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Physical and Biochemical Basis of Resistance in Some Cowpea Varieties against *Callosobruchus maculatus* (F.)

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A B S T R A C T

Four varieties of the cowpea seeds (Local, Dokki331, Kareem7 and Aswany) were screened for their resistance to the bruchid, *Callosobruchus maculatus* F., a serious pest of stored pulses. Morphological characters like seed coat texture, volume and weight, were not found to be responsible for offering resistance to *C. maculatus*. Data revealed that the female weevil preferred the smooth surface for egg oviposition. The results also showed that Aswany was found to reduce the growth and development of *C. maculatus*, which were indicated by different parameters; larval penetration %, adult emergence %, total developmental time, susceptibility index and growth index. Seeds were analysed for protein profiles (SDS-polyacrylamide gel electrophoresis) to study the chemical basis of resistant to bruchid infestation. A negative correlation was indicated between the seed total protein content and the susceptibility index. SDS-PAGE analysis of seeds protein for the fore mentioned varieties revealed the presence of antinutrient chemicals that might impart resistance to *C. maculatus*. The role of these antinutritionals in relation to *C. maculatus* infestation is discussed.

Introduction

The cowpea weevil *Callosobruchus maculatus* (F.) is a major field-to-store pest of many cultivated legumes especially cowpea crop [1-4]. It is well known to cause a considerable damage and economic losses to many pulses that makes them unfit for planting or human utilization either as high protein diet or as an animal feed [2, 4-9].

Cowpea (*Vigna unguiculata* (L.) Walpers) is considered as an important source of plant protein for many poor people in the tropic and is a source of income [2]. Many studies demonstrated the devastating damage of cowpeas during post-harvest storage caused by the cowpea beetle *C. maculatus* in different regions as well as in Egypt [7, 10-11].

Several chemical and non-chemical methods of protecting seeds from bruchid attack are in use among farmers. These methods include using of organic and natural insecticides, exploitation of controlled atmospheres, integration of physical methods such as heating [12], cooling [13] and biological agents such as parasitoids [14]. The researchers have been directed to and emphasized on developing safe methods with less or no effect on food contamination and environmental pollution [2]. One of these methods with a very promising future is using resistant varieties to protect cowpea seeds from *C. maculatus* attack.

As the cowpea beetle *C. maculatus* is one of the most destructive pests for many legumes in Egypt [15], many attempts have been directed to find very simple, easy to use, and not expensive methods to achieve cowpea beetle control in Egypt [7, 10]. Using improved cowpea varieties has contributed to achieve the suppression of *C. maculatus* population [2, 7, 10].

Legume seeds have developed chemical defence compounds that protect them against the attack of different insect pests. There are a wide range of these compounds including tunnins, non-protein amino acids and defensive proteins [15, 16-18].

The present work was conducted to study different biological aspects of *C. maculatus* and how these parameters would vary between different cowpea varieties. Also, it was aimed to study certain biochemical aspects in the resistant and susceptible cowpea cultivars.

Materials and Methods

Insect culture and cowpea varieties

A colony of cowpea weevil, *Callosobruchus maculatus*

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(F.) was obtained from Plant Protection Research Institute, Dokki, Giza, Egypt, where the colony is maintained on cowpea seeds (*Vigna unguiculata* L. Walpers) over about 15 years. In the current study, adult weevils were maintained on cowpea seeds (*Vigna unguiculata* L. Walpers) that were obtained from a local store (Cairo, Egypt), and were frozen by keeping at about -20^{o}C for 2-6 h. to disinfest them from any previous stored-grain pests. The adult weevils were kept at room temperature (29 ± 2^{o}C), relative humidity of 35 ± 5 % and ambient photoperiod of about 16 L: 8 D.

Four cowpea varieties were screened for resistance, Dokki331 (D), Kareem7 (K), Aswany(A) and a Local one (L) that was also used to maintain the weevil colony. The first three ones were obtained from Horticulture Research Institute, Agriculture Research Centre, Dokki, Giza, Egypt, while the last one was obtained from a local store.

Correlation between physical characters of cowpea seeds varieties and some biological aspects of cowpea weevil *C. maculatus*.

The resistance of tested cowpea seeds to infestation with *C. maculatus* was evaluated by two experiments. The first experiment was conducted to determine the correlation between physical parameters (weight, volume and coat texture) of seeds of each variety and female oviposition performance and larval penetration. For each variety four replicates were designed, each contained 15 g seeds and one pair of newly emerged adults that were kept in plastic containers. The number of eggs deposited by each female was recorded and the rate of larval penetration into seeds was determined in relation to the seed morphological characters [19].

For seed weight determination 10 seeds from each variety were weighed individually and the average was calculated, while the seed volume was determined using water displacement in a graduated cylinder (10 ml). To estimate the seed volume 10 seeds from each variety were also used individually.

In the second experiment conventional screening was used to determine the relative susceptibility of cowpea cultivars based on the following parameters according to Singh and Jackai (cited by 15).

- Total developmental time (TDT) = mean developmental time per insect.
- Growth index (GI) =Ln adult emergence % / TDT.
- Susceptibility index (SI) = (GI of tested variety/ GI of the host seeds) X 100; where the host seed is the most susceptible one (D) with the highest (GI).

Four groups, one for each variety, were designed. For each group newly emerged adult weevils were allowed to lay eggs for only 24 h on cowpea seeds. After adult removal all groups were kept for 14 days, then seeds of each group with only one penetrated larva were separated individually in Eppendorf tubes (1.5 ml). For each variety, the one larval penetrated seeds were separated into four groups, each with 10 seeds. At end of the experiment, adult emergence percent (% AE) and total developmental time (TDT) were estimated for each larva/ seed.

For seed moisture content (SMC) measuring, Agrawal [20] method was used. All cowpea seeds of different cultivars were grounded in a mortar then finely grounded in grinding mill. In each group 3-4 replicates were used.

Biochemical studies:

a) Samples collection

Samples of four cowpea seeds varieties (Local, Dokki331, Kareem7 and Aswany) were used for biochemical analysis. Each seed variety was grounded with electrical blender to a very fine powder. Each powder sample was homogenized in ice-cold Tris buffer (1.5 M Tris-Hcl) in sterile glass test tube using glass homogenizer, centrifuged at 10.000 r.p.m. for 10 min. at 4°C. The supernatants of centrifuged tissue (powder) were withdrawn carefully using automatic pipettes and transferred to a new Eppendorf tube and kept frozen at -70 °C till used.

b) Total protein measurement

The total protein concentration of each sample was measured photometrically at 562 nm and compared with standard of bovine gamma globulin using the Bio-Rad protein assay [21]. Results were subjected to analysis of variance using ANOVA one-way as mentioned by Steel and Torrie in [22].

c) Electrophoretic analysis

Total protein was fractioned by SDS polyacrylamide gel electrophoresis (PAGE) as described by Smith [23]; using an acrylamide (10%) gel. High and low MW standards were used for the determination of protein profiles of all fractionated samples. A gel-pro-analyser (version 3.1 Media Cybernetics USA) was used for the protein analysis of tested samples. A comprehensive computer software application is designed to determine the number of molecular weights and the amount of peptide chains as well as scanned graphical presentation of the fractionated bands of each lane.

Data analysis

For data analysis, homogeneity of the variances was tested using Levene's test. When the assumption of homogeneity of the variances was fulfilled, one-way ANOVA was applied followed by pairwise comparisons using LSD-test as mentioned by Steel and Torrie in [22]. When no transformation was able to homogenise the group variances, Welch ANOVA was applied. After that, pairwise comparisons were made using Thamhane-test. Also, coefficient of correlation analysis between variables was performed if needed using the package SPSS program.

Results and discussion

Correlation between physical characters of cowpea seeds and larval penetration of *C. maculatus*

The physical characters of cowpea seeds varieties are shown in Table (1). Data revealed that the seed texture of cowpea was an important factor as an oviposition stimulus for *C. maculatus*. The average number of eggs laid by female on (A) seeds with smooth surface (79.5±5.66) was significantly (P=0.009) higher than those deposited on the other seeds with wrinkled coat. The lowest number of eggs (42.6±6.25) was laid on the seeds of (L) variety followed in order by (D) and (K) (45.6±4.95 and 58.75±5.5) respectively. In agreement with the present results, Sulehrie *et al.* [24] recorded that genotypes of green- and black-grain (Legmuminosae) seeds with smooth seed coats were preferred for oviposition of *C. maculatus*. Abdel Fattah and Ahmed [15] indicated that bean seeds with hard wrinkled coat were less preferable for eggs laying than seeds with soft smooth surface.

No significant correlation was obtained between the numbers of laid eggs, seed weight and volume of different cowpea seeds (r =-0.441, P= 0.076; r =-0.421, P= 0.076, respectively). Among the tested varieties, (A) type had the light and small seed (0.081± 0.005 g/seed) received (79.5±5.66) eggs, while the heaviest large one (D) (0.243±0.014 g/seed) received (45±4.95) eggs (Table 1). This result is in contrast with those of Abdel Fattah and Ahmed [15], El-Shazly, [25] and Cheng [26], who noticed that there is no clear relation between numbers of *C. maculatus* eggs, seed size and weight of different broad bean varieties.

Data also indicated that the lowest larval penetration rate was 93% of (A) type (Table 1). This was significantly lower (P= 0.01) than the other types (L, D and K) which were 99.55%, 97.56% and 98.17, respectively.

No significant correlation (r =218, P=0.4) was found between larval penetration percent and seed weight (Table 1).

The correlation study also demonstrated that the larval penetration percent was inversely correlated with seed texture (r = -0.469, P= 0.043, Table 1). As the larval penetration percent was relatively high (93-99.55) in the four studied varieties where seeds with wrinkled surfaces had higher larval penetration percent than seeds with smooth surfaces, it turns out that the seed coat texture did not play a role in protecting cowpeas from the beetles attack. This disagrees with Abdel Fattah and Ahmed [15] as they found that the seed coat of faba bean was a barrier against *C. maculatus* larval penetration.

Another positive correlation was also seen in larval penetration percent with seed volume although it was not significant (r = 0.91, P= 0.728). Previous studies mentioned that the most preferred cowpea seeds, in terms of total no. of eggs, were the large-seeded cowpeas [2, 15].

Table 1: Relation between physical characters of cowpea seeds varieties and some biological aspects of cowpea weevil *C. maculatus*.

Cowpea seeds varieties	Seed texture and colour	Weight/ seed (g) (Means ± SE.)	Volume/ seed (ml) (Means ± SE.)	Mean of total no. of eggs/ female ± SE	Mean no. of penetrated larvae ± SE	Larval penetration %
Local	slightly wrinkled and creamy (2)	0.129± 0.004 b	0.12±0.01 a	42.6± 6.25 bc	42.4±6.24 b	99.55
Dokki 331	wrinkled and white (1)	0.243± 0.014 a	0.24±0.02 a	45± 4.95 bc	43.75±4.42 b	97.56
Kareem 7	wrinkled and white (1)	0.133±0.007 b	0.1± 0 a	58.75± 5.5 c	57.5±9.02 b	98.17
Aswany	Smooth and brown (3)	0.081± 0.005 c	0.09±0.01 b	79.5± 5.66 a	74.25±6.61 a	93.00

N.B. Different letters indicate significant differences between varieties at the 0.05 level. Numbers between brackets indicate ranks for the correlation test.

Table 2: Different parameters used to screen the susceptibility of different cowpea seeds varieties to infestation of cowpea weevil *C. maculatus*.

Cowpea seed Varieties	Total no. of penetrated larvae	no. of adult emerged (Mean±SE)	Adult emergence % (% AE)	Total developmental time (TDT) (Mean±SE) (range)	Growth index (GI)	Susceptibility Index (SI)	Seed moisture content (SMC) (Mean±SE)	Total protein conc. (mg/g tissue) (TPC) (Mean±SE)
Local	10	6.75±0.25 b	67.5	20.52±0.29 (19-23) a	0.20	88.77	11.03± 0.09 a	177.85±2.37 a
Dokki 331	10	9±0.71a	90.42	19.46±0.14 (17-23) a	0.23	100	11.71± 0.66 a	138.72±1.38 c
Kareem 7	10	5.5±0.25 b	55	23.39±0.22 (20-28) b	0.17	74.09	10.79± 0.083 a	112.35±1.67 d
Aswany	10	3.25±0.29 c	32.5	25.39±0.95 (20-35) b	0.13	59.28	11.07± 0.18 a	154.66±0.55 b

N.B. Different letters indicate significant differences between varieties at the 0.05 level.

Screening of resistance

There was an overall significant difference in mean number of adult emergence of *C. maculatus* and total developmental time (TDT) between the four different varieties (one-way ANOVA: $F_{3,15}$=32.588, *P* =0.0; $F_{3,15}$= 62.414, *P* =0.0), respectively, Table 2). The highest adult emergence % was found to be in beetles reared on (D) (90.42) followed by (L), (K) and finally (A) cultivar (67.5, 55 and 32.5, respectively, Table 2). The TDT was shortest when cowpea beetles were reared on (D) cultivar was (19.46), followed by (L), (K) and finally (A) cultivar (20.52, 23.39 and 25.39, respectively, Table 2). Obiadalla *et al.* demonstrated that both (D) and (K) seeds were among the seeds that had high % AE of *C. maculatus* and shortest TDT [10].

Both growth index (GI) and susceptibility index (SI) were highest in beetles reared on (D) cultivar (0.23, 100) followed by (L) (0.20, 88.77), (K) (0.17, 74.09) and finally (A) cultivar (0.13, 59. 28) (Table 2). The current data shows that the (D) variety is the most susceptible one as the insects reared on it had the highest % AE (90.42) combined with the shortest TDT (19.46) (Table 2), while the (A) seeds are the most resistant seeds as the larval population from this group had the lowest % AE (32.5) combined with the longest TDT (25.39). Data available from other studies also indicate that the resistant seed varieties adversely affect bruchids by reducing adult emergence and elongating TDT [2, 3, 10, 15, 27].

Investigation of cowpea seeds characters revealed that the SMC was not significantly different between all varieties tested (one-way ANOVA: *P* = 0.335, Table 2). At the same time SMC seemed to have no significant effect on larval penetration nor adults production (r = -0.210, *P*= 0.512, r =-0.04, *P* =0.891, respectively), although this relationship was inversely correlated.

Biochemical analysis

a) Total protein content

Data in table (2) indicated that there was an overall significant difference in the total protein contents (TPC) of the four cowpea seeds varieties used in terms of optical density (OD) measurements (one-way ANOVA: $F_{3,11}$= 284.352, *P* =0.0, Fig. 1).

Table (2) shows that the TPC of (L) cowpea seeds was 177.85±2.37 mg/g tissue, while in (D) and (K) varieties the total protein decreased being, 138.72±1.38 and 112.35±1.67 mg/g tissue, respectively. Both (D) and (K) were significantly different compared to that of (L) group (LSD test, *P* =0.0 for both, Fig. 1). The level of protein in (A) variety increased being 154.66±0.55 mg/g tissue, which was significantly different from others (LSD test, *P* =0.0 for all pairwise analysis, Fig. 1). Correlation analysis revealed that TPC have a weak positive correlation with % AE and a weak negative relationship with TDT, although this correlations were not significantly different (r = 0.017, *P*= 0.957, r =-0.198, *P* =0.538, respectively). In contrast Vir (cited by [15]) concluded that the cowpea genotypes with high protein content could be protected from *C. maculatus* attack. Abdel Fattah and Ahmed [15] indicated also that there was a positive correlation between the total storage protein content of faba bean genotypes and the susceptibility index.

b) Protein fraction

In view of the variation observed in the resistant and susceptible type of the tested cowpea seeds varieties to *C. maculatus* infestation, seed protein profiles were analysed for detection of antinutrient compounds. Changes in the total protein profile of cowpea seeds varieties (L), (D), (K) and (A) are presented in figures (2 and 3) as well as in Tables (3, 4 and 5). Data presented in the fore mentioned figures and tables have resulted from the use of gel-pro-analysis for the SDS (PAGE) runs. The achieved results of total protein fraction were compared by electrophoretic runs of proteins extracted from (L) control cowpea seed and other varieties (D), (K) and (A) (Fig. 2). The protein fractions were separated into 33 protein banding patterns fluctuated from 23 to 31 bands. Lanes in Fig. (2) and Table 3 show nineteen identical protein bands of MW 200, 150, 100, 76.6, 70, 60, 50, 40, 35.4, 30, 29.2, 27.2, 25, 22.78, 21.3, 20, 15, 10 and 9.2 kDa in all lanes representing proteins extracted from four cowpea seeds varieties. However, ten (6.1, 5.2, 2.4, 3.3, 4.3, 6.6, 4.3, 3.7, 4.4 and 3.9 % amount) bands differ in the amount of proteins (Table 4) in spite of having the same molecular weights, being lower in the case of (L) control, (D), (K) than in case of (A). This observation may be explained by difference of the protein extracts at a quantitative level.

On reviewing the development of protein bands in all lanes, it came clear that certain bands disappear. The protein banding pattern of MW 24.3 kDa was appeared in all cowpea seeds varieties, but was missing in the (L) cultivar (Table 3). Also, the protein fractions MW 83.907, 26.03, 16.003 with 12.347 kDa appeared in all varieties except (A) type (Table 3). Some other protein fractions MW 90.3 and 17.07 kDa were detected in all cowpea varieties and were missing in the (D) cultivar (Table 3).

Other protein banding patterns appeared only in two cowpea cultivars. The protein band with Rf value 0.17 was detected only in (D) and (K) varieties. The protein fraction with Rf value 0.39 appeared only in (A) and (K) cultivars. Another band with Rf value 0.48 appeared only in (K) and (L) varieties. Results of the comparative electrophoretic analysis showed also appearance of unique protein bands. The protein bands MW 123.39 and 20.37 kDa were produced only in (K) type, while protein fractions with MW 18.47 appeared in the (L) variety. The molecular weights are presented in Table (3) and graphically in Fig, (3) where MWt's scanned against optical densities of each protein band. The optical density of bands is shown in Table (5).

The present investigation focused on chemical composition of seed tissue which might be involved in imparting resistance of cowpeas used. This is confirmed by SDS-(PAGE) of protein fractions.

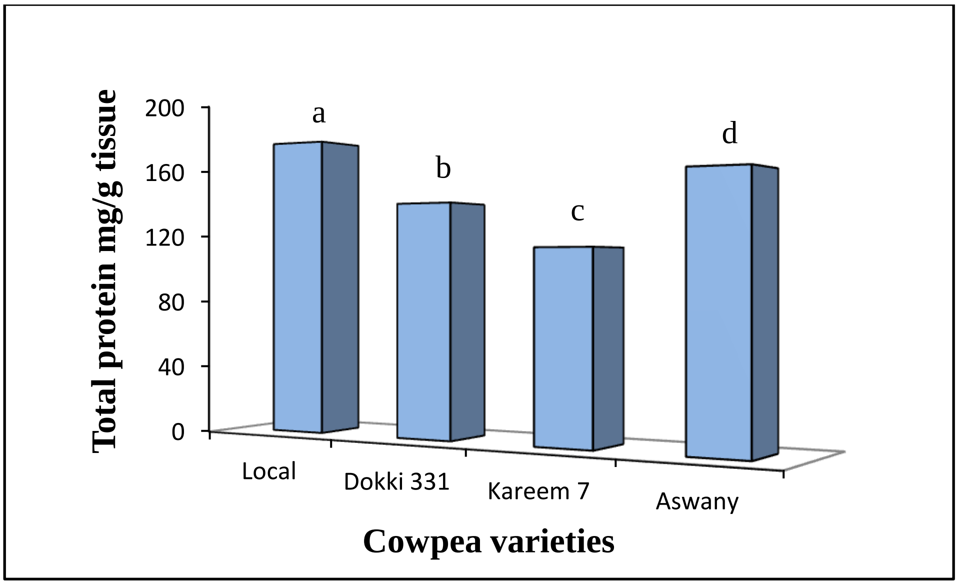

Fig. 1: A histogram showing protein concentration extracted from different cowpea seeds varieties (Local, Dokki331, Kareem7 and Aswany).

The protein profiles of different varieties varied at both the qualitative and quantitative levels. The polypeptide protein bands with MW range between 50-60 kDa were detected in both resistance and susceptible varieties with varied intensities. Protein with molecular weight approximately of 55 kDa is a typical for vicilin subunits [28, 29]. Vicilin could reduce the availability of amino acids necessary for larval growth and development [30]. The vicilin action mechanism was proposed towards *C. maculatus* [17, 18], where the capability to bind to the chitin present in the peritrophic membrane added to their lower digestibility which is likely responsible for the lethal effects of vicilins. Xavier-Filho [31] showed that *C. maculatus* developed successfully in resistant cowpea seeds through the use of its potent enzymatic apparatus.

Sales *et al.* [17] and Abdel Fattah and Ahmed [15] recorded that low level of vicilin was hydrolysed by *C.maculatus* midgut enzymes and it had no clear deleterious effect either on insect development or on insect survival. The protein banding pattern with MW 32 kDa was detected only in the (A) and (K) resistant varieties. Also, protein fraction with MW 17.69 kDa appeared in (A) and (K) resistant types and (L) type which was relatively susceptible. Antinutrient compounds like Lectine (30-32 kDa polypeptides) and α-amylase inhibitors (13-17 kDa polypeptides) have been reported to have a role in imparing resistance to bruchids [15, 29, 32, 33]. In addition, unique bands with MW 123.39 and 20.37 kDa appeared only in (K) resistant cultivars.

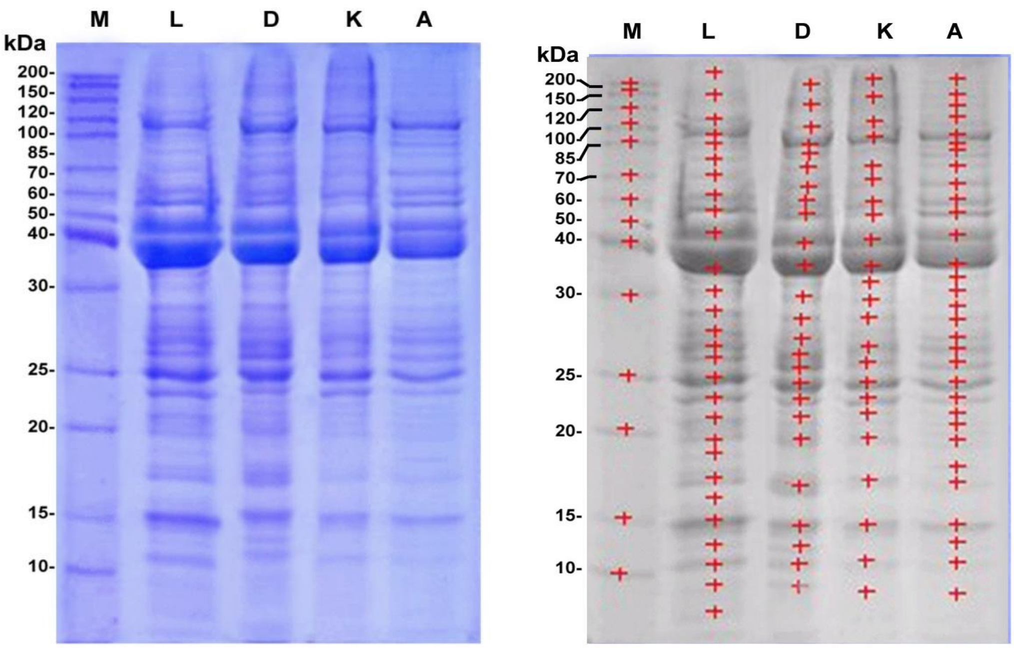

Fig. 2: (A and B) Photograph and diagrammatic illustration of electrophoretic protein pattern of local cowpea seeds (L) as a control and other types of cowpea seeds varieties Dokki331(D), Kareem7(K) and Aswany (A). M= standard molecular weight.

These bands might have a role in reducing the performance of *C. maculatus*. Therefore, it is very important to understand the mechanism underlying the resistant cowpea varieties to improve the development of new breeding lines of cowpea seeds.

In conclusion, planting of (A) could be recommended, where it is a very promising cultivar of breeding programme for improvement of resistance, to cowpea weevil, *Callosobruchus maculatus*.

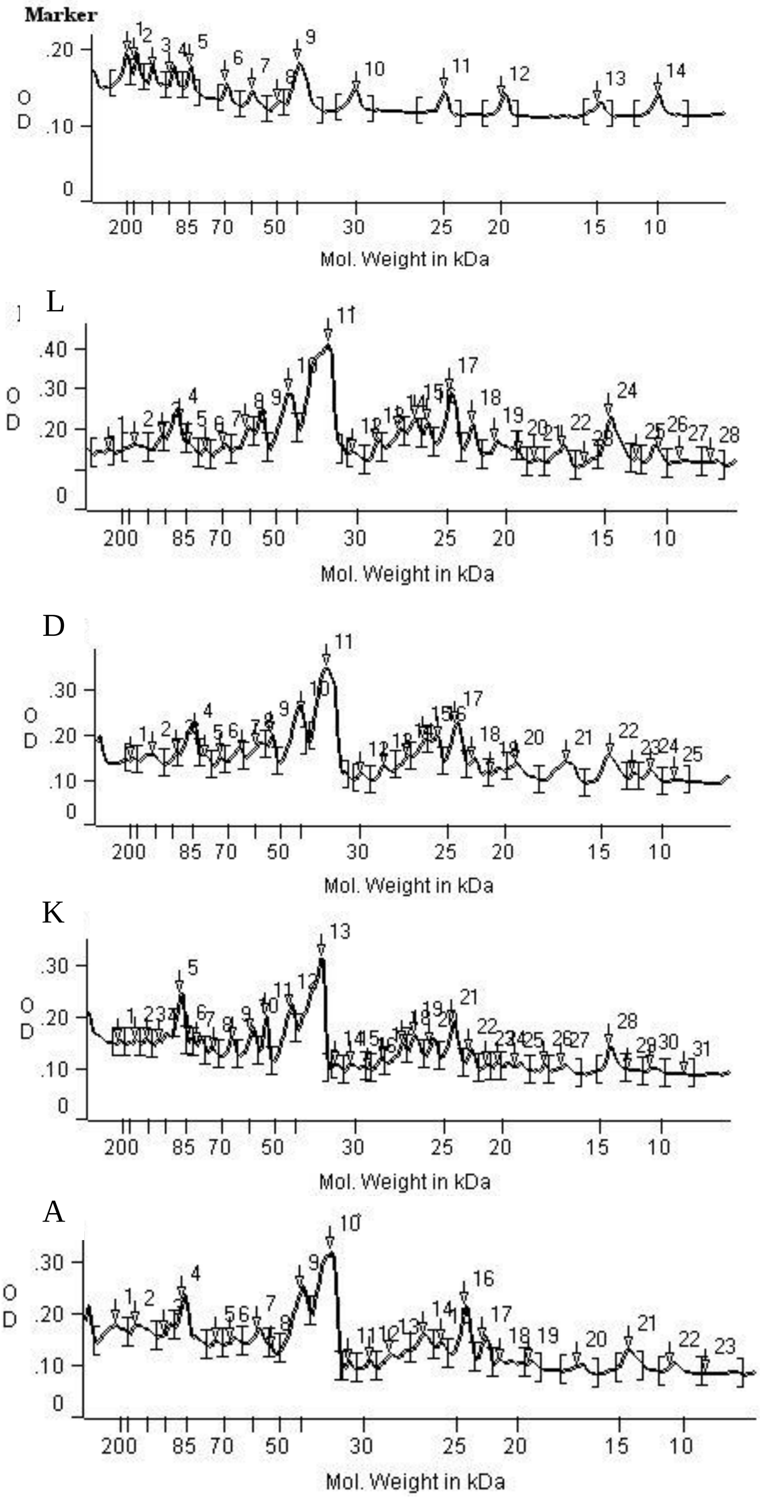

Fig. 3: Optical density (OD) of protein extracted from different cowpea seeds varieties (Local (L), Dokki331 (D), Kareem (K) and Aswany (A)) plotted against the molecular weights of protein bands from the same varieties.

Table 3: Whole tissue protein monitoring of Local (L) and cowpea seed varieties (Dokki331 (D), Kareem7 (K) and Aswany (A)) expressed as molecular weight.

varieties	L	D	K	A	Marker
Rows	(mol.w.)	(mol.w.)	(mol.w.)	(mol.w.)	(mol.w.)
1	323.04	200	242.28	242.28	200
2	141.86	126.88	141.86	137.96	150
3			123.39		120
4	105.35	97.989	108.13	102.64	100
5	90.341		90.341	88.525	
6	83.907	83.907	83.907		85
7		79.673	80.711		
8	76.639	73.72	73.72	73.72	
9	70	65.263	66.184	68.065	70
10	61.705	60	60	58.916	60
11	54.772	53.783	53.783	52.811	50
12	44.17	40	43.089	41.004	40
13	34.641	35.482	35.482	35.059	
14			**33.019**	**32.237**	
15	30.728	30	30.728	29.698	30
16	28.955	28.518	29.25	28.518	
17	27.665		28.374		
18	26.701	27.248	27.248	26.701	
19	26.034	26.299	26.566		
20	24.769	25.512	25.771	25.771	25
21		24.312	24.312	24.312	
22	22.78	22.78	22.78	22.78	
23	20.952	21.147	21.345	21.345	
24			20.375		
25	19.294	19.433	19.294	19.433	20
26	18.479				
27	17.073		17.698	16.951	
28	16.003	16.709	16.829		
29	14.759	14.288	14.288	14.288	15
30	12.347	12.347	12.549		
31	10.67	10.845	10.845	11.022	10
32	9.2211	9.2211	8.6419	8.7832	
33	7.5903				

Table 4: Percent concentrations and relative fragmentation of protein fractions of control (L) and other types of cowpea seeds varieties (Dokki331 (D), Kareem7 (K) and Aswany (A)).

Varieties	L	L	D	D	K	K	A	A	Marker
Rows	%amount	(Rf.)	%amount	(Rf.)	%amount	(Rf.)	%amount	(Rf.)	%amount
1	2.9414	0.0346	1.91	0.0538	2.1983	0.0462	6.1081	0.0462	4.4722
2	3.8974	0.0731	4.2956	0.0885	2.6635	0.0731	5.2627	0.0769	2.5938
3					2.2141	0.0923			4.2699
4	2.2301	0.115	2.3001	0.127	4.134	0.112	3.2891	0.119	3.2862
5	4.1286	0.142			3.1173	0.142	6.4329	0.146	
6	1.7751	0.158	4.4923	0.158	1.358	0.158			3.2229
7			1.9801	0.173	2.2061	0.169			
8	1.6263	0.185	1.8559	0.196	1.9747	0.196	2.4369	0.196	
9	2.6792	0.212	3.2704	0.231	3.2345	0.227	3.3261	0.219	3.6395
10	3.5426	0.246	3.9675	0.254	3.4566	0.254	**4.3415**	0.258	4.2627
11	2.8052	0.273	2.3476	0.277	2.7012	0.277	1.4649	0.281	2.6006
12	6.176	0.312	6.342	0.327	4.9574	0.315	6.6133	0.323	6.8615
13	12.397	0.373	10.427	0.365	8.7753	0.365	8.7933	0.369	
14					1.5387	**0.388**	1.4438	**0.396**	
15	2.1545	0.412	1.9847	0.419	2.7183	0.412	2.2971	0.427	5.7277
16	2.3272	0.446	3.3912	0.458	0.58322	0.438	**4.3262**	0.458	
17	3.5652	0.481			1.9359	0.462			
18	3.2476	0.508	2.2626	0.492	3.3383	0.492	3.7191	0.508	
19	2.1868	0.527	3.1602	0.519	2.9898	0.512			
20	5.3874	0.562	2.8969	0.542	2.479	0.535	2.3606	0.535	6.5068
21			4.7664	0.569	4.3303	0.569	4.5786	0.569	
22	3.1128	0.596	3.0506	0.596	2.114	0.596	2.634	0.596	
23	4.8412	0.631	2.5351	0.627	1.5239	0.623	3.9716	0.623	
24					1.261	0.642			
25	1.226	0.669	4.0693	0.665	3.7004	0.669	1.7741	0.665	5.3567
26	1.9361	0.692							
27	3.9407	0.735			2.3358	0.715	3.7354	0.738	
28	2.4154	0.769	5.9005	0.746	3.7414	0.742			
29	5.1541	0.808	5.4324	0.815	3.9928	0.815	3.5952	0.815	4.2588
30	1.1476	0.85	1.4651	0.85	1.9971	0.846			
31	3.37	0.885	2.8942	0.881	2.5711	0.881	4.4928	0.877	7.7652
32	3.7872	0.919	2.8665	0.919	3.1735	0.933	3.9463	0.93	
33	2.405	0.963							
Sum	96.404		89.864		89.315		90.944		64.825
In Lane	100		100		100		100		100

Table 5: Optical density of protein fractions of control (L) and other types of cowpea seeds varieties (Dokki331 (D), Kareem7 (K) and Aswany (A)).

Varieties	L	D	K	A	Marker
Rows	(max OD)	(max OD)	(max OD)	(max OD)	(max OD)
1	0.15015	0.14764	0.15317	0.18244	0.19319
2	0.16289	0.15843	0.15749	0.17921	0.1865
3			0.15523		0.17344
4	0.183	0.16166	0.15263	0.16257	0.16682
5	0.21737		0.24321	0.22601	
6	0.16331	0.19938	0.15836		0.1785
7		0.15361	0.14584		
8	0.14831	0.15425	0.1356	0.14953	
9	0.1638	0.16488	0.15619	0.15063	0.15674
10	0.2064	0.17682	0.17215	0.16846	0.14609
11	0.21524	0.20363	0.20297	0.13121	0.13152
12	0.28946	0.24636	0.21785	0.24701	0.18346
13	0.41188	0.35004	0.31364	0.32042	
14			0.10914	0.11062	
15	0.14453	0.11682	0.10998	0.11196	0.14265
16	0.16967	0.13243	0.0983	0.12607	
17	0.20006		0.11548		
18	0.22518	0.15745	0.15021	0.16094	
19	0.2175	0.19412	0.16718		
20	0.29254	0.19084	0.14704	0.14687	0.14629
21		0.22625	0.18827	0.21715	
22	0.21269	0.1489	0.1333	0.14967	
23	0.16884	0.11638	0.10932	0.11016	
24			0.10897		
25	0.14136	0.13983	0.10657	0.10787	0.13718
26	0.12706				
27	0.15503		0.10861	0.10153	
28	0.11461	0.14054	0.10701		
29	0.23276	0.15563	0.14382	0.13315	0.12987
30	0.1321	0.11759	0.09774		
31	0.14926	0.1225	0.10287	0.10168	0.14145
32	0.12532	0.1029	0.09058	0.08767	
33	0.12234				
Sum					
In Lane					

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