Effect of salinity and the genetic variation on olive cultivars grown in Sinai based on ISSR, Isozyme and protein markers.

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Abstract

Olive (*Olea europaea* L.) is one of the most important crops in the Mediterranean area and known by high genetic variation. A comparative account of the polymorphic expression of two antioxidative enzymes (Peroxidase and Super oxide dismutase), one hydrolyzing enzyme (Esterase) and protein electrophoresis was detected from the leaves of four olive cultivars irrigated with different concentrations of saline water. Gel electrophoresis experiments revealed that in most of the cases there were extra numbers of protein bands expressed with relatively low molecular weight in trees irrigated with saline water (2 and 3 bands in Manzanillo, 2 and 1 bands in Picual and 2 bands in case of Dolce cultivar). In all salinity imposed trees, there were sharp increases in band intensity and the number of isoforms of investigated enzyme. Peroxidase increment in trees irrigated with saline water ranged from 2 to 3 polymorphic. Similarly, SOD was shown one band only in all the studied cultivars irrigated or no irrigated with saline water. Increments of Esterase revealed that only one polymorphic band appeared in Eggizi cultivar when irrigated with high concentration of saline water. Genetic variability among four common olive cultivars grown in Sinai, Egypt was assessed 78 fragments of which 29 were polymorphic. The number of polymorphic bands per primer varied from 1 to 6. Genetic similarities were calculated using the Jaccard similarity coefficient. The resulting similarity matrix was subjected to the UPGMA clustering method for dendrogram construction and cultivar differentiation. Our results indicate that ISSR can be useful for genetic diversity studies, to provide practical information for parental selection and to assist breeding and conservation strategies Also, the present results along with those of other researchers show that ISSRs can be used for cultivar differentiation in *Olea europaea* L.

Key words: Olive Olea europaea L. Genetic diversity ISSR marker, protein electrophoresis, Isozymes.

Introduction

In many countries of the Mediterranean basin, especially those in the arid zone with high rates of population growth, urbanization and industrialization, water is becoming a scarce resource. In this study, the oil quality parameters of four olive cultivars were measured in trees of four genotypes irrigated with saline water in Ras Sudr station in Sinai, Egypt. The olive tree has been part of Mediterranean civilization since before recorded history. Its domestication goes back6000 years to the East coast of the Mediterranean Sea (**Zohary and Spiegel 1975**).

The genetic diversity could be important resource markers, for the development of modern olive culture towards typical olive oil and fresh productions. From here, the study of less common cultivars represents an important tool to preserve this genetic diversity in respect to genetic erosion due to the introduction of few commercial cultivars in the modern orchards.

Unlike morphological markers, molecular markers are not prone to environmental influences and provide some vital information towards the priority areas for conservation strategies. Therefore, the use of molecular markers (enzymes, DNA) might enhance the understanding of such situations. Enzyme analysis is an added tool for detecting this diversity. The International Union for Protection of New Varieties of Plants (UPOV) have harmonized and adopted test guidelines and procedures for the use of isozyme electrophoresis as a characteristic for establishing the uniqueness of the Plants.

Olive tree germplasm was traditionally evaluated by morphological and phenological parameters .Polymerase Chain Reaction (PCR) – based DNA markers are powerful tools for genetic analysis because of their simplicity and ease handling (**Kojima et al., 1998**) providing an opportunity for direct comparison and identification of olive tree material independently from environment and or developmental stages. ISSRs method is based on the amplification of DNA segments between two microsatellite repeated regions (**Zietkiewicz et al., 1994**) have been used to identify olive cultivars. This work aims to understand the extent of changes of isoforms of two antioxidant enzymes (peroxidase and superoxide dismutase) and one important hydrolyzing enzyme (esterase) in four olive cultivars grown in Sinai, Egypt and irrigated with saline water. Gel electrophoretic study of leaf protein and enzyme, would provide some important clues towards their reverse adaptability to mesophytic condition for postulating proper conservation techniques. The main goal of this work was to construct a molecular data-base using ISSR markers for the four olive cultivars in addition to obtain specific molecular markers for individual identification of the four growing cultivars.

Materials and Methods

Plant Materials: The molecular characterization of four cultivars of olive *Olea europaea* L. was carried out in this study on old olives trees grown at Horticulture Research Station (HRS) in Ras Sudr, Sinai, Egypt. Desert Research Center (DRC) and the Faculty of Agriculture, Ein Shams Univ., Egypt (Table 1).

Table 1. List of olive cultivars studied their origin of production.

No.	Cultivars	Origin
1	**Manzanillo**	**Spain**
2	**Eggizi**	**Egypt**
3	**Picual**	**Spain**
4	**Dolce**	**France**

Table 2. List of primers used for ISSR analysis.

Primer	Sequence 5 -3	Temperature of annealing
A98		**40**
A844	$(CT)_8$ AC	**40**
B844	$(CT)_8$ GC	**40**
B98	$(CA)_6$ G	**40**
HB8	**$(GA)_6$GG**	**48**
HB10	**$(GA)_6$CC**	**40**
HB13	**$(GAG)_3$GC**	**45**
HB14	**$(CTC)_3$ GC**	**40**
HB15	**$(GTG)_3$ GC**	**40**
Ubc26	$(AC)_8$ C	**40**
Ubc801	$(AT)_8$ T	**40**
Ubc825	$(AC)_8$ T	**40**

Protein SDS-PAGE analysis was carried out for four olive cultivars from Sinai (Ras Sudr). Extraction of proteins for gel electrophoresis was done from 2 g of fresh leaf. Leaf samples were macerated in a mortar-pestle, and to this was added 5 ml of extraction buffer (containing10% (w/v) SDS, 10 mM b-Marcapto ethanol, 20% (v/v) glycerol, 0.2 M Tris/HCl (pH 6.8) and0.05% Bromophenol blue). The mixture was centrifuged at 10000 rpm for 20 min. Supernatants were used as samples. Protein samples were resolved in12.5% SDS-PAGE gels following the procedure of **Laemmli (1970)** and stained with Coomassie Brilliant Blue R-250 (Sigma). Molecular weights of the different protein bands were determined in respect to standard protein markers (Bioline Hyper Page prestained protein marker, 10_200 kDa) with the KodakMI software after documentation of the gel slab with Gel-Doc system (Biostep GmbH, Germany).

Extraction of enzymes for native gel electrophoresis and PAGE analysis:

Two grams of young leaf buds were macerated to powder with liquid nitrogen with a mortar-pestle; then 0.1 g PVP and 5 ml of extraction buffer (consisting of 1 M Sucrose, 0.2 M Tris-HCL and 0.056 Mb-Marcapto ethanol; pH adjusted at 8.5) was added and homogenized. The extractants were centrifuged at 10,000 rpm for 20 min at 48C; supernatants were used as samples for gel electrophoresis. Isozymes analysis of three enzymes; Peroxidase, Superoxide dismutase and Esterase was performed for the investigated four cultivars. Equimolar amounts of enzymes were loaded in each well. Samples from the saline and non-saline environment were loaded side by side for precision of polymorphic band expression. Slab gels were stained for definite enzymes following the procedure by **Das and Mukherjee (1997)**. Gels were documented with a Gel-Doc System (Biostep GmbH, Germany) and analysis for band intensity and Relative Mobility Factor (Rmf) were estimated with Kodak-MI software.

Enzyme assay

Peroxidase (PRX)

A total of 200 mg fresh leaf sample was extracted in1_1.5 ml 0.9% KCl and centrifuged at 12,000 rpm for15 min at 48C; the supernatant was used as enzyme sample. Absorbance was taken by Helios g spectrophotometer (Thermo electron Corporation, USA) at460 nm in respect to the standard curve prepared with minute modification.

Superoxide dismutase (SOD)

Cell sap was extracted from 200 mg of leaf and1_1.5 ml 50 mM Phosphate buffer, ph adjusted to7.0; centrifuged at 12,000 rpm for 15 min at 48C.Supernatants were used for enzyme samples. Different aliquots (50, 100, 150, 200, 250 mg/ml) of the standard enzyme samples were also used for

preparing the Pollen samples were collected from 155 cultivars growing at the germplasm collection of the Centro de Investigación standard curve, and absorbance were measured at550 nm.

Esterase (EST)

Enzyme sample was prepared from 200 mg fresh leaf sample extracted with 1_1.5 ml ice cold 0.1 M Tris/HCl buffer adjusted pH 8.0. Extractants were centrifuged at 12,000 rpm for 15 min at 48C. The supernatant was used as sample. Absorbances were noted at 322 nm in respect to the prepared standard curve.

The data presented was the average of 20 readings for each plant, and standard errors were also depicted in the figures. SPSS 12.0 version was used for statistical analysis towards estimating the correlation value, if any, between the total protein amount and quantitatively assayed enzymes. For each enzyme, the pure samples (Sigma chemicals) were used for preparing the standard curves.

Isolation of Plant Genomic DNA: DNA extraction carried out using young tissues collected from three per cultivar. Genomic DNA was extracted and purified.

DNA Extraction: Leaf tissue (0.5 g) was ground in liquid nitrogen and incubated at 65°C for 1 h in 1.5 ml extraction buffer (100 mMTris-HCl, pH 8.0; 50mM EDTA; 0.5 % SDS; 500 mMNaCl and 1% Polyvinyl pyrrolidone). An equal volume of phenol/chloroform (24:1) was added and the whole mixture was centrifuged at 1000 rpm for 10 min. An equal volume of cold chloroform/ isoamyl alcohol (24:1) was added to the supernatant and the mixture was centrifuged at 5000 rpm for 10 min. The precipitation of the upper phase was obtained by adding of 75 µl of 3 M ammonium acetate and 1 volume of cooled isopropanol and centrifugation at 1000 rpm for10 min. The DNA pellet was washed with 70 % ethanol, then dried and dissolved into 400 µL of TE buffer (10mM Tris-HCl, pH 8.0; 1 mM EDTA, pH 8.0). RNA was removed by incubation with 1 µL of RNase (10 mg/ml) at 37°C for 30 min. to have pure DNA and kept at -20° until use. Estimation of DNA concentration and quality were based on **Sambrook et al. (1989)**. DNA concentrations were measured by UV-spectrophotometer (Eppendorf Biophotometer Germany) at a wave length of 260-280 nm.

ISSR Amplification: Inter simple sequence repeats (ISSR) technique was carried out according to procedure described by Martins-Lopes et al. [11]. PCR reactions were performed in a 25 µL volume containing 10 mM Tris of each dNTP; 0.3 µM of a single primer; 20 ng genomic DNA and 2 units of Taq DNA polymerase (Promega, USA). Amplification reactions were performed in a 96-wellBioRad Thermal cycler (U.S.A) under the following conditions: 5 min. initial denaturation step (94°C), 35cycles of 30 S at 94°C; 1 min at 50°C, 1 min at 72°C). The reaction was completed by a final extension step of 7 minat 72°C. Amplification products were separated by electrophoresis in 1 % agarose gels in 1x TBE buffer, stained by ethidium bromide and visualized under UV light. Fragment size was estimated by using a 100 base pairs (bp) molecular size ladder (Promega, U.S.A). The sequences of the twelve ISSR primers (5 - 3 anchored) are presented in Table (2).

Data Analysis: Scoring of ISSR data was achieved C using 1 % agarose gel electrophoresis profile. Clear and distinct fragments were scored as (1) for presence and (0) for absence. Cluster analysis of genetic distances among olive cultivars was performed using the unweighted pair group method with arithmetic average (UPGMA).

Results and Discussion

Biochemical markers of Olive based on SDS-PAGE of total Proteins

In order to estimate the genetic relationships between different Olive proteins extracted from the plant (leaves) with bulk samples. These were fractioned by using one-dimensional SDS-PAGE, Figure (1).

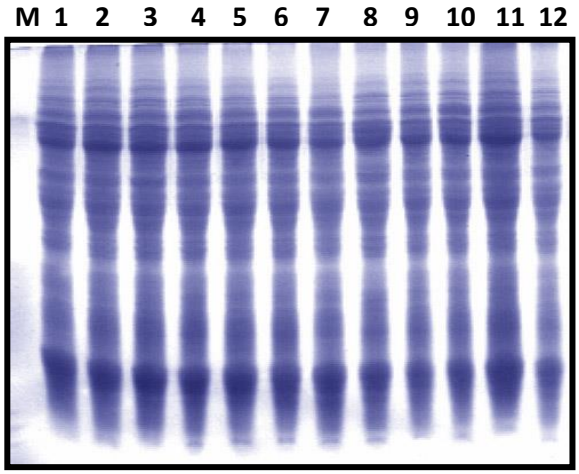


Figure (1) SDS-PAGE of protein banding patterns of four genotypes irrigated with saline water M Protein marker.

1 Manzanillo irrig. With fresh water (300 ppm).
2Manzanilloirrig.with saline water (3000ppm)
3Manzanillo irrig. with saline water (7000ppm).
4Eiggize irrig.with fresh water (300 ppm).
5Eiggize irrigated with saline water (3000ppm).
6Eggize irrig.with saline water (7000ppm).
7 Picual irrigated with fresh water (300 ppm).
8 Picual irrig.with salt water (3000ppm)
9Picual irrigated with salt water (7000ppm).
10 Dolce irrig.with fresh water (300 ppm).
11Dolce irrig.with salt water (3000ppm).

12Dolce irrigated with saline water (7000ppm).

Table 3. Banding patterns and molecular weight (MW) of SDS proteins for four cultivars of olive irrigated with saline water (three concentrations).

Band NO,	MW (KD)	Manzanillo			Eiggize			Picual			Dolce		
		Control 300 ppm	3000 ppm	7000 ppm	Control 300 ppm	3000 ppm	7000 ppm	Control 300 ppm	3000 ppm	7000 ppm	Control 300 ppm	3000 ppm	7000 ppm
1	13234	0	0	0	0	1	0	0	0	0	0	0	0
2	13064	0	0	0	0	0	0	0	0	0	0	1	0
3	12895	1	0	0	0	0	0	0	0	0	0	0	0
4	12798	0	0	1	0	0	0	0	0	0	0	0	0
5	12782	0	0	0	1	0	0	0	0	0	0	0	0
6	12668	0	0	0	0	0	1	0	0	0	0	0	0
7	12216	1	1	1	1	1	1	1	1	1	1	1	1
8	11924	0	1	0	1	0	1	1	0	0	0	1	1
9	11198	1	1	1	1	1	1	1	1	1	1	1	1
10	10180	1	1	1	1	1	1	1	1	1	1	1	1
11	9162	1	1	1	1	1	1	1	1	1	1	1	1
12	8144	1	1	1	1	1	1	1	1	1	1	1	1
13	7126	1	1	1	1	1	1	1	1	1	1	1	1
14	6108	1	1	1	1	1	1	1	1	1	1	1	1
15	5090	1	1	1	1	1	1	1	1	1	1	1	1
16	4072	1	1	1	1	1	1	1	1	1	1	1	1
17	3054	1	1	1	1	1	1	1	1	1	1	1	1
18	2036	1	1	1	1	1	1	0	1	1	1	1	1
19	1636	1	1	1	1	1	1	0	1	1	1	1	1
20	1018	0	1	1	1	1	1	0	1	0	1	0	1
21	506	0	1	1	1	0	1	0	0	0	0	0	1
22	396	0	1	0	1	0	0	0	0	0	0	0	0
Total	7	13	16	15	17	14	16	11	13	12	13	13	15

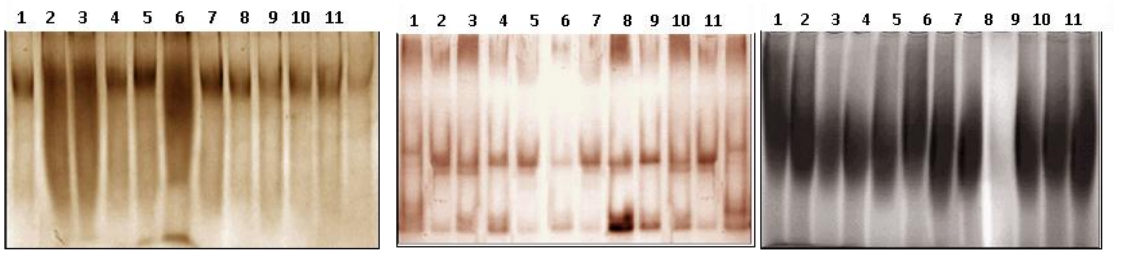
Electrophoretic banding patterns of protein based on SDS-PAGE among four genotypes of Olive cultivars under salinity are illustrated in Figure (1) and Table (3). The total number of bands was 22 bands, which represented in all genotypes with all treatments. The bands were detected with different molecular weights ranged from 132.34 KDa to 3.967 KDa. Band number 1 (MW = 132.34) was identified only in one genotype (Eiggize as irrigated with saline water (3000 ppm)). On the other hand, the band number 2 was appeared only in one genotype (MW = 130.64 in Dolice as irrigated with 3000 ppm) and disappeared in the other genotypes. Moreover, Band number 3 (MW = 128.95) was found only in one genotype (Manzanillo as irrigated with tape water (300 ppm)). Band number 4 (MW = 127.98) was identified only in one genotype (Manzanillo as irrigated with saline water (7000 ppm)). Band number 5 (MW = 127.82) was appeared only in one genotype (Eiggize as irrigated with tape water (300 ppm)). Band number 6 (MW = 126.68) was identified only in one genotype (Eiggize as irrigated with saline water (7000 ppm)). Band number 22 (MW = 3.96) was identified only in two genotypes (Manzanillo as irrigated with saline water (3000 ppm) and Eiggize as irrigated with tape water (300 ppm)). Meanwhile, the bands number 18(MW = 20.36) and the bands number 19 (MW = 16.36) were absent in Dolce genotype. Moreover, some bands like number 8 (MW = 119.24), number 20 (MW = 10.18) and number 21 (MW = 5.06) were appeared in some genotypes and disappeared in others. Results revealed that bands number 9, 10, 11, 12, 13, 14, 15, 16 and 17 were considered as common bands in all the studied genotypes.

Biochemical markers of Olive based on native-gel of Isozymes Electrophoresis:

The genetic information provided by morphological characters is often limited. These limitations have been resulted in the deployment of biochemical techniques such as isozymes and protein electrophoresis **(Crawford, 1989)**. Electrophoretic surveys of proteins play an important role in the quantitative evaluation and management of genetic resources. This is because information concerning the geographical and taxonomic distribution of genetic variation provides guidelines for sampling strategies and germplasm preservation. Gene controlled enzyme variations constitute a rich source of taxonomic characters **(Ayala, 1983).**Isozymes analysis is an economical and effective method for the determination of mutation and recombination in genes and chromosomes. Isozymes are used as genetic markers to observe the recombination and segregation of linked qualitative and quantitative characters **(Fleischmann, 1990)**. In addition, high or low genetic diversity among and within natural populations can be deduced by using different isozymes patterns **(Price et al., 1984**). Isozymes patterns obtained electrophoretically are frequently used as biochemical

markers i) in linkage studies **(Vahl et al., 1987; Melz and Thiele, 1990)**, ii) in establishing whether or not chromosomes or chromosome segments among different individuals are transferred and in identifying different chromosomes **(Salinas and Benito, 1985)**, iii) in detecting the localization of genes on chromosomes **(Drefahl and Buschbeck, 1991)**, iv) in detecting gene expression changes seen in different developmental and differentiation stages **(Chawla, 1988; Drefahl and Buschbeck, 1991)**, v) in providing qualitative and quantitative estimates of gene flow and divergence in switching on and off genes **(Loxdale, 1994)**, vi) in determining spreading limits and species separation of natural plant populations **(Murphy et al., 1990; Jaaska, 1993)**.

Genetic markers generally have contributed to the study of plant biology by providing methods for detecting genetic differences among individuals. There are some important ecological topics, which often use isozymes as powerful markers: Genetic relatedness within and among populations, often with relations to geographic structure. Patterns across a broad range of taxa are mostly consistent with our understanding of the effects of the breeding system (species with self tend to poses lower levels of genetic variation within populations), life history (longer-lived perennials tend to be more variable) and the distribution of genetic diversity within and among populations **(Mahy et al., 1997 and Tarayre et al., 1997)**. There is a narrow linkage between geography and the spatial patterns of genetic variation **(Epperson 1990 and Newton et al., 1999)** as well as the genetics of plant migration and colonization **(Barrett and Husband 1990 and Sun 1997)**.



A α-Esterase (α-Est.) B peroxidase (per) C Super Oxide Dismutase (SOD.)

Figure 2. Zymogram of three isozymes banding patterns among Olive cultivars under different Concentrations of salt.

1 Manzanillo irrig. with fresh water(300ppm) 2 Manzanillo irrig. with saline water(3000ppm)
3 Manzanillo irrig.with saline water (7000ppm) 4 Eggizi irrig. with fresh water(300ppm)
5 Eggizi irrig.with saline water (3000ppm) 6 Eggizi irrig. with saline water(7000ppm)
7 Picual irrig. with fresh water(300 ppm) 8 Picual irrig. with saline water(3000ppm)
9 Picual irrig.with saline water(7000ppm) 10.Dolce irrig .with fresh water(300 -400ppm)
11 Dolce irrig.with saline water (3000ppm) 12 dolce irrig. with saline water(7000ppm).

Table 4. Banding patterns and relative mobilities (RF) of three different isozymes for four cultivars of olive (*Olea europea L*) under saline conditions.

		Cultivar												**Total No. of bands**
Isozyme		**Manzanillo**			**Eiggize**			**Picual**			**Dolce**			
α-Esterase(α-st.)		**300 ppm**	**3000 ppm**	**7000 ppm**	**300 ppm**	**3000 ppm**	**7000 ppm**	**300 ppm**	**3000 ppm**	**7000 ppm**	**300 ppm**	**3000 ppm**	**7000 ppm**	
Band No.	**Rf.**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	
1	0.12	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**2**
2	0.65	**0**	**0**	**0**	**0**	**0**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	
Total		**1**	**1**	**1**	**1**	**1**	**2**	**1**	**1**	**1**	**1**	**1**	**1**	
Peroxidase (Per.)														
1	0.20	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1**	5
2	0.36	**0**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**0**	
3	0.61	**0**	**1**	**1**	**0**	**1**	**0**	**1**	**1**	**0**	**1**	**1**	**0**	

Table 4. Cont.

4	0.82	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1**	
5	0.89	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	
Total		**3**	**3**	**3**	**2**	**3**	**2**	**3**	**3**	**2**	**3**	**3**	**3**	
Super Oxide Dismutase (SOD.)														
1	0.23	1	1	1	1	1	1	1	1	1	1	1	1	1
Total		1	1	1	1	1	1	1	1	1	1	1	1	

Table 5. Number and types of bands as well as the percentage of the total polymorphism generated by three isozymes (α-Esterase, Peroxidase and Super oxide dismutase) for the three cultivars with different concentrations of salt.

Isozymes	Monomorphic Bands	Polymorphic		Total Bands	Polymorphic
		non-unique	Unique		
α-Est.	1	1	0	2	50%
Prx.	1	0	4	5	80%
Sod.	1	0	0	1	0%

Genotype Identification by Unique ISSR Markers: In the present study, Molecular fingerprinting of olive cultivars using 12 ISSRs were tested to explore the genetic diversity among different foreign and local olive genotypes based on the clear scorable band pattern and of good quality. Total number of amplified bands was 78 bands and the number of amplified DNA fragments by each primer ranged from 1-6 bands. The highest number of polymorphic bands was obtained by HB13 and HB14 (6 bands), while A844, B844, B98, and Ubc825 produced the lowest number of polymorphic bands 1 band. The average number of bands/primer was 7 bands/template. All primers produced polymorphic bands ranging in number from 1 to 6 fragments with an average polymorphism/primer of 2.2 (Tables 6). The specific markers (Table 7) ranged from 1 to 3 in Manzanillo and Eggizie. Only one specific marker was detected in Picual. These markers were found to be useful as cultivar specific markers.

Table 6. Number of amplified fragments and specific markers of four olive cultivars based on ISSR-PCR analysis.

			Cultivars								
			Manzanillo		**Eggize**		**Picual**		**Dolce**		
Primer	TAF	PB	AF	SM	AF	SM	AF	SM	AF	SM	TSM
A98	6	2	4	1	5	0	6	0	6	0	1
A844	5	1	4	1	5	0	5	0	5	0	1
B844	7	1	6	1	7	0	7	0	7	0	1
B98	6	1	6	0	6	0	5	1	6	0	1
HB8	7	2	6	0	7	1	6	0	6	0	1
HB10	7	3	3	3	7	0	7	0	7	0	3
HB13	8	6	3	1	7	3	4	0	4	0	4
HB14	9	6	4	2	6	0	8	1	9	0	3
HB15	6	2	4	2	6	0	6	0	6	0	2
Ubc26	5	2	4	0	5	1	2	1	3	0	2
Ubc801	7	2	5	0	7	1	5	0	5	0	1
Ubc825	5	1	4	1	5	0	5	0	5	0	1

TAF = Total amplified fragment, PB = Polymorphic bands, AF = Amplified fragments,
SM = Specific marker, including either the presence or absence in a band in specific cultivar,
TSM = Total no. specific markers across cultivars.

Table7. Cultivar-specific markers in four olive cultivars (*Olea europaea.L*) resulting from ISSR-PCR analysis.

Cultivar	Marker		Total
	Positive	Negative	
Manzanillo	-	**A98-2065,A844-445,B844-980,HB10-980-475-280,HB13-905,HB14-940-490,HB15-780-605,Ubc825-315**	**13**
Eggize	**HB8-1065,HB13-1075-460-215-165, Ubc26-735,Ubc801-1505-1425**	-	**8**
Picual	-	**B98-395,HB14-270,Ubc26-1160**	**3**
Dolice	-	-	**0**
Total			

Table 8. Similarity matrix (%) among the four olive cultivars based on ISSR-PCR analysis

Case	Manazanillo	Eggizi	Picual	Dolce
VAR00001	1.00			
VAR00002	.48	1.00		
VAR00003	.44	.53	1.00	
VAR00004	.46	.65	.88	1.00

Fig ( ): Phenogram demonstrating the relationship among four olive cultivars based on ISSR-PCR analysis.

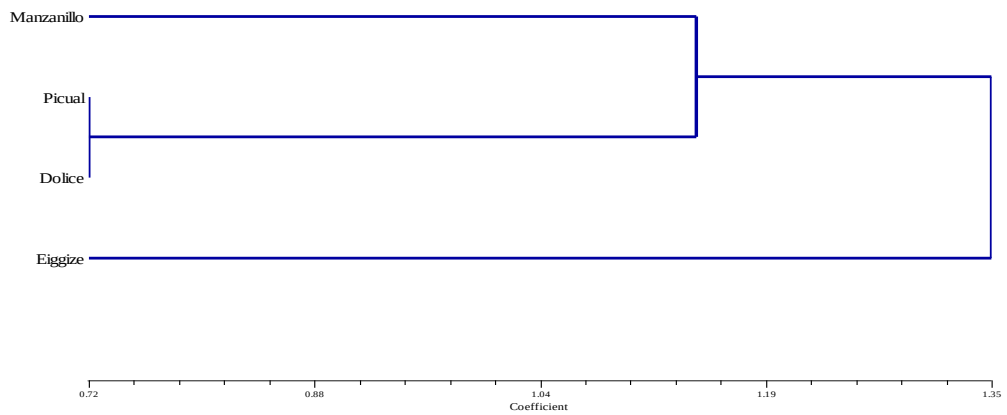
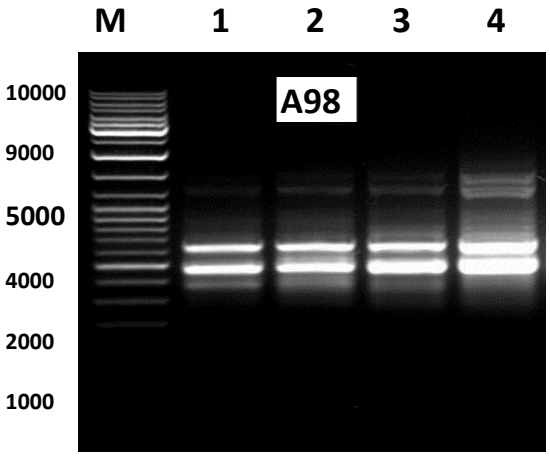
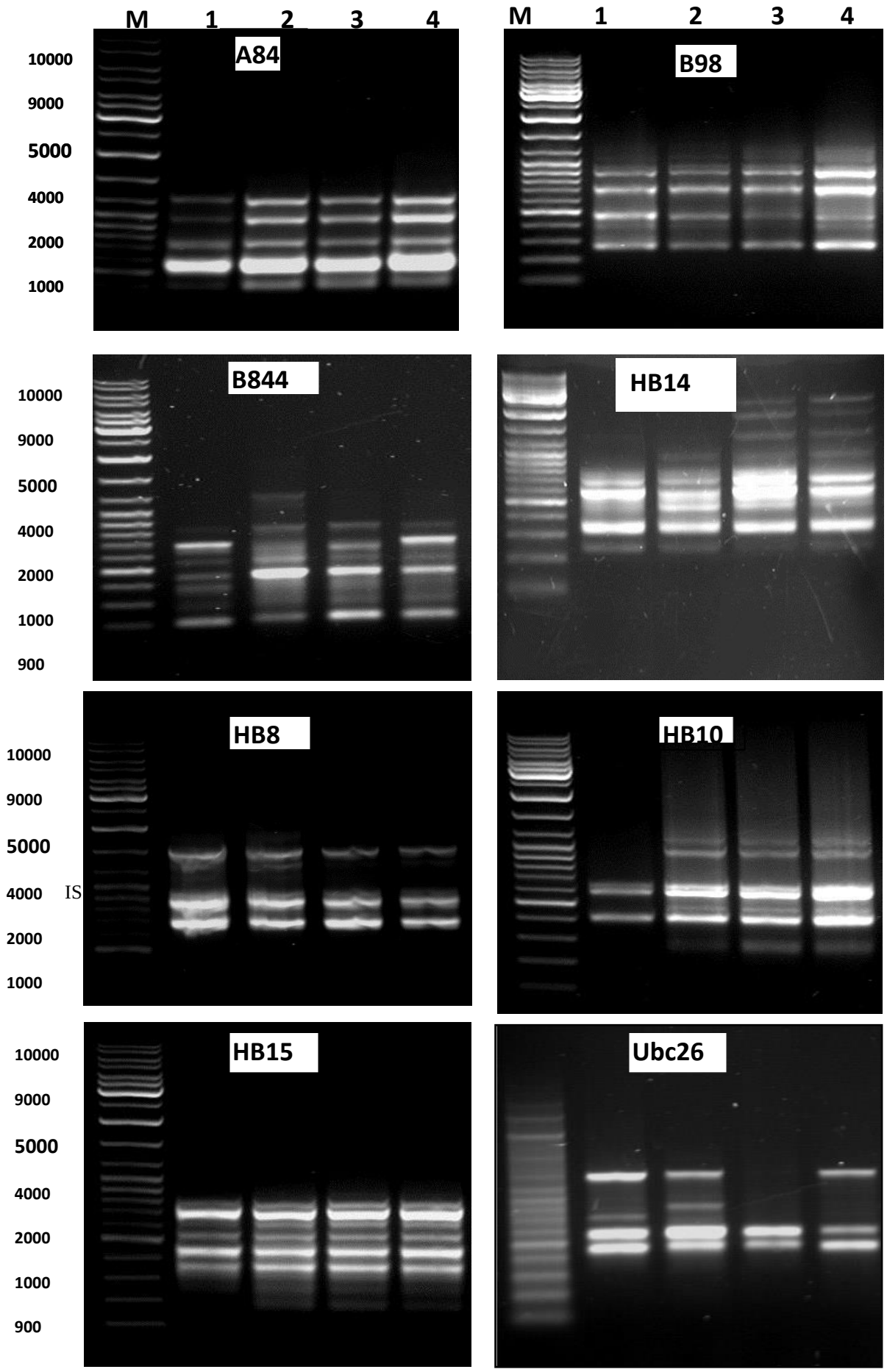


Figure 3. Phenogram demonstrating the relationship among four olives

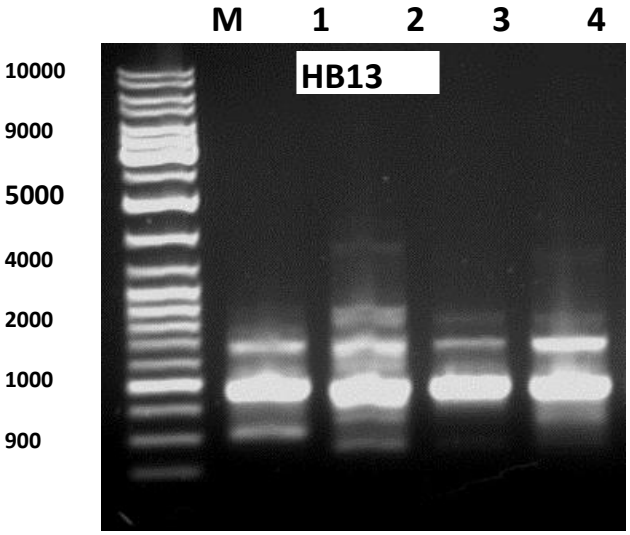
The number of ISSR-PCR fragments generated by using the twelve primers could be used as cultivar specific markers of three cultivars; Manzanillo (A98-2065,A844-445,B844-980,HB10-980-475-280,HB13-905,HB14-940-490,HB15-780-605,Ubc825-315 as a negative markers), Eggizi(HB8-1065,HB13-1075-460-215-165, Ubc26-735,Ubc801-1505-1425 as a positive markers) and Picual(B98-395,HB14-270,Ubc26-1160 as a negative markers).Several authors reported on the usefulness of ISSR for cultivar identifications.

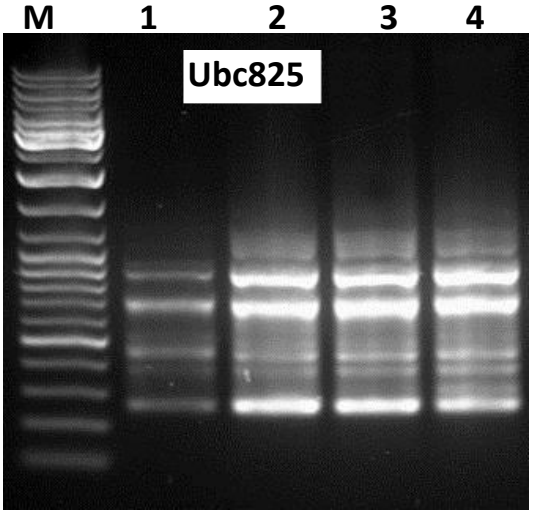
Molecular markers of twelve olive cultivars based on ISSR-PCR using twelve primers.

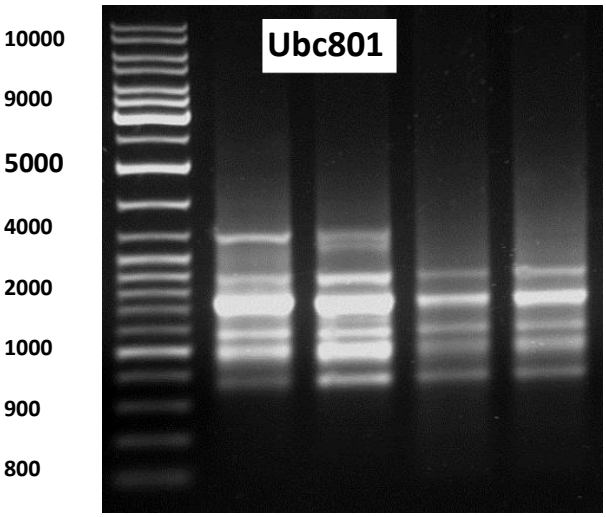



M
1
2
3
4
A84
B98
B844
HB14
HB8
HB10
HB15
Ubc26
10000
9000
5000
4000
2000
1000
900

IS







M DNA marker, 1 cultivar of Manzanillo,
2 cultivar of Eggizi, 3 cultivar of Picual
4 cultivar of Dolce

ISSRs are ideal as markers for genetic mapping and population studies because of their abundance and the high degree of polymorphism between individuals within a population of closely related genotypes (**Lanham and Brennan, 1998**).Molecular markers have been extensively used to derive genetic relationships between olive cultivars(**Besnard ,et al,2001; Gemas et al,2004; Martins lopes et al,2007;Belaj et al.,2003a and b; and Essadki ,et al.,2006**

Moreover, the high level of polymorphism observed in our study was consistent with other comparable studies (**Gemas ,et al., 2004; Martins-Lopes et al., 2007;Gomes, et al., 2008; Sensi, et al.,2003 and Lopes, et al.,2004)**.

ISSR Clustering Analysis: The dendrogram constructed from cluster analysis based on ISSR data is represented in Table (8) and Figure (4). The ISSR Dendrogram obtained by UPGMA analysis grouped the four cultivars into two main clusters. The Jaccard's coefficient ranged from 44% to88 %. The lowest similarity coefficient was observed between two cultivars Manzanillo and Picual cultivar (44 %). While, the highest similarity coefficient was obtained between Dolce and Picual (88 %). Eggizi was ranked in a separated cluster and the other 3 cultivars were clustered into 2 sub-clusters, sub-cluster I comprise done cultivar (Manzanillo), sub-cluster II grouped two cultivars: Picual and Dolce. The results confirm that the olive is a highly variable species which reflect the genetic diversity among olive cultivars. The high diversity found between olive cultivars is probably due to a diverse germplasm origin, which presumably results from crosses between wild and cultivated olives, resulting in new cultivars in different parts of the Mediterranean and low breeding pressures (**Besnard, et al., 2001; Belaj, etal. 2003a and Contento, et al., 2002.**).

Basic oil quality parameters (acidity and peroxides value) were not affected by salinity. No significant differences were found in the oleic acid percentage, while saline water irrigation improves in some varieties olive oil quality increasing antioxidant concentrations.

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