Nat. Volatiles & Essent. Oils, 2021; 8(4): 4723-4732

Identification Of Some Olive (Olea Europaea L.) Cultivars Cultivated In North Of Iraq By Using (RAPD) Markers

[1]Muna O. M. Shehab, [2]Aamer M. Al-Ma'thidy & [3]Talal Taha Ali

[1,2]Biological Department/ College of Education of Pure Science/ University of Mosul/Iraq

[3]. Ministry of Agriculture/Iraq

[1]muna.omar@uomosul.edu.iq, [2]dr.aamer@uomosul.edu.iq&[3]talal_ali612000@yahoo.com

Abstract

In this study the molecular Identification of (6) cultivars Olive (Olea europaea L.) namely "Cormal", Santa Catrina", Khoderiy", "Ducal", "Sorani", Baashiky" used Random Amplified polymorphic DNA (RAPD) markers for identifying the genotype relation among cultivars were analyzed with (10) RAPD which generated (360) random bands. Some of them were variant, and other were distinct. Fragment size ranged from 170- 1737 bp.

The data were enteredinto the computer, and the privates statistical application NTSYS- PC wasutilized for this sort of study,which revealed that the cultivars genetic distance was in the range of (0.191-0.406).The distancebetween "Baashiky" and "Sorani"cultivars has been the greatest (0.406),whereas the distance between "Khoderiy" and "Santa Catrina"cultivarswas the smallest(0.191). UPGMA (i.e. Unweighted pair Group Method Analysis) yielded a dendrogram and similarity matrix, which revealed the cultivars had been separated into sub-major and major groups clustares based on the genetic distance value.

Introduction

Olive (Olea europaea L.) is considered a long lived ever-green tree native to Mediterranean region and the most significant fruit tree species for oil production. (Peyandi et al., 2013; Kangarloo et al., 2016; Kaya, 2015). The technology of the DNA marker is a valuable area of the bio-technology that may significantly improve effectiveness of plant breeding procedures and is a highly significant tool for plant scientists in specifying cultivar similarities and phylogenies (Li,et al., 2001). To increase genetic sources and the understanding regarding their evolution history, one needs to identify genetic links between the wild olives and their cultivated relative types (Llkici and Kockar, 2003; Motawei et al., 2007; Balduni et al., 2006). Various DNA-based marker approaches, like (RFLP, RAPD, SSR), might be used for detecting plant genetic morphism (Owen et al., 2005; Sanz-Cortes et al., 2003). RAPD can be dexcribed as a simple and widely utilized molecular approach for screening genome of living organisms with the use of any primer (Mcclelland and Welsh, 1990; Willams et al., 1993). Many researches have used the approach to efficiently identify various olive varieties (Belaj et al., 2001;Fabbri et al., 1995;

Dil and Sesli, 2009; Yegenoglu and Sesli, 2010; Omar et al, 2012).Hassan et al., (2016) used the (RAPD) marker for assessing the genetic stability related to micro propagated Olive (Olea europaea L.) cultivars. They found that a total of 6 decamer (RAPD) primers have given (39) unique and reproducible bands that range from go to 1500bp. RAPD has been efficiently utilized for the detection of the genetic polymorphism and similarity in both oleasters as well as the cultivated kinds of the olives, indicating that DNA-based markers are adequate for the detection of the polymorphism (Claros et al., 2000;Gemas et al., 2000;Mekuria et al., 2004).

Rallo et al., (2003) reported the molecular study of olive species O. cnspidata, O. crysophilla and O. ferruginae.

The dendrogram obtained according to simple sequence Repeats (SSR) markers showed some degree of genetic difference and joined each other with some distance, indicating different olive forms.

Grati-kamoun et al, (2006) used AFLP markers for characterizing 29 Olive (Olea europaea L.) cultivars,which includethe oil and table olive cultivars, from Tunis and other Mediterranean nations. They generated 410AFLP markers,of which 172 indicated polymorphism.Owen et al., (2005) identify 65 Olive genotypes, which include the majority of main cultivars from Grece, Middle East and Turkey, and few genotypes from western Mediterranean. They were able to get a total of (119). The polymorphismratio for polymorphic markers derived from 5 selective AFLP Primer-Pair combinations was 41.5%.Sensi et al., (2003) used (AFLP) with 6 sets of primers to differentiate 12 Olive cultivars of Italy, totaling (274) markers. This research aimed at finding and determining genetic links between olive cultivars that are grown in Iraq's northwestern region.

Material and Method

1. Plant Material

Healthy fresh leaves of the olive tree were collected from six cultivars cultivated from a different location in the North of Iraq and used for DNA extraction (Table 1).

Table (1) RAPD Primer sequences used for amplification

Primers	Sequences 5' 3'	Resource
OPA1	CAGGCCCTTC	Muzher et al.,(2014)
OPA4	AATCGGGCTG	Teng et al.,(2002)
OPA10	GTGATCGCAG	Lu et al.,(1996)
OPA12	TCGGCGATAG	
OPA19	CAAACGTCGG	
OPH14	ACCAGGTTGG	

OPH16	TCTCAGCTGG	
OPH17	CACTCTCCTC	
OPW7	CTGGACGTCA	
OPW11	CTGATGCGTG	

2. Extraction of the DNA

Total genomic DNA has been obtained based on the kit method (Obtained from the Korean company FAVORGEN/ Biotech. CORP), and Laboratory work was carried out in the RNA lab; the amount of DNA was 50µL/90 mg. The purity ranged between (1.6- 1.8) measured by the Nanodrop device at a wavelength of 260- 280 nm, after which (5) µL of the extracted DNA was migrated into agarose gel at a concentration of 1% as shown in figure (1)

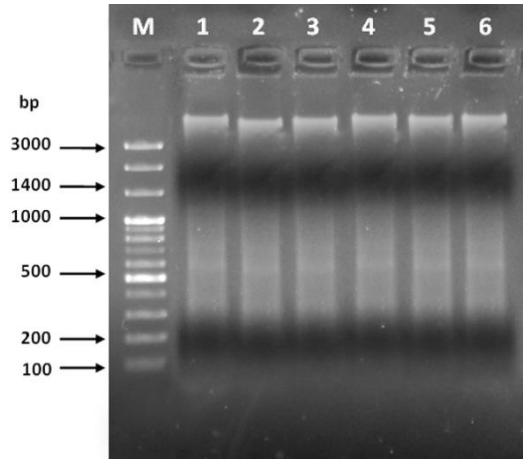


Figure (1) Genomic DNA isolated from the studied Olvie Olea europaea L. cultivars.

1."Cormal" 2. "Santa Catrina" 3. "Khoderiy" 4. "Ducal" 5. "Sorani" 6."Baashiky".

3. Analysis of DNA

(10) Randomly amplified of primers are given in table (1) Supplied by the Korean company macrogen.

The volumes and concentrations of the components of the amplification mixture were determined according to the instructions of the Korean manufacture (Macrogen), and random amplification reactions were performed (Teingey and Tufo Del, 1993).

Then the migration process was carried out on agarose gel and stained with ethidium bromide dye for (20-30) minutes, then, the gel was exposed to a UV. Transiluminature, then the molecular weights were estimated based on the distance traveled by the bands within the gel in comparison with the DNA Ladder.

4. Statistical Analysis:

The data obtained from the RAPD markers that appeared on the agarose gel were analyzed by giving the symbol (1) for the presence of the band and the symbol (0) for its absence, and they were arranged in a

sequential table, then all data were converted to genetic similarity values with utilizing Jaccard's Coefficient and using statistical program (Numerical Taxonomy and Multivar Analysis System) the genetic divergence coefficient was determined using multivariate cluster analysis according to the UN weighted Pair Group Mean Average method (UPGMA) within the same statistical Program (Nei and Li, 1979).

Then a genetic divergence tree that embodies the genetic relationship between these cultivars was determined, and the primer efficiency percentage was calculated using the equation mentioned by (Grudman et al., 1995).

Primers proficiency = (Number of bands for each one of the primer/ Total number of amplification bands for every primer) × 100.

As for the discriminatory ability of each primer, it was renewed according to following eq:

Discriminatory ability =(Number of divergent bands per primer/Total amount of divergent bands for all of the primers) ×100.

Result and Discussion

Primers were evaluated for amplification regarding all cultivar genotypes in RAPD analysis (10). Each primer produced an amplification result that was reproducible and scorable. Codes, number of the polymorphic parts, total amount of amplification fragments from (6) genotypes cultivars, primer proficiency, and discriminatory capacity for each primer are shown in table 2.

Table (2) List of the RAPD primer in addition to their sequences band size, number of monomorphic, polymorphic, and total band. From Olive genotypes

no.	Primer	Polymorphic bands	Monomorphic bands	Total bands	Polymorphism ratio (%)	Primer Proficiency	Discriminatory ability	Band size
1	**OPA- 1**	29	6	35	88.88	9.72	13.42	236-1000
2	**OPA-4**	19	18	37	66.66	10.28	8.80	170-1000
3	**OPA-10**	21	24	45	63.64	12.50	9.72	220-900
4	**OPA-12**	19	12	31	75.0	8.61	8.80	200-1160
5	**OPA- 19**	29	12	41	81.82	11.39	13.42	200-1450
6	**OPH- 14**	9	18	27	50.0	7.50	4.17	282-1160
7	**OPH- 16**	15	6	21	83.33	5.83	6.94	380-1240
8	**OPH- 17**	19	24	43	63.64	11.94	8.80	300-1737
9	**OPW- 7**	29	12	41	80.0	11.39	13.42	370-1340
10	**OPW- 11**	27	12	39	80.0	10.83	12.50	242-1272
		216	144	360	73.30	%100	% 100	

Across six Olive genotypes and cultivars, a total of 360 bands have been collected, with 144 monomorphic and 216 polymorphic bands. The amplified fragments that varied in the size from 170 to 1737bp. Each primer had a different amount of DAN pieces, ranging from 31 (OPA-12) to 45 (OPA-10), with an average fragment of (36). An average of (21.60) and (14.4) monomorphic bands were determined in the polymorphic study fragment per prime. Dilsal and Meltem (2009) found 159 highly polymorphic loci in cultured type olives in Turkey, with average of 4.81 scoreable bands per primer, using RAPD-PCR analyses of the cultivars ("Domat", Manzanilla", "Memecik", and "Gemlik"). The number of poly-morphic fragments to a total amount of the amplified fragments ratio ranged from 50% (OPH-14) to 88.88% (OPA-1), with an average of 73.30%. Different numbers of primers were employed in the investigation of various Olive species, revealing different degrees of polymorphism.

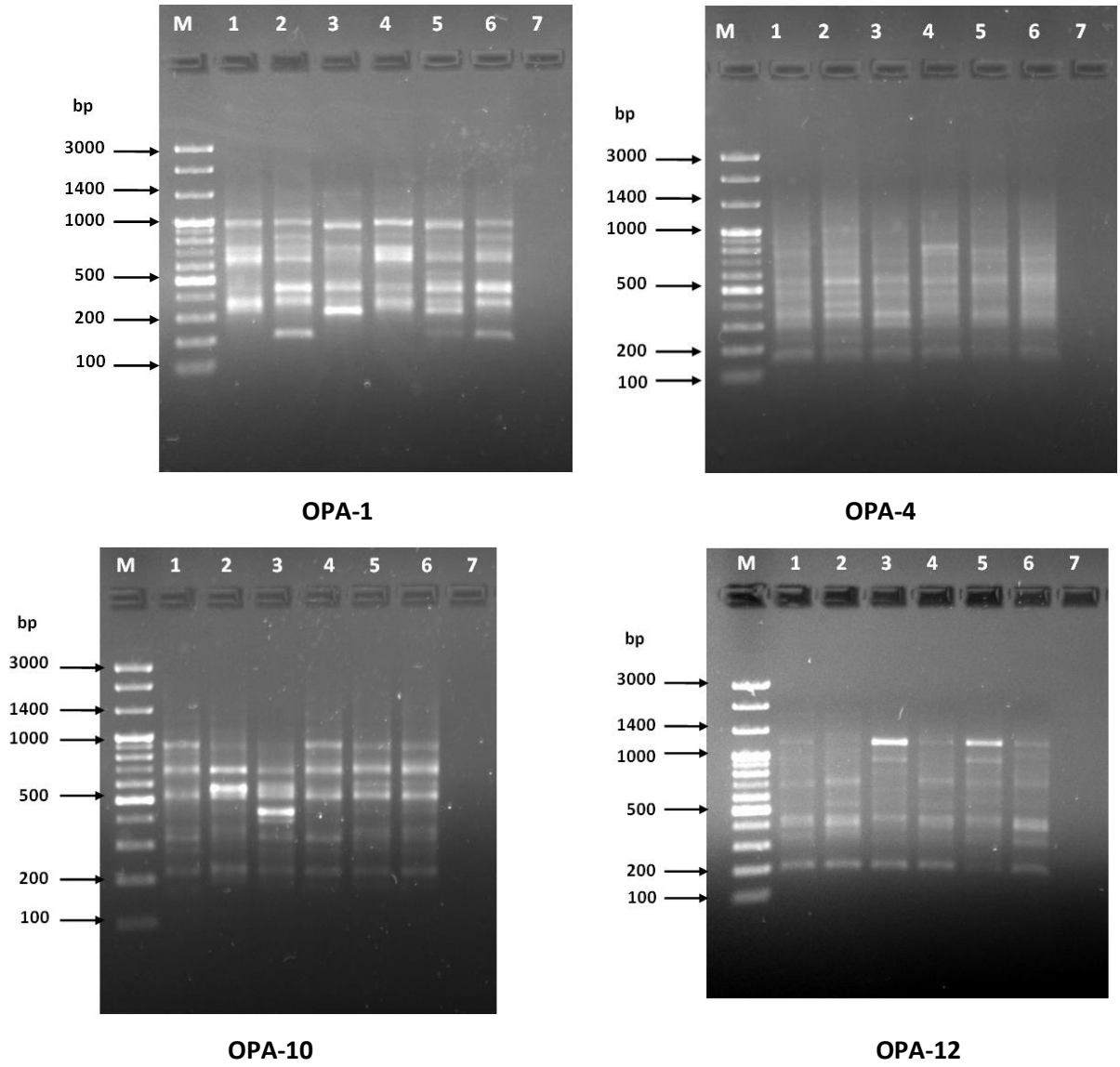
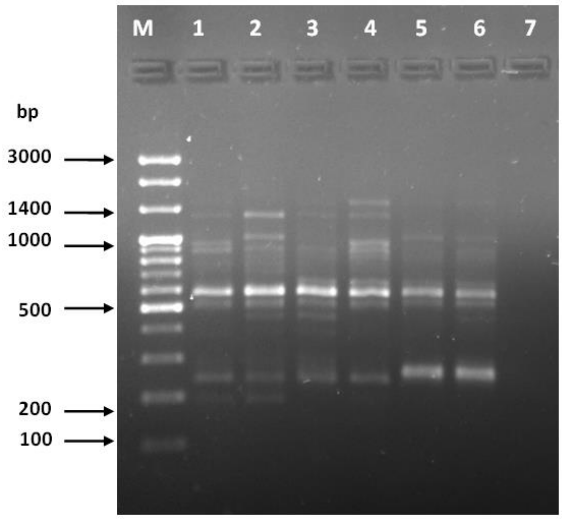
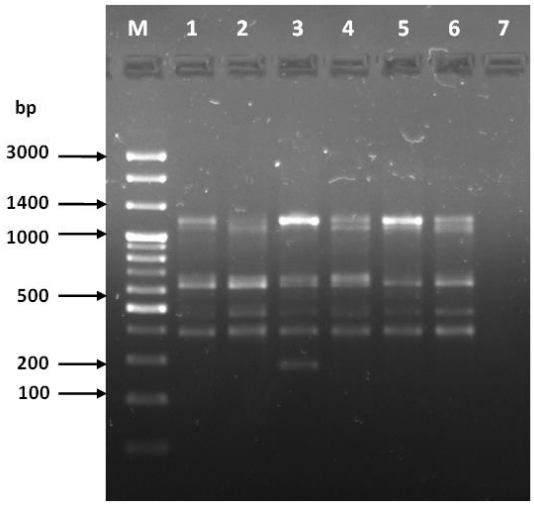


Figure (2) RAPD profiles of six cultivars Olive genotypes with primers.

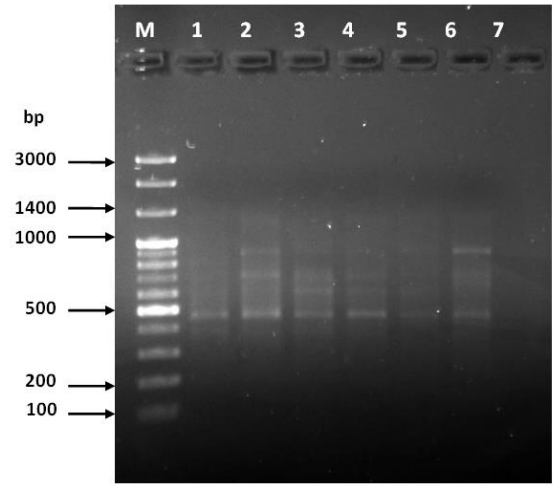
1. "Cormal" 2."Santa Ctrina" 3."Khoderiy" 4."Ducal" 5. "Sorani" 6. "Baashiky".



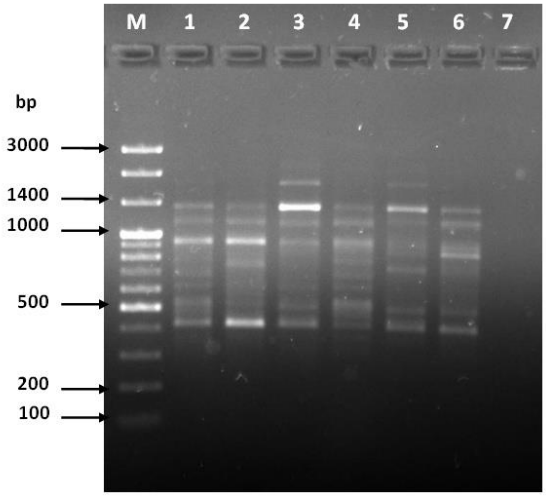
OPA-19



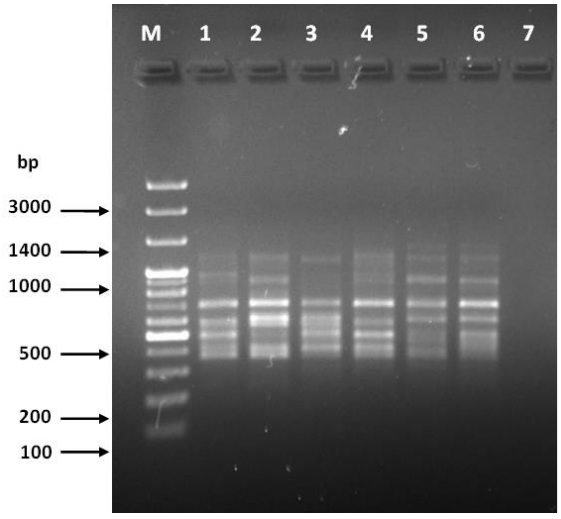
OPH-14



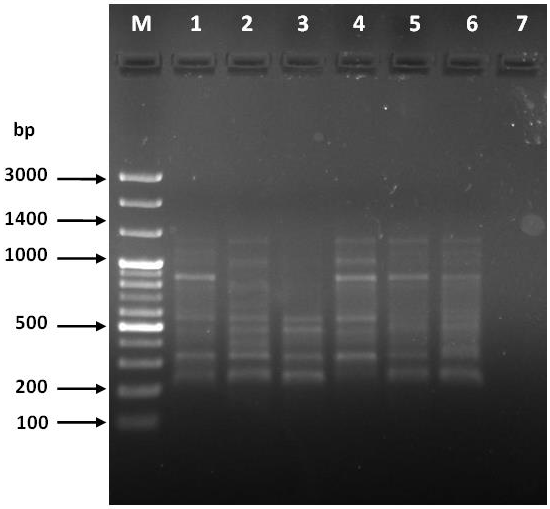
OPH-16



OPH-17



OPW-7



OPW-11

Cont. Figure (2)

The primer proficiency ranged between 5-83 (OPH-16) and 12.50 (OPA-10), while discriminatory ability ranged between 6.94 (OPH-16) and 13.42 (OPA-1,OPA- 19, OPW-7).

The genetic distance among cultivars ranged from 19.1% to 40.6%, Maximum genetic distance estimate was observed between “Khoderiy” and “Santa Catrina” cultivars.

Cultivars displayed low genetic similarity between “Baashiky” and “Sorani” cultivars.

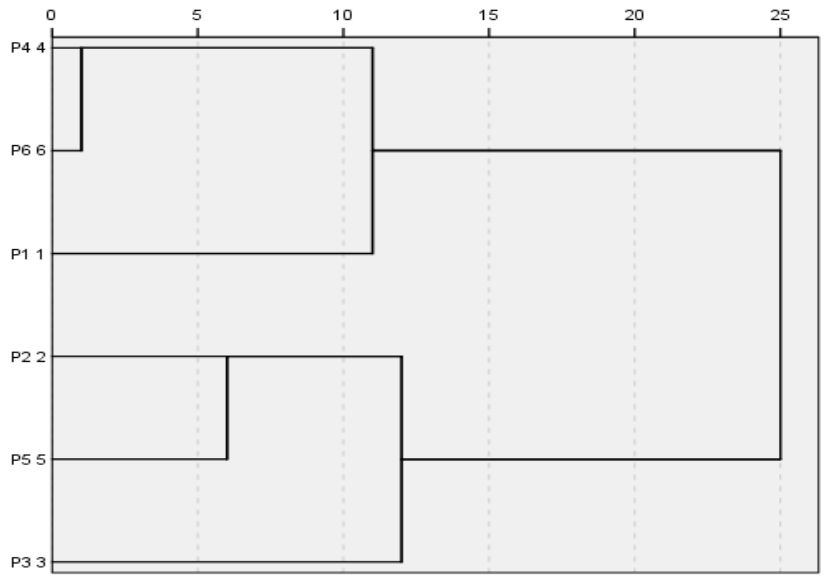
Table (3): The genetic distance of Olive (Olea europaea L.) cultivars.

	P1	P2	P3	P4	P5	P6
P1	**0**					
P2	**0.357**	**0**				
P3	**0.294**	**0.406**	**0**			
P4	**0.214**	**0.251**	**0.266**	**0**		
P5	**0.312**	**0.290**	**0.380**	**0.335**	**0**	
P6	**0.248**	**0.277**	**0.329**	**0.236**	**0.191**	

P1: "Cormal"**P2:** "Santa Catrina" **P3:** "Khoderiy"**P4.** "Ducal" **P5:** " Sorani" **P6:** "Baashiky"

The dendrogram that results from UPGMA culture analyses show that examined cultivars can be divided to 2basic cluster;1st cluster included (Ducal”, “Baashiky”, “Cormal”) cultivars, and this cluster divided into onesub-cluster between “Ducal” and “Baashiky”, Cultivars.

The Second cluster contained (“Santa Catrina”, “Sorani” and “Khoderiy.) cultivars, and this cluster was divided into one sub-cluster between “Santa Catrina” and “Sorani” cultivars.



Figer (3): UPGM dendrogram showing the relationships among Olive genotypes.

P1: "Cormal" **P2:** "Santa Catrina" **P3:** "Khoderiy" **P4.** "Ducal" **P5:** " Sorani" **P6:** "Baashiky"

Conclusion

(RAPD) marker system showed capability to distinguish closely related accessions of Olive (Olea europaea L.) and is a significant tool for finding genetic links across cultivars because it isunaffected by environmental circumstances and allows fordirectgenotype determination.

References

1. Baldoni, L.; Tosti, N.; Ricciolini, C. and Belaj, A. (2006). Genetic structure of wild and cultivated Olives in the central Mediterranean basin. Ann. Bot. 98: 935- 942.
2. Belaj, A.; Rallo, L.; Trujillo, I. and Baldoni, I. (2001). Using RAPD and AFLP markers to distinquish individuals obtained by clonal selection of 'Arbequina and' manzanilla de sevilla' Olive. Hort science 39: 1566- 1570.
3. Claros, G. M.; Crespillo R.; Agilar, M. I. and Canovas, F. M. (2000). DNA fingerprinting and classification of genographically related genotypes of Olive tree (Olea europaea L.). Euphytical 116: 131- 142.
4. Fabbri, A.; Hormaza JI. Polito VS (1995). Random amplified polymorphic DNA analysis of Olive (Olea europaea L.) cultivars. J. Am. Soc. Hort. Sci. 120: 538- 542.
5. Gemas, V. J., Rijo-Johansen, M. J., Tenreiro, R., & Fevereiro, P. (2000). Inter-and intra-varietal analysis of three Olea europaea L. cultivars using the RAPD technique. The Journal of Horticultural Science and Biotechnology, 75(3), 312-319.
6. Grati- Kamoun, N.; Mahmood, F.; Rebai, A. and Gargouri, A. (2006). Genetic diversity of Tunisian Olive tree (Olea europaea L.) cultivars assessed by AFLP markers- Genet. Resour. Crop. Vol 53: 265- 275.
7. Grudman, H.; Schneider, C. D.; Hartung, F. D.; Daschner, and Pith, T. L. (1995). Discriminatory power of three DNA Typing techniques for plant aeruginosn. J. clin.Microbiol. 3: 528- 532.
8. Hassan, S. A.M.; Abd Allaif, A. M.; Heba, A. M. (2016). Assessment of Genetic stability of Micropropagated Olive (Olea europaea L.) cultivars using RAPD marker. International Journal of Pharm Tech Research. Vol 9(12): 816- 825.
9. Kangarloo, B.; Noor, M. Z.; Farahani. F.; Ghasemzadeh- Barakis; sheidai, M. (2016). An assessment of tissue culture induced genetic variability in olive (Olea europaea L.) using chloroplast rp 16 intron sequences and single primer amplification reaction (SPAR) markers, J. Hortic. Sci. Blote chnol. 719- 723.
10. Kaya, E. (2015). ISSR analysis for determination of genetic diversity and relationship in eight Turkish Olive (Olea europaea L.) cultivars. Not. Bot. Hori. Arobo. 43 (1): 96- 99.
11. Kockar, F. and Llikci, R. (2003). Investigations, of genetic variation between Olive (Olea europaea L.) cultivar using arbitrarily primed polymerase chain reaction (AP- PCR). Nature forsch. 58: 837- 842.
12. Li, CD.; Fatokum, C. A.; Vbi, B. and Singh, B. B. (2001). Determining genetic similarities and relation ships among cowpea breeding lines and cultivars by microsatellite markers. Crop. Sci. 41: 189- 197.

13. Lu, Z. X.; Reighard, G. C.; Baird, W.V.; Abbott, A. G. and Rajapakse, S. (1996). Identification of peach root stock cultivars by RAPD marker. Hort. science, 31: 127- 129.
14. Mekuria, G.T.; Collins, G.; Sedgley, M. (2004). Genetic diversity within and isolated Olive (Olea europaea L.) population in relation to teral spread. Sci. Hort. 94: 91- 105.
15. Motawei, M. I., Al-Doss, A. A., & Moustafa, K. A. (2007). Genetic diversity among selected wheat lines differing in heat tolerance using molecular markers. Journal of Food Agriculture and Environment, 5(1), 180.
16. Muzher, B. M.; Sharaf, A. N. and Abdallah, N. A. (2014). Genetic Relationships Among some pears Genotypes Usign RAPD and AFLP Molecular Markers. Scientific Journal of king faisal university, 15(2): 1-17.
17. Nei, M. and Li, W. H. (1979). Mathmatical model for studing genetic variation interms of restriction endonucleases math. Acad. Sci. USA 76: 5269- 5273.
18. Omar, R. J. (2012). Morphological and Genetical Characterisation of the main Palestinian olive (Olea europaea L.) cultivars (Doctoral dissertation). Faculty of Graduate studies, An-Najah National University, Nablus, Palestine.
19. Owen, C. A.; Bita, E. C.; Banila, G. and Hajjar, S. E. (2005). AFLP reveals structural details of genetic diversity within cultivated Olive gerplasm from the Eastern Mediterranean. Theor. APPl. Genet. 110: 1169- 1176.
20. Peyandi, M.; Monsef, M.; Mazinani, M. H. (2013). Assessment of genetic stability of olive in vitro propagated by RAPD marker. J. Cell Mol. Res., 5(2): 81- 86.
21. Rallo, P.; Dorado, G.; Baldoni, L.; and Martin, A. (2003). Explorando el origin del Olivo mediante el so de marcadores microstatelites www.Expoliva.com/expoliva2003/symposium/comunicoiones/Oi/-24-textopdf/Expoliva2003.feria international del accite de Olive. Jan 14-17 de Mavo.
22. Sanz-Cortés, F., Parfitt, D. E., Romero, C., Struss, D., Llácer, G., & Badenes, M. L. (2003). Intraspecific olive diversity assessed with AFLP. Plant breeding, 122(2), 173-177.
23. Sensi, E., Vignani, R., Scali, M., Masi, E., & Cresti, M. (2003). DNA fingerprinting and genetic relatedness among cultivated varieties of Olea europaea L. estimated by AFLP analysis. Scientia Horticulturae, 97(3-4), 379-388.
24. Sesli, M. and Dil, E. (2009). RAPD-PCR analysis of cultured type Olives in Turkey Afric. Biotech. 8(15). 3418- 3423.
25. Sesli, M. and Yegenoglu, E. D. (2010). RAPD assay of wild- type Olives in Turkey. Genet. Mol. Res. 9(2): 966- 972.
26. Tingey, S. V. and Tufo Del, J. P. (1993). Genetic analysis with RAPD markers. Plant physiology. 101: 349-352.
27. Welsh, J. and Meclelland, M. (1990). Fingerprinting genomes using PCR with arbitray primers. Nucleic acids research 18: 7213- 7218.

28. Williams, J. G.; Hanafey, M. K.; Rafalski, J. A. and Tingey, S. V. (1993). Genetic analysis using random amplified polymorphic DNA markers. Methods in Enzymology, Plant Physiol. 218: 704- 741.
29. Wu, S. B.; Collins, G. and Sedglely M. (2004). Amolecular linkagemap of Olive (Olea europaea L.) based on RAPD, micro satellite and SCAR markers Genome 47: 26-35.