GENETIC AND MOLECULAR ANALYSES OF SOME COWPEA (*Vigna unguicualata walp.*) GENOTYPES SELECTED FOR BEAN BEETLS RESISTANCE

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ABSTRACT

The main objective of this investigation was to develop a genotype resistance of cowpea for bean beetls and high yield. Genetic parameters for yield and its component in five genotypes selected from five cultivars of cowpea. RAPD technique was used to confirm the genetic distance among genotypes. Significant and highly significant differences were found between the studied genotypes for all studied traits. The significance of mean squares of genotypes is an indicator for the presence of genetic variation among these genotypes. The mean performance showed that, the cowpea genotype selected (103) show the highest value for green fodder yield per plant (GFY/P) in the first and second cut, dry fodder yield per plant (DFY/P) in the second cut, crude protein (CP%) and digestible protein (DP/P%) in the second cut with the means of 502.0, 339.0, 86.67 GM /p, 19.54, and 15.19 %, respectively. The results revealed that the genotypic variance (VG) relative to environmental variation (VE) was large in magnitude for all traits except for crude protein (CP %) and digestible protein (DP %) in the second cut. The differences between genotypic coefficient of variability (GCV) and phenotypic coefficient of variability (PCV) were low, suggesting lower effects of environments for these traits. The estimated values of heritability in broad sense for all studied traits ranged from 36.96 to 98.23 % for digestible crude protein in the second cut to green fodder yield (GFY/P) in the first cut, respectively. The estimates of expected genetic advanced values for green fodder yield at first and second cut, dry fodder yield at the first and second cut, crude protein at the first and second cut, digestible crude protein in the first and second cut, shoot number, 50% flowering, 100 seed weight, and seed yield per plant are 89.16, 88.39, 83.21, 88.23, 6.76 4.21, 8.23, 5.23, 67.24, 33.2, 52.63, and 59.5 % respectively. This indicated that both additive as well as dominant gene action might involve in controlling these traits. It could be concluded that selection in advanced generations would be used to improve these traits. The pattern produced by ten primers showed a maximum number of 77 DNA bands ranging between 120 to 1050 bp. The primers, OP-C12, OP-C19, OP AX19, OPB11 and OP-B01 gave maximum number of polymorphic bands. All results are in favor at producing promising genotypes resistance to been beetles
Keywords: Cowpea, Genetic variance, Molecular analyses, Lesser bean beetls.

INTRODUCTION

Cowpea has a high leaves: stem ratio with high Juice content but with no glycosides and comarin. Thus it is easy digestible food it has crude protein of 17-19% , crude fiber of 22-24% digestible protein 13-14% and total digestible nutrients of 59-61% as reported by cuts that is an ideal fodder for high performance dairy cows and feeder cattle in the summer season Abd El shafy (1991). Direct losses in seed yield resulting from insects which attack

cowpea in flowering stage period and during storage would be the major limiting in cowpea seed production.

Effective control of the insect pests which are the three bean beetles (Bru. Inc. Boh) and cowpea beetles (call. Chin .l.) family bruchidue would increase from 10 to 30 times the productivity. Been beetles lay its eggs during flowering stage so it causes complete death of seeds in the field or in store house which significantly affect the production cowpea seed.

The genetic variation is of great importance because it is the hereditary portion of the total variation .Muhammad *et al.,* 1994; Shimelis 2006 stated that selection on the basis of grain yield alone is usually not effective , whereas selection including all yield component traits would be more efficient and reliable. Consequently, information on the association between yield and yield components would improve the efficiency of selection in plant breeding programs. The heritability estimates along with genetic gain are useful in predicting the resultant effect through selection of the best individual (Malarvizhi 2000).

Antibiosis as a mechanism of resistance was used according to the method proposed by painter (1951) it is defined as those factors of a resistance plant that cause adverse effects on the insect lifted cycle when the insect uses that plant for feed or one stage of it life cycle by prevent eggs thinning it is the most striking mechanism of insect resistance. High level of it's usually palance great pedigree selection pressure on the insects.

As isozymes and AFLP markers, a larger number of markersfor RAPD data confirmed the single domestication hypothesis, the gap between wild and domesticated cowpea, and the widespread introgression phenomena between wild and domesticated cowpea (Ba *et al.,* 2004).The polymorphism was scored and seen in band sharing analysis to identify genetic relationship. Cluster analysis based on Jaccard's coefficient using UPGMA grouped all the 30 genotypes into three groups at a similarity coefficient of 25 % . Similarity indices ranged from 0.463 to 0.784. The highest similarity coefficient was observed among some genotypes, indicating that the less divergence between genotypes was observed between genotypes which were more divergence .Distinct phenotypes identified using RAPD markers could be potential sources of germplasm for cowpea improvement in breeding program (Prasanthi *et al.,* 2012).

The aim of this investigation was to obtain a cowpea genotype resistant to bean beetles with high yield and to estimated genetic parameters for yield and its component in five selected genotypes from five cultivars of cowpea. RAPD analysis was used to ensure genetic relaions among genotypes.

MATERIALS AND METHODS

Field performance and selection procedure

Varietal screening was done using different genotypes of cowpea (5 genotypes, three of them originated from USA (Buff, cream and upright), Brabham var. from Ghana and the local variety. The names of genotypes were named as follows: Ahmose 101, Ahmose 103, Ahmose 105, Ahmose

107 and Ahmose 109(named by authors). These genotypes were grown during the summer seasons of 2001 and 2002 for purification of seeds on morphological traits at Sers Elliyan Field Crops Research Station, ARE. After define the infection percentages,they are shown in Tables 1 and 2. All recommended agriculture practices were used. Selection for pure genotype was done during summer seasons 2003-2007 by field experimental materials for breeding for bean beetles, large bean beetles (Bni. inc), lesser bean beetle (Bru.Ruf.) and cowpea beetle(cal. Chin.)(The Insect were defined by Plant Protection Research Department (Kindly Personal).

Table 1: infection percentage of cowpea by beam beetles at season 2000

Genotype	Seed number	Total insects	Beetles no.	large and lesser beetles	% total * infection	Cowpea beetles %	% infect by cowpea to the total
Buff	43	76	68	8	176.74	158.4	89.47
Cream	45	56	38	18	124.44	84.44	67.86
Brabham	72	42	24	18	58.33	33.33	57.14
Local	54	130	77	53	240.74	142.59	59.23
Upright	61	90	84	6	147.54	137.7	93.33

(*) One seed had more one insect

Table 2: the total infection percentage at season 2004

genotype	% total * Infection
Buff	2.62
Cream	2.16
Brabham	2.29
Local	2.26
Upright	2.28

The optimal method of controlling these insects is through Host-Plant Resistance and by Antibiosis mechanism which restricts or eliminates damage caused by these insects to have the pure genotype selection under nature infection of bean beetles through the other hosts, *i.e.,* Faba bean, soybean, chick pea, field pea, mang bean, fenugreek and Egyptian lupine. This mechanism depends on maternal-genotype-selection which is considered suitable for cowpea, because it does not increase costs, does not require special equipment it depend on the chemical compounds of the plant which cause exhibit biological activity on more than 100 species of insects and does not environmental pollution by the reduction in pesticides use. In 2008 summer seasons, five promising cowpea genotypes were selected using progeny test and the natural infection of bean beetles. In 2009 and 2010 summer seasons, the selected five genotypes were (Ahmose 101,103,105,107 and Ahmose 109) which were bean beetles resistant to yield and its quality under number of cutting and seed production.

The produced seeds were evaluated at Sers Elliyan Agricultural Research Station in the two years; 2009 and 2010. The experiment was arranged in a randomized complete blocks design with three replications. Plot size was one row, 6m long 80cm apart. Seed was planted in hills at rate of three seeds which spaced at 25cm apart. Seedling was thinned to one plant per hill after 21 day from planting. All agronomic field practices were applied as recommended. Data recorded on 10 guarded plants, which were chosen randomly from each row in two cuts at two seasons for the following forage traits: green fodder yield per plant (GFY/P) dry fodder yield per plant (DFY/P), crude protein percentage(CP%) and digestible crude protein percentage (DP%). Digestible crude protein (DP %) were calculated by, DP %=(CP % × 0.959) - 3.55, according to Bredon et al (1963). Where crude protein percentage was determine by using the Micro-Keldahl Method according to Anonymous, (1962). The first cut was taken after 45 day from the day of sowing and the second cut was taken after 30 days from the first cut. Seed yield traits were days to 50% flowering, number of branches per plant, 100seed weight(g) and seed yield per plant(g).

Analyses of variances were applied in order to the test significance of the differences among the studied genotypes. In addition, a combined analysis of variance across two years was computed for the genotypes according to Cochran and Cox (1980). The differences between any two means were tested for significant using the (LSD) values test at both 5% and 1% levels of probability. Combined analysis among the two years was done on the base of homogeneity test.

The phenotypic and genotypic variance for the character was estimated by the method suggested by Goulden (1952). The genotypic coefficient of variability (GCV) and phenotypic coefficient of variability (PCV) were measured according to Burton (1952).Heritability in broad sense (h^2_b %) is referred to as the ratio of genetic variance to total phenotypic variance as follows according to Johanson *et al.* (1955) as follows Heritability in broad sense (h^2_b %) = $\sigma^2 G / \sigma^2 P \times 100$, where $\sigma^2 G$ is genotypic variance, and $\sigma^2 P$ is phenotypic variance.

Genetic advance under selection (GS) was estimated using a selection intensity of 10% according to formula, GS% = GS unite / Grand mean×100, where GS unite is genetic advance unite which calculated by formula: GS unite = $\sigma^2 P^{\wedge 2} \times H^2/100 \times 2.06$ (Falconer, 1981).

RAPD-PCR analysis
a. DNA extraction

Young and freshly excised leaf were collected separately from all studied genotypes in 2014. Then DNA extraction was performed as described by Dellaporta *et al.* (1983). The DNA pellets were re-suspended in 80ul TE (10 mMTris-HCl pH 8.0 and 1 mM EDTA) buffer.

RAPD -PCR analysis
Polymerase Chain Reaction (PCR):

In order to obtain clear reproducible amplification products, different preliminary experiments were carried out in which a number of factors were optimized. These factors included PCR temperature cycle profile and concentration of each of the template DNA, primer, $MgCl_2$ and Taq

polymerase. A total of ten random DNA oligonucleotide primers were independently used according to Williams *et al*. (1990) in the PCR reaction. Table (3) lists the base sequences of these DNA primers that produced informative polymorphic bands.

The PCR amplification was performed in a 25 µl reaction volume containing the following: 2.5 µl of dNTPs (2.5 mM), 1.5µl of Mg Cl$_2$ (25 mM), 2.5 µl of 10x buffer, 2.0 µl of primer (2.5 µM), 2.0 µl of template DNA (50 ng/µl), 0.3 µl of Taq polymerase (5 U/µl) and 14.7 µl of sterile ddH$_2$O. The reaction mixtures were overlaid with a drop of light mineral oil per sample. Amplification was carried out in Techni TC-512 PCR System. The reaction was subjected to one cycle at 95ºC for 5 minutes, followed by 35 cycles at 94ºC for 30 seconds, 37ºC for 30 seconds, and 72 ºC for 30 seconds, then a final cycle of 72ºC for 12 minutes. PCR products were run at 100 V for one hour on 1.4 % agarose gels to detect polymorphism between genotypes under study. After electrophoresis, the RAPD patterns were visualized with UV transilluminator. RAPD bands were scored from the gels as DNA fragments presence or absence in all lanes. Gels were photographed using a Polaroid camera.

Statistical analysis: The DNA bands generated by each primer were counted and their molecular sizes were compared with those of the DNA markers. The bands scored from DNA profiles generated by each primer were pooled together. Then the presence or absence of each DNA band was treated as a binary character in a data matrix (coded 1 and 0, respectively) to calculate genetic similarity and to construct dendrogram tree among the studied ten cowpea genotypes. Calculation was achieved using Dice similarity coefficients (Dice, 1945) as implemented in the computer program SPSS-17.

Table 3: List of the primer names and their nucleotide sequences used in the study

	NAME	SEQUENCE
1	OP- A02	5´ GAT GAC CGC C 3`
2	OP-A18	5` AGG TGA CCG T 3`
3	OP-AX19	5´ GAT GAC CGC C 3`
4	OP-B01	5´ GTT TCG CTC C 3`
5	OP- B11	5` GGC TGT CCG T 3`
6	OP- C02	5´GTG AGG CGT C
7	OP- C09	5´CTC ACC GTC C 3`
8	OP- C12	5` GGC TGT CCG T 3`
9	OP- C19	5` GGC TGT CCG T 3`
10	OP- E19	5` ACG GCG TAT G 3`

RESULTS AND DISCUSSION

Field Performance

An analysis of variance was separately made for each season (Table 4) and combined analysis of variance over the two seasons in (Table 5). Significant and highly significant differences were found between the studied genotypes for most studied traits. The significance of mean squares of genotypes is an indicator of the presence of genetic variation among these genotypes. However year's mean squares were significant and highly significant for green and dry fodder yield (GFY/P&DFY/P) and seed yield per plant (SY/P).This revealed that these genotypes gave different performances under different years conditions with respect to the studied traits. The mean performances of the studied genotypes were separately determined for each year and the obtained results are present in Table 6. However combined data over the two years for all the studied traits for the ten genotypes appeared in Table 7.

Results in Table (7) showed that, the cowpea genotype (Ahmose103) gave the highest mean values for green fodder yield per plant (GFY/P) in the first and second cut, dry fodder yield per plant (DFY/P) in the second cut, crude protein (CP%) and digestible crude protein (DP/P%) in the second cut, showing the means: 502.0, 339.0. 86.67 GM/p, 19.54, and 15.19 %, respectively. While, the cultivar (cream) gave the highest values for 100 seed weight and seed yield per plant with the means of 13.16 and 52.17 GM/p respectively.

The variances in terms of genotypic (VG) and phenotypic (VP) as well as, genotypic coefficient of variation (G.C.V.) and phenotypic (P.C.V.) coefficient of variability, heritability in broad sense (h²b), and genetic advance under selection using 10% selection intensity are presented in Tables 8, and 9. Similarly, these parameters were determined from the combined data across the two years for all studied traits and are presented in Table 10.

The results revealed that the genotypic variance (VG) relative to environmental variation (VE) was large in magnitude for all traits except for Crude protein (CP %) and digestible crude protein (DP %) in the second cut. The differences between GCV and P.CV were narrow, suggesting little effects of environments on these traits. The data showed that genotypic variances were moderate for green fodder yield (GFY/P), dry fodder yield (DFY/P) at the first and second cut, number of branches per plant (NB/P), and seed yield per plant(SY/P) with the values of 43.67,43.41,42.51,43.94,35.05 and 29.82, respectively. This results are in agreement with Aremu and Adewale(2010). The estimates values of heritability in broad sense for all studied traits ranged from 36.96 to 98.23 % for digestible crude protein in the second cut to green fodder yield (GFY/P) in the first cut, respectively.

514

4-a-b

5-6

7

8-

9

10

These results are in agreement with the results obtained by Rang (1980) and Imam (1991). The estimates of expected genetic advanced value for green fodder yield at first and second cut, dry fodder yield at the first and second cut, crude protein at the first and second cut, digestible crude protein in the first and second cut, shoot number, 50% flowering, 100 seed weight, and seed yield per plant were 89.16, 88.39, 83.21, 88.23, 6.76 4.21, 8.23, 5.23, 67.24, 33.2, 52.63, and 59.5, respectively. This indicated that both additive as well as dominant gene action might be involved in controlling these traits (Panse, 1957). Thus, from the previous results, it would be concluded that selection in advanced generations is good to improve these traits. These results agreed with those obtained by Sakai and Niles (1957**).**

RAPD-PCR analysis

RAPD-PCR was used to investigate the genetic diversity of the ten cowpea genotypes, and to assess their genetic relationships. Ten arbitrary random primers were used to determine RAPD polymorphism in Table 3.Bands were scored as present (1) or absent (0) in Table 11 and figure 1. All the ten primers successfully amplified DNA fragments among all genotypes. The pattern produced by ten primers showed a maximum number of 77 DNA bands ranging between 1050 to 1200bp Tables 11 - 13. The primers: OP-C12, OP-B01,OP-C19,OP AX19,and OPB11 gave maximum number of polymorphic bands. The genotype number1 showed three unique bands at 200, 550, 220 bp with primers OPC19 and OPC12. Local genotypes(balady) showed two bands are seen in Table 15. Also, the genotype number 7 produced two unique bands with OPAX19; two bands which were considered unique bands with OP-A18 primers with the genotypes Ahmose 107, and one band of 1200 bp with OPA02 primer with genotype cream. In addition,, the total number of unique bands were nine bands as presented in Fig.1 Tables 11, 12 and 13.

Based on similarity clustering, the first cluster only had the Genotypes Buff and Cream which showed 73% similarity with other genotypes. The cluster number 2 had the sub cluster 1 included local genotype (balady); the sub cluster 2 had four groups where the first group genotypes Brabham and Upright, the group number two had genotypes Ahmose 101 and the group number three included the subgroup number 1 which had genotypes Ahmose 105 and Ahmose 109 and the sub group number two had genotypes Ahmose 103 & Ahmose 107 as presented in Figure 2 and Table 12.

The highest recorded similarity was 88% between the two genotypes Ahmose 103 and Ahmose 107, while the lowest similarity 58% was observed between the two genotypes Cream and Ahmose 101. The resistances of insects in genotypes were demonstrated and illustrated by bands from the RAPD-PCR technique. The polymorphism of primers and polymorphic percentage appeared as unique bands in genotypes Ahmose 107 with 500 bp and Ahmose 103 with 100bp related to resistant genotypes. In addition to resistant Ahmose 103 also was highest in yield production and its quality. Moreover, the genotypes from Ahmose 101 to 109 showed high yield production and resistance of all insects. The genes of resistance were

accumulated by pedigree selection cowpea breeders were able to develop genotypes resistant to bean beetles.

These results agreed with those by Wafaa M. Sharawy and El-Fiky (2003) who founded the presence of significant differences in morphological and quality traits among genotypes. The Buff genotype showed the highest fresh yield, dry yield, crude protein, crude fiber and ash yield, while the Cream dotted genotype showed the highest plant height and leaves/stem ratio. On the other hand, Upright growing genotype had the lowest values for all traits except leaves/stem ratio. For RAPD-PCR analysis, ten random arbitrary primers were used. Twenty one genotype-specific markers 9 positive and 12 negative were detected which would be used as markers for genetic characterization of the six genotypes used in the present study. They found relationships among the six genotypes of the cowpea as determined by RAP Distance software package, version 1.04. As well as, they studied the dendrogram tree grouped Buff and Upright growing in one cluster with a similarity index of 88.6% and Cream and Cream dotted with a similarity index of 80%. While, Local and Local-improved genotypes were the most genetically distant genotypes with similarity index 65.7%. In conclusion, the significant differences between yield traits, the molecular genetic analysis can be used to identify the different genotypes. Ba *et al.,* (2004) demonstrated that isozymes a larger number of AFLP markers, RAPD data would confirm the single domestication hypothesis, which explain the gap between wild and domesticated cowpea, and the widespread introgression phenomena between wild and domesticated .

LaïtyFall et *al.,* 2003 suggested that random amplified polymorphic DNA (RAPD) technology would be used to reorganize the national germplasm in order to eliminate the putative duplicates, and to identify elite varieties. Feleke*et al.,*2006 screened 54domesticated cowpea accessions and 130 accessions by molecular markers.

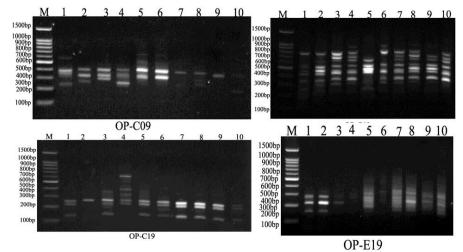**Figure 1: RAPD amplification products of 10 cowpea genotypes generated with ten primers OPA-18, OPA-02,OPB-01,OPA-18OPAX19,OPB-11,OPC-02,OPC-09,OPE-19,OPC-19 and OPA-18,M=DNA marker. The name of genotypes 1= Buff,2= Cream,3= Braham,4= Local,5= upright,6= Ahmose 101,Ahmose 103,Ahmose 105,Ahmose 109 and Ahmose 107. The genotypes from 1 to 5 were susceptible and genotypes from six to ten were resistance.**

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Table 11: Total number of band RAPD-PCR products by OP-Primer from primer B01 to c09 in cowpea genotypes. The name of genotypes 1= Buff,2= Cream,3= Braham,4= Local,5= upright,6= Ahmose 101,Ahmose 103,Ahmose 105,Ahmose 109 and Ahmose 107. The genotypes from 1 to 5 were susceptible and genotypes from six to ten were resistance.

B01

M	1	2	3	4	5	6	7	8	9	10
200	0	1	0	0	0	1	0	1	1	0
300	0	1	0	0	0	1	0	1	0	1
320	1	1	1	1	0	0	1	1	1	1
400	1	1	1	1	1	1	1	1	1	1
420	1	0	1	0	1	1	0	0	0	0
500	1	1	1	1	1	1	1	1	1	1
700	1	1	0	0	1	0	1	1	1	0
800	0	0	0	1	0	1	1	1	1	1
850	1	1	1	1	1	1	1	1	1	1
950	1	1	1	1	1	1	1	1	1	1

A02

M	1	2	3	4	5	6	7	8	9	10
100	1	1	1	1	1	1	1	1	1	1
160	0	1	0	0	0	0	0	0	0	0
250	1	1	1	1	1	1	1	1	1	1
350	1	1	1	1	0	1	1	1	1	1
500	0	0	1	1	0	1	1	1	1	1
600	0	0	0	0	0	1	1	1	1	1
800	0	0	1	0	0	1	1	1	1	1

A18

M	1	2	3	4	5	6	7	8	9	10
120	1	0	1	1	1	1	1	1	1	1
150	1	1	1	1	1	1	1	1	1	1
200	1	1	1	1	1	1	1	1	1	1
250	1	0	0	1	1	1	1	1	1	1
300	1	0	0	1	1	1	1	1	1	1
400	0	0	0	0	0	0	1	1	0	1
500	0	0	0	0	0	0	0	0	0	1

AX19

M	1	2	3	4	5	6	7	8	9	10
100	0	0	0	0	0	0	1	0	0	0
200	1	0	1	1	1	1	1	1	1	1
220	1	1	1	1	1	1	1	1	1	1
260	0	0	1	1	0	1	1	0	0	0
300	0	0	1	0	0	0	1	0	0	0
350	1	0	1	0	1	1	1	1	1	0
500	0	0	1	0	0	1	0	0	0	0
800	1	0	0	0	0	0	0	1	0	0

B11

M	1	2	3	4	5	6	7	8	9	10
250	1	1	0	0	0	0	1	1	0	1
300	0	0	1	1	1	1	1	1	1	1
400	1	1	1	1	1	1	1	1	1	1
500	0	0	0	0	1	1	1	1	0	1
650	0	0	1	0	0	1	0	0	0	0
800	0	0	1	0	1	0	1	0	1	0
1000	0	0	1	0	1	1	1	0	1	1
1050	0	0	1	0	1	1	1	0	1	1

C12

M	1	2	3	4	5	6	7	8	9	10
200	1	0	0	0	0	0	0	0	0	0
230	1	1	1	0	1	0	0	0	0	0
300	0	1	1	1	1	1	1	1	1	1
350	1	1	1	1	0	1	1	1	1	1
400	1	1	1	1	1	1	1	1	1	1
440	1	0	0	1	0	0	0	1	1	0
500	1	1	1	1	1	1	1	1	1	1
550	1	0	0	0	0	0	0	0	0	0
600	0	1	1	1	1	0	1	1	1	1
700	0	0	1	1	0	0	1	1	1	1
800	1	1	1	1	0	1	1	1	0	1
950	0	0	0	1	0	1	0	1	1	1

C19

M	1	2	3	4	5	6	7	8	9	10
120	1	0	1	0	1	1	1	1	1	1
180	0	0	0	0	0	1	0	1	0	0
210	1	0	1	1	1	1	1	1	1	1
250	1	1	1	1	1	1	1	1	1	1
300	0	0	0	1	0	0	0	0	0	0
350	0	0	1	0	0	0	0	1	1	0
400	0	0	0	1	0	1	0	1	0	0
600	0	0	0	1	0	0	0	0	0	0

E19

M	1	2	3	4	5	6	7	8	9	10
160	1	1	0	0	0	0	1	1	0	1
200	0	0	0	0	1	0	1	1	1	1
220	1	1	1	0	1	0	1	1	1	1
290	1	1	0	0	1	1	1	1	1	1
320	0	1	0	0	1	0	1	1	1	1
400	0	0	0	0	0	1	1	1	1	1

C02

M	1	2	3	4	5	6	7	8	9	10
100	1	1	1	1	1	1	1	1	1	1
200	1	1	1	1	1	1	1	1	1	1
300	1	1	1	0	0	0	0	0	0	0
800	1	1	1	1	1	1	1	1	1	1

C09

M	1	2	3	4	5	6	7	8	9	10
220	1	0	0	0	0	0	0	0	0	0
250	0	1	0	1	0	0	0	0	0	1
300	0	0	1	0	0	0	1	1	0	0
350	1	1	1	1	1	1	0	1	0	0
400	1	1	1	1	1	1	1	1	1	1
500	0	0	0	0	1	1	0	0	0	0
600	1	0	0	0	1	1	0	0	0	0

Table 12:Similarity matrix of the all genotypes cowpea based on ten OP-Primers RAPD – PCR, the name of genotypes 1= Buff,2= Cream,3= Braham,4= Local,5= upright,6= Ahmose 101,Ahmose 103,Ahmose 105,Ahmose 109 and Ahmose 107. The genotypes from 1 to 5 were susceptible and genotypes from six to ten were resistance.

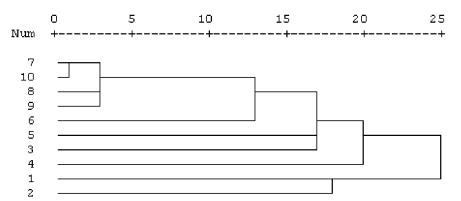|										
	Buff	**Cream**	**Brabham**	**Local**	**Upright**	**Ahmose 101**	**Ahmose 103**	**Ahmose 105**	**Ahmose 109**	**Ahmose 107**
1	100									
2	73	100								
3	69	65	100							
4	67	65	72	100						
5	73	64	73	64	100					
6	67	58	73	74	75	100				
7	68	64	80	70	75	75	100			
8	72	69	70	75	70	80	85	100		
9	68	64	76	75	78	78	86	86	100	
10	66	69	70	75	72	78	88	86	85	100

Figure 2: Dendrogram of the genetic distances between teen genotypes cowpea based of the combined ten primers RAPD-PCR amplification products. The name of genotypes 1= Buff,2= Cream,3= Braham,4= Local,5= upright,6= Ahmose 101,Ahmose 103,Ahmose 105,Ahmose 109 and Ahmose 107. The genotypes from 1 to 5 were susceptible and genotypes from six to ten were resistance.

Table 13: Levels of polymorphism and unique genotypes-specific bands based on RAPD- PCR.

No.	Primer OP	total bands	Poly-morphic	Mono-Morphic	% polymorphism	Unique bands
1	B01	10	6	4	60	-
2	A02	7	5	2	71.43	Genotype 2,MW 160 bp.
3	A18	7	5	2	71.43	Genotype 10,500bp.
4	AX19	8	7	1	87.5	Genotype7,MW 100 bp.
5	B11	8	7	1	87.5	
6	C02	4	1	3	25	-
7	C09	7	6	1	85.7	Genotype1,MW 220 bp.
8	C12	12	10	2	83.3	Genotype1,MW 200&550 bp.
9	C19	8	7	1	87.5	Genotype4,MW 300&600 bp.
10	E19	6	6	0	100	-
Total		77	60	17	77.9	8

CONCLUSION

The selection was the best methods to improve yield and its components of cowpea. The genotype Ahmose 103 was the best genotype and reliable as new variety for commerce use. The RAPD–PCR would be able to distinguish genotypes and to define genotypic resistance

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التحليـل الـوراثي والجزيئـي لـبعض التراكيـب الوراثيـة مـن لوبيـا العلـف منتخبـة لمقاومة الإصابة بحشرة الخنافس

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**قسم بحوث الخلية ــ معهد بحوث المحاصيل الحقلية ــ مركز البحوث الزراعية

تم تقييم خمسة أصناف من لوبيا العلف وخمس تراكيب وراثية منتخبة خلال الفترة من 2001-2007 لمقاومة الاصابة بحشرة الخنافس الكبيرة والصغيرة ثم إكثار هذه البذور في موسم 2008 وتقييمها خلال موسمي 2009 و2010 فى محطة البحوث الزراعية بسرس الليان بمحافظة المنوفية بهدف الحصول على تراكيب وراثية عالية لحاصل العلف ومتحملة لحشرة الخنفساء وقد تم إجراء تحليل التباين المشترك عبر سنتى الدراسة لكل التراكيب الوراثية وذلك بغرض تقدير بعض القياسات الوراثية مثل معامل الإختلاف الوراثى ومعامل الإختلاف المظهري وكذلك التحليل الجزيئ بإستخدام RAPD-PCR لتحديد المسافات الوراثية بين هذه التراكيب الوراثية فى 2014 وقد دلت النتائج على ما يلى:

-وجود فروق معنويـة بين التراكيـب الوراثيـة فى معظـم الصـفات المدروسـة وهى حاصـل العلف الأخضـر والجاف فى الحشة الأولى والثانية ,نسبة البروتين الخام وبروتين المهضوم فى الحشـة الأولى, عدد الأفرع, 50% تزهير, وزن ال100 بذرة, حاصل البذور للنبات.

-التركيب الـوراثى المنتخـب(103) أظهـر أعلـى متوسط لصـفة حاصـل العلف الأخضـر فـي الحشـة الأولـى والثانيـة للنبـات وحاصـل العلف الجـاف للنبـات فـي الحشـة الثانيـة ونسبة البروتين الخـام ونسبة البـروتين المهضـوم بمتوسطات 502.0و339.0و 86.67 جم/نبات 19.54و15.19 % على التوالي.

- معامل التوريث فى المدى الواسع تراوح بين 36.96إلى 98.23 % لنسبة البروتين المهضـوم فى الحشـة الثانيـة وحاصـل العلف الأخضر للنبات في الحشة الأولى على التوالي .

- مستوى التحسين الوراثي المتوقع تدرج من منخفض إلى معتدل ثم مرتفع لبعض الصفات.

- الارتفاع العالي لمعامل التوريث مع القيمة المعتدلة والعالية لمستوى التحسين الـوراثي المتوقع يدل علـى أن كل من الفعل الجيني المضيف والسائد يشتركان فى توريث هذه الصفات لذا فإن الانتخـاب في الأجيـال المتقدمة يعتبر أفضل طريقة لتحسين هذه الصفات.

- وجد أن إستخدام التحليل بواسطة ال RAPD تعتبر دقيقة لتحليل التنوع الجزيئى للتراكيب الـوراثية فى هذه الدراسة.

- نتج عن البادئات العشرة أقصى عدد ل 77 حزمة من ال د ن ا تراوحت بين 1050 إلى 1200نيوكلوتيده . أعطت أقصى عدد من الحزم التعددية OPAX19 , OPB11, OP ,op –c19 , Op –c 12 B01 -البادئات الجزيئية

الكلمات الدالة:لوبيا العلف ــ التباين الوراثي ــ التحليل الجزيئى ــحشرة الخنافس الكبيرة والصغيرة

Table 4-a: Mean squares of yield and yield components traits in 2009 and 2010 season

ANOVA	GFY/p^I		GFY/p^{II}		DFY/p^I		DFY/p^{II}		Shoot No/p		50% Flower		100 Sw		Seed y/p	
	2009	2010	2009	2010	2009	2010	2009	2010	2009	2010	2009	2010	2009	2010	2009	2010
Rep.	308.8	241.03	214.03	149.7	46.3	60.3	73.23	9.63	5.23	0.43	17.73	5.7	11.61	12.25	5.42	4.71
GENO.	29804.9**	34344.89**	13222.7**	14871.0**	2327.78**	1703.0**	853.58*	1067.8*	3.17**	4.21**	77.64**	109.6**	9.98**	11.45*	160.77**	236.3**
Error	563.65	422.85	300.92	297.62	67.11	47.48	20.12	25.07	0.49	0.28	3.40	4.92	0.64	0.22	6.43	6.66
C.V. %	7.5	5.88	8.23	7.43	10.23	8.12	8.31	8.49	16.45	12.52	4.14	4.84	8.3	4.48	7.34	6.33

GF = green fodder and DF=dry fodder *, ** significant at 0.05, 0.01 respectively

Table 4-b: The mean squares for crude protein (CP) and digestible crude protein (DP) in first and second cut during the two years of 2009 and 2010.

ANOVA	Cp1%		Cp2%		Dp1%		Dp2%	
	2009	2010	2009	2010	2009	2010	2009	2010
Reps.	1.78	1.22	5.24	2.44	1.63	1.12	4.81	2.26
Geno	1.42**	0.94**	1.36 ^{NS}	1.06**	1.31**	0.87**	1.25 NS	0.97**
Error	0.307	0.13	1.62	0.13	0.283	0.12	1.49	0.12
C.V. %	2.96	1.73	6.73	1.90	3.28	2.1	8.36	2.37

*, ** significant at 0.05, 0.01 respectively

Table 5: The combined analysis of variance over the two years for all studied traits

ANOVA	GFY/p^{I}	GFY/p^{II}	DFY/p^{I}	DFY/p^{II}	CP1	Cp2	DCP1	DCP2	Shoot N/p	50% F1	100 Sw	Seed y/p
Year	16203.26**	6955.26**	345.6^{NS}	375.00*	1.96^{NS}	0.003^{NS}	1.82^{NS}	0.002^{NS}	0.00^{NS}	24.07^{NS}	11.93^{NS}	583.81**
R/year	274.92	181.86	53.3	41.43	1.5	3.84	1.37	3.54	2.83	11.72	11.93	5.06
Geno	63990.73**	28042.5**	3748.1**	1869.73**	2.12**	2.10^{NS}	1.95**	1.93^{NS}	7.12**	178.63**	21.36**	385.03**
Geno. X year	159.15^{NS}	51.34^{NS}	282.75**	51.67^{NS}	0.24^{NS}	0.314^{NS}	0.22^{NS}	0.29^{NS}	0.26^{NS}	8.66^{NS}	0.06^{NS}	12.09^{NS}
Error	493.25	299.27	57.3	22.6	0.22	0.88	0.20	0.81	0.39	4.16	0.43	6.54
C.V. %	6.67	7.81	9.18	8.42	2.25	4.95	2.74	6.15	14.62	4.52	6.51	6.79

Table 6: Mean performance of the genotypes in 2009 and 2010 for all traits.

Genotypes	GF1		dF1		CP1		DCP1		GF2		DF2		CP2		DCP2		Shoot N/P		50% Flower		100 S w		Seed y/p	
	2009	2010	2009	2010	2009	2010	2009	2010	2009	2010	2009	2010	2009	2010	2009	2010	2009	2010	2009	2010	2009	2010	2009	2010
Buff (USA)	448.33	473.3	138.33	110.00	21.07	21.17	16.67	16.76	286.6	304.0	79.33	77.00	18.91	19.2	14.59	14.87	5.67	6.00	47.33	54.00	9.52	10.46	35.83	42.98
Cream(USA)	278.3	308.3	76.66	79.00	21.11	21.21	16.71	16.8	180.7	199.33	45.67	48.67	19.64	19.2	15.3	14.9	4.33	4.33	47.67	49.00	12.6	13.73	47.44	56.91
Brabham (Ghana)	351.3	393.3	83.3	103.33	20.67	21.77	16.29	17.34	240.00	261.7	57.33	66.67	18.76	19.04	14.45	14.72	4.00	4.33	32.67	32.67	6.7	7.26	21.42	25.69
Local (ARE)	297.6	329.0	70.00	72.33	19.62	20.07	15.27	15.71	200.3	221.00	53.00	51.67	17.8	17.52	13.53	13.26	4.00	3.33	42.67	44.33	9.63	10.4	37.37	36.82
Upright(USA)	411.7	454.3	91.67	111.67	21.10	21.32	16.70	16.91	273.6	305.00	66.67	81.67	19.45	19.32	15.12	14.98	5.67	5.33	43.00	44.67	9.43	10.16	34.4	41.48
101	252.6	282.0	64.00	68.33	20.69	20.86	16.31	16.46	168.0	190.00	41.67	47.00	18.65	18.42	14.35	14.12	4.33	4.00	41.00	41.67	8.24	9.11	27.0	32.38
103	475.0	529.0	113.33	120.0	21.20	21.03	16.79	16.63	323.3	355.00	81.67	91.67	19.98	19.1	15.63	14.76	5.33	6.00	47.33	45.33	9.1	10.23	31.3	37.81
105	246.0	270.0	54.00	68.33	19.81	20.78	15.46	16.39	165.0	181.00	37.00	47.00	18.22	18.82	13.93	14.51	3.33	3.00	48.33	47.00	12.13	12.93	40.8	48.93
107	199.6	220.0	52.3	59.00	21.37	21.57	16.96	17.15	133.3	147.67	38.33	37.67	19.35	19.7	15.02	15.34	3.33	3.67	50.33	53.33	8.3	9.02	31.32	37.51
109	206.3	236.3	57.3	57.00	19.61	20.11	15.26	15.75	136.6	158.33	39.00	40.67	18.57	18.92	14.22	14.6	2.67	2.67	45.00	46.00	11.1	12.3	38.66	47.38
LSD 5%	40.7	35.28	14.04	11.81	0.95	0.62	0.91	0.6	29.74	29.56	7.69	8.6	2.18	0.61	2.09	0.59	1.2	0.91	3.16	3.8	1.36	0.8	4.35	4.42
LSD 1%	55.81	48.38	19.29	16.21	1.29	0.85	1.24	0.82	40.78	40.55	10.55	11.81	2.99	0.85	2.85	0.81	1.64	1.24	4.32	5.21	1.87	1.1	5.96	6.07

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Table 7: The overall mean performances of all traits of the two years

Genotypes	Vegetative traits				Chemical Comp.				Seed yield traits			
	GFY/p^I	GFY/p^{II}	DFY/p^I	DFY/p^{II}	CP1	DP1%	CP2	DP2%	Shoot N/P	50% Flower	100 Sw	Seed y/p
Buff (USA)	460.83	295.33	124.16	78.16	21.12	16.72	19.06	14.73	5.83	50.67	9.99	39.41
Cream(USA)	293.33	190.00	77.83	47.16	21.16	16.75	19.43	15.09	4.33	48.33	13.16	52.17
Brabham (Ghana)	372.33	250.83	93.33	62.00	21.22	16.82	18.89	14.58	4.16	32.67	6.98	23.55
Local (ARE)	313.33	210.67	71.16	52.00	19.84	15.49	17.66	13.4	3.67	43.5	10.01	37.09
Upright(USA)	433.0	289.33	101.67	74.16	21.21	16.81	19.38	15.05	5.5	43.83	9.8	37.94
101	267.33	179.00	66.16	44.33	20.77	16.38	18.54	14.23	4.16	41.33	8.67	29.7
103	502.00	339.16	116.67	86.67	21.11	16.71	19.54	15.19	5.67	46.33	9.66	34.55
105	258.00	173.00	61.17	42.00	20.29	15.93	18.52	14.22	3.16	47.67	12.53	44.87
107	209.83	140.5	55.67	38.00	21.47	17.05	19.52	15.18	3.50	51.83	8.64	34.41
109	221.33	147.5	57.17	39.83	19.86	15.51	18.75	14.44	2.67	45.5	11.7	43.02
LSD 5%	38.1	29.66	12.98	8.15	0.80	0.77	1.60	1.54	1.07	3.5	1.07	4.38
LSD 1%	52.22	40.68	17.8	11.17	1.10	1.06	2.2	2.11	1.47	4.8	1.44	6.05

Table 8: Variances of genotypic (VG), and phenotypic (VP), heritability in broad sense (h^2_b) genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), and genetic advance (Gs) for all studied traits at the first year (2009).

Genetic Parameters	Vegetative traits				Chemical Comp.				Seed yield traits			
	GFY/p^I	GFY/p^{II}	DFY/p^I	DFY/p^{II}	CP1	CP2	DCP1	DCP2	ShootN/p	50% Flower	100 Sw	Seed y/p
G mean	316.7	210.77	80.1	53.93	20.63	18.94	16.24	14.62	4.27	44.53	9.67	34.55
$\sigma^2 e$	563.65	300.92	67.11	20.12	0.31	1.62	0.28	1.49	0.49	3.40	0.64	6.43
$\sigma^2 G$	9747.11	4307.3	753.55	277.82	0.37	-0.08	0.34	-0.08	0.89	24.45	3.11	51.45
$\sigma^2 P$	10310.77	4608.2	820.67	297.94	0.68	1.54	0.62	1.41	1.38	28.15	3.75	57.87
h²%	94.53	93.47	91.82	93.24	54.7	-5.73	54.7	-5.75	64.41	87.92	82.95	88.9
GCV	31.17	31.14	34.27	30.91	2.95	1.5	3.59	1.93	22.1	11.10	18.23	20.76
PCV	32.06	32.22	35.76	32.00	3.99	6.55	4.85	8.12	27.51	11.91	20.02	22.02
GS(unit)	197.73	130.71	54.18	33.15	0.93	0.15	0.89	0.14	1.56	9.61	3.31	13.93
Gs %	62.01	62.01	67.65	61.47	4.5	0.77	5.46	0.96	36.5	21.58	34.22	40.32

Table 9: Variance of genotypic (VG) and phenotypic (VP), heritability in broad sense (h²ᵦ) genotypic coefficient of variation (GCV) phenotypic coefficient of variation (PCV), and genetic advance (Gs) for all studied traits at the second year (2010)

Genetic Parameters	Vegetative traits				Chemical Comp.				Seed yield traits			
	GFY/p^{I}	GFY/p^{II}	DFY/p^{I}	DFY/p^{II}	CP1	CP2	DCP1	DCP2	Shoot n./p	50% Flower	100 Sw	Seed y/p
G mean	349.56	232.3	84.9	58.97	20.98	18.92	16.59	14.61	4.27	45.8	10.56	40.79
$\sigma^2 e$	422.85	297.63	47.48	25.08	0.13	1.06	0.12	0.12	0.28	4.92	0.22	6.86
$\sigma^2 G$	11307.35	4857.81	551.58	347.57	0.27	-0.31	0.25	0.28	1.31	34.91	3.74	76.49
$\sigma^2 P$	11730.2	5155.44	599.33	372.65	0.4	0.75	0.37	0.4	1.59	39.83	3.96	83.35
h²%	96.39	94.23	92.07	93.3	67.5	-41.33	67.56	70.25	82.4	87.65	94.45	91.77
GCV	30.42	30.00	27.67	31.61	2.47	2.94	3.01	3.62	26.8	12.90	18.31	21.44
PCV	30.98	30.91	28.83	32.73	3.01	4.58	3.67	4.33	29.53	13.78	18.84	22.38
GS(unit)	215.05	139.4	46.43	37.1	0.88	0.74	0.85	0.91	2.14	11.39	3.87	17.25
Gs %	61.52	59.99	54.7	62.92	4.2	3.9	5.1	6.26	50.12	24.9	36.66	42.31

Table 10: Variance of genotypic (VG), phenotypic (VP), heritability in broad sense (h^2_b) genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), and genetic advance (Gs) for all studied traits from combined data over the two years

Genetic Parameters	Vegetative traits				Chemical Comp.				Seed yield traits			
	GFY/p^I	GFY/p^{II}	DFY/p^I	DFY/p^{II}	CP1	CP2	DCP1	DCP2	Shoot N/p	50% Flower	100 Sw	Seed y/p
G mean	333.13	221.53	82.5	56.47	20.81	18.93	16.42	14.61	4.27	45.17	10.12	37.67
$\sigma^2 e$	493.25	299.27	57.3	22.6	0.22	0.88	0.2	0.81	0.39	4.16	0.43	6.54
$\sigma^2 g$	21165.83	9247.73	1230.2	615.71	0.63	0.41	0.58	0.37	2.24	58.15	6.97	126.16
$\sigma^2 ge$	-111.36	-82.64	75.15	9.69	0.006	-0.19	0.006	-0.17	-0.04	1.5	-0.12	1.85
$\sigma^2 P$	21547.71	9464.34	1362.71	648.0	0.86	1.09	0.79	1.01	2.59	63.82	7.28	134.55
h^2%	98.23	97.71	90.28	95.01	73.64	37.08	73.84	36.96	86.61	91.13	95.8	93.76
GCV	43.67	43.41	42.51	43.94	3.81	3.38	4.64	4.16	35.05	16.88	26.08	29.82
PCV	44.06	43.91	44.75	45.08	4.46	5.51	5.41	6.88	37.69	17.68	26.67	30.8
GS(unite)	297.03	195.82	68.65	49.82	1.3	0.8	1.35	0.76	2.87	15.0	5.32	22.4
GS %	89.16	88.39	83.21	88.23	6.76	4.21	8.23	5.23	67.24	33.2	52.63	59.5