USING GEL ELECTROPHORESIS TO DIFFERENTIATE BETWEEN SEVEN CULTIVARS OF OLIVE CULTIVATED IN EGYPT

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ABSTRACT

Leave proteins and esterase isozymes have been studied in seven cultivars related to genus *Olea* named Toffahi, Brawey 1; Brawey 2; Khamdy; khosha; Botude and Meraqy cultivated Sewa area, Egypt. Results of proteins on the taxa studied are agreed with isozymes results. A dendogram produced from selected binary characters (49 characters) of the studies cultivars separated both cultivar Toffahi at 87 % dissimilarity level and cultivar Brawey 2 at 71 % (dissimilarity level). On the other hand; the remainder divided into two groups, the first included cultivar khosha, cultivar Brawey 1 and cultivar Botude which separated at 45% and 25% dissimilarity level, respectively. The second included cultivar Meraqy and cultivar Khamdy which delimited at 15% dissimilarity level.

INTRODUCTION

The olive belongs to the genus *Olea*, which is included within the *Oleideae* sub-family and in the *Oleaceae* tribe. The genus Olea includes about thirty-five species, very widely scattered, chiefly over the Old World, from the basin of the Mediterranean to South Africa and New Zealand. (Lavee, 1996). Olive is one of the most important tree crop species of the Mediterranean area and its global importance is rapidly increasing. Two-dimensional electrophoresis (2-DE) gave satisfactory and reproducible results by Wang , *et al*., (2003). There are more than 2000 different olive cultivars this is somewhat

confounded by different cultivars being given the same name in different countries, or even regions within a country (Robbelen *et al*. 1989). The genus is divided into two sub-genera, *Olea* and *Paniculatae*; sub-genus *Olea* is divided into 2 sections, *Olea* (including cultivated olive and its wild relatives) and *Ligustroides*. The section *Olea* includes the complex of *Olea europaea L*., the Mediterranean olive tree (Green and Wickens, 1989). Olive oil is a very important commodity on the world market. Its accounts for only 2% of the world trade in edible vegetable oils, compared with palm oil, which accounts for 45% of the world trade (Luchetti, 2002). Gates (1978) showed that esterase has been found stable for comparison than peptidase, acid phosphatase and peroxidase isozymes. Isozyme considered to be good markers in studying the genetic variation within species and between closely related species (Crawford & Julian, 1983 and Weeden & Wendell, 1989). The main objective of this work to study the variations in genetic variability among the selected cultivars common in the Sewa region of Egypt in light to variation in protein and isozymes electrophoresis profiles.

MATERIAL AND METHODS

In the present work, seven cultivars related to genus *Olea* named Toffahi, Brawey 1; Brawey 2; Khamdy; khosha; Botude and Meraqy cultivated in Sewa, Egypt.

Electrophoretic Investigations:

I- Extraction and analysis of leave proteins:

Extraction of leave proteins examined by using leave protein fractions were carried out by using one dimensional sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE).Samples were prepared for electrophoresis by precipitating protein solution with 5 volumes of cold acetone at 20ºC for 2 hr. Pellets obtained after centrifugation at 10.000 rpm for 30 min were dissolved in 20 µl of sample buffer (80 µl M Tris base (PH 8.2), 50 µl SDS, 20 µl mercaptoethanol, 1 µl bromophenol blue) and denatured by heating at 80ºC for 5-10 min. Preparation and running of gel were carried according to Laemmli (1970) and Stegeman *et al*., (1981). The gel was stained with coomassie brilliant blue stain R- 250.

II- Extraction and analysis of Esterase isozymes:

Leaves of the studied taxa were germinated in Petri dishes under sterilized conditions. Seedlings were macerated in saline buffer solution (0.8 %NaCl, 0.2% NaNO3) and centrifuged at 10.000 rpm for 10 min to prepare the crude tissues extract. 7.5% of Non-dissociating polyacrylamide gel was prepared as described by Lammlie (1970) and aliquots (50µl) of the crude extract were loaded on the gel. After electrophoresis process; the gel were stained for the desire isozyme according to gel procedures used by Salits *et al*.(1983). Gel of esterase isozymes were incubated in 1.0 M Na-phosphate buffer pH 6.0 for 10 min. Then the gel staining solution containing 1%α-naphthyl acetate and 1% fast blue R R and the gels were stained for 2hr at room temperature. Gel bands of protein and esterase isozyme were determined by scanning using Hoefer scanning densitometer GS 300, in Zoology Department, Al-Azhar University.

III- Numerical Studies

This study is dependent upon the application of a total of 88 comparative anatomical characters and their states as a binary character (0 &1), on each of the cultivars of olive. The characters and states have been subjected to numerical analysis under a program using similarity and dissimilarity assessment percentage method (Rohlf, 1989). The method applied is based on cluster analysis by using an UPGMA (unweighted pair-group method with arithmetic means) Dendrogram illustrating the interspecific relationships of the studied cultivaras percent similarity.

RESULTS AND DISCUSSION

Number of protein bands in the extract of between the studied cultivars are represented in Table 1 and photographed in plate I. In extracts of olive cultivars, in total 36 bands have been noticed from scanning of protein gel, where 26 bands have been recorded in cultivar Toffahi, cultivar Brawey 1, and cultivar khosha. 24 bands were detected in cultivar Brawey 2, 25 bands are presented in cultivar Khamdy, 26 bands are recorded in, and 25 bands are represented in cultivar Botude and cultivar Meraqy. From the results, 8 bands no. 3, 5, 8, 11, 19, 20, 27 and 33 with migration distance of 0.29, 0.32, 0.52, 0.88, 3.10, 3.40, 6.10 and 8.62 are present in all cultivars.

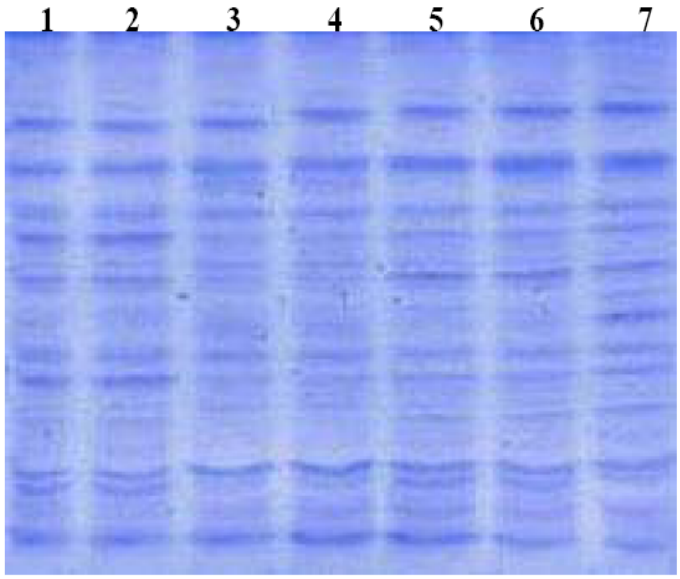

Plate I: SDS-polyacrylamide gel electrophoresis illustrating leaves Protein of the studied cultivars

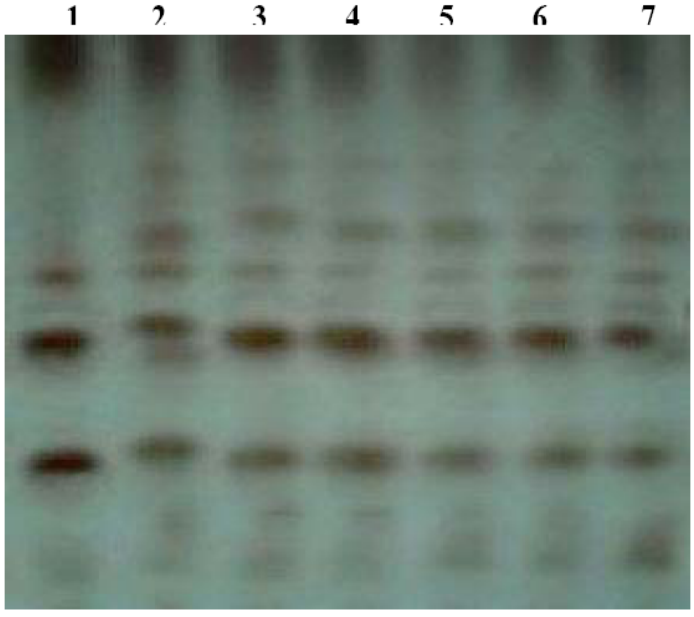

Plate II: SDS-Polyacrylamide gel electrophoresis illustrating esterase isozyme of the studied cultivars

On the other hand, two bands of migration distance of 1.89 and 5.80 are present in cultivar khosha and cultivar Meraqy. It is noticed that, the migration distance of 0.41 and 0.60 are present in cultivar Toffahi, cultivar Brawey 2, cultivar Khamdy and cultivar Meraqy. On the other hand band no. 4 &12 are absent only in cultivar Toffahi only. (Table 1).

Table 1: Relative percentages and position of protein bands extracted from the studied species

Band No.	Relative Position (Cm)	Toffahi	Brawey 1	Brawey 2	Khamdy	khosha	Botude	Meraqy
1	0.19	2.6	3.5	8.7	3.8	5.3	7.8	8.9
2	0.22	--	3.9	2.9	7.6	2.8	7.6	--
3	0.29	6.3	8.6	7.3	5.8	10.6	7.9	11.3
4	0.31	--	2.9	2.0	6.3	10.8	8.9	1.8
5	0.32	2.8	7.3	5.2	7.9	6.8	3.8	5.2
6	0.39	2.8	2.6	--	--	7.8	8.3	--
7	0.41	3.5	--	2.9	--	--	--	5.8
8	0..52	3.8	5.5	5.1	5.5	8.5	5.5	9.1
9	0.60	5.9	--	2.8	--	8.9	--	8.8
10	0.78	--	6.5	--	3.6	6.5	8.1	--
11	0.88	6.8	5.3	2.3	11.2	5.6	4.9	8.3
12	1.19	--	8.6	5.3	7.8	4.3	6.8	5.3
13	1.37	8.6	4.9	--	5.9	--	5.4	--
14	1.86	6.7	--	2.8	4.6	9.8	--	8.7
15	1.89	--	--	--	2.7	--	--	7.3
16	1.93	5.9	--	5.4	8.6	12.3	--	5.9
17	2.23	2.8	5.6	5.9	--	--	2.8	4.9
18	2..50	9.8	--	--	--	6.8	--	--
19	3.10	1.2	1.5	3.6	.5.8	2.8	0.3	4.9
20	3.40	4.5	2.3	0.9	1.2	1.1	5.6	8.9
21	3.51	--	5.2	--	--	--	5.2	--
22	4.48	7.6	4.1	2.9	--	8.2	--	6.7
23	4.60	1.8	--	--	3.8	1.2	--	--
24	4.62	--	9.3	1.8	5.6	--	9.5	8.7
25	5.27	21.4	11.8	--	7.1	2.8	5.9	--
26	5.80	--	--	--	7.1	--	--	8.6
27	6.10	9.3	2.1	5.9	6.8	5.9	1.3	10.9
28	6.32	8.3	--	2.9	--	--	--	--
29	6.83	5.3	2.9	8.9	--	2.9	--	8.9
30	7.01	--	8.6	--	5.8	--	4.6	18.2
31	7.32	8.3	--	2.6	--	2.8	1.3	5.6
32	8.32	--	1.9	1.5	--	1.6	8.9	--
33	8.62	1.3	2.9	5.3	2.9	2.3	8.3	8.3
34	8.63	5.3	2.1	1.9	--		2.3	--
35	8.79	5.3	0. 9	--	.92	0.80	5.3	2.6
36	9.10	8.3	5.6	8.3	2.8	--	5.3	--

Table 2: Relative percentages and position of esterase isozyme bands extracted from the studied species

Band No.	Relative Position (Cm)	Toffahi	Brawey 1	Brawey 2	Khamdy	khosha	Botude	Meraqy
1	0.24	9.3	10.5	--	3.8	6.7	8.6	2.4
2	0.28	2.9	1.8	4.6	6.8	5.9	0.56	7.9
3	0.32	8.9	9.6	2.8	--	--	3.8	0.78
4	0.36	2.9	8.6	0.57	4.6	8.7	6.8	9.4
5	0.47	--	--	5.6	5.8	5.6	8.7	11.6
6	0.80	5.6	7.5	4.2	5.9	8.5	5.6	7.4
7	1.20	--	5.2	1.3	10.6	4.6	7.8	9.1
8	1.33	11.3	12.3	8.6	7.6	5.8	4.6	8.1
9	1.35	5.3	1.8	7.9	4.8	5.3	5.6	4.0
10	1.51	--	7.0	5.8	5.6	4.8	7.3	5.2
11	3.40	--	--	--	9.5	--	--	6.8
12	3.55	9`.6	--	--	--	--	--	--
13	3.98	6.8	4.5	5.9	8.9	5.6	--	--

In this genus in total 13 bands have been recorded, 9 bands have been recorded in cultivar Toffahi, 10 bands are detected in cultivar Brawey 1, 10 bands have been recorded in cultivar Brawey 2., cultivar khosha and cultivar Botude.11 bands were represented in cultivar Khamdy and cultivar Meraqy. On the other hand, bands no. 2,4,6,8 and 9 (with migration distance of 0.28, 0.36, 0.80, 1.33 and 1.35 are found in all studied cultivars. On the other hand, band number 11 with migration distance of 3.40 found in cultivar khamdy and cultivar Meraqy only. It is noticed that, band no. 1, 11 and 12 with migration distance of 0.24, 3.40 and 3.55 are absent in cultivar Brawey 2 only. From the results, band no. 13 with migration distance of 3.98 found in all cultivars except cultivars Botude and Meraqy. Similarly, Band no.

5 with migration distance of 0.47 was absent in cultivar Toffahi and cultivar Brawey 1 (Table 2).

Numerical Analysis

The olive (*Olea europaea* L.) is a polymorphous tree, medium-sized tree (maximum 10-11 m.) with a furrowed trunk. It has fusiform coriaceous grayish-green leaves (generally about 5-6 cm. long and about 1-1.5 cm. wide in the middle of the leaf with smooth edges and a short peduncle. According to seed protein results, cluster analysis based on SDS-PAGE profiles analysis separated the completely morphological similar cultivars of the olive, where each cultivar separated at a distinct % dissimilarity level. From the results, the highest similarity was found between cultivar khamdy and cultivar Meraqy (11 % dissimilarity level).

It is obvious that, leave protein characters fairly do not indicate any differentiation between the different studied cultivars. The tree illustrated from the results by of esterase isozyme characters fairly in congruence with the results obtained from protein analysis where, the cluster showed that, cultivar Toffahi delimited at a separate taxonomic level (82 %). Such cultivar characterized by absence of Band number 7 and 10 with migration distance of 1.20 and 1.51 which found in all cultivars. Also, band no. 12 with migration distance of 3.55 found only in cultivar Toffahi. Such cultivar characterized by absence of bands no. 5,7,10 and 11(with migration distance of 0.47,1.20, 1.51 and 3.40, respectively). Two groups represented from the dendrogram; the first included cultivar Brawey 1, cultivar Khosha and cultivar Brawey 1 while the second group included cultivar Botude, cultivar khamdy and cultivar Meraqy. From the isozyme tree, the highest similarity was found between cultivar khamdy and cultivar Meraqy, such two cultivars have a distinct band (number 11 with migration distance of 3.40). Also, band number 12 with migration distance of 3.55 was found in all cultivars except cultivar khamdy and cultivar Meraqy. On the other hand, such cultivars are near in Sewa area. Dendrogram of leave protein and esterase isozyme (Table 3) showed that, all taxa separated at taxonomic level of 87 %. Cultivar Toffahi and cultivar Brawey 2 are separated at single levels of 87% and 71%, respectively. On the other hand the rest of the cultivars are divided into two groups, the first included, cultivar khosha, cultivar Brawey and cultivar Botude.

Table 3: The resulted 49 binary characters of the studied spp. (Characters & states are symbolized for numerical analysis)

Species / State Recognized	Cultivar 1	Cultivar 2	Cultivar 3	Cultivar 4	Cultivar 5	Cultivar 6	Cultivar 7
I- Migration distance of Protein							
1-0.19: Absent 0 / Present 1	1	1	1	1	1	1	1
2-0.22: Absent 0 / Present 1	0	1	1	1	1	1	0
3-0.29: Absent 0 / Present 1	1	1	1	1	1	1	1
4-0.31: Absent 0 / Present 1	0	1	1	1	0	1	1
5-0.32: Absent 0 / Present 1	1	1	1	1	1	1	1
6-0.39: Absent 0 / Present 1	1	1	0	0	1	1	0
7-0.41: Absent 0 / Present 1	1	0	1	0	1	0	1
8-0..52: Absent 0 / Present 1	1	1	1	1	1	1	1
9-0.60: Absent 0 / Present 1	1	0	1	0	1	0	1
10-0.78: Absent 0 / Present 1	0	1	0	1	1	1	0
11-0.88: Absent 0 / Present 1	1	1	1	1	1	1	1
12-1.19: Absent 0 / Present 1	0	1	1	1	1	1	1
13-1.37: Absent 0 / Present 1	1	1	0	1	0	1	0
14-1.86: Absent 0 / Present 1	1	0	1	1	1	1	0
15-1.89: Absent 0 / Present 1	1	1	0	1	0	1	0
16-1.93: Absent 0 / Present 1	1	0	1	1	1	0	1
17-2.23: Absent 0 / Present 1	0	0	0	1	0	0	1
18-2..50: Absent 0 / Present 1	1	0	1	1	1	0	1
19-3.10: Absent 0 / Present 1	1	1	1	0	0	1	1
20-3.40: Absent 0 / Present 1	1	0	0	0	1	0	0
21-3.51: Absent 0 / Present 1	1	1	1	1	1	1	1
22-4.48: Absent 0 / Present 1	1	1	1	1	1	1	1
23-4.60: Absent 0 / Present 1	0	1	0	0	0	1	0
24-4.62: Absent 0 / Present 1	1	1	1	0	1	--	1
25-5.27: Absent 0 / Present 1	1	0	0	1	1	0	0
26-5.80: Absent 0 / Present 1	0	1	1	1	0	1	1
27-6.10: Absent 0 / Present 1	1	1	0	1	1	1	0
28-6.32: Absent 0 / Present 1	0	0	0	1	0	0	1
29-6.83: Absent 0 / Present 1	1	1	1	1	1	1	1
30-7.01: Absent 0 / Present 1	1	0	1	0	0	0	0
31-7.32: Absent 0 / Present 1	1	1	1	0	1	0	1
32-8.32: Absent 0 / Present 1	0	1	0	1	0	1	1
33-8.62: Absent 0 / Present 1	1	0	1	0	1	1	1
34-8.63: Absent 0 / Present 1	0	1	1	0	1	1	0
35-8.79: Absent 0 / Present 1	1	1	1	1	1	1	1
36-9.10: Absent 0 / Present 1	1	1	1	0	0	1	0
II- Migration distance of EST							
37-0.24: Absent 0 / Present 1	1	1	0	1	1	1	1
38-0.28: Absent 0 / Present 1	1	1	1	1	1	1	1
39-0.32: Absent 0 / Present 1	1	1	1	0	0	1	1
40-0.36: Absent 0 / Present 1	1	1	1	1	1	1	1
41-0.47: Absent 0 / Present 1	0	0	1	1	1	1	1
42-0.80: Absent 0 / Present 1	1	1	1	1	1	1	1
43-1.20: Absent 0 / Present 1	0	1	1	1	1	1	1
44-1.33: Absent 0 / Present 1	1	1	1	1	1	1	1
45-1.35: Absent 0 / Present 1	1	1	1	1	1	1	1
46-1.51: Absent 0 / Present 1	0	1	1	1	1	1	1
47-3.40: Absent 0 / Present 1	0	0	0	1	0	0	1
48-3.55: Absent 0 / Present 1	1	0	0	0	0	0	0
49-3.98: Absent 0 / Present 1	1	1	1	1	1	0	0

The second group included cultivar Meraqy and cultivar Khamdy.

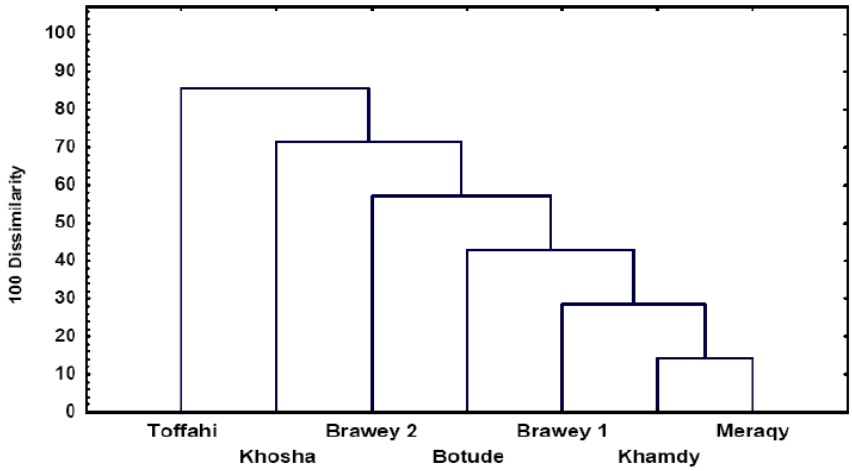

Fig. 3: UPGMA-dendrogram based on 36 protein characters illustrating similarity &dissimilarity distances between the studied cultivar

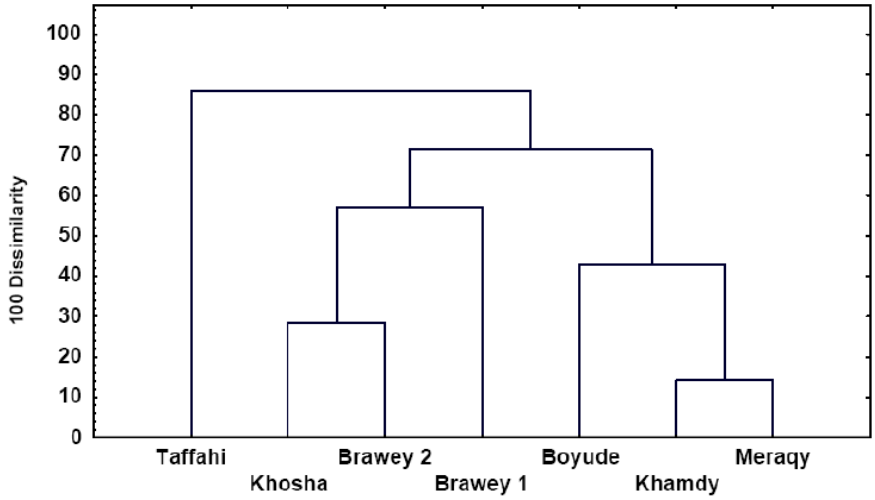

Fig. 4: UPGMA-dendrogram based on 13 EST characters illustrating similarity & dissimilarity distances between the studied cultivar

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استخدام التفريد الكهربي في التميز بين أصناف الزيتون المنزرعه في مصر

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تم دراسة المحتوي البروتيني وكذلك المحتوي الإنزيمي للأوراق سبعة أصناف من الزيتون تم تجميعهم من منطقة سيوه في مصر Toffahi and Brawey 1, Brawey 2, Khamdy, khosha, Botude, Meraqy, باستخدام التفريد الكهربي. ولقد أثبتت الدراسة إن نتائج المحتوي البروتيني تتشابه مع نتائج المحتوي الأنزيمي. لقد استخلصت 49 صفة ثنائية الاحتمالات من نتائج دراسة المحتوي البروتيني والمحتوي الإنزيمي معا والتي حللت عدديا باستخدام برنامج حساب المسافة التصنيفية فأسفر عن رسم هيكلي أدي إلي فصل Toffahi و2 Brawey في مجموعة منفصلة عند مستوي تصنيفي مرتفع (87 % و 71% علي التوالي) بينما تجمعت الأنواع الباقية في مجموعتين الأولي ضمت khosha, Brawey 1 and Botude و والتي انفصلت عند مستوي تصنيفي 45% و28% علي التوالي المجموعة الأخرى اشتملت علي Khamdy & Meraqy عند مستوي تصنيفي 15%