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Random Amplified Polymorphic DNA (RAPD) Analysis of Olive *(Olea europaea* L.) cultivars grown in North west coast region of Egypt.

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***Abstract*: Random Amplified polymorphic DNA (RAPD) fingerprinting technique was applied to evaluate genetic similarity among nine olive (Olea europaea L.) Egyptian and no-Egyptian cultivars, (named: Hamed, Wateken, Maraki, Toffahi, Manzanillo, Picual, Dolce, Kronaki and Coratina) cultivated in experimental ranch, Faculty Agriculture Desert and Envirnmental (Fuka-Matrouh) Alexandia Universtiy, North Eastern Coast. Different polymorphism percentages were recorded for five random primers (MY1-MY2-MY3-MY4-MY5) through this investigation. Generally, all genotypes could be distinguished via five random primers under study. Fifth prime reflected high polymorphic percentage with 81 % of polymorphism. On the other hand, high similarity percentage and lowest polymorphism percentage was recorded for first primer with 27 % of polymorphism. A dendrogram was done to depict the pattern of relationships between the studied cultivars and their genetic diversity**.

Keywords: RAPD, variant identification, molecular fingerprinting, olive, *Olea europaea*

I. Introduction

Olive is cultivated in Egypt from ancient centuries. It is found in pharos tombs and temples as pictures and fruits, the goddess Isis educated the Egyptians about the cultivation of olives and how to produce oil through the pressing of its fruit. Olive cultivation originated in antiquity on the eastern shores of the Mediterranean basin [1].

Nowadays, olive trees play an important role in new orchard establishment, especially in new reclaimed area due to his ability to grow under the stress conditions. Recently it is occupied about 11% from the total fruit area in Egypt, [2]. Olive is widely distributed and grown successfully under the prevailing conditions of North west coast region, Alexandria, North Sinai and Oases.

The genetic patrimony of the Mediterranean Basin's olive trees are very rich and are characterized by an abundance of varieties. Based on estimates by the FAO, the world's olive germplasm contains more than 2.629 different varieties, with many local varieties and ecotypes contributing to this richness, [3].

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Morphological and biological characters have been widely used for descriptive purposes and are commonly used to distinguish olive cultivars. Agronomic characterization also allowed the classification of different olive cultivars. It is a predominant allogamous species showing a high degree of out crossing, which leads to considerable levels of heterozygosity and DNA polymorphism among individuals [4], [5], [6], [7]. The random amplified polymorphic DNA (RAPD) technique has been applied in several studies to successfully distinguish between olive cultivars [8], [9], [10], [11].

Knowledge of genetic diversity in a crop species is fundamental to its improvement. A variety of molecular, chemical and morphological descriptors are used to characterize the genetic diversity among and within crop species. Molecular marker techniques using random amplified polymorphic DNA (RAPD) have been used to study polymorphism in olive. Random Amplified Polymorphic DNA (RAPD) analysis, described by [12], [13], involves the direct analysis of DNA extracted from samples of leaves and has proven a useful tool for genetic typing and mapping. RAPD markers do not depend upon environmental conditions and present a greater degree of polymorphism in olive [14], [15], [16]. Identification of sufficient polymorphic variation among germplasm is necessary to facilitate marker studies.

The analysis of polymorphisms based on the study of DNA, either by restriction or amplification profiling has been used to identify different genotypes of *Olea europaea* L. and other agronomic species. It was possible to discriminate between species and between genotypes, [17], [18]. Intensive modes of production favor the use of a few varieties with a stable and regular yield in olive cultivation, over a wide area associated with acceptable organoleptic characteristics. This selection leads to genetic erosion due to the abandonment of numerous locally adapted olive varieties [19].

[20] Used 16 primers in their study they performed for comparing 19 Albanian olive species and 2 wild olive types. [21] used 43 decamer primers from Bioprobe on five olive species and 8 of the same (A1, A2, A9, C15, C9, E15 and O8) provided characterization of all species and they obtained 76 polymorphic bands.

The first molecular characterization of important Egypt olive cultivars using RAPD, with particular emphasis on the four Egyptians olive cvs. Hamed, Wateken, Maraki, Toffahi, and two Spanish cv. Manzanillo, Picual and one French cv. Dolce and one Greece cv. Kronaki and one Italian cv. Coratina, all varieties were cultivated in Egypt.

The Egyptian olive grove lands, although dominated by three major varieties de origin Siwa Oasis , Hamed, Maraki and Wateken, is rich in cultivars. The objective of this study, we report the use of RAPD markers to distinguish among 9 olive cultivars, and to discuss the use of RAPD to study

relationships among those cultivars of this variety and their genetic diversity.

II. Materials and method

A. Plant material

A total of 9 olives were used in this work, where the three varieties used were taken from Siwa Oasis and the rest of the genotypes from the farm of Faculty of Agriculture desert and environment Fuka – Matrouh, Egypt.

TABLE 1: Origin and traits studied olive cultures [23],[24].

Code	genotypes	sources	Use	Oil content	Fruit size
1	Dolci	French	Table	15-18%	3-6 g
2	Picual	Spanish	Oil and Table	15-22%	3-7 g
3	Manzanell	Spanish	Oil and Table	16-20%	4-6 g
4	Kronaki	Greece	Oil	16-24%	1-1.5 g
5	Toffahi	Egypt	Table	5-7 %	8-16 g
6	Coratina	Italian	Oil	18-22%	3-4 g
7	Hamed	Egypt	Table	16-9 %	5-8 g
8	wateken	Egypt	Oil and Table	18-20%	4-6 g
9	Maraki	Egypt	Oil	25-30%	3-6 g

B. DNA extraction Procedure for total genomic of nine olive cultivars according to manufacturer protocol of Omega Co. (USA.LMt.):

- To 100 mg powdered leaf of nine olive cultivars add 550 µL of lysis buffer solution were added, shacked gently, incubated for 30 min on ice, and centrifuged at 1200 rpm for 10 min at 4°C.
- Supernatant was Removed (tissues waste), 1 ml lysis buffer was added, the pellet was resuspend, and centrifuged for 10 min at 4°C (1200 rpm).
- Supernatant was Removed (tissues waste), 0.5 ml SE-buffer was added, the pellet was resuspend, followed by centrifugation for 10 minutes at 4°C (1200 rpm).
- Supernatant was Removed (tissues waste). (It is possible to store the pellet at -80°C. Centrifugation at 1200 rpm for 10 min at 4°C. The supernatant was Removed and the pellet was freezed). 1 ml SE-buffer was added and the pellet was resuspend, 40 µl proteinase K (10 mg/ml) was added and 250 µl 20% SDS, shaked gently, and incubated overnight at 37°C in a water bath.
- A 5 ml SE-buffer was added and 10 ml phenol shaked by hand for 10 min, and centrifuged at 3000 rpm for 5 min at 10°C.
- The supernatant was transferd into a new tube, 1 ml phenol/chloroform/ isoamyl alcohol (25:24:1) was added, shaked by hand for 10 min, and centrifuged at 3000 rpm for 5 min at 10°C.
- The supernatant Again was transferd into a new tube, 1 ml chloroform/ isoamylalcohol (24:1) was added, shake by hand for 10 min, and centrifuged at 3000 rpm for 5 min at 10°C.
- The supernatant was transferd into a new tube; 100 µl 3 M sodium acetate (pH 5.2) was added and 10 ml isopropanol, shaked gently until the DNA precipitated, use a glass pipette, make a hook over a Bunsen burner, and capture the DNA.
- The DNA was Washed in 70% ethanol and dissolved the DNA in 0.1 ml TE-buffer overnight at 4°C on a rotating shaker. (If the DNA is not dissolved leave it longer at 4°C on the rotating shaker).

C. Preparation of the PCR master mixture:

Preparation of the amplification reaction was done under the biosafety cabinet in a separate room rather than that in which the amplification and the extraction were done. In Eppindorf tube, the components of the PCR were prepared as a master mix containing the reagents needed to amplify the required number of samples as well as positive and negative control, then 5µl (25 ng) of the DNA were added in the PCR tubes and 1.0 µl of random primer was added (random primer listed in table 2). Finally, 20µl of the master mix were dispensed in it, to reach 25µl as a final volume of the reaction.

TABLE 2: Random Amplified Polymorphic DNA primers under study.

Primers	sequence
1 MY1	CTTAGCATGA
2 MY2	ACAATGACGT
3 MY3	ACACAGAATG
4 MY4	TTGTGTACAC
5 MY5	GAGGTCATGA

D. RAPD -PCR amplification:

Total genomic DNA was amplified through GeneAmp Polymerase Chain Reaction (PCR) system cycler. PCR for amplified genomic DNA was carried out. The reaction

consists of 40 cycles; each cycle consisted of denaturation at 94°C for 30 sec followed by annealing at 30°C for 30 sec and extension at 72°C for 30 sec. There was an initial delay for 15 min at 95°C at the beginning of the first cycle and 10 min delay at 72°C at the end of the last cycle as a post extension step [22] The product was stored at –20°C or 4°C.

Table (3): Summarized the obtaining RAPD results

Total amplified band	Polymorphic bands	Monomorphic bands	Polymorphism %
40	11	29	27
27	13	14	48
21	7	15	33
18	10	8	55
16	13	3	81

E. *Agarose gel electrophoresis and detection of the amplification products:*

1.5% agarose solution was prepared by adding 0.75g agarose to 50ml of 1x TBE electrophoresis buffer in 50ml flask. Heating in a microwave oven then dissolved the agarose. The agarose was cooled in 50°C. A comb was inserted in electrophoresis bed and the agarose was poured in it. Great care should be taken during pouring of the agarose to avoid bubbles formation. The gel solidified within 15 min and became cloudy, the electrophoresis apparatus was filled with the electrophoresis buffer and the comb was removed creating 6 or 10 wells for sample application. Electrodes were connected to the power supply and the later was turned on. It was adjusted at 80 Volts for 100 min. The gel was removed from its bed and transferred to the gel staining tray for staining with Ethidium bromide for 30 min followed by 20 min distain in distilled water.

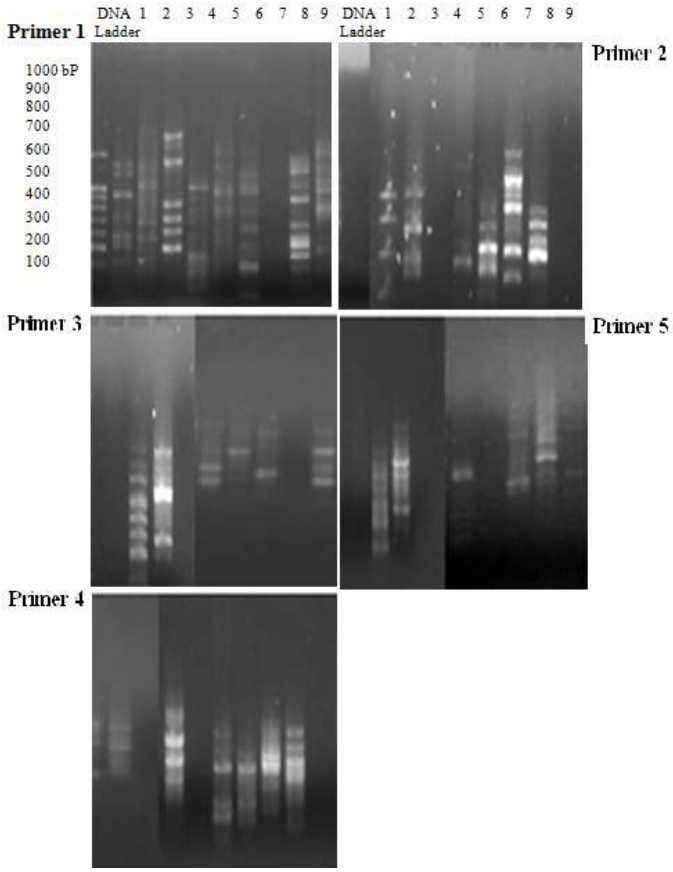
F. *Data analysis:*

Gel documentation system (Geldoc-it, UVP, England), was applied for data analysis using Totallab analysis software, ww.totallab.com, (Ver.1.0.1).

III. Results

Random amplified polymorphic DNA (RAPD) technique:

In this investigation, Random amplified polymorphic DNA (RAPD) technique were performed to detect the differences among nine olive cultivars through five arbitrary primers mentioned in materials and Methods. The numbers and sizes of genomic bands and Polymorphic bands resulted from applying those five primers with nine olive cultivars are shown in Photograph (1), Figure (1) and Table (3). According to Random amplified polymorphic DNA (RAPD) data, 27, 48, 33, 55 and 81 polymorphism percentages were recorded for first, second, third, fourth and fifth random primers. High similarity relation was detecting among cultivars under study as results of polymorphism percentage decreasing. To distinguish variation among cultivars under study, fifth primer with 81 % of polymorphism was recommended. Highly similarity percentage among cultivar was founded through applying first random primer.



Photograph (1): Random Amplified Polymorphic DNA (RAPD) products of five random primers for nine olive cultivars. Where:

1- Dolce	2- Picual	3- Manzanello
4- Kronaki	5- Toffahi	6- Coratina
7- Wateken	8- Hamed	9- Maraki

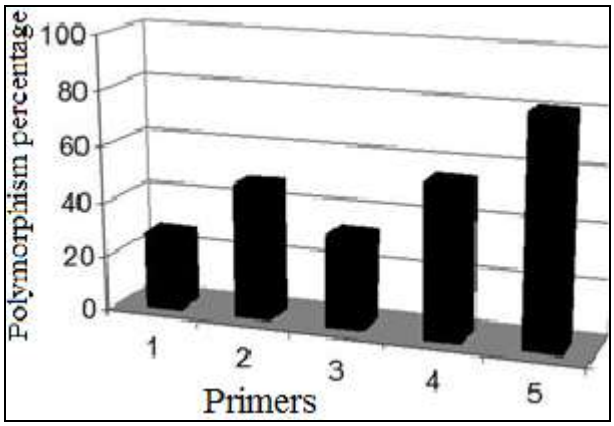


Figure 1: shows Polymorphism percentage of five random primers for nine olive cultivars

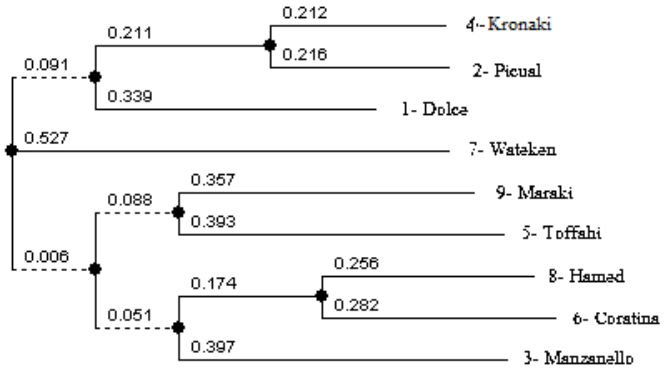


Figure 2: Dendrogram of nine studied olive cultivars using RAPD products.

The dendrogram in Figure (2) depicts the pattern of relationships between the studied cultivars. There is no clear clustering of cultivars in relation with their origin area or end use. Nevertheless, two major clusters can be defined in addition to one Egyptian cultivar i.e. Wateken didn't belong to either of both. The first group corresponds to the six cultivars of the top of the dendrogram composed of the three European cultivars Kronaki , Picual and Dolci. These three cultivars have common oil content (15 to 22) and medium-sized fruits (3 to 6 g) except 'Kronaki' which has small-sized fruits (1 to 1.5 g) [23],[24]. The second group contains 'Maraki Toffahi, Coratina Hamed Manzanello ', all having medium to big-sized fruits (3 to 16 g) and high oil content except Toffahi [23],[24]. Maraki and Toffahi cultivars corresponded to subgroup as two Egyptians genotypes, while the Italian and Spanish cultivars Coratina and Manzanello, respectively with Hamed from Egypt form another subgroup which reflects the probability that they have common genetic background and the same origin [25].

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