Characterization of some olive cultivars (*Olea europaea* L) in Egypt using isozyme, RAPD and ISSR markers

Aly, M. A.[1]; Ezz, Thanaa M.[1], Rasmy, M. R.[2] and Khodier, Shimaa M.[3]

1 Fac. Agric., Saba Bacha, Alex. Univ.
2 Plant Pathology Research Institute (ARC)
3 Postgraduate student: e-mail: shimaa_khodier@yahoo.com

ABSTRACT

This investigation was carried on nine olive cultivars (*Olea europea*, L.), namely: Chemlaly, Kalamata, Maraky, Egizy, Watikin, Meloky, Hamedy, Manzanillo and Kronaky, grown in the experimental farm, Faculty of Agriculture (Saba-Basha), Alexandria University, Egypt. Isozyme, random amplified polymorphic DNA (RAPD-PCR) and inter simple sequence repeats (ISSR) were used to characterize the nine studied olive cultivars. Three isozyme systems [α- Esterase (α Est), Peroxidase (Prx) and Poly Phenol Oxidase (PPO)] amplified a total of 29 bands of which, 15 bands were polymorphic; ten RAPD primers amplified 436 bands of which 420 were polymorphic; and five ISSR primers amplified 123 bands of which 107 bands were polymorphic. The percentages of polymorphism detected by isozyme, RAPD and ISSR markers were 51.72, 96.3 and 86.9%, respectively. Data were used for clustering analysis to assess the genetic relationships among olive cultivars analyzed.

Keywords: olive, *Olea europaea*, cultivars, Characterization, Egypt isozyme, RAPD, ISSR, markers.

INTRODUCTION

Olives are one of the most extensively cultivated fruit crops in the world. In 2009 there were 9.9 million hectares planted with olive trees, which is more than twice the amount of land devoted to apples, bananas or mangoes, produced about 18.24 million tons (FAO STAT, 2010). Statistics of Egyptian ministry of agriculture for the year 2009 indicated that a total area of about 158,058 feddan are grown to olive of which 90,344 feddan outside the Nile valley as newly reclaimed land. The total production is about 449,009 tons. Distribution of incorrectly labeled olive and the global spread of vegetative propagated cuttings over hundreds of years changing their names have caused the current problem of homonyms and synonyms (Cimato and Attilio, 2008). Moreover, Cavagnaro and Masuelli (2002) evaluated genotypic homogeneity in olive nurseries using RAPD. They found that only one of five Argentine olive nurseries plant materials was in complete genetic homogeneity with its respective materials kept in the germplasm bank of INTA Juin. It is difficult to identify and classify olive (*Olea europaea* L.) varieties, which are estimated as being close to 2,000 in number. Morphological and physiological traits are widely used for this

purpose, but they are strongly dependent on the environmental factor, consequently it is difficult to resolve the issues that arise concerning synonymy and homonymy (Vergari *et al.*, 1999). Phenotype is the combination of individual traits resulting from a genotype and its interaction with the environment. Assessment of phenotypic variation focuses on morphological traits-those characteristics that define the shape and appearance of a set of individuals. Isozymes are used for cultivars identification and to determine the genetic relations between them. The first attempts at the use of biochemical markers for understanding the domestication process in the olive started in the 80s by using isozymes extracted from pollen or from leaves, and they were able to distinguish the polymorphism between the cultivars (Pontikis *et al.*, 1980; Trujillo *et al.*, 1990; Ouazzani *et al.*, 1993). Assessment of genotypic variation is at the level of the DNA molecule responsible for transmitting genetic information. The advantage of using DNA molecular markers in variation assessment that it is not subjected to environmental influences. The major disadvantage is the need for technically more complex equipment (De Vicente and Fulton, 2003). The molecular markers used in the Germplasm Bank of Córdoba (isozymes and RAPDs) have shown to be useful for identification purposes (Belaj *et al.*, 2008). RAPD analysis was able to distinguish clearly between olive cultivars (Perri *et al.*, 1999) which provides a new method for cultivar identification in olive (Trujillo *et al.*, 1999). The use of RAPD primers (Williams *et al.*, 1990) has given environment independent markers. This procedure has been performed in olive to identify varieties, to study olive genetic diversity and used to determine the relationships between varieties (Belaj *et al.*, 2001; Ozkaya *et al.*, 2004; Ganino *et al.*, 2007; Helally, 2008; Durgac *et al.*, 2010; Sheidai *et al.*, 2010). However, RAPD markers proved to be highly effective in discriminating cultivars analysed by (Belaj *et al.*: 2001, Sanz-Cortés *et al.*, 2001 and Lamantia *et al.*, 2006) demonstrating the reliability of RAPDs to identify all studied olive cultivars and to reveal the degree of their relatedness to each other. Furthermore, the results of (Hdeib and Hassawi 2006 and Sesli and Yeğenoğlu 2009) confirmed the usefulness of RAPD markers as a powerful tool for olive genotypic identification. Moreover, Terzopoulos *et al.* (2005) and Kattmah *et al.* (2011) reported that ISSR technique can be used for cultivar differentiation in *O. europaea* L.

The present study aimd to characterize and identify 9 olive cultivars using different characterization and identification approaches (isozymes, RAPD-PCR and ISSR). Also, to establish the relative relatedness between each of these varieties.

MATERIALS AND METHODS

Nine olive cultivars (*Olea europea*, L.) namely Chemlaly, Kalamata, Maraky, Egizy, Watikin, Meloky, Hamedy, Manzanillo and Kronaky, grown in the experimental farm, Faculty of Agriculture (Saba-Basha), Alexandria University, were used for this experiment. Polyacrylamide gel electrophoresis (Native-PAGE) was used to study different isozyme variations. Peroxidase (Prx), α-esterases (α Est) and Poly Phenol Oxidase (PPO) were prepared from young leaves and were separated in 9% (pH 8.6) polyacrylamide gel electrophoresis according to (Stegeman *et al.*, 1985).

Total DNA was extracted from 50 mg of frozen leaves using a Bioflux Kit (from china). The gel solution was poured on the vertical slab of the electrophoretic apparatus manufactured by Biorad.

However, RAPD-PCR reactions were performed according to Williams *et al.* (1990). Ten primers (Operon Technologies, Inc) were used in this study. Amplification was carried out by Techni PCR model 512 programmed as reported by (Sambrook *et al.*, 1989). The run was performed for one hour at 100 volt using Biometra gel electrophoresis submarine (20 cm x l0 cm). Bands were detected on UV-transilluminator and photographed by gel documentation system (Biometra Bio Doc Analyze).

ISSR - PCR reactions were conducted using 5 specific primers. Amplification was carried out by Techni PCR model 512 which was programmed as follows: Denaturation (one cycle) 94°C for 2 min, followed by 45 cycles: as follows 94°C for 30 sec, 44°C for 45 sec, 72°C for 1.5 min and finally one cycle at 72°C for 7 min, and 4°C (infinitive). PCR- products (15 µl) were resolved in 1.5 % agarose gel electrophoresis with 1X TAE running buffer. The run was performed at 80 V for 3 h and the gel was stained with Ethidium Bromide (Pasqualone *et al.*, 2001). A 1- Kb plus DNA Ladder (750 ng /3 µl) (GeNetBio) was used which was separated into forty bands ranging from 12000 to 100 bp. Bands were detected on UV-transilluminator and photographed by gel documentation system (BioRad Bio Doc Analyze). All DNA electrophoretic patterns were analyzed by EgyGeneGelAnalyzer version three software to determine relative mobility (RF), molecular size by base pairs (bp) and presence (1) or absence (0) of each fragment.

RESULTS AND DISCUSSION

Isozyme markers

Three isozyme systems including α- Esterase (αEst), Peroxidase (Prx) and Poly Phenol Oxidase (PPO) were used to test the genetic variability among the studied cultivars. As can be seen from the results in

Table 1, a total of 29 bands were detected using the three isozyme systems, 14 bands were monomrphic and the other 15 bands were polymorphic among all studied olive cultivars. The obtained results were as follow: generally, α-esterase exerted highly polymorphic patterns among the studied cultivars. Duarte *et al.* (2007) stated that esterases are recommended for hydrolyses of ester bond of lipid so as to alleviate energy level for other metabolisms especially under drought conditions. Two unique bands were found (no. 2 and 4). Unique band no.2 belonged to the cultivar Chemlaly while unique band no.4 belonged to cultivar Watikin. Potes *et al.* (1999) observed polymorphism in pollen of the cultivars 'Azeitoneira' (synAzeiteira), 'Cordovil de Serpa', 'Galega Vulgar'. Zymograms for EST revealed patterns consisting of several bands, allowing differentiation among the three *O. europaea* cvs.

Regarding Peroxidase electrophoresis, patterns, resulted peroxidase patterns of the nine olive cultivars revealed a high polymorphism. Among the studied cultivars all bands (four bands) were polymorphic. This peroxidase patterns showed a unique band (no.4) for the cultivar Kronaky. These results are in agreement with the results of Seker *et al.* (2008) who found that gel electrophoresis for Peroxidase (PRX) isozymes allowed differentiation among 43 olive genotypes. Whereas, Potes *et al.* (1999) observed no reaction indicating of PRX in any of olive cultivars examined.

Poly phenol oxidase (PPO) electrophoresis showed highly polymorphic pattern among the studied cultivars. Results indicated that the pattern revealed a total of four bands. No monomorphic bands were found. All of the bands were polymorphic. Based on the three isozyme systems, polymorphism percentages were generated Table (1). The highest polymorphism percentages were 100% by Prx and PPO while the lowest polymorphism percentage was 83.3% by α-Est. These results are in agreement with the results of Belaj *et al.* (2008) and Seker *et al.* (2008) who found that the isozymes markers were useful for identification purposes in olive cultivars.

Based on isozyme polymorphisms, similarity matrix was developed by SPSS computer package system in Table (1). The closest relationship was scored between Hamedy and Manzanillo with similarity %100, followed by Chemlaly and Egizy which had a similarity of 92.3%, while the lowest was found between Hamedy and Kronaky and the same between Manzanillo and Kronaky with similarity of 18.2%.

The dendrogram based on isozyme separated the nine cultivars into two main clusters. Hamedy, Manzanillo and Maraky were clustered together. While remain cultivars clustered in the second cluster as shown in Fig. (1).

Table (1): Polymorphism percentages and number of monomorphic and polymorphic bands generated by three isozyme systems in the nine olive cultivars

Type of isozyme	No of monomorphic bands	No. of polymorphic bands	Total bands	% polymorphism
α-Est	1	5	6	83.3%
Prx	0	4	4	100%
PPO	0	4	4	100%
Total	14	15	29	51.72%

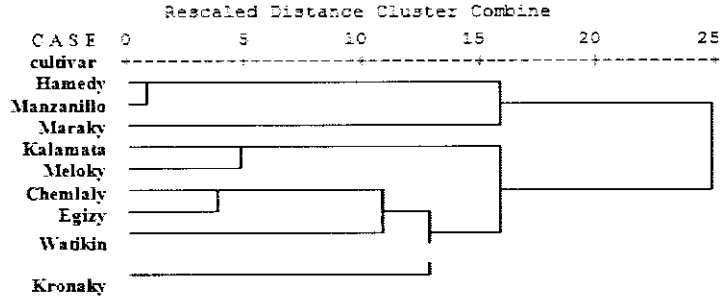

Fig. (1): Dendrogram based on three isozyme analysis among nine olive cultivars

RAPD–PCR analysis

Ten RAPD primers were used in the present study in Table (2). They amplified 436 bands of which 420 bands were polymorphic (96.3% of polymorphism) as shown in Table (3). The highest polymorphism percentage (100%) could be observed in primers OPC-01, OPC-02 (Fig.2), OPC-03, OPC-04, OPE-02 and OPE-04. That was followed by primers OPE-01 and OPE-05 which had polymorphism percentage of 98.3% and 98%, while the lowest polymorphism percentage (52.2%) were observed for primer OPC-05. In general, the results indicated that RAPD-PCR markers gave adequate distinctions among the nine olive cultivars. These results were in partial agreements with the finding of Fabbri *et al.*, (1995) they screened seventeen olive (*Olea europaea* L.) using (RAPD) markers. They found a high degree of polymorphism is evident in the olive cultivars.

The dendrogram based on RAPD separated the nine cultivars into two main clusters. Manzanillo and Kronaky were clustered together in one subcluster. While remain cultivars clustered in the second cluster. The closest relationship was found between Kalamata and Maraky as shown in Fig. (3). The results agree with the results of Belaj *et al.* (2001) and Sanz-Cortés *et al.* (2001) which revealed that adequate use of RAPD technology offers a valuable tool to distinguish between olive cultivars. Also, these results agree with the results of, Sheidai *et al.* (2007), Belaj *et al.* (2008), Ganino and Fabbri (2008), Helaliy (2008), Sesli and Yeğenoğlu (2009), Durgac *et al.* (2010), Sesli and Yeğenoğlu (2010) Who demonstrated the reliability of RAPDs to identify all studied olive cultivars and to reveal the degree of their relatedness to each other.

Furthermore, primer1 (OPC-01) gave 32 unique bands. As shown in Table (4), the fragment size of unique bands ranged from 200 to 1550 bp. Chemlaly could be distinguished from other cultivars by the presence of three unique bands at fragment size of 835, ٦٢٩ and 509 bp. Also, the other cultivars could be distinguished from the others by the presence of unique bands. These unique bands could be considered as specific markers for each cultivar.

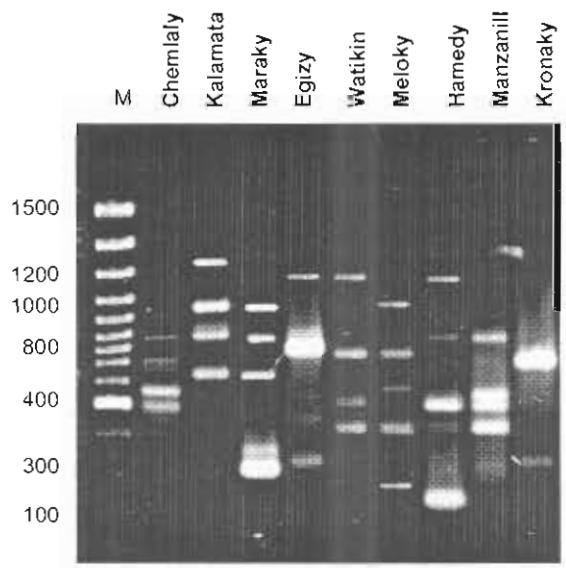

Fig. (2): DNA profile generated by primer 2 (OPC-02) among nine olive cultivars

Table (2): Primers code and nucleotide sequences.

No.	Primer code	Sequences
1	OPC-01	TTCGAGCCAG
2	OPC-02	GTGAGGCGTC
3	OPC-03	GGGGGTCTTT
4	OPC-04	CCGCATCTAC
5	OPC-05	GATGACCGCC
6	OPE-01	CCCAAGGTCC
7	OPE-02	GGTGCGGGAA
8	OPE-03	CCAGATGCAC
9	OPE-04	GTGACATGCC
10	OPE-05	TCAGGGAGGT

Table (3): Polymorphism percentages, number of monomorphic and polymorphic bands detected by each primer (Kits C and E) among nine olive cultivars

No.	Kit	Primer	Monomorphic Bands	Polymorphic bands	Tota l	Polymorphism %	Unique Bands
1		OPC-01	0	39	39	100%	32
2		OPC-02	0	40	40	100%	36
3	C	OPC-03	0	31	31	100%	16
4		OPC-04	0	47	47	100%	33
5		OPC-05	11	12	23	52.2%	9
6		OPE-01	1	58	59	98.3%	44
7		OPE-02	0	51	51	100%	35
8	E	OPE-03	3	39	42	92.9%	28
9		OPE-04	0	53	53	100%	30
10		OPE-05	1	50	51	98%	37
Total			16	420	436	96.3%	300

Table (4): Molecular weight (bp) of unique bands of the nine olive cultivars in RAPD profile of primer OPC-01

Chemlaly	Kalamata	Maraky	Egizy	Watikin	Meloky	Hamedy	Manzanillo	Kronaky
٨٢٥	٨٦٠	٤١٠	١٥٥١	1396	783	1364	1060	--
١٢٩	514	297	١٤٥٤	1123	699	922	806	--
509			562	354	390	608	671	--
				316	329	535	492	--
					307	452	303	--
						362		--
						314		--
						200		--

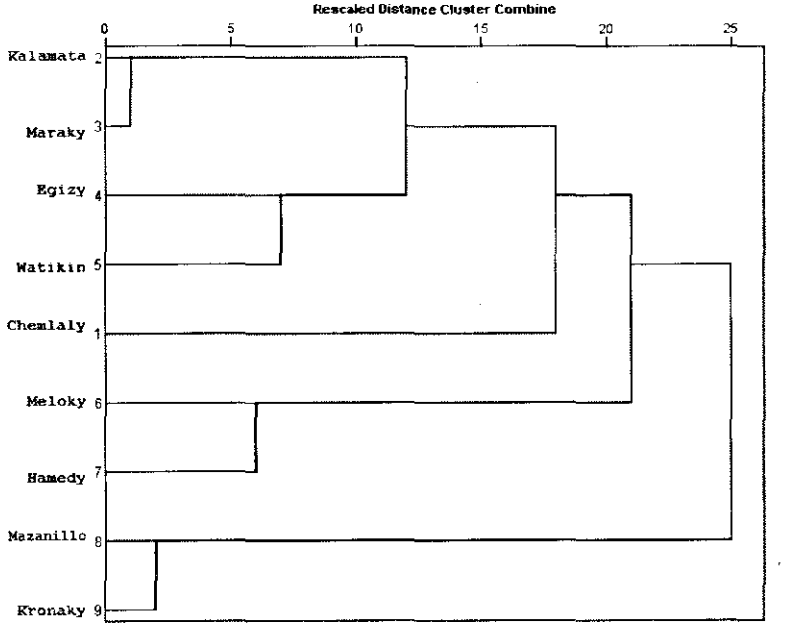

Fig. (3): Dendrogram based on ten RAPD primer profiles among nine olive cultivars

ISSR-PCR amplification analysis

Five ISSR primers were used in the present study to identify nine cultivars of olive as shown in Table (4). Sixteen monomorphic and 107 polymorphic distinct fragments (86.9% of polymorphism) were revealed in the nine tested cultivars with these primers. The results showed that primers B17898, B17899 and HB15 were highly polymorphic (100%, 92.5%

and 91.1% polymorphism, respectively). On the other hand, primers A844 and B844 showed the lowest polymorphism (25%). The overall results of five primers were illustrated in Table (4). Primers HB15 and B17899 gave the highest total number of bands 45 and 40 bands. While, primers A844 and B844 gave the lowest total number of bands (8 and 4 bands). Moreover, seventy three out of 123 ISSR markers were found to be cultivar specific .These markers were scored for the presence of unique bands for a given species. As shown in Table (4), the highest number of unique bands (27 bands) which scored one or more unique bands for each cultivar was found with the primer HB15 (Fig. 4) while primer B844 gave no unique bands. The ISSR marker technique was useful in identifying most of these cultivars by one or more unique DNA band. In general, the result indicated that ISSR markers gave adequate distinguishing among studied olive cultivars.

These results were in partial agreements with the finding of Wolfe and Morgan-Richards (1998) who reported that ISSR markers have recently become widely used in species and population studies due to high variability, less investment of time, money and labor than other methods. Also, Pasqualone *et al.* (2001) found that ISSR markers were effective in discriminating between ten olive studied cultivars. Moreover, the present results agreed with the result of Terzopoulos *et al.*, (2005) who used two inter-simple sequence repeat (ISSR) markers for the differentiation of 31 *Olea europaea* L. cultivars grown in Greece. Both ISSR primers showed high degrees of polymorphism. They suggested that their results along with those of other researchers show that ISSRs can be used for cultivar differentiation in *O. europaea* L. the results agreed with the results of Essadki *et al.* (2006). Also, Martins-Lopes *et al.* (2007) screened thirty Portuguese and eight foreign olive (*Olea europaea* L.) cultivars using Inter-Simple Sequence Repeat (ISSR) markers. The percentage of polymorphic bands detected by ISSR was 88%. Seven ISSR primers were able to distinguish individually all 38 olive cultivars. Moreover, Kattmah *et al.* (2011) conducted a fingerprint experiment using ISSR technique on eight phenotypes of cultivated wild olive in Mesief region –Hamah Province, Syria. They concluded that ISSR technique was able to discriminate among the wild types included in the study.

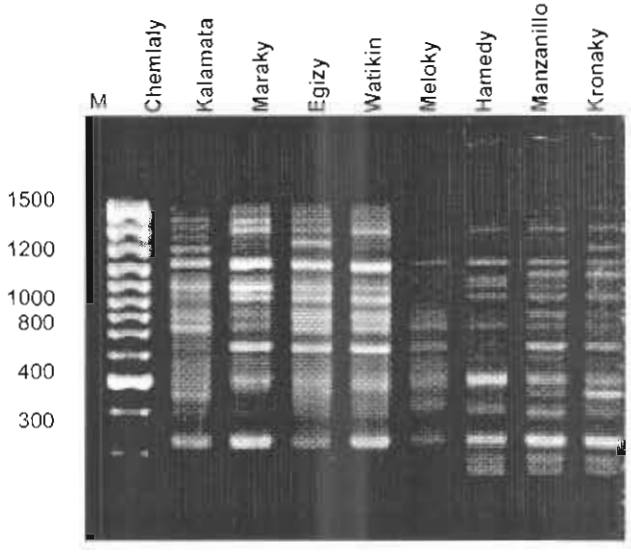

Fig. (4): DNA profile generated by primer HB15 among the nine olive cultivars

Based on ISSR polymorphisms, similarity matrix was developed by SPSS computer package system. The closest relationship was scored between Maraky and Egizy with similarity 74.3%. That was followed by Chmelaly and Kalamata with similarity 67.6%. Moreover, cultivars Maraky and Kalamata showed 66.7% similarity. While Watikin and Kronaky gave the lowest similarity of 33.3% which were considered not closely related cultivars.

The dendrogram based on ISSR (Fig.5) separated the nine cultivars into two main clusters. Moreover, Kronaky cultivar was separated alone in one cluster and remain cultivars were clustered together.

Table (5): Polymorphism percentages, number of monomorphic and polymorphic bands detected with each ISSR primers among nine olive cultivars

Primer Code No.	No. of monomorphic bands	No. of polymorphic bands	Unique Bands	Total bands	Polymorphism %
HB15	4	41	27	45	91.1%
A844	6	2	1	8	25%
B844	3	1	0	4	25%
B17899	3	37	23	40	92.5%
B17898	0	26	22	26	100%
Total	16	107	73	123	86.9%

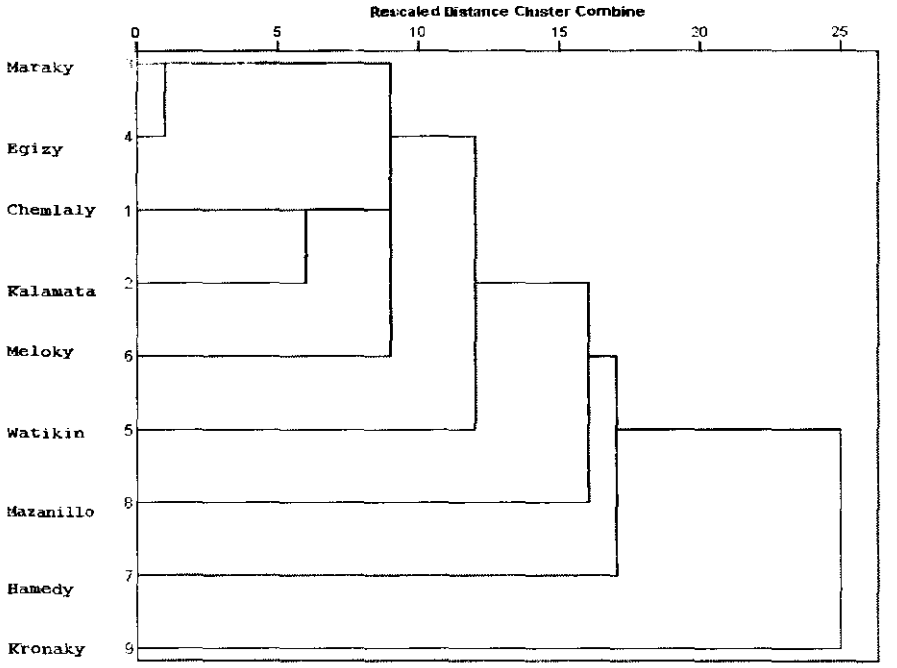

Fig. (5): Dendrogram based on five ISSR primer profiles among nine olive cultivars

Moreover, similarity matrix based on isozymes, RAPD- PCR and ISSR polymorphism among the nine olive cultivars was developed by SPSS computer package system. The closest relationship was scored between Maraky and Egizy with similarity 45.7%. That was followed by Maraky and Kalamata with similarity of 45.1% then 43.5% similarity was found between cultivars Chmelaly and Kalamata. On the other hand, cultivars Chemlaly and Manzanilo gave the lowest similarity 30.3% which were considered not closely related cultivars.

The dendrogram based on isozymes, RAPD and ISSR data (Fig.6) was constructed, clearly indicated that there was correlation between the nine cultivars of olive by clustering them together and gave us another dimension to detect the genetic variability by showing the accurate differentiations and demonstrated significant levels of variation. In this study dendrogram separated the cultivars into two clusters. Cultivars Manzanillo and Kronaky clustered together in one cluster. While the remain cultivars clustered in the other cluster.

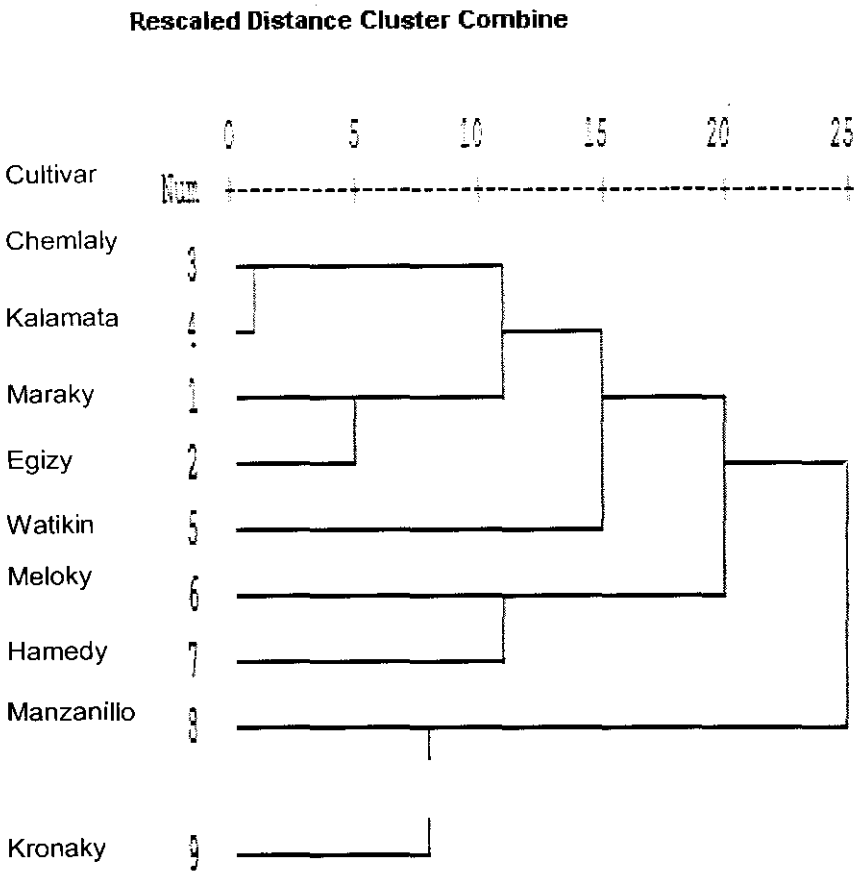

Fig. (6): Dendrogram based on three systems (isozymes, RAPD and ISSR-PCR) of the nine studied olive cultivars.

Finally, the results revealed that the dendrograms obtained from the analysis showed the genetic relationship among the nine studied olive cultivars. The results demonstrated the reliability of isozymes, RAPDs and ISSRs to identify all studied olive cultivars and to reveal the degree of their relatedness to each other. The analysis also reveals the presence of an interesting amount of genetic diversity among the studied individuals.

REFERENCES

Belaj A., Trujillo I., De la Rosa R. and Rallo L. 2001. Polymorphism and discrimination capacity of randomly amplified polymorphic markers in an olive germplasm bank. J. Soc. Hort. Sci., 126 (1): 64-71.

Belaj A., Ojeda A., Muñoz C., Trujillo I., Barranco D., Rallo L., de la Rosa R., Rallo P., Diaz A., Martin A. and Baldoni L. 2008. Molecular markers in olive: An integrated approach. Acta Hort. 791:45-48.

Cavagnaro P. F. and Masuelli R. W. 2002. Evaluation of genotypic homogeneity in olive nurseries using molecular markers. Revista de la Facultad de Ciencias Agrarias, 34(2): 17-26.

Cimato A. and Attilio C. 2008. Conservation, characterization, collection and utilization of the genetic resources in olive. Technical paper by Common Fund for Commodities (CFC), the International Olive Council (IOC) and the Istituto per la Valorizzazione del Legno e delle Specie Arboree (WALSA).

De Vicente M.C. and Fulton T. 2003. Using molecular marker technology in studies on plant genetic diversity. [Online] IPGRI, Rome, Italy and Institute for Genetic Diversity, Ithaca, New York, USA.

Duarte V. M. A., P. S. Garcia, A. B. E Garcia, J. R. Whitaker and Regalado C. T. 2007. Broccoli processing wastes as a source of peroxidase. J. Agri. & Food Chem., 55(25): 10396-10404.

Durgac C., Y. Kiyga and Ulas M. 2010. Comparative molecular analysis of old olive (*Olea europaea* L.) genotypes from Eastern Mediterranean Region of Turkey. Afri. J. Biotech., 9(4): 428-433.

Essadki M., Ouazzani N., Lumaret R. and Moumni M. 2006. ISSR variation in olive-tree cultivars from Morocco and other western countries of the Mediterranean basin. Gen. Res. & Crop Evol., 53(3): 475-482.

Fabbri A., Hormaza J. I. and Polito V. S. 1995. Random amplified polymorphic DNA analysis of olive (*Olea europaea* L.) cultivars. J. Amer. Soc. Sci., 120: 538-542.

FAO Stats 2010. Database. http://faostat.fao.org.

Ganino T. and Fabbri A. 2008. Genetic characterization of *Olea europaea* L. germplasm in northern Italy. Acta Hort., 791:95-102.

Ganino T., Beghè D., Valenti S., Nisi R. and Fabbri A. 2007. RAPD and SSR markers for characterization and identification of ancient cultivars of *Olea europaea* L. in the Emilia region, Northern Italy. Gen. Res. & Crop Evol., 54(7): 1531-1540.

Hdeib T. M. and Hassawi D. S. 2006. Genetic analysis of Jordanian olive (*Olea europea* L.) using random amplified polymorphic DNA (RAPD). Jord. J. Agri. Sci., 2(2): 95-102.

Helally A. M. 2008. Evaluation and identification of nine olive cultivars (*Olea europaea* L.) grown in Nobaria region of Egypt using agrobbiological traits and RAPD markers. Ph.D Thesis, Alex. University, Fac. Agri., Egypt.

Kattmah G., Hamed F. and Makhool S. 2011. Studying the genetic relationship of some cultivated wild olive types in Mesiaf region by using ISSR technique. Jord. J. Agri. Sci. (in Arabic), 7(1):139-147.

Lamantia M., Guerin J., Sedgley M. and Barone E. 2006. Identification of olive (*Olea europea* L.) genotypes using SSR and RAPD markers. Actes Editions, Rabat, 9-14.

Martins-Lopes P., Lima-Brito J., Gomes S., Meirinhos J., Santos L. And Guedes-Pinto H. 2007. RAPD and ISSR molecular markers in *Olea europaea* L. genetic variability and molecular cultivar identification. Gen. Res. & Crop Evol., 54(1): 117-128.

Ouazzani N., Lumart R., Villemur P. and Di Giusto F. 1993. Leaf allozyme variation in cultivated and wild olive trees (*Olea europaea* L.). J. Hered., 84(1): 34-42.

Ozkaya M.T., Ergülen E., Ülger S. and Özilbey N. 2004. Genetic and Biologic characterization of some olive (*Olea europaea* L.) cultivars grown in Turkey. Tarim Bilimlerl Dergisi, 10 (2): 231-236.

Pasqualone A., Caponio F. and Blanco A. 2001. Inter-simple sequence repeat DNA markers for identification of drupes from different *Olea europaea* L. cultivars. Eur. Food Res. & Tech., 213 (3): 240-243.

Perri E., Sirianni R., Godino G. and Tartarini S. 1999. Characterization of Itallian olive (*Olea europaea* L.) cultivars by statistical and molecular methods. Acta Hort., 474: 489-493.

Pontikis C.A., Loukas M. and Kousounis G. 1980. The use of biochemical markers to distinguish olive cultivars. The J. Hort. Sci. & Biotech., 55(4): 333-344.

Potes M. F., Leitao F., Serrano J. F. and Ivone Clara M. 1999. Preliminary studies on isoenzyme polymorphism in cvs. Of *Olea europaea* and *Olea oleaster.* Acta Hort., 474: 499-504.

Sambrook J., Fritschi E. F. and Maniatis T. 1989. Molecular cloning: a laboratory manual, Cold Spring Harbor Laboratory Press, New York.

Sanz-Cortés F., Badenes M. L., Íñiguez A. and Llácer G. 2001. Molecular characterization of olive cultivars using RAPD markers. J. Amer. Soc. Hort. Sci., 126(1): 7-12.

Seker M., Dulger S. and Kaynas N. 2008. Investigation of genetic diversity in olive (*olea europaea* l.) Cultivars and types using isozyme analysis. Acta Hort., 791:165-171.

Sesli M. and Yeğenoğlu E. D. 2009. RAPD-PCR analysis of cultured type olives in Turkey. Afri. J. Biotech., 8(15): 3418-3423.

Sesli M. and Yeğenoğlu E. D. 2010. Comparison of Manzanilla and wild type olives by RAPD-PCR analysis. Afri. J. Biotech., 9(7): 986-990.

Sheidai M., Vaezi-Joze S., Shahhriari Z. H., Noormohammadi Z., Parsian H. and Farahanei F. 2007. Study of genetic diversity in some olive (*Olea europea* L.) cultivars by using RAPD markers. Pakistan J. Bio. Sci., 10(17): 2972-2975.

Sheidai M., Noormohammadib Z., Dehghania A., Parvinia F., Hoshiar-Parsiana H. and Hosseini-Mazinanic M. 2010. Intra-specific morphological and molecular diversity in brown olive (*Olea cuspidata*) of Iran. Science Asia, 36: 187–193.

Stegemann H., A.M. Afify and K.R. Hussein 1985. Cultivar identification of dates (*Phoenix dactylifera*) by protein patterns. Proceeding of 2nd International Symposium Biochemical Approaches Identification of cultivars, p.44, Brounschweig, Germany.

Terzopoulos P. J., Kolano B., Bebeli P. J., Kaltsikes P. J. and Metzidakis I. 2005. Identification of *Olea europaea* L. cultivars using inter-simple sequence repeat markers. Sci. Hort., 105(1): 45-51.

Trujillo I., Rallo L., Carbonell E.A. and Asins M.J. 1990. Isoenzymatic variability of olive cultivars according to their origin. Acta Hort., 286:137-139.

Trujillo I., De la Rosa R., Rallo L. and Belaj A. 1999. Selection of RAPD markers for olive (*Olea europaea* L.) cultivars identification. Acta Hort., 474: 495-498.

Vergari G., Patumi M. and Fontanazza G. 1999. Varietal discrimination of 15 genotypes of olive (*Olea europaea* L.) belonging to cv. Manzailla. Acta Hort., 474: 141-145.

Williams, J. G. K., Kubelik A. R., Livak K. J., Rafalski J.A. and Tingey S.V. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acid Res., 18: 6531-6535.

Wolff, K. and Morgan-Richards M. 1998. PCR markers distinguish plantago major subspecies. Theo. Appl. Gen., 96: 282-286.

الملخص العربي

توصيف بعض أصناف الزيتون بمصر باستخدام تقنيات الisozyme وال RAPD و الISSR

محمود أحمد علي[١]، ثناء مصطفى عز[١]، محمد رفعت رسمي[٢] ،
شيماء مصطفى خضير[٣]
١ كلية الزراعة سابا باشا جامعة الاسكندرية
٢ معهد بحوث أمراض النبات (مركز البحوث الزراعية)
٣ طالبة دراسات عليا- كلية الزراعة سابا باشا

أجريت هذه الدراسة على تسعة أصناف من الزيتون (*Olea europea*, L.) وهي شملالي، كلاماتا، مراقي، عجيزي، وطيقين، ملوكي، حامضي، منزانيلو وكروناكي النامية في المزرعة التجريبية لكلية زراعة سابا باشا، جامعة الإسكندرية، مصر لاختبار الاختلافات الوراثية وتوصيف وتمييز أصناف الزيتون محل الدراسة. أستخدمت كل من طرق التوصيف البيوكميائية (باستخدام مشابهات الانزيمات isozymes) والطرق الجزيئية باستخدام كل من تكنيك RAPD و ISSR. استخدمت ثلاثة أنظمة انزيمية (α استريز، بيروكسيديز وبولي فينول أوكسيديز) انتجت ٢٩ حزمة منها ١٥حزمة polymorphic، عشرة بوادئ RAPD انتجت ٤٣٦ حزمة منها ٤٢٠ حزمة polymorphic، وخمسة بوادئ ISSR انتجت ١٢٣حزمة منها ١٠٧ polymorphic. النسب المئوية للاختلافات التي وجدت باستخدام مشابهات الانزيمات وتحليل الRAPD و ISSR كانت٥١,٧٢ ، ٩٦,٣ و ٨٦,٩ ٪ على التوالي. استخدمت البيانات المتحصل عليها من كل تحليل في عمل التحليل العنقودي لتقدير العلاقات الوراثية بين أصناف الزيتون التسعة محل الدراسة.