruchid (C. maculatus) which was able to cause severe damage to a resistant genotype TVu 2027, which was otherwise resistant to the bruchid strain in Nigeria.

One important resistant character is seed hardness which may interfere with the larva establishment stage of the insect pest. Its softening on exposure to air with different water contents, its age (since its constituents chemicals may degrade thereby producing or destroying toxins) that means its storage can affect any of its resistant attributes to insect pest infestation (Thiery, 1984).

An important challenge of breeding for resistant genotypes is durability (Johnson, 1984). Because it is easy to utilize, costs little and is compatible with other control tactics, genotype resistance emerges as a potential option to minimize losses caused by *C. maculatus* during storage, especially because cowpea is a crop of low economic return. The extent to which achieving durable resistance is difficult and is highlighted by the fact that most cultivars deployed possessing monogenic resistance had been rapidly overcome because of changes in pathogen/pest populations (Leach *et al.*, 2001). The development of resistant cultivars is however still very limited, since few high-resistance sources have been identified (Singh *et al.*, 1985; Dongre *et al.*, 1996). Another problem that can jeopardize its utilization when it occurs frequently and in high proportions is the genetic variability of *C. maculatus*. This variability is capable of changing the plant-insect relationship as a result of selection pressure imposed by genotypes after several generations, resulting in biotype development (Messina and Renwick, 1985; Dick and Credland, 1986a,b; Mbata, 1993; Shade *et al.*, 1996).

The durability of the resistance may be improved, among other strategies, by the use of multiple cultivar or varietal mixtures (Wolfe 1985, Garrett and Mundt 2000, Zhu *et al.*, 2000) or by pyramiding genes (McDonald and Linde, 2002). In any case, a sound knowledge of the biological and genetic components of the insect pest system is important to design the most appropriate strategy for deployment of the different resistance genes (McDonald and Lande, 2002) because the use of resistant varieties against storage insect pests, when successful, has a number of comparative advantages over other control measures, particularly the use of chemical insecticides (Keneni *et al.*, 2011). Lima *et al.* (2004) stated that the frequent utilization of genotypes with high levels of resistance could promote the development of biotypes resulting from selection pressure, especially when the insect shows high genetic variability, as it is the case with *C. maculatus*. Therefore, the alternation of hosts with different degrees of resistance is very important as a strategy to avoid the occurrence of resistant populations.

However, knowledge on *C. maculatus* biotypes has not been adequately utilized in evaluating resistant genotypes of cowpea. Cowpea genotypes that exhibit significant resistance levels of a single Nigerian population were produced at the International Institute of Tropical Agriculture (IITA), Ibadan (Ofuya and Credland, 1995). These authors, working with three populations of the bruchid from Cameroon, Brazil and Burkina Faso, observed variability in bruchid response to cowpea genotypes, demonstrating that resistance assays must be performed periodically using different bruchid biotype populations. The existence of biotypes capable of developing normally on resistant plants has frequently hampered pest management programs. An efficient strategy to avoid the development of these populations is to periodically control alternation of genotypes with different degrees of resistance or distinct background, or their association in the same area (Beck and Schoonhoven, 1984).

#### 5. Conclusion

In conclusion, seed resistance is a valuable tool against bruchid infestation but must be carefully deployed to avoid the rapid development of a virulent cowpea bruchid biotype. Also, the occurrence of a perfect positive correlation between the number of undamaged seeds and percentage pest tolerance in this study suggest that the use of undamaged seeds as a criterion for ascertaining the resistant status of cowpea genotypes. Periodic evaluation should therefore be conducted on resistant genotypes to ascertain the durability of their resistance still intact.

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**Table 1:** Mean Number of cowpea weevils recorded in four cowpea genotypes from 19-37 days after infestation (DAI)

Genotype	19 DAI	22 DAI	25DAI	28 DAI	31 DAI	34 DAI	37 DAI
IT99K-494-6	0.00	0.00	0.25	1.50	2.50	7.750	17.00
IT84S-2246-4	0.00	5.33	7.33	10.67	15.00	18.00	18.67
TVx 3236	0.00	7.33	16.00	20.67	22.67	24.33	24.33
IT81D-994	0.00	0.00	2.00	2.50	4.00	6.00	8.50

Cumulative number of bruchid emergence

**Table 2:** Effect of cowpea weevil on initial seed weight, residual seed weight, weight loss, damaged seeds, undamaged seeds and genotype tolerance of the four genotypes

Genotype	Initial seed weight (g)	Residual seed weight (g)	% weight loss	Number of damaged seeds	Number of undamaged seeds	% Pest Tolerance
IT99K-494-6	1.73±0.04 <sup>a</sup>	1.50±0.13 <sup>a</sup>	22.75±10.66 <sup>a</sup>	8.00±1.41 <sup>a</sup>	2.00±1.41 <sup>a</sup>	20.00 <sup>a</sup>
IT84S-2246-4	$1.32{\pm}0.03^{b}$	1.10±0.19 <sup>b</sup>	21.67±15.93 <sup>a</sup>	7.67±1.20 <sup>a</sup>	2.33±1.20 <sup>a</sup>	24.44 <sup>a</sup>
TVx 3236	$1.31 \pm 0.01^{b}$	$1.07{\pm}0.20^{b}$	25.33±21.33 <sup>a</sup>	$9.00{\pm}1.00^{a}$	$1.00{\pm}1.00^{a}$	11.11 <sup>a</sup>
IT81D-994	1.59±0.15 <sup>a</sup>	1.51±0.09 <sup>a</sup>	$9.00{\pm}7.00^{b}$	$4.00 \pm 2.00^{b}$	$6.00 \pm 2.00^{b}$	60.00 <sup>b</sup>

\*Mean  $\pm$  SE followed by the same letter are not significantly different from each other at p<0.05

Table 3:	Correlation	coefficients	(r) fo	r experiment	parameters,	under	Callosobruchus	maculatus	artificial
infestation	n on the four	genotype san	nples						

	Initial seed weight (g)	Residual seed weight (g)	% weight loss	Number of damaged seeds	Number of undamaged seeds	% Pest Tolerance
Initial Seed weight (g)		0.751**	-0.116	-0.263	0.249	0.249
Residual Seed weight (g)			-0.595*	0.518	0.322	0.322
% weight loss				0.621*	-0.055	-0.055
Number of damaged seeds					-0.485	-0.485
Number of undamaged seeds						1.000**
% Pest Tolerance						

\*\* Significant at p<0.01; \* Significant at p<0.05

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