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EVALUATION OF F_3 GENERATIONS OF THE HYBRID CROSSES BETWEEN VEGETABLE AND FIELD COWPEAS AND THEIR PARENTS FOR RESISTANCE TO THE COWPEA BRUCHIDS (CALLOSOBRUCHUS MACULATUS (F))

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ABSTRACT

An evaluation of crosses between vegetable and field cowpea for resistance to the cowpea Bruchids, [Callosobruchus maculatus (F)], was carried out at the Enugu State University of Science and Technology, Enugu southeastern Nigeria. There was a significant difference in the field infestation of the cowpea lines by C. maculatus. AD-36-W_R had the highest percentage field infestation by the insect pest, whereas, AE-36-C X AN-16-D, and AN-14-D were free from the field infestation by the pest. Vegetable cowpea lines tested had some levels of resistance to the cowpea Bruchid and the gene responsible for the resistance were successfully transferred from vegetable cowpea to the susceptible field cowpea by cross-breeding techniques. No vegetable cowpea tested had complete resistance to the cowpea bruchid, however, they had lower susceptibility than the field cowpea tested. Reduction in the relative susceptibility of the cowpea to C. maculatus was achieved by cross breeding field cowpea with vegetable types. For instance, in crosses between AD-36-W (field cowpea) and AE-36-C (vegetable cowpea), AD-36-W_B (field cowpea) and AN-14-D (vegetable cowpea), AD-36-W (field cowpea) and AE-36-F (vegetable cowpea), AD-36-W_B (field cowpea) and AE-36-C (vegetable cowpea), AN-14-D and AE-36-F reduced susceptibility of AD-36-W and AD-36-W_B to C. maculatus from 63.59% to 51.09%, 68.46% to 59.09%, 63.59% to 47.38% and 68.48% to 38.84% respectively. Also, gene responsible for susceptibility to the storage insect pest were successfully transferred from field cowpea to the vegetable cowpea through cross-breeding techniques. A cross between two vegetable types namely; AE-36-C and AN16-D had the least susceptibility level of 24.32%. There was a significant difference in mean number of eggs laid per cowpea line with AD-36-WB recording the highest mean number of 134.75 eggs and AE-36-F recording the least mean number of 4.75 eggs. There was also a significant difference in the percentage adults emergence per cowpea line with AD-36W_R having the highest percentage of 68.46% adults, and AE-36-C X AN-16-D having the least percentage of 24.32% adults. The result of the experiment also showed a significant difference in mean longevity of female adults reared from cowpea lines with those reared from AD-36W_B X An-14-D, and AE-36-C having the highest mean longevity of 14 days each whereas those from AD-36WXAE-36-C had the least mean longevity of 5.6 days. Also, there was a significant difference in mean testa thickness of the seeds tested with AE-36-C having the highest mean testa thickness of 0.20mm, whereas AD-36-W_R had the least mean testa thickness of 0.02mm. However, there was no correlation between either testa thickness or moisture content and levels of infestation by the storage insect pest. Again, there was a significant difference between mean weight loss of seeds tested after infestation by C. maculatus, with AD-36W_B recording the highest mean weight loss of 0.38g, whereas AE-36-C and AE-36-F, had the least mean weight loss of 0.07g. There was a positive correlation between the weight loss of the adult C. maculatus and the weight (g) of the seeds from which the insect pest was reared (r = 0.03).

Key words: Cowpea lines, cross-breeding, *Callosobruchus maculatus*, resistance, susceptibility

INTRODUCTION

Cowpea (Vigna unguiculata (L.) Walp) is an important legume in many developing countries (Adams and Baidoo 2008) and are 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 fish and meat (Traver, et al., 2005, Olakojo et al., 2007, Lephale et al., 2012). Cowpea production is favoured by farmers because of its ability to maintain soil fertility (Blade et al., 1997), provide income (Singh, 2003), (Timko et al., 2007), its use as animal fodder (Deshpande et al., 2011) and comparatively high yields in harsh environment where other food legumes do not thrive (Shimingani and Shimelis, 2011). Cowpea, an important legume is faced with a wide range of biotic constraints. Among them is infestation of stored grains Callosobruchus maculatus, an insect capable of causing high grain loss both in quality and quantity. 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 Doust, 1986, Deshpande et al., 2011). Estimates of storage losses are highly variable ranging widely from 4 - 90% (IITA, 1989, Umeozor, 2005) due to perforation by this weevil, thus reducing the degree of usefulness and making the seeds unfit for planting or human consumption (IITA, 1989, Ali et al., 2004).

Several attempts 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 (Oluwafemi et al. 2013). It is in this direction that this research work was designed to (i) test crosses between vegetable and field cowpeas for resistance to the cowpea bruchid (Callosobruchus maculatus) to ascertain whether vegetable cowpeas actually have some resitance to this storage pests (ii). find out whether the resistant traits (genes) can be transferred to the susceptible ones (field cowpeas) by cross breeding as a means of finding a lasting solution to the menacing activities of this storage pest of cowpeas. (iii) attempt to elucidate the nature of resistance through studying the effect of the cowpea lines on the longevity of the insect pest.

MATERIALS AND METHODS

The experiment was carried out at the Enugu State University of Science and Technology. The University lies between latitude 06° 50′ N, and longitude 07° 15′ E, with a mean elevation of

450 m above sea level (Anikwe et al; 2005). The treatments (vegetable cowpea locally called "akidi") and (field cowpea locally called "Agwa") were procured from stock of successful crosses done at the Department of Crop Science, University of Nigeria Nsukka. The F₂ generations were supplied which were planted to obtain F₃ generations with which the study was carried out, together with the parents as a check or base for references. The field cowpea is the type of cowpea that can only be consumed when processed as dry grains whereas the vegetable cowpea is the type of cowpea that can be consumed either as processed dry grains, fresh grains or pods. The genotypes were named based mainly on the physical characteristics of the seeds such as seed colour. For example, black (D), chocolate (C), speckled (S), fawn (F), white (W). Subscripts after fifth digits had meaning as follows: Suture (S), Brown splash (B). For example AD-36-W_B. The experiment was carried out in a complete randomized design (CRD) replicated four (4) times using four wire mesh cages having a wooden bottom on four stands dipped in water troughs.

Six (6) crosses of seven (7) cowpea lines namely: AE-36-C X AD-36-W, AE-36-C X AN-14-D, AD-36-W X AE-36-F, AD-36-W_B X An-14-D, AD-36-W X AE-36-F, AD-36-W_B X AE-36-C were screened for resistance to cowpea bruchid [*Callosobruchus maculatus* (F)] in the laboratory of Agronomy and Ecological Management, Enugu State University of Science and Technology with mean laboratory temperature of 28±2.65°C throughout the period of study in June 2008.

Twenty (20) seeds were placed in plastic petri dishes into which a pair each of newly emerged adult females and males was restrictedly introduced. The newly emerged adult C. maculatus used for this study were obtained from already existing culture in the post graduate research laboratory of the Department of Agronomy and Ecological Management Enugu State University of Science and Technology, Enugu, Nigeria. They were subsequently reared inside 1 litre kilner jars, on un-infested Potiskum cowpea variety obtained from International Institute for Tropical Agriculture, Ibadan, Oyo State, Nigeria. The culture was placed in an insect rearing cage at a mean ambient temperature of $28\pm2.65^{\circ}$ C. A thermometer was installed for the purpose of determining the average daily temperature for the period of study. Parameter assessed included percentage field infestation level seed weight loss (g), mean number of eggs laid per cowpea line, percentage adult emergence out of total number of eggs laid per cowpea line. Mean number of adult per seed, mean adult weight reared from each line, mean

number of holes created by the insect pest per seed, testa thickness, moisture content and infestation levels. The data collected were subjected to analysis of variance (ANOVA) according to the procedure for complete randomized design (CRD) experiment as outlined by Steel and Torrie (1980) and Obi (2001) at 5% probability level. Detection of differences between treatment means was done by the use of F-LSD (Obi 2001).

RESULTS

Percentage field infestation of cowpea lines by Callosobruchus maculatus

Post harvest investigation showed that all the cowpea lines except AE-36-C X AN-16-D and AN-14-D were infested in the field by the insect pest. There were significant differences in the mean levels of infestation by the pest. AD-36-W_B (field cowpea) recorded the highest mean field infestation level of 1.68% which differed significantly (P=0.05) from its crosses with vegetable cowpeas such as AD-36-W_B X AN-14-D and AD-36-W_B X AE-36-C which recorded field infestation of 0.38% and 0.25% respectively. This result showed susceptibility of AD-36-W_B (field cowpea) was reduced by crossing it with AE-36-C (vegetable cowpea) which was known to be less susceptible to C. maculatus (personal information from Prof. J.O Uzo, Department of Crop Science, University of Nigeria Nsukka in the year 1991 during my interview). He observed that vegetable cowpeas were known to have some levels of resistance to cowpea weevils than field cowpeas and that the genes responsible for the resistance could be transferable by cross breeding techniques between two cowpea varieties (Table 1).

Number of eggs laid per cowpea line by Callosobruchus maculatus

Results of the experiment showed significant differences (P=0.05) between mean number of eggs laid on the seeds with AD-36-W_B having the highest mean number of 134.75 eggs followed by AD-36-W (Field cowpea) having a mean number of 91.25 eggs whereas AE-36-F (vegetable cowpea) recorded the least mean

number of 4.75 eggs, followed by AN-16-D (vegetable cowpea) which had a mean number of 6.75 eggs. The result also showed that all the crosses of field cowpeas with the vegetable cowpeas recorded lower number of eggs laid than their parent lines. For examples; AD-36-W X AE-36-C recorded mean number of 68.50 eggs as against AD-36-W (Maternal parent) that recorded mean number of 91.75 eggs; AD-36-W_B X AN-14-D recorded mean number of 27.50 eggs as against AD-36-W_B (maternal parent) that recorded mean number of 134.75 eggs; AD-36-W X AE-36-F had mean number of 11.25 eggs as against AD-36-W that had mean number of 91.75 eggs and lastly AD-36-W_B X AE-36-C which had a mean number of 36.50 eggs as against AD-36-W_B which recorded a mean number of 134.75 eggs. All the mean number of eggs laid on the crosses by C. maculatus were significantly different (P=0.05) from those of the maternal parents (Table 1).

Percentage Adults Emergence per Cowpea line

There was significant difference (P=0.05) between mean percentage adult emergence per cowpea line with AD-36- W_B (field cowpea) recording the highest percentage adult emergence of 68.46% followed by AD-36-W (field cowpea) with 63.59% and lastly AE-36-C X AN-16-D with 24.32% followed by AN-16-D with 25.00. The result of the experiment on the percentage adult emergence on the maternal cowpea parents and their crosses followed the same trend with the earlier discussion on the mean number of eggs laid per cowpea line (Table 1).

Table 1: Mean percentage field infestation, mean number of eggs laid, percentage adult emergence

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Cowpea lines	Field Infestation (%)	Number of eggs la	aid Adult mergence (%)
AE-36-S X AD-36-W	0.10	30.00	50.00
AE-36-C X AN-16-D	0.00	9.25	24.32
AD-36-W X AE-36-C	0.25	68.50	51.09
AD-36-W _B X AN-14-D	0.38	27.50	59.09
AD-36-W X AE-36-F	0.13	11.25	43.00
AD-36-W _B X AE-36-C	0.25	36.50	42.00
AN-14-D	0.00	8.00	25.00
AD-36-W	0.50	91.75	63.59
AE-36-C	0.50	21.25	30.59
AN-16-D	0.38	6.75	29.63
AE-36-F	0.13	4.75	26.32
AE-36-S	0.18	27.50	31.82
$AD-36-W_B$	1.68	134.75	68.46
F-LSD(0.05)	0.08	2.52	5.12

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Number of Adults per Seed

Statistical analysis of the experiment showed a significant difference (P=0.05) in the mean number of adult of C. maculatus per seed with AD-36-W_B (field cowpea) recording the highest mean number of 4.61 adults per seed followed by AD-36-W with mean number of 2.94 adults and lastly AE-36-F Vegetable cowpea with mean number of 0.06 adult followed by AN-14-D (Vegetable cowpea) with mean number of 0.10 adult per seed. The result of the experiment also showed that a significant (P=0.05) lower number of adults of C. maculatus was obtained among field cowpea crosses than among their maternal parents. For example: AD-36-W (field cowpea) X AE-36-C (vegetable cowpea) had mean number of 1.75 adult per seed as against AD-36-W which had mean number of 2.94 adults per seed; AD-36-W_B (field cowpea) X AN-14-D (vegetable cowpea) recorded mean number of 0.81 adult, whereas AD-36-W_B (maternal parent) had mean number of 4.61 adults per seed. The mean differences of the number of adults per seed among the cowpea crosses and their maternal parents were significant (P=0.05) (Table 2).

Weight of adults reared from 100 seed weight of each cowpea line

Statistical analysis of the experiment indicated that AN-14-D (vegetable cowpea) had the highest seed weight of 19.46g and 0.0029g adult weight. This was followed by AD-36-WXAE-36-C with 100 seed weight of 12.86 and adult weight of 0.0029g and lastly AE-36-S with 100 seed weight of 7.79g and adult weight of 0.0016g. There was a positive correlation (r=0.03) between 100 seed weight and weight of adult *C. maculatus* reared from them (Table 3).

Table 2. Mean number of adults per seed, 100 seed weight (g) and mean weight of adults (g) reared from each cowpea line

Cowpea lines	Number of Adults	100 seed weight (g)	Weight of adult (g)
AE-36-S X AD-36-W	0.75	11.17	0.0028
AE-36-C X AN-16-D	0.11	10.86	0.0027
AD-36-W X AE-36-C	1.75	12.86	0.0029
$AD-36-W_BX$ $AN-14-D$	0.81	11.96	0.0029
AD-36-W X AE-36-F	1.65	10.49	0.0027
AD-36-W _B X AE-36-C	2.78	12.63	0.0029
AN-14-D	0.10	19.46	0.0029
AD-36-W	2.94	11.39	0.0029
AE-36-C	0.33	10.22	0.0026
AN-16-D	0.10	9.44	0.0025
AE-36-F	0.06	11.13	0.0027
AE-36-S	0.44	7.79	0.0016
$AD-36-W_B$	4.61	9.27	0.0024
F-LSD(0.05)	0.07	1.10	0.002

Table 3. Correlation between seed weight and weight of adult

Cowpea lines	100 seed weight (g) X	Weight of adult (g) Y
AE-36-S X AD-36-W	11.17	0.0028
AE-36-C X AN-16-D	10.86	0.0027
AD-36-W X AE-36-C	12.86	0.0029
$AD-36-W_BX$ $AN-14-D$	11.96	0.0029
AD-36-W X AE-36-F	10.49	0.0027
AD-36-W _B X AE-36-C	12.63	0.0029
AN-14-D	19.46	0.0029
AD-36-W	11.39	0.0029
AE-36-C	10.22	0.0026
AN-16-D	9.44	0.0025
AE-36-F	11.13	0.0027
AE-36-S	7.79	0.0016
$AD-36-W_B$	9.27	0.0024
Mean	11.44	0.0027

Seed weight loss after infestation by C. maculatus, mean longevity of female adults per cowpea line and mean number of holes created by C. maculatus per cowpea seed

The results of the experiment showed a significant difference (P=0.05) in mean seed weight loss after infestation by the pest with line AD-36-W_B (field cowpea) recording the highest seed weight loss of 0.38g followed by AD-36-W (field cowpea) with 0.28g seed weight loss and lastly AE-36-F (vegetable cowpea) with 0.08g seed weight loss. The result of the experiment also showed that all the crosses of maternal parents of field cowpeas with vegetable cowpeas had lower seed weight loss than their uncrossed maternal parent lines. For instance, AD-36-W X AE-36-C had mean seed weight loss of 0.13g as against its maternal parent that recorded a mean seed weight loss of 0.28g, AD-36-W_B X AN-14-D recorded a mean seed weight loss of 0.26g as

against its maternal parent that had a mean seed weight of 0.38g, AD-36-W X AE-36-F recorded a mean seed weight loss of 0.19g, whereas its maternal parent recorded a mean seed weight loss of 0.28g, AD-36- W_B X AE-36-C had a mean seed weight loss of 0.20g, whereas its maternal parent recorded a mean seed weight loss of 0.38g. All the mean seed weight losses of crosses were significantly different (P= 0.05) from those of their maternal parents. Also, there was a significant (P=0.05) longevity (days) of female adults reared from the cowpea lines with AD-36-W_R X AN-14-D and AE-36-C recording the longevity of adult females of 14 days each. On the mean number of holes per seed, field cowpeas crossed with vegetable cowpeas maintained recording significant (P=0.05) lower holes per seed than their maternal parents as in the other parameters previously assessed. (Table 4).

Table 4 Mean seed weight loss after infestation by C. maculatus, mean longevity of female adults reared from each cowpea line and mean number of holes created by the storage nest per seed

by the storage pest per seed.			
Cowpea lines	Seed weight loss (g)	Longevity of female adults (days)	Number of holes per seed
AE-36-S X AD-36-W	0.27	10.67	0.75
AE-36-C X AN-16-D	0.14	8.67	0.11
AD-36-W X AE-36-C	0.13	5.67	1.75
AD-36-W _B X AN-14-D	0.26	14.00	0.81
AD-36-W X AE-36-F	0.19	10.67	0.10
AD-36-W _B X AE-36-C	0.20	7.00	0.10
AN-14-D	0.13	9.33	0.10
AD-36-W	0.28	7.67	2.94
AE-36-C	0.18	14.00	0.33
AN-16-D	0.12	11.67	0.10
AE-36-F	0.07	12.67	0.06
AE-36-S	0.08	12.33	0.44
$AD-36-W_B$	0.38	8.67	4.61
F-LSD(0.05)	0.01	4.12	0.07

Table 5: Mean testa thickness (mm), moisture content (%) and levels of infestations (%)

Cowpea lines	Testa Thickness (mm)	Moisture Content (%)	Levels of Infestation (%)
AE-36-S X AD-36-W	0.15	16.67	50.00
AE-36-C X AN-16-D	0.18	14.12	24.32
AD-36-W X AE-36-C	0.13	9.11	51.09
$AD-36-W_B X AN-14-D$	0.15	14.51	59.09
AD-36-W X AE-36-F	0.18	16.67	47.38
$AD-36-W_B X AE-36-C$	0.17	14.42	38.84
AN-14-D	0.14	9.09	25.00
AD-36-W	0.16	13.96	63.59
AE-36-C	0.20	15.09	30.59
AN-16-D	0.18	13.23	29.63
AE-36-F	0.16	15.37	26.32
AE-36-S	0.18	14.72	31.82
$AD-36-W_B$	0.12	14.56	68.46
F-LSD(0.05)	0.02	2.61	6.52

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Table 6. Correlation between Testa thickness and levels of infestation

Cowpea lines	Testa Thickness (mm) X	Levels of Infestation (%) Y
AE-36-S X AD-36-W	0.15	50.00
AE-36-C X AN-16-D	0.18	24.32
AD-36-W X AE-36-C	0.13	51.09
AD-36-W _B X AN-14-D	0.15	59.09
AD-36-W X AE-36-F	0.18	47.38
$AD-36-W_B X AE-36-C$	0.17	38.84
AN-14-D	0.14	25.00
AD-36-W	0.16	63.59
AE-36-C	0.20	30.59
AN-16-D	0.18	29.63
AE-36-F	0.16	26.32
AE-36-S	0.18	31.82
$AD-36-W_B$	0.02	68.46
Mean	0.154	42.01

Table 7. Correlation between moisture content and levels of infestation by C. maculatus.

Cowpea lines	Moisture Content (%) X	Levels of Infestation (%) Y
AE-36-S X AD-36-W	16.67	50.00
AE-36-C X AN-16-D	14.12	24.32
AD-36-W X AE-36-C	9.11	51.09
$AD-36-W_B X AN-14-D$	14.51	59.09
AD-36-W X AE-36-F	16.67	47.38
AD-36-W _B X AE-36-C	14.42	38.84
AN-14-D	9.09	25.00
AD-36-W	13.96	63.59
AE-36-C	15.09	30.59
AN-16-D	13.23	29.63
AE-36-F	15.37	26.32
AE-36-S	14.72	31.82
$AD-36-W_B$	14.56	68.46
Mean	13.96	42.01

Testa thickness (mm) moisture content (%) and levels of infestation (%)

The experiment showed significant difference (P=0.05) in mean testa thickness with AE-36-C recording the highest mean testa thickness of 0.20mm followed by two vegetable cowpea lines – AN-16-D and AE-36-S with 0.18mm each and two cross breeds – AD-36-W_B X AE-36-F and AE-36-C X AN-16-D with 0.18mm each whereas AD-36-W_B had the least mean testa thickness of 0.12mm (Table 5). There was no correlation between the testa thickness and levels of infestation by *C. maculatus* (table 6). There was also no correlation between moisture content and levels of infestation by *C. maculatus* (table 7).

DISCUSSION

Investigation of percentage field infestation of the cowpea lines by *Callosobruchus maculatus* helped to ensure that only seeds free from the insect pest were used for the experiment. The result of the experiment showed that the higher the seed weight, the higher the weight of the insect pest reared from it. This means that the weight of the insect pest is positively correlated with the weight of the seed in which the insect pest was reared (r=0.03), (Table 3). The observation is in agreement with the finding of

El-halfway et al; (1972), who stated that there was a positive correlation between the weight of the seeds in which the insects were reared and the weight of resulting adults. The non correlation between either testa thickness or moisture content of the cowpea seeds and levels of infestation by C. maculatus could mean that chemical characteristics influenced the growth and development of the weevil forming the basis for resistance in the vegetable cowpea parent lines. Breeding programmes utilizing the resistance characters in certain vegetable cowpea genotypes are therefore suggested. The result of this experiment however disagreed with the report of Lephale et al; (2012) that seed size, testa thickness and moisture content (hardness) of cowpea lines may influence cowpea seed response to bruchid attacks. Vegetable cowpea parent lines showed lower mean numbers of eggs laid and percentage adult C. maculatus emergence than the field cowpea parent lines and a cross between field cowpeas and vegetable cowpeas brought a reduction in mean number of eggs laid and percentage adult emergence in field cowpeas hybrids. It therefore means that increase in the resistance of field cowpeas to C. maculatus was reduced by cross-breeding them with vegetable cowpeas. In other words, susceptibility of field cowpeas to C. maculatus was reduced by cross-breeding them with

vegetable cowpeas. For example, AD-36-W_B (field cowpea) that had mean number of 4.61 adults per seed was reduced to 0.81 adult per seed by cross-breeding it with AN-14-D (vegetable cowpea). The higher mean number of holes created by C. maculatus per seed observed in field cowpeas was an index of high susceptibility of the lines to storage pests which was reduced by cross-breeding the lines with vegetable cowpeas. This result agreed with the observation made by Oluwafemi et al; 2013 who noted that most of the resistant cowpea genotypes studied were susceptible when crossbred with TV_x3236 being the most susceptible. These findings therefore showed that the genes responsible for either resistance or susceptibility of cowpeas to Callosobruchus maculatus are transferable through cross-breeding techniques. This was evident in the parental lines of field cowpeas recording higher significant (P=0.05) values in all the parameters assessed than their hybrids (cross-breeds with vegetable cowpeas). The non significant effect of testa thickness and moisture contents of the cowpea seeds on the levels of infestation by C. maculatus however disagreed with the observation of Lephale et al; (2012) who stated that testa thickness and (moisture content) of hardness cowpea genotypes may influence cowpea seed response to bruchid attacks. The result of the experiment showing a significant difference between means of female adults longevity therefore suggests that the chemical component (antibiotic nature of the seed) may be responsible for the resistant nature of vegetable cowpeas to C. maculatus. since seed testa thickness and moisture content (hardness) of the seed were not correlated with levels of infestation by the insect pest.

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