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GENETIC CONTROL OF SEED YIELD, PROTEIN CONTENT AND RESISTANCE TO *Callosobruchus maculates* F IN COWPEA (*Vigna unguiculata* (L.) Walp)

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Abstract: A full-diallel cross was established between six morphologically divergent cultivars of cowpea in order to estimate the genetic parameters of seed yield/plant and percentage of protein content. Moreover, laboratory experiments were also established to determine the types of gene action controlling the inheritance of resistance to *C. maculatus*, percentage of seeds weight loss and total number of the emerged adult insects. The results reveled that both additive and non-additive gene effects were involved in the control of the studied traits except total number of the emerged adult insects where additive gene effect was non-significant. The highest positive general combining ability (G.C.A) values were recorded for Black Crowder and Tvu21 improved for seed yield/plant and Sudani for protein content. However, the highly negative GCA values were recorded for Sudani which exhibited the minimum array mean of seed weight loss and Black Crowder for minimum number of emerged adult insects. The Wr/Vr analysis revealed that non-allelic gene interaction of the duplicate type was operating for % protein content and percentage seed weight loss, while complementary type was operating for seed yield/plant. For total number of the emerged adult insects, a major gene(s) differentiated Black Crowder from the other six cultivars which confer resistance to *C. maculatus*, expressed as a recessive character. The maternal effects present for percentage of protein content and seed yield/plant, but absent for seeds weight loss and total emerged adult insects.

Key words: Cowpea, *Callosobruchus maculates* , seed yield and protein percentage.

Introduction

Cowpea (*Vigna unguiculata* (L.) Walp.) is one of the most ancient species crop which belongs to the genus *Vigna* Savi. (Subgenus *Vigna* sect. Catiang) in the Phaseoleae (Marechal *et al.* 1978). Cowpea is a multipurpose and multiseason crop. It can be used at all stages of its growth (Moroso, 2003).

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It is cultivated around the world primarily for seeds which are nutritious with a protein content of about 23% and rich in the essential amino acids mainly lysine (El-Shaikh, 1999 and Abd El-Hady, 2003) and grown extensively in 16 African countries including Egypt (Rashwan, 1997 and Fery, 2002). Cowpea is attacked by many destructive pests that cause a great damage to the crop (Singh *et al.*, 1978). The bean pod borer, *Etiella zinckenella* treit, is one of the most serious pests that cause a severe economic decrease in both the quality and the yield (Metwally, 1993). Also, several insects belonging to family Bruchidae of order Coleoptera attacks stored cowpeas. The most important insect pest attacking cowpea seeds is *Callosobruchus maculatus* (F.), which causes weight loss, decreased germination potential and a reduction in commercial value (Dick and Credland, 1986a and Amro, 1999). According to Painter (1951) plant resistance phenomena were divided into three categories: non-preference (antixenosis), antibiosis and tolerance. Antibiosis is the possession of some property by the plant which directly or indirectly affects the performance of the pest, whereas tolerance is a reduce plant response (usually in terms of yield loss) to a given pest burden. A resistance, whether genetic or environmentally induced, is very often a combination of two or even all three of these phenomena (Knipling, 1979). Breeding procedures for improving cowpea is mainly dependant on the type of gene action and relative amount and components of the genetic variance in population.

The present investigation was conducted in order to partition the genetic variance into its components and to estimate the types of gene action controlling the inheritance of some economic characters in cowpea and the resistance to Cowpea weevil *Callosobruchus maculates.*

Materials and Methods

Plant materials:

Six different varieties of cowpea *(Vigna unguiculata L. Walp)*, were used in this study (Table A).

Table(A): Source, testa color and growth habit of the tested cowpea cultivars.

Genotypes	Source	Testa color	Growth habit
Black Crowder	USA	Black	Indeterminate
Sudani	Sudan	Black	Indeterminate
Tvu21 improved	IITA*	Yellowish-white	Indeterminate
Monarch Blackeye	EAO#	White with black eye	Determinate
Dokki331	EAO	White with black eye	Determinate
Kahal	EAO	Yellowish-white	Determinate

* IITA, International Institute of Tropical Agriculture, Ibadan, Nigeria.

\# EAO, Egyptian Agricultural Organization, Egypt.

Field experiment conditions:

In the summer season of 2003, the six parental genotypes of cowpea were sown into the field of the Experimental Farm of the Faculty of Agriculture, Assiut University. A full-diallel cross was done to obtain a total of 30 F_1 crosses. In the fall season (10^{th} August 2003), seeds of the six parental varieties and the 30 F_1 hybrids were sown into the field in order to produce the F_2 seeds. Crosses were also made to produce more F_1 seeds. Pods were harvested twice (early December, and early January) as soon as they ripened to avoid shattering and loss of seeds. In the summer season of 2004, seeds of the six parents, the 30 F_1's and the 30 F_2's of the six-parent full diallel cross were sown into the field for evaluation. The experimental lay out was a complete randomized block design with three replications. The parents and the 30 F_1 hybrids were represented each by one row of plants per block, while three rows per block were used for each of the 30 F_2 populations. Each row was 2 meters long, spaced 60 cm apart with plants spaced 20 cm within rows. The pods were picked twice in the summer season (early July and early August). Measurements were recorded on a random sample of 10 guarded plants for parents and the F1 hybrids and 30 guarded plants for each F2 populations in each replicate. The following characters were recorded for each plant: Seed yield/plant (g) and Protein content (%), which was determined by micro Kjeldahl method **(A.O.A.C., 1984)** directly after harvest.

Laboratory experiments:

Dry seeds of the six cowpea cultivars and the 30 F_2's were tested for susceptibility to infestation and the damage by insect pest *C. maculatus* under laboratory conditions at a temperature of 32 ± 2°C, relative humidity of 60 % and photoperiod of 15:9 (day to night) in order to study the inheritance of resistance to cowpea weevil in the tested genotypes. Only antibiosis and tolerance were tested in the present study.

I- Antibiosis:

Three replicates were used to study the effect of cowpea genotypes on laid eggs and the developmental time of *C. maculatus*. In each replicate, dried 10 clean seeds of each genotype were weighed then submitted to infestation with one freshly emerged adult male and female (0-24 hr. old) of *C. maculatus* in glass vials (10x2 cm). After 48 hr. the insects were removed and laid eggs were counted. Then all eggs were removed except one egg on each seed. The emergence of first and last insects were counted and the number of days elapsed were recorded.

II- Tolerance:

Ten infested seeds were dried and weighed after the emergence of the last insect. Seed weight loss percentage was recorded as the difference between the weight before and after infestation.

Statistical analysis:

The data collected were analyzed using the diallel analysis as developed by Hayman (1954 a and b) and Mather and Jinks (1971). General combining ability (GCA) was calculated as the deviation of array mean from the mean of all crosses for F_1 and F_2 generations (Ghosh and Das, 2004).

Results and Discussion

The means of the seed yield/plant, protein content percentage, seeds weight loss percentage and number of emerged adult insects of the six parental cultivars, the 15 F_1 hybrids and 15 F_2 populations (averaged over reciprocals) are presented in Table (1). The parental means of seed yield/plant ranged from 55.44 (P1) to 118.37 (P4) grams with an average of 85.05 grams. The means of the F_1 hybrids ranged from 60.29 to 259.15 grams with an average of 107.54 grams indicating the occurrence of heterotic effects arising from dominance or dominance plus non-allelic gene interaction. The means of the F_2's range from 33.49 to 108.56 grams with an average of 74.96 grams. Black Crowder (P1) and Tvu21 improved (P3) showed the highest positive GCA values in the F_1 generation while, the highest positive GCA value in the F_2 generation was recorded for Sudani (P2). For protein content percentage, the parental average was 26.5 %. The parent Sudani (P2) showed the highest positive GCA value for protein content. The average of seeds weight loss percentage for the parents was 5.48 %. Whereas the average of seeds weight loss percentage for the F_2 generation seeds was 5.31 %. The highest negative GCA was recorded for P1 (Black Crowder) and P2 (Sudani) which displayed the minimum array mean of seeds weight loss percentage (5.13 %). The resistance of Black Crowder and Sudani is in agreement with the results of Gatehous et al., (1979); Dick and Credland, (1986 a & b); Piergiovanni et al., (1991 and 1994); Ussuf et al., (2001); Lawrence and Koundal, (2002) and Sales et al., (2005), but in variance with Baker et al., (1989). The cultivar Dokki 331 showed the least weight loss percentage, indicating that it may be tolerant to *C. maculatus* (Painter, 1951; Redden and McGuire, 1983 and Jackai and Asante, 2001). For number of emerged insects, the parental mean was 8.33 whereas that of the F_2 generation seeds was 9.74 insects. The highest negative GCA was recorded for Black Crowder (P1) which show the minimum number of adult insect emergence, and the highest positive GCA was recorded for Tvu21 improved (P3) which

Table(1): Means of seed yield/plant (upper value), protein content % (second value), seed weight loss (third value) and number of emerged adult insects (lower value) of the parental varieties (diagonal) and their F_1 crosses of seed yield/plant (upper right) and F_2 populations (lower lift) averaged over reciprocals.

Genotypes	P1	P2	P3	P4	P5	P6	Array mean F_1	GCA F_1
P1 (Black Crowder)	55.44 24.44 5.63 5.00	103.74	259.15	161.46	87.38	63.36	121.75	17.96
P2 (Sudani)	96.63 29.92 5.01 9.66	92.31 29.06 5.57 7.33	158.06	72.01	95.65	74.08	99.31	-4.5
P3 (Tvu21 improved)	89.95 33.28 5.21 9.83	81.68 28.5 5.25 9.5	62.47 30.83 5.33 10.0	99.68	82.56	63.77	120.95	17.16
P4 (Monarch blackeye)	74.47 27.27 5.48 9.33	99.11 30.12 4.81 9.16	51.42 26.64 5.32 10	118.37 26.00 5.48 9.67	113.57	118.43	113.92	10.13

Table(1): continued

Genotypes	P1	P2	P3	P4	P5	P6	Array mean F_1	GCA F_1
P5 (Dokki331)	85.02 30.47 5.55 9.83	108.56 31.91 5.07 9.5	67.67 23.05 5.43 10	59.82 24.01 5.47 9.83	108.65 25.23 5.31 9.33	60.29	91.35	-12.44
P6 (kahal)	45.47 27.89 5.38 10	99.67 28.62 5.1 9.67	33.49 27.13 5.54 10	93.69 29.93 5.48 10	37.86 32.01 5.55 9.83	73.08 23.44 5.54 8.67	75.5	-28.3
Array mean F_2	0074.49 28.88 5.37 8.94	96.32 29.69 5.13 9.13	64.44 28.24 5.34 9.88	82.81 27.33 5.34 9.66	77.93 27.78 5.39 9.72	63.87 28.17 5.43 9.69	Total array mean	
GCA F_2	-2.15 0.53 0.04 -0.56	19.68 1.34 -0.2 -0.31	-12.2 -0.11 0.01 0.38	6.17 -1.02 0.01 0.16	1.29 -0.57 0.06 0.22	-12.77 -0.18 0.1 0.13	F_1= 103.79 F_2= 76.64 28.35 5.33 9.5	
Means P= 85.05 F_1= 107.54 F_2= 74.96 26.5 28.72 5.48 5.31 8.33 9.74								

show maximum number of adult insect emergence suggesting that Black Crowder seeds may have some degree of resistance to *C. maculatus* (Dongre et al., 1996; Ofuya and Adeduntan, 1999 and Lale and Mustapha, 2000).

The analysis of variance (Table 2) revealed highly significant differences among the genotypes for all characters studied.

The diallel analysis of variance revealed that, both "a" and "b" items measuring additive and non-additive gene effects, respectively, were highly significant for both F_1 and F_2 generations, except the "a" item for number of emerged adult insects (Table 2). Directional dominance towards greater expression was operating for protein content percentage Haridy (2005) seed yield/plant, and towards the least percentage of seeds weight loss as indicated by the significance of the "b_1" item. The 'b_2" item was significant for all characters, except for number of emerged adult insects, indicating unequal distribution of dominance and recessive alleles among the parents. The significance of "b_3" item for all studied traits, except for number of emerged adult insects, indicated further dominance due to specific cross combinations and/or epistasis. Results also revealed highly significant "c" and "d" items for seed yield/plant and protein content percentage indicating the presence of maternal effects. Meanwhile, for seed weight loss percentage and number of emerged adult insects, 'c" item was non-significant indicated the absence of maternal effects. However, the significance of "d" item in seed weight loss indicated the presence of reciprocal differences between crosses.

The interpretation of the Wr/Vr graph:

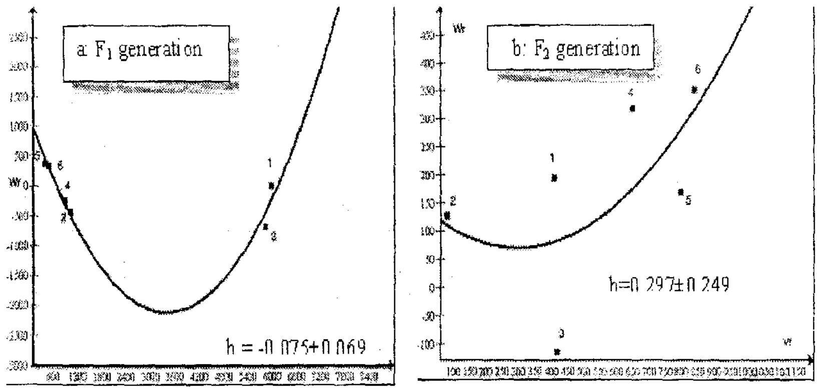
The results of the analysis of the variance of the (Wr+Vr) and (Wr-Vr) values (Table 2) reveled significant array differences in the (Wr+Vr) values for the F_1 and F_2 generations confirming the presence of non- additive genetic variation for all studied characters. The differences in the magnitude of the (Wr-Vr) values over arrays were significant for all studied characters, except for number of emerged adult insects, indicating the presence of either non-allelic gene interaction or epistatic effects. The Wr/Vr relationship is graphically illustrated in Figure (1). The slope of the Wr/Vr regression line was not significantly different from zero, but significantly different from unity for the F_1 and F_2 generations of seed yield/plant (b = -0.075 ± 0.069 and 0.297 ± 0.249, respectively), % Protein content (b = -0.130 ± 0.344) and also for seed weight loss percentage (b = -0.130 ± 0.344), indicating inadequacy of the simple additive-dominance model and that duplicate type of non-allelic gene interaction may be involved. The shape of Wr/Vr was concave upwards for the F_1 and F_2

Table (2): The diallel analyses of variance of the six parents, F_1 and their F_2 generations in a complete diallel cross.

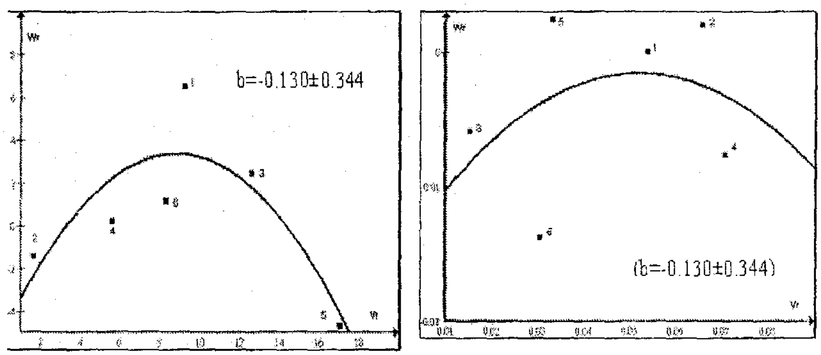
Items	d.f.	Seed yield/plant		Protein content%	Seed weight loss %	Number of emerge adult insects
		F_1 (M.S)	F_2 (M.S)	F_2 (M.S)	F_2 (M.S)	F_2 (M.S)
Blocks	2	22.75	4.59	0.41	0.0014	0.23
Genotypes	35	7602.3**	2150.09**	40.18**	0.15**	2.66**
a #	5	12202.9**	5353.0**	25.1**	0.39**	5.07
b	15	12420.3**	2293.9**	50.19**	0.2**	4.25**
b_1	1	7591.3**	1524.6**	73.8**	0.41*	29.87
b_2	5	11076.4**	2467.5**	36.1**	0.3**	6.4
b_3	9	13703.4**	2282.97**	55.4**	0.1**	0.21
c	5	809.97**	1149.7**	46.9**	0.006	0.2
d	10	1471.2**	833.03**	29.4**	0.03*	0.28
bxa	10	17.4	12.2	0.26	0.003	1.6
bxb	30	6.64	4.99	0.17	0.004	1.19
bxb_1	2	3.27	0.2	0.19	0.006	2.58
bxb_2	10	5.28	6.7	0.16	0.002	2.6
bxb_3	18	7.77	4.57	0.17	0.006	0.24
bxc	10	9.57	6.65	0.09	0.005	0.13
bxd	20	9.9	6.08	0.12	0.003	0.22
Block interaction	70	9.53	6.57	0.16	0.004	0.82
Wr+Vr	5	0.0000001**	476277.4**	107.04**	0.002**	56.42*
Wr-Vr	5	0.0000002**	182147.7**	150.5**	0.0001**	0.53

*, ** Significant at 0.05 and 0.01 levels, respectively.

Each item was tested against its own block interaction.

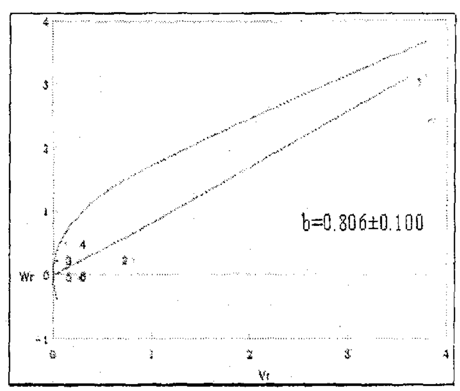


The Wr/Vr graphs for seed yield/plant for the six cowpea parents, F_1 and F_2 generations.



The Wr/Vr graph for cowpea protein content percentage of the F_1 generation seeds in a complete diallel cross.

The Wr/Vr graph for seeds weight loss% for the six parents and their F_2 generation seeds in a complete diallel cross.



The Wr/Vr graph for total number of emerged *C. maculatus* insect adult for the six parents and their F_2 generation cowpea seeds in a complete diallel cross.

Fig.(1):

of seed yield/plant indicating complementary non-allelic gene interaction, meanwhile it was concave downwards for % protein content and seed weight loss percentage indicating duplicate type of non-allelic gene interaction (Sherif et al., 1991; Sherif and Damarany, 1992; Bhor and Dumbre, 1998; Sangwan and Lodhi, 1998; Sangwan et al., 1998 and Mehta, 2000). For number of emerged adult insects, the slope of the Wr/Vr regression line was significantly deviating from zero but not from unity ($b = 0.806 \pm 0.1$) indicating the adequacy of the additive-dominance model which is in agreement with Fatunla and Badaru (1983); Redden (1983) and Adjadi et al. (1985). The regression line intercepted the Wr axis at the origin point indicating complete dominance. Meanwhile, there is a sharp discontinuity in the distribution of the points representing parents along the Wr/Vr graph which indicated that a major recessive gene(s) for greater number of emerged adult insects might differentiate parent 1 (Black Crowder) from the other group of parents.

Genetic parameters:

The estimates of various component of genetic variation are given in Table (3). For number of emerged adult insects, the "D" parameter estimating the additive effect was much smaller than the dominance parameter "H_1" confirming those revealed by the Wr/Vr graph. The average degree of dominance as measured by the $(H_1/D)^{1/2}$ ratio reached (1.19) indicating complete dominance. The "F" parameter is positive indicating that there were more dominant then recessive alleles in parental seeds. The $H_2/4\ H_1$ value indicated that the UV value was not equal to 0.25 indicating non-equal distribution of the dominant and recessive alleles among the analyzed parents. Broad- sense heritability value was 0.89 indicating that the major proportion of the total phenotypic variation was genetic variation. The narrow-sense heritability reached 0.48 indicating that the additive gene effects were as important as dominance effects for number of emerged adult insects. The parent Black Crowder, which posses the minimum number of emerged insects, suggested that the resistance was expressed as a recessive character as indicated from Wr/Vr graph (Redden, 1983 and Rusoke and Fatunla, 1987).

Table(3): Genetic parameters of the number of emerged adult insects from 10 cowpea seeds of the six parents and their F_2 generation.

Components	F_2 generation seeds	
	Values	S.E.
D	2.7336	0.2870
F	6.6377	0.7011
H_1	15.5709	0.7286
H_2	4.9471	0.6508
E	0.8219	0.1084
$(H_1/D)^{1/2}$	1.1933	
UV	0.0794	
Gene ratio	3.0708	
Broad heritability	0.8876	
Narrow heritability	0.4778	

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التحكم الوراثي في محصول البذور ونسبة البروتين في اللوبيا والمقاومة لخنفساء اللوبيا

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أجري التهجين الدائري الكامل بين ستة أصناف متفارقة مورفولوجيا للوبيا (*Vigna unguiculata* (L.) Walp) وذلك لتقدير الثوابت الوراثيـة لمحـصول البذور للنبات ونسبة محتوي البروتين. علاوة علي ذلك اجريت التجارب المعملية لتقدير طرز الفعل الجيني المتحكم في توارث *Callosobruchus maculatus* المقاومة لخنفساء اللوبيا ونسبة الفقد في وزن البذور نتيجة الاصابة والعدد الكلي للحشرات البالغة المتحصل عليها. أظهرت النتائج أن الفعل المضيف وغير المضيف يتحكم في الصفات المدروسة ما عدا صفة العدد الكلي للحشرات البالغة حيث كان الاثر الجيني المضيف غير معنويا. أعلي قيمة موجبة للقدرة العامة علي الائتلاف (GCA) سجلت للصنفين Black Crowder , Tvu21 improved لوزن البذور للنبات ، وللصنف Sudani لمحتوي البروتين. بالنسبة لمعدل فقد الوزن للحبوب المصابة سجلت أعلي قيمة سالبة للـ (GCA) للصنف Sudani ، والصنف Black Crowder لاقل عدد من الحشرات البالغة. تحليل الـ Wr/Vr يشير الي وجود التفاعل الجيني الغير أليلي من الطراز المتضاعف بالنسبة لمحتوي البروتين ونسبة فقد وزن البذور نتيجة الاصابة بينما كان التفاعل الجيني من الطراز المكمل متحكما في محصول البذور للنبات. بالنسبة للعدد الكلي للحشرات البالغة المتحصل عليها وجد جين رئيسي أو مجموعة جينات تفصل الاصناف الستة إلي مجموعتين أحداهما تحتوي علي الصنف Black Crowder فقط الذي يمتلك درجة من المقاومة لخنفساء اللوبيا وهذه المقاومة تم التعبير عنها كصفة متنحية والمجموعة الاخري تحتوي علي باقي الاصناف. تأثير الام ظهر في المحتوي من البروتين ومحصول البذور للنبات ولكنه غاب في فقد الوزن للبذور نتيجة الاصابة وكذلك بالنسبة للعدد الكلي للحشرات البالغة التي تم الحصول عليها.