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MORPHOLOGICAL AND BIOCHEMICAL IDENTIFICATION OF SOME NEW FABA BEAN (*Vicia faba* L.) VARIETIES.

BY

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ABSTACT

This study was carried out at Laboratory Seed Technology Dep. to determine the actual characterizing differences between various genotypes of faba bean. Five faba bean genotypes (Sakha 1, Sakha 2, Misr 1, Misr 2 and Nobaria 1) were used in this study. Certain quantitative and qualitative morphological characteristics were investigated on seed seedling and adult plant stages. The results revealed great differences in certain chemical and biotechnological characters between various genotypes which could be used to assist in the quality control and seed testing. According to the UPOV Guideline could used about 10 characteristics such as plant height, anthocyanin coloration, time of flowering, pod characteristics and seed shape can be used to distinguished between the genotypes. Chemical composition including crude protein, total carbohydrates and crude oil were tested in seeds. Moreover, the patterns of seed protein were studied by using SDS-PAGE and RAPD Technique. It was found that each genotype was characterized by proteins with specific molecular weight. Therefore, electrophoretic and DNA analysis is an important tool for the varietal identification. The chemical composition of faba bean seeds was significantly affected by genetic makeup. The protein percent range between 25.83% to 30.98, carbohydrates between 61.85-69.37% but oil % showed no significant between genotypes (3.53-4.95%). The anti-nutritional factors showed significant differences between genotypes such as phenols (61.47-69.83 mg/100g), vicine (17.47-23.97 mg/g) and trypsin inhibitor (69.45-84.49 TIU).

INTRODUCTION

Food legumes play an important role in increasing the quantity of food as well as in enhancing the quality of cereal based diets. The high-protein content in leguminous products makes them a valuable source of food for both human and animals. Faba bean (*Vicia faba L .)* is considered the world's fifth food legume after dry bean, dry pea, chick pea and lintel. In Egypt, Faba bean (*Vicia faba* L). is also the most seed legume crops used for human consumption as well as for animal feeding and more than 0.3 million fedans are cultivated yearly

The Morphological characteristics could be used to identify between faba bean varieties and assist in quality control and seed testing. horse bean cultivars could be distinguished according to seed characters such as seed color and seedling characters. The different varieties of the crop could be distinguished

on the basis of their morphological as well as biochemical characters (Abd El-Gawad *et al.*, 1981). While, Nasib (1984) described faba bean cultivars in Egypt using the flowering data, plant height, No of stem/plant, maturity data, seed size, seed color and number of seed/pod. Kotecki (1994) and Schafferman (1994)) tested 16 varieties of broad beans and found that all genotypes had large kernels, and differs for flowering dates, plant height and yield. Mudzana *et al.* (1995) studied variety discrimination in 12 faba bean varieties using visual assessment of plant morphology and biochemical characters. These assessment included seed characters (hilum color, presence or absence of testa tannin and testa color), plant characters (growth types, plant height at maturity), stem characters (number of branching and presence or absence of anthocyanin coloration), flower characters (number of days to 50% flowering, flower length and extent of anthocyanin anthocyanin) and pod characters (attitude, length, breadth and number of seed/pod). Nemat A. (2000) identify Seven faba bean genotypes (Giza 461, Giza 402, Giza 716, Giza Blanka, Line 40193 Triple white and Bakestani). The results revealed great differences in certain morphological characters between various genotypes. Faba bean seeds with 18.6, 37.8% protein content are considered an important protein source (Kaul and Vaid 1996; Tewati and Virk 1996). Protein content ranged from 18 to 23% in the seeds of legumes (Seidl *et al.*, 1969). Filippetti and Azadegan (1994) measured viability in protein and trypsin inhibitor content for 113 lines/ varieties of faba bean (*Vicia faba L.*), A broad rang of variability was observed for both character especially for trypsin inhibitor content (CV = 22%). There was no correlation between trypsin inhibitor content and protein content (r = -0.19). In other study conducted on 134 lines / varieties of chickpea and 113 lines / varieties of faba bean of various origins were evaluated for protein and trypsin inhibitor content, A broad range of variability was observed especially for trypsin inhibitor content (cv = 17% for chick pea and 22% for faba bean (Filippetti and Azadegan 1998). Kamel and El-Mashad (1999) found that evolutionary relationship between among 16 different species representing the four major sections of the genus *vicia faba L.* was studied using the seed protein electrophoresis technique. Protein bands of the examined species had been considered as attributes and were used for numerical analysis. Sammour (1992) analyzed seed proteins of both single seed and composite samples of some vicia faba cultivars using SDS – PAGE. Each seed exhibited a distinctive electrophoretic pattern. The variation was detected amongst the polypeptides with molecular weight greater than 45 Kd. The electrophoretic pattern of the bulk extracted samples was characteristic for each cultivar. Therefore, this was recommended for cultivars identification. Zimniak and Przybylska (1995) investigated 173 vicia species accessions for electrophoretic seed albumin pattern. In the examined material, 38 well-defined polypeptide bands, in the relative to molecular mass range of 19-61 Kd, were distinguished. The electrophoretic data were used for hierarchical grouping of the examined taxa.El- Shanshoury and Soliman (1996) determined electrophoretic profiles of native seed protein of 26 species of the genus vicia using SDS-PAGE. Numerical analysis of the results indicated that the delimitation of the majority of the examined species agree with their previous classification based on morphological characters. Hamman (1996) studied the gross composition mineral content and functional properties of five genotypes broad bean. A little difference recorded among the genotypes varieties in the chemical composition ranging between 23.4 to 26.45% for protein, 1.58 to

1.88% for fat, 68.32 to 71.88% for total carbohydrates. Under temperature alternation, a thermo stable DNA polymerase is able to synthesize discrete DNA products that can be resolved on an agarose gel following electrophoresis. Each primer has the capability to amplified of several using DNA fragment in the genome. Some of these amplified fragment (patterns) may be unique to a genotype and then they can be useful in varietal identification (McDonald *et al.*, 1994). The aim of the present investigation was to determine the most distinguished morphological and biochemical traits of some faba bean varieties cultivated under Egyptian conditions.

MATIRIAS AND METHODS

Seed samples of five faba bean (*Vicia faba* L.) cultivars namely: Sakha1, Sakha2, Misr1, Misr2 and Nobaria1 were obtained from Legume Research Department, Agriculture Research Center. Laboratory experiments was carried out at Seed Technology Department ARC and Field trails conducted at Kalubia governorate. All seed samples planted next to each other, during Nov.2003. The plot size for quality control contained at least 1000 plants that were to make plant examination easy. Observation was made at all stages of plant growth in order to find out a reliable morphological and biochemical characters for varietal identification.

Optimal cultural practices were carried out to safeguard the full expression of all plant characteristics.

Morphological identification: The Morphological identification was conducted using the UPOV (The International Union for the Protection of New Varieties of Plants) descriptors. The Decimal code for the growth stage of legumes according to Tottman (1987) was also used to standardize the growth stages of varieties during morphological description and identification.

Biochemical characters: For seed chemical composition, untreated seed samples were randomly taken to determine crude protein percent (calculated by multiplying the total nitrogen by 6.25), total carbohydrates and crude oil percentages according to (A.O.A.C., 1990). Total phenols were determined by using Folin- Denis reagent according to the method of Swain and Hillis (1959) where Vicine content I.e., vicine and convicine were extracted from one g sample using 4% meta- phosphoric acid (Collier, 1976). Trypsin Inhibitor content (TI) and Trypsin Inhibitor activity (TIA) was determinate by using the method of Roy and Bhat (1974). Sodium dodecyle sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was used to identify extracted protein from seeds according to the method of Laemmli (1970). The DNA amplification protocol was performed as described by Williams *et al.* (1990) with some modifications. DNA isolation from plant tissues was done using DNeasy plant Mini Kit (QIAGEN Hilden, Germany).Randomly Amplified Polymorphic DNA (RAPD). RAPD – PCR reactions were conducted using 5 arbitrary 10-mer primers. Their names and sequences are shown in Table (1).

Table (1): Primer names and sequences in 5 to 3 direction.

Primer code	Sequence (5 to 3)
OPA-07	GAAACGGGTG
OPB-10	CTGCTGGGAC
OPB-12	CCTTGACGCA
OPB-17	AGGGAACGAG
BOP-18	CCACAGCAGT

The reaction conditions were optimized according to Williams *et al.* (1990) and mixtures (30µl total volume) consisted of the following were used: DNTPs (2.5 mM) 0.2 Mm, $Mgcl_2$ (25 mM) 1.5 mM, 10x buffer 3.0 µl, Primer (10 µM) 0.2 µM, Template DNA (50 ng /µl) 2.0 µl, Taq (5 u/µl) 0.3 µl and distilled H_2O up to 30 µl. Amplification was carried out in a PTC–200 thermal cycle (MJ Research, watertown, USA) programmed for 40 cycles as follows: 94°C/4 min (1 cycle); 94°C/30 sec, 36°C/1 min, 72°C/2 min (40 cycles); 72°C/10 min (1 cycle) and 4°C (infinitive).

Data were subjected to statistical analysis of variance using Mstatc Computer Program (1994). Multiple rang test was used for comparison between means of faba bean varieties. Different alphabetical letters in the column are significantly differed at 5% level of significance according to Sendecor and Cochran (1969).

RESULTS AND DISCUTION

1-Morphological characteristics:

According to the UPOV guidelines which included 32 characteristics for the conduct of DUS for faba bean. The data in Table (2) include 10 characteristic, which show differences between the tested five faba bean varieties. Regarding plant height, the varieties Sakha1 and Sakha2 were characterized by shortest plant height, whereas the variety Nubria1 was characterized by the longest plant height. The previous results should that plant height, may be used as a trait to identify among faba bean varieties. These results are in harmony with those obtained by Nemat (2000). The anthocyanin coloration in stem can be used to identify among the tested varieties, the Sakha1 variety doesn't have anthocyanin coloration in the stem, whereas this coloration was present in other tested varieties. Concerning the time of flowering (50% of the plants with at least one flower) of the tested faba bean varieties, the variety Misr 2 was the earliest, while the variety Nobaria 1 was the latest. Pod attitude was erect in Sakha1 and Semi-erect in Sakha2, Misr1 and Misr2, while the Nobaria1 characterized by Semi-pendulous pod attitude. The melanin width at pods can be used to identify Nobaria1 variety which is broad, whereas the other tested varieties are medium. Regarding pod degree of curvature at green shell stage, three categories were recorded. The first category included varieties Misr1 and Nobaria1 with strong degree of curvature. The second category included varieties Sakha2 and Misr2 with medium degree of curvature. The third category included variety Sakha2, which was characterized by slight pod degree of curvature. The variety Nobaria1 has light pod intensity of green color whereas the other faba bean varieties have medium pod intensity of green

color. Concerning seed shape of median longitudinal section, the faba bean variety Misr1 was characterized by circular seed shape while the other tested varieties have ovate seed shape. On the other hand, the cross section of seed shape showed that the varieties Sakha2 and Misr2 have elliptic seed shape while the varieties Sakha1 and Nobaria1 have broad elliptic seed shape. Regarding 100-seed weight, the tested faba bean varieties could be classified into three classes. The first one included the varieties Nobaria1 and Sakha 2 with heaviest 100-seed weight (91.2 and 90.3 g), respectively. The second class included the varieties Misr 2 and Misr 1 with lowest 100-seed weight (67.1 and 68.5 g) respectively. The third class included the variety Sakha1 with medium 100-seed weight (74.4 g).

2- Biochemical characteristics:

Results in table (3) indicated that chemical composition were significantly differences among the tasted faba bean seeds. The variety Sakha 1 had the greatest crude protein value (30.98%), while the variety Misr 2 had the lowest crude protein (25.83%). The other varieties had an intermediate values ranged between 26.26 to 30.90%. Such significant differences in crude protein trait could be used as a varietal identification between varieties under study. Total carbohydrates ranged between the lowest value 61.9% for the variety Misr1 compared with the greatest value for the variety Sakha 2. The slight variation between the other studied varieties in total carbohydrate could be used to identification between the studied varieties. Crude oil content indicated that faba bean seeds of studied varieties had low concentration ranging between 3.53 to 4.95%. The slight differences in crude oil content made it unable to use it as a description factor. This observation is agreed with that obtained by (Kaul and Vaid 1996)

Tables (3) show the anti-nutritional factors such as vicine, trypsin inhibitor and total phenols. It is well known that anti-nutritional factors play an important role in human food digestibility and the quantitative and qualitative data of these components may help in identification different faba bean varieties under study. The total phenols were ranged between 69.83 – 61.47 mg /100 g. Concerning the vicine content of the faba bean varieties, The variety Misr 1 had the highest level of vicine 23.96 mg /g, while the lowest vicine content was 17.47 mg /g in variety Nobaria1. Such differences in the vicine content of various varieties could be used as mean in varietal identification. The trypsin inhibitors are important factors that effect the nutritional value and protein digestibility in human. The Trypsin inhibitor activity (TIA) was significantly varied among the different faba bean varieties. The high significant TIA was found for the variety Nobaria 1 (84.49 TIU/mg dry seed), while the lowest TIA level was recorded by the variety Sakha 1 (69.45 TIU/mg dry seed). These findings indicate clearly the low pancreatic hypertrophy effect of raw faba bean Sakha 1, Sakha 2 and Misr 2 varieties (Brik 1974).

Table (2): Morphological characteristics of some Faba Bean varieties

Characteristics	Degree	Sakha 1	Sakha2	Misr1	Misr2	Nubaria1
1- Plant: height	Very low (1) Low (3) Medium (5) High (7) Very high (9)	3	3	5	5	7
2-Stem: anthocyanin coloration	Absent (1) Present (9)	1	9	9	9	9
3-Time of flowering (50 of the plants with at least one flower)	Early (3) Medium (5) Late (7)	5	5	5	7	3
4-Pod: attitude	Erect (1) Semi-erect (3) Horizontal (5) Semi-pendulous(7) Pendulous (9)	1	3	3	7	3
5-Pod:melanin width	Very narrow(1) Narrow (3) Medium (5) Broad (7) Very broad (9)	5	5	5	7	5
6-Pod: degree of curvature at green shell stage	Absent or very slight (1) Slight (3) Medium (5) Strong (7) Very strong (9)	3	5	7	7	5
7-Pod: intensity of green color	Light (3) Medium (5) Dark (7)	5	5	5	3	5
8-Seed: shape of median longitudinal section	Elliptic (1) Broad elliptic (3) Circular (5) Oblong (7) Square (5) Ovate (6)	6	6	5	6	6
9-Seed: shape of cross section	Narrow elliptic (1) Elliptic (2) Broad elliptic (3)	3	2	2	3	2
10-1000 seed weight	Very small (1) Small (3) Medium (5) Large (7) Very large (9)	7 (744)	9 (903)	5 (685)	9 (912)	5 (671)

Table (3): Seed chemical composition of studied faba bean varieties.

Traits / varieties	Protein %	Carbohydrates %	Oil %	TIA (TIU/mg dry seed)	Phenols mg/100g	Vicine mg/g
Sakha 1	30.98 a	62.85 c	4.95 a	69.45 d	67.13 ab	17.73 c
Sakha 2	26.26 b	69.37 a	4.37 a	72.74 c	69.83 a	17.90 c
Misr 1	30.90 a	61.85 c	4.02 a	76.67 b	63.10 b	23.97 a
Misr 2	25.83 b	63.22 bc	3.53 a	71.43 cd	62.78 b	19.99 b
Nobaria 1	29.76 a	65.80 b	4.31 a	84.49 a	61.47 b	17.47 c
L.S.D at 5%	2.39	2.82	N.S	2.41	5.78	2.06

Protein fractionation was done for the five faba bean varieties (Table 4). Results indicated that number of bands ranged from 13 to 17 bands. There were distinct differences in seed protein banding patterns between the various varieties All tested varieties had the ten major bands at molecular weight 114.469, 111.678, 108.166, 101.412, 98.081, 66.200, 61.128, 59.487, 40.549 and 29.636 Kd. It could be identify the faba bean variety as following, seeds of Sakha1 contained protein with molecular weight of 200.238 Kd while, the variety Sakha 2 characterized by protein with molecular weight of 149.806 Kd. Misr1 characterized by proteins with molecular weight of 206.113, 136.474 and 52.413 Kd. Protein detected in faba bean seeds of Misr2 variety were 104.204 and 63.764 Kd. Nobaria1 seeds characterized by protein with molecular weight of 158.615 Kd.

The electrophoretic differences observed in this study should provide a supplemental means for cultivar identification. These findings indicated clearly that electrophoretic analysis is an important tool for the identification of faba bean cultivars. These observations are compatible with those obtained by Sammour (1992), Kamel and El- Mashad (1999) and Nemat (2000).

Identification of RAPD molecular markers:

A total number of 100 DNA bands were detected as generated by the five random primers for the five faba bean varieties used in the present study. The least number of polymorphic bands was detected for primer A07, while the largest number of polymorphic bands was detected for primer B10. However, two bands were common for all varieties. 63 out of the 100 (0.63 %) RAPD-PCR bands were found to be useful as genotype-specific markers. A number of 62 positive specific markers was scored for the presence of unique bands for a given genotype, while one negative specific marker was scored for the absence of a common band.

In conclusion, the five primers which used in the present study allowed enough distinction among the faba bean varieties. These genotype-specific markers can be used in subsequent experiments to detect molecular markers for polymorphic genes with economic importance among these and other varieties. This results are shown in Tables (5 to 9) and Figures (a to e).

Table (4): Molecular weights of soluble protein bands extracted from faba bean varieties by polyacrylamie gel electrophoresis.

M.W	Sakha1	Sakha2	Misr1	Misr2	Nobaria1
222.473	-	+	-	+	+
206.113	-	-	+	-	-
200.238	+	-	-	-	-
158.615	-	-	-	-	+
149.806	-	+	-	-	-
136.474	-	-	+	-	-
114.469	+	+	+	+	+
111.678	+	+	+	+	+
108.166	+	+	+	+	+
104.204	-	-	-	+	-
101.412	+	+	+	+	+
98.081	+	+	+	+	+
90.805	-	-	+	-	-
80.905	+	-	-	-	+
75.477	-	+	-	+	+
66.200	+	+	+	+	+
63.764	-	-	-	+	-
61.128	+	+	+	+	+
59.487	+	+	+	+	+
55.410	-	+	+	+	+
52.413	-	-	+	-	-
48.745	+	-	-	+	+
40.549	+	+	+	+	+
29.636	+	+	+	+	+
Total of bands	13	14	15	16	17

Table (5): DNA polymorphism of five faba bean varieties using randomly amplified polymorphic DNA with primer A07

Band No.	M.W	Varieties				
		Sakha1	Sakha 2	Misr 1	Misr 2	Nobaria1
1	626.600	-	+	-	-	-
2	539.360	+	+	-	-	-
3	513.070	-	-	+	+	-
4	464.260	+	+	-	-	-
5	441.630	-	-	+	-	-
6	380.140	-	+	-	-	-
7	292.435	+	+	+	+	-
8	254.917	+	+	+	+	-
9	219.380	+	-	-	-	-
10	192.097	+	+	+	+	+
11	111.510	-		-	+	-
12	88.370		-	+	+	-
Total of bands		6	7	6	6	1

Table (6): DNA polymorphism of five faba bean varieties using randomly amplified polymorphic DNA with primer B12

Band No.	M.W	Varieties				
		Sakha1	Sakha 2	Misr 1	Misr 2	Nobaria1
1	1299.01	-	+	+	+	+
2	1278.22	+	-	-	-	-
3	925.63	+	+	-	-	-
4	881.88	-	-	-	-	+
5	800.50	-	-	-	-	+
6	623.37	-	-	+	+	+
7	608.44	+	-	-	-	-
8	453.45	-	-	-	-	+
9	526.19	+	+	-	-	-
10	473.81	-	+	+	+	+
11	447.77	+	-	-	-	-
12	406.45	-	-	+	+	-
13	387.29	-	+	-	-	+
14	322.58	+	-	+	+	+
15	246.49	-	-	+	-	+
16	211.49	+	-	-	-	+
Total of bands		7	5	6	5	10

Table (7): DNA polymorphism of five faba bean varieties using randomly amplified Polymorphic DNA with primer B10

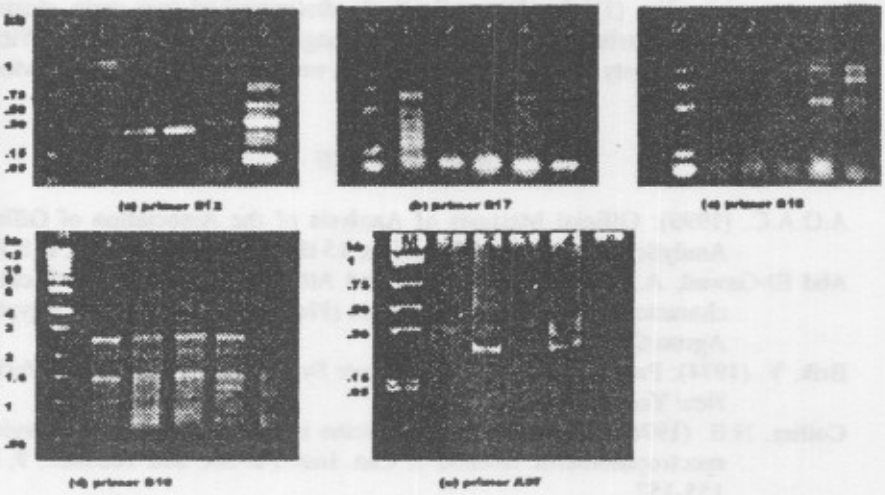
Band No.	M.W	Varieties				
		Sakha1	Sakha 2	Misr 1	Misr 2	Nobaria1
1	2967.33	-	-	-	-	+
2	2908.82	-	-	+	+	-
3	2851.45	-	+	-	-	-
4	2740.10	+	-	-	-	-
5	2245.25	-	+	+	+	-
6	2157.57	-	-	-	-	+
7	2115.02	+	-	-	-	-
8	1953.05	-	-	+	-	-
9	1914.54	-	+	-	-	-
10	1839.77	+	-	-	-	-
11	1568.78	-	-	-	-	+
12	1537.85	-	-	-	+	-
13	1507.52	-	+	+	-	-
14	1420.08	+	-	-	+	-
15	1392.07	-	-	-	-	+
16	1364.62	-	+	-	-	-
17	1285.47	-	-	+	-	-
18	1235.27	-	-	-	+	+
19	1163.62	+	-	-	-	-
20	1074.51	-	+	+	+	+
21	972.66	+	-	-	-	-
22	916.24	-	-	+	-	-
23	898.17	-	+	-	+	-
24	863.10	-	-	-	-	+
25	829.39	+	-	-	-	-
26	813.04	-	+	-	-	-
27	781.28	-	-	+	-	-
28	750.77	-	-	-	-	+
29	735.97	-	+	-	+	-
30	707.23	+	-	-	-	-
31	659.64	-	+	+	-	-
32	640.19	+	-	-	-	-
33	489.275	+	+	-	-	-
34	470.17	-	-	+	+	+
35	429.84	-	+	+	+	-
Total of bands		10	12	11	10	9

Table (8): DNA polymorphism of five faba bean varieties using randomly amplified polymorphic DNA with primer B1

Band No.	M.W	Varieties				
		Sakha1	Sakha 2	Misr 1	Misr 2	Nobaria1
1	3176.09	+	-	-	+	-
2	2339.00	-	-	-	+	-
3	2268.52	+	-	-	-	-
4	1722.53	+	-	-	-	-
5	1670.63	-	-	-	+	-
6	1307.94	-	-	-	+	-
7	1268.54	+	+	-	-	-
8	687.98	-	-	-	+	-
9	647.15	+	-	-	-	-
10	627.65	-	+	-	-	-
11	402.825	+	+	-	-	-
12	320.20	-	-	-	+	-
13	283.32	+	-	-	-	-
14	243.13	-	-	-	+	-
15	208.65	+	-	-	-	-
16	168.42	-	-	-	+	-
17	144.53	+	-	-	-	-
18	120.30	-	-	+	-	+
19	81.71	+	+	+	+	+
Total of bands		10	4	2	9	2

Table (9): DNA polymorphism of five faba bean varieties using randomly amplified polymorphic DNA with primer B18

Band No.	M.W	Varieties				
		Sakha1	Sakha 2	Misr 1	Misr 2	Nobaria1
1	1473.81	-	+	-	-	-
2	1403.96	-	-	-	+	-
3	1184.53	-	+	-	-	-
4	1156.11	+	-	-	-	-
5	1128.38	-	-	+	-	-
6	1101.32	-	-	-	+	+
7	1023.95	-	+	-	-	-
8	975.42	-	-	+	-	-
9	929.18	+	-	-	-	-
10	863.91	-	-	-	+	-
11	843.19	-	-	-	-	+
12	661.43	-	-	-	-	+
13	558.05	+	-	-	-	-
14	531.60	-	-	+	-	+
15	518.85	-	+	-	+	-
16	448.51	-	-	-	-	+
17	387.71	-	-	-	-	+
18	311.61	-	-	-	-	+
Total of bands		3	4	3	4	7



Fig(a-e) : RAPD fingerprints of five faba bean varieties generated by the five primers (B12, B17, B18, B10 andA07) (Line 1-5) represent Sakha 1, Sakha 2, Misr1, Misr2 and Nubria1 and M refers to ladder marker.

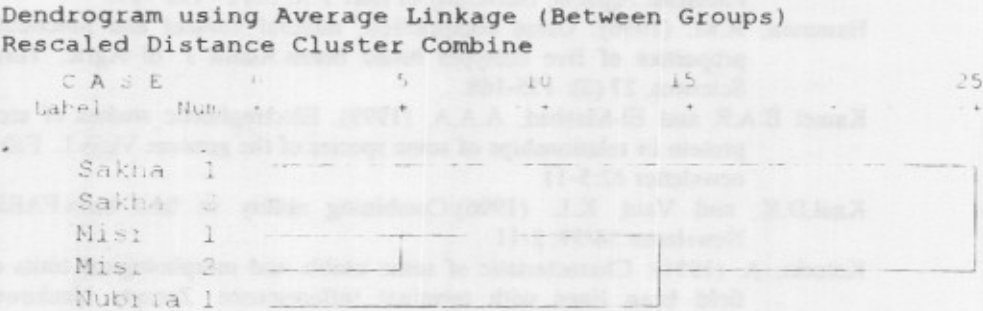


Fig. (1): Dendrogram illustrating genetic distance between the five faba bean Varieties based on RAPD data

The dendogram constructed from cluster based on RAPD data is represented in Fig (1) the data collectively distinguished two main clusters. Except variety Nubaria 1 all varieties were belonging to the same cluster. Within the first cluster variety Sakha 1 and Sakha 2 as well as variety Misr 1 and Misr 2 were closely related.

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التوصيف المورفولوجى والبيوكيماوى لبعض الأصناف الجديدة من الفول البلدى *

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قسم بحوث تكنولوجيا البذور – معهد بحوث المحاصيل الحقلية – مركز البحوث الزراعية

تهدف هذه الدراسة إلى التمييز بين بعض الأصناف الجديدة لمحصول الفول البلدى (سخا١، سخا٢، مصر ١، مصر ٢، نوبارية١) والتي تمت زراعتها تحت الظروف المصرية بالقليوبية وذلك من خلال تحديد بعض الصفات المورفولوجية مثل طول النبات ، وجود صبغة الانثوسيانين، ميعاد التزهير، صفات القرن، شكل البذور وكذلك تحديد بعض الصفات الكيميائية المميزة لها مثل تقدير البروتين- الكربوهيدرات- الزيت- بعض المواد المضادة للتغذية مثل الفينولات، نشاط إنزيم التربسين، الفيسين وكذلك التفريد الكهربي للبروتينات واستخدام التقنيات الحديثة في التمييز مثل الـ PCR وكانت أهم النتائج المتحصل عليها:

- أمكن التمييز بين الخمسة الأصناف الجديدة لمحصول الفول البلدىباستخدام عشرة صفات مورفولوجية من بين ٣٢. صفة يحتويها دليل التوصيف المورفولوجى لمحصول الفول الصادر عن الأتحاد الدولى لحماية حق المربى (UPOV).
- هناك فروق معنوية بين أصناف محصول الفول البلدى تحت الدراسة فى نسبة البروتين والكربوهيدرات و نسبة المواد المضادة للتغذية ويمكن استخدام هذه التقديرات كأداة مساعدة فى تمييز الأصناف بينما لم يكن الفرق معنويا بين الأصناف فى نسبة الزيت.
- أمكن تمييز مجموعة من البروتينات المختلفة فى البذور الأصناف تحت الدراسة باستخدام التحليل الكروماتوجرافى الكهربائي، كما وجد اختلاف فى الوزن الجزيئى للبروتينات الموجودة فى بذور الأصناف تحت الدراسة لذلك فهي وسيلة هامة للتمييز بين الأصناف المختلفة.
- باستخدام تكنيك الـ PCR أمكن تمييز الأصناف تحت الدراسة باستخدام خمس أنواع من البادئات المختلفة لذلك يعتبر هذا التكنيك من الوسائل الفعالة والهامة لتمييز الأصناف تحت الدراسة