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Genetic and Chemical Analysis of Seed Coat of Cowpea and Bean Cultivars in Relation with Resistance to Weevil Pest

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ABSTRACT

The objective of this work was to evaluate the genetic variability among 6 cowpea (*Vigna unguiculata*) cultivars differing in their resistance to *Callosobruchus maculatus* (F.) weevil. Two resistant bean cultivars were used to compare between the sensitive, moderate tolerant and high tolerant cowpea cultivars. The differentiations were performed by using random amplified polymorphic DNA (RAPD) marker, protein concentration and organic and non organic components in seed coat. Six polymorphic primers were identified, resulting in different informative bands. Based on polymorphic profiles, 3 clusters were formed. Clustering was mainly affected by the resistance to weevil pest. The sensitive cowpea cultivars were separated in one group, the moderate tolerant and high tolerant cultivars came in separate group and finally, the resistant bean cultivars separated clearly in one distinct group. The most interesting result was represented by concentration of total protein in the seed coat. The protein concentration in the resistant bean cultivars were approximately 50 % less than concentration in each of the moderate tolerant and sensitive cultivars of cowpea. ferric ions were about 25 % less than the moderate tolerant and sensitive cultivars. The concentrations of calcium and potassium in seed coats were higher in the resistant beans than in cowpea cultivars. Cobalt was about 4 times higher in resistant bean than in the sensitive and moderate tolerant cowpea cultivars, which may play a major role in seed resistance to weevil.

Key Words: Cowpea (*Vigna unguiculata*), *callosobruchus maculates* (F.) weevil, RAPD marker, chemical analysis, total protein, seed coat.

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INTRODUCTION

Old World legume Cowpea, *Vigna unguiculata* (L.) Walp. is a tropical grain legume which plays an important nutritional role in developing countries of the tropics and subtropics, especially in sub-saharan Africa, Asia, Central and South America *(Singh et al., 1997)*. Because of its high protein content (20-25%), cowpea has been referred to as "poor man's meat." Cowpea young leaves, pods and peas contain vitamins and minerals which have fuelled its usage for human consumption and animal feeding *(Nielsen et al., 1997)*.

On the other hand, the New World legume common bean (*Phaseolus vulgaris* L.) and lima bean (*Phaseolus lunatus*) are a widely cultivated, legumes originated in the New World which have been domesticated both in Mesoamerica and South America and are currently cultivated in many tropical regions of the World *(Smartt 1990)*. *Callosobruchus maculatus* F. (Coleoptera: Bruchidae), a pest of the seeds of *Vigna unguiculata* (L.) Walp. (Cowpea), do not attack the seeds of *Phaseolus vulgaris (Applebaum et al., 1970)*. Bruchids are major threats to stored cowpea grains *(Singh et al., 2000)* and infestations by the most prominent species, *C. maculatus* and *C. chinensis* are responsible for grain losses estimated at 20–60% *(Tarver et al., 2007)*.

The reason why *C. maculatus* does not develop in the seeds of the common bean may be explained by their mutual isolation since the bruchid is an Old World species while *P. vulgaris* has its origin in the New World. Like other food crops, cowpea is attacked by a variety of microbial, viral and fungal pathogens and is a host for a number of insect pests *(Ouédraogo et al., 2002)*.

In Egypt and in many places the seeds of both legumes (*Vigna unguiculata and Phaseolus* spp) are stored together and cause major losses during storage of cowpea. *C. maculatus*, which is associated with *Vigna unguiculata*, completely destroys cowpea seeds within a very short time after infestation and it does not attack (*Phaseolus* spp.) seeds *(Simmonds et al., 1989)*. An explanation for this apparent specificity was given by *Janzen (1977)*, who suggested that *C. maculatus* larvae die shortly after passing through the testa of wild *Phaseolus* spp. due to the high levels of HCN originating from the hydrolysis of the glucoside linamarin. Simmonds, et al. *(1989)* showed that *C. maculatus* larvae die on entering the cotyledons of *P. lunatus* and suggested that this happens due to the delayed consequences of the ingestion of toxins present in the testa. Thirty years ago *Janzen (1977)* demonstrated that the seed coat can be a barrier to bruchid infestation and suggested that this could complicate the analysis of the toxicity of seed contents, emphasizing the need to analyze seed contents separately from seed coats.

The seed coat plays a vital role in the life cycle of plants by controlling the development of the embryo

and determining seed dormancy, germination as well as tolerance/or resistance to weevil pest. The seed coat synthesizes a wide range of novel compounds that may serve the plant in diverse ways, including defense and control of development. *Edde and Amatobi (2003)* revealed that seed coat has no value in protecting cowpea seed against attack by *C. maculatus* (F.). In contrast, *Akintola and Oyegoke (2004)* indicated that seed coat texture plays significant role in inducing ovipositional response. Non-preference was suspected to be the resistance mechanism. Vincenzo, et al. *(2005)* revealed that seed coat tannins must also be considered in biochemical defence mechanisms, which can deter, poison or starve bruchid larvae that feed on cowpea seeds.

Molecular markers reveal differences of natural sites at the DNA level. These variations are not seen in the phenotype and each might be a single nucleotide difference in a gene or a piece of repetitive DNA *(Johns et al., 1997)*. Thus, they are much more numerous than morphological markers and do not disturb the organisms physiology. One of the most widely used PCR-based marker techniques is Random Amplified Polymorphic DNA (RAPD). RAPD marker is generated by PCR amplification of random genomic DNA fragments with single oligonucleotide primers of arbitrary sequence and it's useful for the assessment of genetic diversity (*Williams et al., 1990*), owing to their simplicity, speed and, relatively low-cost *(Rafalski and Tingey 1993)* compared to other types of molecular markers.

The RAPD assay has been used to generate genetic maps of different crop species such as cowpea *(Menéndez et al., 1997 and Ouédraogo et al. 2002)*, *Citrullus lanatus (Levi et al., 2001b, 2002 and Zhang et al., 2004)*, *Cucurbita* sp. *(Lee, et al. 1995 and Brown and Myers 2002)* and *Lycopersicon esculentum (Saliba-Colombani, et al. 2000)*. In addition, RAPD markers were used to study the genetic diversity of the germplasm collection, cultivar identification and the plant genetic relationship in different crop species *(Levi et al., 2000, 2001a; Davis and Myers 2002; Fall et al., 2003; Ba et al., 2004; Cunha et al., 2004; Diouf and Hilu 2005 and Obiadalla-Ali et al., 2006 and Tanttawi et al., 2007)*.

The seed coat synthesizes a wide range of novel compounds that may serve the plant in diverse ways, including defense and control of development *(Moïse et al., 2005)*. Chemical analysis of seed coat in cowpea and common bean has been studied by many investigators *(Xavier-Filho et al., 1989, 1996; Macedo et al., 1993, 1995; Yunes et al., 1998; Moraes et al., 2000; Sales et al., 2000; Seifelnasr, 1991; Silva et al., 2004 and Gayan et al., 2006)*.

Obiadalla-Ali, et al. *(2007)*, screened 21 cultivars of cowpea for dry-seed yield and some resistance characters to weevil pest (number of adults emerged, percentage of damaged seeds, developmental period (days), average life span of female (days), number of eggs laid on seeds, mean of egg laid per female, percentage of adult emergence, percentage of loss in seed weight. They mentioned that cowpea cultivars classified into three groups, sensitive (Dokki 331 and Creamy7), moderate tolerant (Black eye and IT 90 K 2840-2) and high tolerant (IT 93 K 12904 and IT 81 D-1064).

Here, in this study we analyzed the seed coat in each group to identify and compare both organic and non-organic component that may lead to the resistance of weevil pest. As well as RAPD marker carried out to determine the genetic relationships among all studied cultivar groups.

MATERIALS AND METHODS

Seed materials:
Sensitive (Dokki 331 and Creamy 7), moderate tolerant (Black eye and IT 90 K 2840-2) and high tolerant (IT 93 K 12904 and IT 81 D-1064) to weevil cowpea cultivars were used. In this investigation, we used also two resistant bean cultivars (Lima bean and Common bean).

Data of resistance characters:
Preference and non preference tests were conducted according to *Messina and Renwick (1985)*, antibiosis tests were conducted according to *Van Emden (1987) and Ofuya (1987)* and tolerance tests were conducted as described by *Van Emden (1987) and Nakhla (1988)*. Seed resistance to cowpea weevil (*Callosobruchus maculatus*), has been explained in details previously *(Obiadalla-Ali et al., 2007)*.

DNA extraction and RAPD assay:
Total genomic DNA was extracted from fresh young leaves following the cetyltrimethylammonium bromide (CTAB) protocol *(Doyle and Doyle, 1990; Poresbski et al., 1997)*. The quality of the DNA was checked by electrophoresis in 1% agarose gels containing ethidium bromide (0.5 mg/mL) in ½ × TBE (89 mM Tris-HCl, 89 mM boric acid and 2mM EDTA). Twenty three RAPD primers (decamer oligonucleotides obtained from Operon Technologies U.S.A.) were tested as single primers for the amplification of a genomic sequence of cowpea. Of these, 6 primers (Table 1) produced polymorphic band patterns among genotypes. PCR reaction mixes consisted of 2.5 µl of 10×PCR buffer, 0.5 µl of dNTPs (0.2 mM) (Promega, Madison, USA) 2 µl of primer (25 pmol), 0.3 µl of Taq DNA polymerase (0.3U) (Promega, Madison, USA), 4 µl of $MgCl_2$ (25 mM), 9.7 µl of sterile ultrapure deionized water and 1µl of 100 ng DNA template, all reaction volumes were 20 µl. A negative-DNA control was performed by adding 1 µl of sterile ultrapure deionized water.

Amplification was carried out in the (Primus 25 Thermal Cycler, Germany). The Thermal Cycler was programmed by: 1 cycle (an initial denaturing step) of 4 min at 90°C, 35 cycles of 1 min at 90°C (denaturation step), 1 min at 33°C (annealing step), 2 min at 72°C (elongation step) and 10 min at 72°C (final extension), then kept at 4°C. The amplification products were electrophoresed at 60 V for 2 h. in 1.6% agarose (Himedia, India) gels containing 0.2µl Ethidium Bromide (0.5 mg/mL) in ½ × TBE. The amplified fragments were visualized under ultraviolet light (BXT-20-M, France) and photographed by digital Camera (Olympus SP-510UZ).

Data analysis:
The DNA banding patterns generated from RAPD experiments were analyzed by a computer program, Gene Profiler (version 4.03). Whereas, the presence (1) or absence

Table1: RAPD primers generating polymorphic bands, total number of fragments detected by each primer and polymorphism found among cultivars studied.

RAPD Primer Name	Sequences	Amplified bands		Polymorphic bands
		Total number of fragments	Number of polymorphic fragments	
OPA-2	5'-TGCCGAGCTG-3'	10	9	90%
OPA-3	5'-AGTCAGCCAC-3'	12	9	75%
OPA-13	5'-CAGCACCCAC-3'	16	16	100%
OPB-9	5'-TGGGGGACTC-3'	7	7	100%
OPC-18	5'-TGAGTGGGTG-3'	12	11	91.7%
OPI-9	5'-GGACACCACT-3'	15	13	86.7%
Total		72	65	
Mean		12	10.8	90%

(0) of each band was recorded for each line for the six primers used. Genetic similarity estimates were determined using Jaccard's coefficient *Jaccard (1908).* Dendrograms were generated with the unweighted pair group method with arithmetic mean (UPGMA) algorithm using the computational package MVSP version 3.1.

In order to detect patterns of genetic relationship in the genotypes, data analysis on the means of the resistance to weevil pest characters was initially performed based on the Euclidean distance matrix. The output was analysed using an agglomerative hierarchical clustering method with complete linkage strategy. Firstly, the data was subjected to analysis to produce a matrix of dissimilarity values and the phenotypic distance between each pair of genotypes was estimated as Euclidean distance. Secondly, cluster analysis was conducted on the Euclidean distance matrix with un-weighted pair-group method based on arithmetic averages (UPGMA) to develop a dendrogram using computer program NTSYS-pc version 2.1 *(Rolhf 2000).*

Combining the euclidean distance and RAPD distance:
The Mantel test is a statistical test of the correlation between two matrices. The matrices must be of the same rank.The similarity matrix of RAPD was converted to dissimilarity matrix. A cophenetic matrix was derived from each matrix to test goodness of fit of the clusters by comparing the two matrices using the Mantel test (*Mantel 1967*). Finally, the correlation between each distance pair using computer program NTSYS-pc version 2.1 was calculated.

Chemical analysis of seed coat:
Protein assay:
The protein contents were colorimetrically determined according to *Lowery, et al. (1951).* A bovine serum albumin was used for making standard curve and the data was expressed as mg/g d.w. The following equation was used in calculating of protein concentration:
Concentration of protein = Abs. x 0.5 x V_{ex} / V_{sample} x d.w. = mg/g (d.w.)

Non organic component contents:
Plant samples (Coat of Seed) were separated mechanically and washed with diluted HCl and twice with water, dried in an aerated oven at 70°C until constant weight was reached. Grind in porcelain mortar and preserved for analysis. One half gram plant sample materials were digested using concentrated H_2SO_4 and $HCLO_4$ (Pericloric acid) according to *Black (1982).* The digested sample was filtered and raised to 50 ml in volumetric flask. Plant digested was analyzed using atomic absorption spectrophotometer.

Statistical analysis:
All chemical analysis recorded data were tabulated and statistically analyzed according to *Snedecor and Cochran (1967), using Duncan (1955)* for comparing various treatment means.

RESULTS AND DISCUSSION

Level of polymorphism:
Six out of twenty three primers used generated different degrees of genetic polymorphism among cultivars studied About 90% (65) of the 72 visible bands were polymorphic (Figure 1), with a mean of 10.8 bands per primer. This level of variation is much higher than that observed in Malawian Sorghum landraces *(Nkongolo and Nsapato, 2002). Nkongolo (2003)* showed that about 80% of the scored bands were polymorphic in cowpea cultivars. In cowpea, about 54% polymorphism was found when applying 6 different primer combinations using AFLP technique *(Fang et al., 2007).*

The percentage of total polymorphic bands detected ranged from 75 to 100% with an average of 90% (Table 2). *Sarutayophat et al. (2007)* used 5 primers and reported that polymorphic fragments percent ranged from 50 to 71.4% from yardlong bean and cowpea accessions. On the contrary, a low proportion of polymorphic bands 18.5% among 13 cowpea landraces was reported by *Tosti and Negri (2002).* The size of the amplified fragments ranged from approximately 200 bp to 2500 bp. *Pooprompan et al. (1996)* identified various varieties of yardlong bean by RAPD and reported that fragment sizes ranged from 500 to 2200 bp, while fragment sizes of 940 to 1100 bp were reported by *Phansak et al. (2001).* Also, Sarutayophat et al. *(2007)* obtained 225 to 1650 bp amplified bands in RAPD cowpea experiments. However, primers used by the three research groups were different from primers used in our experiment.

Cluster analysis of the cultivars based on RAPD analysis:

RAPD similarity of *Jaccard (1908)* coefficient matrix for 6 cowpea and 2 bean species was calculated and used for UPGMA cluster analysis. Similarity coefficient ranged from 0.11 to 0.92 between Dokki331 and Lima bean and between Black eye and IT 90 K 2840-2, respectively (Table 2). The genetic distance values among accessions of cowpea varied from 0.09 to 0.59 (*Nkongolo, 2003*). Sarutayophat et al. *(2007)* reported a similarity coefficient varying from 0.548 to 1.000 among cowpea cultivars.

The eight cultivars separated into two distinct clusters. The first cluster contains two resistant bean species (Lima bean and Common bean) with similarity coefficient 0.82 (Figure 2). The second cluster contains cowpea cultivars and it is subdivided into two sub clusters, moderate tolerant and high tolerant cultivars grouped in first sub-cluster and the second sub-cluster contains the two sensitive cultivars. The highest value of similarity coefficient (0.92) was observed between the moderate tolerant cultivars (Black eye and IT 90 K 2840-2), the similarities coefficient between the high tolerant (IT 93 K 12904 and IT 81 D-1064) and the sensitive cultivars (Dokki 331 and Creamy 7) were 0.89 and 0.91, respectively. It is worth to note that the results of RAPD fingerprint support the classification of the tested entries based on their genus and resistance to *C. maculatus* (F.) weevil *(Obiadalla et al., 2007)*.

Cluster analysis based on resistance to weevil pest traits of cowpea:

The dendrogram constructed on the basis of the genetic distances among cowpea cultivars was calculated based on the means of characters of resistance to *C. maculatus* (F.) that indicates the systematic relationships among cultivars studied.

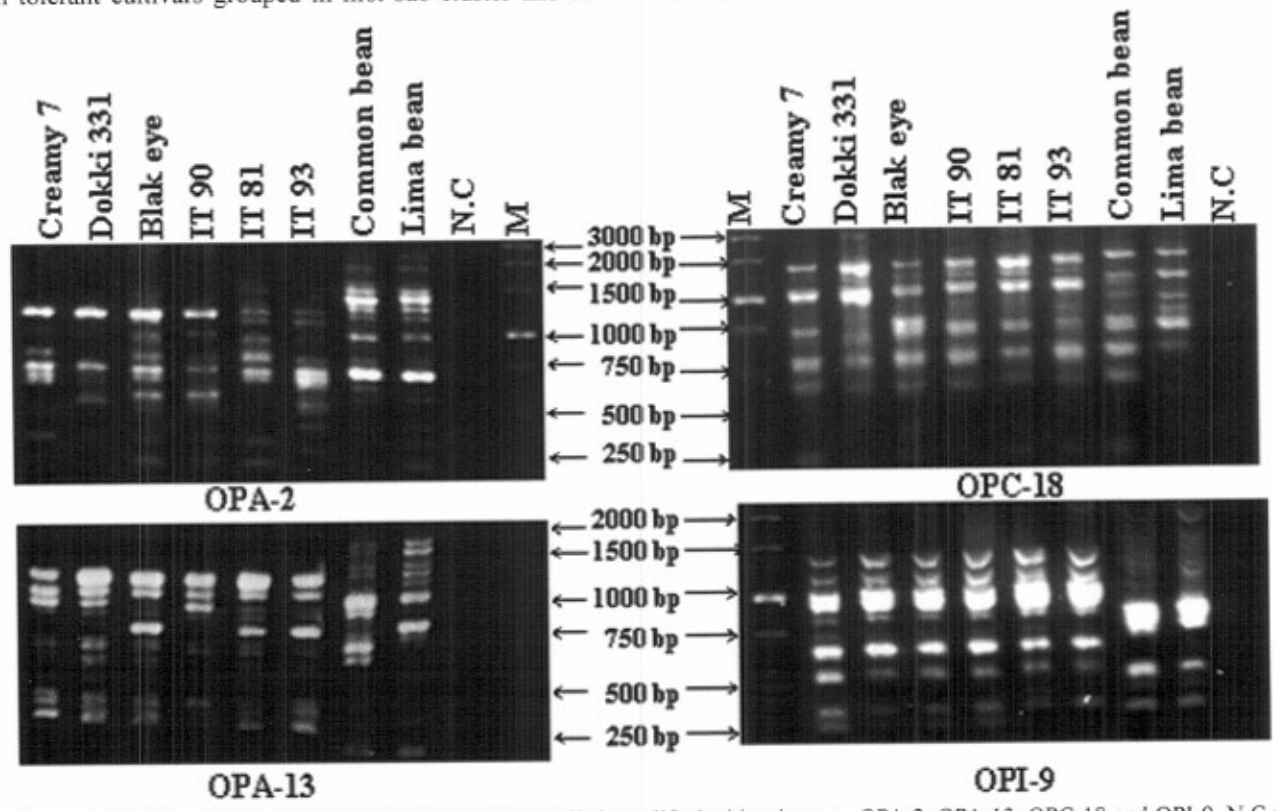

Figure 1: RAPD profiles obtained for eight cultivars studied amplified with primers OPA-2, OPA-13, OPC-18 and OPI-9, N.C = Negative Control and M= 1000 bp ladder size marker.

Table 2: Similarity matrix calculated for cowpea and bean cultivars according to Jaccard coefficient obtained from 72 RAPD fragments.

Cultivars	Creamy 7	Dokki 331	Black eye	IT 90 K 2840-2	IT 81 D-1064	IT 93 K 12904	Common bean	Lima bean
Creamy7	1.00							
Dokki 331	0.91	1.00						
Black eye	0.77	0.74	1.00					
IT 90 K 2840-2	0.79	0.81	0.92	1.00				
IT 81 D-1064	0.70	0.63	0.82	0.80	1.00			
IT 93 K 12904	0.65	0.63	0.77	0.74	0.89	1.00		
Common bean	0.18	0.15	0.21	0.19	0.25	0.22	1.00	
Lima bean	0.12	0.11	0.17	0.15	0.21	0.21	0.82	1.00

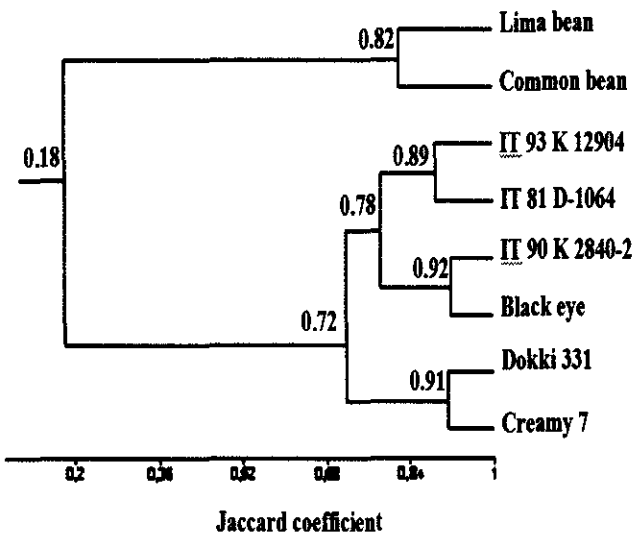

Figure 2: Pairwise similarities between accessions were calculated using Jaccard's coefficient obtained from 72 RAPD fragments.

The Euclidean distance ranged from 7.14 between Black eye and IT 90 K 2840-2 cultivars to 181.25 between Creamy 7 and IT 81 D-1064 cultivars (Table 3). The range of Euclidean distance among the cultivars (7.14 – 181.25) was, relatively wide. This result indicated that the amount of phenotypic variation among these cultivars was, relatively high and reflects the genetic diversity of the genes controlling these characters. Bootstrap values on the dendrogram (Figure 3) indicated a high genetic variation pattern. The cultivars created two distinct clusters at high Euclidean distance of 141.95 between the two sensitive cultivars in first cluster and the four other resistant cultivars that formed second cluster (Figure 3). The second cluster created two sub-clusters, which were separated at 49.31 Euclidean distance (Table 3). The dendrogram also revealed that the morphological diversity was less than within all separated groups (Table 3).

The results obtained based on the two dendrograms were similar. The agronomic characterization and RAPD based on dendrogram were some what similar, indicating that the agronomic characterizing information will continue to be useful to identify diverse germplasm in breeding programs of lupine and/or other species (*Abd EL-Ghani et al., 2007*). Pandey, et al. *(2008)* showed that DNA markers are preferable to morphological ones because they relate variability directly at the genetic level and provide reliable and enormous data that permit a reproducible estimate of genetic diversity in the germplasm.

Correlation between the two distance matrices generated by mean of traits and RAPD marker was calculated. The correlation between Euclidean distance and RAPD distance was low (r = – 0.80357, P = 0.0014). The low association between these traits and RAPDs markers was not surprising since the estimation of genetic relationship among different germplasm was based on different approaches. The lack of correlation between morphological traits and other genetic markers such as isozyme markers has been documented in cowpea and other crops *(Doebley, 1989; Vaillancourt et al., 1993)*. The correlation between Euclidean coefficient (r) distance and RAPD distance was – 0.40 (P = 0.11) in faba bean *(Tanttawi et al., 2007)*. Schut et al. *(1997)* reported a correlation of -0.1 for AFLP and agronomic data in Barley varieties. RAPD markers are randomly distributed throughout the genome, but most regions of the genome (90 %) are not expressed at the phenotypic level *(Williams et al., 1990; Joyee et al., 1999; Dahlberg 2000)*. As a result, markers like RAPDs may accurately assay the degree of genetic change between two genomes, but they may not necessarily reflect the divergence in terms of changes in traits of agronomic importance. Pandey et al. *(2008)* showed that the comparison of Jaccard's similarity based on RAPD markers and average taxonomic distance based on quantitative characters revealed a non-significant (r = 0.0477) correlation between the two matrices in Indian ash gourd.

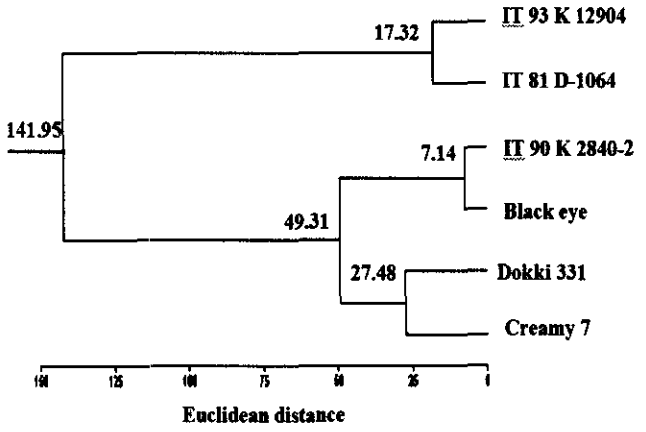

Figure 3: Dendrogram based on UPGMA cluster analysis showing the genetic distances among cowpea cultivars.

Table 3: Euclidean distance matrix of 6 cowpea cultivars using means of resistance characters to Callosobruchus maculatus (F.).

Cultivars	Creamy 7	Dokki 331	Black eye	IT 90 K 2840-2	IT 81 D-1064	IT 93 K 12904
Creamy7	00.00					
Dokki 331	27.48	00.00				
Black eye	61.68	35.47	00.00			
IT 90 K 2840-2	62.92	37.17	7.14	00.00		
IT 81 D-1064	181.25	155.40	123.26	124.07	00.00	
IT 93 K 12904	172.87	147.39	115.53	115.81	17.83	00.00

Total Protein:

The results in (Table 4) clearly explain that protein concentration differed significantly in each group cultivars. Resistant bean species were the least in protein concentration (0.492 mg/g). While, sensitive cowpea cultivars were the greatest in protein concentration (1.294 mg/g). The most interesting result is, protein concentration in the resistant bean cultivars were approximately half only in comparison with both moderate tolerant and sensitive cowpea cultivars, indicating that the increase of protein concentration in seed coat may play a major role towards susceptibility to weevil pest. *Khokhar and Gupta (1974)* revealed that high protein content was linked to susceptibility to the stored- product insect.

Table 4: Organic and non-organic (Heavy Metals) component in coat seed of some cowpea and bean cultivars.

Susceptibility	Sensitive	Moderate tolerant	High Tolerant	Resistant
Protein	1.294 a	1.146 b	1.019 c	0.492 d
Mg++	2082.6 a	2018.9 a	1910.2b	1880.0 b
Ca++	3539.0 d	3791.5 c	5210.2 b	7173.2 a
Na+	1421.6 a	1566.9 a	1378.6 a	1021.1 b
K+	8282.3 b	495.8 d	5770.7 c	10203.7 a
Co++	2.227 d	5.251 b	2.877 c	8.198 a
Mn++	16.44 a	14.83 b	17.02 a	10.05 c
Fe++	450.13 a	367.13 b	378.87 b	97.67 c

Means within each rows followed by the same letter(s) are not significantly different at the 0.05 probability level.

Magnesium (Mg^{++}):
Chemical analysis results (Table 4) clearly show that magnesium concentration differed significantly in resistant bean cultivars and sensitive cowpea cultivars. The magnesium concentrations ranged from (1880.0 ppm) to (2082.6 ppm) for resistant bean cultivars and sensitive cowpea cultivars, respectively, indicating that the presence of magnesium in seed coat may have a role in susceptibility to weevil pest.

Calcium (Ca^{++}):
Calcium concentration (Table 4) differed significantly in each group cultivars. Resistant bean cultivars had the greatest concentration calcium (7173.2 ppm). While, sensitive cowpea cultivars had the least concentration calcium (3539.0 ppm). The most important result is, calcium concentration in the resistant bean cultivars were approximately twice in comparison with both moderate tolerant and sensitive cowpea cultivars, indicating that the presence of calcium in seed coat may have a major role in weevil pest tolerance/or resistance.

Sodium (Na^{+}):
The results of Sodium concentration analysis (Table 4) differed significantly in resistant bean cultivars and all group of cowpea cultivars. The concentration of sodium in seed coat was lower (1021.1 ppm) in the resistant bean cultivars than in all cowpea cultivars group (1566.9, 1421.6 and 1378.6 ppm) moderate tolerant, sensitive and high tolerant cowpea cultivars, respectively.

Potassium (K^{+}):
Results in (Table 4) show that potassium concentration differed significantly in each group cultivars. The concentration of potassium in seed coat was higher (10203.7 ppm) in the resistant bean cultivars than in all cowpea cultivars group (8282.3, 5770.7 and 495.8 ppm) sensitive, high tolerant and moderate tolerant cowpea cultivars, respectively.

Cobalt (Co^{++}):
Resistant bean cultivars exhibited the greatest cobalt concentration (8.198 ppm, Table 4). While, sensitive cowpea cultivars were the least in cobalt concentration (2.227 ppm). The Cobalt concentration was about 4 times higher in resistant bean than that in sensitive and high tolerant cowpea cultivars, which may play a major role in seed resistance to weevil.

Manganese (Mn^{++}):
The manganese concentration (Table 4) differed significantly in each group cultivars. The concentration of manganese in seed coat was lower (10.05 ppm) in the resistant bean cultivars than in all cowpea cultivars group (17.02, 16.44 and 14.83 ppm) high tolerant, sensitive and moderate tolerant cowpea cultivars, respectively.

Iron (Fe^{++}):
Resistant bean cultivars were the least in iron concentration (97.67 ppm, Table 4). While, sensitive cowpea cultivars were the greatest (450.13 ppm). The most interesting result is, a ferric ion in the resistant beans was about 25 % less than that in the moderate tolerant and high cultivars, indicating that iron concentration in seed coat may play a major role towards resistant weevil.

Gayan, et al. *(2006)* showed that Na, K, Mg and Ca were the major metal elements in cowpea seeds. These minerals were rich in the husk portion of the seed; therefore, decrease in above properties may be due to formation of emergence holes in the husk by the *C. maculatus* larvae.

CONCLUSION

the Ca, K and Cu metals were higher in resistant been than those in cowpea cultivars. But, the protein, Mn and Fe metals were less in resistant been cultivars than those cowpea cultivars which may play a role in seed resistance to weevil.

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