Assessment of Bruchid (Callosobruchus maculatus) Tolerance of Some Elite Cowpea (Vigna unguiculata) Varieties

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Assessment of Bruchid (*Callosobruchus maculatus*) Tolerance of Some Elite Cowpea (*Vigna unguiculata*) Varieties

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Abstract: The resistance of cowpea to bruchid infestation has been a major concern to plant breeders as some elite cowpea varieties become susceptible to the polymorphic nature of this storage insect pest. The current status of ten bruchid resistant varieties collected from the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria was evaluated for bruchid tolerance. Each of the varieties was infested with two pairs of bruchids and comparative data was taken for 60 days. Results showed a delay in bruchid emergence with mean development period for successful adult emergence ranging from 32-47 days. Nine of the varieties studied showed percentage seed damage above 80% and percentage pest tolerance below 50%. Susceptibility index indicates that seven of the studied varieties to be moderately resistant and the remaining three to be resistance to the bruchid infestation with TVu 11953 being the most resistant of all with index 1.78. Analysis of seed coat resistance indicated no significant difference in number of eggs laid, mean bruchid development time, percentage bruchid emergence, percentage seed damage and susceptibility index between the smooth and rough seed coats. The study indicates other factors, not seed coat nature to be responsible for bruchid resistance in cowpea.

Keywords: Bruchid resistant, Cowpea, *Callosobruchus maculatus*, Susceptibility index, *Vigna unguiculata*.

1.0 Introduction

Cowpea, an important legume in many developing countries, is face with varieties of biotic stresses among which *Callosobruchus maculatus* (cowpea beetle, weevil or bruchid), a storage insect pest capable of causing high grain loss both in quantity and quality constitute a major problem to cowpea production (Jackai and Daoust, 1986; Deshpande et al. 2011).

Infestation by this insect pest starts on the field, but heavy damage is done in storage. The pest generates exceedingly high levels of infestation even when they pass only one or two generations on the host plant. The larvae of the bruchid feed on the seed contents (Ali et al. 2004; Swella and Mushobozy, 2007) and estimates of storage losses are highly variable ranging widely from 4 - 90% due to perforations, thus reducing the degree of usefulness and making the seeds unfit either for planting or human consumption (IITA, 1989; Ali et al. 2004; Umeozor, 2005).

Several attempts to preserve the seeds through chemical means are expensive and have sometimes result in food poisoning and environmental toxicity (Olakojo et al., 2007). This suggests the need for alternative management method that would protect both the crop and also the environment. The International Institute of Tropical Agriculture (IITA) and John Innes Centre, UK in a collaborative research project attempt to modify cowpea genetically for insect pests' resistance (IITA, 1990). This resulted in the release of several improved cultivars or varieties of cowpea seeds with different levels of resistance to infestation by *C. maculatus* to date (Lale and Kolo, 1998, Maina et al., 2006).

However, with the increasing polymorphic nature of the *C. maculatus* as reported by Credland (1994) and, George and Verma (1999), the durability of resistance or tolerance of improved cowpea varieties to this insect pest becomes a major priority. Shade et al. (1999) have reported a virulent biotype capable of breaking resistance to an already established resistant line TVu 2027. Amusa et al. (2013) also reported the breakdown of genetic resistance of some improved varieties to this insect pest. It therefore becomes necessarily important to re-evaluate the earlier reported bruchid resistant varieties of cowpea. Hence, the objective of this study was to evaluate the resistance level of ten elite cowpea varieties to ascertain their resistance to this insect pest.

2.0 Materials and Methods

2.1 Sample Collection

Seeds of ten cowpea varieties (Table 2.1) were collected from the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. The seeds collected were planted in the screen house at the Institute of Agricultural Research and Training (IAR&T), Ibadan to obtain fresh seeds used for the evaluation. Bruchid tolerance evaluation was carried out at the Central Laboratory, Department of Cell Biology and Genetics, University of Lagos, Akoka, Lagos, Nigeria (6.514^{o}N 3.397^{o}E).

Table 2.1: Description of Cowpea Varieties

Variety	Physical Characteristics	Current Status	Reference
TVu 2027	Rough, White	Resistant	Singh et al., 1985
TVu 11953	Rough, mottled brown and white	Resistant	Singh et al., 1985
TVu 11952	Rough, White	Resistant	Singh et al., 1985
IT97K-499-8	Rough, White	Resistant	IITA Germplasm
IT99K-429-2	Rough, White	Resistant	IITA Germplasm
IT81D-1032	Smooth, Blood red	Resistant	Singh and Singh, 1990
IT97K-1042-8	Smooth, Black	Resistant	IITA Germplasm
IT97K-1042	Smooth, Black	Resistant	IITA Germplasm
IT81D-1064	Smooth, Blood red (Pale)	Resistant	Singh and Singh, 1990
IT81D-994	Rough, white	Resistant	Norris 1996

2.2 Insect Culture

Bruchid cultures were established according to Beck and Blumer (2011). Cowpea grains already infested with bruchids were collected from Minna market, Niger State, Nigeria. Twenty adult bruchids (10 males and 10 females) were introduced into setup culture jars. They were removed 5 days after introduction. Resulting colonies were established at 28-30^{o}C and 55-60% RH.

2.3 Bioassay

The screening evaluation was done according to Lephale et al. (2012) with little modifications. Seeds collected were oven dried at 30^{o}C for 24 hours to kill off any bruchid eggs or larvae that might have been in the seeds and also to dry the seeds to uniform and safe moisture content. Ten seeds from each variety were weighed and put into petri dish of 90x15mm. Two pairs of newly emerged adult bruchids (2 males and 2 females) were introduced into each petri dish containing the cowpea seeds. The insects were left in the dishes and arranged in the dark for 3 days at 28^{o}C and 60% RH, to allow for mating and oviposition before being removed. The experiment was laid in randomized complete block design with four replicates. A control was setup for each cowpea variety with no weevil introduction.

2.4 Data Collection

Data were obtained on the following parameters from the infested samples for the duration of 60 days.

a. Initial seed weight (g): weight of samples before experiment

b. Residual seed weight (g): weight of seed after the experiment

c. Percentage weight loss (%): $\frac{Initial\ Seed\ Weight - Residual\ Seed\ Weight}{100}$

d. Number of eggs laid: eggs counted on eggs after 3 days bruchid infestation

e. Mean development period (days): $\frac{x1+x2+x3+x4}{100}$ (where x = development period of adult insects in each replicate)

f. Percentage bruchid emergence (%): $\frac{Number\ of\ emerged\ adult\ insect}{Number\ of\ eggs\ laid}$ X100

g. Percentage seed damage (%): $\frac{Number\ of\ damaged\ seeds}{Number\ of\ undamaged\ seeds} X100$, (a damaged seed is recorded as a seed with at least a perforation from an adult emergence)

h. Percentage pest tolerance (%): $\frac{Total\ number\ of\ initial\ seeds - Number\ of\ damaged\ seeds}{Total\ number\ of\ initial\ seeds} X100$

i. The susceptibility index for each variety was calculated according to Dobie (1974) using formula below;

$$SI = \frac{\log_e F}{D} \times 100$$

Where F = Total number of F_1 progeny emerged

D = Median development period (which is calculated as the time in days from the middle of the oviposition period to the emergence of 50% of the F_1 progeny)

The susceptibility index, ranging from 0 to 11, was used to classify the cowpea varieties; where; 0-3 = resistant, 4-7 = moderately resistant, 8-10 = susceptible and ≥10 = highly susceptible (Dobie, 1974).

2.5 Statistical Analysis

Data from the four replicates were pooled together and statistically analyzed using IBM SPSS Statistics 20 Software (2011). Difference in means of the varietal parameters were separated by Duncan's multiple range test. Analysis of variance was used to compare F-values of the performances of the varieties. Correlation coefficients (r) for measured parameters were also evaluated.

3.0 Results

3.1 Tolerance evaluation of samples against 60 days bruchid infestation

There were significant difference between the initial seed weight, residual seed weight and percentage seed weight loss of the seed samples (Table 3.1). There was no significant differences in percentage seed damage between the cowpea varieties evaluated after 60 days of bruchid infestation. The least percentage seed weight loss (6.02%) with percentage seed damage (40%) was observed in TVu 11953. IT81D-1064 and IT81D-994 recorded the highest percentage of seed damage (100%). IT81D-1064 showed the highest percentage seed weight loss of 83.20%. Majority of cowpea samples however showed percentage seed damage more had more than 80% seed damage. The highest mean number of eggs recorded was on IT81D-994 from which percentage bruchid emergence recorded was 83.40% and the least mean number of eggs recorded was on TVu 2027 from which percentage bruchid emergence recorded was 84.47%. There was no significant difference observed in the mean bruchid development period between the samples evaluated. TVu 11952 showed the least bruchid development time of 32 days while IT97K-1042 showed the highest bruchid development time (47 days). Percentage pest tolerance level was 0% in four varieties (TVu 11952, IT97K-1042-8, IT81D-1064, and IT81D-994) after 60 days bruchid infestation while percentage pest tolerance level was highest in TVu 11953 (60%). Susceptibility index analysis revealed that most of the varieties used showed moderately resistance after 60days bruchid infestation (Table 2).

Table 3.1: Analysis of varietal performance before and after 60 days infestation

Variety	ISW(g)	RSW(g)	PWL(%)	NEL	MDP(Days)	PBE(%)	PSD(%)	PPT(%)	SI (Status)
TVu 2027	2.58^{e}	1.54^{c}	39.68^{bc}	32.50^{a}	42^{ab}	84.47^{bc}	94.44^{b}	5.55^{a}	3.78 (MR)
TVu 11953	2.59^{e}	2.44^{d}	6.02^{a}	57.00^{b}	44^{ab}	10.37^{a}	40.00^{a}	60.00^{c}	1.78 (R)
TVu 11952	2.60^{e}	0.91^{b}	64.97^{cd}	86.00^{bc}	32^{a}	98.43^{c}	100^{b}	0.00^{a}	6.02 (MR)
IT97K-499-8	1.89^{d}	1.07^{b}	45.05^{bc}	56.00^{b}	39^{ab}	74.88^{bc}	87.50^{b}	37.50^{ab}	4.40 (MR)
IT99K-429-2	1.37^{a}	0.80^{b}	41.18^{bc}	40.50^{ab}	47^{b}	77.72^{bc}	95.00^{b}	5.00^{a}	3.30 (R)
IT81D-1032	1.58^{bc}	0.99^{b}	37.54^{bc}	57.00^{b}	39^{ab}	73.35^{bc}	82.50^{b}	17.50^{ab}	3.89 (MR)
IT97K-1042-8	1.78^{cd}	0.70^{b}	60.48^{bcd}	112.50^{cd}	38^{ab}	73.33^{bc}	100^{b}	0.00a	5.66 (MR)
IT97K-1042	1.57^{b}	1.10^{bc}	29.68^{b}	67.00^{bc}	42^{ab}	45.01^{ab}	85.00^{b}	15.00^{ab}	3.27 (R)
IT81D-1064	1.19^{a}	0.20^{a}	83.20^{d}	111.00^{cd}	35^{ab}	48.49^{ab}	100^{b}	0.00^{a}	5.06 (MR)
IT81D-994	1.74^{bcd}	0.65^{b}	62.80^{cd}	120.00^{d}	33^{a}	83.40^{bc}	100^{b}	0.00^{a}	6.61 (MR)

Means followed by the same letters are not significantly different at P>0.05; ISW: Initial seed weight, RSW: Residual seed weight, PWL: Percentage weight loss, NEL: Number of eggs laid; MDP: Mean bruchid development period; PBE: Percentage bruchid emergence, PSD: Percentage seed damage, PPT: Percentage pest tolerance, SI: Susceptibility index; R: Resistant; MR: Moderately Resistant

3.2 Correlation analysis of performance parameters

The result presented in Table 3.2 indicated no significant correlation between initial seed weight and the rest of the parameters observed except for residual seed weight at 0.01 significant level (r=0.64). Percentage weight loss showed significant positive correlation with the number of eggs laid (0.56), percentage bruchid emergence (0.49), and percentage seed damage (0.76) at 1% significant level. The number of eggs laid was positively correlated with percentage seed damage (r=0.36). Percentage bruchid emergence was also positively and significantly correlated with percentage seed damage (r=0.66). The table also indicated that apart from the initial seed weight (r=-0.04), susceptibility index showed significant positive correlation with the remaining parameters and, a significant negative correlation with percentage pest tolerant (r=-0.53) and mean development period (r=-0.75) at 0.01 significantly level.

Table 3.2: Phenotypic correlation of seed performance after 60 days bruchid infestation

Parameters	ISW	RSW	PWL	NEL	MDP	PBE	PSD	PPT	SI
ISW	1	0.64**	-0.23	-0.13	-0.11	0.07	-0.24	0.17	-0.04
RSW		1	-0.86**	-0.45*	0.39*	-0.48*	-0.76**	0.64**	0.67**
PWL			1	0.56**	-0.58**	0.49**	0.76**	-0.63**	0.84**
NEL				1	-0.40*	-0.51	0.36	-0.35	0.70**
MDP					1	-0.42	-0.51**	0.30	-0.75**
PBE						1	0.66**	-0.43*	0.53**
PSD							1	-0.71**	0.76**
PPT								1	-0.53**

** Correlation is significant at 1% level, * Correlation is significant at 5% level, ISW: Initial seed weight, RSW: Residual seed weight, PWL: Percentage weight loss, NEL: Number of eggs laid; MDP: Mean bruchid development period; PBE: Percentage bruchid emergence, PSD: Percentage seed damage, PPT: Percentage pest tolerance, SI: Susceptibility index

3.3 Seed coat analysis after 60 days bruchid infestation

Analysis of seed coat parameters on bruchid infestation indicated that there was no significant difference between the rough and smooth coated seeds on all the parameters evaluated except for initial and final seed weight at 5% significant level (Table 3.3).

Table 3.3: Sample seed coat performance before and 60 days after bruchid infestation

Parameters	Rough testa	Smooth testa	F (P)
Variety (Replicates)	6 (24)	4 (16)	
Initial weight (g)	1.97	1.54	6.97*(0.01)
Final weight (g)	1.10	0.79	2.18 (0.15)
Percentage seed weight loss (%)	45.41	49.69	0.23 (0.64)
Number of eggs	67.89	80.90	0.58 (0.45)
Mean development period	39.63	38.73	0.12 (0.74)
Percentage bruchid emergence (%)	73.92	62.71	0.95 (0.34)
Seed damage (%)	88.83	90.00	0.02 (0.89)
Pest tolerance (%)	16.73	10.00	0.40 (0.53)
Susceptibility index	4.46	4.35	0.03 (0.87)

Values represented in mean of sample replicates, *F-value is not significant at P>0.05

4.0 Discussion

Genotype resistance emerges as a potential option to minimize losses caused by *C. maculatus* during storage because it is easy to utilize, costs little and is compatible with other control tactics and most 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).

In this study, a seed with at least a perforation from an adult bruchid emergence is considered as a damaged seed. The majority of the cowpea varieties in this study showed a high percentage seed damage but susceptibility index indicated that most of these cowpea varieties were moderately resistant to bruchid infestation. Previous studies showed that most of the cowpea varieties have a combined resistant ability not only to *Callosobruchus* beetle but also to other insect pests and weeds (Singh et al., 2002). Such may indicate moderate resistance to this insect pest as shown in the study. However, the highest resistant variety TVu 11953 had been reported to be unanimously resistant to only bruchid infestation (Norris, 1996).

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 were less preferable and therefore more resistant than rough seeded varieties. The present study showed that higher number of eggs was laid on the smooth coated seeds than the rough coated seeds, consequently, higher number of bruchid emergence was observed in smooth coated seeds than the rough coated seeds. Although, this may suggest an oviposition preference for the smooth seeded coats as reported by some authors, there was no significant difference in the bruchid tolerance performance between the rough and smooth coated seeds. The resistance in cowpea to bruchid infestation may not be attributed to the seed coat nature as suggested in earlier reports. Edde and Amatobi (2003) in a similar report had indicated that seed coat plays no role in the resistance of cowpea to bruchid infestation in their study.

The average life cycle of the *C. maculatus* beetle ranged between 21-25 days on a susceptible variety (Beck and Blumer, 2011). However, this study showed a delay in adult emergence in all the varieties studied irrespective of the oviposition preference. There was no significant difference observed in the mean development period for adult bruchid emergence among the evaluated varieties. The longest development period observed was 47 days (IT97K-1042) for adult bruchid emergence while the shortest mean development period for adult bruchid emergence was 32 days which was observed in two varieties, TVu 11952 and IT81D-994. This study also showed no significant difference for the bruchids' oviposition preference, percentage adult emergence, percentage pest tolerance and susceptibility index between the rough and smooth coated seeds. These observations suggest antibiosis, not antixenosis as reported by many authors to be responsible for bruchid resistant or tolerance in cowpea. Sales et al. (2001, 2005) and Edde and Amatobi (2003) have also suggested that the seed's biochemical compounds may be responsible for the protection against the bruchid infestation in earlier studies.

Singh et al. (1974) have previously 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. In the present study, significance difference was observed in initial seed weight between the rough and smooth coat seeds but did not affect the tolerance of these varieties to bruchid infestation. This corroborates earlier work of Amusa et al. (2013) who reported no significant difference in adult population emergence in the larger or smaller seeds evaluated.

5.0 Conclusion

Different parameters or index have been used to identifying bruchid resistant cowpea varieties which include high percentage pest tolerance level, reduce seed weight loss, low level of seed damage, oviposition preference and delay in insect emergence due to prolonging the insect's life cycle. This study demonstrated that there is a variation between the elite cowpea varieties in their expression of resistance to bruchid infestation. However, only TVu 11953 can be regarded as a true bruchid resistant variety. It showed a high percentage pest tolerance, reduce seed weight loss, low level of seed damage, delay bruchid emergence and a moderate oviposition preference. The other elite cowpea varieties showed high seed damage and low percentage pest tolerance although the susceptibility index showed them to be resistant and moderately resistant varieties. We therefore recommend TVu 11953 a promising alternative for breeding programmes concerning bruchid infestation management to minimized postharvest losses of stored cowpea grains. However, periodic evaluation of seed resistance to bruchid infestation is essential for continuous establishment of the durability and intactness of cultivar or varietal resistance to this storage insect pest.

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